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Determination of veterinary drugs in foods of animal origin by QuEChERS coupled with ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS)

Qianran Sun [a], Jun Liu [a,*], Yuan Gou [a], Tieyuan Chen [a], Xiaofang Shen [b], Tao Wang [a], Yongli Li [a], Huizhen He [a], Huidan Deng [a], Yi Hua [a]

[a] Technology Center, Chengdu Customs, Chengdu 610041, China
[b] School of Food Science and Technology, Jiangnan University, Wuxi, 214122, China

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ABSTRACT

A method using QuEChERS coupled with ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was developed for the determination of the residues of 19 veterinary drugs in ten animal-derived matrices, including beef, pork, sheep, horse, chicken, prawn, fish, liver, milk, and fat. This method was based on the enactment of veterinary drug compounds by Korea, Canada, the United States, and the European Union in recent years. The samples were extracted using 85% acetonitrile and separated on an ACQUITY UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm) with a gradient elution of methanol-0.2% formic acid water as the mobile phase. The detection of the analytes was achieved through the use of positive ion electrospray ionization (ESI) and multiple reaction monitoring (MRM) modes, while the quantification was conducted via the matrix-matched external standard method. Following optimization, the linearity of the target veterinary residues in the ten matrices was observed to be satisfactory, having a range of 0.5–50.0 ng/mL ($R^2 > 0.991$). The limits of detection (LOD) were in the range of 0.01–1.29 μg/kg, while the limits of quantification (LOQ) were in the range of 0.02–4.31 μg/kg. The recoveries were observed to be in the range of 60.6–117.7 %, with relative standard deviations (RSDs) of ≤20.6 %. The method is straightforward and highly sensitive, and it satisfies the maximum limits set by the relevant standards of Korea, Canada, the USA, and the EU. It is well-suited for the rapid screening, qualitative, and quantitative analyses of metomidate, acetanilide, dl-methylephedrine, and other substances in foods of animal origin, providing technical assistance for cross-border food safety and testing.

1. Introduction

As the global demand for foodstuffs derived from animals continues to escalate, the concern surrounding the residues of veterinary drugs also rises. The presence of drug residues in food is typically attributed to the use of veterinary drugs in animal husbandry. In recent times, there have been a number of instances where veterinary drugs have been misused and abused, with the intention of enhancing the growth and feed efficiency of livestock [1]. Veterinary drug residues are defined as drug prototypes, metabolites, and drug impurities of toxicological significance. These residues accumulate or are stored in the edible parts of the food obtained from animals, including cells, tissues, or organs, or that enter the eggs of laying poultry or the milk of lactating animals, following the administration of veterinary drugs (including drug additives). Prolonged ingestion can result in toxic effects on the human body [2], including carcinogenesis, organ dysfunction, drug resistance, and other adverse consequences [3–5]. To prevent these outcomes, pertinent regulations and standards regarding the maximum residue limit (MRL) of veterinary drugs have been promulgated in numerous countries(European, [6]).

The ongoing development and approval of novel veterinary pharmaceuticals has led to an expansion in the range of veterinary drugs present in foodstuffs. Consequently, the MRL for these drugs are subject to frequent updates and dynamic changes. To illustrate, drugs such as acetanilide, pentetrazol, methyl ephedrine hydrochloride, antipyrine, and guaifenesin are listed in the Korean Positive List with the corresponding specified limits in the respective animal-derived matrices. However, this is not the case in China, the European Union (EU),

* Corresponding author.
E-mail address: liujuncdhg@163.com (J. Liu).

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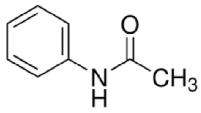
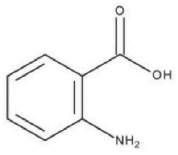
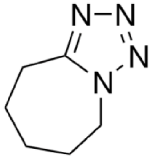
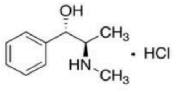
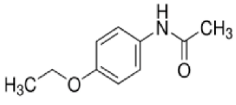
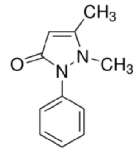
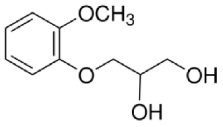
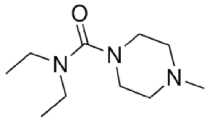
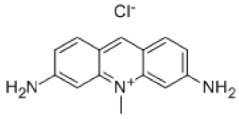
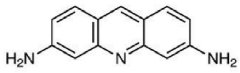
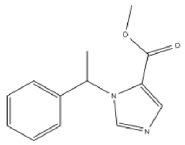
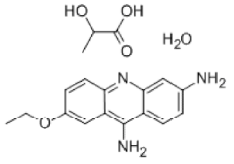
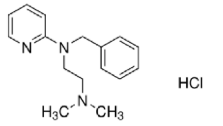
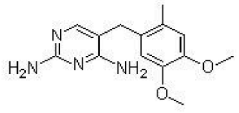
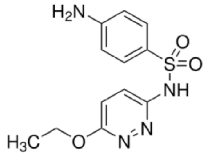
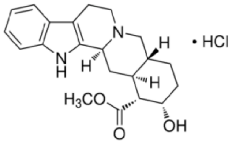
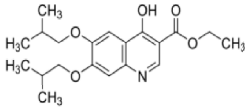
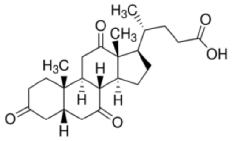
Table 1
CAS number, molecular weight, solvent, chemical structure, and pharmacologically use of 19 veterinary residues.

NO.	Compound	CAS	Formula	Purity(%)	Solvent	Molecular weight	Chemical structure	Pharmacologically use
1	Acetanilide	103-84-4	C_8H_9NO	99.9	Acetonitrile	135.06		Aniline derivatives with analgesic and antipyretic properties
2	o-Aminobenzoic acid (Anthranilic acid)	118-92-3	$C_7H_7NO_2$	99.9	Methanol	137.04		Intermediate for the synthesis of various drugs such as anti-inflammatory drugs and hypnotic drugs
3	Pentetrazol (Pentylenetetrazol)	54-95-5	$C_6H_{10}N_4$	99.9	Acetonitrile	138.09		A circulatory and respiratory stimulant
4	Methyl ephedrine hydrochloride(dl-Methylephedrine HCl)	18760-80-0	$C_{11}H_{17}NO$* HCl	99.9	Methanol	215.72		A non-selective adrenergic receptor agonist
5	Phenacetin	62-44-2	$C_{10}H_{13}NO_2$	99.9	Methanol	179.09		An analgesic and fever-reducing drug
6	Antipyrine (Phenazone)	60-80-0	$C_{11}H_{12}N_2O$	98.0	Methanol	188.09		A nonsteroidal anti-inflammatory drug
7	Guaifenesin	93-14-1	$C_{10}H_{14}O_4$	99.3	Methanol	198.02		Expectorants
8	Diethylcarbamazine	90-89-1	$C_{10}H_{21}N_3O$	99.8	Methanol	199.16		Treatment of certain worm infections

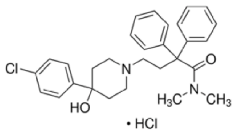
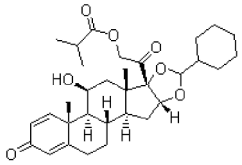
(*continued on next page*)

Table 1 (*continued*)

9	*Acriflavine(8048-52-0)	3,6-diamino-10-methylacridinium chloride	86-40-8	$C_{14}H_{14}N_3Cl$	99.9	Methanol	259.73		An antiseptic agent
		3,6-Acridinediamine(Proflavine)	92-62-6	$C_{13}H_{11}N_3$		Methanol	209.25		
10	Metomidate		5377-20-8	$C_{13}H_{14}N_2O_2$	99.0	Methanol	230.1		A sedative-hypnotic drug
11	Ethacridine lactate monohydrate (Acrinol)		6402-23-9	$C_{15}H_{15}N_3O*C_3H_6O_3*H_2O$	94.6	Methanol	361.39		An antiseptic agent
12	Tripelennamine hydrochloride		154-69-8	$C_{16}H_{21}N_3*HCl$	99.9	Methanol	291.82		An antipruritic and first-generation antihistamine
13	Ormetoprim		6981-18-6	$C_{14}H_{18}N_4O_2$	99.9	Methanol	274.14		An antimicrobial agent
14	Sulfaethoxypyridazine		963-14-4	$C_{12}H_{14}N_4O_3S$	99.0	Acetonitrile	294.07		An antimicrobial agent
15	Yohimbine hydrochloride		65-19-0	$C_{21}H_{26}N_2O_3*HCl$	97.7	Methanol	390.91		An alpha 2 adrenergic receptor antagonist
16	Buquinolate		5486-03-3	$C_{20}H_{27}NO_5$	98.3	Methanol	361.43		An anticoccidial agent
17	Dehydrocholic acid		81-23-2	$C_{24}H_{34}O_5$	99.6	Acetonitrile	402.24		A synthetic bile acid,hydrocholeretic

(*continued on next page*)

Table 1 (*continued*)

18	Loperamide hydrochloride	34552-83-5	$C_{29}H_{33}ClN_2O_2$*HCl	98.7	Methanol	513.5		An antidiarrheal agent
19	Ciclesonide	126544-47-6	$C_{32}H_{44}O_7$	99.3	Acetonitrile	540.3		A glucocorticoid used to treat asthma and allergic rhinitis

*Commercially purchased acriflavine is a mixture of 3,6-diamino-10-methylacridinium chloride and 3,6-Acridinediamine (Proflavine).

Canada. Limits have been established for tripelennamine hydrochloride in foods of animal origin in Korea and the United States (US). However, these limits are not mentioned in the Chinese standards GB 31,650–2019, GB 31,650.1–2022, and Announcement No. 250 of the Ministry of Agriculture and Rural Affairs. Furthermore, there is a dearth of corresponding detection methods for these drug residues. The abovementioned limits are also not covered in Canada and the EU. The limits for ormetoprim and buquinolate are included in Canada's Maximum Residue Limits (MRLs) for veterinary drugs in foods. However, these limits have not been established in Korea, the EU, or the US. Ciclesonide is only referenced in the EU Regulation (EU) No 43/2020, and it has not been identified in other countries. In order to enhance the efficacy of veterinary drug testing standards and facilitate the acceleration of global trade, it is imperative to address the technical barriers to trade encountered by enterprises engaged in export activities. In this context, the standards of the corresponding veterinary drug detection

Table 2
Retention times and optimum operating mass spectrometric parameters for analysis of target veterinary drugs.

NO.	Analyte	Retention time	Ion source	Adduct ion composition	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
1	Diethylcarbamazine	4.94	ESI+	$[M+H]^+$	200.07	100.07*	2	14
					200.07	72.06	2	22
2	Methyl ephedrine hydrochloride	5.61	ESI+	$[M\text{-HCL}+H]^+$	180.10	147.04*	4	16
					180.10	117.07	4	18
3	Pentetrazol	6.17	ESI+	$[M+H]^+$	138.99	69.08*	28	22
					138.99	55.10	28	20
4	Ormetoprim	6.47	ESI+	$[M+H]^+$	275.03	123.08*	58	24
					275.03	81.03	58	42
5	Tripelennamine hydrochloride	6.99	ESI+	$[M\text{-HCL}+H]^+$	256.05	119.18*	2	32
					256.05	91.04	2	30
6	Antipyrine	7.07	ESI+	$[M+H]^+$	189.01	56.08*	2	22
					189.01	104.06	2	26
7	o-Aminobenzoic acid	7.13	ESI+	$[M+H]^+$	137.94	92.07*	12	20
					137.94	65.05	8	24
8	3,6-Acridinediamine	7.14	ESI+	$[M+H]^+$	209.96	193.03*	44	30
					209.96	182.02	44	30
9	Yohimbine hydrochloride	7.25	ESI+	$[M\text{-HCL}+H]^+$	355.03	144.05*	6	28
					355.03	212.11	6	22
10	Acetanilide	7.37	ESI+	$[M+H]^+$	135.96	77.02*	18	20
					135.96	94.06	18	22
11	Sulfaethoxypyridazine	7.45	ESI+	$[M+H]^+$	295.08	156.00*	30	19
					295.08	140.00	30	20
12	Guaifenesin	7.52	ESI+	$[M+H]^+$	198.99	125.03*	28	8
					198.99	163.06	28	6
13	3,6-diamino-10-methylacridinium chloride	7.65	ESI+	$[M\text{-HCL}+H]^+$	223.91	182.02*	20	36
					223.91	209.05	20	30
14	Phenacetin	8.28	ESI+	$[M+H]^+$	180.10	110.20*	30	20
					180.10	138.00	30	30
15	Ethacridine lactate monohydrate	8.45	ESI+	$[M\text{-}C_3H_6O_3\text{–}H_2O+H]^+$	253.96	197.11*	20	30
					253.96	225.97	20	34
16	Metomidate	8.56	ESI+	$[M+H]^+$	230.97	126.99*	2	20
					230.97	95.01	2	8
17	Loperamide hydrochloride	9.31	ESI+	$[M\text{-HCL}+H]^+$	477.23	266.20*	50	25
					477.23	210.00	50	45
18	Dehydrocholic acid	9.67	ESI+	$[M+H]^+$	403.08	349.22*	18	20
					403.08	367.23	18	16
19	Buquinolate	11.3	ESI+	$[M+H]^+$	362.02	204.00*	10	38
					362.02	260.04	10	28
20	Ciclesonide	12.9	ESI+	$[M+H]^+$	541.31	323.20*	30	10
					541.31	523.20	30	20

* Monitored transition ions for quantification.

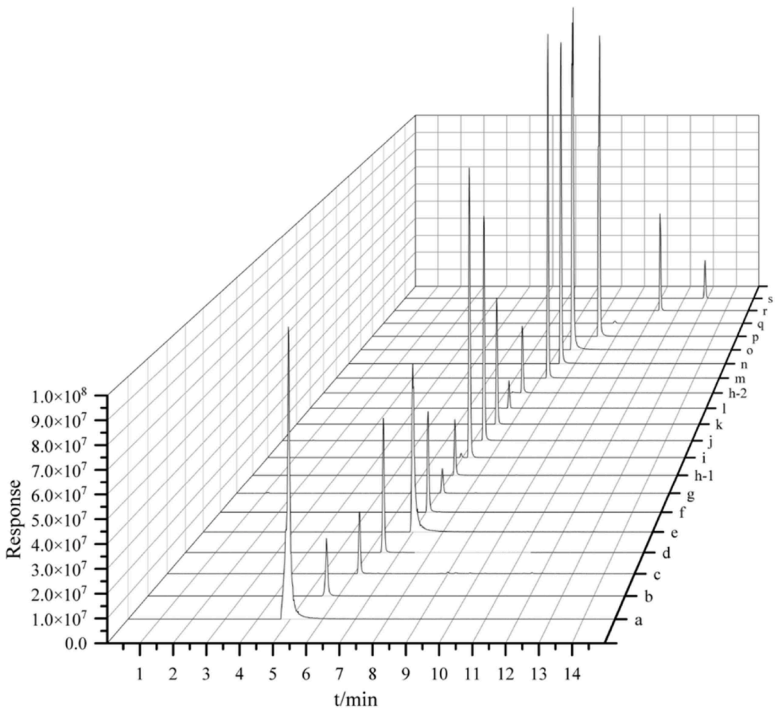


Fig. 1. The 19 target veterinary drug residues were separated effectively on the chromatogram (50ng/ml).
a. Diethylcarbamazine; b. Methyl ephedrine hydrochloride; c. Pentetrazol; d. Ormetoprim; e. Tripelennamine hydrochloride; f. Antipyrine; g. o-Aminobenzoic acid; h-1. 3,6-Acridinediamine; i. Yohimbine hydrochloride; j. Acetanilide; k. Sulfaethoxypyridazine; l. Guaifenesin; h-2. 3,6-diamino-10-methylacridinium chloride; m. Phenacetin; n. Ethacridine lactate monohydrate; o. Metomidate; p. Loperamide hydrochloride; q. Dehydrocholic acid; r. Buquinolate; s. Ciclesonide

methods need to be instantly updated and improved simultaneously. Based on the differences in the regulatory limits between different countries, it is imperative to establish a universal analysis method for the residues of these veterinary drugs which have established maximum residue limits in only some countries and lack matching detection method standards. In this regard, Chae et al. [7] conducted a study on the detection of dl-methylephedrine hydrochloride in porcine muscle using LC-MS/MS. A study on the detection of flumethasone, dl-methylephedrine, and 2-hydroxy-4,6-dimethylpyrimidine in porcine muscle and pasteurised cow milk using the LC-MS/MS method was conducted by Zhang et al. [8]. Compared to this study, the above-mentioned investigations detected fewer veterinary substances, and the justification of the matrix was not as comprehensive. This study is therefore prospective in terms of both the types of veterinary drugs and the matrices tested. To the best of the authors' knowledge, no studies have been published currently on the use of high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) methods for the detection of ciclesonide residues in foods of animal origin. Further comparative analyses with other relevant literature were presented in the comparison section(3.8) below.

A majority of pre-treatment purification methods for veterinary residues are based on solid-phase extraction (SPE), dispersive solid-phase extraction (DSPE), magnetic-solid phase extraction (M-SPE), dispersive liquid-liquid microextraction (DLLME), and liquid-liquid extraction (LLE) techniques [9–11]. At present, the utilisation of solid-phase extraction columns is relatively widespread. However, the series of operations, such as column activation, loading, washing, and elution, used in this pretreatment method are time-consuming and require a large number of reagents. Therefore, QuEChRES-DSPE was proposed as a pretreatment clean-up material [10]. This method requires straightforward operation, involves less solvent, effectively prevents sieve plate clogging due to complex matrices, and enhances the efficiency of pretreatment and purification of large-volume samples. The study involved ten matrices, namely beef, chicken, pork, prawn, fish, etc. The influencing factors, such as chromatographic conditions, column selection, extraction solvent, purification powder, and centrifugation temperature, were optimized. The evaluation of specificity, linearity, matrix effect, limit of detection (LOD), limit of quantification (LOQ), recovery, and precision was employed as the basis for establishing a QuEChERS DSPE-UPLC-MS/MS method for the determination of 19 veterinary drug residues in foods of animal origin. This method provides a basis for the safety detection and market supervision of foods of animal origin and offers technical support for strengthening cross-boundary food safety assurance. The method is efficient, accurate, economical, and suitable for the rapid screening, confirmation, and quantitative detection of a wide range of matrices in animal-derived foods.

2. Material and methods

2.1. Reagents and chemicals

Methanol (MeOH) and acetonitrile (ACN) of LC-MS grade were purchased from CNW Technologies GmbH (Dusseldorf, Germany). Ammonium acetate (CH_3COONH_4), ammonium formate($HCOONH_4$), formic acid (HCOOH), and ammonium hydroxide ($NH_3{\cdot}H_2O$) were of HPLC grade, obtained from Chengdu Kelong Chemical Co. Ltd. (Sichuan, China). Analytical-grade sodium chloride (NaCl), C18 adsorbent (40–63 μm, C18), anhydrous sodium sulfate (Na_2SO_4) and anhydrous magnesium sulphate ($MgSO_4$) were purchased from Anpel Laboratory Technologies (Shanghai, China). Additionally, the water used in the analyses was obtained from a Mili-Q Integral 3 ultrapure water device manufactured by Millipore Corporation (Boston, USA).

Buquinolate (10mg, 98.3%, CAS 5486–03–3), and Ciclesonide(100 mg, 99.3%, CAS 126,544–47–6) were procured from Bejing manhage bio-technology company(Bejing, China). All the other standards were liquid reference materials with a purity of $\geq$ 94.6%, sourced from Alta scientific co. ltd. (Tianjin, China). Further details regarding the target veterinary residues, including the chemical abstract service (CAS) number, molecular weight, solvent, chemical structure, and

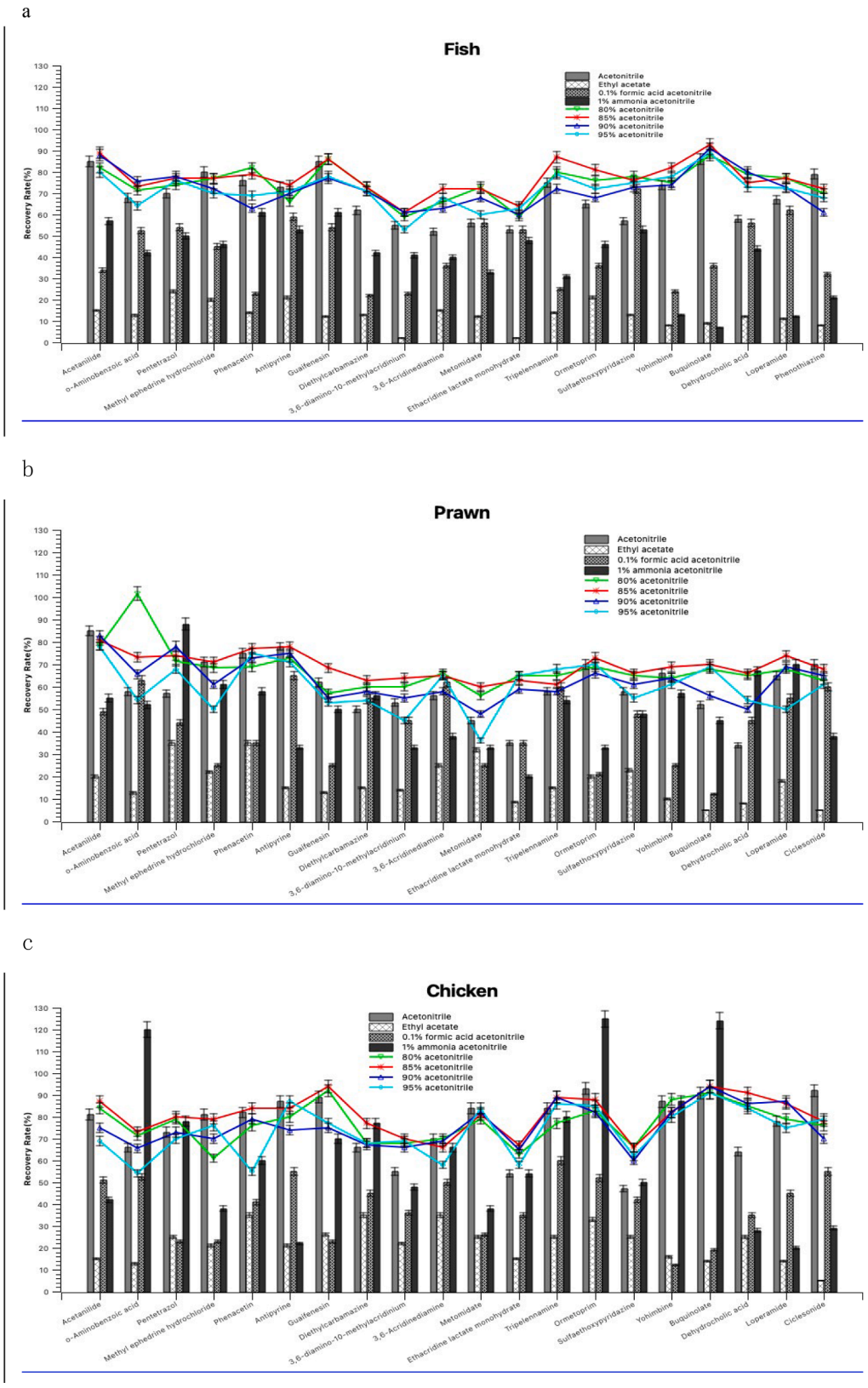
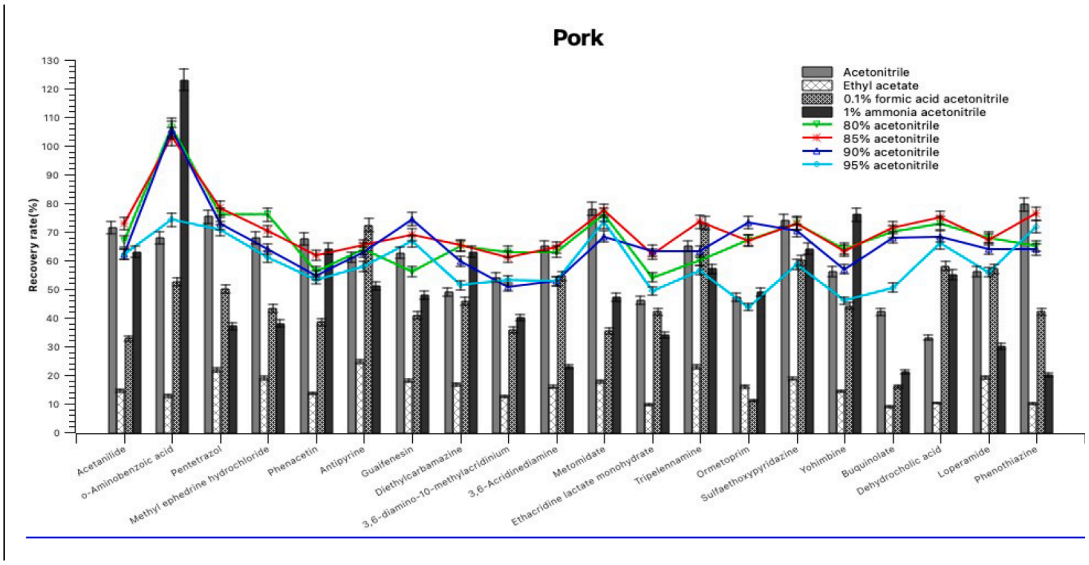


Fig. 2. Effect of optimising the choice of extraction solvents on the recovery of 19 veterinary drugs.

d



e

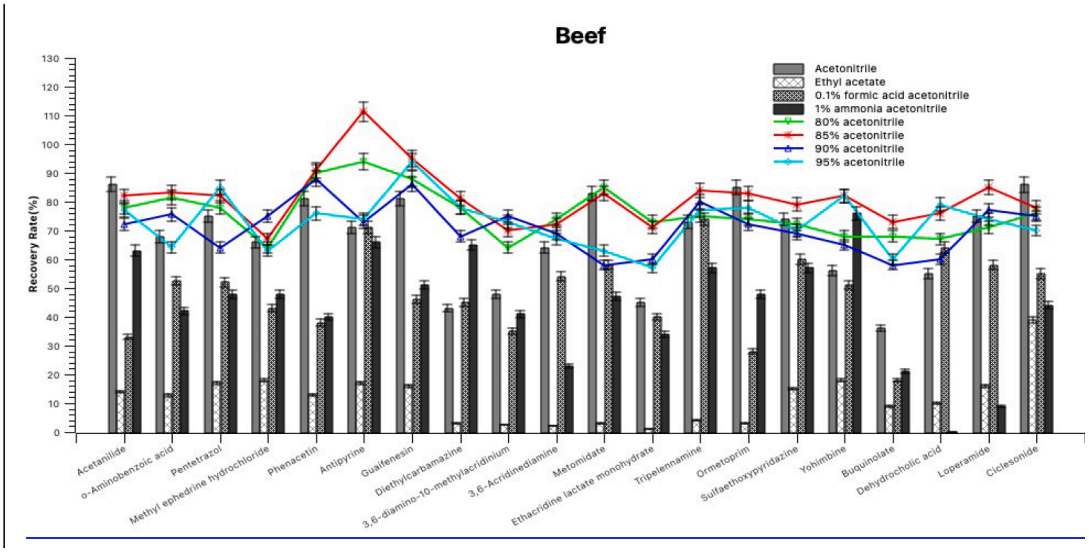


Fig. 2. (*continued*).

pharmacologically use are provided in Table 1.

Most notably, acriflavine is a known mixture of the parent compound (3,6-diamino-10-methylacridinium chloride) and the demethylated derivative, proflavine, as indicated by the purchased standard [12,13]. The concentration ratio of chlorinated 3,6-diamino-10-methylacridine to 3, 6-acridinediamine was 2:1. The chromatographic columns exhibited distinct peaks and retention times. Consequently, these substances were treated as two separate substances in the following analyses.

2.2. Standard solutions

2.2.1. Preparation of standard stock solution

0.01 g of ciclesonide and buquinolate were weighed accurately in a 10 mL volumetric flask. Acetonitrile and methanol were added to dissolve and dilute the standard to the scale, respectively. The individual standard stock solution was prepared at a mass concentration of 1000 μg/mL and stored at −20 °C, protected from light. The remaining liquid standards were accurately pipetted at 1 mL each, with the addition of methanol or acetonitrile to achieve a final volume of 10 mL. The standard stock solutions were then prepared with a mass concentration of either 10 μg/mL or 100 μg/mL, and stored at −20 °C in a dark environment for future use. An appropriate amount of the standard stock solutions of the abovementioned veterinary residues was accurately measured, diluted with methanol, and formulated into a mixed standard working stock solutions with a mass concentration of 1 μg/mL. Subsequently, a series of working standard solutions were formulated at concentrations of 0.5, 1.0, 2.0, 3.0, 5.0, 10.0, 20.0, and 50.0 ng/mL, for UPLC-MS/MS detection.

2.2.2. Preparation of blank matrix curves

The mixed standard working solution was quantified and incorporated into the residues of eight extracted and purified blank samples (the pretreatment method was identical to that described in Section 2.3), and then blown to dryness under nitrogen at 50 °C. 1 ml of methanol was

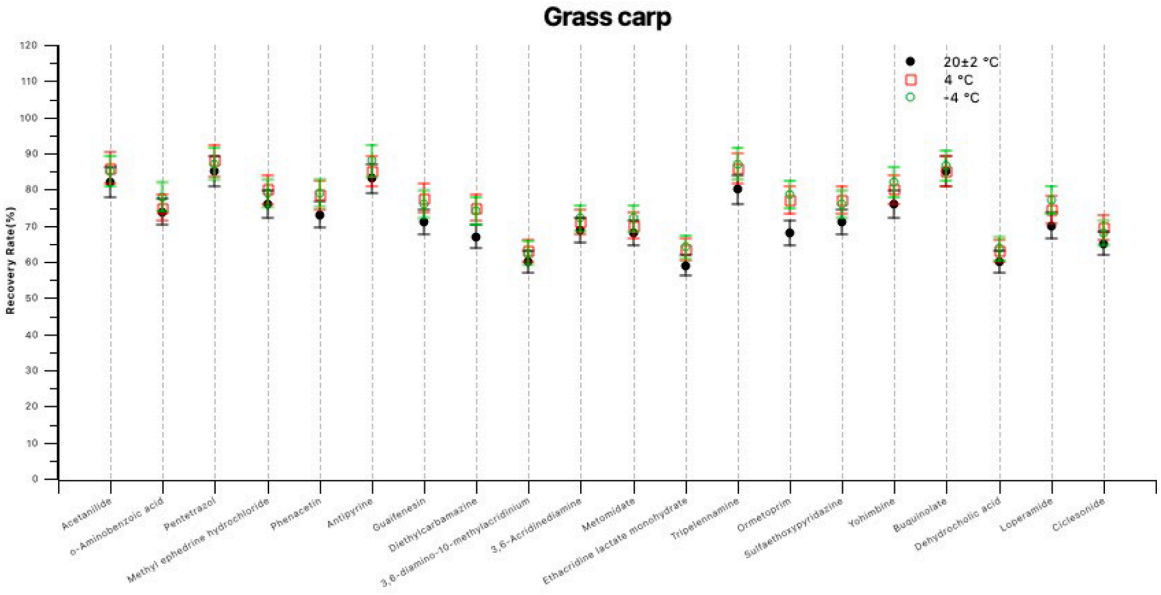


Fig. 3. Recoveries of 19 veterinary residues at different optimized centrifugation temperatures (take grass carp for example).

added to the resulting samples, followed by vortexing and dissolution. The resulting solutions were then synthesized to form a matrix-matched series of mixed standard solutions at concentrations of 0.5, 1.0, 2.0, 3.0, 5.0, 10.0, 20.0, and 50.0 ng/mL. The solutions were poured through a 0.22-μm filter membrane for UPLC-MS/MS on-line determination. The standard curve was plotted with the measured characteristic ion peak area as the vertical coordinate and the corresponding standard solution concentration as the horizontal coordinate.

2.3. Sample preparation

A total of 50 or more batches of muscle samples of cattle(Muscle), swine(Muscle), sheep(Muscle), horse(Muscle), chicken(Muscle), fish (Grass carp, Skin and Muscle), prawn(Muscle), liver(pork liver), milk (cow milk), and fat(lard oil) were used for the experiments. All the samples were obtained from local markets or supermarkets. Each sample was homogenized in a homogenizer and stored at −20 °C.

2.3.1. Beef, sheep, horse, chicken, fish, prawn, liver, milk, and fat samples

2.0 g of the sample was accurately weighed and placed individually into 50-ml screw-capped centrifuge tubes. Subsequently, the tubes were spiked with an appropriate quantity of mixed standard solutions and kept in 4 °C overnight. This process enables the solution to penetrate completely into the tissue cells(the fat samples were heated at 60 °C in a water bath until they melted). A volume of 10 mL of 85% acetonitrile was added to the sample(the addition of 1 g NaCl for milk samples), which was then extracted by shaking for 5 mins. The sample was then extracted by an ultrasonic water bath for 15 mins, after which it was centrifuged at 10,000 r/min at 4 °C for 5 mins.

The supernatant was added to a purification tube containing 100 mg of C18 and 1100 mg of anhydrous magnesium sulfate, vortexed and mixed for 2 mins, and then subjected to freeze-centrifugation at 10,000 r/min and 4 °C for 5 mins. The final supernatant was transferred to a 15 mL glass nitrogen blowing tube and blown to dryness at 45 °C. Finally, 1 mL of methanol was accurately added, vortexed to dissolve the residue, and filtered through a 0.22-μm microporous membrane for UPLC-MS/MS on-line analysis.

2.3.2. Pork samples

2.0 g of the sample was accurately weighed and placed individually into 50-ml screw-capped centrifuge tubes. Subsequently, the tubes were spiked with an appropriate quantity of mixed standard solutions and allowed to stand for 30 mins at room temperature, which enables the solution to penetrate completely into the tissue cells. A volume of 10 mL of 85% acetonitrile was added to the sample, which was then extracted by shaking for 5 mins. The sample was subsequently extracted by an ultrasonic water bath for 15 mins, after which it was centrifuged at 10,000 r/min at 4 °C for 5 mins. The supernatant was combined with 5 ml of hexane saturated with acetonitrile, vortexed for 2 min, and then freeze-centrifuged at 10,000 r/min 4 °C for 5 mins.

The acetonitrile layer was transferred to a purification tube containing 100 mg of C18 and 1100 mg of anhydrous magnesium sulfate, vortexed for 2 min, and then freeze-centrifuged at 10,000 r/min 4 °C for 5 min. The final supernatant was pipetted into a 15-mL glass nitrogen blowing tube and nitrogen-blown to a clean dry state at 45 °C. 1 mL of methanol was added accurately to dissolve the residue and then passed through a 0.22-μm filter membrane for UPLC-MS/MS analysis.

2.4. Instrumentation

2.4.1. Chromatographic conditions

The detection of 19 veterinary drug residues was performed on a UPLC system (UPLC l-class-XEVOTQ-XS, Waters Corporation, USA). The chromatographic separation was performed on an Acquity UPLC HSS T3 (2.1 mm*100 mm, 1.8 μm, Waters Corporation, USA).

Methanol (mobile phase A) was used as the organic phase, and 0.2% formic acid (HCOOH) in distilled water (mobile phase B) was used as the aqueous phase. The optimal elution conditions for the mixture of veterinary drug residues used were 5 % A at 0–2 min, 5–90 % A at 2–10 min, 90–95 % A at 10–10.1 min, 95 % A at 10.1–12 min, 95–5 % A at 12–12.5 min, and 5 % A at 12.5–15 min. The total duration of the experiment was 15 mins. Moreover, the flow rate was 0.25 mL/min, and the injection volume was 3.0 μL.

2.4.2. Detection conditions

Mass spectrometry was conducted in the electrospray positive ion (ESI+) mode. The electrospray capillary voltage was set at 3.00 kV, while the ESI+ cone voltage was fixed at 30 V. The dissolution gas flow rate was adjusted to 1000 L/h, while the collision gas flow rate was set at 0.15 mL/min at a dissolution temperature of 500 °C. Additionally, a qualitative analysis of each compound was conducted in the multiple reaction monitoring mode (MRM).

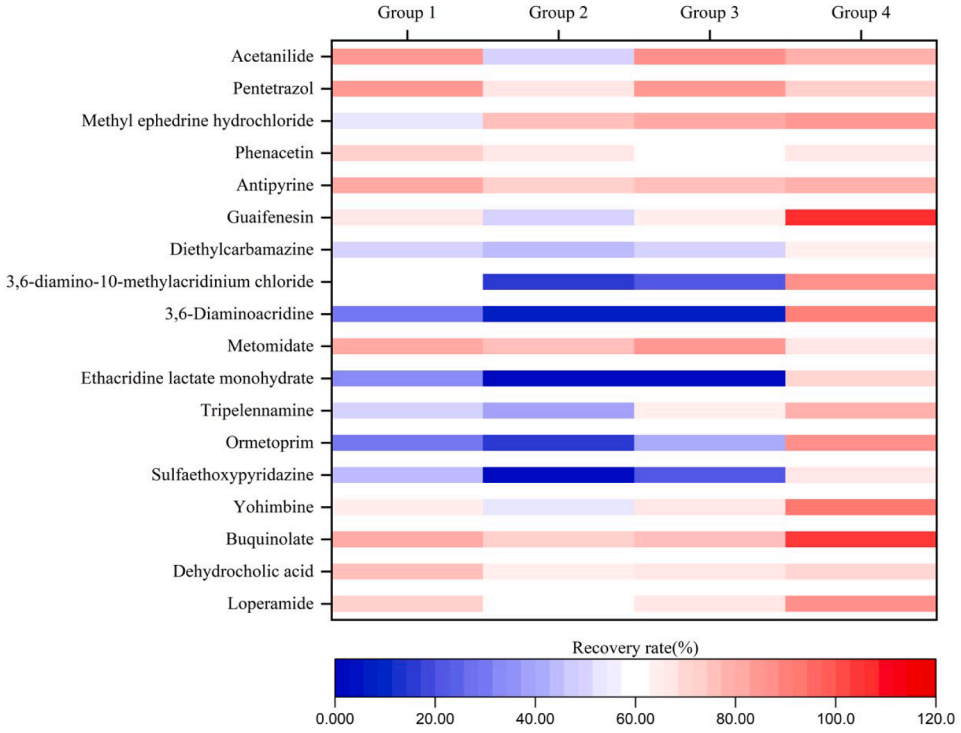


Fig. 4. Recoveries of veterinary residues at different types of purified powder(take liver for example). Group1: 80 mg C18+1000 mg $MgSO_4$+80 mg PSA; Group2: 80 mg C18+1000 mg $MgSO_4$+40 mg GCB; Group3: 80 mg C18+1000 mg $MgSO_4$+80 mg PSA+40 mg GCB; Group4: 80 mg C18+1000 mg $MgSO_4$.

The UPLC-MS/MS system was controlled by using the MassLynx™ software. Data were processed through the TargetLynx™ software (both from Waters) to obtain standard curves, linear equations, and calculated results. Microsoft Excel was employed to process the data and draw graphs. The spectra were plotted by using SciDAVis and Origin.

3. Results and discussion

3.1. Optimisation of detection conditions

A single standard solution (100 ng/mL) was selected for the experiment to determine the precursor ions of the analyte in both positive and negative ion modes. Based on the optimised detector voltage conditions, the two highest-responding fragment ions were selected as their respective product ions. As per a previous study, the most common ion adducts observed in positive ion electrospray ionization (ESI+) mass spectrometry are $[M + H]+$, $[M+NH_4]+$, $[M+Na]+$, $[M + K]+$ $[2M+H]+$, $[2M+NH_4]+$, $[2M+Na]+$ [14]. Ionised acidic residues(Sulfonylamino, carboxyl, phenolic hydroxyl, etc.) readily form ion pairs with positive ions such as Na^+ and NH_4^+[15–17].The results revealed that most of the target veterinary drug residues formed $[M + H]+$ adducts (Table 2), with higher responses in the positive ion mode. The selection of the positive ion mode for the detection of substances with similar aniline or amino (-NH_2) structures was reported by Palm et al. [18], and Mezghich et al. [19]. The optimal mass spectrometry parameters of each target substance are presented in Table 2.

3.2. Chromatographic column selection

The separation of 19 target veterinary residues on ACQUITY UPLC BEH C18 (100 mm × 2.1 mm, 1.7 μm, Waters, USA), ACQUITY UPLC HSS T3 (2.1 mm × 100 mm, 1.8 μm, Waters, USA), CAPCELL PAK C18 MGIII (2.0 mm × 100 mm, 5 μm, Shiseido, Japan), and InertSustain C18 (2.1 mm × 150 mm, 3 μm, GL Science, Japan) columns was compared. Although the ACQUITY UPLC BEH C18 column is commonly employed as a general-purpose ultra-high performance chromatographic column for a diverse array of analytes, the separation of the target veterinary residues was found to be suboptimal. The compounds demonstrated comparable performance on both the InertSustain C18 and MGIII columns. Specifically, dehydrocholic acid and 3,6-acridinediamine presented elevated baselines on the InertSustain C18 column. In contrast, the HSS T3 column demonstrated a superior peak shape and response. The HSS T3 stationary phase is a C18 stationary phase that is compatible with a 100% aqueous mobile phase [20,21], which is more suitable for the retention of water-soluble and polar small organic compounds. A majority of the target veterinary residues possess a high degree of polarity. Walter et al. [22] employed a combination of six polar compounds to assess the retention and selectivity of distinct column stationary phases. The authors state that for the positively charged polar analytes, the HSS T3 column exhibited the most retention. Moreover, Medellín-Martínez et al. [23] used ACQUITY UPLC HSS T3 to separate clenbuterol residues from beef. Therefore, the T3 column was chosen as the analytical column for the experiment.

3.3. Optimisation of mobile phases

In general, polar solvents (e.g., water, methanol, acetonitrile, etc.) are employed in ESI-MS experiments owing to their propensity to undergo electrochemical reactions in the spraying nozzle [24]. The peak shape behaviors obtained using methanol and acetonitrile were compared. It was observed that the methanol/water mobile phase exhibited a lower elution capacity than the acetonitrile/water mobile phase. Consequently, the retention time of the target veterinary residues using the methanol/water mobile phase was prolonged, thereby facilitating the separation of the chromatographic peaks. Following a comprehensive evaluation, methanol was selected as the organic phase.

The separation effects and response discrepancy of different water phases for each target were investigated using 0.1% HCOOH, 0.2% HCOOH, 5 mmol/L $HCOONH_4$, 5 mmol/L CH_3COONH_4, and 5 mmol/L CH_3COONH_4 + 0.1% HCOOH as the mobile aqueous phase,

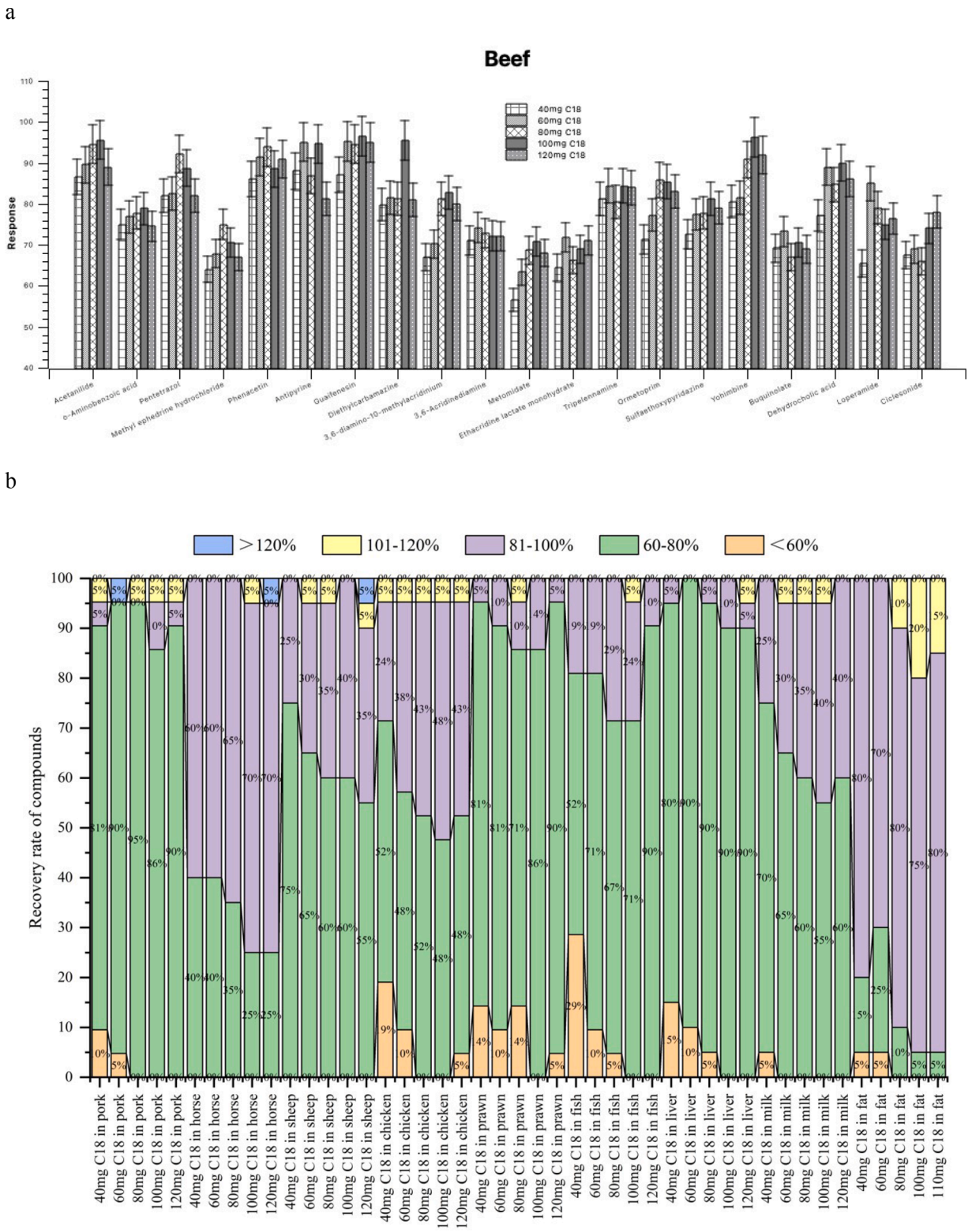


Fig. 5. Optimisation of C18 dosage for 19 veterinary residues in ten matrices.

respectively. The results demonstrated that the addition of HCOOH could effectively improve the peak shape and protonation ability, thereby facilitating the formation of the $[M+H]+$ peak and augmenting the response [25]. It was observed that compared with 0.1% HCOOH, 0.2% HCOOH exhibited a higher response and good peak shape. Therefore, methanol-0.2% formic acid water was finally selected as the mobile phase. Under optimal conditions, the rapid and complete separation of the 19 target veterinary drug residues was achieved on the chromatograms as shown in Fig. 1.

3.4. Optimization of sample extraction

3.4.1. Extraction solvent

Extraction reagents demonstrate a significant effect on the recovery,

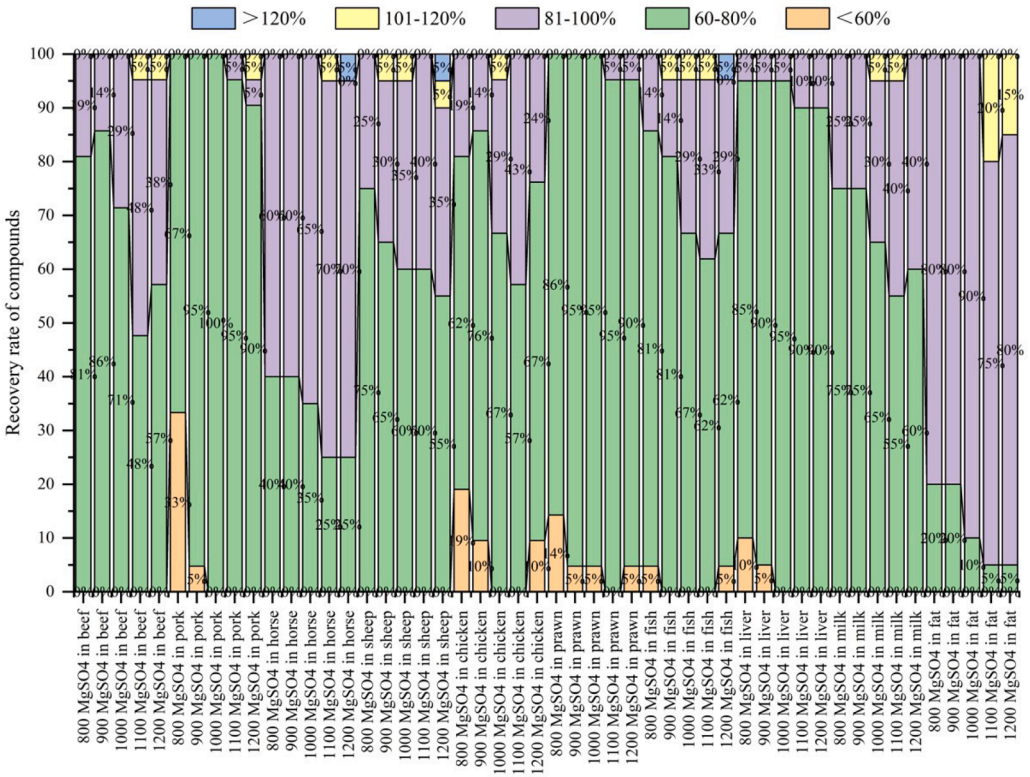


Fig. 6. Optimisation of anhydrous magnesium sulfate ($MgSO_4$) for 19 veterinary residues in ten matrices.

the time of appearance of the target peaks, and the peak shape of several veterinary drug residues. Commonly used extraction solvents in foods of animal origin include methanol, acetonitrile, and ethyl acetate [26,27]. Furthermore, the acidity or alkalinity of the extraction environment influences the efficiency of the extraction process with regard to the target substances. In order to compare the extraction efficiency of the substances in question, the study employed formic acid and ammonium acetate as buffer solutions [28]. Acetonitrile, ethyl acetate, 0.1% formic acid acetonitrile, and 1% ammonia acetonitrile were chosen as extraction solvents to compare the extraction effect. The results presented in Fig. 2a reveal that acetonitrile serves as an effective extraction solvent in fish matrices, exhibiting notable benefits. The recoveries of metomidate and acrinol in acetonitrile and 0.1% formic acid acetonitrile were comparable, whereas sulfaethoxypyridazine exhibited a higher recovery in the latter solvent. In the case of prawn(Fig. 2b), o-aminobenzoic acid, 3,6-acridinediamine, and tripelennamine were better recovered when 0.1% formic acid acetonitrile was used as the extraction solvent. However, guaifenesin, metomidate, buquinolate, ormetoprim, methyl ephedrine hydrochloride, and yohimbine were not suitable for extraction in acidic environments, with recoveries below 30%. The extractions of pentetrazol, diethylcarbamazine, dehydrocholic acid, and loperamide were more effective when ammonia acetonitrile was used, while the remaining substances demonstrated higher recoveries under acetonitrile conditions. In chicken(Fig. 2c), o-aminobenzoic acid, ormetoprim, and buquinolate showed an extremely high recovery efficiency (up to 120% or more) when extracted by 1% ammonia acetonitrile. On the other hand, acetanilide, methyl ephedrine hydrochloride, metomidate, antipyrine, and loperamide demonstrated lower recovery efficiencies (<50%). The recovery of all substances by using ethyl acetate was limited to a maximum value of 40 % by ethyl acetate. A majority of substances demonstrated a good recovery efficiency in acetonitrile. Similarly, acetonitrile demonstrated high extraction efficiencies in pork and beef matrices. However, such high values were not observed for buquinolate and dehydrocholic acid in pork(Fig. 2d), and diethylcarbamazine, 3,6-diamino-10-methylacridinium chloride, acrinol, and buquinolate in beef matrices(Fig. 2e).

Ethyl acetate also exhibited the worst recovery efficiency, which was hardly higher than 20%. Given that animal matrices contain a large amount of lipids, and many lipophilic compounds are readily extracted by ethyl acetate, the degree of matrix interference is high, resulting in a low extraction efficiency [29]. The extreme emulsification of ethyl acetate as an extraction solvent is also another possible factor. Acetonitrile exhibits good solubility and strong penetration, which can cause precipitation of proteins and effectively avoid over-extraction of lipids [30,31]. Therefore, it has the advantage of the ability to extract the target more effectively than other solvents in most matrices. However, proteins are subject to agglutination in organic solvents, and the recoveries of certain substances under acetonitrile extraction conditions were found to be unsatisfactory. The addition of an appropriate amount of purified water to the extraction reagent can reduce the proportion of acetonitrile and promotes sample dispersion, as well as decrease the rate of homogeneous agglomeration [32]. Furtherrmore, a mixture of acetonitrile and water is capable of extracting a wide polarity range of analytes from the matrix [33]. Therefore, the experiment was continued to compare the extraction efficiencies of 80%, 85%, 90% and 95% acetonitrile in Fig. 2. The results revealed that 85% acetonitrile exhibited good recovery efficiencies for the target substances in all the studied matrices, especially for buquinolate and dehydrocholic acid, with a significant enhancement effect. The subsequent optimized treatment could also improve the sensitivity of the method. Thus, 85% acetonitrile in water was finally chosen as the extraction solvent.

The high lipid content of pork necessitated the implementation of an additional extraction step. Animal fats are composed primarily of long-chain saturated fatty acids, which are distinguished by the presence of two groups: a polar carboxyl group and a non-polar hydrocarbon group. The solubility of the fatty acids in water is influenced by the length of the hydrocarbon group, with longer hydrocarbon groups exhibiting reduced polarity and increased non-polarity. The target veterinary residues are predominantly polar substances with a high polarity. Therefore, it is advisable to avoid over-extraction with highly polar solvents in order to

Table 3
Matrix effects of targeted analytes in ten matrices.

No.	Analyte	Solvent standard curve	Correlation coefficient(r^2)	Matrix	Matrix-matched calibration curve	Correlation coefficient(R^2)	Matrix effect (ME/%)
1	Acetanilide	Y = 61,204.3X+12,316.2	0.998	Beef	Y = 12,924.03X-6334.18	0.999	−78.88
				Pork	Y = 29,574.1X-7721.23	0.992	−51.68
				Sheep	Y = 55,623.2X+16,368.8	0.999	−9.12
				Horse	Y = 52,837.3X+22,144.1	0.997	−13.67
				Chicken	Y = 24,353.8X+75,795.2	0.995	−60.21
				Prawn	Y = 7768.35X-2955.34	0.993	−87.31
				Fish	Y = 19,834.3X+1309.34	0.998	−67.59
				Liver	Y = 31,928.4X+14,333.1	0.994	−47.83
				Milk	Y = 58,934.3X+43,451.2	0.999	−3.71
				Fat	Y = 68,368.8X+50,030.5	0.999	11.71
2	o-Aminobenzoic acid	Y = 12,189.2X+8143	0.996	Beef	Y = 6983.77X+73,455.6	0.993	−42.71
				Pork	Y = 7938.26X+25,469.2	0.993	−34.87
				Sheep	Y = 11,356.3X+234,554	0.999	−6.83
				Horse	Y = 11,981.1X+96,009.3	0.996	−1.71
				Chicken	Y = 6425.02X+18,739.3	0.993	−47.29
				Prawn	Y = 3483.35X+9345.87	0.994	−71.42
				Fish	Y = 7309.32X+39,285.6	0.992	−40.03
				Liver	/	/	/
				Milk	Y = 11,021.5X+198,743	0.995	−9.58
				Fat	Y = 13,364.9X+19,809	0.998	9.65
3	Pentetrazol	Y = 21,827.3X+923.434	0.997	Beef	Y = 8234.43X+8782.33	0.999	−62.27
				Pork	Y = 6543.33X-532.384	0.997	−70.02
				Sheep	Y = 16,748.8X+9833.29	0.999	−23.27
				Horse	Y = 14,537.4X+18,384.3	0.994	−33.40
				Chicken	Y = 7473.45X+38,921.3	0.994	−65.76
				Prawn	Y = 3974.87X+12,242.5	0.997	−81.79
				Fish	Y = 5476.86X+6393.44	0.995	−74.91
				Liver	Y = 7783.22X+4894.36	0.999	−64.34
				Milk	Y = 18,739.2X-3338.35	0.999	−14.15
				Fat	Y = 23,293.7X+2344.54	0.999	12.82
4	Methyl ephedrine hydrochloride	Y = 26,538.4X+2534.4	0.997	Beef	Y = 5283.59X+9123.67	0.998	−80.09
				Pork	Y = 3928.5X+2896.88	0.996	−85.20
				Sheep	Y = 18,274.8X-2122.61	0.999	−31.14
				Horse	Y = 8763.08X+672.34	0.997	−66.98
				Chicken	Y = 6473.23X-1673.57	0.996	−75.61
				Prawn	Y = 7364.23X+11,245.2	0.993	−72.25
				Fish	Y = 7683.7X-2456.78	0.999	−71.05
				Liver	Y = 3789.71X-1778.63	0.993	−85.72
				Milk	Y = 18,739.3X-8123.89	0.999	−29.39
				Fat	Y = 39,893.5X-2332.74	0.999	50.32
5	Phenacetin	Y = 108393X+2631.71	0.997	Beef	Y = 12,783.1X-14,238.1	0.998	−88.21
				Pork	Y = 17,849.4X-8877.55	0.997	−83.53
				Sheep	Y = 89,837.1X-2337.6	0.999	−17.12
				Horse	Y = 79,803.2X-9762.95	0.995	−26.38
				Chicken	Y = 14,757.32X-18,780.3	0.995	−86.39
				Prawn	Y = 9793.84X-387.554	0.994	−90.96
				Fish	Y = 15,243.42X-28,767.6	0.992	−85.94
				Liver	Y = 49830X+10,983.1	0.997	−54.03
				Milk	Y = 68,973.7X-7674.56	0.999	−36.37
				Fat	Y = 118238X-4333.14	0.999	9.08
6	Antipyrine	Y = 48,372.1X+1359.72	0.998	Beef	Y = 7832.43X+1976.64	0.997	−83.81
				Chicken	Y = 33,243.9X+56,752.9	0.995	−31.27
				Pork	Y = 46,453.3X-8909.54	0.994	−3.97
				Sheep	Y = 44,354.1X+7808.32	0.999	−8.31
				Horse	Y = 31,232.5X+8797.73	0.998	−35.43
				Prawn	Y = 7342.65X+2309.43	0.997	−84.82
				Fish	Y = 13,245.7X-7909.6	0.997	−72.62
				Liver	Y = 27,242.2X+5098.23	0.993	−43.68
				Milk	Y = 32,343.3X+2654.89	0.998	−33.14
				Fat	Y = 54,675.5X-4984.45	0.998	13.03
7	Guaifenesin	Y = 8435.31X+2423.65	0.998	Beef	Y = 5865.13X-209.453	0.993	−30.47
				Pork	Y = 1453.32X-988.032	0.996	−82.77
				Sheep	Y = 7435.22X+2988.65	0.994	−11.86
				Horse	Y = 6675.71X+3767.77	0.999	−20.86
				Chicken	Y = 2149.42X+9943.3	0.999	−74.52
				Prawn	Y = 7345.96X-715.209	0.999	−12.91
				Fish	Y = 1764.43X-2123.69	0.994	−79.08
				Liver	Y = 4324.6X-885.232	0.999	−48.73
				Milk	Y = 7453.32X-402.143	0.999	−11.64
				Fat	Y = 11,234.8X-2541.06	0.999	33.19
8	Diethylcarbamazine	Y = 167543X+32,468.9	0.998	Beef	Y = 25,432.8X+178,633	0.999	−84.82
				Pork	Y = 37,643.2X+3789.45	0.995	−77.53
				Sheep	Y = 45,643.4X-5981.2	0.999	−72.76

(*continued on next page*)

Table 3 (*continued*)

No.	Analyte	Solvent standard curve	Correlation coefficient(r^2)	Matrix	Matrix-matched calibration curve	Correlation coefficient(R^2)	Matrix effect (ME/%)
				Horse	$Y = 59{,}869.9X+17{,}867.5$	0.998	−64.27
				Chicken	$Y = 65{,}436.5X+139{,}733$	0.999	−60.94
				Prawn	$Y = 75{,}355.7X+208{,}945$	0.995	−55.02
				Fish	$Y = 37{,}549.1X+8554.53$	0.993	−77.59
				Liver	$Y = 65{,}413.8X-5234.61$	0.993	−60.96
				Milk	$Y = 134636X-48{,}891.1$	0.999	−19.64
				Fat	$Y = 223{,}411.3X+16{,}230.9$	0.998	33.35
9	3,6-diamino-10-methylacridinium chloride	$Y = 21{,}928.9X+134{,}761$	0.998	Beef	$Y = 4342.87X+1323.21$	0.992	−80.20
				Pork	$Y = 7324.43X-402.334$	0.992	−66.60
				Sheep	$Y = 19{,}876.7X-2543.87$	0.999	−9.36
				Horse	$Y = 12{,}321.3X-343.724$	0.997	−43.81
				Chicken	$Y = 7342.89X-7342.72$	0.995	−66.52
				Prawn	$Y = 5342.23X+7897.66$	0.998	−75.64
				Fish	$Y = 6342.95X+3972.34$	0.993	−71.07
				Liver	$Y = 4843.99X+5698.25$	0.995	−77.91
				Milk	$Y = 15{,}462.9X-6988.53$	0.997	−29.49
				Fat	$Y = 23{,}343.2X-4874.78$	0.998	6.45
10	3,6-Acridinediamine	$Y = 31{,}288.7X+4341.08$	0.997	Beef	$Y = 5231.24X+387.564$	0.999	−83.28
				Pork	$Y = 7651.32X+3987.21$	0.998	−75.55
				Sheep	$Y = 24{,}533.6X-1987.72$	0.999	−21.59
				Horse	$Y = 24{,}324.6X-711.89$	0.994	−22.26
				Chicken	$Y = 15{,}343.2X+38{,}973.1$	0.998	−50.96
				Prawn	$Y = 6451.16X+4897.34$	0.999	−79.38
				Fish	$Y = 7923.67X-5782.13$	0.998	−74.68
				Liver	$Y = 9874.46X+3768.71$	0.998	−68.44
				Milk	$Y = 26{,}548.6X-8972.19$	0.999	−15.15
				Fat	$Y = 36{,}433.2X+9553.8$	0.999	16.44
11	Metomidate	$Y = 192{,}831.2X+11{,}113.4$	0.998	Beef	$Y = 23{,}255.2X-49{,}807.9$	0.998	−87.94
				Pork	$Y = 48{,}984.3X-46{,}578$	0.994	−74.60
				Sheep	$Y = 229{,}381.2X-20{,}391.1$	0.999	18.95
				Horse	$Y = 218{,}307.2X-11{,}135.2$	0.998	13.21
				Chicken	$Y = 38{,}324.1X-19{,}875.3$	0.991	−80.13
				Prawn	$Y = 24{,}342.2X+28{,}795.9$	0.999	−87.38
				Fish	$Y = 21{,}732.7X-34{,}098$	0.996	−88.73
				Liver	$Y = 132458X+2223.23$	0.997	−31.31
				Milk	$Y = 167{,}434.3X-57{,}686.3$	0.999	−13.17
				Fat	$Y = 255332X-17{,}817.2$	0.998	32.41
12	Ethacridine lactate monohydrate	$Y = 158373X+23{,}879$	0.995	Beef	$Y = 26{,}732.2X+737.092$	0.999	−83.12
				Pork	$Y = 40{,}342.6X-6094.42$	0.996	−74.53
				Sheep	$Y = 130293X-19{,}878.6$	0.999	−17.73
				Horse	$Y = 149303X-2898.1$	0.998	−5.73
				Chicken	$Y = 33{,}442.6X-26{,}664.3$	0.995	−78.88
				Prawn	$Y = 23{,}244.9X+28{,}904$	0.993	−85.32
				Fish	$Y = 36{,}553.3X-56{,}375.5$	0.994	−76.92
				Liver	$Y = 49{,}736.8X+25{,}986.4$	0.996	−68.60
				Milk	$Y = 149280X-48{,}277.8$	0.994	−5.74
				Fat	$Y = 198{,}374.1X-17{,}866.6$	0.998	25.26
13	Tripelennamine hydrochloride	$Y = 212357X+14{,}531.2$	0.997	Beef	$Y = 47{,}638.4X+3873.5$	0.997	−77.57
				Pork	$Y = 68{,}394.1X-4034.3$	0.994	−67.79
				Sheep	$Y = 224{,}221.2X-4164.3$	0.999	5.59
				Horse	$Y = 209238X-10{,}989.8$	0.998	−1.47
				Chicken	$Y = 97{,}384.7X+189{,}775$	0.997	−54.14
				Prawn	$Y = 41{,}211.8X+49{,}811.2$	0.994	−80.59
				Fish	$Y = 58{,}927.5X-34{,}532.7$	0.998	−72.25
				Liver	$Y = 49{,}383.7X+28{,}934.3$	0.991	−76.74
				Milk	$Y = 183948X-54{,}453$	0.999	−13.38
				Fat	$Y = 283{,}943.2X-267.986$	0.996	33.71
14	Ormetoprim	$Y = 20{,}928.1X+2132.16$	0.997	Beef	$Y = 10{,}096.23X+21{,}133.7$	0.999	−51.76
				Pork	$Y = 14{,}533.3X-1360.45$	0.998	−30.56
				Sheep	$Y = 8453.23X+378.123$	0.997	−59.61
				Horse	$Y = 6782.43X+354.100$	0.999	−67.59
				Chicken	$Y = 21{,}537.4X+48{,}938.2$	0.998	2.91
				Prawn	$Y = 14{,}539.3X+6549.7$	0.993	−30.53
				Fish	$Y = 16{,}758.8X+4343.32$	0.999	−19.92
				Liver	$Y = 5340.64X+694.122$	0.992	−74.48
				Milk	$Y = 20{,}246.1X-5193.2$	0.996	−3.26
				Fat	$Y = 30{,}322.2X-2123.46$	0.999	44.89
15	Sulfaethoxypyridazine	$Y = 48{,}764.1X+1233.2$	0.992	Beef	$Y = 13{,}452.6X+2389.37$	0.999	−72.41
				Pork	$Y = 17{,}654.4X-7776.68$	0.998	−63.80
				Sheep	$Y = 53{,}420.4X-8123.56$	0.997	9.55
				Horse	$Y = 50{,}424.2X-3111.64$	0.998	3.40
				Chicken	$Y = 28{,}764.7X+63{,}452$	0.992	−41.01
				Prawn	$Y = 9975.54X+21{,}523.1$	0.996	−79.54
				Fish	$Y = 15{,}439.1X-756.657$	0.999	−68.34
				Liver	$Y = 16{,}532.2X+23{,}576.8$	0.998	−66.10

(*continued on next page*)

Table 3 (*continued*)

No.	Analyte	Solvent standard curve	Correlation coefficient(r^2)	Matrix	Matrix-matched calibration curve	Correlation coefficient(R^2)	Matrix effect (ME/%)
				Milk	$Y = 53,424.5X-25,779.9$	0.999	9.56
				Fat	$Y = 79,342.5X+27,774.5$	0.998	62.71
16	Yohimbine hydrochloride	$Y = 129301X+6028.2$	0.992	Beef	$Y = 24,938.5X+21,187.3$	0.997	−80.71
				Pork	$Y = 38,971.4X+5435.2$	0.998	−69.86
				Sheep	$Y = 88,394.1X-15,647.2$	0.998	−31.64
				Horse	$Y = 76,473.2X-4988.52$	0.998	−40.86
				Chicken	$Y = 78,326.9X+62,222$	0.999	−39.42
				Prawn	$Y = 21,122.4X+112,435$	0.998	−83.66
				Fish	$Y = 33,242.4X-1879.23$	0.993	−74.29
				Liver	$Y = 21,113.6X+14,561.3$	0.993	−83.67
				Milk	$Y = 98,337.6X-49,876.9$	0.995	−23.95
				Fat	$Y = 133459X+2777.25$	0.999	3.22
17	Buquinolate	$Y = 117865X+7686.45$	0.993	Beef	$Y = 13,242.4X-189.335$	0.995	−88.76
				Pork	$Y = 63,243.6X+18,971.6$	0.992	−46.34
				Sheep	$Y = 155432X-33,457.1$	0.999	31.87
				Horse	$Y = 85,334.7X-9878.41$	0.996	−27.60
				Chicken	$Y = 41,234.2X+18,666.8$	0.997	−65.02
				Prawn	$Y = 34,345.1X+15,779.3$	0.999	−70.86
				Fish	$Y = 64,242.1X-1223.12$	0.997	−45.50
				Liver	$Y = 58,758.7X+7534.88$	0.996	−50.15
				Milk	$Y = 104246X-31,231.2$	0.999	−11.55
				Fat	$Y = 135334X+564.735$	0.999	14.82
18	Dehydrocholic acid	$Y = 4123.21X+802.332$	0.994	Beef	$Y = 3346.14X-278.712$	0.995	−18.85
				Pork	$Y = 1768.31X-454.567$	0.996	−57.11
				Sheep	$Y = 4340.11X-945.762$	0.999	5.26
				Horse	$Y = 3976.34X-1564.67$	0.998	−3.56
				Chicken	$Y = 2568.37X+3432.08$	0.993	−37.71
				Prawn	$Y = 5785.37X+567.098$	0.997	40.31
				Fish	$Y = 2345.15X-218.225$	0.994	−43.12
				Liver	$Y = 2123.21X-543.334$	0.999	−48.51
				Milk	$Y = 2531.11X-521.567$	0.991	−38.61
				Fat	$Y = 4345.45X-967.204$	0.997	5.39
19	Loperamide hydrochloride	$Y = 354353X+43,516.5$	0.997	Beef	$Y = 50,962.1X-2112.34$	0.999	−85.62
				Pork	$Y = 84,532.2X-34,347.3$	0.993	−76.14
				Sheep	$Y = 414243X-64,341.5$	0.999	16.90
				Horse	$Y = 371461X-1435.7$	0.998	4.83
				Chicken	$Y = 155322X+245,609$	0.998	−56.17
				Prawn	$Y = 55,740.1X+187,675$	0.998	−84.27
				Fish	$Y = 49,234.1X-69,821.9$	0.994	−86.11
				Liver	$Y = 193241X+48,977.5$	0.997	−45.47
				Milk	$Y = 265309X-91,123$	0.993	−25.13
				Fat	$Y = 425632X+89,856.4$	0.999	20.12
20	Ciclesonide	$Y = 10,654.3X-6236.54$	0.994	Beef	$Y = 4532.42X-309.819$	0.993	−57.46
				Pork	$Y = 6342.17X-235.345$	0.996	−40.47
				Sheep	$Y = 4344.23X-563.074$	0.999	−59.23
				Horse	$Y = 4453.22X-193.321$	0.997	−58.20
				Chicken	$Y = 7543.64X-112.133$	0.992	−29.20
				Prawn	$Y = 4764.21X-242.456$	0.997	−55.28
				Fish	$Y = 3985.23X-135.411$	0.996	−62.60
				Liver	/	/	/
				Milk	$Y = 8982.1X-10,788.2$	0.993	−15.70
				Fat	$Y = 6563.31X+1358.46$	0.998	−38.40

minimize losses. According to the similarity compatibility principle, nonpolar organic solvents interact with the long hydrophobic chains of fatty acids and neutral lipids through hydrophobic interactions (van der Waals forces), thereby solubilizing these classes of lipids [34,35]. Hexane, the most widely used solvent for lipid removal, although highly flammable, is an effective extraction solvent for nonpolar/neutral lipids [34,36], while reducing matrix effects [30,37]. In a study by Zhang et al. [8], acetonitrile combined with hexane liquid-liquid extraction was used to remove proteins and fats from pork and milk. Similarly, in another study by Zhang et al. [38], n-hexane was employed to remove fat from milk, eggs, and porcine muscle matrices with highly satisfactory outcomes. Thus, an acetonitrile-hexane saturated with an acetonitrile liquid-liquid extraction step was added to the pork matrix to eliminate large amounts of lipids [34] . Satisfactory recovery was achieved in this process.

O-aminobenzoic acid was not detected in the liver due to its inability to be linear in the liver matrix, thereby precluding quantification. Additionally, no blank liver matrix was identified. This analysis may also consider the interference from endogenous substances, resulting from the complex composition of the liver matrix. The matrix is composed of a multitude of endogenous substances, including proteins, enzymes, and metabolites. These substances may interact with o-aminobenzoic acid in varied ways. For instance, o-aminobenzoic acid may bind with liver proteins through hydrogen bonding, hydrophobic interaction, etc. Such interactions modify its behavior in the detection system, and hence, a linear relationship cannot be obtained between the concentration and the response signals. In other cases, metabolic transformations may also occur. It should be noted that the liver is rich in a variety of metabolic enzymes, such as the cytochrome P450 enzyme system. Following the entry of o-aminobenzoic acid into the liver matrix, it may undergo rapid catalysis with the aid of these enzymes. This results in metabolic transformations, such as the oxidation of the amino group and the hydroxylation of the benzene ring. Consequently, the metabolic processes alter the detected concentration of o-aminobenzoic acid, preventing the linear relationship between the concentration and the detection signal.

Table 4
Validation parameters and MRLs reference regulation of target veterinary drugs in beef, pork, sheep, horse, chicken, prawn, fish, liver, milk, fat.

No.	Analyte	Matrix	Matrix-matched calibration curve	Correlation coefficient(R^2)	Linear range/ (μg/L)	LOD/ (μg/ kg)	LOQ/ (μg/ kg)	MRLs reference regulation
1	Acetanilide	Beef	$Y = 12{,}924.03X-6334.18$	0.999	0.5~50	0.07	0.22	Pig, Horse, Cattle, Sheep, Goat Muscle: 0.01 mg/kg; Milk: 0.01 mg/kg
		Pork	$Y = 29{,}574.1X-7721.23$	0.992	1~50	0.09	0.30	Source: Applicability of Veterinary Drug MRLs
		Sheep	$Y = 55{,}623.2X+16{,}368.8$	0.999	0.5~50	0.04	0.12	for food in general (Korean) https://residue.foodsafetykorea.go.kr/vd/mrl
		Horse	$Y = 52{,}837.3X+22{,}144.1$	0.997	0.5~50	0.03	0.12	
		Chicken	$Y = 24{,}353.8X+75{,}795.2$	0.995	0.5~50	0.04	0.15	
		Prawn	$Y = 7768.35X-2955.34$	0.993	1~50	0.13	0.42	
		Fish	$Y = 19{,}834.3X+1309.34$	0.998	1~50	0.11	0.37	
		Liver	$Y = 31{,}928.4X+14{,}333.1$	0.994	0.5~50	0.06	0.20	
		Milk	$Y = 58{,}934.3X+43{,}451.2$	0.999	0.5~50	0.03	0.10	
		Fat	$Y = 68{,}368.8X+50{,}030.5$	0.999	0.5~50	0.02	0.06	
2	o-Aminobenzoic acid	Beef	$Y = 6983.77X+73{,}455.6$	0.993	0.5~50	0.03	0.09	Pig Muscle: 0.01 mg/kg
		Pork	$Y = 7938.26X+25{,}469.2$	0.993	1~50	0.12	0.41	Source: Applicability of Veterinary Drug MRLs for food in general (Korean) https://residue.foodsafetykorea.go.kr/vd/mrl
		Sheep	$Y = 11{,}356.3X+234{,}554$	0.999	2~50	0.18	0.62	
		Horse	$Y = 11{,}981.1X+96{,}009.3$	0.996	0.5~50	0.07	0.23	
		Chicken	$Y = 6425.02X+18{,}739.3$	0.993	1~50	0.16	0.54	
		Prawn	$Y = 3483.35X+9345.87$	0.994	1~50	0.16	0.53	
		Fish	$Y = 7309.32X+39{,}285.6$	0.992	1~50	0.10	0.33	
		Liver	/	/	/	/	/	
		Milk	$Y = 11{,}021.5X+198{,}743$	0.995	0.5~50	0.06	0.20	
		Fat	$Y = 13{,}364.9X+19{,}809$	0.998	1~50	0.09	0.31	
3	Pentetrazol	Beef	$Y = 8234.43X+8782.33$	0.999	1~50	0.10	0.35	Pig, Horse, Cattle Muscle: 0.01 mg/kg
		Pork	$Y = 6543.33X-532.384$	0.997	0.5~50	0.06	0.22	Source: Applicability of Veterinary Drug MRLs
		Sheep	$Y = 16{,}748.8X+9833.29$	0.999	2~50	0.25	0.84	for food in general (Korean) https://residue.foodsafetykorea.go.kr/vd/mrl
		Horse	$Y = 14{,}537.4X+18{,}384.3$	0.994	2~50	0.23	0.75	
		Chicken	$Y = 7473.45X+38{,}921.3$	0.994	1~50	0.09	0.31	
		Prawn	$Y = 3974.87X+12{,}242.5$	0.997	2~50	0.21	0.68	
		Fish	$Y = 5476.86X+6393.44$	0.995	1~50	0.13	0.45	
		Liver	$Y = 7783.22X+4894.36$	0.999	3~50	0.46	1.53	
		Milk	$Y = 18{,}739.2X-3338.35$	0.999	2~50	0.25	0.83	
		Fat	$Y = 23{,}293.7X+2344.54$	0.999	1~50	0.13	0.43	
4	Methyl ephedrine hydrochloride	Beef	$Y = 5283.59X+9123.67$	0.998	0.5~50	0.04	0.12	Pig, Horse, Cattle, Sheep, Goat Muscle:
		Pork	$Y = 3928.5X+2896.88$	0.996	0.5~50	0.05	0.18	0.01mg/kg; Milk: 0.005 mg/kg
		Sheep	$Y = 18{,}274.8X-2122.61$	0.999	0.5~50	0.05	0.17	Source: Applicability of Veterinary Drug MRLs
		Horse	$Y = 8763.08X+672.34$	0.997	0.5~50	0.05	0.18	for food in general (Korean)
		Chicken	$Y = 6473.23X-1673.57$	0.996	2~50	0.18	0.60	https://residue.foodsafetykorea.go.kr/vd/mrl
		Prawn	$Y = 7364.23X+11{,}245.2$	0.993	0.5~50	0.04	0.14	
		Fish	$Y = 7683.7X-2456.78$	0.999	0.5~50	0.04	0.14	
		Liver	$Y = 3789.71X-1778.63$	0.993	2~50	0.29	0.97	
		Milk	$Y = 18{,}739.3X-8123.89$	0.999	0.5~50	0.03	0.09	
		Fat	$Y = 39{,}893.5X-2332.74$	0.999	0.5~50	0.02	0.05	
5	Phenacetin	Beef	$Y = 12{,}783.1X-14{,}238.1$	0.998	0.5~50	0.03	0.10	Chicken, Pig, Horse, Deer, Cattle, Sheep, Goat,
		Pork	$Y = 17{,}849.4X-8877.55$	0.997	0.5~50	0.05	0.16	Duck, Turkey, Rabbit Muscle: 0.01mg/kg;
		Sheep	$Y = 89{,}837.1X-2337.6$	0.999	0.5~50	0.02	0.06	Poultry Eggs: 0.01mg/kg; Milk: 0.01mg/kg;
		Horse	$Y = 79{,}803.2X-9762.95$	0.995	0.5~50	0.02	0.05	Source: Applicability of Veterinary Drug MRLs
		Chicken	$Y = 14{,}757.32X-18{,}780.3$	0.995	0.5~50	0.05	0.17	for food in general (Korean) https://residue.foodsafetykorea.go.kr/vd/mrl
		Prawn	$Y = 9793.84X-387.554$	0.994	5~50	0.88	2.94	
		Fish	$Y = 15{,}243.42X-28{,}767.6$	0.992	5~50	0.85	2.84	
		Liver	$Y = 49830X+10{,}983.1$	0.997	0.5~50	0.03	0.10	
		Milk	$Y = 68{,}973.7X-7674.56$	0.999	0.5~50	0.02	0.08	

(*continued on next page*)

Table 4 (*continued*)

No.	Analyte	Matrix	Matrix-matched calibration curve	Correlation coefficient(R^2)	Linear range/ (μg/L)	LOD/ (μg/ kg)	LOQ/ (μg/ kg)	MRLs reference regulation
		Fat	$Y = 118238X-4333.14$	0.999	0.5~50	0.01	0.04	
6	Antipyrine	Beef	$Y = 7832.43X+1976.64$	0.997	0.5~50	0.08	0.26	Pig, Horse, Cattle, Sheep, Goat Muscle: 0.01
		Pork	$Y = 33,243.9X+56,752.9$	0.995	0.5~50	0.02	0.06	mg/kg; Milk: 0.01 mg/kg
		Sheep	$Y = 46,453.3X-8909.54$	0.994	0.5~50	0.02	0.06	Source: Applicability of Veterinary Drug MRLs for food in general (Korean)
		Horse	$Y = 44,354.1X+7808.32$	0.999	0.5~50	0.02	0.05	https://residue.foodsafetykorea.go.kr/vd/mrl
		Chicken	$Y = 31,232.5X+8797.73$	0.998	0.5~50	0.04	0.12	
		Prawn	$Y = 7342.65X+2309.43$	0.997	0.5~50	0.04	0.14	
		Fish	$Y = 13,245.7X-7909.6$	0.997	0.5~50	0.03	0.12	
		Liver	$Y = 27,242.2X+5098.23$	0.993	0.5~50	0.05	0.16	
		Milk	$Y = 32,343.3X+2654.89$	0.998	0.5~50	0.02	0.07	
		Fat	$Y = 54,675.5X-4984.45$	0.998	0.5~50	0.02	0.05	
7	Guaifenesin	Beef	$Y = 5865.13X-209.453$	0.993	3~50	0.52	1.74	Chicken, Pig, Horse, Cattle Muscle: 0.01 mg/kg
		Pork	$Y = 1453.32X-988.032$	0.996	3~50	0.43	1.45	Source: Applicability of Veterinary Drug MRLs
		Sheep	$Y = 7435.22X+2988.65$	0.994	3~50	0.31	1.03	for food in general (Korean)
		Horse	$Y = 6675.71X+3767.77$	0.999	3~50	0.40	1.33	https://residue.foodsafetykorea.go.kr/vd/mrl
		Chicken	$Y = 2149.42X+9943.3$	0.999	5~50	0.74	2.45	
		Prawn	$Y = 7345.96X-715.209$	0.999	5~50	0.63	2.10	
		Fish	$Y = 1764.43X-2123.69$	0.994	5~50	0.68	2.27	
		Liver	$Y = 4324.6X-885.232$	0.999	5~50	0.83	2.78	
		Milk	$Y = 7453.32X-402.143$	0.999	2~50	0.29	0.96	
		Fat	$Y = 11,234.8X-2541.06$	0.999	2~50	0.22	0.72	
8	Diethylcarbamazine	Beef	$Y = 25,432.8X+178,633$	0.999	0.5~50	0.01	0.03	Horse, Cattle, Sheep, Goat Muscle: 0.01 mg/kg
		Pork	$Y = 37,643.2X+3789.45$	0.995	0.5~50	0.01	0.03	Source: Applicability of Veterinary Drug MRLs for food in general (Korean)
		Sheep	$Y = 45,643.4X-5981.2$	0.999	0.5~50	0.03	0.10	https://residue.foodsafetykorea.go.kr/vd/mr
		Horse	$Y = 59,869.9X+17,867.5$	0.998	0.5~50	0.02	0.07	
		Chicken	$Y = 65,436.5X+139,733$	0.999	0.5~50	0.03	0.11	
		Prawn	$Y = 75,355.7X+208,945$	0.995	0.5~50	0.01	0.03	
		Fish	$Y = 37,549.1X+8554.53$	0.993	0.5~50	0.02	0.06	
		Liver	$Y = 65,413.8X-5234.61$	0.993	0.5~50	0.02	0.05	
		Milk	$Y = 134636X-48,891.1$	0.999	0.5~50	0.01	0.02	
		Fat	$Y = 223,411.3X+16,230.9$	0.998	0.5~50	0.01	0.02	
9	3,6-diamino-10-methylacridinium chloride	Beef	$Y = 4342.87X+1323.21$	0.992	1~50	0.17	0.50	Horse, Cattle, Sheep Muscle: 0.01 mg/kg; Milk:
		Pork	$Y = 7324.43X-402.334$	0.992	0.5~50	0.01	0.04	0.01 mg/kg
		Sheep	$Y = 19,876.7X-2543.87$	0.999	0.5~50	0.02	0.07	Source: Applicability of Veterinary Drug MRLs
		Horse	$Y = 12,321.3X-343.724$	0.997	0.5~50	0.03	0.12	for food in general (Korean)
		Chicken	$Y = 7342.89X-7342.72$	0.995	1~50	0.10	0.33	https://residue.foodsafetykorea.go.kr/vd/mrl
		Prawn	$Y = 5342.23X+7897.66$	0.998	1~50	0.10	0.35	
		Fish	$Y = 6342.95X+3972.34$	0.993	0.5~50	0.07	0.25	
		Liver	$Y = 4843.99X+5698.25$	0.995	0.5~50	0.08	0.27	
		Milk	$Y = 15,462.9X-6988.53$	0.997	0.5~50	0.05	0.17	
		Fat	$Y = 23,343.2X-4874.78$	0.998	0.5~50	0.01	0.04	
10	3,6-Acridinediamine	Beef	$Y = 5231.24X+387.564$	0.999	1~50	0.13	0.43	
		Pork	$Y = 7651.32X+3987.21$	0.998	0.5~50	0.03	0.10	
		Sheep	$Y = 24,533.6X-1987.72$	0.999	0.5~50	0.03	0.09	
		Horse	$Y = 24,324.6X-711.89$	0.994	0.5~50	0.02	0.08	
		Chicken	$Y = 15,343.2X+38,973.1$	0.998	1~50	0.11	0.37	
		Prawn	$Y = 6451.16X+4897.34$	0.999	1~50	0.11	0.35	
		Fish	$Y = 7923.67X-5782.13$	0.998	1~50	0.08	0.26	
		Liver	$Y = 9874.46X+3768.71$	0.998	1~50	0.15	0.49	
		Milk	$Y = 26,548.6X-8972.19$	0.999	0.5~50	0.03	0.09	
		Fat	$Y = 36,433.2X+9553.8$	0.999	0.5~50	0.01	0.05	
11	Metomidate	Beef	$Y = 23,255.2X-49,807.9$	0.998	0.5~50	0.02	0.06	Pig Muscle: 0.01 mg/kg
		Pork	$Y = 48,984.3X-46,578$	0.994	0.5~50	0.01	0.03	Source: Applicability of Veterinary Drug MRLs
		Sheep	$Y = 229,381.2X-20,391.1$	0.999	0.5~50	0.01	0.02	for food in general(Korean)
		Horse	$Y = 218,307.2X-11,135.2$	0.998	0.5~50	0.01	0.02	https://residue.foodsafetykorea.go.kr/vd/mrl
		Chicken	$Y = 38,324.1X-19,875.3$	0.991	0.5~50	0.01	0.04	
		Prawn	$Y = 24,342.2X+28,795.9$	0.999	0.5~50	0.08	0.26	
		Fish	$Y = 21,732.7X-34,098$	0.996	0.5~50	0.01	0.04	

(*continued on next page*)

Table 4 (*continued*)

No.	Analyte	Matrix	Matrix-matched calibration curve	Correlation coefficient(R^2)	Linear range/ (μg/L)	LOD/ (μg/ kg)	LOQ/ (μg/ kg)	MRLs reference regulation
		Liver	$Y = 132458X+2223.23$	0.997	0.5~50	0.01	0.02	
		Milk	$Y = 167,434.3X-57,686.3$	0.999	0.5~50	0.01	0.03	
		Fat	$Y = 255332X-17,817.2$	0.998	0.5~50	0.01	0.03	
12	Ethacridine lactate monohydrate	Beef	$Y = 26,732.2X+737.092$	0.999	0.5~50	0.01	0.05	Pig, Horse, Cattle Muscle: 0.01 mg/kg Source: Applicability of Veterinary Drug MRLs for food in general (Korean) https://residue.foodsafetykorea.go.kr/vd/mrl
		Pork	$Y = 40,342.6X-6094.42$	0.996	0.5~50	0.01	0.03	
		Sheep	$Y = 130293X-19,878.6$	0.999	0.5~50	0.01	0.03	
		Horse	$Y = 149303X-2898.1$	0.998	0.5~50	0.01	0.02	
		Chicken	$Y = 33,442.6X-26,664.3$	0.995	0.5~50	0.03	0.10	
		Prawn	$Y = 23,244.9X+28,904$	0.993	0.5~50	0.01	0.03	
		Fish	$Y = 36,553.3X-56,375.5$	0.994	0.5~50	0.02	0.08	
		Liver	$Y = 49,736.8X+25,986.4$	0.996	0.5~50	0.01	0.03	
		Milk	$Y = 149280X-48,277.8$	0.994	0.5~50	0.01	0.02	
		Fat	$Y = 198,374.1X-17,866.6$	0.998	0.5~50	0.01	0.02	
13	Tripelennamine hydrochloride	Beef	$Y = 47,638.4X+3873.5$	0.997	0.5~50	0.01	0.04	Pig, Horse, Cattle Muscle: 0.01 mg/kg; Milk: 0.01 mg/kg Source: Applicability of Veterinary Drug MRLs for food in general (Korean) https://residue.foodsafetykorea.go.kr/vd/mrl Edible tissues of Cattle: 0.2 mg/kg; Cattle milk: 0.02 mg/kg Source: Code of Federal Regulations Title 21(US Food and Drug Administration) www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=556&showFR=1
		Pork	$Y = 68,394.1X-4034.3$	0.994	0.5~50	0.01	0.03	
		Sheep	$Y = 224,221.2X-4164.3$	0.999	0.5~50	0.01	0.04	
		Horse	$Y = 209238X-10,989.8$	0.998	0.5~50	0.02	0.06	
		Chicken	$Y = 97,384.7X+189,775$	0.997	0.5~50	0.06	0.21	
		Prawn	$Y = 41,211.8X+49,811.2$	0.994	0.5~50	0.01	0.04	
		Fish	$Y = 58,927.5X-34,532.7$	0.998	0.5~50	0.01	0.04	
		Liver	$Y = 49,383.7X+28,934.3$	0.991	0.5~50	0.02	0.06	
		Milk	$Y = 183948X-54,453$	0.999	0.5~50	0.01	0.05	
		Fat	$Y = 283,943.2X-267.986$	0.996	0.5~50	0.01	0.03	
14	Ormetoprim	Beef	$Y = 10,096.23X+21,133.7$	0.999	0.5~50	0.02	0.07	Muscle and skin of salmonids in natural proportions: 0.1mg/kg Source: Maximum Residue Limits (MRLs) for Veterinary Drugs in Foods (Canada) https://www.canada.ca/en/health-canada/services/drugs-health-products/veterinary-drugs/maximum-residue-limits-mrls/list-maximum-residue-limits-mrls-veterinary-drugs-foods.html
		Pork	$Y = 14,533.3X-1360.45$	0.998	0.5~50	0.01	0.03	
		Sheep	$Y = 8453.23X+378.123$	0.997	0.5~50	0.16	0.53	
		Horse	$Y = 6782.43X+354.100$	0.999	3~50	0.37	1.24	
		Chicken	$Y = 21,537.4X+48,938.2$	0.998	0.5~50	0.03	0.09	
		Prawn	$Y = 14,539.3X+6549.7$	0.993	0.5~50	0.03	0.10	
		Fish	$Y = 16,758.8X+4343.32$	0.999	0.5~50	0.03	0.12	
		Liver	$Y = 5340.64X+694.122$	0.992	1~50	0.15	0.50	
		Milk	$Y = 20,246.1X-5193.2$	0.996	1~50	0.08	0.28	
		Fat	$Y = 30,322.2X-2123.46$	0.999	0.5~50	0.04	0.12	
15	Sulfaethoxypyridazine	Beef	$Y = 13,452.6X+2389.37$	0.999	0.5~50	0.01	0.04	Fat, kidney, Liver, Muscle of cattle; Kidney, Liver, Muscle, Skin and fat of swine: 0.1mg/kg - singly or in combination with other sulfonamides listed. Milk of cattle: 0.01mg/kg - singly or in combination with other sulfonamides listed. Source: Maximum Residue Limits (MRLs) for Veterinary Drugs in Foods (Canada) https://www.canada.ca/en/health-canada/services/drugs-health-products/veterinary-drugs/maximum-residue-limits-mrls/list-maximum-residue-limits-mrls-veterinary-drugs-foods.html Edible tissues (excluding milk): 0.1 mg/kg; Milk: Zero; Swine. Edible tissues: Zero. Source: Code of Federal Regulations Title 21(US Food and Drug Administration) www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=556&showFR=1
		Pork	$Y = 17,654.4X-7776.68$	0.998	0.5~50	0.02	0.07	
		Sheep	$Y = 53,420.4X-8123.56$	0.997	0.5~50	0.01	0.04	
		Horse	$Y = 50,424.2X-3111.64$	0.998	0.5~50	0.01	0.04	
		Chicken	$Y = 28,764.7X+63,452$	0.992	0.5~50	0.04	0.15	
		Prawn	$Y = 9975.54X+21,523.1$	0.996	0.5~50	0.06	0.21	
		Fish	$Y = 15,439.1X-756.657$	0.999	0.5~50	0.05	0.17	
		Liver	$Y = 16,532.2X+23,576.8$	0.998	0.5~50	0.03	0.08	
		Milk	$Y = 53,424.5X-25,779.9$	0.999	0.5~50	0.01	0.04	
		Fat	$Y = 79,342.5X+27,774.5$	0.998	0.5~50	0.01	0.02	
16	Yohimbine hydrochloride	Beef	$Y = 24,938.5X+21,187.3$	0.997	0.5~50	0.01	0.04	Deer Muscle: 0.01 mg/kg Source: Applicability of Veterinary Drug MRLs for food in general(Korean) https://residue.foodsafetykorea.go.kr/vd/mrl
		Pork	$Y = 38,971.4X+5435.2$	0.998	0.5~50	0.03	0.11	
		Sheep	$Y = 88,394.1X-15,647.2$	0.998	0.5~50	0.01	0.03	
		Horse	$Y = 76,473.2X-4988.52$	0.998	0.5~50	0.01	0.03	
		Chicken	$Y = 78,326.9X+62,222$	0.999	0.5~50	0.04	0.15	
		Prawn	$Y = 21,122.4X+112,435$	0.998	0.5~50	0.01	0.03	
		Fish	$Y = 33,242.4X-1879.23$	0.993	0.5~50	0.05	0.15	

(*continued on next page*)

Table 4 (*continued*)

No.	Analyte	Matrix	Matrix-matched calibration curve	Correlation coefficient(R^2)	Linear range/ (μg/L)	LOD/ (μg/ kg)	LOQ/ (μg/ kg)	MRLs reference regulation
		Liver	Y = 21,113.6X+14,561.3	0.993	0.5~50	0.02	0.06	
		Milk	Y = 98,337.6X-49,876.9	0.995	0.5~50	0.01	0.03	
		Fat	Y = 133459X+2777.25	0.999	0.5~50	0.01	0.03	
17	Buquinolate	Beef	Y = 13,242.4X-189.335	0.995	0.5~50	0.07	0.24	Kidney, Liver, Skin and fat of chickens: 0.4 mg/ kg
		Pork	Y = 63,243.6X+18,971.6	0.992	0.5~50	0.06	0.20	Muscle of chickens: 0.1 mg/kg
		Sheep	Y = 155432X-33,457.1	0.999	0.5~50	0.01	0.03	Source: Maximum Residue Limits (MRLs) for
		Horse	Y = 85,334.7X-9878.41	0.996	0.5~50	0.01	0.02	Veterinary Drugs in Foods (Canada)
		Chicken	Y = 41,234.2X+18,666.8	0.997	10~50	1.26	4.20	https://www.canada.ca/en/health-canada/services/drugs-health-products/veterinary-drugs/maximum-residue-limits-mrls/list-maximum-residue-limits-mrls-veterinary-drugs-foods.html
		Prawn	Y = 34,345.1X+15,779.3	0.999	3~50	0.50	1.68	
		Fish	Y = 64,242.1X-1223.12	0.997	0.5~50	0.06	0.22	
		Liver	Y = 58,758.7X+7534.88	0.996	0.5~50	0.01	0.03	
		Milk	Y = 104246X-31,231.2	0.999	0.5~50	0.01	0.02	
		Fat	Y = 135334X+564.735	0.999	0.5~50	0.01	0.03	
18	Dehydrocholic acid	Beef	Y = 3346.14X-278.712	0.995	3~50	0.43	1.43	Fish: 0.01 mg/kg
		Pork	Y = 1768.31X-454.567	0.996	10~50	1.29	4.31	Source: Applicability of Veterinary Drug MRLs
		Sheep	Y = 4340.11X-945.762	0.999	2~50	0.32	1.07	for food in general(Korean)
		Horse	Y = 3976.34X-1564.67	0.998	5~50	0.69	2.30	https://residue.foodsafetykorea.go.kr/vd/mrl
		Chicken	Y = 2568.37X+3432.08	0.993	5~50	0.69	2.31	
		Prawn	Y = 5785.37X+567.098	0.997	10~50	0.88	2.94	
		Fish	Y = 2345.15X-218.225	0.994	10~50	0.83	2.78	
		Liver	Y = 2123.21X-543.334	0.999	10~50	0.91	3.05	
		Milk	Y = 2531.11X-521.567	0.991	3~50	0.37	1.25	
		Fat	Y = 4345.45X-967.204	0.997	3~50	0.37	1.23	
19	Loperamide hydrochloride	Beef	Y = 50,962.1X-2112.34	0.999	0.5~50	0.01	0.04	Chicken, Pig, Cattle Muscle: 0.01 mg/kg
		Pork	Y = 84,532.2X-34,347.3	0.993	0.5~50	0.02	0.06	Source: Applicability of Veterinary Drug MRLs
		Sheep	Y = 414243X-64,341.5	0.999	0.5~50	0.01	0.03	for food in general(Korean)
		Horse	Y = 371461X-1435.7	0.998	0.5~50	0.01	0.04	https://residue.foodsafetykorea.go.kr/vd/mrl
		Chicken	Y = 155322X+245,609	0.998	0.5~50	0.01	0.04	
		Prawn	Y = 55,740.1X+187,675	0.998	0.5~50	0.02	0.08	
		Fish	Y = 49,234.1X-69,821.9	0.994	0.5~50	0.02	0.07	
		Liver	Y = 193241X+48,977.5	0.997	0.5~50	0.01	0.03	
		Milk	Y = 265309X-91,123	0.993	0.5~50	0.02	0.05	
		Fat	Y = 425632X+89,856.4	0.999	0.5~50	0.02	0.07	
20	Ciclesonide	Beef	Y = 4532.42X-309.819	0.993	0.5~50	0.15	0.50	Muscle, Liver, Kidney of Equidae: 0.6 μg/kg;Fat
		Pork	Y = 6342.17X-235.345	0.996	0.5~50	0.15	0.60	of Equidae: 4 μg/kg;
		Sheep	Y = 4344.23X-563.074	0.999	0.5~50	0.15	0.60	Not for use in animals from which milk is
		Horse	Y = 4453.22X-193.321	0.997	0.5~50	0.15	0.60	produced for human consumption.
		Chicken	Y = 7543.64X-112.133	0.992	0.5~50	0.14	0.47	Source: Regulation (EU) No 43/2020;
		Prawn	Y = 4764.21X-242.456	0.997	0.5~50	0.03	0.10	Regulation (EC) No 470/2009
		Fish	Y = 3985.23X-135.411	0.996	0.5~50	0.15	0.50	
		Liver	/	/	/	/	/	
		Milk	Y = 8982.1X-10,788.2	0.993	0.5~50	0.16	0.52	
		Fat	Y = 6563.31X+1358.46	0.998	0.5~50	0.16	0.53	

Table 5
Peak areas, noise signals and spiked concentrations for some typical substances.

Matrix	Analyte	Peak area	Noise signal	Spiked concentration(μg/kg)	Test concentration(μg/kg)
Chicken	Sulfaethoxypyridazine	186,501.375	610.77	5	4.49
	Buquinolate	6550.171	11.937	5	5.32
Beef	Sulfaethoxypyridazine	171,544.5	2386.815	10	8.63
	Buquinolate	20,621.824	426.114	10	8.38

3.4.2. *Optimization of centrifugal temperature*

The samples were subjected to centrifugation at room temperature (20 ± 2 °C), at 4 °C, and at −4 °C, respectively, and the results are shown in Fig. 3. In fish (grass carp), for instance, there was a negligible difference in the recoveries of the target veterinary residues at 4 °C and at −4 °C. The recoveries of o-Aminobenzoic acid, antipyrine, and loperamide at −4 °C were found to be slightly higher than those at 4 °C, although the deviation did not exceed 2%. However, the recovery efficiency of a majority of the compounds centrifuged at room temperature exhibited a slight decline in comparison to the latter two, with ormetoprim demonstrating notable decreases (< 9%). Centrifuges generate a considerable amount of heat when operated at high speeds, and proteins or fats present in centrifuged samples may undergo qualitative changes at elevated temperatures. According to Schmid et al. [39], the difference in temperature demonstrates a significant effect on fats, and their composition changes over time. Furthermore, Escorsim et al. [34] also states that as temperature rises, fat extraction becomes more facile, which will impact the outcome of the experiments and introduce interference with the target to be measured. Given that there was minimal distinction between the recoveries obtained at the

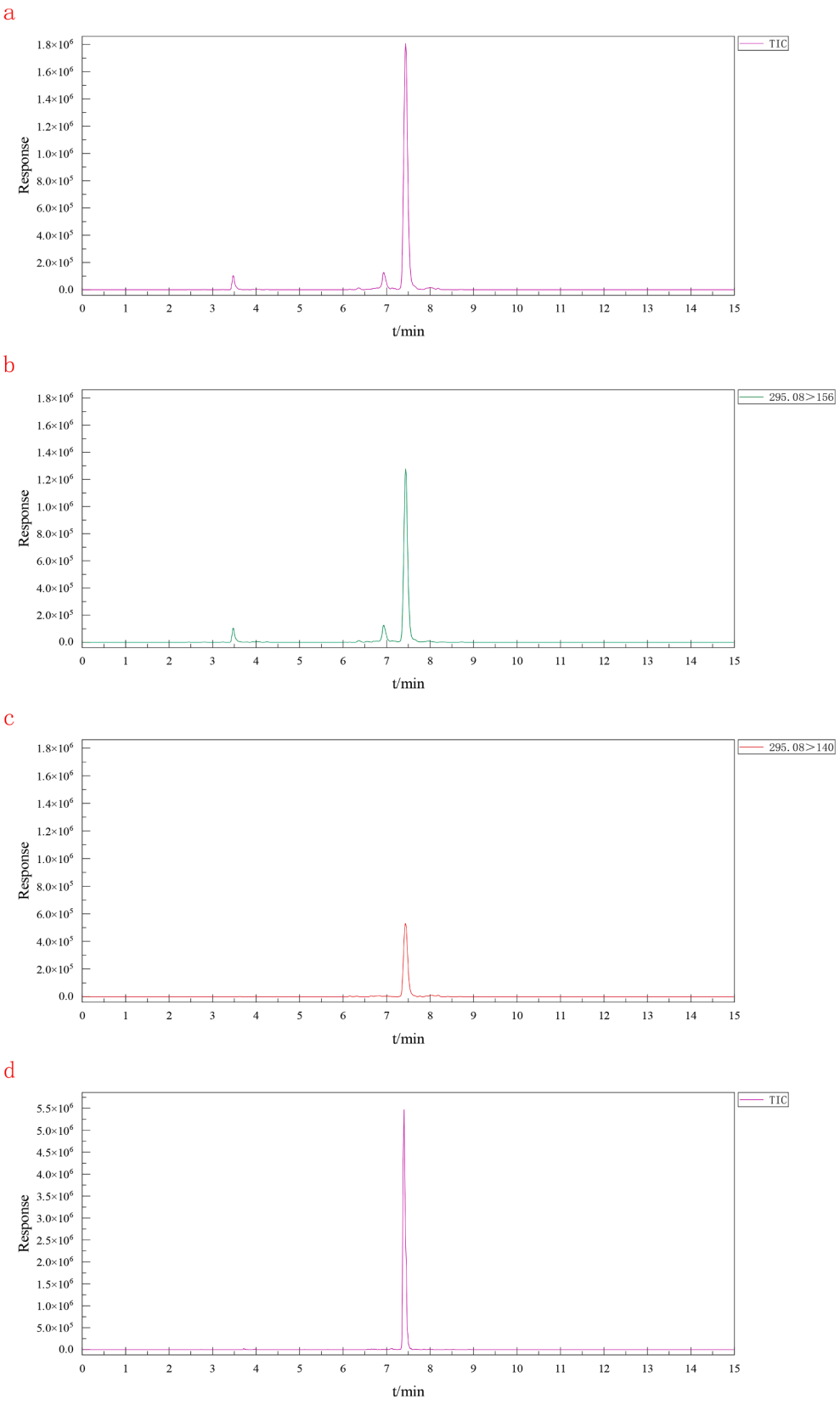


Fig. 7. Total ion chromatograms (TIC) and extracted ion chromatography (EIC) of sulfaethoxypyridazine (spiked conc. 5 μg/kg) in chicken(a, b, c) and (spiked conc. 10 μg/kg) in beef (d, e, f).

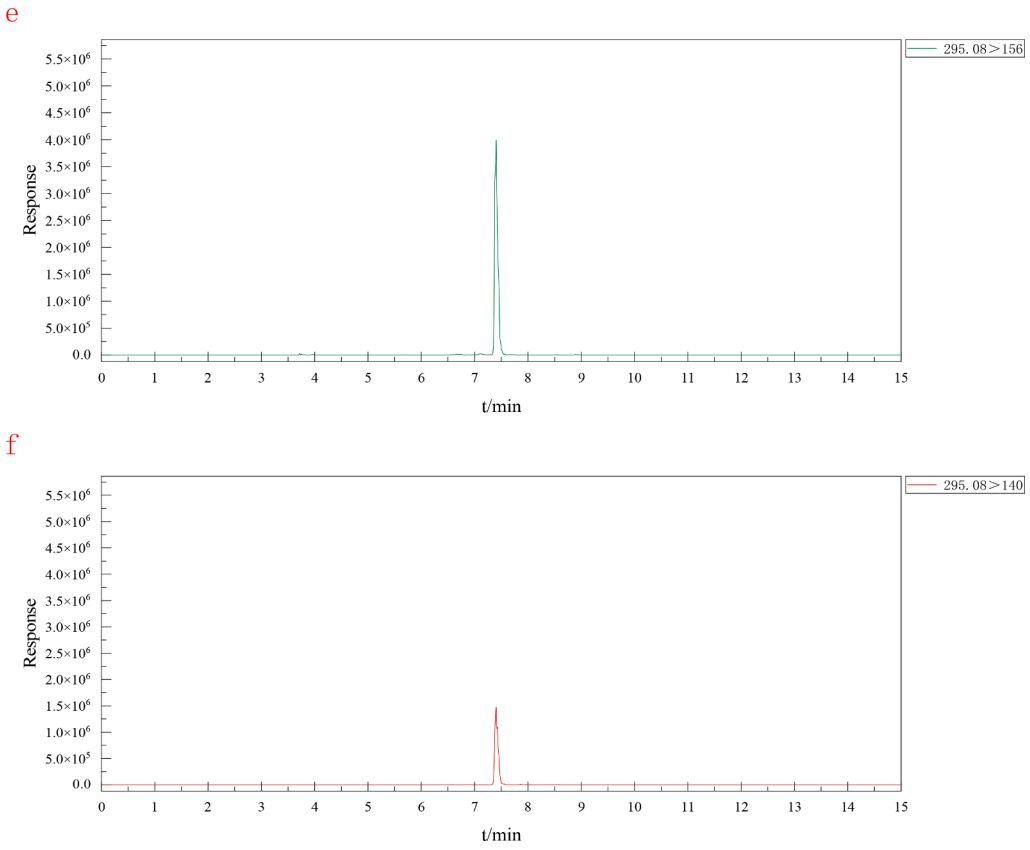


Fig. 7. (*continued*).

temperatures of 4 °C and −4 °C, the final parameter decided for the experiment was cryo-centrifugation at 4 °C.

3.5. Optimization of the purification step

3.5.1. Selection of purification powder and C18 dosage

The composition of animal food matrices is intricate, comprising a multitude of fats and phospholipids that are readily extracted by acetonitrile. However, direct injection into the column may result in adsorption and subsequent difficulty in elution. Long-term utilisation of the system may result in increased stress, necessitating the removal of impurities such as fats and phospholipids prior to mass spectrometry. As a typical dispersive solid-phase extraction (DSPE) technique, QuEChERS employs the interaction between the adsorbent filler and the interfering matrix to achieve the purpose of decontamination and clean-up. This method exhibits the advantages of simple operation, high recovery, accurate detection results, and a wide range of applications [10]. The principal purification materials employed in the QuEChERS technique include N-(n-propyl)ethylenediamine (PSA), octadecyl-bonded silica gel (C18), graphitised carbon black (GCB), anhydrous magnesium sulphate ($MgSO_4$), etc. PSA is employed for the removal of organic acids, pigments, and metal ions [40]. On the other hand, C18 has a greater capacity to remove non-polar substances and can effectively remove long-chain lipids, such as fats and fat-soluble pigments [41]. GCB is primarily utilized to adsorb substances such as pigments [42]. A series of experiments were conducted with the objective of comparing the efficacy of different purified powder formulations at varying ratios: 1) 80 mg C18+1000 mg $MgSO_4$+80 mg PSA; 2) 80 mg C18+1000 mg $MgSO_4$+40 mg GCB; 3) 80 mg C18+1000 mg $MgSO_4$+80 mg PSA+40 mg GCB; and 4) 80 mg C18+1000 mg $MgSO_4$, as shown in Fig. 4. The outcomes demonstrated that the inclusion of PSA enhanced the recovery rate of certain substances to a certain degree. Nevertheless, its effect was less pronounced than that of the group 4 with respect to the overall recovery rate of substances. The incorporation of GCB notably decreased the recuperation of several substances, with a distinct impact on 3,6-diamino-10-methylacridinium and 3,6-acridinediamine. Consequently, C18 and $MgSO_4$ were selected as purification reagents, and their dosages were optimized. A mixture with a concentration level of 10 ng/ml was added to beef, pork, chicken, prawn, and fish under the same conditions to investigate the purification effect of the target species of veterinary residues at 40 mg, 60 mg, 80 mg, 100 mg, and 120 mg C18, respectively. Six replicates were conducted for each matrix, and the mean recoveries of the ten matrices are presented in Fig. 5. The results demonstrated that a majority of the targets exhibited superior recoveries and purification in ten representative matrices when C18 was 100 mg. Conversely, the recoveries were significantly lower when the amount of C18 powder used was 40 mg or less. The beef matrix (Fig. 5a) was used as an example to illustrate the results. The data indicated that pentetrazol, methyl ephedrine hydrochloride, and phenacetin exhibited slightly higher recoveries (not exceeding 4%) at 80 mg C18 than at 100 mg C18. Loperamide demonstrated the highest recovery at 60 mg C18, while ciclesonide exhibited the highest recovery at 120 mg C18. When C18 was set at 40 mg, the majority of substances exhibited reduced recoveries, with the exception of buquinolate and antipyrine.

3.5.2. Dosage of mgso4

The presence of water reduces the rate of concentration. Extracted by the QuEChERS method, salt is usually added at the time of extraction in order to reduce the solubility of the veterinary drug in the aqueous phase by means of salting out. Therefore, the content of the veterinary drug in the organic phase is increased, facilitating the separation of the aqueous phase from the organic phase and reducing the amount of water in the organic phase [33]. The most commonly used salts are $MgSO_4$, Na_2SO_4 and NaCl. Na_2SO_4 is particularly challenging as it can easily release a large amount of heat when exposed to water. However, veterinary residues should be kept at a relatively low temperature during the

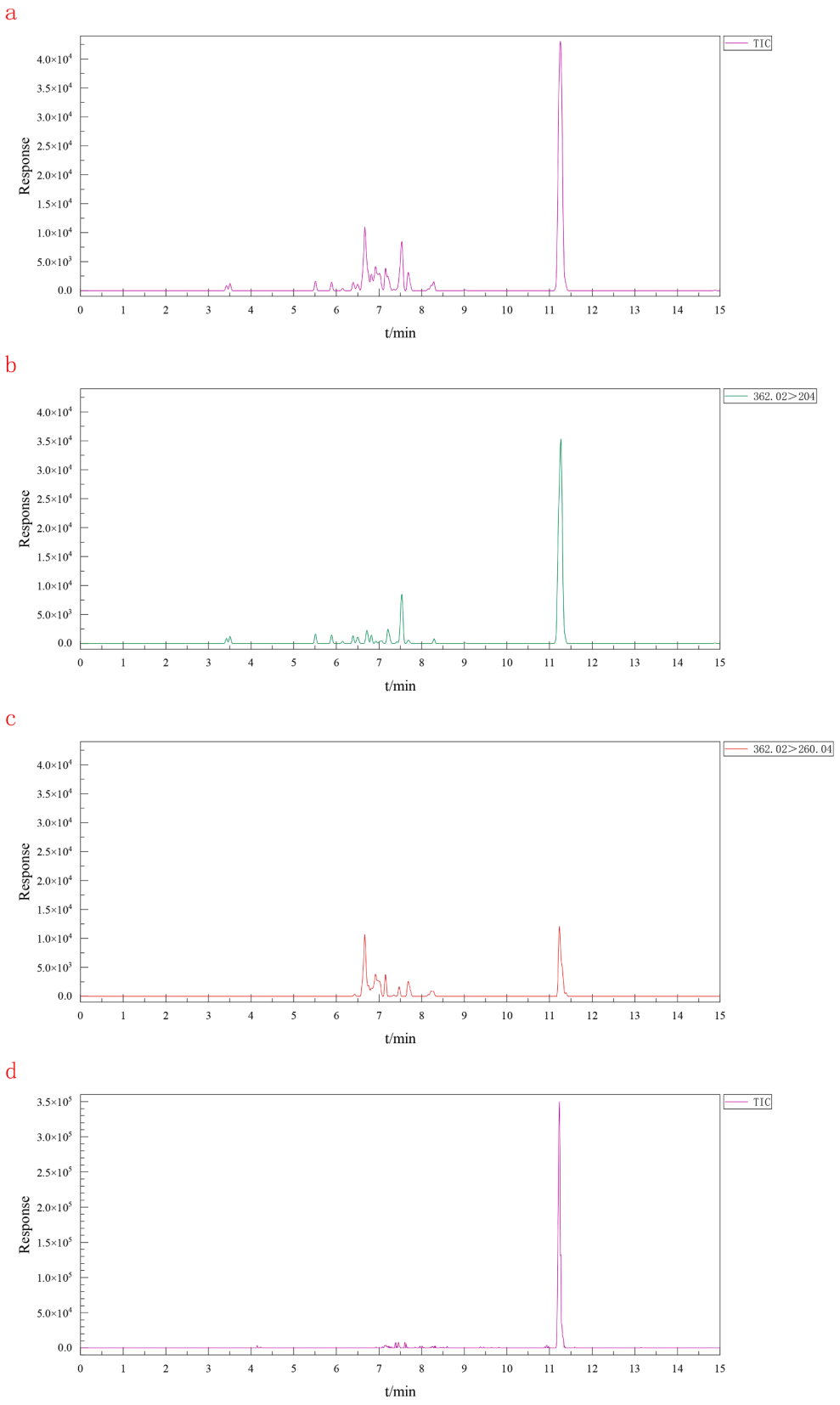


Fig. 8. TIC and EIC of buquinolate (spiked conc. 5 μg/kg) in chicken(a, b, c) and (spiked conc. 10 μg/kg) in beef (d, e, f)

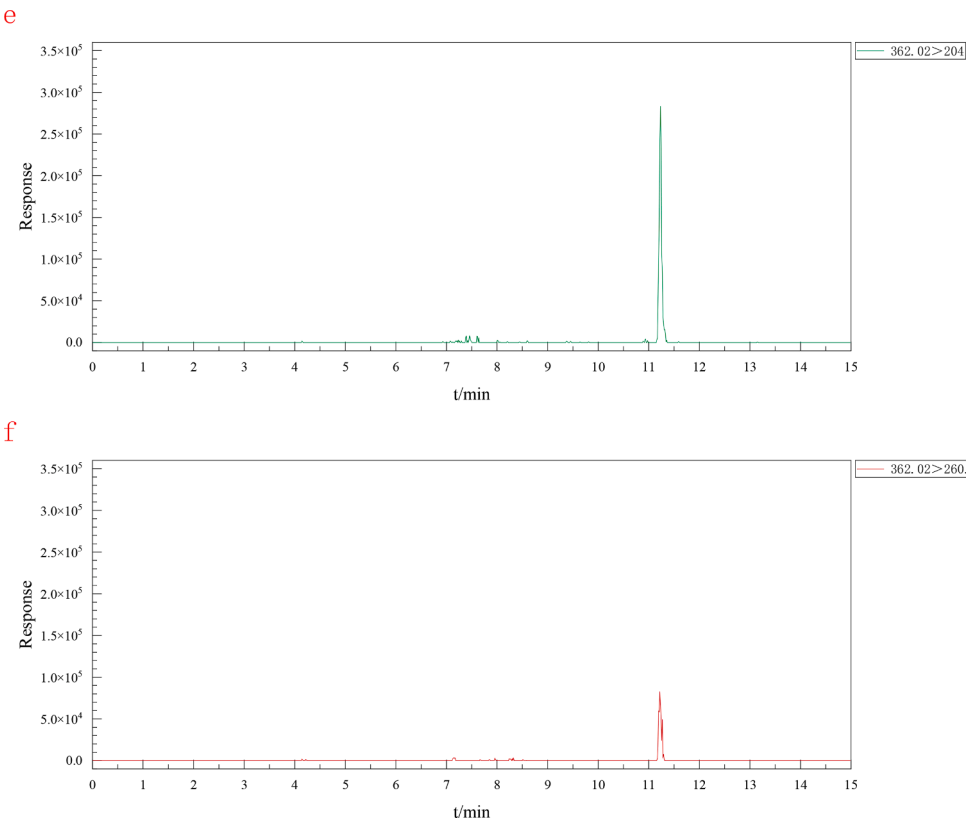


Fig. 8. (*continued*).

extraction process in order to minimise fat leaching [34]. Furthermore, sodium chloride may remove some water-soluble compounds. In this regard, Schenck et al. [43] have reported that $MgSO_4$ is a more effective drying agent than Na_2SO_4 with respect to the removal of residual water from organic solvents. Following a comparison of the various dehydrating salts, $MgSO_4$ was finally selected as the dehydrating salt in this study, and its content was optimised (in Fig. 6). Under the conditions of 1100 mg of $MgSO_4$, 85% acetonitrile water achieved the maximum extraction efficiency as the extraction solvent, while also removing water rapidly and effectively reducing the nitrogen blowing time. Consequently, the final dosage for the purification step was determined to be 100 mg of C18 and 1100 mg of $MgSO_4$.

3.6. Evaluation of the matrix effects

Following the extraction and purification of the samples, it is possible that some common extracts may affect the ionisation efficiency of the target veterinary residues. This phenomenon may result in the enhancement or inhibition of the signal response of the target substances, which is known as the matrix effect (ME). During the electrospray process, compounds may be affected by ionisation and the transfer of impurities, coeluting analytes, or degradation products from the formation of charged droplets from the sample solution ejected by the capillary tip to the entry of charged ions into the cone pore [44,45]. Matrix compounds can modify the physical properties of the droplet, including surface tension. A competition between the matrix and the analyte for the limited elemental charge on the droplet may occur. This phenomenon can interfere with the detection or recovery of the target substance [45]. This interference manifests as reduced recovery or response. The matrix enhancement or inhibition effect may also cause false-positive or false-negative results.

Consequently, the majority of researchers endeavour to employ a multitude of techniques to minimise or negate the influence of the matrix. These method include optimisation of sample preparation [46,47], adjustment of chromatographic parameters [48,49], utilisation of internal standards [50], setting of flow splitting [51] or the necessity to employ standard additions [44,52,53]. Therefore, this study conducted a preliminary investigation into the matrix effect of the method. The negative samples were extracted and purified in accordance with Section 2.3, resulting in the production of the blank matrix extract. The blank matrix extract and methanol were employed in the preparation of mixed standard solutions, which were used to generate matrix-matched calibration solutions and solvent standard solutions. These solutions were analyzed using the same method. The MEs were evaluated by using the following equation:

$$ME = \left(\frac{A}{B} - 1\right) \times 100\%$$

where A is the slope of the matrix standard curve and B stands for the slope of the pure solvent standard curve.

The matrix effect varies for different kinds of substances, with ME<0 representing matrix inhibition and vice versa for matrix enhancement. A larger absolute value indicates a stronger matrix effect. When |ME|< 20%, a weak matrix effect is indicated, which suggests that the matrix effect is not significant; when |ME|<50%, a moderate matrix effect is indicated; and when |ME|>50%, a strong matrix effect is implied, which is considered to be significant [54]. Typical matrices employed in this study included poultry (chicken), livestock (beef, pork, sheep, horse), aquatic (Grass carp, prawn), liver, fat, and milk matrices. These matrices were selected to evaluate the matrix effects of the test targets across different product categories. As illustrated in Table 3, the targets exhibited varying degrees of matrix enhancement or matrix inhibition in all ten matrices. The target veterinary residues exhibited significant matrix inhibition in the majority of matrices, with the majority also exhibiting moderate or strong matrix effects. For instance, acetanilide, pentetrazol, methyl ephedrine hydrochloride, phenacetin, antipyrine, and diethylcarbamazine in beef; metomidate, tripelennamine,

Table 6
Mean recoveries and RSD of the targeted veterinary residues at different spiked levels, $n = 6$.

No.	Compound	Spiked level (μg/kg)	Beef			Pork			Sheep			Horse			Chicken			Prawn			Fish			Liver			Milk			Fat		
			Recovery(%)	RSD(%)		Recovery(%)	RSD(%)		Recovery(%)	RSD(%)		Recovery(%)	RSD(%)		Recovery(%)	RSD(%)		Recovery(%)	RSD(%)		Recovery(%)	RSD(%)		Recovery(%)	RSD(%)		Recovery(%)	RSD(%)		Recovery(%)	RSD(%)	
				Inter day	Intra day		Inter day	Intra day		Inter day	Intra day		Inter day	Intra day		Inter day	Intra day		Inter day	Intra day		Inter day	Intra day		Inter day	Intra day		Inter day	Intra day		Inter day	Intra day
1	Acetanilide	5	89.3	8.5	10.6	69.6	2.3	4.6	92.1	7.5	9.9	109.2	9.8	10.3	86.1	9.4	10.5	71.2	9.6	10.5	92.1	8.3	9.5	77.3	10.3	14.6	75.2	6.9	9.8	95.7	13.0	16.5
		10	95.6	2.5	4.1	70.4	5.3	7.6	80.2	10.5	13.6	86.6	2.3	5.3	83.4	4.3	6.7	70.8	5.6	7.6	92.9	3.9	3.5	70.9	8.4	10.5	76.3	1.0	2.3	114.5	3.5	5.8
		50	86.7	5.4	9.5	72.7	7.4	10.2	76.8	1.4	3.4	82.8	4.0	6.4	87.2	5.4	7.5	70.6	3.6	4.6	94.5	3.4	5.7	69.7	5.4	6.4	76.6	1.3	6.5	66.4	5.8	6.8
2	o-Aminobenzoic acid	5	84.0	3.5	7.6	62.3	2.0	5.7	66.8	3.4	4.5	63.9	1.3	2.3	77.7	6.5	8.5	72.4	7.5	9.3	67.0	3.5	6.7	/	/	/	63.5	1.2	2.3	107.9	8.4	9.5
		10	78.9	9.6	11.2	113.0	8.6	11.5	67.6	3.8	5.8	69.5	5.9	7.9	105.1	7.6	9.3	65.4	2.5	3.5	65.4	4.6	5.4	/	/	/	67.7	4.5	5.7	112.4	4.2	5.3
		50	113.2	2.8	5.6	116.2	1.6	1.8	65.1	2.9	3.4	67.3	3.6	4.5	103.2	5.4	8.6	103.2	7.6	9.7	70.2	5.4	6.3	/	/	/	63.7	2.5	3.3	109.7	2.5	3.5
3	Pentetrazol	5	72.3	10.4	12.8	74.3	11.6	12.6	64.0	1.8	3.3	113.6	3.6	4.4	73.2	5.3	7.8	71.2	8.5	9.5	87.0	7.6	8.5	79.8	16.9	20.6	81.0	3.7	4.5	99.8	16.4	17.4
		10	88.7	6.5	8.7	80.6	4.4	5.8	68.9	4.2	5.3	110.1	7.6	8.1	82.5	8.5	9.7	69.2	5.6	7.6	89.4	7.2	9.3	73.2	8.4	10.5	84.0	2.5	3.4	95.7	3.2	5.4
		50	82.0	6.5	9.8	73.2	3.6	6.5	70.6	3.5	6.6	92.1	4.0	5.6	80.5	9.5	10.6	73.9	6.8	8.7	112.6	2.6	5.6	65.9	2.7	3.5	79.3	3.6	6.5	65.7	2.2	3.5
4	Methyl ephedrine hydrochloride	5	67.4	4.5	6.7	65.4	3.4	4.5	97.0	10.4	13.4	98.0	5.1	6.1	82.3	3.4	5.6	72.3	6.5	9.8	77.0	3.4	5.4	83.7	3.8	4.3	65.1	4.2	5.3	90.1	3.0	6.5
		10	70.6	7.5	8.9	75.6	2.0	4.5	74.6	5.6	6.4	85.9	10.5	12.4	76.5	4.5	7.5	75.2	4.6	6.7	82.4	5.4	6.7	75.0	7.2	8.5	67.3	4.6	4.5	95.3	1.4	2.5
		50	69.5	4.7	6.8	70.2	6.5	7.5	62.9	1.6	3.4	85.3	1.5	2.6	69.2	5.4	8.6	71.1	5.4	8.7	76.5	7.3	8.6	75.2	5.7	5.6	66.6	3.2	3.8	85.8	1.5	4.2
5	Phenacetin	5	93.4	8.4	9.8	65.3	4.2	4.3	70.1	6.2	8.4	112.2	6.0	8.4	74.2	5.4	7.5	78.5	8.7	9.8	79.0	9.2	11.5	62.1	1.9	2.4	76.3	6.9	7.1	93.8	2.5	3.5
		10	88.6	8.7	10.4	66.5	6.7	5.2	97.8	2.6	3.2	99.4	2.2	5.6	84.4	6.6	6.9	76.5	5.4	6.7	72.1	5.4	6.5	67.7	4.7	5.9	79.4	2.3	3.3	95.8	4.7	5.6
		50	91.8	5.6	6.8	71.8	6.0	5.7	67.2	2.3	4.5	89.2	7.7	9.5	81.6	5.6	9.6	77.0	4.7	8.9	78.5	3.4	7.6	67.3	5.6	6.5	82.1	1.2	2.1	87.7	12.3	13.6
6	Antipyrine	5	91.4	13.5	18.7	66.3	3.9	5.4	74.4	7.4	11.2	98.1	16.4	18.8	87.5	5.6	8.7	69.0	7.8	8.7	88.2	10.5	13.5	73.0	9.3	11.2	82.3	6.3	6.9	97.0	6.3	7.6
		10	94.7	11.6	12.4	67.6	5.0	6.6	79.9	2.6	3.4	103.4	2.5	2.3	92.3	4.6	5.6	65.5	4.6	6.7	73.4	3.5	3.5	70.2	7.1	8.5	85.9	1.5	1.9	98.1	5.5	6.5
		50	111.4	5.8	5.7	70.3	4.9	6.5	70.3	3.3	6.5	92.1	2.4	5.4	84.4	5.6	7.6	68.6	5.7	6.8	86.5	4.5	7.6	79.7	2.4	3.5	87.0	2.3	3.3	67.6	3.7	4.5

(*continued on next page*)

Table 6 (*continued*)

7	Guaifenesin	5	95.0	6.7	7.6	71.2	6.1	6.4	101.9	14.1	17.4	92.5	3.9	3.2	82.4	8.5	12.4	75.0	10.7	11.8	76.5	9.5	10.3	107.9	15.9	17.3	78.0	6.7	6.8	87.3	2.9	3.3
		10	96.6	8.9	9.8	76.7	4.9	5.7	73.1	6.5	8.4	95.6	12.7	15.2	89.6	6.4	8.6	75.4	5.7	6.7	84.3	4.5	5.4	71.3	7.4	11.9	85.3	2.5	3.5	89.6	2.5	3.2
		50	97.5	8.5	9.7	69.0	4.3	4.9	64.4	1.3	2.3	86.5	5.7	11.2	94.6	6.8	7.5	68.5	4.6	5.8	86.7	3.5	6.7	74.6	3.3	5.6	77.2	3.4	4.5	82.4	1.7	2.5
8	Diethylcarbamazine	5	81.6	6.7	8.9	68.3	3.0	5.4	86.6	3.9	5.4	90.5	7.0	9.6	67.4	5.8	6.8	72.5	7.5	8.6	73.0	4.5	7.6	65.4	4.0	4.6	63.4	1.2	2.3	86.5	2.7	3.5
		10	95.5	7.6	9.6	76.5	2.0	3.6	71.1	2.5	3.4	88.6	4.9	5.7	66.9	2.4	4.5	74.2	8.5	9.6	71.2	2.5	3.5	63.7	2.1	3.1	64.2	1.3	2.5	89.6	5.5	6.5
		50	81.0	8.7	7.8	67.4	4.2	5.8	67.4	2.4	5.4	78.7	2.1	7.3	73.4	4.2	7.6	66.4	2.6	3.8	74.3	3.2	6.5	64.6	2.4	2.5	66.4	2.5	3.5	64.7	1.7	1.9
9	3,6-diamino-10-methylacridinium chloride	5	70.3	7.5	8.9	68.6	3.7	5.6	88.4	10.5	13.5	65.2	2.5	3.8	71.3	15.6	18.7	69.5	4.6	5.9	67.2	5.3	7.6	64.1	3.2	4.5	65.1	2.4	3.6	88.8	1.8	2.5
		10	82.8	4.6	7.8	68.9	2.5	3.4	72.3	7.1	9.5	69.6	4.2	6.6	73.5	4.6	5.8	66.2	4.6	5.7	66.4	3.4	4.4	73.7	2.3	2.8	68.5	1.6	2.3	84.4	4.7	6.5
		50	70.0	7.7	9.5	70.2	4.0	6.4	65.3	2.2	3.4	66.5	1.2	2.5	64.2	2.5	3.6	64.3	2.8	3.6	67.4	2.6	5.8	88.0	3.3	5.4	67.5	2.3	3.5	80.5	13.3	15.6
10	3,6-Acridinediamine	5	72.4	8.5	10.8	67.5	3.7	7.6	64.6	3.5	3.6	76.4	6.0	7.0	75.3	6.4	7.7	66.0	4.9	5.7	71.4	7.3	10.8	64.6	3.3	3.8	68.4	5.6	6.5	88.5	1.2	1.5
		10	72.2	8.6	9.8	69.3	5.7	6.4	73.5	3.5	5.6	85.4	1.8	2.3	84.3	5.4	8.6	67.9	4.7	5.6	73.4	7.5	5.4	67.1	4.1	5.4	73.7	1.2	2.5	85.2	1.3	1.9
		50	72.7	4.5	7.6	64.5	2.5	4.5	70.6	5.0	6.6	69.2	1.8	1.9	70.5	5.5	9.6	69.2	6.5	6.7	82.7	7.5	9.8	89.8	7.2	9.5	72.1	1.0	3.5	76.6	3.7	4.5
11	Metomidate	5	83.4	11.5	14.9	69.1	2.8	5.7	74.6	8.6	9.8	108.5	7.0	8.8	77.6	7.2	10.5	64.0	2.4	4.9	75.3	11.5	17.6	62.3	1.9	2.5	86.0	5.4	6.5	96.0	1.8	1.3
		10	70.8	3.5	6.7	71.2	3.0	4.5	80.4	1.3	2.5	106.2	3.9	5.6	72.4	4.6	6.7	67.3	8.9	11.7	72.0	3.2	4.5	68.3	4.9	5.6	90.9	2.5	2.3	97.8	1.2	1.9
		50	83.3	6.6	8.7	67.4	5.6	6.5	69.8	5.4	6.5	96.1	1.7	3.5	81.5	7.4	8.7	69.9	6.4	7.8	76.3	4.5	7.6	75.8	5.4	7.5	87.2	1.3	3.2	85.3	4.8	5.6
12	Ethacridine lactate monohydrate	5	71.8	8.4	9.7	64.5	2.9	3.7	63.9	2.7	3.1	63.9	1.9	2.8	67.3	3.4	4.7	62.3	1.5	1.9	64.0	3.6	3.7	69.5	5.4	6.5	70.1	6.6	8.5	64.9	3.5	3.8
		10	68.9	6.5	8.8	69.6	8.7	9.8	67.6	1.2	3.5	62.5	2.3	5.9	63.4	1.7	3.3	68.9	2.5	3.7	68.4	3.3	3.5	64.4	2.5	3.4	79.4	3.5	5.6	66.0	2.6	3.5
		50	71.2	8.6	9.8	70.1	3.2	6.4	68.1	3.8	6.5	69.2	4.4	5.6	68.9	2.4	3.2	67.5	4.6	5.6	68.2	2.6	4.8	70.3	1.4	3.2	72.9	2.5	3.5	65.6	3.8	3.8
13	Tripelennamine hydrochloride	5	82.3	5.7	7.6	72.3	6.7	8.3	91.1	6.9	8.9	113.4	3.8	7.4	84.4	9.4	10.5	68.7	5.9	7.6	87.8	9.4	12.5	68.9	5.2	6.6	66.2	1.7	3.5	86.6	3.3	5.6
		10	84.4	8.6	8.8	70.6	2.5	4.3	77.8	3.5	5.4	112.2	4.4	6.5	73.3	6.5	8.6	69.4	5.6	6.7	83.2	5.4	8.6	78.8	8.8	8.9	72.5	9.5	11.5	87.3	5.9	6.1
		50	80.2	3.6	5.7	73.4	7.5	8.6	67.2	2.8	2.6	99.1	2.7	3.5	89.5	8.6	9.7	71.3	5.8	7.6	84.5	6.4	8.7	75.3	5.7	6.4	70.5	3.5	6.5	80.3	7.4	8.9
14	Ormetoprim	5	79.8	7.6	8.9	81.7	12.4	15.6	83.0	15.4	19.6	112.1	5.4	6.6	70.2	6.5	7.7	75.4	6.8	8.6	88.6	11.3	17.5	66.5	3.1	4.6	82.9	9.3	10.5	86.1	1.8	2.5
		10	85.4	11.7	12.4	74.8	3.5	5.4	78.6	3.7	5.7	107.4	8.1	10.5	74.4	7.6	8.9	83.5	4.6	7.6	74.3	7.3	8.4	78.8	7.8	8.7	85.6	4.5	5.5	91.9	9.3	12.6
		50	84.4	9.6	11.2	76.9	5.6	6.8	75.2	5.2	6.6	93.3	4.9	6.4	82.1	9.4	10.2	73.4	8.6	9.4	81.5	6.4	8.7	87.2	13.5	15.6	91.2	3.5	5.6	77.5	2.7	3.5

(*continued on next page*)

Table 6 (*continued*)

15	Sulfaethoxypyridazine	5	78.7	7.8	8.8	65.6	3.1	4.0	74.8	11.7	12.6	68.2	5.3	5.9	91.7	6.5	8.7	73.2	4.7	5.6	77.6	5.3	8.7	65.9	3.3	4.6	81.0	8.8	9.8	85.7	6.0	7.6
		10	81.2	7.6	8.6	76.9	4.6	5.7	65.1	1.9	2.3	72.2	5.7	6.8	82.3	6.8	8.7	82.5	10.6	13.8	70.9	2.4	4.5	72.4	8.3	9.4	79.8	2.4	3.5	77.1	2.7	3.8
		50	78.9	7.5	7.8	81.0	3.3	5.4	63.1	1.3	3.2	67.9	1.5	2.5	86.4	8.7	9.6	76.4	6.5	7.6	76.2	5.4	6.9	69.3	6.3	8.9	90.6	7.7	8.5	72.4	5.6	6.5
16	Yohimbine hydrochloride	5	86.4	12.6	17.2	70.6	9.7	10.8	88.0	6.6	6.8	103.8	12.9	15.2	85.8	15.6	17.8	68.5	2.7	3.4	82.2	2.5	4.5	78.1	8.9	9.8	81.1	5.9	6.5	86.6	2.4	3.5
		10	96.3	5.6	7.8	70.0	4.0	3.5	74.8	3.3	4.5	106.4	4.0	6.2	93.4	4.6	6.7	69.4	5.5	7.6	74.9	5.5	6.5	75.5	10.2	12.3	74.4	3.6	3.5	88.9	3.5	6.5
		50	81.8	6.7	8.8	69.3	5.4	6.5	66.4	5.1	5.8	96.2	5.8	6.7	82.4	6.8	8.7	65.5	2.5	2.7	84.3	6.3	7.5	91.6	8.7	8.9	85.8	1.3	2.4	71.9	7.4	7.8
17	Buquinolate	5	73.5	7.6	11.5	65.7	4.5	5.4	88.5	5.3	6.5	106.9	9.3	10.5	87.5	14.5	16.9	75.5	8.6	8.7	86.6	11.7	16.7	84.2	6.3	6.7	66.1	3.0	4.6	86.3	13.3	16.6
		10	70.5	4.6	5.7	76.4	4.9	6.5	71.4	1.9	1.2	110.2	3.6	4.6	87.5	7.3	8.9	71.5	8.7	9.4	93.0	2.2	4.3	90.0	9.2	11.1	62.8	1.4	1.8	92.6	8.7	9.8
		50	76.7	6.8	7.6	74.3	6.3	7.8	68.1	1.8	3.5	92.8	1.8	2.3	104.4	6.9	8.7	70.5	6.5	7.6	95.4	3.2	5.4	104.1	5.4	6.8	64.5	2.4	3.5	87.4	3.6	6.5
18	Dehydrocholic acid	5	78.5	8.6	13.7	71.2	6.7	8.7	102.8	14.6	15.4	106.8	13.2	14.5	66.9	1.9	5.6	69.5	3.6	4.3	67.0	4.3	6.5	67.2	4.4	5.1	63.5	1.3	1.9	83.9	2.0	3.5
		10	90.0	10.5	11.2	77.0	7.6	9.7	85.7	11.6	13.9	101.4	4.6	6.8	71.5	7.8	8.3	68.6	5.7	6.5	70.4	6.5	8.7	68.6	4.4	6.5	67.7	4.3	5.5	97.9	11.5	15.9
		50	75.3	8.7	9.6	75.9	9.4	11.8	71.8	1.6	2.2	96.5	6.3	7.2	72.4	8.5	9.8	66.6	2.5	3.8	66.5	2.6	5.6	63.3	1.7	2.4	70.1	4.5	3.9	107.2	12.9	13.5
19	Loperamide hydrochloride	5	85.6	9.6	10.5	68.6	2.1	3.8	91.4	1.5	2.5	93.9	15.6	18.6	84.2	10.8	12.7	80.4	9.5	10.7	76.5	12.4	13.9	72.3	8.2	9.5	85.9	11.3	12.5	67.1	6.2	7.8
		10	75.0	6.7	7.6	69.6	4.7	5.0	81.2	1.0	2.5	112.3	1.6	5.4	83.4	7.6	8.7	85.3	5.6	8.6	83.5	8.5	9.1	87.9	11.4	12.5	91.0	3.5	3.6	68.3	3.1	4.6
		50	86.4	8.4	8.6	67.2	4.5	8.0	73.3	4.2	4.7	99.2	5.9	9.0	76.5	5.7	7.9	74.4	7.6	8.7	77.0	3.4	5.4	73.1	2.4	3.7	94.0	2.5	2.8	70.6	2.4	3.5
20	Ciclesonide	0.6	87.5	14.5	16.7	74.5	8.7	11.6	78.0	7.7	9.4	69.2	3.5	5.4	71.2	4.7	6.8	80.5	16.5	19.7	75.6	5.3	6.4	/	/	/	103.4	10.7	11.5	101.7	9.0	11.9
		1.2	74.0	7.6	8.7	79.6	5.7	6.6	69.8	3.2	6.6	83.5	11.0	15.4	76.4	6.6	8.7	93.6	7.8	6.8	74.0	7.4	8.9	/	/	/	106.6	9.8	10.8	106.0	1.7	2.6
		10	78.5	8.6	9.8	83.4	5.7	8.7	77.1	9.9	11.2	78.2	10.8	11.5	81.7	8.9	9.7	88.6	6.8	7.9	87.6	5.4	7.6	/	/	/	109.1	2.5	3.5	94.2	4.4	5.8

Table 7
Comparison of this method with other detection methods using LC-MS/MS.

NO.	Compound	Method	Pretreatment	LOQ(μg/kg)	Recovery(%)	RSD (%)	Foodstuffs	References
1	Acetanilide, Antipyrine, Anthranilic acid, Diphenhydramine, Cyproheptadine, DL-methylephedrine, Phenacetin	LC-MS/MS	Acetonitrile -QuEChERS	0.9–7.1	71.7–102.4	≤8.6	Milk	[61]
2	Tetramisole, Diethylcarbamazine, Guaifenesin	LC/MS/MS	Acetonitrile	0.2–2	67.47–97.38	<20	Milk, eggs, and porcine muscle	[38]
3	Flumethasone, Dl-Methylephedrine, 2-Hydroxy-4,6-dimethylpyrimidine	LC-MS/MS	Acetonitrile-QuEChERS	2–10	73.62–112.70	≤20.33	Porcine muscle, and pasteurized cow milk	[8]
4	Acetanilide, Pentylenetetrazole, Phenacetin, Tetramethrin	LC/ESI-MS/MS	0.1 % formic acid in acetonitrile-QuEChERS	0.5–2.5	63.75–89.30	≤15.78	Porcine muscle, milk, and table eggs	Zhang et al. 2016c
5	64 compounds(Acetanilide, Dl-Methylephedrine HCl, Phenacetin, Antipyrine, Diethylcarbamazine, Tripelennamine,Yohimbine, Loperamide etc.)	LC/MS/MS	Acetonitrile/water (4:1, v/v)-QuEChERS	0.03–3	60.0–120.4	≤30.8	Flat fish, eel, shrimp	[56]
6	Scopolamine, Metoclopramide, Acriflavine, Berberine, Tripelennamine, Diphenhydramine, Acrinol, Triamcinolone, Loperamide, and Roxithromycin	LC/MS/MS	0.1% formic acid acetonitrile	0.5–10	70.1–93.3	19.8	Pork, milk, and eggs	[68]
7	Sulfadimethoxine, and Ormetoprim	LC-MS/MS	Acetonitrile	100	97–99	<16	Fish feed and fish fillets	[69]
8	Nequinate, and Buquinolate	LC-MS/MS	Acetonitrile	0.001	89.5–108.6	<20	Chicken muscle, chicken liver, chicken heart, swine muscle, swine heart, cattle muscle, sheep muscle, egg	[67]
9	115 Drugs	LC-MS/MS	1% (v/v) acetic acid acetonitrile -QuEChERS	0.73–745.15	67.3–117.9	<19.6	Beef	[31]
10	Metoserpate, Buquinolate, and Diclofenac	LC-MS/MS	0.1% formic acid in acetonitrile	1–2	74.06–108.65	13.67	Pork, milk, and eggs	[66]
11	Anesthetics, and Sedatives(Metomidate etc.)	LC-ESI/MSMS	Acetonitrile and 0.1 % ammonium acetate in acetonitrile	0.5–5	64.7–112.5	1.0–8.6	Flatfish, eel, shrimp	[70]
12	19 Compounds	LC-MS/MS	Acetonitrile-QuEChERS	0.02–4.31	60.6–117.7	≤20.6	Beef, pork, sheep, horse, chicken, prawn, fish, liver, milk, fat	This work

sulfaethoxypyridazine, yohimbine hydrochloride, and loperamide hydrochloride in prawn demonstrated strong matrix effects (|ME|>50%). On the other hand, o-aminobenzoic acid, ormetoprim, buquinolate, and ciclesonide showed a moderate matrix effect (50%>|ME|>20%) in pork.

Given the inherent complexity of animal-derived food matrices, the matrix-matched standard curve external standard method was employed in this study to mitigate the impact of matrix effects on the determination of the actual samples and to enhance the accuracy of the method for quantification.

3.7. Method validation

A series of matrix standard working solutions of 0.5, 1.0, 2.0, 3.0, 5.0, 10.0, 20.0, and 50.0 ng/mL, prepared in accordance with Section 2.2.2, was utilized. The matrix-matched calibration curves of the 19 target veterinary residues were plotted. The horizontal coordinate x represented the concentration of the analyte and the vertical coordinate y represented the measured peak area. The results are shown in Table 4. The correlation coefficients (R^2) of the working curves in the range of 0.5–50.0 ng/ml were 0.991–0.999, indicating a good linear relationship.

It is important to note that the limits of detection (LODs) and limits of quantitation (LOQs) values varied across different matrices for the same analyte. The LODs and LOQs were determined by analysing the spiked samples with signal-to-noise(S/N) ratios of 3 and 10, respectively. The peak areas, noise signals, and spiked concentrations of some typical substances were shown in Table 5. Representative transition chromatograms of spiked standard of chicken and beef samples were shown in Figs. 7 and 8. The LOD in cattle, chicken, prawn, fish, pork, sheep, hourse, liver, fat, and milk were 0.01–0.52, 0.01–1.26, 0.01–0.88, 0.01–0.85, 0.01–1.29, 0.01–0.32, 0.01–0.69, 0.01–0.91, 0.01- 0.37, and 0.01–0.29 μg/kg ($S/N \geq 3$) and the LOQ were 0.03–1.74, 0.04–4.20, 0.03–2.94, 0.04–2.84, 0.03–4.31, 0.02–1.07, 0.02–2.30, 0.02–3.05, 0.02–1.23, and 0.02–0.96 μg/kg ($S/N \geq 10$), respectively(Table 4). Upon comparison, the LOQs of all the analytes in the method were found to be lower than the veterinary drug limits set by Korea, Canada, US, and the European Union. This indicates that the method meets the requirements for routine testing of veterinary drug residues in multi-matrix animal-derived foods. The specificity of the method was analyzed by using five blank samples of each matrix. None of the chromatographic peaks were detected at the retention times of the target analytes. These results indicate that there were no matrix compounds in the purified liquids of blank samples that might have produced a false positive signal.

In addition, the recoveries, and relative standard deviations (RSDs) were investigated to evaluate the application accuracy and precision for the determination of 19 veterinary residues. The method was validated in accordance with the CODEX guidelines(CAC/GL 71–2009) [55,56] and the Ministry of Food and Drug Safety's Guidelines for Analysis of Residual Animal Drugs of Korea [57]. Recoveries of 60–120% and relative standard deviations of 20–30% or lower served as the validation criteria for the determination of the recoveries and RSD. The spiked levels were determined according to the requirements of the limits for the target substances in the Applicability of Veterinary Drug MRLs for food in general [58], Maximum Residue Limits (MRLs) for Veterinary Drugs in Foods [59], Regulation (EU) No 43/2020, Regulation (EC) No 470/2009, and Code of Federal Regulations Title 21 [60]. The samples

were spiked with 5, 10, and 50 μg/kg of the analyte (0.6, 1.2, and 10 μg/kg for ciclesonide). Six replicates of three quality control samples were analyzed on the same day and on three consecutive days, respectively.

The results showed that the target animal residues could achieve satisfactory recovery rates (60.6–117.7 %) in ten matrices. The RSD for both intra-day and inter-day precision, shown in Table 6($n = 6$), were ≤14.5 % and ≤16.7 % in beef, ≤12.4% and ≤15.6 % in pork, ≤15.4 % and ≤19.6 % in sheep, ≤16.4 % and ≤18.8 % in hourse, ≤15.6 % and ≤18.7 % in chicken, ≤16.5 % and ≤19.7 % in prawn, ≤12.4 % and ≤17.6 % in fish, ≤16.9 % and ≤20.6 % in liver, ≤11.3 % and ≤12.5 % in milk, ≤16.4 % and ≤17.4 % in fat, respectively. From the collected data, it can be inferred that the developed UPLC-MS/MS method was precise and accurate.

The LC-ESI(+)-MS/MS method described in this study is capable of complying with the daily detection requirements for target veterinary residues, which is significantly lower than the maximum limit level compared with the corresponding standards. Nevertheless, the lower limit requirements for ciclesonide in the EU regulations indicate a necessity for further optimisation of the conditions and modes of detection for ciclesonide. Atmospheric pressure chemical ionisation (APCI) or atmospheric pressure photoionization (APPI) sources are employed primarily for the analysis of compounds with medium/non polarity. In certain instances, analytes are unable to generate ions with sufficient strength via electrospray ionisation (ESI) due to structural and polarity constraints. In such cases, the utilisation of APCI can enhance the ion yield, thereby serving as a valuable complement to ESI. Ciclesonide is a neutral compound, and it is difficult to detect it with adequate sensitivity(Yamamoto et al. 2021). Detection using LC-APCI-MS/MS(Su et al. 2011) or LC-APCI-MS/MS modes ([7], Mascher et al. 2008), which can assist ionization, may result in lower detection or quantitation lines.

3.8. Comparison with other reported methods

The principal parameters of the proposed method were contrasted with other LC-MS/MS methods reported in the literature for the detection of veterinary residues in foods of animal origin (Table 7). In a study conducted by Kim et al. [61], LC-MS/MS was employed to detect seven veterinary residues in milk. Five of these were found to be identical to those identified in the present study. Similarly, Zhang et al. [38] employed LC/ESI-MS/MS to detect four veterinary residues in porcine muscle, milk, and table eggs. Three of these were found to be identical to those identified in the present study. Nevertheless, the assay developed in this study is capable of achieving a lower limit of quantification and encompasses a broader range of matrices, including beef, chicken, and fish, which renders it more widely adaptable. Kim et al. [56] employed LC-MS/MS to identify 64 veterinary residues, including acetanilide, dl-methylephedrine HCl, phenacetin, antipyrine, diethylcarbamazine, tripelennamine, yohimbine, loperamide, and others, in flatfish, eel, and shrimp. Nevertheless, this study was able to achieve lower quantification limits for a number of compounds, including acetanilide, dl-methylephedrine HCl, loperamide, and diethylcarbamazine. In order to comply with the regulatory limits set by Korea, Canada, the EU, and the USA, a number of different matrix assays were employed. The results demonstrated satisfactory accuracy and precision. Yohimbine is an α2-adrenergic receptor antagonist that is most commonly used in veterinary medicine to reverse the effects of the α2-receptor agonists, xylazine and detomidine [62] . However, the focus of the current detection methods is on botanicals, barks and dietary supplements [63–65], with limited research having been conducted on the detection of this substance in food matrices of animal origin. Nakajima et al. [66] developed an LC-ESI-MS/MS method for the determination of nequinate and buquinolate in eight matrices(chicken muscle, liver, heart etc.). In contrast, this method can detect a greater number of substances and involves a greater number of matrices. In a study published by Jung et al. [67], an LC-MS/MS method was developed for the determination of 115 veterinary drug residues in beef. Of these, 10 overlapped with those tested in this paper, but had higher LOQs (acetanilide: 1.2 μg/kg, dl-methylephedrine: 1.0μg/kg, phenacetin: 1.3μg/kg, antipyrine: 1.7μg/kg, diethylcarbamazine: 1.2μg/kg, acriflavine: 2.7μg/kg, tripelennamine: 1.18μg/kg, ormetoprim: 1.6μg/kg, yohimbine: 1.4μg/kg, loperamide: 0.8μg/kg)and did not adequately cover their matrices. The authors of the paper also noted that their LOQs were higher than those reported in previous studies of multi-residue analyses of beef. As per the discussion, it can be concluded that the QuEChERS combined with the UPLC-MS/MS method proposed in this work has more advantages over other methods.

3.9. Determination of veterinary drugs in real samples

The applicability of the optimized UPLC-MS/MS method was evaluated on authentic samples procured from local markets. A total of 50 batches (5 batches per matrix) of the samples were subjected to analysis and quantification using matrix-matched analytical calibration curves. The results demonstrated that none of the aforementioned substances were detected.

4. Conclusions

In this study, a QuEChRES coupled with UPLC-MS/MS method was developed for the determination of 19 veterinary drug residues in foods of animal origin. This method enabled the simultaneous detection of multiple veterinary residues in multiple matrices (beef, pork, sheep, horse, chicken, prawn, fish, liver, milk, fat). At present, there is a paucity of literature pertaining to the detection of 19 target veterinary drug residues in animal-derived foods. It should be noted that some countries (e.g. China) have yet to establish relevant limits and testing standards. In comparison to existing reports, the method proposed here is relatively simple, highly sensitive, and applicable to a variety of matrices. The method complies with the limit requirements of relevant matrices in the food safety standards of South Korea, the United States, Canada, and other countries. It has been successfully used to detect a variety of animal-derived foods in the local market, providing valuable technical assistance for cross-border food safety testing.

CRediT authorship contribution statement

Qianran Sun: Writing – review & editing, Writing – original draft. **Jun Liu:** Project administration, Funding acquisition. **Yuan Gou:** Formal analysis, Data curation. **Tieyuan Chen:** Investigation, Formal analysis. **Xiaofang Shen:** Methodology, Investigation. **Tao Wang:** Validation, Supervision. **Yongli Li:** Software, Resources. **Huizhen He:** Validation, Software. **Huidan Deng:** Visualization, Validation. **Yi Hua:** Visualization, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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