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Simultaneous Determination of 61 Veterinary Drug Residues in Animal Derived Food by Clean-Up LPAS Combined with LC-MS/MS

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Abstract

A rapid analytical method was established for the simultaneous determination of 61 veterinary drug residues, covering 13 different drug categories, in five animal-derived food matrices (pork, pork liver, chicken, egg, and beef). The method integrated Low-Pressure Automated Solid-phase extraction (LPAS) as a clean-up procedure with liquid chromatography-tandem mass spectrometry (LC-MS/MS) for detection. The samples were extracted using 8 mL of acetonitrile:water (90:10, containing 0.2% formic acid), followed by purification with LPAS, and were directly analyzed using LC-MS/MS without an evaporation step. The experimental conditions, including LC-MS/MS parameters, extraction solvents and volumes, as well as the clean-up procedure for all 61 veterinary drugs, were systematically optimized. Method validation parameters, such as accuracy, precision, limits of detection (LOD), limits of quantitation (LOQ), linearity, and matrix effects, were thoroughly evaluated to ensure the reliability and robustness of the developed method. The results demonstrated good linearity for all analytes in the concentration range of 0.5 μg/L - 50 μg/L, with correlation coefficients (R^2) ≥ 0.995. Recoveries at three spiked levels (5 μg/kg, 10 μg/kg and 50 μg/kg) in the 5 tested matrices ranged between 70.9% and 119.0%, with relative standard deviations (RSD) of 0.1% - 10.9%. The method showed low detection and quantitation limits, with LODs ranging from 0.03 μg/kg to 1.5 μg/kg and LOQs from 0.1 μg/kg to 5.0 μg/kg. When applied to analyze 731 real samples, 13 drug categories were detected in a total of 64 instances, among which 12 categories of restricted drugs were identified 57 times, and two types of prohibited drugs were found seven times; no other veterinary drugs were detected. These findings suggest that the

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simplified LPAS clean-up procedure is superior to the conventional QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, making it highly suitable for batch analysis and monitoring of multiple veterinary drug residues in animal-derived foods.

Keywords

Clean-Up LPAS, LC-MS/MS, Animal Derived Food, Multiple Veterinary Drug Residues, Rapid Test

1. Introduction

Veterinary drugs, which are essential for preventing and treating animal diseases, are used not only to prevent, treat, and diagnose diseases but also to regulate physiological functions in animals, including antibiotics, antiparasitic drugs, antifungal drugs, bronchodilators and more [1]-[3]. Owing to the perceived benefits of veterinary drugs, some breeders employ them irrationally, driven by the desire to enhance economic returns or due to insufficient awareness of the potential hazards associated with these drugs [4] [5]. However, these veterinary drugs remain in animals and enter the human body through the food chain and further exacerbates risks to human health [6]-[8]. The harm of veterinary drug residues to human health is multifaceted. Residues of antibiotic veterinary drugs may induce drug resistance in humans, thereby diminishing the efficacy of antibiotics in treating infections, which can lead to therapeutic failures and increased medical costs [9]. Additionally, certain veterinary drug residues may also trigger allergic reactions, causing symptoms such as rashes, itching, and breathing difficulties, and endangering life in severe cases. Long-term consumption of animal-derived foods containing veterinary drug residues may also have adverse effects on the human immune system, nervous system, and endocrine system, increasing the risk of chronic diseases such as cancer and cardiovascular diseases [10] [11]. Moreover, veterinary drug residues can also pollute the ecological environment, affect the quality of soil, water, and air, and disrupt the ecological balance [12]. Therefore, China, EU, USA, and others have set the maximum residue limits (MRL) [13]-[15] of veterinary drugs in animal derived food and they initiate legal procedures in any excessive cases; to meet the monitoring objectives and regulatory requirements, it is of great necessity to provide accurate test results and develop effective and reliable veterinary drug residue testing methods.

Veterinary drugs are mainly composed of involatile polar or low-polar compounds. Gas chromatography (GC) or gas chromatography-tandem mass spectrometry (GC-MS/MS) is not suitable for testing such components [2]. The liquid chromatography (LC) also has limitations in detecting veterinary drugs and their metabolites, as well as in classifying certain veterinary drugs, due to its relatively low sensitivity and selectivity. In recent years, the methods have been largely superseded by chromatography-tandem mass spectrometry techniques, which offer enhanced selectivity and improved detection efficiency [16]-[18]. The advancements in test

equipment have significantly increased the capacity for simultaneous detection of veterinary drugs. For instance, the method established by Zhao *et al.* [19] enabled the analysis of 80 veterinary drugs. Similarly, Yin *et al.* [20] established a method for the detection of 210 veterinary drugs in pork, while Alcantara-Duran *et al.* [6] reported a method for the analysis of 77 veterinary drugs in chicken. However, these methods are mostly based on analysis on one and the same matrix in the pretreatment method of QuEChERS, and have limitations in analyzing multiple matrices. Li *et al.* developed a screening method for 204 veterinary drugs [21], while Desmarchelier *et al.* established a qualitative LC-MS/MS method for the detection of 154 veterinary drugs [22]. Although these methods enable the detection of multiple matrices, they are incapable of performing quantitative analysis. This limitation is particularly significant given that veterinary drug residues in food typically exist at concentrations in the low microgram or even nanogram range [16], which presents a major challenge for residue testing in animal-derived food products.

The matrices of livestock and poultry products are complex due to the presence of substantial amounts of lipids, proteins, and other macromolecular substances [23]-[25], resulting in false positives or false negatives test results. Consequently, sample preparation is still a challenging process for testing drug residues in animal derived foods. In this study, the sample pretreatment methods were improved and the instrumental analysis conditions were optimized to address the deficiencies of existing technologies. As a result, an efficient, accurate, and sensitive method for detecting veterinary drug residues in animal-derived foods was established. By enabling the simultaneous detection of multiple veterinary drug residues, the developed method enhances detection efficiency and reduces analytical costs, providing strong technical support for the quality and safety supervision of animal-derived foods. Clean-up LPAS columns made of novel high-polymer materials were used to rapidly filter and decontaminate multiple animal derived food. In conjunction with LC-MS/MS, a high-throughput test method was established that enables the simultaneous, rapid, accurate, and efficient detection of 61 veterinary drugs across 13 categories, including 21 sulfonamides, 10 quinolones, 9 β-receptor agonists, 4 tetracyclines, 4 amphenicols, 3 macrolides, 2 sedatives, 2 lincosamide antibiotics, 2 penicillin and 1 each of nitroimidazole, polyether anticoccidials, corticosteroid and antiviral agents. The method was verified with satisfactory results obtained and successfully applied in testing 731 real samples to investigate the safety conditions of veterinary drug residues in livestock and poultry products.

2. Experiments

2.1. Drugs and Reagents

1290-6470 LC-MS (Agilent, USA), TGL-16 High-speed Refrigerated Centrifuge (Sichuan Shuke Instrument Co., Ltd., China), KNS-2500 Multi-Tube Vortex Mixer (Krownus Scientific Experimental Instrument Co., Ltd., China), KH-500B Ultrasonic Cleaner (Kunshan Hechao Ultrasonic Instruments Co., Ltd., China), UPT-II-100L ULUPURE-series Ultrapure Water Machine (ULUPURE, China),

Milli-Q Ultrapure Water Machine (Millipore, USA).

Carbinol (chromatographically pure, Fisher Chemical), acetonitrile (chromatographically pure, Fisher Chemical), formic acid (chromatographically pure, Fisher Chemical), ammonium acetate (chromatographically pure, Tianjin Kemiou Chemical Reagent Co., Ltd.), ammonium formate (GR (guarantee reagent)), clean-up column-LPAS (Beijing Knorth Technology Co., Ltd., China), and pure water used in this experiment were produced by the ultrapure water machines in the laboratory. All standard substances are standard solid substances with a purity of over 95% and were purchased from Dr. Ehrenstorfer GmbH. The names, CAS No. and groups of all standard substances are given in Table 1.

Table 1. Name of each target compound and the MS/MS acquisition conditions.

NO.	veterinary drug (Types)	CAS number	Quantitative ion (M/z) &Collision Energy (V)	Qualitative ion (M/z) & Collision Energy (V)	Fragment (V)	Retention time (min)	Polarity
1	Florfenicol amine (Am)	76639-93-5	248.1/230 (5)	248.1/130 (21)	100	0.650	Positive
2	Amoxicillin (Pe)	26787-78-0	366/349 (4)	366/114 (20)	90	1.400	Positive
3	Sulfaguanidine (Su)	57-67-0	215/156 (10)	215/108 (20)	100	1.424	Positive
4	Sulfacetamid (Su)	144-80-9	215.1/156.1 (5)	215.1/92 (20)	70	1.430	Positive
5	Benzonitrile (β-R)	54239-37-1	220.1/202.1 (5)	220.1/160.1 (13)	100	1.540	Positive
6	Terbutalinee (β-R)	23031-25-6	226.1/152.1 (12)	226.1/125 (24)	92	1.910	Positive
7	Salbutamole (β-R)	18559-94-9	240/148 (15)	240/222.1 (5)	80	2.060	Positive
8	Metronidazole (N)	443-48-1	172.1/128 (12)	172.1/82 (26)	90	2.061	Positive
9	Sulfadiazine (Su)	68-35-9	251/156 (20)	251/108 (25)	100	2.139	Positive
10	Sulfathiazole (Su)	72-14-0	256/156 (20)	256/108 (25)	100	2.966	Positive
11	Sulfapyridine (Su)	144-83-2	250.1/156 (10)	250.1/184 (15)	110	3.470	Positive
12	Sulfamerazine (Su)	127-79-7	265/156 (20)	265/172 (20)	100	4.090	Positive
13	Fenoterole (β-R)	13392-18-2	304.1/135.2 (15)	304.1/286.2 (8)	120	4.570	Positive
14	Sulfameter (Su)	651-06-9	281/156 (15)	281/108 (25)	130	4.885	Positive
15	Lincomycin (L)	154-21-2	407.2/126 (30)	407.2/359 (15)	150	4.928	Positive
16	Sulfamoxole (Su)	729-99-7	268/156 (13)	268/113 (16)	110	4.990	Positive
17	Sulfamethizole (Su)	144-82-1	271/156 (20)	271/108 (26)	100	5.030	Positive
18	Sulfamethazine (Su)	57-68-1	279.1/186.1 (15)	279.1/156.1 (16)	120	5.120	Positive
19	Trimethoprim (Pe)	738-70-5	291.1/230.1 (25)	291.1/123 (25)	120	5.137	Positive
20	Sulfamethoxypyridazine (Su)	80-35-3	281.1/156 (15)	281.1/108 (25)	105	5.289	Positive
21	Fleroxacin (Q)	79660-72-3	370.1/326 (15)	370.1/269 (25)	130	5.300	Positive
22	Sulfachloropyridazine (Su)	80-32-0	285/156 (20)	285/108 (25)	100	5.447	Positive
23	Ofloxacin (Q)	82419-36-1	362/318.1 (15)	362/261.1 (26)	130	5.470	Positive
24	Pefloxacin (Q)	70458-92-3	334.1/316.2 (20)	334.1/290.2 (16)	130	5.504	Positive
25	Sulfamethoxazole (Su)	723-46-6	254.1/108 (25)	254.1/156 (10)	100	5.540	Positive
26	Tetracycline (T)	60-54-8	445.2/410 (19)	445.2/427.1 (12)	125	5.550	Positive
27	Sulfamonomethoxine (Su)	1220-83-3	281.1/156.1 (15)	281.1/108.1 (26)	100	5.565	Positive
28	Norfloxacin (Q)	70458-96-7	320/302.1 (20)	320/276.1 (15)	130	5.586	Positive
29	Amantadine(Aa)	768-94-5	152.2/135 (18)	152.2/93 (30)	100	5.588	Positive
30	Ractopamine hydrochloridee (β-R)	97825-25-7	302/121 (22)	302/164.1 (10)	110	5.634	Positive

Continued

31	Clorprenalinee (β-R)	3811-25-4	214.1/154 (13)	214.1/196.1 (5)	80	5.666	Positive
32	Oxytetracycline (T)	79-57-2	461.2/426 (21)	461.2/443.1 (13)	125	5.670	Positive
33	Ciprofloxacin (Q)	85721-33-1	332.1/314.1 (20)	332.1/231 (42)	135	5.690	Positive
34	Enrofloxacin (Q)	93106-60-6	360/316.2 (20)	360/245 (30)	125	5.750	Positive
35	Sulfadoxine (Su)	2447-57-6	311/156 (20)	311/108 (25)	130	5.750	Positive
36	Danofloxacin mesylate (Q)	119478-55-6	358.1/340.1 (25)	358.1/255 (46)	140	5.780	Positive
37	Sulfisoxazole (Su)	127-69-5	268/156 (10)	268/108 (10)	100	5.808	Positive
38	Lomefloxacin (Q)	98079-51-7	352.1/265.1 (20)	352.1/308.1 (10)	130	5.822	Positive
39	Benzenemethanole (β-R)	37148-27-9	277.1/203 (12)	277.1/259.1 (5)	100	5.848	Positive
40	Sulfabenzamide (Su)	127-71-9	277.1/156 (10)	277.1/108 (25)	80	5.980	Positive
41	Sarafloxacin (Q)	98105-99-8	386.1/342.1 (18)	386.1//299 (35)	132	6.010	Positive
42	Tulobuterole (β-R)	41570-61-0	228.1/154 (13)	228.1/172 (5)	100	6.190	Positive
43	Sulfaphenazole (Su)	526-08-9	315/158 (30)	315/222 (20)	130	6.260	Positive
44	Chlorotetracycline (T)	57-62-5	479.1/444 (19)	479.1/462 (16)	130	6.473	Positive
45	Sulfadimethoxine (Su)	122-11-2	311/156 (20)	311/108 (26)	130	6.490	Positive
46	Sulfaquinoxaline (Su)	59-40-5	301.1/156 (11)	301.1/108 (22)	110	6.640	Positive
47	Oxolinic acid (Q)	14698-29-4	262.1/216 (30)	262.1/160 (40)	90	6.720	Positive
48	Doxycycline (T)	6543-77-7	445.1/428 (15)	445.1/321 (33)	130	6.920	Positive
49	Tilmicosin (M)	108050-54-0	869.6/174 (50)	869.6/696.4 (45)	260	7.090	Positive
50	Clindamycin (L)	18323-44-9	426/126 (30)	426/378 (20)	120	7.140	Positive
51	sulfanitran (Su)	122-16-7	336/294 (10)	336/156 (10)	110	7.374	Positive
52	Tylosin (M)	1401-69-0	917/174 (42)	917/101 (54)	240	7.770	Positive
53	Erythromycin (M)	114-07-8	734.5/158.1 (30)	734.5/576.3 (14)	170	7.800	Positive
54	Dexamethasone (Co)	1950/2/2	393.1/373.1 (5)	393.1/355 (10)	100	8.100	Positive
55	Chlorpromazine hydrochloride (Se)	69-09-0	319.2/86 (15)	319.2/246 (20)	120	8.120	Positive
56	Penbutolole (β-R)	36507-48-9	292.1/236 (12)	292.1/201 (20)	110	8.140	Positive
57	Diazapam (Se)	439-14-5	285.1/193 (32)	285.1/153.9 (25)	170	8.460	Positive
58	Thiamphenicol (Am)	15318-45-3	354/185 (20)	354/290 (6)	120	4.535	Negative
59	Florfenicol (Am)	73231-34-2	356/336 (5)	356/185 (15)	120	5.610	Negative
60	Chloramphenicol (Am)	56-75-7	321/257 (10)	321/152 (15)	117	6.330	Negative
61	Nicarbazin (Po)	587-90-6	301/137 (15)	301/107 (45)	70	8.760	Negative
62	Amantadine-D15	33830-10-3	167.2/150 (18)	/	100	5.511	Positive
63	Norfloxacin-D5	1015856-57-1	325.1/307.2 (17)	/	130	5.588	Positive
64	Ciprofloxacin-D8	1130050-35-9	340.2/322.2 (20)	/	135	5.670	Positive
65	Enrofloxacin-D5	1173021-92-5	365.2/321.2 (18)	/	120	5.750	Positive
66	Sulfadoxine-D3	1262770-70-6	314/156 (20)	/	130	5.750	Positive
67	Tulobuterol-D9	1325559-14-5	237.1/155 (13)	/	100	6.160	Positive
68	Sulfadimethoxine-D6	73068-02-7	317/156 (20)	/	130	6.460	Positive
69	Chlorpromazine-d6 hydrochloride	1228182-46-4	325.2/92.2 (15)	/	100	8.118	Positive
70	Chloramphenicol-D5	202480-68-0	326/157 (15)	/	117	6.310	Negative
71	Nicarbazin-d8	1156508-87-0	309/141 (15)	/	70	8.740	Negative

Note: In Table 1, * marks quantitative ions, 62 - 71 are internal standard substance, () means drug classification: (Am), Amide alcohols; (Pe), Penicillins; (Su), Sulfonamides (β-R), β-Receptor agonists; (N), Nitroimidazoles; (L), Lincosamide antibiotics; (Q), Quinolones; (Co), corticosteroid; (T), Tetracyclines;(An), Antiviral agents; (M), Macrolides; (Se), Sedatives; (Po), Polyether anti-coccidials.

2.2. Preparation of Standard Solutions

Standards at 10 mg in each target group were accurately weighed and placed in 10 mL brown volumetric flasks, sufficiently dissolved with methanol to a constant volume, and then transferred to brown standard sample bottles after uniform mixing to prepare single standard stock solutions at a concentration of 1 mg/mL and stored at −20˚C. Amoxicillin was dissolved in acetonitrile: water (1:1) to a constant volume; quinolones were dissolved in an appropriate amount of sodium hydroxide solution at a concentration of 0.03 mol/L and then dissolved in methanol to a constant volume. A mixed standard solution with a concentration of 10 μg/mL containing all the diluted single standard methanol was prepared, and a series of working curves with seven concentrations of 0.5 μg/L, 1.0 μg/L, 2.0 μg/L, 5.0 μg/L, 10.0 μg/L, 20.0 μg/L, and 50.0 μg/L were prepared after diluting the mixed standard solution with ultra-pure water or blank matrices.

2.3. Instruments

2.3.1. Instruments Information

1290-6470 LC-MS (Agilent, USA), TGL-16 High-speed Refrigerated Centrifuge (Sichuan Shuke Instrument Co., Ltd., China), KNS-2500 Multi-Tube Vortex Mixer (Krownus Scientific Experimental Instrument Co., Ltd., China), KH-500B Ultrasonic Cleaner (Kunshan Hechao Ultrasonic Instruments Co., Ltd., China), UPT-II-100L ULUPURE-series Ultrapure Water Machine (ULUPURE, China), Milli-Q Ultrapure Water Machine (Millipore, USA).

2.3.2. LC-MS/MS Instrument Conditions

In the test, detection was performed using Agilent 1290-6470 HPLC-MS/MS combined with Agilent Zorbax SB-C18 and 50 mm × 0.30 mm × 1.8 μm chromatographic columns, with the following specific conditions: sample volume 2.0 μL; mobile phase A is methanol and B is 5 mmol/L ammonium acetate solution containing 0.1% formic acid; gradient elution program: 0 min - 2 min; 10% A; 2 min - 7 min; 10% - 80% A; 7 min - 7.5 min, 80% A; 7.5 min - 8 min, 80% - 95% A; 8 min - 9 min, 95% A; 9 min - 9.5 min, 95% - 10% A; 9.5 min - 14 min, 10% A; column temperature: 35˚C; flow rate: 0.3 mL/min; electrospray ionisation (ESI) source, positive and negative ion modes; drying gas temperature: 325˚C; capillary drying gas flow: 7 L/min; nebulizer pressure: 35 psi; sheath gas flow: 12 L/min; sheath gas heater: 300˚C; the chromatographic conditions for dynamic multiple reaction monitoring (dMRM) detection, internal standard rations and each target veterinary drug of interest are shown in Table 1.

2.4. Sample Preparation

The collection, preparation and storage of pork, pig liver, chicken, egg and beef samples were carried out according to the methods of sample preparation and storage of animal and poultry products according to [26]. The above samples, which were accurately weighed at 2.00 ± 0.05 g, were instilled with 100 μL of

internal standard mixture solution at a concentration of 0.2 μg/mL, mixed by vortexing, and then placed in a 50 mL centrifuge tube and left in the dark for 20 min. Then the samples were instilled with 8mL acetonitrile/water solution (90:10) containing 0.2% formic acid, under vortex shaking for 2 min at the speed of 2000 r/min and ultrasonic extraction after 20 min of ice water bath, and then centrifuged at 4˚C for 5 min at the speed of 5000 r/min. 2 mL of supernatant was injected into the clean-up LPAS column at the rate of 1 drop per second, a 0.22 μm organic filter head was installed at the lower end of the column to collect the filtrate into the sample bottle for analysis by LC-MS/MS, with the specific operation as shown in Figure 1.

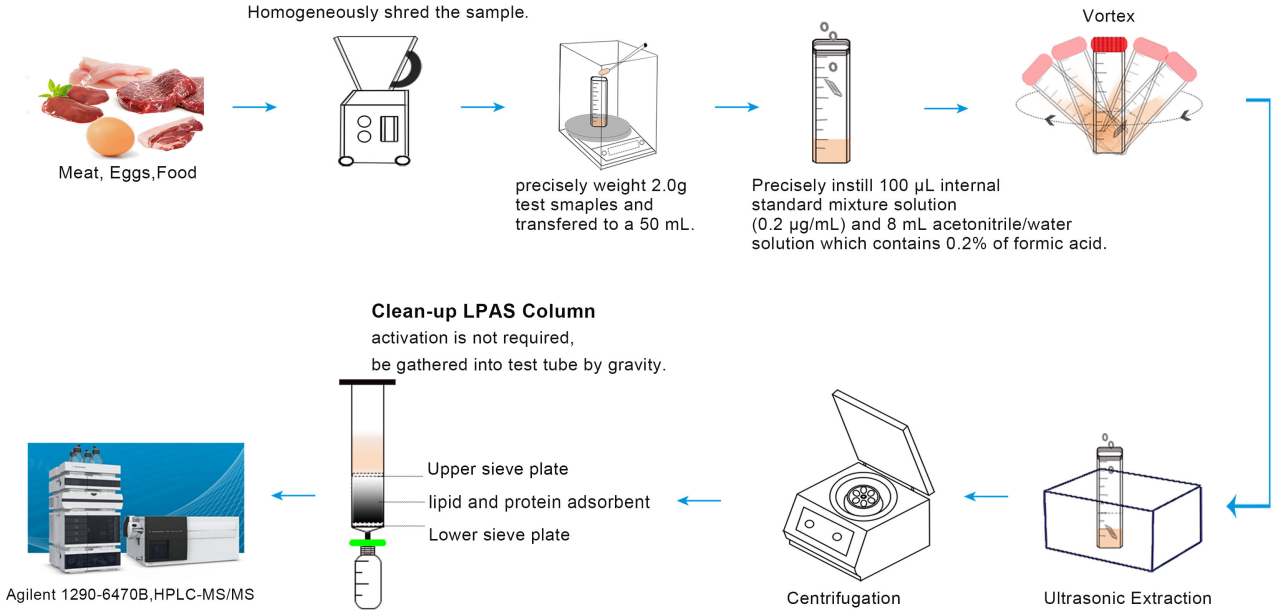


Figure 1. Sample pretreatment main process.

2.5. Method Validation

Method validation includes accuracy, precision, LOD, LOQ, linear range and matrix effect. Linearity was evaluated using the correlation coefficient (R^2) of the matrix-matched calibration curve of each veterinary drug. Accuracy and precision were obtained in the recovery tests at the three addition levels (low, medium and high: 5 μg/kg, 10 μg/kg, and 50 μg/kg) ($n = 3$). In accordance with the standards set up in (EU) 2021/808 [27], the LOQ is defined so that the accuracy and precision of the quantitative results are at the lowest peak levels in the acceptable range. Therefore, in the experiment, 0.25 times MRL standard solutions were added to blank matrix samples ($n = 10$) (reference value 0.05 mg/kg for MRL-free pesticides). The LOD of each matrix was calculated using the following formula [4].

$$\text{LOD} = 3.3 \times (SD_{MRL} * 0.25/\text{S})$$

$$\text{LOQ} = 10 \times (SD_{MRL} * 0.25/\text{S})$$

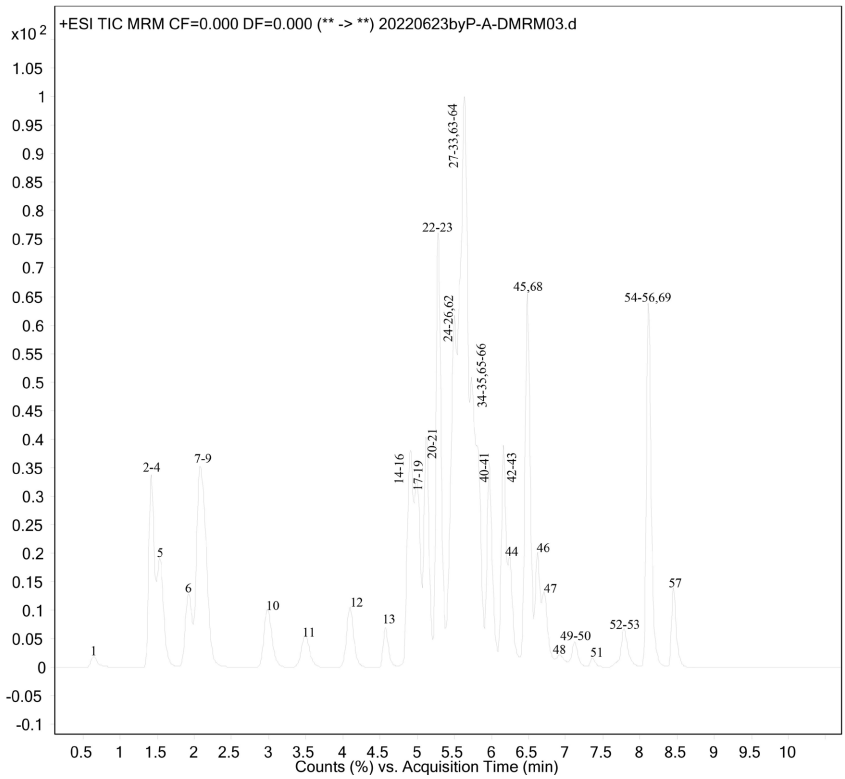
ME was calculated from the ratios of the slopes of the matrix-matched calibration curves to the solvent calibration curves [27] [28].

3. Results and Discussions

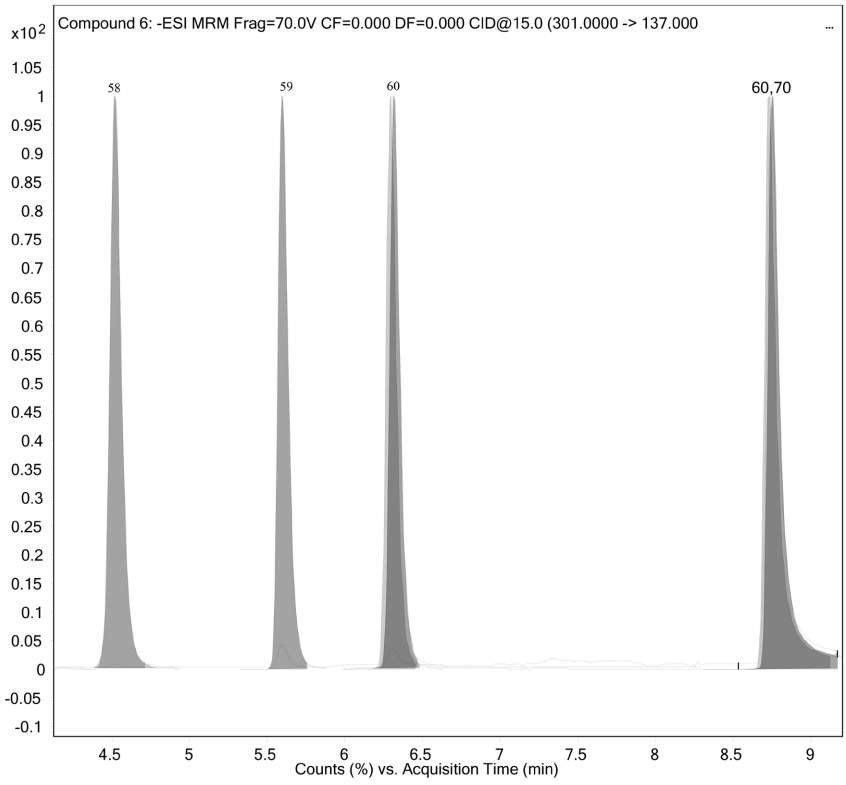
3.1. Instrument Condition Optimization

To optimise the LC conditions, mobile phases, gradient elution programmes and other key LC parameters were repeatedly adjusted. Comparisons were conducted between methanol and acetonitrile as organic phases, and between aqueous solutions of 0.1% formic acid and 5 mmol/L ammonium acetate containing 0.1% formic acid as aqueous phases, and the mobile phase gradients were constantly adjusted to ensure that each parameter could peak normally. It was observed that when the organic phase was acetonitrile, tetracyclines and certain quinolones exhibited significant peak tailing or fronting, along with reduced signal responses. It was observed that when acetonitrile was used as the organic phase, tetracyclines and certain quinolones exhibited significant peak tailing or fronting, along with reduced response values. Similarly, when using only 0.1% formic acid aqueous solution as the aqueous phase, compounds such as chloramphenicol, thiamphenicol florfenicol, and nicarbazin exhibited either no signal or extremely low responses in negative ion monitoring mode. After several experiments, it was found that the response of the target objects was significantly enhanced after adding 5 mmol/L ammonium acetate solution. Considering peak patterns, test sensitivity and isomeric separation of each target compound, methanol combined with 5 mmol/L ammonium acetate aqueous solution containing 0.1% formic acid was ultimately selected as the optimal mobile phase. After further optimization of the gradient elution conditions, the gradient described in section 2.3.1 was finally confirmed to be suitable to simultaneous determination and satisfactory separation of the 61 veterinary drug residues.

Under optimized chromatographic conditions, the parent ions of target compounds were identified through full-scan mode (MS_2Scan) using single standard solution (2 mg/L). Multiple fragment voltages (Fragment/V) were systematically set, and the ion with a mass-to-charge ratio (*m/z*) corresponding to the molecular weight of each compound was selected as the precursor ion by comparing the molecular weights obtained from individual standard solutions, and the fragment voltage that yielded below the maximum ion response was determined as optimal. Then the product ion scan was performed, with the collision energy (CE) optimized, the characteristic daughter ions and their optimal CE values were discovered simultaneously to ensure that each parameter, except for internal standards, possessed at least two characteristic ion pairs. Ultimately, the optimized parameters were applied in dynamic multiple reaction monitoring (dMRM) analysis. And the detailed retention times, MRM ion pairs, monitoring modes, and optimal CE values for all 61 veterinary drug residues are summarized in **Table 1**, with the total MRM ion diagrams shown in **Figure 2(a)**, **Figure 2(b)**.



(a)



(b)

Figure 2. Total MRM ion diagram of 61 veterinary drugs and 10 internal standards (10 μg/L): (a). Positive Polarity; (b). Negative Polarity.

3.2. Optimization of Extraction Reagents

Common extraction agents used in the pretreatment of samples for veterinary drug residue analysis typically include solutions of acetonitrile, acidified acetonitrile, ethyl acetate, phosphate buffer [29]. Animal-derived food matrices genarally contain substantial amounts of lipids, proteins, and other macro-molecular components. Acetonitrile demonstrates favorable characteristics as an extraction solvent due to its ability to minimize lipid co-extraction and facilitate albumin precipitation through protein denaturation [30]. However, its strong dehydration capacity may induce rapid drying and aggregation of samples with low moisture content, thereby compromising extraction efficiency. To address this limitation, extraction efficiency of pure acetonitrile and acetonitril with various proportions of water (10%, 20%, 30%, and 40%, v/v) were compared. As illustrated in **Figure 3**, acetonitrile containing 10% water (v/v) exhibited the highest extraction efficiency, with 47 target objects demonstrating recoveries within the range of 60-120%. This might be because an appropriate amount of water can improve the dispersibility of the sample, preventing the sample from caking due to excessive dehydration, thereby enhancing the extraction efficiency of veterinary drugs. However, when the water content is too high, it may lead to an increased distribution of veterinary drugs in the aqueous phase, thus reducing the extraction rate in the acetonitrile phase.

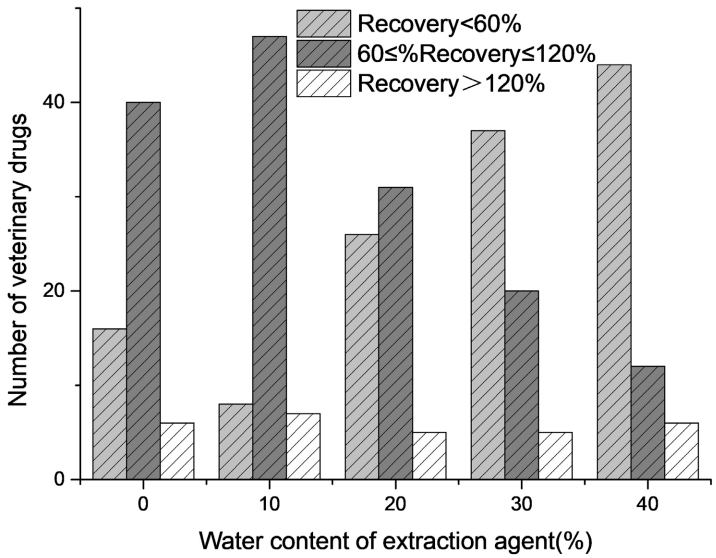


Figure 3. Recovery of veterinary drugs with different water contents in the extraction agent.

To improve the extraction efficiency of each target object, different extraction solvents, including pure acetonitrile and acetonitrile solutions containing 0.1%, 0.2%, 0.5%, and 1% formic acid (FA), were systematically compared. As shown in **Figure 4**, acetonitrile containing 0.2% FA provided optimal extraction performance, with 54 veterinary drugs achieving satisfactory recoveries ranging from 60% to 120%. The addition of formic acid adjusted the pH of the solution,

promoting the ionization of veterinary drugs, making them more soluble in acetonitrile and thus enhancing the extraction efficiency. However, when the formic acid content was too high, it might affect the stability of some veterinary drugs, leading to a decline in the recovery rate. Therefore, after comprehensive consideration, the acetonitrile solution containing 0.2% formic acid was chosen as the optimal extraction reagent.

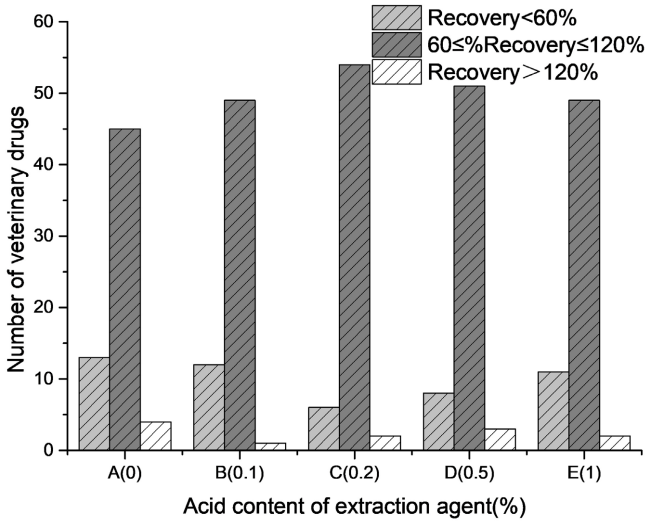


Figure 4. Recovery of veterinary drugs with different acid content in the extraction agent. Note: A: acetonitrile; B: 0.1% FA in Acetonitrile; C: 0.2% FA in Acetonitrile; D: 0.5% FA in Acetonitrile; E: 1% FA in Acetonitrile.

3.3. Optimization of Extraction Volumes

After selecting the optimal extraction solvent composition, the dosage of extraction solvent was also optimized. Generally, small extraction volumes resulted in high concentrations of matrix components, which ultimately complicated subsequent purification steps and increased matrix interference. However, extracting in excessive volumes can pose several issues. During the clean-up and detection procedures, it significantly escalates experimental costs. This not only diminishes the recovery rates but also results in the waste of reagents and raises environmental concerns. To systematically investigate the effect of extraction solvent volume on analyte recovery and matrix interference, 35 analytes were randomly selected and extracted using four extraction volumes (4 mL, 6 mL, 8 mL and 10 mL). As shown in **Figure 5**, as the extraction volume increased, the matrix effect was gradually reduced and the overall recovery rate decreased. When the extraction volume was 10 mL, recoveries for 26 analytes dropped to the range of 60% - 80%, with the recovery of metronidazole notably falling below 60%. This reduction was likely caused by excessive dilution of the analytes. Since the extraction volume is too large, the concentration of the target substances in the extraction liquid is relatively reduced. This leads to an increase in losses during the subsequent purification and detection processes, thereby affecting the recovery rate. When the

extraction volume was 8 mL, 30 analytes showed recoveries within the range of 70% - 120%. However, smaller extraction volumes of 6 mL and 4 mL led to excessively high recoveries (>120%) for 8 and 25 analytes, respectively, indicating significant co-extraction of interfering matrix substances due to higher matrix concentrations. This phenomenon could negatively impact detection accuracy. This could be due to the fact that when the extraction volume is too small, the matrix concentration is too high, causing some target substances to be coxtracted with impurities during the extraction process, resulting in a high recovery rate. However, in this case, there may also be high matrix interference, affecting the accuracy of the detection. After comprehensively considering the matrix effect and recovery rate, it was finally determined that 8 mL of acetonitrile/water (90:10) solution containing 0.2% formic acid was used as the extraction volume in this experiment. This choice not only ensures the extraction effect but also minimizes the use of reagents, reduces experimental costs, and is also conducive to reducing environmental pollution, which is in line with the concept of green chemistry.

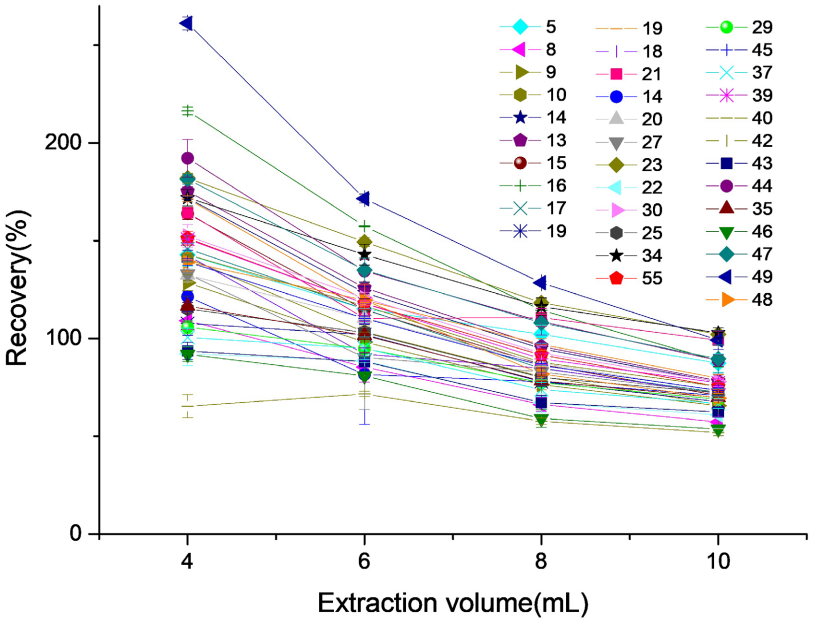


Figure 5. Effects of the different extraction volumes on the extraction effect. Note: The names of corresponding veterinary drugs matching No. in the Figure are available in **Table 1**.

3.4. Optimization of Clean-Up Processes

Solid phase extraction (SPE) is widely used in the detection of veterinary drug residues in animal derived foods due to its high purification efficiency and reliable recovery rates. However, SPE also presents certain inherent disadvantages. Different analyte classes require the specialized adsorbents such as MAX, MCX, HLB, C18, PSA, and GCB, whose separation mechanisms typically involve silica-based adsorption or ion exchange processes. The conventional SPE procedure includes multiple complex steps such as cartridge activation, equilibration, sample loading, washing, analyte elution, nitrogen evaporation, and redissolution. These extensive

operations result in increased analytical time, reagent consumption, and operational costs. Additionally, repeated extraction and solvent evaporation steps demand skilled operators and specialized equipment, thus limiting SPE's suitability for high-throughput applications.

The emergence of QuEChERS methodology addressed these constraints through streamlined liquid-liquid extraction coupled with dispersive SPE (d-SPE), which turns out to be a simpler and more efficient solution for simultaneously detecting multiple veterinary drugs [4] [16]. This method combines liquid-liquid extraction (LLE) with dispersive solid-phase extraction (d-SPE), enabling rapid separation of analytes from matrix interferences through a simple centrifugation step. Common adsorbents utilized in d-SPE include GCB, C18, PSA, and silica gel [31] [32]. Despite being advantageous over traditional SPE in terms of operational simplicity, speed, and cost-effectiveness, QuEChERS purification often exhibits higher matrix effects, reduced recoveries for certain analytes, and elevated costs associated with disposable purification tubes. Consequently, d-SPE is predominantly employed for qualitative screening of large sample sets rather than precise quantitative analyses. Thus, in addition to effective extraction, efficient removal of matrix interferences remains a critical challenge in achieving rapid and accurate quantitative detection of veterinary drugs.

Based on d-SPE and SPE, needle-cylinder clean-up columns were adopted in the test. The needle-cylinder clean-up columns are pre-filled with LPAS (lipid and protein adsorbent) prepared by the chemical bond modification technique, which can absorb interfering substances of lipid and protein better than C18, PSA and GCB, and is more suitable determination of multiple veterinary drug residues in animal derived food. The clean-up procedures of stripping, vortex clean-up, secondary centrifugation and secondary transfer of the supernatant have been eliminated compared with the traditional QuEChERS method, and the clean-up is completed by extracting the supernatant into clean-up tubes, gently pressing the tubes and transferring it to LPAS cartridges at the rate of 1 drop per second, it is easier and faster (specific procedures are shown in **Figure 1**).

The experiment compared the reagent consumption, time efficiency, waste liquid generation, etc. of two commonly used pretreatment methods with the LPAS syringe type purification (**Table 2**). As can be seen from **Table 2**, the LPAS syringe-type purification reduces the consumption of solution reagents, vessels, and reagents compared with SPE and QuEChERS. The amount of waste liquid generated is 6.0 mL, which is only one-fourth of that of SPE, showing a significant advantage in reducing pollution emissions. Additionally, the LPAS syringe-type purification method simplifies the operational procedure, with the single-sample detection time not exceeding 30 minutes. This is only one-fourth of the time required for SPE and half of that for QuEChERS. Therefore, compared with SPE, LPAS has obvious advantages in the purification steps. It can process larger batches of samples in a short time and is friendly to both the health of testers and the environment.

Table 2. Comparison of consumption among three pretreatment methods.

Items	SPE [33]	QuEChERS [34]	LPAS
Purification materials	Solid-phase extraction cartridges, such as HLB, C18, PCA, PCX, etc. 1 piece for each category of drugs	QuEChERS purification tube (containing adsorbents such as neutral aluminum oxide (NA), primary secondary amine (PSA), octadecylsilyl (C18), and polar enhanced polymer (PEP), etc.) 1 piece	1 piece of LPAS filtration type cartridge (containing adsorbents such as lipid and protein adsorbent)
Organic reagent usage amount	15 mL of Methanol	11.5 mL of Acetonitrile	8 mL of Acetonitrile
Inorganic reagent usage amount	18 mL of EDTA buffer	4 g of sodium sulfate	None
Amount of waste liquid (in liquid state)	Approximately 20.0 mL	Approximately 10.0 mL	Approximately 5.0 mL
Consumption of vessels	Many (such as stoppered measuring cylinders, conical flasks, beakers, etc. for preparing lots of solutions)	Less (1 for each of 15 mL, 10 mL, and 5 mL centrifuge tubes are used)	Few (only 1 15 mL centrifuge tube is used)
Average processing time per sample	Approximately 200 min	Approximately 60 min	Approximately 30 min

In the test, commercially available clean-up columns were selected because their fillers are fixed and therefore the clean-up effect is dependent on the volume of clean-up liquid added. In the test, 1 mL, 1.5 mL, 2 mL and 2.5 mL of pork supernatant (the addition amount was 10 μg/kg) samples were extracted and filter liquid was collected through clean-up columns; it was found that when the volume of liquid to be cleaned up was 2 mL, there was the best clean-up effect with less interference from blank matrix impurities and the best recoveries of the 61 veterinary drugs, therefore 2 mL of sample liquid was selected for filtration clean-up in the method. At the same time, a comparison of speeds through the cartridges was made and there was the best clean-up effect when the clean-up speed was 1 drop per second.

In the experiment, 21 sulfonamides were added to pork at an addition of 10 μg/kg (n = 3). The recovery effects of these 21 sulfonamides after purification by SPE, QuEChERS, and LPAS were compared respectively. The results are presented in Figure 6.

Figure 6 shows that for the pork samples purified by SPE, the average recovery rate of sulfonamides was 88.8% with a relative standard deviation (RSD) of 3.3%. When purified by QuEChERS, the average recovery rate was 87.6% with an RSD of 8.2%. After purification by LPAS, the average recovery rate was 92.4% with an RSD of 5.0%. Both the accuracy and precision of these results met the requirements specified in (EU) 2021/808 [27]. The average recovery rates of 3 purification methods all fell within the range of 75% - 110%, and the RSDs ranged from 0.5% to 10.3%. After purification with LPAS and SPE, the precision of sulfonamide recovery was relatively high, with average RSDs both within 5%. Although the recovery performance of SPE was slightly better than that of QuEChERS and LPAS, it had several drawbacks. These included long pretreatment times, high

consumption of reagents and consumables, and the limitation of being able to process only one type of drug at a time, making it an unadvisable choice. Although QuEChERS could purify a relatively wide variety of drugs, the recovery data for sulfonamides were relatively scattered. The average RSD was 8.2%, and the RSDs of three sulfonamides exceeded 10%, indicating relatively low precision. This highlights the advantages of LPAS. In addition to its simple operation and low reagent and consumable consumption, LPAS can achieve good recovery and repeatability.

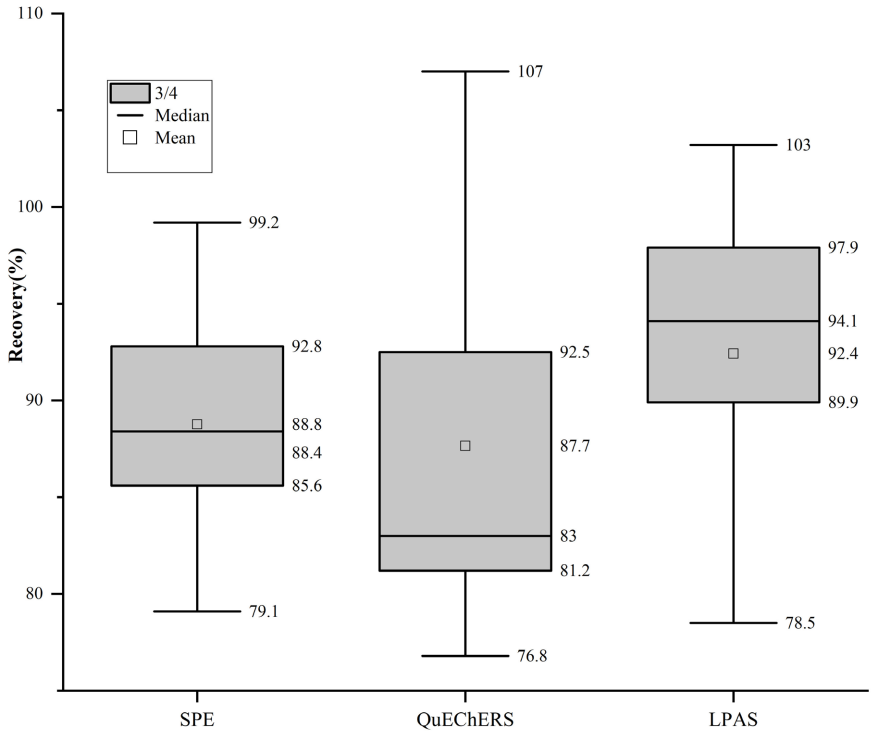


Figure 6. Effects of different purification on the recovery of sulfonamides in pork.

3.5. Method Validation

3.5.1. Accuracy and Precision

Different animal derived foods have different substances and different interferences with veterinary drug residue testing; to eliminate the interfering factors, the 5 types of animal derived food: pork, pig liver, chicken, egg and beef, which are widely consumed by the residents in daily life and tend to be positive, were selected as samples, adding recovery experiments were conducted by adding low, medium and high (5 μg/kg, 10 μg/kg and 50 μg/kg) levels to their blank samples ($n = 3$), their average recovery and RSD were calculated to verify the applicability, accuracy and precision of the method. The results have shown (**Table 3**) that at the low, medium and high addition levels, the total recoveries of the 61 veterinary drugs were in the range of 70.9% - 119.0%, and their RSDs were in the range of 0.1% - 10.9%. The high levels of accuracy and precision achieved demonstrate that this method is fully capable of meeting the detection requirements for 61

veterinary drug residues in various animal-derived foods, providing a reliable technical means for veterinary drug residue detection.

Table 3. Average recoveries and RSDs of 61veterinary drugs in the five matrices (n = 3).

Add levels (μg/kg)	Average Recoveries (%)					RSD (%)				
	pork	pig liver	chicken	egg	beef	pork	pig liver	chicken	egg	beef
5	71.4 - 116.7	71.3 - 119.0	71.3 - 117	71.6 - 113	70.9 - 107.1	1.1 - 9.9	0.5 - 9.5	0.1 - 9.0	0.5 - 10.2	0.3 - 10.8
10	73.9 - 113.2	71.9 - 114.2	71.8 - 112.6	70.9 - 113	70.9 - 113.6	0.4 - 10.9	0.7 - 10.3	0.5 - 9.9	0.1 - 10.7	0.9 - 10.7
50	73.5 - 108.1	71.2 - 112.6	73.1 - 111.5	72.0 - 111.5	72.6 - 113.4	0.1 - 6.9	0.6 - 9.5	0.5 - 9.6	0.2 - 9.8	0.6 - 10.1

3.5.2. LOD, LOQ and Linear Range

Calibration working curves were drawn with the response value of the target compounds as the vertical coordinate and quality concentration as the horizontal coordinate. The results showed that the 61 veterinary drugs had strong linearity in the range of 0.5 μg/L - 50 μg/L with correlation coefficients (R^2) > 0.995. The total LODs of the 61 veterinary drugs in 5 animal derived food were in the range of 0.03 μg/kg - 1.5 μg/kg, and their LOQs were in the range of 0.1 μg/kg - 5.0 μg/kg, which were much lower than the MRLs set by a majority of countries; therefore, it could meet the demand for the detection of multiple veterinary drugs in multiple livestock and poultry products. As β-receptor agonists are hardly used in livestock and poultry breeding, and many countries have not yet set the MRLs of such drugs in livestock and poultry products, no LOD or LOQ was investigated in chicken and eggs in the study.

3.5.3. Matrix Effects

Matrix effects (ME) refer to phenomena where other interfering substances present in matrices lead to varying degrees of signal enhancement or attenuation of analytes [28]. Matrix effects are widespread in instrumental analysis, such as GC, GC-MS/MS, LC-MS/MS, etc. [35], and affect the accuracy and precision of the determination results. When LC-MS/MS was used to analyse complex samples, such as pig liver and egg, especially in the ESI mode, matrix effects were particularly evident and directly affected quantitative accuracy, unless such matrix effects were minimised or compensated [24].

Matrix effects were obtained by comparing the slope ratio of the calibration curve prepared by matrix blank solutions (k_1) and that of the reagent calibration curve (k_2) (ME = k_1/k_2). Assuming that matrix enhancement occurs when ME > 1, that matrix attenuation occurs when ME < 1, and that no matrix effect occurs when ME = 1. Comparisons were made between the matrix effects of the 61 veterinary drugs in the 5 types of animal derived food of pork, pig liver, chicken, egg and beef in the test with the results as shown in **Table 4**.

It is known from **Table 4** that the ME of 55 veterinary drugs in pork matrix, 38 types in chicken matrix and 37 types in beef matrix were in the range of 0.8 - 1.2, those of 26 veterinary drugs in pig liver were less than 0.8 and those of 4 types

Table 4. The MRLs, MEs, LODs and LOQs of 61 veterinary drugs in 5 matrices.

Number	veterinary drugs	MRLs (μg/kg)															ME					pork				pig liver				chicken				egg				beef			
		pork			pig liver			chicken			egg			beef									LOD		LOQ		LOD		LOQ		LOD		LOQ		LOD		LOQ		LOD		LOQ	
		CHN/CAC/EU			CHN/CAC/EU			CHN/CAC/EU			CHN/CAC/EU			CHN/CAC/EU			pork	pig liver	chicken	egg	beef	(μg/kg)		(μg/kg)		(μg/kg)		(μg/kg)		(μg/kg)		(μg/kg)		(μg/kg)		(μg/kg)		(μg/kg)	
1	Florfenicol amine	300	/	300	2000	/	2000	100	/	100	CND	/	/	200	/	100	0.89	0.84	0.82	0.85	0.83	1.27		4.24		1.50		5.00		1.25		4.17		1.46		4.87		1.42		4.72	
2	Amoxicillin	50	50	50	50	50	50	50	/	50	CND	/	/	50	50	50	0.88	0.83	0.67	0.93	0.75	1.46		4.85		1.50		5.00		1.50		5.00		1.44		4.80		1.50		4.98	
3	Sulfaguanidine	100	/	100	100	/	100	100	/	100	CND	/	/	100	100	/	0.84	0.73	0.83	0.71	0.80	0.32		1.06		0.61		2.02		0.43		1.43		0.55		1.83		0.34		1.13	
4	Sulfacetamide	100	/	100	100	/	100	100	/	100	CND	/	/	100	100	/	0.82	0.63	0.83	0.67	0.82	0.14		0.45		0.15		0.50		0.14		0.45		0.15		0.51		0.15		0.49	
5	Benzonitrile	CND	/	/	CND	/	/	/	/	/	/	/	/	CND	/	/	0.88	0.87	/	/	0.91	0.89		2.97		1.05		3.50		/		/		/		/		1.15		3.85	
6	Terbutaline	CND	/	/	CND	/	/	/	/	/	/	/	/	CND	/	/	0.81	0.80	/	/	0.87	0.45		1.50		0.75		2.50		/		/		/		/		0.74		2.46	
7	Salbutamol	CND	/	/	CND	/	/	/	/	/	/	/	/	CND	/	/	0.74	0.76	/	/	0.80	0.24		0.80		0.50		1.67		/		/		/		/		0.51		1.70	
8	Metronidazole	CND	CND	/	CND	CND	/	CND	CND	/	CND	CND	/	CND	CND	/	0.94	0.82	0.86	7.26	0.37	0.14		0.45		0.27		0.89		0.25		0.83		0.03		0.10		0.18		0.61	
9	Sulfadiazine	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.97	0.75	0.91	0.63	0.99	0.11		0.37		0.38		1.25		0.09		0.30		0.13		0.44		0.08		0.28	
10	Sulfathiazole	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.73	0.42	0.62	0.47	0.52	0.11		0.35		0.24		0.79		0.12		0.39		0.12		0.40		0.18		0.60	
11	Sulfapyridine	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.96	0.56	0.89	0.65	0.72	0.14		0.48		0.28		0.92		0.15		0.49		0.26		0.86		0.28		0.93	
12	Sulfamerazine	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.94	0.67	0.81	0.64	0.69	0.16		0.55		0.22		0.74		0.13		0.43		0.23		0.77		0.22		0.72	
13	Fenoterol	CND	/	/	CND	/	/	/	/	/	/	/	/	CND	/	/	0.88	0.59	/	/	0.69	0.60		2.00		0.70		2.34		/		/		/		/		0.73		2.42	
14	Sulfameter	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.85	0.72	0.91	0.82	0.78	0.25		0.84		0.43		1.44		0.31		1.02		0.36		1.18		0.41		1.35	
15	Lincomycin	200	200	100	500	500	500	200	200	/	50	/	50	100	/	100	0.96	1.11	0.91	0.67	0.80	0.47		1.56		0.89		2.98		0.82		2.72		0.63		2.09		0.85		2.82	
16	Sulfamoxole	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.89	0.73	0.79	0.70	0.81	0.24		0.81		0.20		0.68		0.29		0.96		0.47		1.56		0.31		1.04	
17	Sulfamethizole	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.87	0.67	0.80	0.79	0.79	0.40		1.32		0.88		2.92		0.41		1.36		0.95		3.18		0.47		1.56	
18	Sulfamethazine	100	100	100	100	100	100	100	100	100	CND	/	/	100	100	100	0.90	0.66	0.80	0.64	0.80	0.10		0.34		0.31		1.03		0.15		0.50		0.33		1.11		0.24		0.81	
19	Trimethoprim	50	/	100	50	/	100	50	/	100	CND	/	/	50	/	100	0.93	0.95	0.86	2.88	0.88	0.21		0.69		0.18		0.59		0.12		0.41		0.06		0.20		0.13		0.43	
20	Sulfamethoxypyridazine	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.90	0.69	0.80	0.79	0.78	0.16		0.53		0.23		0.76		0.16		0.53		0.26		0.88		0.21		0.71	
21	Fleroxacin	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	1.07	0.95	0.99	0.95	1.07	0.21		0.71		0.64		2.14		0.31		1.03		0.41		1.37		0.32		1.08	
22	Sulfachloropyridazine	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.85	0.62	0.80	0.76	0.78	0.75		2.50		0.96		3.19		0.65		2.17		0.88		2.93		0.69		2.29	
23	Ofloxacin	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	1.02	0.91	0.93	1.00	1.00	0.30		1.00		0.38		1.26		0.28		0.94		0.19		0.64		0.31		1.03	
24	Pefloxacin	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	1.20	2.15	1.23	1.05	1.61	0.94		3.13		1.02		3.40		1.01		3.36		1.00		3.35		0.82		2.73	
25	Sulfamethoxazole	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.88	0.70	0.79	0.62	0.76	0.61		2.04		0.68		2.27		0.68		2.26		0.81		2.68		0.78		2.60	
26	Tetracycline	200	200	100	600	600	300	200	200	100	400	400	200	200	200	100	0.92	0.90	0.82	0.73	0.81	1.15		3.85		1.35		4.50		1.45		4.84		1.48		4.92		1.36		4.53	
27	Sulfamonomethoxine	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	1.41	1.05	0.71	0.77	0.71	0.40		1.33		0.50		1.67		0.45		1.49		0.53		1.76		0.59		1.97	
28	Norfloxacin	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	1.10	1.17	1.02	0.80	1.13	0.58		1.93		1.07		3.57		0.96		3.19		0.84		2.79		1.00		3.33	

Continued

29	Amantadine	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	0.96	0.89	0.85	0.83	0.99	0.55	1.83	0.59	1.97	0.58	1.93	0.64	2.13	0.51	1.71
30	Ractopamine hydrochloride	CND	/	/	CND	/	/	/	/	/	/	/	/	CND	/	/	0.94	0.84	/	/	0.91	0.14	0.48	0.17	0.57	/	/	/	/	0.20	0.68
31	Clorprenaline	CND	/	/	CND	/	/	/	/	/	/	/	/	CND	/	/	0.97	0.92	/	/	1.00	0.45	1.50	0.50	1.67	/	/	/	/	0.18	0.59
32	Oxytetracycline	200	200	100	600	600	300	200	200	100	400	400	200	200	200	100	1.04	0.99	1.00	0.83	0.97	1.00	3.35	0.76	2.53	0.94	3.14	1.00	3.33	0.83	2.75
33	Ciprofloxacin	100	/	/	200	/	/	100	/	/	CND	/	/	100	/	/	1.00	1.11	0.98	1.14	1.04	0.26	0.87	0.29	0.97	0.25	0.85	0.12	0.41	0.39	1.29
34	Enrofloxacin	100	/	100	200	/	200	100	/	100	CND	/	/	100	/	100	1.04	1.09	0.97	0.94	1.09	0.50	1.67	0.68	2.25	0.57	1.91	0.61	2.05	0.70	2.34
35	Sulfadoxine	100	/	100	100	/	100	100	/	100	CND	/	/	/	/	100	0.88	0.77	0.71	0.57	0.65	0.24	0.81	0.27	0.89	0.29	0.98	0.31	1.04	0.28	0.93
36	Danofloxacin mesylate	100	100	100	50	50	200	200	200	200	CND	30	/	200	200	100	1.25	1.90	1.03	0.84	1.37	0.65	2.16	0.92	3.05	1.09	3.62	1.19	3.98	0.66	2.21
37	Sulfisoxazole	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.94	0.86	0.82	0.65	0.78	1.37	4.55	1.50	5.00	1.36	4.55	1.29	4.29	1.33	4.42
38	Lomefloxacin	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	0.97	0.82	0.89	0.84	0.97	0.94	3.13	1.17	3.91	0.85	2.83	1.14	3.80	0.96	3.19
39	Benzenemethanol	CND	/	/	CND	/	/	/	/	/	/	/	/	CND	0.2	0.1	0.93	0.91	/	/	0.94	0.16	0.54	0.26	0.86	/	/	/	/	0.03	0.10
40	Sulfabenzamide	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.85	0.63	0.72	0.64	0.75	0.44	1.48	0.79	2.63	0.61	2.03	0.64	2.13	0.55	1.82
41	Sarafloxacin	/	/	/	/	/	/	10	10	/	CND	/	/	/	/	/	0.93	0.76	0.84	0.68	0.83	1.05	3.50	1.20	4.01	1.15	3.85	1.23	4.09	1.08	3.60
42	Tulobuterol	CND	/	/	CND	/	/	/	/	/	/	/	/	CND	/	/	0.94	0.90	/	/	0.97	0.29	0.97	0.54	1.81	/	/	/	/	0.57	1.88
43	Sulfaphenazole	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.83	0.59	0.79	0.77	0.71	0.87	2.88	0.82	2.75	0.70	2.34	1.03	3.42	0.79	2.65
44	Chlorotetracycline	200	200	100	600	600	300	200	200	100	400	400	200	200	200	100	0.88	0.79	0.83	0.76	0.79	1.25	4.17	1.37	4.57	1.19	3.97	1.33	4.42	1.33	4.42
45	Sulfadimethoxine	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.88	0.77	0.71	0.57	0.65	0.27	0.89	0.35	1.16	0.22	0.74	0.26	0.88	0.31	1.03
46	Sulfaquinoxaline	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.79	0.48	0.63	0.49	0.67	0.38	1.27	0.95	3.18	0.36	1.19	0.28	0.93	0.60	2.00
47	Oxolinic acid	100	/	/	150	/	/	100	/	/	CND	/	/	100	/	/	1.08	0.95	0.94	0.75	1.08	0.58	1.92	1.08	3.60	0.65	2.17	0.79	2.63	0.66	2.21
48	Doxycycline	100	/	100	300	/	300	100	/	100	CND	/	/	100	/	100	0.91	0.87	0.90	0.85	0.93	1.50	5.00	1.50	5.00	1.47	4.89	1.50	4.98	1.50	5.00
49	Tilmicosin	100	100	50	1500	1500	1000	150	150	75	CND	/	/	100	100	50	1.27	2.82	1.41	0.92	1.83	0.68	2.27	0.93	3.09	0.79	2.63	0.94	3.13	0.71	2.38
50	Clindamycin	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	0.91	0.93	0.89	0.86	0.96	1.49	4.95	1.50	5.00	1.49	4.97	1.50	5.00	1.32	4.41
51	sulfanitran	100	/	100	100	/	100	100	/	100	CND	/	/	100	/	100	0.88	0.70	0.78	0.66	0.90	1.46	4.85	1.50	5.00	1.50	5.00	1.50	5.00	1.47	4.90
52	Tylosin	100	100	100	100	100	100	100	100	100	300	300	200	100	100	100	1.12	1.18	0.91	0.89	1.14	1.50	5.00	1.50	5.00	1.32	4.41	1.50	5.00	1.43	4.76
53	Erythromycin	200	/	200	200	/	200	100	100	200	50	50	150	200	/	200	1.28	1.24	1.09	0.84	1.19	0.17	0.56	0.26	0.86	0.28	0.94	0.20	0.68	0.27	0.89
54	Dexamethasone	1	1	0.75	2	2	2	/	/	0.75	/	/	/	1	1	0.75	0.91	0.71	0.88	0.74	0.88	0.21	0.70	0.35	1.17	0.22	0.73	0.29	0.97	0.22	0.74
55	Chlorpromazine hydrochloride	CND	CND	/	CND	CND	/	CND	CND	/	CND	CND	/	CND	CND	/	0.95	0.91	0.89	0.84	1.01	0.31	1.03	0.37	1.24	0.31	1.04	0.35	1.15	0.28	0.95
56	Penbutolol	CND	/	/	CND	/	/	/	/	/	/	/	/	CND	/	/	0.99	0.95	/	/	1.02	0.28	0.94	0.25	0.82	/	/	/	/	0.30	1.01
57	Diazapam	CND	/	/	CND	/	/	CND	/	/	CND	/	/	CND	/	/	0.87	0.64	0.78	0.62	0.84	0.31	1.02	0.42	1.41	0.28	0.95	0.33	1.11	0.28	0.94
58	Thiamphenicol	50	/	/	50	/	/	50	/	50	CND	/	/	50	/	50	0.98	0.96	1.06	1.17	1.12	0.30	1.00	0.47	1.58	0.36	1.19	0.42	1.40	0.42	1.40
59	Florfenicol	300	/	300	2000	/	2000	100	/	100	CND	/	/	200	/	100	0.95	0.98	0.95	0.98	1.01	0.29	0.98	0.39	1.30	0.29	0.95	0.31	1.03	0.35	1.16
60	Chloramphenicol	CND	CND	/	CND	CND	/	CND	CND	/	CND	CND	/	CND	CND	/	0.98	0.98	1.03	1.04	1.04	0.29	0.96	0.30	1.01	0.27	0.89	0.24	0.81	0.32	1.07
61	4,4'-Dinitrocarbanilide	/	/	/	/	/	/	200	200	/	/	/	/	/	/	/	0.99	1.01	0.97	0.96	0.98	0.31	1.03	0.39	1.30	0.38	1.28	0.42	1.40	0.46	1.55

were greater than 1.2, those of 28 veterinary drugs in eggs were below 0.8 and those of 2 types were greater than 1.2. This might be caused by the fact that pig liver and eggs contain a huge amount of protein, lipid etc., more complex components resulted in more severe matrix interferences. In order to effectively compensate for matrix effects, the test was calibrated with blank matrices to ensure that the detection data can truly reflect the actual situation of veterinary drug residues in animal-derived foods.

3.6. Real Sample Testing

The method established in the test was used to detect 731 animal derived samples randomly selected from the market, slaughterhouses and plants in the years 2024-2025, including: 272 pork samples, 85 pig liver samples, 140 chicken samples, 172 egg samples, and 62 beef samples. The results showed (Table 5) that 13 veterinary drugs were detected 64 times in 61 veterinary drugs; 12 restricted veterinary drugs were detected 57 times, which were below the limits of China, USA and EU, and were determined as qualified samples; 2 prohibited veterinary drugs were detected 7 times and were determined as unqualified samples, no other veterinary drugs were detected, and the total percentage is 99.3%.

Table 5. The real samples detected results.

Samples & quantity	Detected drugs	Detected times	Results (μg/kg)	MRLs (μg/kg) CHN/CAC	Samples & quantity	Detected drugs	Detected times	Results (μg/kg)	MRLs (μg/kg) CHN/CAC
pork (272)	Doxycycline	4	6.89 - 40.9	100/	pig liver (85)	Doxycycline	1	44.6	300/
	Tetracycline	2	7.18 - 30.3	200/200		Ciprofloxacin	2	2.11 - 56.3	CND/-
	Oxytetracycline	2	6.22 - 23.8	200/200		Enrofloxacin	3	1.38 - 3.31	CND/-
	Chlorotetracycline	1	26.0	200/200	egg (172)	Ofloxacin	1	1.65	/
	Sulfadimethoxine	6	0.55 - 4.40	100/-		Fleroxacin	2	1.30 - 1.48	/
	Sulfaquinoxaline	4	0.72 - 5.50	100/-		Pefloxacin	2	2.56 - 3.26	/
	Metronidazole	2	0.21 - 0.42	CND/- CND		Doxycycline	1	46.0	100/
chicken (140)	Ciprofloxacin	10	1.99 - 9.33	100/-	beef (62)	Oxytetracycline	1	25.8	200/200
	Enrofloxacin	8	1.26 - 2.05	100/-		Chlorotetracycline	1	42.7	200/200
	Ofloxacin	1	1.93	/		Sulfadimethoxine	1	1.86	100/-
	Norfloxacin	9	3.19 - 10.1	/					

The veterinary drugs with the highest detection rate were the quinolones (ciprofloxacin, enrofloxacin, etc.), with 38 times; tetracyclines (doxycycline, tetracycline, etc.) were second to the quinolones with 13 times; sulfonamides (sulfadimethoxine, sulfaquinoxaline, etc.) were in the third place with 11 times. The results showed that the veterinary drugs are most commonly used in the treatment of livestock and poultry diseases.

It is worth noting that ciprofloxacin and enrofloxacin were detected twice and 3 times with the detection value ranges of 2.11 μg/kg - 56.3 μg/kg and 1.38 μg/kg - 3.31 μg/kg in egg samples respectively, but the two veterinary drugs are stipulated as prohibited drugs during egg production periods in GB 31650-2019 [36] and by CAC and EU [8] [14]; Metronidazole was detected twice in pork samples, and it is prohibited in animal derived food as stipulated in GB 31650-2019 [32] and by CAC and EU [8] [14], so the 7 samples were unqualified. These results suggest that although, overall, the safety of animal-derived food is relatively high, there are still some farmers who use prohibited veterinary drugs in violation of regulations. It is necessary to strengthen the supervision of veterinary drug use as well as publicity and education to ensure the quality and safety of animal-derived foods. Through the testing of actual samples, the detection method established in this study can accurately detect veterinary drug residues in animal-derived foods, providing strong technical support for food safety supervision.

4. Conclusions

In this study, a novel clean-up LPAS column was used to rapidly filter and purify a wide range of animal foods food matrices, achieving efficient decontamination. Coupled with LC-MS/MS, a high throughput detection method was established which can simultaneously detect 61 veterinary drug residues across 13 categories in various animal-foods. Compared with the traditional QuEChERS, the process of salt stripping, liquid transfer, secondary centrifugation and other steps were reduced, which is simpler and faster.

In the experiment, the conditions of LC-MS/MS, extraction reagents, extraction volumes and clean up process of 61 kinds of veterinary drugs were optimized. The performance of the method including accuracy, precision, detection limit, quantitative limit, linear range and 5 matrix effects were verified. The results show that 61 kinds of veterinary drugs get good recovery rates in 5 kinds of animal foods ranged from 70.9% to 119.0%, RSD ranged from 0.1% to 10.9%; had a good linear relationship: $R^2 \geq 0.995$; the total LODs ranged from 0.03 μg/kg to 1.5 μg/kg, and the LOQs ranged from 0.1 μg/kg to 5.0 μg/kg; far below the MRLs set by most countries. There are 50.8% of the veterinary drugs ME in the 5 samples: ranged 0.8 - 1.2 can be acceptable. This approach has been triumphantly utilized for the detection of 731 samples related to livestock and poultry.

The test results indicate that quinolone, tetracycline, and sulfonamide are the most frequently used veterinary drugs in the treatment of livestock and poultry diseases. Meanwhile, they also reflect the problem that some livestock and poultry products are sold during the withdrawal period after being treated with prohibited drugs. This finding provides important data support for food safety supervision departments, which is conducive to strengthening the supervision of the quality and safety of animal products and safeguarding the health of consumers.

In conclusion, the clean-up LPAS combined with LC-MS/MS detection method established in this study enables the rapid, accurate, and efficient detection of multiple veterinary drug residues in animal-derived foods. It has the advantages of

simple operation, low cost, and environmental friendliness. It provides strong technical support for the quality and safety supervision of animal-derived foods and has broad application prospects.

Although this study has successfully established a method for detecting multiple veterinary drug residues in animal-derived foods based on Clean-up LPAS combined with LC-MS/MS technology and achieved satisfactory results, there are still some deficiencies that need to be further improved and refined in future research. This study mainly focuses on the detection of 61 veterinary drugs in 13 categories. However, there is a wide variety of veterinary drugs, and new varieties of veterinary drugs are constantly emerging on the market. To more comprehensively ensure the safety of animal-derived foods, future research can further expand the types of veterinary drugs to be detected, covering more new veterinary drugs and metabolites. This requires further optimization and verification of the detection method to ensure that the newly included veterinary drugs can be accurately detected within the existing detection system, improving the comprehensiveness and accuracy of detection.

In addition, in practical applications, animal-derived foods in different regions may have different veterinary drug use situations and matrix characteristics. Although this study has analyzed common samples such as pork, pig liver, chicken, eggs, and beef, for animal-derived foods with other special matrices, such as mutton, duck meat, dairy products, etc., for samples from different regions and with different matrices, the detection method needs to be optimized and adjusted accordingly to ensure the accuracy and reliability of the method. In the future, multi-center and large sample studies can also be carried out to collect animal-derived food samples from different regions, conduct more extensive verification and evaluation of the detection method, and provide a basis for establishing universal veterinary drug residue detection standards.

Future research should also focus on the integration of veterinary drug residue detection technology and the food safety supervision system. By establishing a complete veterinary drug residue monitoring network, real-time monitoring and early warning of the whole chain of animal-derived foods from breeding, processing to sales can be realized: strengthen cooperation with relevant departments, apply detection technology to actual supervision work, provide a scientific basis for formulating reasonable veterinary drug use policies and food safety standards, and thus better ensure public food safety.

Authors' Contributions

Data curation, Yu Zhang, and Qiaohui Yang; Formal analysis, Xiaxue Li; Yunhua Zheng, Funding acquisition, Xiaxue Li and Yan Zeng; Investigation, Qiaohui Yang; Methodology, Qiaohui Yang and Yan Zeng; Resources, Jie Chen; Supervision, Jie Chen and Yan Zeng; Validation, Qiaohui Yang and Xin Zhou; Visualization, Xiaxue Li and Yu Zhang; Writing original draft, Qiaohui Yang; Writing review & editing, Yan Zeng, Yunhua Zheng and Xin Zhou. All authors have read

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Data Availability Statement

The data were released to the researchers without access to any personal data.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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