Journal of Food and Nutrition Science **ISSN: 2832-9457**
Volume 5 (2025) 15 Pages
DOI: http://dx.doi.org/10.53043/2832-9457.JFNS.5.001

Research Article* | *Open Access

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

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Received: December 21, 2024;
Accepted: February 11, 2025;
Published: February 14, 2025

Citation: Zeng Y, Yang Q, Li X, Zhang Y, Chen Y, Yang J, et al. Simultaneous Determination of 61 Veterinary Drug Residues in Animal Derived Food by Clean-up LPAS Combined with LC-MS/MS. J Food Nutr Sci. (2025);5(1): 1-15

Key words: clean-up LPAS, LC-MS/MS, animal derived food, multiple veterinary drug residues, rapid test

Abstract

A method of rapid test of 61 veterinary drugs which can divide into 13 types in 5 kinds of animal derived food, namely, pork, pork liver, chicken, egg and beef by Clean-up LPAS combined with LC-MS/MS. The animal derived samples, after preparation, are extracted from 8 ML acetonitrile: water (90:10, with 0.2% of formic acid), and can determine by a mass spectrometer after Clean-up LPAS without stripping. The test is carried out to optimize the LC-MS conditions, extraction reagents, extraction volumes, clean-up ways of the 61 veterinary drugs, etc.; to verify the method performance, including accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), linear range and matrix effects, and to achieve satisfactory method performance. The results have shown that the 61 veterinary drugs have strong linearity in the range of 0.5~50μg/L with the correlation coefficient (R^2) ≥0.995, and have obtained satisfactory recovery efficiency in 5 matrix samples under the three high, medium and low addition levels of (5, 10 and 50μg/kg) with the overall recovery rates in the range of 70.9%~119.0% and RSD in the range of 0.1%~10.9%. The overall LOD is in the range of 0.03-1.5 μg/kg, and the LOQ is in the range of 0.1-5.00 μg/kg. When the method was applied to test 731 real animal derived samples, a total of 13 types of drugs were detected 64 times; 12 types of restricted drugs were detected 57 times; 2 types of prohibited drugs were detected 7 times, and no other veterinary drug were detected . Thus, it is suggested that the simple Clean-up LPAS is superior to the traditional QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method and can be used for testing batch samples of multiple veterinary drugs in animal derived food.

1. Introcuction

Veterinary drugs are substances used for the prevention, treatment and diagnosis of animal diseases or for the targeted regulation of physiological functions in animals [1, 2], including but not limited to antibiotics, antiparasitic drugs, antifungal drugs and bronchodilators, and are essential for

the prevention and treatment of animal disease [3]. Because of their many benefits, some breeders use veterinary drugs irrationally and even abuse prohibited drugs illegally in order to improve economic returns or due to lack of cognition of drug hazards or scientific guidance on their use [4, 5]. These veterinary drugs remain in animals and enter the human body through the food chain , further jeopardize human health [6-8]. Therefore, China, EU, USA, and others have set the maximum residue limits (MRL) [9-11] 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], and the pure liquid chromatography (LC) performs limited detection of veterinary drugs and their metabolites and classified detection of some veterinary drugs due to its low sensitivity and poor selectivity, and has great limitations for simultaneous detection of f different veterinary drugs. In recent years, the method has been replaced by methods of chromatography tandem-mass spectrometry methods with better selectivity and higher testing efficiency [12-14]. The improvement of the test equipment has greatly increased the number of veterinary drugs that can be detected simultaneously: 80 drugs could be analysed by the method established by Zhao et al [15]; 210 drugs in pork could be analyzed by the method established by Yin et al [16]; 77 drugs in chicken could be analyzed by the method established by Alcantara-Duran et al [6]; 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 Xiaoqin et al established a method to screen 204 veterinary drugs [17]; Desmarchelier A established a qualitative LC-MS/MS method to detect 154 veterinary drugs [18], these methods could detect multiple matrices but cannot perform quantitative analysis. There are only several micrograms and even nanograms of veterinary drugs in food [12], and this is a major challenge for residue testing in animal derived food.

The matrices of livestock and poultry products are complex due to the large amount of lipids, proteins and other macromolecular substances present in the products [19-21], resulting in false positives or false negatives test results. Therefore, sample preparation is still a challenging process for testing drug residues in animal derived foods. In this study, Clean-up LPAS columns made of novel high-polymer materials were used to rapidly filter and clean up multiple animal derived food, and by combining LC-MS/MS, a high-throughput test method was established that can rapidly, accurately and efficiently test 61 veterinary drugs in 13 categories simultaneously, including 22 types of sulfonamides, 10 types of quinolones, 9 types of β-receptor agonists, 4 types of tetracyclines, 4 types of amphenicol, 3 types of macrolides, 2 types of sedatives, 2 types of lincosamide antibiotics, and 1 type of nitroimidazole, globigerina, corticosteroid, penicillin and antiviral drug respectively. 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.

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, 20.0μg/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

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: 0~2 min; 10% A; 2~7 min; 10%~80% A; 7~7.5 min, 80% A; 7.5~8 min, 80%~95% A; 8~9 min, 95% A; 9~9.5 min, 95%~10% A; 9.5~14 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 [22]. 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 0.22μm 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.

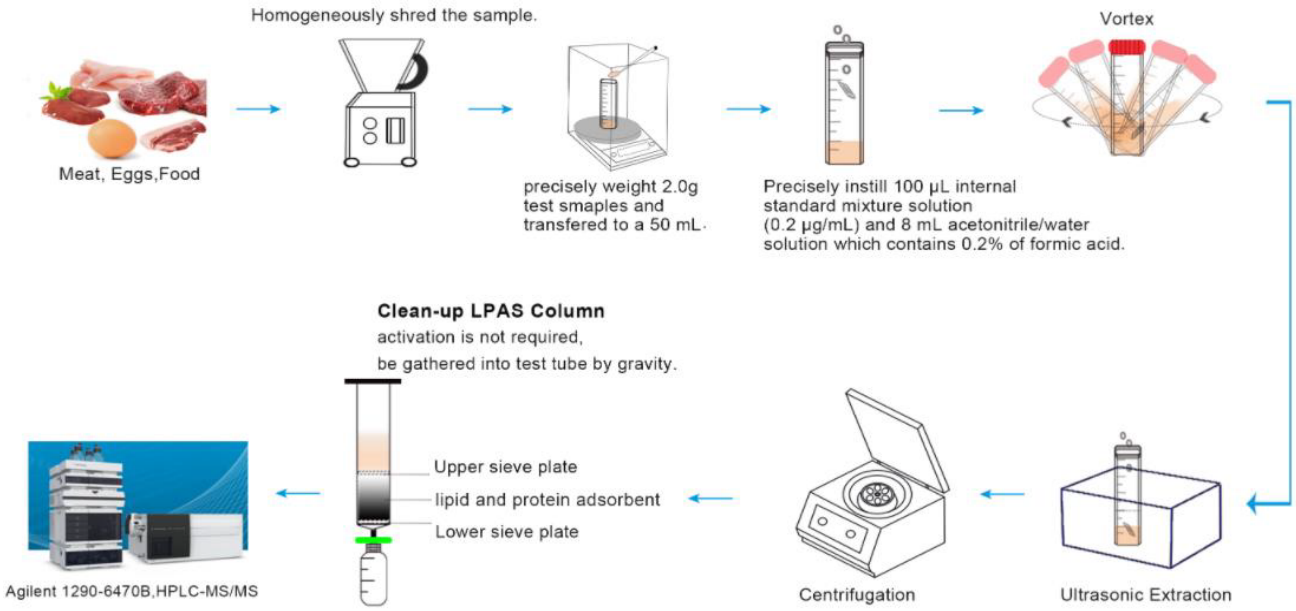


Figure 1: Sample pretreatment main process.

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 [23], 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 0.05mg/kg for MRL-free pesticides). The LOD of each matrix was calculated using the following formula [4].

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

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

ME were calculated from the ratios of the slopes of the matrix-matched calibration curves to the solvent calibration curves [23, 24].

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. The test focused the emphases were put on comparisons between methyl alcohol and acetonitrile for organic phases, and between 0.1% formic acid water and 5mmol/L ammonium acetate solution containing 0.1% formic acid for water phases, and the mobile phase gradients were constantly adjusted to ensure that each parameter could peak normally. When the organic phase was acetonitrile, tetracyclines and some quinolones tended to have leading or tailing peaks and low response values. When the water phase was 0.1% formic acid water, drugs such as chloramphenicol, thiamphenicol, florfenicol, nicarbazin, etc., had no response or extremely low response when they were scanned in the negative ion monitoring mode. After several experiments, it was found that the response of the target objects improved significantly after the addition of 5 mmol/L ammonium acetate solution. Through the above experiments and considering the peak patterns, test sensitivity and isomeric separation of each target compound, it was finally confirmed that the optimum mobile phases for the test were methyl alcohol-5mmol/L ammonium acetate solution containing 0.1% formic acid. After adjusting the mobile phase gradient elution programme several times during the test, the gradient elution conditions in 2.3.1 were finally confirmed to test 61 veterinary drugs simultaneously and to achieve satisfactory separation effects.

In the well-optimised chromatographic conditions, parent ions were under full scan (MS_2Scan) with a single standard solution (2mg/L), and several groups of different fragment voltages (Fragment/V) were set, a mass-to-charge ratio identical to a relative molecular weight was considered as the parent ion of the latter after comparing the relative molecular weight of each standard sample, and a fragment voltage below the maximum response value was selected as the subsequent optimal fragment voltage. Then the product ion scan was performed, the collision energy (CE) optimised, the characteristic daughter ion and its optimal CE were discovered simultaneously to ensure that each parameter, except for the internal standards, possessed at least two characteristic ion pairs. Finally, the well-optimised conditions were adopted to perform dynamic multiple reaction monitoring (dMRM) analysis, and the retention time of 61 veterinary drug components, MRM ion pairs, monitoring mode and optimal CE areprovided in Table 1, and the total MRM ion diagrams in Figure 2A and B.

3.2 Optimization of Extraction Reagents

The common extraction agents used in the pretreatment of veterinary drug residue tests include solutions of acetonitrile, acidified acetonitrile, ethyl acetate, phosphate buffer [25], among others. There are many lipids, proteins and so on in animal derived food; when used as an extraction agent, acetonitrile could reduce lipid extraction and facilitate albumin precipitation [26], and has a certain dehydration; samples with low water content are rapidly dehydrated and caked, which works against extraction. Therefore, the extraction efficiency was compared between acetonitrile and acetonitril:water solutions with water contents of 10%, 20%, 30% and 40% respectively. The results of the experiment is shown in Figure 3 and shows that there was the best extraction effect when the acetonitrile/water solution with water content of 10% was the extraction agent as there were 47 target objects whose recoveries were in the range of 60-120%.

To improve the extraction efficiency of each target object, comparisons were made between acetonitrile, 0.1% FA, 0.5% and 1% FA in acetonitrile in the experiment. The result of experiment is shown in Figure 4. As shown in the figure, when the extraction agent was 0.2% FA in acetonitrile, there were 54 veterinary drugs whose recovery rate was in the range of 60%-120%, which means that the recovery effects were satisfactory.

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 (Su)	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
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 (G)	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 (A)	15318-45-3	354/185 (20)	354/290 (6)	120	4.535	Negative
59	Florfenicol (A)	73231-34-2	356/336 (5)	356/185 (15)	120	5.610	Negative
60	Chloramphenicol (A)	56-75-7	321/257 (10)	321/152 (15)	117	6.330	Negative
61	Nicarbazin (An)	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; (T), Tetracyclines;(An), Antiviral agents; (M), Macrolides; (Se), Sedatives; (P), Polyether anticoccidials.

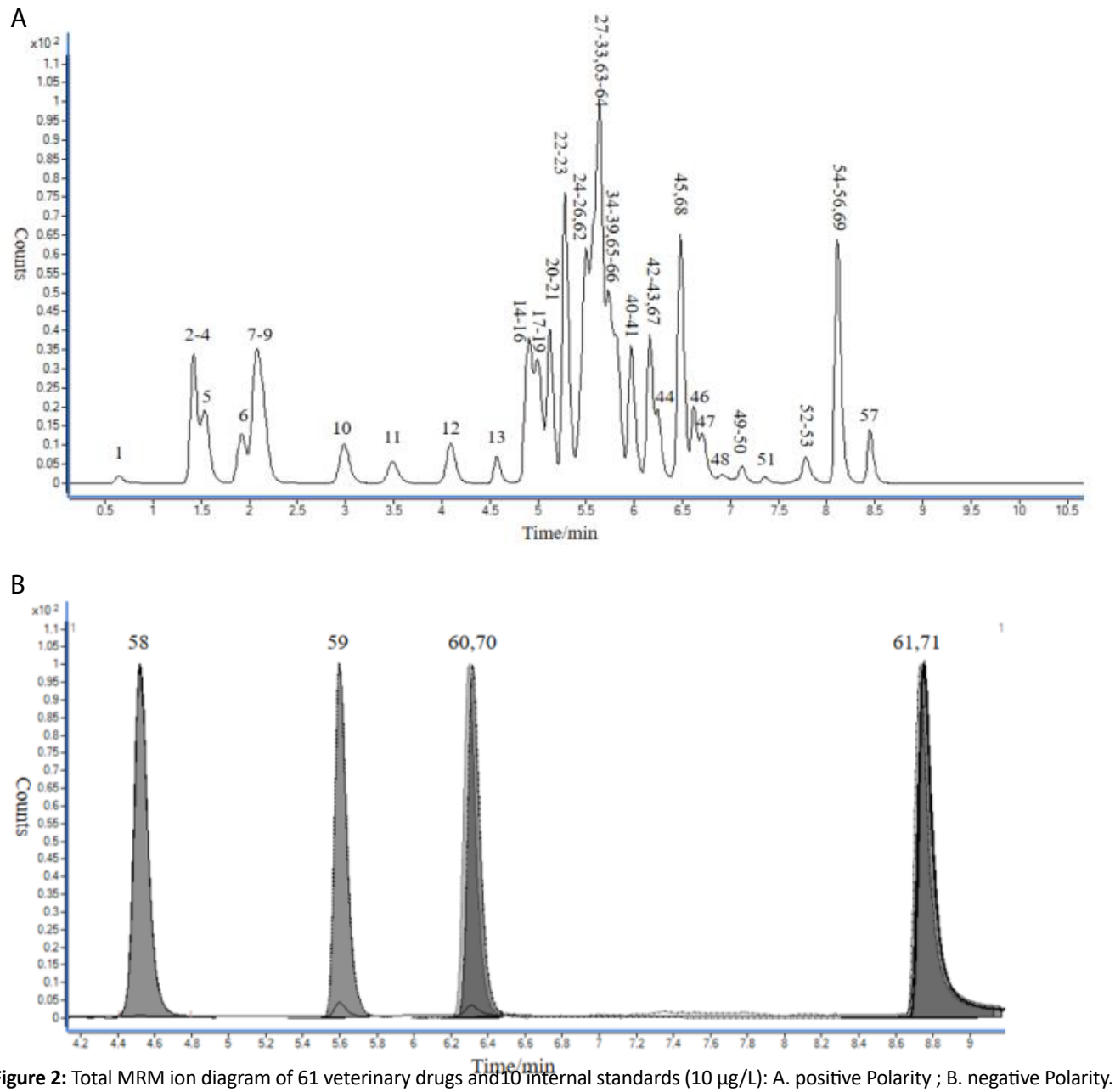


Figure 2: Total MRM ion diagram of 61 veterinary drugs and10 internal standards (10 μg/L): A. positive Polarity ; B. negative Polarity.

3.3 Optimization of Extraction Volumes

Having optimised the best extraction solvent, the dosage of extraction solvent has also been optimized. Typically, the smaller the extraction volume is, the higher the matrix concentration and the more difficult the subsequent clean-up, but too large an extraction volume wastes reagents, increases economic costs and is not environmentally or human friendly. Therefore, half (35 types) of the target objects were randomly selected to be extracted in extraction volumes of 4 mL, 6 mL, 8 mL and 10 mL and comparisons were made, with the experimental result shown in figure 5.

As shown in the figure, as the extraction volume increased, the matrix effect was gradually reduced and the overall recovery rate decreased. When the extraction volume was 10 mL, the recoveries of 26 target objects were reduced to the range of 60%~80%, and that of metronidazole was below 60%; when it was 8mL, the recoveries of 30 target objects were between 70% and 120%, showing a satisfactory recovery effect; when it was 6mL and 4mL respectively, 8 and 25 target objects showed recoveries of over 120%. Therefore, 8 mL of 0.2% FA in acetonitrile was used to extract the samples in the test.

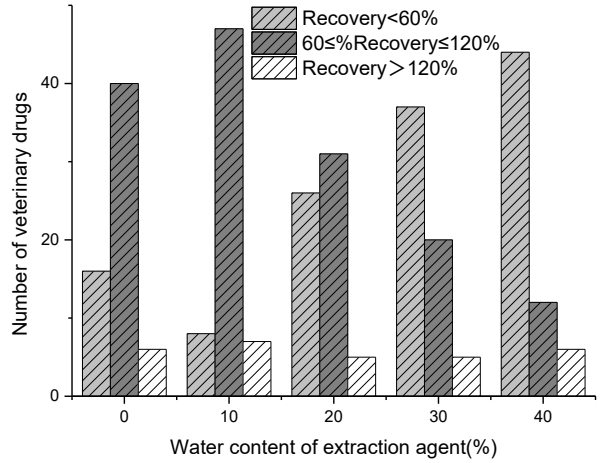
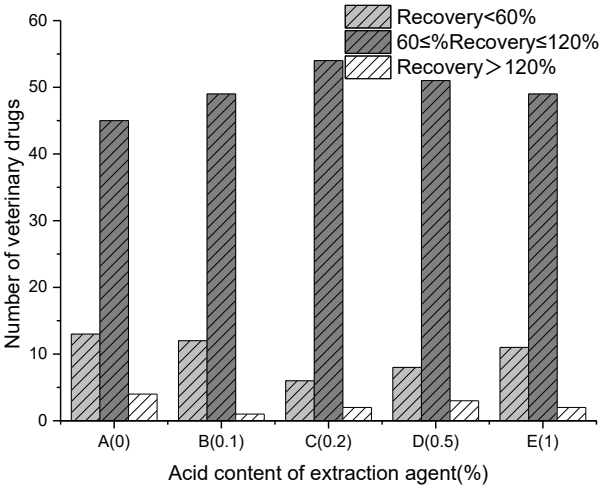
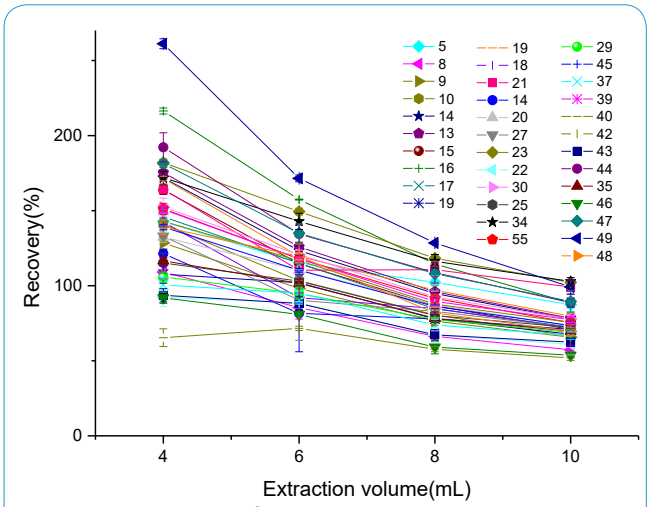


Figure 3: Recovery of veterinary drugs with different water content in the extraction agent.



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

Figure 4: Recovery of veterinary drugs with different acid content in the extraction agent.



Note: The names of corresponding veterinary drugs matching No. in the Figure are available in Table 1.

Figure 5: Effects of the different extraction volumes on the extraction effect

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. Despite its effectiveness, SPE has obvious drawbacks. Different adsorbent columns are required depending on the veterinary drugs being detected. Adsorbents such as MAX, MCX, HLB, C18, PSA, and GCB are selected based on their adsorption properties. They operate through silica - based or ion - exchange mechanisms to separate target compounds from complex biological matrices. The standard SPE procedure includes column activation, sample loading, equilibration, washing, elution, and subsequent nitrogen evaporation and re - dissolution of the analyte. This results in a multi - step process that increases both time and cost. In addition, repeated extractions with various organic solvents not only complicate the operation but also require high - level professional skills. As a result, SPE is labor - intensive and costly, limiting its application in high - throughput detection.

Subsequently, QuEChERS has been developed as an alternative method. It is a simpler and more efficient solution for simultaneously detecting multiple veterinary drugs [4, 12]. This method combines liquid - liquid extraction (LLE) with dispersive solid - phase extraction (d-SPE), and then, through centrifugation, rapidly separates the target compounds from interfering substances. Commonly used adsorbents include GCB, C18, PSA, and silica gel [27, 28]. Although compared with solid - phase extraction, it has significant advantages in terms of ease of use, speed, and cost effectiveness, the matrix effect after purification is significantly increased, the recovery rate of some target drugs is relatively low, and the purification tubes are expensive. Therefore, d-SPE is mainly used for the screening (qualitative detection) of a large number of samples. Thus, in addition to effective extraction, effectively removing interfering substances is another crucial sample preparation step, as the complex matrix effect poses a major challenge to the rapid and accurate 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 C_{18}, 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 consumption, waste liquid generation, etc. of two commonly used pretreatment methods with the LPAS syringe type purification, as listed in 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, only one - fourth of that of SPE, showing a significant advantage in reducing pollution emissions. The operation is simpler. The single sample detection time of the LPAS syringe - type purification does not exceed 30 minutes, only 1/4 of that of SPE and1/2 of that of 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.

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, 1mL, 1.5mL, 2mL and 2.5mL of pork supernatant (the addition amount was 10μg/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 2mL, there was the best clean-up effect with less interference from blank matrix impurities and the best recoveries of the 61 veterinary drugs, therefore 2mL 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.

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%,

Table 2: Comparison of consumption among three pretreatment methods

Items	SPE[29]	QuEChERS[30]	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 reagents usage amount	15 mL of Methanol	11.5 mL of Acetonitrile	8 mL of Acetonitrile
Inorganic reagents 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,10mL, and 5mL 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

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

Add levels	Average Recoveries (%)					RSD (%)				
(μg/kg)	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

and their RSDs were in the range of 0.1%~10.9%; with satisfactory accuracy and precision, the method could satisfy the demand for testing 61 veterinary drug residues in multiple animal derived food.

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 0.5~50 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 0.03~1.5 μg/kg, and their LOQs were in the range of 0.1~5.0 μg/kg, which wre much lower than the MRLs set by the 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 [24]. Matrix effects are widespread in instrumental analysis, such as GC, GC-MS/MS, LC-MS/MS, etc. [31], 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 [20].

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 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.

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 years 2021-2022, 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 99.32%.

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 2.11~56.3 μg/kg and 1.38~3.31 μg/kg in egg samples respectively, but the two veterinary drugs are stipulated as

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

No.	veterinary drugs	MRLs (μg/kg)																ME					pork		pig liver		chicken		egg		beef	
		pork			pig liver			chicken			egg			beef																				
		CHN/CAC/EU			CHN/CAC/EU			CHN/CAC/EU			CHN/CAC/EU			CHN/CAC/EU			pork	pig liver	chicken	egg	beef	LOD (μg/kg)	LOQ (μg/kg)	LOD (μg/kg)	LOQ (μg/kg)	LOD (μg/kg)	LOQ (μg/kg)	LOD (μg/kg)	LOQ (μg/kg)	LOD (μg/kg)	LOQ (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
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

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	egg (172)	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		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	beef (62)	Doxycycline	1	46.0	100/
chicken (140)	Ciprofloxacin	10	1.99-9.33	100/-		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	/					

prohibited drugs during egg production periods in GB 31650-2019 [32] and by CAC and EU [8, 10]; 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, 10], so the 7 samples were unqualified. The above test results have shown that the sampled animal derived food is safe as a whole, some livestock and poultry products were still sold within the withdrawal period after the treatment of livestock and poultry diseases with prohibited drugs, therefore, the departments concerned shall strengthen the publicity of drug use and the supervision of the quality safety of animal products.

4. Conclusion

In this experiment, a new clean up LPAS column was used to rapidly filter and purify a variety of animal foods, and obtained a good purification effect. In combination with LC-MS-MS, a high throughput detection method was established which can simultaneously detect 13 kinds of 61 veterinary drug residues in variety of animal foods. Compared with the traditional QuEChERS, the process of salt stripping, liquid transfer, secondary centrifugation and other steps were reduced , which is more simpler and faster.

The established method consumed less reagents, consumables and equipments, but can simultaneously detect more kinds of veterinary drugs in different animal foods, It can greatly reduce the economic cost and time cost of detection and reduce the waste liquid, It's more friendly to the environment and the health of personnel, and less professional requirements for personnel.

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 rusults shown that 61 kinds of veterinary drugs get good recovery rate 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: R2≥0.995; the total LODs ranged from 0.03 to 1.5 μg/kg, and the LOQs ranged from 0.1 to 5.0 μg/kg; far bleow the MRLs seted by most countries. There are 50.81% of the veterinary drugs ME in the 5 samples: ranged 0.8-1.2 can be acceptable. The method has been successfully applied to detecte 731 samples of livestock and poultry, and can meet the requirements for the detection of various veterinary drugs in a variety of livestock and poultry products, It can be used as a qualitative and quantitative method to detecte various veterinary drug residues in high-throughput animal foods in the quality and safety supervision.

5. Author Contributions

Data curation, Yu Zhang, and Jian Yang; Formal analysis, Xia xue Li; Funding acquisition, Xia xue Li and Yan Zeng; Investigation, Jian Yang; Methodology, Qiao hui Yang and Yan Zeng; Resources, Ya Chen; Supervision, Ya Chen and Yan Zeng; Validation, Qiao hui Yang and Xin Zhou; Visualization, Xia xue Li and Yu Zhang; Writing – original draft, Qiao hui Yang; Writing – review & editing, Yan Zeng and Xin Zhou. All authors have read and agreed to the published version of the manuscript.

6. Conflflicts of Interest

The authors declare that they have no conflflict of interest.

7. Funding

Agricultural Product Quality Safety Risk Monitoring Project of Sichuan Province of China in 2024 (Chuan Agricultural Letter [2024] No. 98)

8. Acknowledgement

We acknowledge the financial support from Agricultural Product Quality Safety Risk Monitoring Project of Sichuan Province of China in 2024 (Chuan Agricultural Letter [2024] No. 98);

9. Data Availability Statement

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

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