Food Science of Animal Products

Research Article

Determination of 24 tranquilizer drugs and 27 anesthetic drugs in livestock meat by QuEChERS combined with ultra-high performance liquid chromatography-quadrupole-orbitrap high resolution mass spectrometry

Liangna He, Lixin Fan, Xiaoqian Dong, Junmei Ma, Sufang Fan, Henan Xu, Qiang Li ✉

Hebei Food Safety Key Laboratory, Key Laboratory of Special Food Supervision Technology, State Administration for Market Regulation, Hebei Engineering Research Center for Special Food Safety and Health, Hebei Food Inspection and Research Institute, Shijiazhuang 050227, China

✉Address correspondence to Qiang Li, liqiang@nepp.com.cn

Received: January 5, 2026; Revised: February 3, 2026; Accepted: February 27, 2026

Abstract: This study established a method for detecting 24 tranquilizer drugs and 27 anesthetic drugs in livestock meat by combining the QuEChERS method with ultra-high performance liquid chromatography-quadrupole-orbitrap high resolution mass spectrometry (UPLC-Q-Orbitrap HRMS). The results demonstrated that the 51 target compounds exhibited good linear relationships within their respective ranges ($R^2 \geq 0.995\ 2$), low detection and quantification limits, satisfactory spike recovery rates (84.0%–111.9%), and good precision (relative standard deviation: 2.5%–8.5%), meeting the requirements for quantitative analysis. This approach combines the convenience and speed of QuEChERS with the high accuracy of UPLC-Q-Orbitrap HRMS, enabling efficient qualitative confirmation and reliable quantitative analysis of 51 target compounds. With its rapid, accurate, and straightforward advantages, this method holds significant potential for application in food safety monitoring of livestock meat, providing technical support for regulating the illegal use of sedatives and anesthetics in animal-derived foods.

Keywords: ultra-high performance liquid chromatography-quadrupole-orbitrap high resolution mass spectrometry; tranquilizer drugs; anesthetic drugs; screening and identification

Citation: L. N. He, L. X. Fan, X. Q. Dong, et al., Determination of 24 tranquilizer drugs and 27 anesthetic drugs in livestock meat by QuEChERS combined with ultra-high performance liquid chromatography-quadrupole-orbitrap high resolution mass spectrometry, Food Sci. Anim. Prod. 4 (2026) 9240164. https://doi.org/10.26599/FSAP.2026.9240164.

1 Introduction

Tranquilizer drugs refer to a class of medications that inhibit excessive excitement in the central nervous system and alleviate emotions such as anxiety and tension, thereby inducing a state of relaxation and exerting anti-convulsant effects[1–3]. Some unscrupulous merchants add these drugs to animal feed during breeding, aiming to increase the weight of the animals[4–6]. Anesthetic drugs can anesthetize the central nervous system, with the advantages of rapid onset and short recovery time[7–9]. In recent years, some farmers inject such drugs into the animals ready for sale and then infuse a large volume of water to increase their weight[10–11]. Moreover, administration of these two types of drugs during animal transportation can reduce the stress response and mortality rate, allowing farmers to gain higher profits[12–13]. If these drugs are abused in animal breeding and transportation, they will eventually accumulate in the human body through the food chain and threaten human health[14–15]. Therefore, it is crucial to establish a rapid qualitative and quantitative method for the simultaneous determination of tranquilizer drugs and anesthetic drugs in livestock meat.

Currently, the detection methods for tranquilizer drugs and anesthetic drugs mainly include gas chromatography-mass spectrometry (GC-MS)[16–20], liquid chromatography-mass spectrometry (LC-MS)[21–24], tandem high-resolution LC-MS[25–27], and ion chromatography (IC)[28–30]. However, the samples detected by GC-MS require derivatization treatment, which is complex and inefficient; LC-MS and IC both suffer from problems such as strong matrix interference and low sensitivity; tandem high-resolution LC-MS can achieve simultaneous rapid qualitative and quantitative analysis of multiple target compounds. After obtaining abundant ion fragment information, a corresponding database can be established, enabling qualitative screening of positive samples without the use of standard samples in subsequent analyses. At the same time, it can avoid the shortcoming of traditional LC-MS that requires optimization of MS parameters for each target compound, and has higher sensitivity and accuracy.

The detection of tranquilizer drugs primarily focuses on matrices such as feed, hair, blood, and livestock meat[31–32]. The detection of anesthetic drugs primarily focuses on matrices such as aquatic products and cosmetics[33–34]. However, there are few reports on the simultaneous detection of tranquilizer drugs and anesthetic drugs in livestock meat. In this study, ultra-high performance liquid chromatography-quadrupole-orbitrap high resolution mass spectrometry (UPLC-Q-Orbitrap HRMS) was used as the analytical instrument, and the QuEChERS method was selected for the purification of livestock meat matrix. A qualitative screening and quantitative analysis methods was established for 24 tranquilizer drugs and 27 anesthetic drugs in livestock meat, together with

MS/MS spectral library of 51 target compounds. This method can be widely applied for the detection and analysis of anesthetic and sedative drug residues in livestock meat, providing robust technical support and methodological guidance for the risk assessment of such compounds.

2 Materials and methods

2.1 Materials and reagents

The samples used in this experiment comprised pork muscle samples, beef muscle samples, and mutton muscle samples, with 10 samples from each animal type, totaling 30 samples. All samples were sourced from local farmers' markets and promptly refrigerated at 4 °C after collection for subsequent experimental analysis.

The standard substances (100 μg/mL) were all purchased from Tianjin Alta Technology Co., Ltd. (Tianjin, China) (Table 1). Acetonitrile (chromatographic grade) and methanol (chromatographic grade) were purchased from Thermo Fisher Scientific (USA); formic acid (chromatographic grade) was purchased from Sigma (USA); ultra-pure water (with a resistivity of 18.2 MΩ·cm at 25 °C) was purchased from Millipore (USA); 0.22 μm nylon membrane was purchased from Tianjin Agela Co. (China); 150 mg $MgSO_4$ + 25 mg primary secondary amine (PSA) and 150 mg $MgSO_4$ + 50 mg PSA + 50 mg C_{18} were purchased from NanoChrom Technologies (China) Co., Ltd.; 50 mg petroleum coke (PC) + 50 mg C_{18} + 50 mg $MgSO_4$ was purchased from Tianjin Agela Co. (China).

2.2 Instruments and equipment

The ultimate 3000 UPLC-Q-Orbitrap HRMS was purchased from Thermo Fisher Scientific, USA; the CR22N high-speed refrigerated centrifuge was purchased from Hitachi, Germany; the KQ-500E ultrasonic cleaner was purchased from Kunshan Ultrasonic Instruments Co., Ltd., China; the Vortex Genius 3 vortex mixer was purchased from, IKA, Germany.

2.3 Method

2.3.1 Sample preparation

A 5 g portion of previously homogenized at 10 000 r/min for 5 min at room temperature sample (pork muscle, beef muscle, and mutton muscle samples) was weighed into a 50 mL plastic centrifuge tube. Added 5 mL of acetonitrile, vortexed for 1 min to mix, ultrasonicated for 20 min at 500 W, and then centrifuged at 10 000 r/min for 5 min. Following this, 2 mL of supernatant was transferred into a 2 mL QuEChERS purification tube containing 150 mg of $MgSO_4$, 50 mg of PSA, and 50 mg of C_{18}. The mixture was mixed on a vortex for 1 min and then centrifuged at 13 000 r/min for 5 min. The supernatant was filtered through a 0.22 μm nylon filter membrane into the LC vial for UPLC-Q-Orbitrap HRMS analysis.

2.3.2 Preparation of the mix-standards stock solution

The mix-standards stock solution were prepared in methanol at a concentration of 1 μg/mL and were stored at −20 °C in a dark place.

2.3.3 Preparation of blank matrix solution

The livestock meat without the target compound was pretreated according to the above sample preparation, and the blank matrix solution was obtained, which was used for the preparation of the standard curve.

2.3.4 Chromatographic conditions

The chromatographic separation was performed on a XBridge BEH C_{18} chromatographic column (2.1 mm × 100 mm, 2.5 μm, Waters, USA), aqueous solution containing 0.1% (*V*/*V*) formic acid (phase A) and 0.1% formic acid methanol-acetonitrile (1:1, *V*/*V*) (phase B) were used as mobile phase. The flow rate was 0.3 mL/min. The injection volume was 5 μL. The gradient elution program was as follows: 0–3.0 min, 98% to 70% A; 3.0–15.0 min, 70% to 50% A; 15.0–18.0 min, 50% to 5% A; 18.0–20.0 min, 5% A; 20.0–20.1 min, 5% to 98% A; 20.1–22.0 min, 98% A.

Table 1 MS parameters of 24 tranquilizer drugs and 27 anesthetic drugs.

Category	Compound	Molecular formula	CAS	Retention time (min)	Adduct/ charge	Parent ion *m*/*z*		Relative mass deviation (× 10^{-6})	Fragment ions *m*/*z*
						Theoretical value	Measured value		
Tranquilizer drugs	Azaperol	$C_{19}H_{24}FN_3O$	2804-5-9	4.43	$[M+H]^+$	330.197 6	330.198 2	1.82	312.187 38, 192.118 71, 149.076 45, 121.076 17
	Desloratadine	$C_{19}H_{19}ClN_2$	100643-71-8	6.51	$[M+H]^+$	311.131	311.131 8	2.57	294.104 95, 282.105 13, 259.135 89
	Hexanitrodiphenylamine	$C_{16}H_{19}ClN_2$	131-73-7	6.69	$[M+H]^+$	275.131	275.132 1	4.00	230.074 08, 202.042 42, 167.073 67
	Azaperone	$C_{19}H_{22}FN_3O$	1649-18-9	5.04	$[M+H]^+$	328.182	328.181 4	−1.83	264.873 41, 208.883 54, 165.071 33, 121.076 19
	Propionylpromazine	$C_{20}H_{24}N_2OS$	3568-24-9	15.21	$[M+H]^+$	341.168 2	341.169 3	3.22	296.111 66, 268.079 59, 86.096 56
	Cyproheptadine	$C_{21}H_{21}N$	41354-29-4	13.14	$[M+H]^+$	288.174 7	288.174 4	−1.04	191.086 18, 110.096 53, 96.081 02, 82.065 41
	Diazepam	$C_{16}H_{13}ClN_2O$	439-14-5	16.39	$[M+H]^+$	285.078 9	285.079 1	0.70	257.084 44, 222.115 17, 193.088 68, 154.041 87
	Chlorpromazine	$C_{17}H_{19}ClN_2S$	50-53-3	15.83	$[M+H]^+$	319.103	319.103 4	1.25	246.014 45, 86.096 52, 58.065 22
	Terfenadine	$C_{32}H_{41}NO_2$	50679-08-8	18.16	$[M+H]^+$	472.321	472.320 9	−0.21	454.310 70, 436.300 11, 57.070 76
	Haloperidol	$C_{21}H_{23}ClFNO_2$	52-86-8	11.32	$[M+H]^+$	376.147 4	376.149	4.25	358.137 94, 165.071 73, 123.024 56
	Droperidol	$C_{22}H_{22}FN_3O_2$	548-73-2	8.47	$[M+H]^+$	380.176 9	380.178 3	3.68	194.098 31, 165.071 66, 123.024 48
	Carazolol	$C_{18}H_{22}N_2O_2$	57775-29-8	7.13	$[M+H]^+$	299.175 4	299.176 4	3.34	222.092 29, 184.076 40, 116.107 41, 74.060 32
	Prochlorperazine	$C_{21}H_{26}ClN_3OS$	58-38-8	15.81	$[M+H]^+$	404.155 8	404.157 2	3.46	246.014 56, 171.149 70, 143.118 33, 70.065 32
	Diphenhydramine	$C_{17}H_{21}NO$	58-73-1	9.45	$[M+H]^+$	256.169 6	256.168 5	−4.29	211.121 64, 119.059 66, 91.053 86, 72.081 05
	Promethazine	$C_{17}H_{20}N_2S$	60-87-7	11.74	$[M+H]^+$	285.142	285.142 6	2.10	240.084 96, 198.037 72, 86.096 66, 71.073 17
	Acepromazine	$C_{19}H_{22}N_2OS$	61-00-7	12.21	$[M+H]^+$	327.152 6	327.153 3	2.14	254.064 15, 222.091 90, 86.096 60, 58.065 27
	Astemizole	$C_{28}H_{31}FN_4O$	68844-77-9	7.21	$[M+H]^+$	459.255 5	459.257 5	4.35	308.156 31, 218.154 42, 135.080 73, 105.070 05
	Hydroxyzine	$C_{21}H_{27}ClN_2O_2$	68-88-2	13.46	$[M+H]^+$	375.183 4	375.182 9	−1.33	219.148 57, 201.046 65, 166.078 08
	Fluphenazine	$C_{22}H_{26}F_3N_3OS$	69-23-8	17.27	$[M+H]^+$	438.182 1	438.180 3	−4.11	396.167 39, 280.038 15, 171.148 19, 143.117 16
	Xylazine	$C_{12}H_{16}N_2S$	7361-61-7	5.10	$[M+H]^+$	221.110 7	221.110 8	0.45	164.053 09, 147.091 93, 90.037 33, 58.065 19
	Loratadine	$C_{22}H_{23}ClN_2O_2$	79794-75-5	16.33	$[M+H]^+$	383.152 1	383.150 3	−4.70	337.108 12, 294.102 54, 259.133 94
	Cetirizine	$C_{21}H_{25}ClN_2O_3$	83881-51-0	14.30	$[M+H]^+$	389.162 6	389.162 3	−0.77	201.046 66, 166.077 79, 149.023 44
	Brompheniramine	$C_{16}H_{19}BrN_2$	86-22-6	7.35	$[M+H]^+$	319.080 4	319.079 8	−1.88	274.023 28, 244.983 17, 194.096 83, 167.073 35
	Tripelennamine	$C_{16}H_{21}N_3$	91-81-6	5.94	$[M+H]^+$	256.180 8	256.180 5	−1.17	211.123 02, 119.060 41, 91.054 43, 72.081 53

Table 1 (*Continued*)

Category	Compound	Molecular formula	CAS	Retention time (min)	Adduct/ charge	Parent ion *m/z*		Relative mass deviation (× 10^{-6})	Fragment ions *m/z*
						Theoretical value	Measured value		
Anesthetic drugs	Procaine	$C_{13}H_{20}N_2O_2$	59-46-1	3.61	$[M+H]^+$	237.159 8	237.158 8	−4.22	164.069 60, 120.043 93, 100.111 98, 72.081 07
	Procainamide	$C_{13}H_{21}N_3O$	51-06-9	2.64	$[M+H]^+$	236.175 7	236.174 9	−3.39	163.085 60, 120.043 92, 100.111 90
	Chloroprocaine	$C_{13}H_{20}Cl_2N_2O_2$	133-16-4	18.39	$[M+H]^+$	271.272 2	271.273 32	4.13	254.246 44, 240.230 80, 158.153 20, 123.116 62
	Prilocaine	$C_{13}H_{20}N_2O$	721-50-6	5.34	$[M+H]^+$	221.164 8	221.163 82	−4.43	205.157 53, 193.157 39, 136.074 84, 111.080 28
	Ropivacaine	$C_{17}H_{26}N_2O$	84057-95-4	6.19	$[M+H]^+$	275.211 8	275.211 1	−2.54	126.128 14, 98.096 76, 84.081 06
	Bupivacaine	$C_{18}H_{28}N_2O$	2180-92-9	7.74	$[M+H]^+$	289.227 4	289.228 2	2.77	140.143 86, 98.096 69, 84.081 05
	Butacaine	$C_{18}H_{30}N_2O_2$	149-16-6	7.85	$[M+H]^+$	307.238	307.238 9	2.93	178.086 55, 142.159 32, 120.044 53, 100.112 18
	Tetracaine	$C_{15}H_{24}N_2O_2$	94-24-6	9.04	$[M+H]^+$	265.191 1	265.190 1	−3.77	220.131 99, 176.106 02, 72.081 11
	Benzocaine	$C_9H_{11}NO_2$	94-09-7	9.32	$[M+H]^+$	166.086 3	166.086 3	0.57	138.055 18, 120.044 49, 94.065 23, 77.038 59
	Pramoxine	$C_{17}H_{27}NO_3$	637-58-1	11.28	$[M+H]^+$	294.206 4	294.207 2	2.72	151.075 88, 128.107 44, 123.044 48, 100.075 97
	Cinchocaine	$C_{20}H_{29}N_3O_2$	85-79-0	13.95	$[M+H]^+$	344.233 3	344.233 6	0.87	271.145 32, 215.082 50, 172.040 12, 144.045 06
	Risocaine	$C_{10}H_{13}NO_2$	94-12-2	12.63	$[M+H]^+$	180.101 9	180.101 9	0.22	138.055 01, 120.044 14, 94.065 14, 77.038 57
	3-Aminobenzoic acid	$C_7H_7NO_2$	99-05-8	2.55	$[M+H]^+$	138.055 0	138.055 1	0.91	120.044 45, 94.651 4, 77.038 62, 56.964 80
	4-Aminobenzoic acid	$C_7H_7NO_2$	150-13-0	12.63	$[M+H]^+$	138.055 0	138.055 1	0.94	120.044 41, 105.044 84, 94.065 13, 77.038 63
	4-Acetamidobenzoic acid	$C_9H_9NO_3$	556-08-1	3.55	$[M+H]^+$	180.065 5	180.065 6	0.60	162.054 14, 136.075 07, 94.065 75
	Etidocaine	$C_{17}H_{28}N_2O$	36637-18-0	6.80	$[M+H]^+$	277.227 4	277.227 5	0.36	232.070 59, 128.143 88, 86.096 70
	Mepivacaine	$C_{15}H_{22}N_2O$	22801-44-1	5.00	$[M+H]^+$	247.180 5	247.179 9	−2.43	98.069 34, 84.513 90, 70.065 41
	Trimecaine	$C_{15}H_{24}N_2O$	616-68-2	6.22	$[M+H]^+$	249.196 1	249.195 2	−3.61	86.096 57, 224.925 93, 193.121 46, 98.984 23
	Oxybuprocaine	$C_{17}H_{28}N_2O_3$	99-43-4	8.84	$[M+H]^+$	309.217 3	309.218	2.26	236.128 88, 192.102 60, 136.039 81, 100.112 37
	Proparacaine	$C_{16}H_{26}N_2O_3$	298-50-0	6.02	$[M+H]^+$	295.201 6	295.202 2	2.03	222.113 34, 178.086 94, 136.039 84, 100.112 39
	Articaine	$C_{13}H_{20}N_2O_3S$	23964-58-1	5.03	$[M+H]^+$	285.126 7	285.126 9	0.70	240.062 61, 198.036 07, 86.096 56
	Lidocaine	$C_{14}H_{22}N_2O$	137-58-6	4.91	$[M+H]^+$	235.180 5	235.180 7	0.85	217.193 36, 179.105 61, 57.070 33
	Tricaine methanesulfonate	$C_9H_{11}NO_2$	886-86-2	9.02	$[M+H]^+$	166.086 3	166.086 4	0.94	138.055 01, 120.044 34, 94.065 14, 77.037 61
	Methyl eugenol	$C_{11}H_{14}O_2$	93-15-2	16.15	$[M+H]^+$	179.106 7	179.106 7	0.01	164.082 43, 149.022 42, 138.066 73, 116.971 71
	Methyl isoeugenol	$C_{11}H_{14}O_2$	93-16-3	16.97	$[M+H]^+$	179.106 7	179.106 6	−0.41	151.074 58, 138.054 24, 116.971 55, 107.049 10
	1-Acetoxy-2-methoxy-4-(1-propenyl)benzene	$C_{12}H_{14}O_3$	93-29-8	17.42	$[M+H]^+$	207.101 6	207.101 9	1.45	165.090 16, 149.022 60, 133.064 22, 105.069 77
	Eugenol acetate	$C_{12}H_{14}O_3$	93-28-7	16.95	$[M+H]^+$	207.101 6	207.101 8	0.97	189.089 98, 165.090 21, 149.022 67, 84.959 91

2.3.5 MS conditions

The MS analysis was performed using an electrospray ionization (ESI) source in positive ionization mode. The optimized parameters of ion source were as follows: the positive ion voltage was 3 500 V, the ion transfer tube temperature was 320 °C, the vaporizer temperature was 350 °C, the sheath gas pressure was 35 arb, the aux gas pressure was 10 arb. MS conditions were as follows: scan type was Full Scan-ddMS^2, scan range was 60–600 *m/z*, the duration was 22 min, the collision energy was 30 eV, Full Scan orbitrap resolution was 70 000, MS^2 orbitrap resolution was 17 500. Automatic gain control target was 1×10^5, isolation window was 4.0 *m/z*. The MS parameters of 24 tranquilizer drugs and 27 anesthetic drugs were shown in Table 1.

2.3.6 Calculation of matrix effects (ME)

ME was calculated using the following formula:

$$\mathrm{ME}\ (\%) = \frac{B-A}{A} \times 100$$

In the formula: *A* represents the slope of the standard curve prepared with acetonitrile; *B* represents the slope of the matrix-matched standard curve prepared with the blank matrix extract.

2.3.7 Establishment of database

Under the chromatographic and MS conditions specified in sections 2.3.4 and 2.3.5, data were collected for 51 mixed standard solutions. The original data were imported into the Thermo mzVault software, and a secondary spectral library including 51 compounds was created.

2.3.8 Method validation

The quantitative analysis of 51 target compounds was carried out using the external standard method. The standard solutions prepared in section 2.3.2 were diluted with blank matrix extract solutions to form standard series working solutions of different concentrations (1, 2, 5, 10, 20, 50, 100, 200 ng/mL), with the peak area of the parent ion of the target compound as the ordinate (*y*) and the corresponding standard solution concentration (ng/mL) as the abscissa (*x*), and a standard curve was plotted. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by adding the standard solutions to the blank matrix. The signal-to-noise ratio (R_{SN}) was measured for each analyte, with LOD and LOQ defined as the concentrations achieving $R_{SN} \geq 3$ and $R_{SN} \geq 10$, respectively.

Accuracy and precision determination: the mix-standards stock solution were added to blank beef, mutton and pork matrix for the spiked recovery experiment. Each matrix was spiked with three concentration levels: LOQ, 2LOQ, and 10LOQ, with each concentration level tested independently six times.

2.4 Data processing

Data for target compounds were acquired in Full Scan-ddMS^2 mode, with precise quantitative results analyzed in the Quan Browser module of Xcalibur software. Qualitative confirmation was achieved using a combination of Tracefinder and mzVault software. Data processing and statistical analysis were conducted in Microsoft Excel, including data organization, calculation, and graphical presentation.

3 Results and discussion

3.1 Optimization of chromatographic conditions

In this experiment, XBridge BEH C_{18} chromatographic column (2.1 mm × 100 mm, 2.5 μm) was used to investigate the separation degree, peak shape and response values of 51 target compounds. The experimental results showed that the XBridge BEH C_{18}

chromatographic column could effectively separate four groups of isomers: tricaine methanesulfonate and benzocaine, 3-aminobenzoic acid and 4-aminobenzoic acid, methyl eugenol and methyl isoeugenol, and 1-acetoxy-2-methoxy-4-(1-propenyl) benzene and eugenol acetate. The peak shape was better and sharper. The extraction ion chromatograms of the 51 compounds were shown in Figure S1.

Since all 51 compounds were detected using ESI^+, 0.1% formic acid acetonitrile-0.1% formic acid water, 0.1% formic acid methanol-0.1% formic acid water, and 0.1% formic acid methanol-acetonitrile (1:1, *V*/*V*)-0.1% formic acid water were selected as the mobile phases to investigate the separation degree and peak shape of the target compounds. The experimental results showed that when acetonitrile was used as the mobile phase, the chromatographic peaks of methyl eugenol and methyl isoeugenol could not be separated; however, when acetonitrile was used as the organic phase, the peak shape of the tranquilizer drugs was better than that of methanol as the organic phase. Therefore, 0.1% formic acid methanol-acetonitrile (1:1, *V*/*V*) was adopted as the organic phase for this experiment. In addition, adding formic acid to the mobile phase can effectively improve the response of the target compounds. Therefore, 0.1% formic acid methanol-acetonitrile (1:1, *V*/*V*)-0.1% formic acid water was selected as the mobile phase for this experiment.

3.2 Optimization of MS parameters

The UPLC-Q-Orbitrap HRMS simultaneously collects the primary parent ion and secondary fragment ion information of 51 target compounds in the Full Scan-ddMS^2 mode. The theoretical accurate mass number of the molecular ion peak $[M + H]^+$ is calculated based on their chemical formulas, and then the corresponding ion chromatogram and MS/MS spectral library are extracted, as shown in Figures S1–S2. The relative deviation of the accurate mass numbers of the 51 compounds is all less than 5.0×10^{-6}, as shown in Table 1. This complies with the regulations of European Union for LC/MS[35], and can be used for qualitative and quantitative analysis of the target compounds.

3.3 Optimization of pre-treatment conditions

3.3.1 Optimization of the extraction solvent

Since livestock meat contains a considerable amount of fat and protein, and the polarities of various tranquilizer drugs and anesthetic drugs are quite different, while improving the extraction efficiency of different compounds, it is necessary to minimize the influence of fat, protein and other substances on the experimental results. In this experiment, the commonly used extraction reagents acetonitrile, ethyl acetate and methanol reported previously were selected as extraction solvents[16,21]. Each extraction solvent was tested in triplicate in parallel, and the experimental results were shown in Figure 1. The protein precipitation effect of methanol and ethyl acetate is not as good as that of acetonitrile. Moreover, the average recovery rate of 51 compounds in acetonitrile is between 60.4% and 98.4%; the average recovery rate in methanol is between 8% and 81.3%, and the recovery rate of 20 compounds is below 50%; the average recovery rate in ethyl acetate is between 12% and 90%, and the recovery rate of 23 compounds is below 50%. Based on the above experimental results, acetonitrile was selected as the extraction solvent in this experiment.

3.3.2 Optimization of purification methods

In recent years, many scholars have applied the QuEChERS method, which is commonly used for pesticide residue detection and analysis, to the experiments of veterinary drug residue detection and analysis[36–39]. The common QuEChERS usually includes fillers such as C_{18}, PSA, $MgSO_4$, and PC. C_{18} mainly adsorbs non-polar macromolecular impurities, such as proteins and fats, which are liposoluble substances; PSA can adsorb organic acids; $MgSO_4$ mainly serves to strongly absorb water, preventing the emulsification of the organic phase; PC can strongly adsorb pigments. In this experiment, the recovery rates of three different fillers and specifications of QuEChERS (A: 150 mg $MgSO_4$ + 25 mg PSA; B: 150 mg $MgSO_4$ + 50 mg PSA + 50 mg C_{18}; C: 50 mg PC + 50 mg C_{18} + 50 mg $MgSO_4$) were compared through the blank matrix spiked recovery experiment. Each purification material was tested in triplicate in parallel, the experimental results were shown

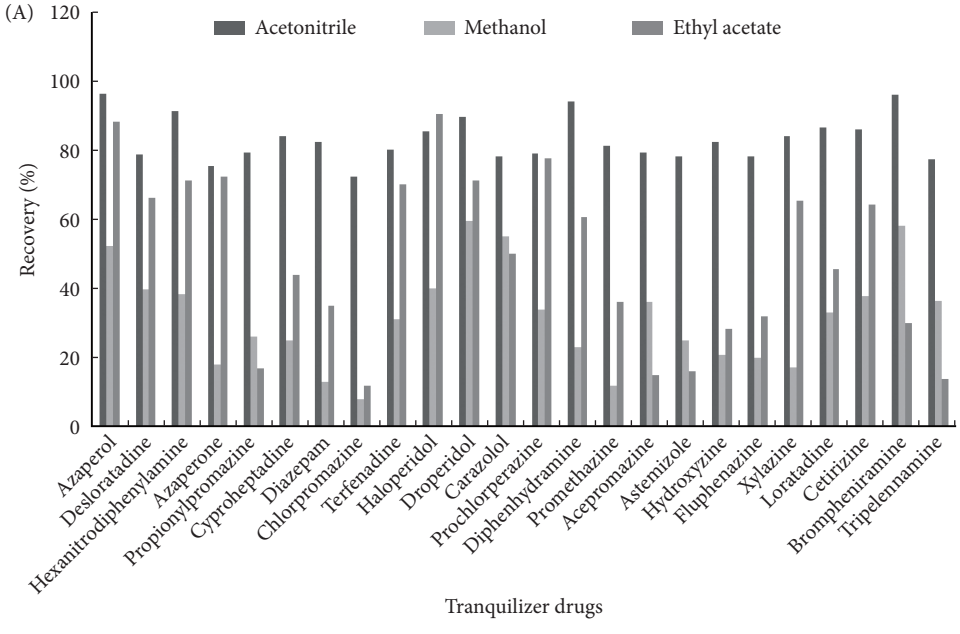

Figure 1 Effects of different extraction solvent on recovery rates of (A) 24 tranquilizer drugs and (B) 27 anesthetic drugs.

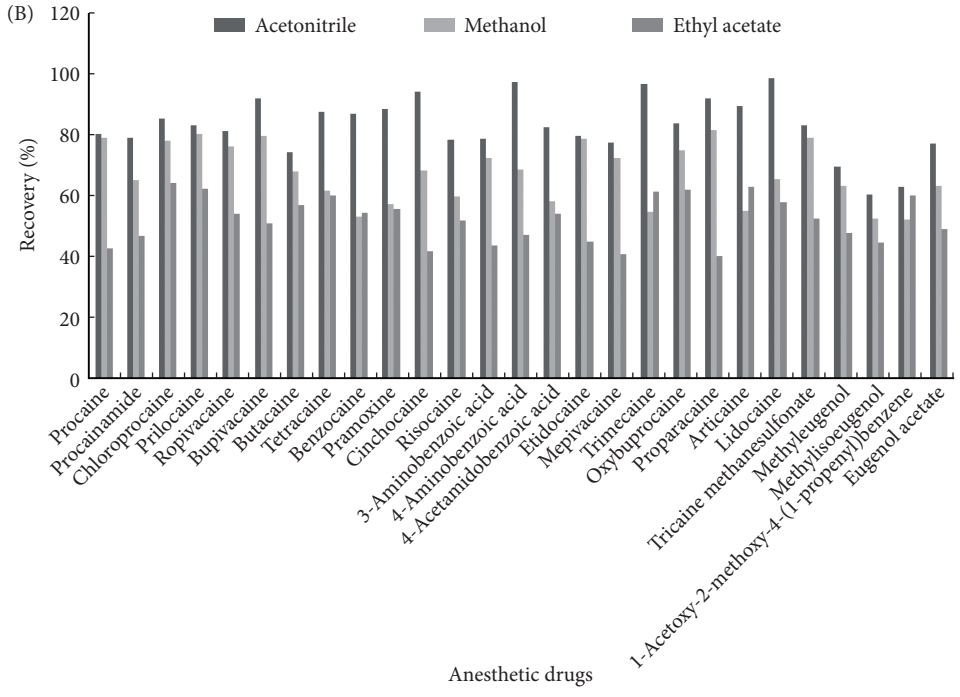

Figure 1 (*Continued*)

in Figure 2. The recovery rates of the fillers in A, B, and C were within the range of 28.0% to 132.5%, 71.0% to 116.0%, and 5.0% to 118.5%, respectively. The results demonstrated that the average recovery rates for all 51 compounds obtained using B fell within the acceptable range of 70% to 120%, meeting the validation requirements for quantitative analysis. Therefore, B was selected as the purifying agent. The samples were purified using the QuEChERS method, which offers the advantages of simplicity and speed compared to solid phase extraction (SPE)[17–18].

3.4 ME

The matrix of livestock meat which rich in fat and protein is relatively complex and can easily interfere with the reproducibility and accuracy of the test results. Therefore, this study examines the degree of interference of the ME on the experimental results. When the |ME| ≤ 15%, it is considered that the ME can be ignored[21]. The results of the ME for beef, mutton and pork in this experiment were shown in Table 2. Only 10 compounds in the beef matrix, 26

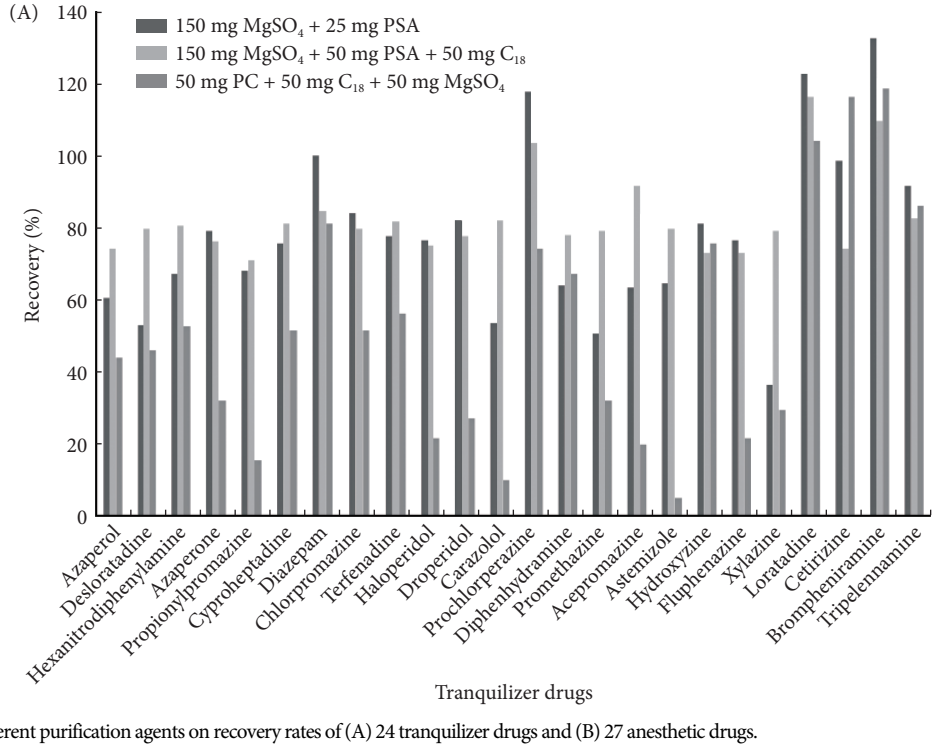

Figure 2 Effects of different purification agents on recovery rates of (A) 24 tranquilizer drugs and (B) 27 anesthetic drugs.

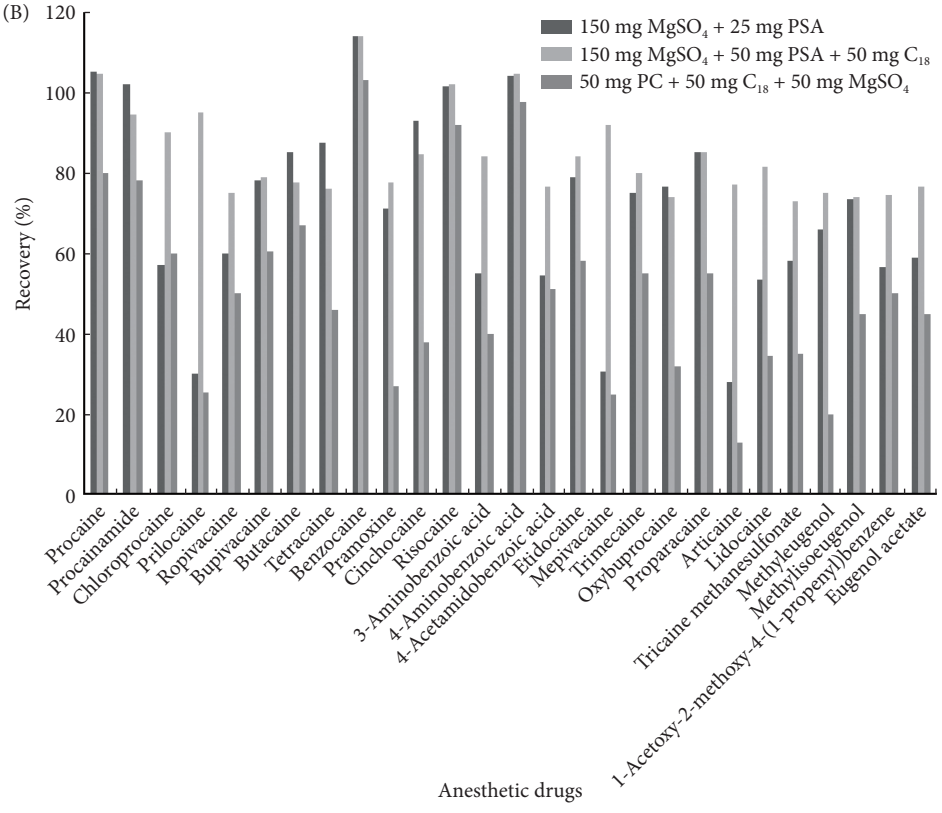

Figure 2 (*Continued*)

Table 2 Linearity, LOD and LOQ of 24 tranquilizer drugs and 27 anesthetic drugs.

No.	Compound	Linear equation	Linear range (ng/mL)	R^2	LOD (μg/kg)	LOQ (μg/kg)	ME (%)		
							Beef	Mutton	Pork
1	Azaperol	$y = -1.262\ 3 \times 10^6 + 362\ 111x$	1–100	0.996 3	1	2	−39.9	46.1	6.4
2	Desloratadine	$y = -837\ 746 + 265\ 786x$	1–200	0.995 6	1	2	47.6	−28.2	−15.7
3	Hexanitrodiphenylamine	$y = -1.700\ 07 \times 10^6 + 469\ 457x$	1–100	0.995 2	1	2	12.5	34.5	−20.5
4	Azaperone	$y = -985\ 469 + 186\ 263x$	2–100	0.995 6	2	5	9.2	61.3	−0.9
5	Propionylpromazine	$y = -2.090\ 36 \times 10^6 + 1.149\ 94 \times 10^6x$	1–200	0.999 8	1	2	18.4	−2.6	−9.3
6	Cyproheptadine	$y = 258\ 681 + 1.618\ 64 \times 10^6x$	1–200	0.999 4	1	2	1.7	−1.9	45.4
7	Diazepam	$y = -4.755\ 43 \times 10^6 + 1.728\ 09 \times 10^6x$	1–200	0.997 8	1	2	38.4	60.6	16.9
8	Chlorpromazine	$y = -8.964\ 45 \times 10^6 + 1.033\ 3 \times 10^6x$	5–200	0.997 0	5	10	37.3	0.8	18.5
9	Terfenadine	$y = -243\ 647 + 836\ 102x$	1–200	0.998 9	1	2	−27.9	−8.4	−42.5
10	Haloperidol	$y = 17\ 961.2 + 793\ 821x$	1–200	0.999 8	1	2	−48.3	−1.4	−26.3
11	Droperidol	$y = -20\ 724.3 + 325\ 624x$	1–200	0.999 9	1	2	50.9	54.6	5.0
12	Carazolol	$y = 444\ 213 + 947\ 604x$	1–200	0.999 2	1	2	−32.9	11.3	−39.0
13	Prochlorperazine	$y = -5.689\ 94 \times 10^6 + 522\ 372x$	5–200	0.996 8	5	10	30.0	20.9	−18.5
14	Diphenhydramine	$y = -273\ 287 + 931\ 218x$	1–200	0.999 9	1	2	47.1	−4.5	47.3
15	Promethazine	$y = -5.248\ 37 \times 10^6 + 1.299\ 28 \times 10^6x$	1–200	0.998 5	1	2	−7.4	−16.8	39.5
16	Acepromazine	$y = -1.873\ 11 \times 10^6 + 1.116\ 64 \times 10^6x$	1–200	0.999 7	1	2	34.9	−8.6	16.3
17	Astemizole	$y = -53\ 196 + 199\ 573x$	1–200	0.997 8	1	2	−44.5	−12.7	30.0
18	Hydroxyzine	$y = -57\ 330.7 + 694\ 885x$	1–200	0.999 6	1	2	−35.7	−6.9	26.8
19	Fluphenazine	$y = -193\ 966 + 787\ 070x$	1–200	0.999 6	1	2	−49.5	10.5	−47.6
20	Xylazine	$y = 578\ 396 + 1.915\ 12 \times 10^6x$	1–200	0.999 7	1	2	−24.8	−5.8	−44.8
21	Loratadine	$y = 89\ 214.3 + 530\ 582x$	1–200	0.999 8	1	2	−15.1	52.6	−42.4
22	Cetirizine	$y = 195\ 066 + 300\ 047x$	1–200	0.999 1	1	2	34.9	61.3	−26.7
23	Brompheniramine	$y = -988\ 913 + 268\ 569x$	1–100	0.995 6	1	2	−25.5	18.5	−16.7
24	Tripelennamine	$y = -1.647\ 87 \times 10^6 + 478\ 574x$	1–100	0.996 7	1	2	41.2	26.4	−35.3
25	Procaine	$y = -1.772\ 5 \times 10^6 + 1.294\ 52 \times 10^6x$	1–200	0.998 5	1	2	−53.7	−43.8	−11.3
26	Procainamide	$y = -653\ 290 + 695\ 696x$	2–200	0.999 9	2	5	−37.6	−38.4	−23.5
27	Chloroprocaine	$y = 9.206\ 36 \times 10^6 + 1.106\ 32 \times 10^7x$	5–200	0.999 4	5	10	27.8	−58.7	43.9
28	Prilocaine	$y = 1.320\ 72 \times 10^6 + 755\ 569x$	1–200	0.997 6	1	2	−1.4	1.9	48.3
29	Ropivacaine	$y = -844\ 839 + 1.727\ 93 \times 10^6x$	1–200	1.000 0	1	2	40.3	−9.7	42.3

Table 2 (*Continued*)

No.	Compound	Linear equation	Linear range (ng/mL)	R^2	LOD (μg/kg)	LOQ (μg/kg)	ME (%)		
							Beef	Mutton	Pork
30	Bupivacaine	$y = -683\,552 + 933\,327x$	1–200	0.999 9	1	2	47.9	−6.6	38.8
31	Butacaine	$y = 1.090\,95 \times 10^7 + 1.106\,38 \times 10^7 x$	1–200	0.999 2	1	2	15.8	14.5	−14.2
32	Tetracaine	$y = -911\,930 + 1.322\,55 \times 10^6 x$	1–200	0.999 6	1	2	−43.0	−1.6	−4.9
33	Benzocaine	$y = -872\,679 + 1.216\,04 \times 10^6 x$	1–200	0.999 7	1	2	−45.4	23.3	9.7
34	Pramoxine	$y = 968\,869 + 1.559\,65 \times 10^6 x$	1–200	0.999 0	1	2	−27.2	−1.4	−11.3
35	Cinchocaine	$y = -3.503\,39 \times 10^6 + 1.291\,68 \times 10^6 x$	1–200	0.999 5	1	2	−19.2	2.5	−1.7
36	Risocaine	$y = -1.436\,14 \times 10^7 + 1.237\,96 \times 10^7 x$	1–200	0.999 7	1	2	17.8	14.7	−21.2
37	3-Aminobenzoic acid	$y = -594\,665 + 597\,934x$	1–200	0.999 5	1	2	−8.9	−3.9	25.5
38	4-Aminobenzoic acid	$y = -1.367\,06 \times 10^6 + 1.467\,43 \times 10^6 x$	1–200	0.999 9	1	2	−50.9	18.7	−32.2
39	4-Acetamidobenzoic acid	$y = -229\,741 + 253\,536x$	1–200	0.999 1	1	2	−14.1	48.4	−8.9
40	Etidocaine	$y = -558\,096 + 1.432\,98 \times 10^6 x$	1–200	0.999 4	1	2	−11.9	−3.9	37.3
41	Mepivacaine	$y = 1.504\,96 \times 10^6 + 1.451\,05 \times 10^6 x$	1–200	0.999 1	1	2	46.6	−1.5	−51.0
42	Trimecaine	$y = 937\,145 + 1.553\,98 \times 10^6 x$	1–200	0.999 0	1	2	31.8	−1.4	30.8
43	Oxybuprocaine	$y = -561\,302 + 1.222\,87 \times 10^6 x$	1–200	0.999 4	1	2	−2.5	−4.3	21.1
44	Proparacaine	$y = -9.252\,28 \times 10^6 + 9.298\,99 \times 10^6 x$	1–200	0.999 3	1	2	35.2	16.6	−47.0
45	Articaine	$y = 1.637\,83 \times 10^6 + 950\,915x$	1–200	0.998 3	1	2	44.4	−22.5	−13.3
46	Lidocaine	$y = -449\,967 + 1.655\,79 \times 10^6 x$	1–200	0.999 6	1	2	24.8	−5.9	−45.3
47	Tricaine methanesulfonate	$y = 15\,702 + 534\,459x$	1–200	0.999 6	1	2	54.0	−34.7	−47.1
48	Methyl eugenol	$y = 721\,513 + 27\,097.3x$	10–200	0.997 8	5	10	48.9	−57.8	30.1
49	Methyl isoeugenol	$y = 47\,596.3 + 59\,668.8x$	5–200	0.996 7	5	10	35.1	−47.6	−22.4
50	1-Acetoxy-2-methoxy-4-(1-propenyl)benzene	$y = 449\,587 + 194\,123x$	2–200	0.996 8	2	5	19.4	−32.8	−28.7
51	Eugenol acetate	$y = 452\,803 + 146\,710x$	2–200	0.995 8	2	5	−8.7	−30.9	−44.1

compounds in the mutton matrix and 12 compounds in the pork matrix can be considered to have no ME. The response intensity of the remaining compounds is significantly enhanced or inhibited by the matrix. To eliminate this influence, the quantitative analysis of 51 target compounds uses a blank matrix-matched standard curve.

3.5 Linearity, LOD and LOQ of tranquilizer drugs and anesthetic drugs

As shown in Table 2, within the concentration range of 1–200 ng/mL, the linear relationship of the 51 target compounds was good, with a correlation coefficient (R^2) ≥ 0.995 2, and the LOD was 1–5 μg/kg, the LOQ was 2–10 μg/kg.

3.6 Accuracy and precision

As shown in Table 3, the average spike recovery rates of beef, mutton and pork ranged from 84.0% to 111.9%, 85.1% to 107.1% and 84.1% to 105.9% respectively, and and the relative standard deviation (RSD) ranged from 2.5% to 7.5%， 2.7% to 7.4% and 2.6% to 8.5% respectively. The results indicate that the average spike recovery rates and RSDs of the 51 target compounds all meet the corresponding detection requirements[40].

Table 3 Recoveries and RSDs of 24 tranquilizer drugs and 27 anesthetic drugs ($n = 6$).

Compound	Spiked level (μg/kg)	Beef		Mutton		Pork	
		Average spike recovery rate (%)	RSD (%)	Average spike recovery rate (%)	RSD (%)	Average spike recovery rate (%)	RSD (%)
Azaperol	2	94.8	4.6	97.2	5.9	97.3	3.1
	4	87.0	7.4	105.7	6.1	91.6	6.3
	20	107.7	5.4	94.8	7.0	102.8	3.7
Desloratadine	2	86.7	4.7	85.9	4.9	91.8	7.0
	4	87.8	3.6	85.9	5.7	92.8	4.5
	20	86.3	6.2	101.6	4.7	91.1	6.0
Hexanitrodiphenylamine	2	87.2	3.0	99.7	5.7	86.7	3.2
	4	105.3	4.6	101.1	6.5	101.1	4.6
	20	105.5	5.4	85.3	7.3	97.3	5.9
Azaperone	5	85.9	6.7	99.8	5.4	100.8	5.5
	10	110.5	4.5	101.2	5.2	88.1	5.9
	50	105.0	7.1	102.6	4.2	87.6	2.7
Propionylpromazine	2	108.7	7.4	89.5	3.5	104.1	4.2
	4	108.1	3.4	94.4	5.7	102.9	6.5
	20	87.6	3.7	106.8	3.7	94.0	4.9
Cyproheptadine	2	86.5	5.5	85.3	4.2	105.6	6.9
	4	107.7	5.2	101.0	6.6	97.5	3.7
	20	100.9	3.2	87.2	4.7	89.3	6.5
Diazepam	2	89.0	2.9	96.4	4.1	99.4	6.7
	4	96.0	2.8	93.6	3.0	99.6	4.0
	20	88.3	5.9	104.1	6.4	103.2	6.7

Table 3 (*Continued*)

Compound	Spiked level (μg/kg)	Beef		Mutton		Pork	
		Average spike recovery rate (%)	RSD (%)	Average spike recovery rate (%)	RSD (%)	Average spike recovery rate (%)	RSD (%)
Chlorpromazine	10	90.5	5.0	91.9	6.7	94.1	7.0
	20	111.2	5.4	104.0	6.8	91.1	6.3
	100	111.2	4.5	100.7	3.6	90.4	7.1
Terfenadine	2	88.5	6.4	96.4	6.2	92.3	6.9
	4	93.6	3.8	105.9	4.2	86.4	4.5
	20	92.2	4.0	95.6	2.8	94.5	3.9
Haloperidol	2	111.4	3.0	102.0	5.4	105.6	3.8
	4	99.6	5.2	86.4	3.8	103.6	3.3
	20	106.7	2.6	96.0	6.6	88.7	3.3
Droperidol	2	106.0	3.1	105.2	3.8	103.5	5.0
	4	102.6	4.6	88.2	3.2	98.7	5.9
	20	86.1	2.9	102.9	2.7	92.0	6.4
Carazolol	2	89.3	4.8	91.1	7.1	105.3	3.1
	4	102.0	3.9	93.0	5.5	96.6	6.5
	20	92.1	5.6	106.6	4.7	100.5	3.6
Prochlorperazine	10	111.9	2.7	93.7	3.0	90.4	7.0
	20	111.6	4.0	97.0	5.1	88.4	4.2
	100	102.5	4.7	102.6	5.9	103.3	3.4
Diphenhydramine	2	95.8	5.8	98.0	3.2	104.2	5.0
	4	96.5	6.8	85.4	4.3	95.5	2.9
	20	88.0	5.8	106.6	3.8	92.7	4.0
Promethazine	2	86.8	4.1	100.2	3.6	92.3	3.7
	4	94.8	5.7	99.1	7.1	95.4	6.8
	20	91.4	5.4	96.8	6.4	102.0	7.4
Acepromazine	2	89.5	5.9	85.4	3.1	91.2	6.8
	4	92.0	3.0	99.6	3.5	97.6	4.2
	20	105.4	4.9	93.6	5.5	99.9	6.9
Astemizole	2	98.5	7.5	88.8	4.5	95.1	3.5
	4	111.4	5.4	87.3	6.1	103.9	3.9
	20	102.4	5.0	106.1	6.8	92.3	5.2
Hydroxyzine	2	104.7	3.5	101.8	5.5	91.2	5.3
	4	85.6	5.6	104.0	5.4	90.0	3.7
	20	108.3	5.7	102.7	7.0	96.4	3.7
Fluphenazine	2	99.3	2.8	107.1	5.1	101.6	5.6
	4	88.0	5.0	95.5	6.5	100.2	3.1
	20	99.6	6.5	105.5	4.1	86.7	7.2
Xylazine	2	87.9	7.4	85.9	4.9	105.9	8.5
	4	98.9	4.9	104.1	6.5	95.6	5.5
	20	110.7	5.8	102.8	6.4	87.7	6.7
Loratadine	2	94.9	3.5	95.2	3.0	86.4	4.3
	4	105.6	4.5	85.6	3.4	99.7	3.8
	20	102.8	4.8	102.6	7.1	104.1	7.3
Cetirizine	2	89.2	3.1	96.2	6.5	97.0	7.0
	4	106.3	4.7	100.1	6.0	92.9	6.6
	20	111.4	6.6	105.6	5.1	100.2	3.8
Brompheniramine	2	91.2	7.3	103.8	4.5	89.2	5.3
	4	100.5	4.4	86.7	4.5	86.8	7.4
	20	106.5	7.1	88.9	3.5	87.2	2.9
Tripelennamine	2	90.1	6.9	98.0	6.6	90.8	6.0
	4	85.6	6.0	103.4	3.5	101.2	6.0
	20	89.0	5.6	95.6	4.7	90.3	4.2
Procaine	2	93.2	2.5	93.5	5.3	90	3.1
	4	86.4	3.7	87.5	4.8	94.7	4.3
	20	93.6	6.7	88.5	6.6	89.3	2.8
Procainamide	5	92.3	2.5	89.9	3.6	84.2	3.2
	10	94.2	6.5	93.3	5.8	91.1	3.7
	50	88.2	5.2	90.0	6.5	90.3	5.6
Chloroprocaine	10	92.0	7.5	87.8	5.4	89.7	3.3
	20	92.4	6.6	85.5	5.1	94.5	5.6
	100	94.3	6.7	92.9	3.8	92.8	4.0

Table 3 (*Continued*)

Compound	Spiked level (μg/kg)	Beef		Mutton		Pork	
		Average spike recovery rate (%)	RSD (%)	Average spike recovery rate (%)	RSD (%)	Average spike recovery rate (%)	RSD (%)
Prilocaine	2	85.8	2.8	86.2	5.0	90.4	5.3
	4	88.3	6.6	91.3	7.0	84.8	3.8
	20	89.5	3.4	88.3	4.5	87.4	4.2
Ropivacaine	2	91.9	6.1	87.6	6.1	94.3	2.7
	4	84.2	4.8	92	2.8	84.4	8.3
	20	88.9	6.8	85.3	5.3	87.5	5.1
Bupivacaine	2	87.1	2.8	92.8	7.3	92.0	6.3
	4	87.2	4.8	87	5.7	93.8	6.7
	20	90.1	6.8	87.7	4.4	88.8	5.0
Butacaine	2	86.5	4.9	85.6	3.3	84.6	6.7
	4	90.0	3.1	88.7	6.6	87.4	7.2
	20	84.1	3.8	92.5	3.3	100.5	6.7
Tetracaine	2	91.3	4.7	86.8	5.5	84.5	5.6
	4	93.1	3.0	85.9	5.1	94.8	3.9
	20	92.4	5.0	87.2	5.6	94.5	4.0
Benzocaine	2	84.5	7.1	88.1	6.7	93.7	7.1
	4	86.2	4.6	85.9	4.8	91.1	5.1
	20	85.3	3.5	89.0	6.3	91.3	6.4
Pramoxine	2	91.5	5.5	90.9	5.3	93.9	6.4
	4	85.3	5.9	87.0	3.5	95.0	4.0
	20	90.8	3.8	86.4	6.7	85.5	6.8
Cinchocaine	2	87.5	3.1	85.9	6.0	94.8	3.5
	4	101.8	3.6	85.1	5.5	94.8	4.7
	20	85.9	6.5	94.5	3.9	87.3	4.7
Risocaine	2	91.1	7.5	93.8	5.2	87.9	6.9
	4	90.6	5.6	85.5	6.0	93.3	2.6
	20	89.9	5.0	92.4	7.4	94.1	5.2
3-Aminobenzoic acid	2	94.1	4.8	92.5	6.5	87.2	6.6
	4	90.3	3.5	88.1	7.3	84.7	3.3
	20	94.3	6.2	89.7	6.2	87.6	7.4
4-Aminobenzoic acid	2	86.9	3.3	90.9	3.9	93.3	6.1
	4	90.1	4.5	92.5	7.3	88.2	5.4
	20	91.1	6.6	90.0	5.8	90.2	5.7
4-Acetamidobenzoic acid	2	86.7	4.7	87.4	3.9	88.6	4.1
	4	85.4	3.7	86.9	5.7	91.3	4.3
	20	89.5	3.4	90.3	2.8	86.6	4.0
Etidocaine	2	91.4	4.0	92.6	5.5	88.2	4.4
	4	93.5	4.9	93.3	5.2	89.4	2.6
	20	95.0	5.3	88.5	4.8	85.8	3.2
Mepivacaine	2	91.6	4.8	86.1	5.0	87.6	4.2
	4	92.2	7.5	89.4	5.5	90.9	7.3
	20	84.8	5.7	91.3	5.6	91.2	4.0
Trimecaine	2	87.7	6.6	88.6	7.2	84.5	3.2
	4	86.9	6.9	93.3	3.0	90.7	4.0
	20	92.3	3.1	85.1	4.6	85.1	3.0
Oxybuprocaine	2	88.5	6.6	89.1	5.5	90.7	5.0
	4	91.7	5.6	85.5	4.0	89.7	3.5
	20	92.9	6.4	88.0	5.4	84.3	3.2
Proparacaine	2	89.6	7.4	90.8	6.2	86.5	8.1
	4	86.9	5.8	90.1	6.4	85.3	4.0
	20	88.7	3.9	86.8	7.1	88.5	3.7
Articaine	2	92.8	3.7	87.4	2.8	88.9	3.3
	4	91.2	7.2	91.0	3.3	88.0	5.9
	20	88.7	7.2	93.7	4.1	84.2	3.5
Lidocaine	2	92.3	2.9	93.0	4.6	86.1	5.1
	4	93.8	3.4	86.0	6.6	93.8	3.9
	20	90.4	5.3	85.3	5.3	91.5	6.6
Tricaine methanesulfonate	2	92.8	6.5	90.5	2.7	89.6	4.2
	4	86.2	3.1	90.5	4.9	92.9	4.4
	20	91.5	3.4	85.7	4.0	93.8	5.0

Table 3 (*Continued*)

Compound	Spiked level (μg/kg)	Beef		Mutton		Pork	
		Average spike recovery rate (%)	RSD (%)	Average spike recovery rate (%)	RSD (%)	Average spike recovery rate (%)	RSD (%)
Methyl eugenol	10	87.5	4.8	94.0	4.5	90.4	4.2
	20	94.5	3.8	85.9	2.9	84.1	5.1
	100	92.5	2.7	85.4	3.6	89.1	5.4
Methyl isoeugenol	10	87.2	4.1	90.2	7.3	84.8	3.9
	20	88.9	3.2	85.8	7.0	87.0	3.8
	100	92.5	2.8	93.0	6.2	85.1	7.2
1-Acetoxy-2-methoxy-4-(1-propenyl)benzene	5	93.3	4.2	94.6	7.3	93.4	4.6
	10	84.9	6.1	94.5	6.5	88.6	5.3
	50	84.0	6.8	88.9	3.3	91.0	5.4
Eugenol acetate	5	90.9	4.3	85.7	4.3	90.6	7.2
	10	92.1	7.2	87.2	2.7	94.6	5.3
	50	85.7	6.1	90.9	5.1	88.0	4.4

3.7 Sample analysis

In order to investigate the applicability of the method, 10 beef samples, 10 mutton samples and 10 pork samples were tested for the residues of 24 tranquilizer drugs and 27 anesthetic drugs. It was found that the content of cyproheptadine in one batch of pork was 11 μg/kg, while none of the other compounds were detected. None of the 51 target substances were detected in other samples.

4 Conclusions

This study focused on beef, mutton and pork as the research subjects. By optimizing the purification methods for pre-treatment, chromatographic and MS conditions, a method for simultaneously detecting 24 tranquilizer drugs and 27 anesthetic drugs in livestock meat was established. Methodological validation and actual sample testing were also conducted. The samples were purified using the QuEChERS method, which has the advantages of simplicity and rapidity compared to traditional purification methods (SPE). It is an environmentally friendly and highly versatile analytical method. This study provides important data and technical support for the rapid screening and precise quantification of 24 tranquilizer drugs and 27 anesthetic drugs in livestock meat.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors gratefully acknowledge the financial support from the Science and Technology Plan Project of Hebei Provincial Administration for Market Regulation (2024ZC08) and the Science and Technology Project of State Administration for Market Regulation (2024MK012). Additionally, we would like to thank the Hebei Food Inspection and Research Institute for assistance and technical support in this study.

Author contributions

Liangna He: investigation; formal analysis; writing (original draft, review and editing); visualization; software. Lixin Fan: methodology; investigation. Xiaoqian Dong: methodology; investigation. Junmei Ma: methodology; investigation. Sufang Fan: supervision; resources. Henan Xu: supervision; resources. Qiang Li: supervision; conceptualization; investigation; project administration.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at http://doi.org/10.26599/FSAP.2026.9240164.

References

[1] M. I. Gold, H. H. Stone, The tranquilizing drugs: a classification and pharmacological survey, Anesthesiology 1 (1957) 1–16. https://doi.org/10.1097/00000542-195705000-00001.

[2] A. Hordern, Psychiatry and the tranquilizers, New Engl. J. Med. 265 (1961) 584–588. https://doi.org/10.1056/NEJM196109212651206.

[3] K. Munkholm, A. Ussing, M. Brink, et al., Minor tranquillizers for short-term treatment of newly onset symptoms of anxiety and distress: a systematic review with network meta-analysis of randomized trials, Eur. Arch. Psy. Clin. N. 274 (2024) 475–486. https://doi.org/10.1007/s00406-023-01680-0.

[4] J. C. Fritz, F. D. Wharton, L. J. Classen, Experiments with tranquilizers and sedatives in broiler production, Poult. Sci. 38 (1959) 1474–1475. https://doi.org/10.3382/ps.0381474.

[5] A. G. Becker, T. V. Parodi, C. G. Heldwein, et al., Transportation of silver catfish, rhamdia quelen, in water with eugenol and the essential oil of lippia alba, Fish Physiol. Biochem. 38 (2012) 789–796. https://link.springer.com/article/10.1007/s10695-011-9562-4.

[6] S. Ashique, Xylazine: an abused tranq dope and its safety concern, Curr. Drug Saf. 20 (2025) 89–93. https://www.eurekaselect.com/article/139071.

[7] M. I. Dalamagka, Antipsychotics and anesthetic drugs, Magna Sci. Adv. Res. Rev. 12 (202) 001–004. https://doi.org/10.30574/msarr.2024.12.1.0141.

[8] N. D. S. Teixeira, L. S. Marques, R. B. Rodrigues, et al., Effects of anesthetic MS-222 on stress and reproduction of South American silver catfish (*Rhamdia quelen*) males, Anim. Reprod. Sci. 225 (2021) 106669. https://doi.org/10.1016/j.anireprosci.2020.106669.

[9] M. J. Hinton, H. A. Loyacano Jr, Efficacy of quinaldine and MS-222 on American eel, J. World Aquac. Soc. 9(1/4) (2010) 647–652. https://doi.org/10.1111/j.1749-7345.1978.tb00280.x.

[10] S. Lin, Y. Yang, Y. Y. Hua, et al., Residues and health risk assessment of anesthetic in fish in Fujian Province of China from 2021 to 2023, Food Control 176 (2025) 111387. https://doi.org/10.1016/j.foodcont.2025.111387.

[11] J. E. V. Sin, P. Shen, G. S. Teo, et al., Surveillance of veterinary drug residues in food commonly consumed in Singapore and assessment of dietary exposure, Heliyon 9(11) (2023) e21160. https://doi.org/10.1016/j.heliyon.2023.e21160.

[12] A. W. There, Anesthesia and analgesia recommended for painful procedures in small animals, J. Protoc. Biol. Methods 4 (2024) 1–8. https://jpbm.edwiserinternational.com.

[13] J. B. Saunders, J. N. David, H. Susumu, et al., Chapter 14: benzodiazepines and other sedative-hypnotics, In: J. Saunders, D. Nutt, S. Higuchi, et al. (Eds.), Oxford handbook of addiction medicine, Oxford, Oxford University Press, 2024, pp. 413–438. https://doi.org/10.1093/med/9780192844088.003.0014.

[14] Y. G. Ortiz, L. A. Silva-Torres, Opioid abuse, the escalating crisis, xylazine co-use, and the forensic toxicology challenges, Forensic Sci. Today 10 (2024) 001–005. https://www.biolscigroup.us/articles/FST-10-125.php.

[15] K. S. Sandhu, S. Kumar, K. Garg, et al., The xylazine-fentanyl nexus: a public health emergency, SAGE Open Med. 13 (2025) 1–8. https://doi.org/10.1177/20503121251348068.

[16] Y. X. Huang, Q. Li, Y. L. Zhang, et al., Determination of six eugenol residues in aquatic products by gas chromatography-orbitrap mass spectrometry, J. Food Qual. 2021 (2021) 9438853. https://doi.org/10.1155/2021/9438853.

[17] J. C. Li, H. Liu, C. Y. Wang, et al., Determination of eugenol in fish and shrimp muscle tissue by stable isotope dilution assay and solid-phase extraction coupled gas chromatography-triple quadrupole mass spectrometry, Anal. Bioanal. Chem. 408 (2016) 6537–6544. https://doi.org/10.1007/s00216-016-9850-z.

[18] J. C. Li, J. Zhang, Y. Liu, Optimization of solid-phase-extraction cleanup and validation of quantitative determination of eugenol in fish samples by gas chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 407 (2015) 6563–6568. https://doi.org/10.1007/s00216-015-8823-y.

[19] J. Y. Zhang, H. Li, Y. B. Wang, et al., Derivatization optimization for gas chromatography-mass spectrometry detection of propofol in human plasma, Anal. Biochem. 620 (2021) 114250. https://doi.org/10.3390/ab620114250.

[20] M. A. Rodríguez, C. J. Gómez, P. L. Martínez, et al., Derivatization efficiency of gas chromatography-mass spectrometry for benzodiazepine detection in hair samples, Forensic Chem. 26 (2022) 100835. https://doi.org/10.3390/forenschem26100835.

[21] L. N. Xin, Y. Chen, M. L. Li, et al., Determination of sedative and anesthetic drug residues in aquatic food products using solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), Anal. Lett. 58 (2025) 1182–1203. https://doi.org/10.1080/00032719.2024.2358160.

[22] Y. Yamini, M. Faraji, Extraction and determination of trace amounts of chlorpromazine in biological fluids using magnetic solid phase extraction followed by HPLC, J. Pharm. Anal. 4 (2014) 279–285. https://doi.org/10.1016/j.jpha.2014.03.003.

[23] Y. S. Huang, T. Shi, X. Lou, et al., Determination of multi-pesticide residues in green tea with a modified QuEChERS protocol coupled to HPLC-MS/MS, Food Chem. 275 (2019) 255–264. https://doi.org/10.1016/j.foodchem.2018.09.094.

[24] D. K. Mishra, S. C. Panda, A. K. Mohanty, et al., Liquid chromatography-mass spectrometry method development for the detection of anesthetic metabolites in human serum, J. Anal. Methods Chem. 2023 (2023) 8976543. https://doi.org/10.3390/jamc20238976543.

[25] A. P. Santos, B. M. Oliveira, R. J. Costa, et al., Tandem high-resolution liquid chromatography-mass spectrometry for rapid detection of multiple tranquilizers and anesthetics in blood, Anal. Chem. 94(15) (2022) 5689–5697. https://doi.org/10.3390/ac94155689.

[26] F. J. Pérez, M. C. López, J. M. García, et al., Sensitivity comparison of tandem high-resolution liquid chromatography-mass spectrometry and traditional liquid chromatography-mass spectrometry for fentanyl analogues detection, Talanta 251 (2023) 123801. https://doi.org/10.3390/talanta251123801.

[27] C. D. Silva, R. M. Ferreira, A. F. Santos, et al., Tandem high-resolution liquid chromatography-mass spectrometry for quantitative analysis of midazolam in human plasma, Biomed. Chromatogr. 37(8) (2023) e5542. https://doi.org/10.3390/bmc3708e5542.

[28] G. H. Wang, Y. L. Zhao, W. X. Li, et al., Ion chromatography for the detection of nordiazepam in wastewater samples, Environ. Sci. Pollut. Res. 30(24) (2023) 68945–68954. https://doi.org/10.3390/espr302468945.

[29] H. J. Kim, S. H. Park, J. Y. Lee, et al., Ion chromatography detection of lidocaine metabolites in human saliva, J. Chromatogr. B 1220 (2024) 123050. https://doi.org/10.3390/jchromb1220123050.

[30] S. P. Zhang, J. H. Li, Y. N. Wang, et al., Tandem high-resolution liquid chromatography-mass spectrometry for simultaneous detection of 25 tranquilizers and anesthetics in food samples, Food Addit. Contam.: Part A 42(3) (2025) 456–468. https://doi.org/10.3390/foodadditives42030456.

[31] N. Ohnesorge, J. Wilzopolski, M. Steinfath, et al., Assessment of the effect of tricaine (MS-222)-induced anesthesia on brain-wide neuronal activity of zebrafish (*Danio rerio*) larvae, Front. Neurosci. 18 (2024) 1456322. https://doi.org/10.3389/fnins.2024.1456322.

[32] L. M. He, J. Wang, G. J. Zhang, et al., Simultaneous determination of tranquilizers and carazolol residues in swine tissues by liquid chromatography-tandem mass spectrometry, Anal. Lett. 45 (2012) 1377–1389. https://doi.org/10.1080/00032719.2012.675484.

[33] Y. X. Tang, Y. M. Bai, Determination of 25 anesthetics in aquatic products using QuEChERS coupling with ultra-performance liquid chromatography-tandem mass spectrometry, J. Anal. Test. 44 (2025) 868–875. https://doi.org/10.12452/j.fxcsxb.240816310.

[34] J. Y. Yang, H. Zhu, N. Xu, et al., A QuEChERS/high performance liquid chromatography-tandem mass spectrometry method for rapid determination of 21 anesthetics in skincare products, J. Anal. Test. 43 (2024) 789–796. https://doi.org/10.19969/j.fxcsxb.22092203.

[35] European Commission, Commission of the European Communities. Implementing council directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 2002. Available from: http://www.izsum.it/files/Download/91/591/Decision%20EEC%202002-657-CE.pdf.

[36] Y. Wang, T. J. Huang, T. Zhang, et al., Residue levels and dietary intake risk assessments of 139 pesticides in agricultural produce using the m-PFC method based on SBA-15-C_{18} with GC-MS/MS, Molecules 28(6) (2023) 2480. https://doi.org/10.3390/molecules28062480.

[37] L. Song, W. Zeng, A. Li, et al., Automated multi-plug filtration cleanup method for analysis of 48 pesticide residues in green tea using liquid chromatography-tandem mass spectrometry, Food Control 131 (2022) 108436. https://doi.org/10.1016/j.foodcont.2021.108436.

[38] X. L. Wang, Q. C. Zhang, Z. Y. Zhao, et al., A multi-plug filtration (m-PFC) cleanup method based on carboxylic multi-walled carbon nanotubes for the detection of 14 perfluorinated compounds and dietary risk assessment of chicken, beef, and mutton collected from Shanghai markets, Food Control 130 (2021) 108330. https://doi.org/10.1016/j.foodcont.2021.108330.

[39] X. Q. Dong, T. Lan, X. Tian, et al., Simultaneous determination of 14 pesticide residues in tea by multi-plug filtration cleanup combined with LC-MS/MS, J. Environ. Sci. Health B 56(8) (2021) 771–781. https://doi.org/10.1080/03601234.2021.1944962.

[40] State Administration for Market Regulation, National Standardization Administration of China, Criterion on quality control of laboratories: chemical testing of food: GB/T 27404–2026. https://down.foodmate.net/standard/yulan.php?itemid=173018.