Screening for Illegally Added Drugs in Self–Formulated Feeds Using High–Resolution Mass Spectrometry

Xing Qianwen[1], Xa Cui[2], Zhao Fang[2], Cui Shasha[2], Wu Liyong[2], Zhang Liang[3], Li Yandong[1*], Han Xue[2*]

1. Hebei Provincial Station of Veterinary Drug and Feed, Shijiazhuang 050035, China; 2. College of Food and Biology, Hebei University of Science and Technology, Shijiazhuang 050018, China; 3. Hebei Provincial Animal Husbandry Station, Shijiazhuang 050035, China

Abstract **[Objective]** The paper aimed to effectively reduce the occurrence of bacterial resistance associated with breeding practices and to mitigate food safety risks by controlling the illegal use of veterinary drugs in self–formulated feed at the source. **[Method]** A screening database comprising 274 illegally added chemical drugs in self-formulated feed was established utilizing ultra-performance liquid chromatography coupled with quadrupole/electrostatic field orbitrap high-resolution mass spectrometry (HPLC-Q-Exactive Focus/MS). Subsequently, 253 batches of self-formulated feed samples from various farms in Hebei Province were screened and quantitatively analyzed. **[Result]** The screening results indicated the presence of 8 pharmaceutical components across 10 batches of self-formulated feed samples, with a detection rate of 3.2% and concentrations ranging from 0.06 to 28 851.8 μg/g. **[Conclusion]** The application of high-resolution mass spectrometry is feasible and highly significant for the risk monitoring of illegally added drugs in self-formulated feed.

Keywords High-resolution mass spectrometry; Self-formulated feed; Illegal addition

With the rapid advancement of intensive breeding models, the number of practitioners utilizing self-formulated feed is increasing steadily. Feed company technicians and self-media channels have disseminated knowledge regarding premixes and highlighted the benefits and features of self-formulated feed. When combined with the practical experience of industry professionals, this has contributed to the growing adoption of self-formulated feed. Self-formulated feed primarily entails combining purchased premixes or concentrated feeds with raw feed materials such as corn and bran. This approach is characterized by its simplicity, low processing costs, and operational flexibility, making it highly favored among farmers. However, some farmers, aiming to increase economic gains and reduce expenses, utilize substandard raw materials, which leads to inconsistent quality in self-formulated feed[1–2]. Among these, certain unscrupulous breeders incorporate veterinary drugs intended for the prevention and treatment of animal diseases, as well as prohibited substances, into the feed to mask potential adverse reactions in livestock and poultry resulting from the inferior quality of the raw materials themselves[3]. To enhance the supervision of the breeding process, promote rational and scientific breeding practices, and further reduce the illegal addition of veterinary drugs in self-formulated feed, the Ministry of Agriculture and Rural Affairs of the People's Republic of China issued Announcement No.307. This announcement explicitly stipulates that when farmers produce self-formulated feed for daily use, they are prohibited from adding veterinary drugs other than those permitted for use in commercial feed, such as anticoccidials and traditional Chinese medicinal drugs[4]. However, owing to the diverse forms of self-formulated feed, some feed preparers exhibit limited legal awareness and insufficient understanding of relevant laws and regulations. Consequently, illegal and irregular practices persist, including drug abuse, unauthorized addition of veterinary drugs, and even the incorporation of prohibited substances during the feed preparation process, posing significant challenges to the regulatory oversight conducted by feed management authorities[5–6].

In this study, a database comprising 274 drugs was established using ultra-performance liquid chromatography coupled with quadrupole/electrostatic field orbitrap high-resolution mass spectrometry (HPLC-Q-Exactive Focus/MS). This database was utilized to investigate the presence of illegally added drugs in self-formulated feed products, identify the specific drugs and illegal additives present, and analyze the potential causes or sources of their occurrence. The findings aim to provide technical support for subsequent regulatory supervision.

1 Sample Source

The samples were obtained from multiple breeding enterprises located in Hebei Province. Detailed information regarding the feed samples is provided in Tab.1.

Received: 2025–07–29 Accepted: 2025–08–16
Supported by the Earmarked Fund for Hebei Agriculture Research System (HBCT2024260407).
*Corresponding author. E–mail: 2379112966@qq.com

Tab.1 Information of collected samples

Collection site	Sample type	Collection quantity batch
Langfang, Hebei	Self-formulated feed for fattening pigs	2
Baoding, Hebei	Self-formulated feed for laying hens, self-formulated feed for livestock and poultry	24
Xingtai, Hebei	Self-formulated feed for laying hens, self-formulated feed for broilers	30
Cangzhou, Hebei	Self-formulated feed for laying hens at youth period, self-formulated feed at egg laying period	31
Hengshui, Hebei	Self-formulated feed for laying hens	21
Tangshan, Hebei	Self-formulated feed for laying hens	38
Qinhuangdao, Hebei	Self-formulated feed for livestock and poultry	15
Shijiazhuang, Hebei	Self-formulated feed for laying hens	52
Handan, Hebei	Self-formulated feed for broilers, self-formulated feed for fattening pigs	23
Zhangjiakou, Hebei	Self-formulated feed for laying hens	10
Chengde, Hebei	Self-formulated feed for laying hens	7

2 Materials and Methods

The analytical method initially developed by the research group[6] was adopted and suitably modified to enhance its sensitivity and accuracy to fulfill the requirements for qualitative and quantitative analysis. The specific details were as follows.

2.1 Instrument UltiMate 3000-Q-Exactive Focus system was utilized, equipped with an Accucore RP-MS chromatographic column (100 mm×2.1 mm, particle size 2.6 μm) manufactured by Thermo.

2.2 Sample pretreatment A sample weighing (2.00±0.01) g was added 10 mL of 50% acetonitrile aqueous solution. The mixture was vortexed at 2 500 rpm for 3 min, followed by ultrasonic extraction for 30 min, and then centrifuged at 12 000 rpm for 10 min. Subsequently, 1 mL of the supernatant was collected and diluted with 9 mL of 50% acetonitrile aqueous solution. This solution was vortexed thoroughly for 3 min, after which 1 mL of the supernatant was filtered through a 0.22 μm microporous membrane prior to mass spectrometry analysis.

2.3 Chromatographic conditions Chromatographic separation was performed using an Accucore RP-MS column (100 mm×2.1 mm, particle size 2.6 μm) maintained at 30 ℃. The mobile phases consisted of 0.05% formic acid in water (phase A) and methanol (phase B). The flow rate was set at 0.3 mL/min, and the injection volume was 5 μL. The gradient elution conditions are detailed in Tab.2.

2.4 Mass spectrometry conditions The mass spectrometry analysis was conducted using an electrospray ionization (ESI) source, with simultaneous monitoring of positive and negative ions (ESI^+ and ESI^-). The scanning mode employed was a first-stage full scan/data-dependent second-stage mass spectrometry scan (Full MS/$ddMS^2$). The primary source parameters were set as follows: sheath gas flow rate 9 arb, spray voltage 3 kV, capillary (ion transport tube) temperature 320 ℃, S-lens voltage 55 kV, and auxiliary gas heating temperature 30 ℃. The primary scanning mode employed in mass spectrometry was Full MS, with a scanning range (m/z) of 100–1 200, a primary resolution of 70 000, an ion capacity of 1×10^6 entering the C-Trap, and a maximum ion injection time of 100 ms. The secondary scanning mode utilized was $ddMS^2$, featuring a secondary resolution of 35 000 and a variable excitation time ranging from 3 to 9 s. The stepped collision energies were set at 20, 40, and 60 kV, with a dynamic exclusion duration of 8 s. Prior to data acquisition, mass axis calibration was performed using both positive and negative ion correction solutions.

Tab.2 Elution gradient of the mobile phase

Time//min	Volume ratio of mobile phase	
	Phase A	Phase B
0	98	2
2.5	95	5
5.8	90	10
6.5	85	15
7.5	80	20
9.5	70	30
10.5	60	40
12	55	45
14	45	55
18	5	95
21	5	95
22.5	98	2
25	98	2

2.5 Qualitative and quantitative methods The samples underwent Full MS/$ddMS^2$ scanning, and target compounds were identified by comparing accurate mass number, retention time, daughter ions, and isotope abundance against those in the established screening database. When the peak time deviation of the unknown substance was within ±0.5 min, the parent ion response value was 10^5 or higher, the precise mass number deviation was within 5 ppm, the daughter ion response value was 10^4 or higher, the precise mass number deviation was within 5 ppm, and the isotope abundance ratio was within 70%, it could be concluded that the sample contained illegally added drugs. During quantitative analysis, the total ion current chromatogram was extracted based on the exact mass number of the primary parent ion, and the chromatographic peak corresponding to the target compound was integrated. Subsequently, quantification was conducted using single-point calibration.

3 Results and Analysis

3.1 Establishment of the screening database A mixed standard solution was prepared, and analysis was conducted according to the established chromatographic conditions (Section **2.4**) and mass spectrometry parameters (Section **2.5**). The exact mass number of each compound was determined using the high-resolution mass spectrometry data analysis software, Xcalibur. When $ddMS^2$ was employed in conjunction with dynamic exclusion mode, secondary data were automatically acquired once the response intensity of the parent ions in the list reached the predetermined threshold. The target was subjected to collisions at three different collision energies (20, 40, and 60 eV), and the secondary spectra were obtained by summation. The exact mass number of the primary secondary fragments and the retention time of the compounds were determined using Xcalibur software. Subsequently, the Chinese and English names, molecular formulae, retention time, precise mass number, and fragment ion mass number of all identified compounds were imported into TraceFinder 4.0 software to construct the screening database. The database information is presented in Tab.3.

3.2 Screening results of actual feed samples The experiment involved the selection of 253 batches of self-formulated feed samples collected during the breeding process for screening and analysis. Among these, 10 batches tested positive for 8 different drugs, resulting in a detection rate of 3.2%. The identified substances included monensin, tiamulin, albendazole, albendazole sulfone, albendazole oxide, tilmicosin, amoxicillin, and zearalenone, with concentrations ranging from 0.06 to 28 851.8 μg/g, as detailed in Tab.4.

Among the detected drugs, monensin is an anticoccidial agent that is effective in preventing and treating coccidiosis in domestic animals, including chickens and calves[7]. According to Announcement No.307 issued by the Ministry of Agriculture and Rural Affairs of China, monensin is classified as an insecticidal agent permitted for inclusion in self-formulated feed[4]. In this study, samples containing monensin were identified as self-formulated feed for laying hens, with a detected concentration of 25.6 μg/g. This concentration is below the recommended dosage range of 90–110 g of monensin per 1 000 kg of feed, as specified in Announcement No.665 issued by the Ministry of Agriculture and Rural Affairs of China[8]. The analysis of the cause is twofold: first, farmers incorporated low levels of monensin into their self-formulated feed as a preventive measure; second, the feed became contaminated with monensin residues originating from other samples processed on the same production line during the mixing procedure. Except for monensin, the substances detected in the screening, including tiamulin, albendazole, tilmicosin, and amoxicillin, are antibacterial and anthelmintic drugs that are prohibited from being added.

In addition to the detection of veterinary drugs, this screening also identified the mycotoxin zearalenone. Zearalenone is commonly present in mold-contaminated wheat, corn, and other grains. The use of grains contaminated with zearalenone as feed for livestock and poultry can adversely affect their health and pose safety risks to consumers[9]. The zearalenone content detected in this screening was 13.5 μg/g, which exceeds the maximum limit of 500 ng/g established by the GB 13078–2017 *Feed Hygiene Standards*[10]. It is hypothesized that this exceedance may result from the use of grains contaminated with zearalenone as raw materials in the production of self-formulated feed, or from the storage of these feed products in humid conditions, thereby facilitating zearalenone contamination. Consequently, it is imperative for farmers to rigorously regulate the quality of feedstuffs and to store the finished feed in an appropriate environment.

4 Discussion

China exhibits a substantial demand for meat, eggs, and dairy products, which consequently results in significant feed requirements for livestock farming, with a wide variety of feed types available[11]. Among livestock farmers, particularly those operating on a larger scale, self-formulated feed are commonly preferred due to their high flexibility, functioning as a crucial supplement to commercial feed. The utilization of self-formulated feed offers two primary advantages. Firstly, it enables farmers to reduce expenses substantially by eliminating costs related to packaging, transportation, and storage. Secondly, its flexible application permits farmers to adjust the feed composition promptly in response to the growth status of their livestock, thereby enhancing production efficiency. Concurrently, the extensive and flexible use of self-formulated feed presents substantial challenges for regulatory oversight, particularly due to the difficulty in effectively controlling the improper use of veterinary drugs. This situation poses a considerable risk to the quality and safety of livestock and poultry products[12–13]. Effectively controlling the illegal use of veterinary drugs in self-formulated feed at the source, reducing the emergence of bacterial resistance originating from the breeding stage, minimizing food safety risks, and preventing harm to human health at the end of the food chain constitute a novel challenge for regulatory authorities[14].

Currently, the primary techniques for detecting veterinary drugs in feed are liquid chromatography and liquid chromatography-tandem mass spectrometry. These methods involve relatively complex detection procedures, including intricate sample pretreatment and limited detection targets. The development and application of high-resolution mass spectrometry screening technologies have addressed these limitations. By utilizing the high resolution and mass accuracy of this equipment, it is possible to simultaneously analyze hundreds of compounds and conduct non-targeted drug screening[15]. This study employed HPLC-Q-Exactive Focus/MS to screen for drugs

Tab.3 Database information

No.	Drug name	Retention time//min	Parent ion m/z	Daughter ion 1//m/z	Daughter ion 2//m/z	No.	Drug name	Retention time//min	Parent ion m/z	Daughter ion 1//m/z	Daughter ion 2//m/z
1	Ractopamine hydrochloride	10.77	302.175 1	107.049 51	164.106 96	138	Haloperidol	15.07	376.147 4	123.024 23	165.070 98
2	Salbutamol hemisulfate	5.95	240.159 4	148.075 70	91.055 01	139	Azaperol	10.22	330.197 6	149.076 14	121.076 20
3	Terbutaline sulfate	5.45	226.143 8	152.070 63	125.059 94	140	Azaperone	11.20	328.182 0	165.071 06	121.076 26
4	Cimaterol	4.71	220.144 4	116.049 91	143.060 41	141	4-Aminoantipyrine	7.20	204.113 1	105.935 00	189.089 80
5	Clenbuterol hydrochloride	11.23	277.086 9	132.068 34	168.044 83	142	4-Methylaminoantipyrine	6.83	218.128 8	97.076 53	56.050 37
6	Tulobuterol hydrochloride	12.09	228.115 0	118.065 47	154.041 76	143	Acepromazine maleate	15.52	327.152 6	254.062 88	239.075 45
7	Penbutolol	16.91	292.227 1	236.164 43	74.060 83	144	Carbadox	11.40	263.077 5	130.052 80	231.051 16
8	Propranolol hydrochloride	14.02	260.164 5	116.107 30	183.080 46	145	Chlorpromazine	16.84	319.103 0	246.013 61	86.097 07
9	Clorprenaline	10.80	214.099 3	154.041 81	196.088 87	146	Dapsone	9.93	249.069 2	92.050 05	108.044 76
10	Bromchlorbuterol hydrochloride	11.75	321.036 3	246.963 20	168.044 89	147	Desoxycarbadox	14.44	231.087 7	199.061 43	143.060 44
11	Brombuterol	12.15	364.985 8	211.994 40	290.912 60	148	Flunixin meglumine	18.23	297.084 5	264.050 38	279.073 79
12	Isoxsuprine hydrochloride	12.49	302.175 1	284.164 40	107.049 57	149	Imidocarb	9.01	349.177 1	162.102 54	188.081 82
13	Mabuterol hydrochloride	12.35	311.113 3	237.039 92	217.033 78	150	Isometamidium chloride	12.53	460.224 4	312.149 32	298.144 62
14	Mapenterol hydrochloride	13.25	325.128 9	237.039 90	217.033 78	151	Olaquindox	5.44	264.097 9	212.081 85	143.060 52
15	Bambuterol	12.77	368.218 0	294.144 50	72.045 20	152	Propionylpromazine hydrochloride	16.53	341.168 2	58.066 03	86.097 05
16	Formoterol	12.00	345.180 9	121.065 00	149.096 10	153	Valnemulin hydrochloride	17.20	565.367 0	164.073 94	263.142 33
17	Zilpaterol	5.65	262.155 0	185.070 90	202.097 40	154	Diminazene aceturate	7.68	282.146 2	135.079 20	119.060 60
18	Cimbuterol	7.46	234.160 1	160.086 90	216.149 60	155	Zearalanone	17.93	321.169 7	161.059 74	205.085 21
19	Clenproperol	9.89	263.071 2	132.068 30	168.044 80	156	Zearalenone	18.01	319.154 0	185.059 81	175.075 23
20	Ritodrine	8.85	288.159 4	150.091 40	121.065 00	157	2,4-Dimethylaniline	7.68	122.096 4	107.073 37	105.070 34
21	Clencyclohexerol	9.32	319.097 5	203.013 60	301.086 60	158	Amantadine	10.38	152.143 4	79.055 03	135.116 90
22	Salmeterol	16.98	416.279 5	91.054 80	380.258 30	159	Cyproheptadine hydrochloride	15.92	288.174 7	96.081 33	191.085 54
23	Labetalol	11.12	329.186 0	91.054 80	162.054 90	160	3-Ethoxyaniline	7.56	138.091 3	109.052 60	110.060 50
24	Arformoterol	14.31	345.180 9	118.065 40	150.091 40	161	N-Acetyl dapsone	11.96	291.079 8	198.021 93	156.011 32
25	Phenylethanolamine A	11.87	345.180 9	327.170 59	149.096 21	162	Nitrovin hydrochloride	14.48	361.089 1	58.040 90	222.062 47
26	Benzoyl sulfonamide	12.18	277.064 1	156.011 31	108.044 78	163	Rifamycin S	18.85	696.301 5	123.080 76	151.075 55
27	Sulfacetamide	3.87	215.048 5	156.011 37	92.050 06	164	Rimantadine hydrochloride	13.85	180.174 7	81.070 55	163.148 03
28	Sulfachloropyridazine	10.83	285.020 8	108.044 78	92.050 05	165	Apraclonidine hydrochloride	4.27	245.035 5	174.090 19	209.059 02
29	Sulfaclozine	12.83	285.020 8	92.050 05	108.044 87	166	Brimonidine	6.18	292.019 2		212.093 05
30	Sulphadiazine	6.00	251.059 7	108.044 75	156.011 31	167	Clonidine hydrochloride	7.08	230.024 6	186.982 45	212.997 92
31	Sulfadimethoxypyrimidine	13.22	311.080 9	156.076 78	108.044 78	168	Tizanidine hydrochloride	6.40	254.026 2	209.988 14	185.989 03
32	Sulfamethazine	6.61	279.091 0	124.087 07	204.043 73	169	Pentadecafluorooctanoic acid	18.72	412.965 3	218.985 60	118.991 07
33	Sulfadoxine	11.61	311.080 9	108.044 80	156.011 32	170	Perfluorooctanesulfonic acid	19.22	498.929 1	79.955 76	98.954 22
34	Sulfaguanidine	1.40	215.059 7	156.011 25	108.044 76	171	Ceftiofur	13.82	524.036 3	241.039 00	125.004 51
35	Sulfamerazine	8.54	265.075 4	108.044 81	156.011 34	172	Cefalexin	10.55	348.101 3	158.027 02	174.054 89
36	Sulfamethoxydiazine	11.35	281.070 3	126.06 64	108.044 90	173	Cefaclor	14.9	332.070 0	175.037 67	191.032 71
37	Sulfamethizole	10.06	271.031 8	156.011 34	108.044 79	174	Cefapirin	8.60	424.063 2	292.057 16	152.016 46
38	Sulfamethoxazole	11.20	254.059 4	108.044 76	156.011 37	175	Cephradine	11.06	350.116 9	192.046 50	160.041 80
39	Sulfamethoxypyridazine	10.46	281.070 3	108.044 75	92.050 01	176	Cefetamet pivoxil	16.54	512.126 8	241.039 29	398.058 65
40	Sulfamonomethoxine	9.75	281.070 3	156.011 28	108.044 75	177	Cefotaxime	10.77	456.063 7	396.042 60	167.027 20

Tab.3 Database information

No.	Drug name	Retention time// min	Parent ion m/z	Daughter ion 1// m/z	Daughter ion 2// m/z	No.	Drug name	Retention time// min	Parent ion m/z	Daughter ion 1// m/z	Daughter ion 2// m/z
41	Sulfamoxole	11.86	268.075 0	108.044 78	113.071 27	178	Cephalonium	9.15	459.079 14	152.016 50	123.055 70
42	Sulfaphenazole	12.77	315.091 0	158.071 27	156.011 35	179	Doxycycline hyclate	13.82	445.160 50	410.123 26	427.150 09
43	Sulfapyridine	7.99	250.064 5	108.044 82	156.011 38	180	Metacycline hydrochloride	13.34	443.144 90	426.118 23	201.054 57
44	Sulfaquinoxaline	13.54	301.075 4	156.011 32	108.044 80	181	Oxytetracycline dihydrate	10.60	461.155 50	426.118 04	337.070 19
45	Sulfathiazole	7.62	256.020 9	156.011 34	108.044 79	182	Tetracycline hydrochloride	10.38	445.160 50	428.133 85	321.075 04
46	Sulfadimidine	9.98	279.091 0	149.023 32	156.011 29	183	Amoxicillin trihydrate	4.73	366.111 80	208.042 77	114.001 10
47	Sulfisoxazole	9.86	268.075 0	113.071 27	108.044 81	184	Ampicillin	11.06	350.116 90	106.065 56	192.042 69
48	Trimethoprim	9.72	291.145 2	123.066 75	230.116 20	185	Chlortetracycline	12.52	479.121 60	462.095 12	154.049 93
49	Diaveridine	9.19	261.134 6	123.066 87	245.103 58	186	Piperacillin	15.36	518.170 40	143.086 17	160.042 83
50	Ormethoprim	10.55	275.150 3	123.066 84	259.119 23	187	Pipemidic acid	9.32	304.140 40	286.128 51	215.093 41
51	Cinoxacin	12.8	263.066 2	189.029 34	245.056 06	188	Tenoxicam	13.05	338.026 40	121.039 88	95.060 92
52	Ciprofloxacin	10.72	332.140 5	288.150 60	245.108 46	189	Promethazine hydrochloride	15.44	285.142 00	198.037 20	240.084 38
53	Decoquinate	19.82	418.258 8	372.216 55	204.029 07	190	Sodium cloxacillin monohydrate	16.93	458.054 79	182.024 28	299.018 74
54	Difloxacin hydrochloride	11.38	400.146 7	299.098 85	356.156 59	191	Triamcinolone	14.56	395.186 44	147.080 23	225.126 91
55	Enoxacin	10.26	321.135 7	234.103 64	206.071 90	192	Penicillin G	11.89	335.106 01	160.042 60	176.070 50
56	Enrofloxacin	10.92	360.171 8	316.181 61	245.108 31	193	Hydroxymetronidazole	4.10	158.056 00	55.042 60	140.045 50
57	Fleroxacin	9.86	370.137 3	269.089 45	326.147 28	194	Benximidazole	7.48	164.045 50	118.052 90	134.047 60
58	Flumequine	15.61	262.087 4	244.076 90	220.040 18	195	5-Chloro-1-methyl-4-nitroimidazole	7.37	162.006 50	116.972 20	145.003 90
59	Gatifloxacin	11.89	376.166 7	261.103 06	289.134 22	196	2-Methyl-5-nitroimidazole	2.88/4.99	128.045 50	98.048 00	111.043 10
60	Gemifloxacin mesylate	12.91	390.157 2	328.120 12	372. 148 32	197	Mequindox	10.83	219.076 42	143.060 20	185.070 80
61	Lomefloxacin Hydrochloride	11.03	352.146 7	265.114 69	308.156 68	198	Thioridazine	17.31	371.161 02	126.127 90	98.096 90
62	Marbofloxacin	9.72	363.146 3	72.081 52	320.103 88	199	Perphenazine	17.52	404.155 80	171.148 90	143.117 80
63	Moxifloxacin hydrochloride	12.46	402.182 4	261.103 30	384.171 70	200	Furantoitone	5.31	325.114 26	100.076 10	252.097 60
64	Nalidixic acid	15.13	233.092 1	187.050 22	215.081 41	201	Nitrofurantoin	9.12	237.025 45	152.008 60	124.002 60
65	Nequinate	18.82	366.170 0	334.143 59	201.042 04	202	Furazolidone	9.25	226.045 85	95.037 00	122.011 10
66	Norfloxacin	10.48	320.140 5	233.108 35	276.150 67	203	Furancilin	9.08	197.030 53	124.026 20	150.029 20
67	Ofloxacin	10.29	362.151 1	261.103 24	318.161 04	204	Sulindac	17.56	357.095 52	233.075 80	340.092 10
68	Orbifloxacin	11.15	396.153 0	295.105 07	352.163 02	205	Tolmetin	17.53	258.112 47	119.049 30	166.049 90
69	Oxolinic acid	13.42	262.071 0	216.029 27	244.060 27	206	Indometacin	18.67	358.084 06	138.994 50	174.091 20
70	Pefloxacin mesylate	10.32	334.156 1	290.166 20	316.144 99	207	Meloxicam	17.34	352.042 03	115.032 60	141.011 50
71	Sarafloxacin hydrochloride	11.61	386.131 1	342.141 14	299.098 88	208	Mefenamic acid	19.32	242.117 56	224.106 60	209.083 30
72	Sparfloxacin	12.20	393.173 3	292.125 37	149.050 93	209	Piroxicam	15.20	332.069 95	95.060 80	121.039 70
73	Danofloxacin	10.95	358.156 1	340.161 14	82.065 83	210	Ketoprofen	17.59	255.101 57	209.096 00	105.033 90
74	Trenbolone	17.65	358.156 1	340.161 14	82.065 83	211	Diflunisal	18.47	249.035 78	205.045 70	185.039 00
75	Testosterone	18.17	271.169 3	253.158 49	199.111 83	212	Carprofen	8.34	272.047 28	228.057 50	226.041 90
76	Methyltestosterone	18.48	289.216 2	97.065 31	109.065 19	213	Flufenamic acid	19.09	280.058 00	236.067 34	216.060 96
77	Nandrolone	17.87	303.231 9	97.065 35	109.065 22	214	Indoprofen	16.85	282.112 50	236.107 07	218.096 60
78	Megestrol acetate	18.62	275.200 6	109.065 20	257.189 85	215	Salsalate	17.52	257.044 40	187.038 85	155.048 39
79	Prednisone	15.98	385.237 3	325.216 09	267.174 50	216	Tolfenamic acid	19.37	260.047 30	216.056 58	214.041 14
80	Prednisolone	16.00	359.185 3	341.175 75	147.080 41	217	Flunixin meglumine	18.24	297.084 45	279.074 22	264.050 84

Tab.3 Database information

No.	Drug name	Retention time// min	Parent ion m/z	Daughter ion 1// m/z	Daughter ion 2// m/z	No.	Drug name	Retention time// min	Parent ion m/z	Daughter ion 1// m/z	Daughter ion 2// m/z
81	Hydrocortisone	16.39	361.201 0	343.189 15	147.080 51	218	Diclofenac	18.62	294.008 30	250.017 7	214.041 4
82	Methylprednisolone	17.20	363.216 6	121.065 03	147.116 81	219	Nabumetone	17.99	229.122 30	170.700 0	128.000 0
83	Dexamethasone	17.09	375.216 6	161.095 98	121.064 93	220	Tylosin	16.16	916.526 40	174.112 5	145.086 2
84	Betamethasone	6.11	393.207 2	147.080 41	355.190 37	221	Erythromycin	16.25	734.468 50	158.117 7	576.373 3
85	Cortisone	16.39	393.207 2	112.112 50	345.202 30	222	Lincomycin	9.54	407.221 00	126.127 9	359.217 7
86	Flumethasone	16.95	361.201 0	163.111 76	121.064 97	223	Tiamulin	15.77	494.329 90	119.016 4	192.105 2
87	Triamcinolone acetonide	17.37	411.197 7	121.065 11	253.122 30	224	Tilmicosin	13.92	869.573 30	174.112 5	132.102 1
88	Sodium nifurstylenate	17.41	435.217 7	339.159 06	397.199 95	225	Roxithromycin	17.25	837.531 90	158.100 0	116.200 0
89	Beclomethasone	17.28	258.039 7	108.019 91	184.051 71	226	Methandrostenolone	18.12	301.216 21	121.064 9	283.205 3
90	Chlormadinone acetate	18.59	409.177 6	279.174 13	337.180 02	227	Stanozolol	19.09	329.258 74	81.045 5	121.101 4
91	Cortisone acetate	17.28	405.182 7	345.161 47	309.184 33	228	Dehydroepiandrosterone	18.29	289.216 21	253.149 7	213.163 8
92	Estradiol benzoate	19.76	403.211 5	163.111 68	343.190 49	229	Epiandrosterone	18.73	291.231 90	105.070 8	145.101 8
93	Fludrocortisone acetate	17.23	377.211 1	105.033 76	77.039 29	230	Memantine	14.57	180.174 68	163.148 0	107.086 0
94	Flurogestone acetate	17.59	423.217 7	239.142 88	325.181 40	231	Oseltamivir	14.68	313.212 18	166.086 3	208.096 7
95	Norgestrel	18.31	407.222 8	225.163 62	267.174 22	232	Imiquimod	13.00	241.144 77	185.082 2	186.065 9
96	Medroxyprogesterone acetate	18.65	313.216 2	245.190 17	109.065 25	233	Clindamycin	14.51	425.187 15	126.127 9	377.183 5
97	Melengestrol acetate	18.70	387.253 0	285.220 98	327.231 69	234	Pirlimycin	14.25	411.171 50	112.112 4	363.167 5
98	Nandrolone phenylpropionate	19.88	397.237 3	337.216 25	279.174 41	235	Dienestrol	16.58	265.122 31	249.090 7	235.075 3
99	Norethisterone	17.87	407.258 1	105.070 32	257.189 94	236	Monensin	20.26	693.418 43	461.287 1	479.298 2
100	Progesterone	18.82	299.200 6	109.065 15	231.174 09	237	Salinomycin	20.61	773.481 03	431.240 2	531.328 9
101	Fludrocortisone	16.42	315.231 9	109.065 20	97.065 33	238	Narasin	20.97	787.496 69	431.240 0	531.328 9
102	Testosterone propionate	19.51	381.207 2	239.142 80	267.173 30	239	Lasalocid	20.34	613.371 10	377.266 0	359.255 7
103	2-Aminoflubendazole	13.23	256.088 1	123.024 26	108.044 70	240	Nigericin	20.89	747.465 40	697.426 8	501.317 7
104	5-Hydroxymebendazole	13.18	298.118 6	266.092 32	220.088 03	241	Maduramicin ammonium	20.36	934.573 40	431.200 0	531.400 0
105	5-Hydroxy thiabendazole	8.95	218.038 3	191.027 44	147.055 04	242	Virginiamycin M1	17.28	526.254 78	355.129 0	337.118 1
106	Albendazole	16.61	266.095 8	234.069 43	191.014 70	243	Oxymetholone	19.40	333.242 40	99.044 4	279.210 6
107	Albendazole-2-aminosulfone	8.84	240.080 1	198.033 19	133.063 49	244	Danazol	18.95	338.211 50	91.054 7	148.081 0
108	Albendazole sulfone	13.35	298.085 6	266.059 23	224.012 33	245	Androsta-1,4-diene-3,17-dione	17.25	285.184 90	121.064 9	151.111 8
109	Albendazole oxide	12.78	282.090 7	208.017 47	240.043 53	246	Dexamethasone acetate	17.87	435.217 70	147.080 3	237.127 2
110	Cambendazole	13.85	303.091 0	217.054 21	261.043 98	247	Diphenhydramine	14.48	256.169 60	167.085 3	165.069 9
111	Dimetridazole	5.92	142.061 1	95.060 84	81.045 40	248	Carbamazepine	16.25	237.102 20	194.096 2	192.080 5
112	Fenbendazole	17.76	300.080 1	268.053 77	159.042 66	249	Levamisole	7.46	205.079 40	146.096 4	178.068 2
113	Flubendazole	16.72	314.093 5	282.067 08	123.024 24	250	Trifluoperazine	18.10	408.171 60	113.107 7	70.066 0
114	Ipronidazole	12.02	170.092 4	124.099 72	109.076 37	251	Isothipendyl	14.31	286.137 20	241.079 3	199.032 6
115	Mebendazole	16.28	296.103 0	264.076 78	105.033 98	252	Levomepromazine	16.44	329.168 20	58.066 0	100.112 6
116	Aminomebendazole	12.87	238.097 5	77.039 81	105.033 94	253	Fluphenazine sulfoxide	14.65	454.177 10	266.024 4	143.118 0
117	Metronidazole	5.04	172.071 7	128.045 55	82.053 23	254	Midazolam	14.25	326.085 50	291.116 7	290.063 4
118	Hydroxymetronidazole	3.22	188.066 6	123.055 46	126.029 95	255	Flurazepam	14.31	388.158 60	89.060 3	133.086 1
119	Oxfendazole	14.47	316.075 0	159.042 50	191.032 49	256	Triflupromazine	17.40	353.129 40	58.066 0	86.097 0
120	Fenbendazole sulfone	14.98	332.070 0	300.043 64	159.042 69	257	Dioxopromethazine	12.17	317.131 80	86.097 0	71.073 7

Tab.3 Database information

No.	Drug name	Retention time// min	Parent ion m/z	Daughter ion 1// m/z	Daughter ion 2// m/z
121	Oxibendazole	13.97	250.118 6	218.092 36	176.045 39
122	Ronidazole	5.47	201.061 8	140.045 44	55.042 58
123	Thiabendazole	9.77	202.043 3	175.032 41	131.060 35
124	Tinidazole	8.47	248.070 0	121.032 01	128.045 62
125	Triclobendazole	19.04	358.957 4	343.933 87	273.996 12
126	Chloramphenicol	13.05	321.004 0	152.034 21	257.033 75
127	Florfenicol	11.33	355.992 1	185.026 84	335.986 97
128	Florfenicol amine	1.50	248.075 1	230.064 44	129.994 98
129	Thiamphenicol	9.30	353.996 4	185.026 90	227.037 75
130	Quinoxaline-2-carboxylic acid	11.06	175.050 2	129.044 95	104.050 05
131	3-Methylquinoxaline-2-carboxylic acid	11.09	189.065 9	145.075 97	143.060 41
132	Diclazuril	18.47	404.970 7	333.971 25	334.971 25
133	Clopidol	7.82	191.997 7	174.031 62	101.015 72
134	Dinitolmide	10.4	224.030 2	181.024 49	77.038 03
135	Ethopabate	14.67	238.107 4	206.081 19	136.039 41
136	Nicarbazin	18.10	301.056 8	137.034 23	107.036 15
137	Carazolol	12.61	299.175 4	116.107 24	222.091 26
258	Oxychlorpromazine	13.52	335.097 90	58.066 00	86.097 10
259	Metopimazine	13.94	446.156 70	141.102 20	126.091 50
260	Hydroxyzine	16.47	375.183 40	201.046 60	166.077 70
261	Cetirizine	16.98	389.162 60	201.046 50	166.077 70
262	Diazepam	17.51	285.078 90	154.041 80	193.088 70
263	Perphenazine sulfoxide	13.75	420.150 70	143.117 90	231.998 00
264	Promethazine	15.74	285.142 00	86.097 00	198.037 30
265	Promethazine sulfoxide	11.85	301.136 90	86.097 00	198.037 40
266	Droperidol	13.43	380.176 90	165.071 00	123.024 20
267	Doxycycline	13.76	445.160 54	428.134 22	98.060 61
268	Oxytetracycline hydrochloride	10.57	461.155 45	426.118 32	201.054 70
269	Demeclocycline	11.39	465.105 91	448.079 62	289.026 49
270	Eprinomectin	19.75	936.507 90	352.172 90	490.277 20
271	Ivermectin	20.50	897.497 00	183.062 90	329.208 60
272	Doramectin	20.14	921.497 00	353.209 30	183.063 10
273	Quinocetone	15.78	307.107 71	131.049 30	143.060 50
274	Dihydropyridine	15.78	254.138 68	208.096 90	108.081 20

in self-formulated feed samples collected throughout the province. Among 253 batches of self-formulated feed products analyzed, illegal additions of tiamulin, albendazole, albendazole sulfone, albendazole oxide, tilmicosin, and amoxicillin were detected. Albendazole sulfone and albendazole oxide, recognized as metabolic derivatives of albendazole [16], were excluded from the list of illicit additives. The analysis further indicated that excessively high concentrations of albendazole produced strong instrument signal responses, which interfered with detection accuracy. This phenomenon warrants careful consideration in future experimental designs. Another possibility is that the feed contained metabolites originating from other feedstuffs, which necessitates further testing for confirmation. Additionally, screening revealed the presence of two or more veterinary drugs in self-formulated feed products, indicating that multiple veterinary drugs were likely added concurrently during the breeding process. Regulatory authorities ought to enhance guidance and professional training for livestock farmers within their jurisdictions, thereby promoting scientifically grounded and environmentally sustainable farming practices. Concurrently, these authorities should intensify risk assessments concerning the presence of illegal drug additives in self-formulated feed, implement targeted supervision and inspection programs, and impose stringent penalties on farmers who unlawfully incorporate veterinary drugs into feed. Such measures will contribute to the establishment of standards and regulations governing the environmentally responsible use of self-formulated feed in the livestock farming industry.

5 Conclusions

This study developed a database comprising 274 drugs utilizing HPLC-Q-Exactive Focus/MS and subsequently screened 253 batches of self-formulated feed samples against this database. The screening results indicated that 10 batches contained 8 distinct drugs, corresponding to a detection rate of 3.2%. The identified compounds included monensin, tiamulin, albendazole, albendazole sulfone, albendazole oxide, tilmicosin, amoxicillin, and zearalenone, with concentrations ranging from 0.06 to 28 851.8 μg/g. The screening results demonstrate that this method effectively accomplishes rapid, high-throughput, non-targeted screening of illegal drug additives in self-formulated feed. It offers robust technical support for the supervision of feed product quality and serves as a valuable reference for governmental decision-making.

Tab.4 Screening results of actual feed samples

Sample No.	Sample source	Sample type	Drugs	Effects	Drug content//μg/g
ZPL4	Handan, Hebei	Self-formulated feed for fattening pigs	Tiamulin	Antimicrobial	1 376.2
ZPL16	Shijiazhuang, Hebei	Self-formulated feed for laying hens	Monensin	Anticoccidial	25.6
ZPL19	Qinhuangdao, Hebei	Self-formulated feed for pigs	Albendazole, albendazole sulfone, albendazole oxide	Deworming	14.2, 0.06, 0.6
ZPL21	Qinhuangdao, Hebei	Self-formulated feed for cattle	Albendazole, albendazole sulfone, albendazole oxide	Deworming	185.8, 1.7, 164.6
ZPL36	Cangzhou, Hebei	Self-formulated feed for laying hens	Tiamulin	Antimicrobial	262.3
ZPL41	Xingtai, Hebei	Self-formulated feed for laying hens	Tiamulin	Antimicrobial	340.5
ZPL42	Xingtai, Hebei	Self-formulated feed for laying hens	Tiamulin	Antimicrobial	5 808.4
ZPL48	Baoding, Hebei	Self-formulated feed for livestock and poultry	Tilmicosin, amoxicillin	Antimicrobial	28 851.8, 7.4
ZPL49	Baoding, Hebei	Self-formulated feed for livestock and poultry	Tilmicosin, amoxicillin	Antimicrobial	707.3, 9.7
ZPL50	Baoding, Hebei	Self-formulated feed for laying hens	Zearalenone	Mycotoxin	13.5

References

[1] Li SP, An CY, Liu KZ, *et al*. Current status and recommendations for self-mixed feed for Taihang chickens [J]. Northern Pastoral, 2021 (21): 20–22.

[2] Xiao MM, Li HC. Problems and countermeasures in the supervision of self-made feed [J]. China Animal Health Inspection, 2021, 38(6): 48–51.

[3] Wen W. Thoughts on reducing feed costs in pig farms: analysis of the advantages and disadvantages of full price feed and self made feed[J]. Animals Breeding and Feed, 2021, 20 (5): 70–71.

[4] The Ministry of Agriculture and Rural Affairs of the People's Republic of China. No. 307 of Bulletin[J]. Gazette of the Ministry of Agriculture and Rural Affairs of the People's Republic of China, 2020(7): 125.

[5] Zou XR, He GC, Jia XX, *et al*. Determination of 33 veterinary drugs in feed by liquid chromatography tandem mass spectrometry [J]. Journal of Green Science and Technology, 2023, 25 (10): 146–156.

[6] Huo NN, Li YD, Huo HL, *et al*. Screening of illegal additives in compound premix feed by high resolution mass spectrometry [J]. China Feed, 2023(1): 105–113.

[7] Zhu JH, Liu XN, Wu XH, *et al*. Research progress of Monensin in ruminants [J]. Journal of Domestic Animal Ecolog, 2022, 43(4): 8–13.

[8] The Ministry of Agriculture and Rural Affairs of the People's Republic of China. No. 665 of Bulletin[J]. Gazette of the Ministry of Agriculture and Rural Affairs of the People's Republic of China, 2023(6): 82–89.

[9] Huang ZW, Zhao PP, Zhang H, *et al*. Investigation on the contamination of zearalenone in different plant-based feedstuffs [J]. Animals Breeding and Feed, 2024, 23(6): 45–48.

[10] GB 13078 –2017. Hygienical standard for feeds [S]. Beijing: China Agricultural Press, 2017–10–14.

[11] Kenjeric, Lidija. Extention and interlaboratory comparison of an LC-MS/MS multi-class method for the determination of 15 different classes of veterinary drug residues in milk and poultry feed [J]. Food Chemistry, 2024, 449:138834.

[12] Qian S. Analysis of common veterinary drugs and their detection techniques in feed [J]. Livestock and Poultry Industry, 2020, 31(2): 17.

[13] Liu XF. Study on the detection of multiple veterinary drug residues in pig feed and muscle by LC QqTOF MS method [D]. Hunan Agricultural University, 2018.

[14] Boti, Vasiliki. Target and suspect screening approaches for the identification of emerging and other contaminants in fish feeds using high resolution mass spectrometry[J]. Environmental Research, 2024, 251: 118739.

[15] Wang Y, Huang YF, Han F, *et al*. Screening 175 veterinary drugs in fishery feed by ultra-high performance liquid chromatography-orbitrap high resolution mass spectrometry [J]. Chinese Journal of Analysis Laboratory, 2018, 37(9): 1013–1019.

[16] Liu Y, Gu BB, Wang XT, *et al*. Determination of Albendazole and its metabolites residues in poultry eggs by high performance liquid chromatography-quadrurpole/electrostatic field orbitrap high-resolution mass spectrometry [J]. Modern Food Science and Technology, 2024, 2(7): 1–9.