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Multi-Residue Determination of 244 Chemical Contaminants in Chicken Eggs by Liquid Chromatography-Tandem Mass Spectrometry after Effective Lipid Clean-Up

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Abstract: In this study, we aimed to establish a multi-residue analytical method for the simultaneous detection of chemical contaminants in eggs. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS), we developed an analytical method that can separate 244 compounds (including β-agonists (25), imidazole and benzimidazoles (31), sulfonamides (22), antihistamines (10), β-lactam (5), insecticides (7), quinolones (24), non-steroidal anti-inflammatory drugs (13), and steroidal hormones (38)) within 30 min. A new enhanced matrix removal-lipid (EMR-Lipid) material was used as a purified sorbent in the QuEChERS clean-up method. Excellent linearity ($r > 0.9905$) was achieved. Additionally, recoveries ranged between 51.33% and 118.28%, with repeatability (RSD_r) and reproducibility (RSD_{wR}) in the range of 1.01–14.22% and 1.08–14.96%, respectively. In all of the compounds, low limits of quantification (LOQs) ≤ 5 μg kg^{-1} were found. Meanwhile, the detection limit (CCα) and detection capability (CCβ) were 1.88–40.60 μg kg^{-1} and 2.85–407.19 μg kg^{-1}, respectively. In conclusion, the evaluated method was shown to provide reliable screening, quantification, and identification of 244 multi-class chemicals in eggs and was successfully applied in real samples.

Keywords: eggs; multi-residue; EMR-Lipid; LC-MS/MS

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1. Introduction

Chemical contaminant residues in agricultural products are one of the most serious problems that can affect food safety and quality [1,2]. Usually, veterinary drugs are used at therapeutic levels or as food additives to maintain animals' health and promote animal growth [3]. For example, antibiotics such as quinolones are widely used by farmers against the growth of various microorganisms [4]. Other families of veterinary drugs, such as anthelmintics [5] and coccidiostats [6], can be used to kill or inhibit the growth of microscopic protozoan parasites. Furthermore, the fipronil egg scandal, which occurred in the European Union in 2017, showed that pesticides could be detected in animal-derived foods [7]. The use of these products has been shown to enhance the risk of residues in tissues or eggs and create potential hazards for human health, such as drug resistance and allergies, poisoning, carcinogenicity, or teratogenicity through the paths of the environment and the food chain [8–10]. Many researchers have focused on the development of detection methods for poultry tissue such as muscle, liver, and eggs (Table S1). Bilandzic et al. developed a method to detect 178 pesticides in eggs using C18 sorbent (C18) and primary secondary amine (PSA) as clean-up sorbents and obtained good accuracy [11]. Dasenaki et al. developed a multi-residue method for the determination of 115 veterinary drugs in eggs. For over 80% of the compounds, the recoveries were between 50% and 120%. However, the time taken for sample preparation was too long because the lipids and remaining

proteins needed to be precipitated at 23 °C for 12 h [3]. Ping et al. used a method for the simultaneous determination of 169 veterinary drugs in chicken eggs, and their results did not suggest that pesticides or other chemical contaminants may exist in eggs [12]. To summarize, all of these methods require a pretreatment procedure that is relatively tedious, they can be quite time-consuming, and they have low detection sensitivity for some analytes. Additionally, using these methods, it is not possible to provide a technical guarantee of the quick and efficient identification of a plurality of mixed pollutants such as pesticides, veterinary drugs, and illegal additives that may exist in eggs at present. Additionally, these methods cannot meet the requirements of quality and safety supervision under new guidelines [13]. Hence, the development of an efficient and universal analytical method is important to ensure the quality and safety of eggs and to protect consumers' health.

At present, the methods used for multi-residue detection in animal-derived foods are mainly liquid chromatography-tandem mass spectrometry (LC-MS/MS) [14,15], liquid chromatography–time of flight mass spectrometry (LC-TOF/MS) [16,17], as well as gas chromatography-mass spectrometry (GC-MS) [18,19]. Gasification must be performed when analyzing samples using GC-MS; thus, the detection of thermal instability, polarity, and macromolecular compounds is limited. Comparatively, LC-MS/MS combines the advantages of chromatography and mass spectrometry and has dramatically increased sensitivity and selectivity, making it a more general and powerful method for qualitative and quantitative analysis. Additionally, it has been widely used for the detection of chemical contaminants in animal-derived foods. For example, the method used by Jadhav et al. [20] was shown to simultaneously detect 78 drugs and 238 pesticides, including penicillins, quinolones, and tetracyclines, in bovine milk using ultra-fast liquid chromatography-tandem mass spectrometry (UFLC-MS/MS). Meanwhile, Jamie et al. developed an accurate quantitative analytical method to examine the mycotoxin, hormone, and fat-soluble vitamin content in hen egg yolks using a Shimadzu LCMS-8040 (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) triple quadrupole instrument [21]. LC-MS/MS can also be used for the detection of β-agonists in pork meat [22,23], as well as fipronil in chicken egg and muscle [24], multi-class antibiotic residues [25] and pesticides [26] in honey, and quinolones and tetracyclines in aquatic products [27,28]. All of these methods have shown relatively high accuracy and sensitivity. Additionally, these methods indicate that most analytes ranging from non-polar organochlorine pesticides to polar drugs are suitable for LC-MS/MS analysis. It is a relatively advanced and popular detection method worldwide.

In addition to advanced instruments, the sample pretreatment process is also a crucial link in the analysis of chemical contaminant residues, which can directly decrease the interference of impurities and improve the detection sensitivity and selectivity to obtain satisfactory analysis results. The QuEChERS methodology, introduced by Anastassiades et al. in 2003, is a green chemical extraction and clean-up method used for the detection of veterinary residues [29]. d-SPE sorbents are always used in clean-up methods. For example, C18 and PSA can be used to determine the levels of fipronil and its metabolites [30] and other pesticides in chicken eggs [31]. Zirconium-dioxide-based sorbents have been widely applied in the extract purification of fat-rich samples by different researchers [32–34].

In this work, different traditional d-SPE sorbents mixtures (C18, PSA, and ZrO_2), as well as enhanced matrix removal-lipid (EMR-Lipid), a novel selective sorbent for lipid removal [35], were compared in order to obtain a sensitive, high throughput, and reliable method for screening multiple drugs in eggs. The method covered a wide range of compounds, including veterinary drugs, pesticides, additives, and other drugs. The recovery and matrix effect was fully evaluated to select the optimal extract conditions. Linearity, limits of detection (LODs), quantification (LOQs), the detection limit (CCα), and detection capability (CCβ) analyses were also carried out for method validation according to the SANTE/11813/2017 [36] and the EU Commission Decision 2002/675/EC [37]. This method provides a powerful method for the Ministry of Agriculture and Rural Affairs to monitor a wide range of potential hazards and guarantee the quality and safety of agro-products in China.

2. Materials and Methods

2.1. Chemicals and Reagents

All standard solutions for chemical contaminants were purchased from Alta Scientific Co., Ltd. (Tianjin, China), which were configured, respectively, with concentrations of 100 mg L^{-1} according to the categories listed in Table S2. and stored at −20 °C. Working standard solutions needed to be prepared before use at the concentration of 5 mg L^{-1}, and they were prepared for each group of analytes by dissolving an appropriate amount of standard stock solutions in methanol.

High-performance liquid chromatography (HPLC)-grade acetonitrile (>99.5%) was supplied by Fisher Scientific (Fair Lawn, NJ, USA), and MS-grade methanol was obtained from Merck (Darmstadt, Germany). Formic acid (>96%) and ammonium acetate (>99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water was provided by a Milli-Q purification apparatus (Millipore Direct-Q UV, Bedford, MA, USA). Bond Elut dSPE Enhanced Matrix Removal EMR–Lipid (p/n 5982-1010) and Bond Elut EMR-Lipid Polish Pouch, anhydrous $MgSO_4$ only (p/n 5982–0102) were purchased from Agilent Technologies (Santa Clara, CA, USA).

2.2. Sample Preparation

Firstly, 5.00 g of homogenized egg sample were weighed (accurate to 0.01 g) into a 50 mL centrifuge tube, and 10 mL of acetonitrile solution containing 5% formic acid were accurately added. Then, the tissue was dispersed by shaking it manually (for approximately 10 s), and then it was vortexed (2000 rpm) for 5 min and centrifuged for 5 min (4000 rpm).

Then, 3.0 mL of 5 mM ammonium acetate solution were added to the 15 mL EMR-Lipid dSPE tube and were shaken for 2 min using a vortex to create an activated EMR-Lipid dSPE tube. A total of 7.0 mL of acetonitrile extract from the first step were transferred to the activated EMR-Lipid dSPE tube, and then they were mixed by shaking, vortexed for 2 min, and centrifuged for 5 min (4000 rpm). All of the supernatant was poured into an empty 50.0 mL centrifuge tube. Then, the EMR polish powder bag was added to the supernatant, which was shaken rapidly and vortexed for 2 min (2000 rpm), and then centrifuged for 5 min (4000 rpm). Finally, 1 mL of supernatant was drained using a disposable syringe and filtered through a 0.2 μm organic filter membrane into the sample vial for analysis.

2.3. LC-MS/MS Analysis

An Agilent 1290 Infinity II LC system (Agilent Technologies, Santa Clara, CA, USA) was used. A gradient elution program separated the analytes on a 150 × 3.0 mm, 1.8 μm Zorbax Eclipse XDB-C18 column (Agilent Technologies, Santa Clara, CA, USA). The elution solvents were 2 mM ammonium acetate in water containing 0.2% formic acid (A) and 0.2% formic acid in methanol (B) with the following gradient: initial, 5% B; 0–0.5 min, 5% B; 0.5–3 min, 15% B; 3–10 min, 40% B; 10–18 min, 100% B; 18–22 min, 100% B; 22–22.1 min, 5% B. The post-run time was 3 min. The column temperature was maintained at 40 °C, and the injection volume and the mobile phase flow rate were 2 μL and 0.4 mL min^{-1}, respectively. Typical chromatograms are shown in Figure 1.

MS spectrometry analysis was performed on an Agilent 6470 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The dynamic multiple reaction monitoring modes (DMRM) and fast real-time to switch electrospray ion source positive (ESI^+) and electrospray ion source negative (ESI^-) were used in order to ensure the correct number of compounds were detected in a single run while reducing its time. The instrument conditions were as follows: gas temperature, 250 °C; gas flow, 7 L/min; nebulizer gas, 35 psi; capillary voltage, 3.5 kV; sheath gas heater, 325 °C; sheath gas flow, 11 L/min. The fragmentor voltage and collision energy of each compound are presented in Table S2.

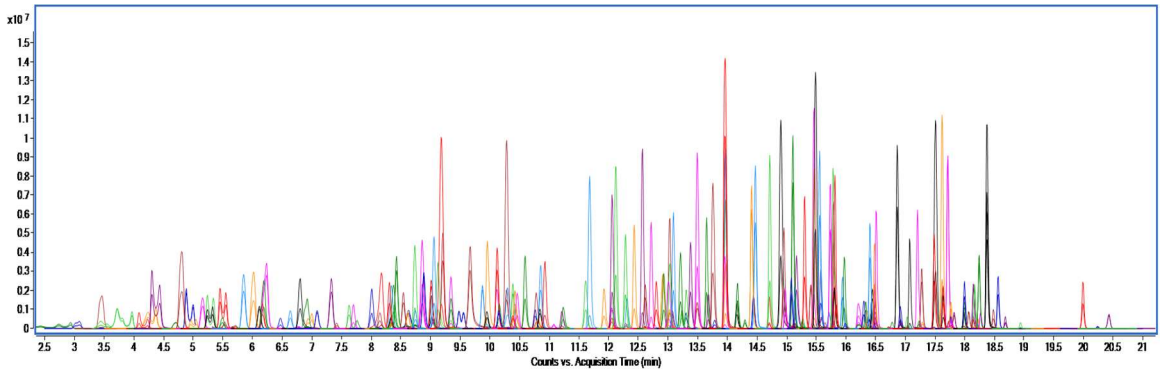


Figure 1. Typical chromatograms of 244 chemical contaminants in egg samples at the spiked concentration of 50 μg kg^{-1}.

2.4. Method Validation

An analyte was identified when the following parameters were achieved: (1) at least 2 product ions were detected, as defined by the SANTE/11813/2017; (2) the retention time of 2 product ions in the extract corresponded with the calibration standard within a tolerance of ±0.1 min; (3) the ion ratio between multiple reaction monitoring(MRM) 1 and MRM2 from sample extracts was within ±30% (relative) in accordance with the calibration standards. The selectivity was determined by analyzing blank samples from different resources, and the result indicated that there was no endogenous interference showing similar properties with analytes.

In addition, measured parameters including trueness, repeatability (RSD_r) and reproducibility (RSD_{wR}), linearity, the limit of quantitation (LOQ), the limit of detection (LOD), decision limit (CCα), detection capability (CCβ), and matrix effect (ME) were evaluated by conducting the recovery experiment at three concentrations (10, 50, and 100 μg kg^{-1}) with six replicates for each level on three separate days. A six-point calibration curve was constructed for all the compounds injected in duplicates for each batch. All of the results were in accordance with the guideline of SANTE/11813/2017 as well as the EU Commission Decision 2002/675/EC for validating methods, in which mean recoveries should be in the range of 70−120% with RSD_r of ≤20% and RSD_{wR} of ≤20%. The linearity of the calibration curves for each analyte was studied by injecting calibration solutions at concentrations of 0.1, 0.5, 1, 2, 5, 10, 25, 50, and 100 μg L^{-1} in both acetonitrile and matrix extracts. The matrix effect (ME) was calculated using the calibration curve of the solvent standard and matrix-matched standards. The calculation formula is as follows:

$$ME\% = (b_{matrix}/b_{solvent} - 1) \times 100\%$$

where b_{matrix} and $b_{solvent}$ are the slopes of the compound in the calibration curves of the matrix and solvent, respectively.

3. Results and Discussion

3.1. Optimization of Pretreatment Methods

3.1.1. Selection of Extract Solvent

To reduce the handing procedure and achieve compounds with high throughput, a universal extract method based on QuEChERS needed to be selected. Various factors can impact the efficiency of extraction. Based on previous reports, the acetonitrile extraction system has generally been used with a mixture of different amounts of water or formic acid (FA) [38–41]. The addition of water aids the extraction of hydrophilic compounds. As shown in our previous study, by using a mixture of MeOH/water (3:2, *v*/*v*) with 1% acetic acid, a maximum rate of recovery between 70% and 120% [42] was achieved in eggs. However, for multi-residue extraction, this method was also shown to cause the incomplete extraction of hydrophobic compounds as well as cloudy and foaming sample

extraction due to the low solubility of proteins and lipids in water. In addition, when appropriate formic acid or acetic acid was added to acetonitrile, the ionization efficiency was improved, and the recovery rate was also increased due to the weak alkalinity of most compounds. In this study, we investigated four extraction solutions, including 1% acetic acid in acetonitrile and 1%, 5%, and 10% formic acid in acetonitrile. Meanwhile, the number of compounds was compared with recovery rates in the range of 50–130% and 70–120% in the spiking level of 100 μg kg^{-1}, respectively.

As shown in Figure 2, when 1% acetic acid in acetonitrile and 1% formic acid (FA) in acetonitrile were used, there were 122 and 136 compounds with recovery rates between 70 and 120%, respectively. In particular, the recovery rates of quinolones that were extracted with formic acid in acetonitrile were higher than those extracted with acetic acid. Interestingly, there were no significant differences in the three ratios of formic acid in acetonitrile. The number of compounds with recovery rates between 50% and 130% was approximately equivalent in these three extracts. Especially when extracted with 5% formic acid in acetonitrile, the recovery rates of 158 compounds were between 51.74% and 105.66%, nearly 90% of them were ranged from 70.42% to 105.66%, which means that the extraction method that used 5% formic acid in acetonitrile was shown to enable the maximum number of compounds to meet the recovery range. Therefore, in this study, 5% formic acid in acetonitrile was chosen as an extract solvent.

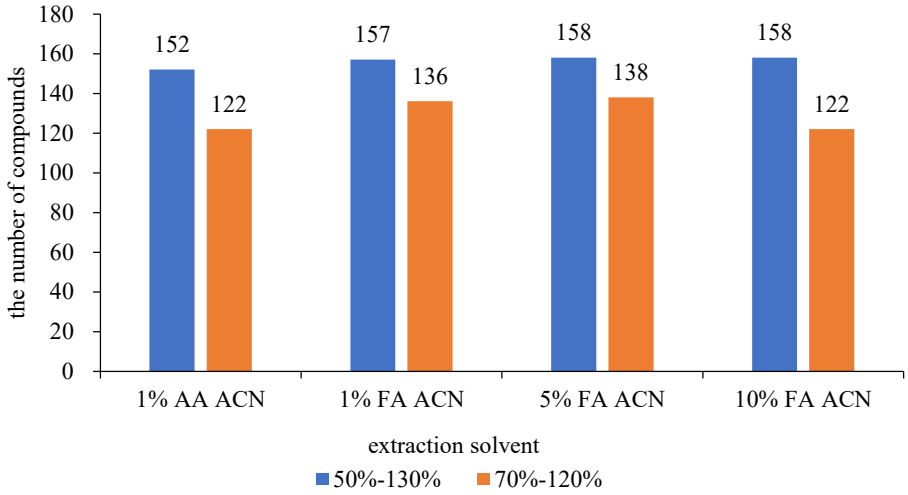


Figure 2. The number of compounds with recoveries between 50 and 130 (%) and 70 and 120 (%) using different extraction solvents. Abbreviations: AA: acetic acid, FA: formic acid, ACN: acetonitrile.

3.1.2. Selection of Clean-Up Method

To obtain a better recovery result for more compounds, the clean-up method is fundamental. Traditional QuEChERS methods use C18 and PSA as dispersive solid-phase extraction (d-SPE) clean-up sorbents and add different amounts of magnesium sulfate ($MgSO_4$) to remove water [15,43]. Research has shown that PSA can remove interferences such as fatty acids, organic acids, and pigments effectively, while C18 can reasonably remove non-polar components, such as lipids and waxes [44]. However, all of these d-SPE sorbents are non-selective, as their mechanisms are hydrophobic interactions between interferences and sorbents [35]. They not only trap and remove unwanted impurities but also cause the loss of hydrophobic analytes. Zirconia (ZrO_2) is another type of material that utilizes Lewis acid/base interactions to selectively retain fatty non-polar interferers. It was successfully used to analyze 30 pesticides in milk, which significantly reduced matrix interference [45]. Meanwhile, it was shown to retain analytes of interest such as tetracyclines, fluoroquinolones, and macrolides [46]. EMR-Lipid is an original chemistry sorbent. By combining size exclusion and hydrophobic interactions mechanism, it selectively removes straight, unbranched hydrocarbon chains and lipid-like molecules in fatty foods such as animal tissue and edible oil [19]. As these sorbents have different

characteristics, four clean-up combinations were compared to determine the most efficient clean-up method. The combinations were as follows: (A) 50 mg of ZrO_2 + 50 mg of C18, (B) 50 mg of PSA + 50 mg of C18, (C) 150 mg of $MgSO_4$ + 50 mg of PSA + 50 mg of C18, and (D) EMR-Lipid. The recovery rates were calculated, and the number of compounds per 10 percent interval was counted, starting from 50% to 130%, and the distribution of the different recovery rates of the compounds is shown in Figure 3.

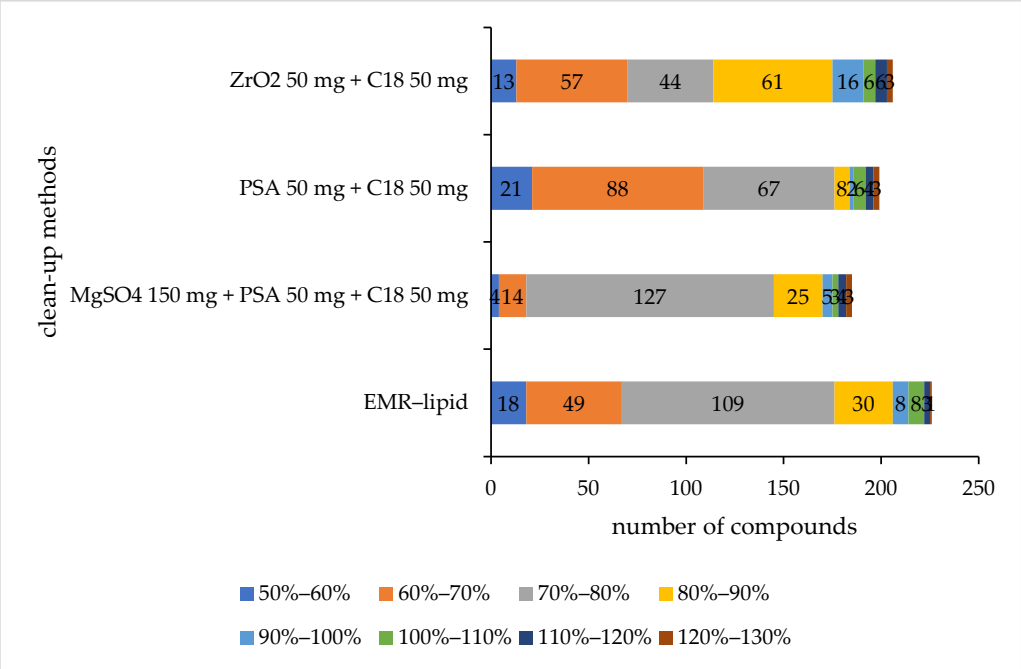


Figure 3. Number of compounds in recovery ranges with different clean-up methods.

Compared to the combination of 50 mg of PSA and 50 mg of C18, when $MgSO_4$ was added, the number of compounds with recovery rates between 70% and 120% was increased to 164. However, 90% of sulfonamides obtained a poor recovery rate that was below 50%, which means adding $MgSO_4$ can strongly affect the extraction of sulfonamides. This result is in accordance with the results of the study performed by Chunna Guo et al., which indicated that when d-SPE was used with a combination of PSA and C18, an obvious improvement in the recovery rates of sulfonamides was seen [47]. In addition, when ZrQ_2 combined with C18 instead of PSA was used for purification, there was a slight increase in recovery rates between 50% and 130%. Most importantly, recovery rates in the range of 70–120% (especially between 80% and 90%) were dramatically increased. When EMR-Lipid was used, recovery rates between 50% and 130% and 70% and 120% increased to a total number of 226 and 158, respectively. Additionally, all of the recovery rates of sulfonamides after clean-up with EMR were in the range of 67.51% to 91.69%, which was better than the combination of 50 mg of PSA and 50 mg of C18. Zhao's results also showed that the use EMR-Lipid cartridge clean-up for the analysis of 39 veterinary drugs in meat matrices could effectively absorb lipids in the matrix, thus improving recovery rates [35]. Above all, the results indicated that EMR-Lipid is an effective sorbent that can simultaneously enable multi-residue screening and obtain satisfactory quantitative results. Hence, EMR-Lipid was chosen as a clean-up sorbents for the following optimization.

After cleaning up with EMR-Lipid sorbent, it is essential to remove the redundant water. Thus the effects of EMR polish and sodium chloride (NaCl) on chemical contaminants were compared. As shown in Figure S1, in the spiked concentration of 100 μg kg^{-1}, the number of compounds used by EMR Polish with recovery rates of 50–130% (198) and 70–120% (179) were both higher than the number of compounds used by NaCl. So, the EMR Polish powder package was finally selected to achieve better results.

3.2. Method Validation

3.2.1. Matrix Effects

In this study, matrix inhibition and matrix enhancement were observed in different compounds in eggs (Figure 4). There were 203 compounds with matrix effects between −18.9% (budesonide) and 19.9% (pefloxacin), which indicated no noticeable matrix enhancement or inhibition; 8 compounds had strong matrix-enhancing effects, namely ampicillin, ciprofloxacin, desloratadine, flubendazole, maduramycin, nigericin, norfloxacin, and pirbuterol acetate. Among them, the maximum enhancement effect of desloratadine was 94.4%; 33 compounds had apparent matrix inhibition, mostly benzimidazoles, and the value was between −68.1% (Nimorazole) and −20.4% (Miconazole). The effect of the matrix could effectively be reduced by minimizing possible interference, improving the chromatographic separation ability, changing the strength of the mobile phase, and changing gradient conditions. Meanwhile, the use of internal standards can effectively correct the ion suppression (or enhancement) induced by co-eluting components present in the sample extracts and improve the quantitative accuracy. However, the internal standard is expensive, and this method involves too many different kinds of drugs; it is difficult to find a suitable internal standard that meets a large number of drug screening conditions. Therefore, matrix-matched calibration standards were used to equalize the response enhancement or exhibition for calibration standards and sample extracts.

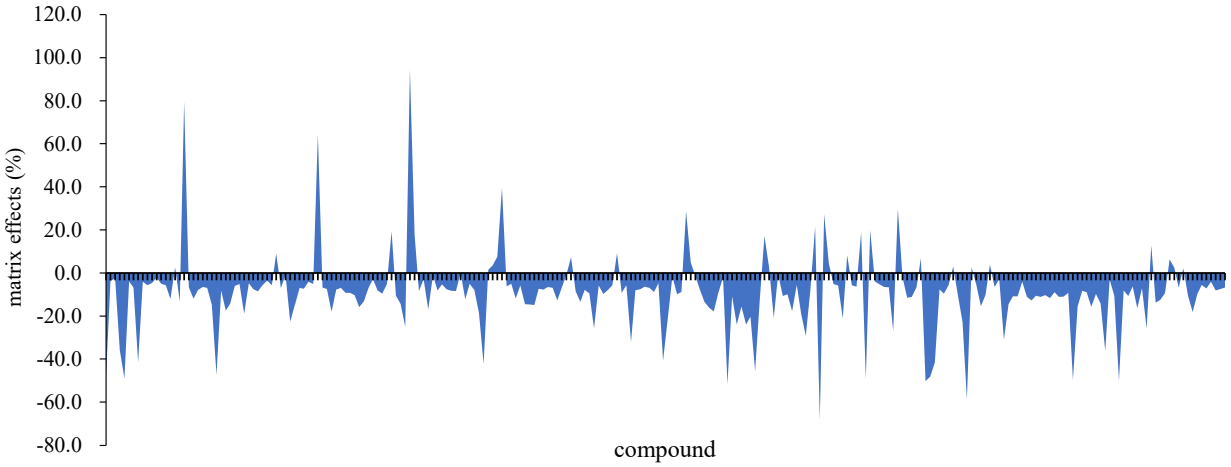


Figure 4. Matrix effects of 244 chemical contaminants in egg.

3.2.2. Linearity

The regression equation and the correlation coefficient were calculated according to the standard curve of the peak area value and the corresponding solution concentration ratio (μg L^{-1}). The matrix-matched standard curve showed excellent linearity in the entire range (0.1, 0.5, 1, 2, 5, 10, 25, 50, and 100 μg L^{-1}) and the Scorrelation coefficient (r) of all drugs was higher than 0.9905. The specific linear equations and correlation coefficients are shown in Table S3.

3.2.3. LOQs and LODs

The mixture of chemical contaminants was added to samples, and the fortified concentration was 0.2, 0.5, 1, 2, 5, 10, 25, 50, and 100 μg kg^{-1}. The pretreatment method described above was used for the sample for the LC-MS/MS analysis. The LODs and LOQs of this method were determined by the signal-to-noise (S/N) ratios of 3 and 10, with the recovery of LOQs in the range of 50% to 130%, respectively. As is shown in Table 1, the LODs and LOQs ranged from 0.2 to 2 μg kg^{-1} and 0.5 to 5 μg kg^{-1}, respectively. The LODs of 193 compounds were 0.2 μg kg^{-1}, and the LOQs of 162 compounds were 0.5 μg kg^{-1}. This method showed a high sensitivity for the determination of chemical contaminants.

Table 1. The results of method validation for determination of 244 chemical contaminants in egg.

Compound Name	Fortified Concentration (n = 6)									LOQ	LOD	CCα	CCβ
	10 μg kg^{-1}			50 μg kg^{-1}			100 μg kg^{-1}						
	Recovery	RSD_r	RSD_{wR}	Recovery	RSD_r	RSD_{wR}	Recovery	RSD_r	RSD_{wR}				
Unit	%									μg kg^{-1}			
2-Aminobenzimidazole	71.8	3.7	3.8	75.5	5.5	6.0	73.9	2.1	3.0	5.0	2.0	11.2	17.4
2-Mercaptobenzimidazole	54.7	9.8	11.7	58.2	9.4	11.5	56.4	7.0	10.4	5.0	2.0	16.1	27.2
2-Methyl-4 (5)-nitroimidazole	56.9	4.0	6.7	55.6	5.5	6.1	59.8	3.8	4.0	2.0	1.0	5.1	8.2
2-Methyl-5-nitroimidazole	58.8	1.6	1.7	61.0	2.2	2.4	64.4	1.5	1.6	0.5	0.2	12.7	24.9
3-Methoxytyramine (3-MT)	52.5	1.9	2.0	53.8	2.7	3.0	60.8	1.4	1.9	0.5	0.2	11.4	22.4
4-Acetamido antipyrine	72.3	2.5	3.2	75.0	4.0	6.4	71.5	2.3	2.4	0.5	0.2	15.5	30.5
4-Formylamino antipyrine	65.5	2.1	2.4	63.1	4.1	4.3	64.7	1.6	1.8	0.5	0.2	4.5	8.6
4-Nitroimidazole	97.0	2.0	2.5	97.6	4.7	5.9	93.1	6.0	8.6	0.5	0.2	22.9	45.3
4-Nitrophenol	69.8	9.1	9.2	65.9	6.7	6.7	64.9	3.3	4.0	5.0	2.0	11.6	18.1
5-Hydroxymebendazole	67.4	3.1	3.9	70.0	3.9	4.6	67.6	4.9	7.8	0.5	0.2	5.3	10.0
5-Hydroxy-thiabendazole	65.9	1.5	1.8	66.9	2.3	2.6	69.1	1.9	2.4	0.5	0.2	12.2	23.9
5-Nitrobenzimidazole	69.4	2.7	2.7	72.4	1.2	2.1	68.8	5.0	7.8	2.0	1.0	5.1	8.1
Albendazole	69.2	2.0	2.4	72.5	5.8	6.7	69.8	4.0	6.1	0.5	0.2	9.9	19.3
Albendazole sulfone	71.2	3.1	3.9	75.3	3.9	4.6	77.6	4.9	7.8	0.5	0.2	5.3	10.0
Alclomethasone dipropionate	73.0	3.7	4.1	73.5	3.6	4.8	73.0	3.8	4.3	0.5	0.2	18.7	36.9
Amantadine	76.2	2.0	2.5	75.6	3.9	5.3	75.7	3.8	4.0	0.5	0.2	14.7	28.9
Amcinonide	79.5	6.3	7.6	81.7	4.8	6.9	73.8	3.4	3.7	0.5	0.2	15.6	30.7
Ampicillin	58.4	8.5	11.1	53.8	10.2	11.2	53.3	7.6	9.3	5.0	2.0	12.9	20.9
Antipyrine	77.5	1.5	1.9	78.6	1.3	1.3	83.1	1.6	2.0	0.5	0.2	13.5	26.5
Azaperol	76.4	2.3	3.5	77.8	1.2	1.9	78.6	1.7	2.3	0.5	0.2	4.1	7.7
Azaperone	76.1	3.1	3.2	76.8	2.1	3.0	79.5	2.3	3.0	0.5	0.2	14.0	27.5
Bambuterol	86.8	1.9	2.2	84.4	1.9	2.7	87.2	1.4	1.8	0.5	0.2	8.1	15.6
Beclomethasone	73.4	4.5	6.1	73.9	3.5	4.0	69.2	3.3	6.2	1.0	0.5	14.7	28.3
Beclomethasone dipropionate	82.7	7.4	9.0	83.8	2.4	4.3	84.3	3.5	3.5	0.5	0.2	25.6	50.7
Benzimidazole	76.7	3.5	4.3	80.3	3.5	4.6	80.5	1.2	1.7	2.0	1.0	7.4	12.7
Betamethasone	76.8	3.4	3.8	75.7	3.7	3.9	77.1	2.6	2.7	0.5	0.2	18.7	36.8
Betamethasone valerate	86.2	5.7	7.4	71.8	6.6	9.7	76.1	4.4	4.9	0.5	0.2	4.4	8.3
Betamethasone dipropionate	85.3	2.8	3.1	81.5	4.0	4.4	80.7	3.1	4.0	0.5	0.2	16.2	31.9
Bromchlorbuterol	77.1	1.8	2.1	80.6	1.5	2.1	84.4	1.8	2.5	0.5	0.2	19.4	38.2

Table 1. *Cont.*

Compound Name	Fortified Concentration (n = 6)									LOQ	LOD	CCα	CCβ
	10 μg kg^{-1}			50 μg kg^{-1}			100 μg kg^{-1}						
	Recovery	RSD$_r$	RSD$_{wR}$	Recovery	RSD$_r$	RSD$_{wR}$	Recovery	RSD$_r$	RSD$_{wR}$				
Unit	%									μg kg^{-1}			
Brompheniramine	55.3	3.6	4.2	58.0	4.2	4.4	57.4	3.1	3.6	0.5	0.2	8.1	15.6
Budesonide	84.2	5.2	6.0	73.4	5.7	8.2	71.3	1.4	3.8	0.5	0.2	10.1	19.7
Cambendazole	65.7	2.6	3.1	68.1	2.7	2.8	67.3	5.0	7.5	0.5	0.2	5.9	11.3
Carazolol	86.3	1.7	1.8	87.8	2.7	3.3	86.0	1.1	1.6	0.5	0.2	3.3	6.1
Carbadox	104.2	4.0	5.9	107.8	4.2	6.0	108.1	2.5	3.1	1.0	0.5	12.5	24.1
Carbamazepine	76.1	1.1	1.3	74.6	2.6	2.8	81.1	1.3	1.8	0.5	0.2	4.9	9.3
Carbofuran	74.0	1.8	2.4	71.3	3.0	4.7	77.6	1.3	1.9	0.5	0.2	14.7	28.8
Cefapirin	104.4	7.0	7.4	91.3	11.9	12.4	110.7	3.3	4.7	2.0	1.0	14.6	27.2
Cefotaxime	101.6	6.7	8.1	101.4	7.1	9.0	105.4	1.9	3.1	5.0	2.0	15.9	26.8
Ceftiofur	72.8	5.2	5.7	76.6	6.7	7.2	83.4	10.0	12.4	2.0	1.0	8.7	15.3
Chlordimeform	70.9	4.5	4.9	75.3	3.4	4.4	80.5	3.7	5.5	2.0	1.0	7.7	13.4
Chlormadinone acetate	77.3	6.8	7.4	74.2	4.7	5.9	76.9	1.6	3.9	0.5	0.2	18.0	35.5
Chloroprocaine	72.1	2.0	2.0	71.6	2.3	2.8	72.4	1.3	1.4	0.5	0.2	13.9	27.4
Chlorpheniramine	58.3	2.6	3.2	53.9	4.5	5.8	55.0	1.5	2.8	0.5	0.2	7.9	15.2
Chlorpromazine	75.8	2.0	3.6	74.8	2.7	3.4	74.1	2.4	3.4	0.5	0.2	3.1	5.7
Cinchocaine	80.5	2.3	2.4	81.8	2.5	3.8	88.3	2.0	2.2	0.5	0.2	10.1	19.7
Cinoxacin	61.3	3.1	3.5	59.7	2.6	2.9	60.1	2.7	3.0	0.5	0.2	11.0	21.5
Ciprofloxacin	74.5	1.8	2.1	75.6	1.6	2.3	78.4	1.6	4.3	2.0	1.0	4.5	7.0
Clenbuterol	84.1	2.0	2.5	83.0	1.7	2.4	84.7	1.3	1.6	0.5	0.2	6.4	12.3
Clenbuterol hydroxymethyl	76.9	2.0	2.2	82.0	1.5	2.8	81.4	1.1	1.2	0.5	0.2	6.7	12.9
Clencyclohexerol	73.7	1.7	2.1	80.7	1.8	2.3	83.5	1.6	1.9	0.5	0.2	10.4	20.3
Clenhexerol	80.8	1.7	2.2	81.3	2.0	2.3	80.2	1.6	2.2	0.5	0.2	6.7	12.8
Clenisopenterol	76.8	2.6	3.2	77.9	2.5	3.3	76.7	1.8	2.0	0.5	0.2	5.2	9.8
Clenpenterol	78.9	3.9	4.6	83.1	1.1	2.6	83.3	1.2	1.9	0.5	0.2	8.6	16.6
Clenproperol	75.5	1.9	2.1	77.8	2.0	2.5	81.1	1.7	1.9	0.5	0.2	7.3	14.1
Clindamycin	72.1	4.1	4.9	71.2	3.5	4.3	73.2	1.1	1.2	0.5	0.2	7.8	15.1
Clobetasol 17-propionate	79.1	4.8	5.5	83.6	3.3	3.9	77.2	3.9	5.2	0.5	0.2	19.1	37.6
Clobetasone 17-butyrate	83.4	5.0	5.6	80.6	2.7	3.2	76.0	4.6	5.5	0.5	0.2	23.1	45.7
Clopidol	72.5	1.1	2.7	73.5	1.2	2.7	78.8	1.8	1.9	0.5	0.2	9.6	18.8
Clorprenaline	71.7	2.7	2.7	71.6	2.1	3.0	72.1	2.4	2.4	0.5	0.2	7.5	14.5
Cortisone	66.3	8.6	9.0	64.5	3.2	4.4	64.4	2.1	2.3	0.5	0.2	6.1	11.8
Coumaphos	96.1	1.6	1.7	91.2	4.5	6.9	83.9	4.3	5.8	0.5	0.2	7.0	13.5

Table 1. *Cont.*

Compound Name	Fortified Concentration (n = 6)									LOQ	LOD	CCα	CCβ
	10 μg kg^{-1}			50 μg kg^{-1}			100 μg kg^{-1}						
	Recovery	RSD_r	RSD_{wR}	Recovery	RSD_r	RSD_{wR}	Recovery	RSD_r	RSD_{wR}				
Unit	%									μg kg^{-1}			
Cyproheptadine	77.6	1.7	2.4	77.6	2.9	3.0	78.4	2.9	3.9	0.5	0.2	10.7	21.0
Danofloxacin	76.2	2.8	2.9	77.9	2.5	2.7	76.3	1.6	1.7	0.5	0.2	4.1	7.7
Dapsone	61.9	1.6	1.7	66.6	2.0	2.1	63.1	1.8	2.2	0.5	0.2	8.5	16.5
Deflazacort	82.9	3.9	4.8	79.0	2.8	3.6	80.7	4.7	5.3	0.5	0.2	25.1	49.7
Demeclocycline	58.2	5.8	6.1	56.9	9.7	10.3	67.3	7.3	9.1	5.0	2.0	18.6	32.2
Desloratadine	74.7	4.3	6.9	70.7	7.2	9.4	74.3	4.0	5.7	2.0	1.0	7.1	12.3
Desoxycarbadox	51.3	3.3	4.1	52.3	3.0	4.6	55.0	3.7	4.9	0.5	0.2	5.4	10.2
Dexamethasone	77.8	5.4	5.7	72.3	4.6	6.7	74.3	3.3	3.9	0.5	0.2	18.0	35.4
Dichlorvos	59.9	4.1	4.7	60.9	5.9	7.6	64.6	1.6	1.6	5.0	2.0	10.4	15.7
Diflorasone diacetate	71.5	5.3	6.3	77.0	6.0	8.5	80.2	4.4	5.5	0.5	0.2	6.7	13.0
Difloxacin	62.4	3.9	4.0	66.6	3.6	3.7	69.9	4.9	7.1	0.5	0.2	16.5	32.5
Dimetridazole	71.4	2.8	3.2	71.4	3.0	4.4	74.1	2.2	2.5	0.5	0.2	27.3	54.1
Diphenhydramine	83.6	1.0	1.7	83.1	2.6	2.9	82.2	1.3	1.6	0.5	0.2	4.4	8.3
Doxepin	76.8	5.3	6.5	80.2	3.8	4.4	79.9	7.5	11.1	2.0	0.2	10.2	18.4
Econazole	67.8	4.9	6.0	69.9	3.9	4.2	69.2	5.2	8.1	0.5	0.2	12.1	23.6
Enoxacin	73.5	1.6	1.6	73.2	3.7	5.6	72.7	1.4	1.9	5.0	2.0	9.0	13.1
Enrofloxacin	64.8	1.5	1.7	66.1	2.2	2.8	69.2	4.6	7.0	0.5	0.2	16.3	32.1
Epitestosterone	65.1	3.1	3.6	64.5	2.6	2.6	66.2	3.9	4.3	0.5	0.2	12.6	24.7
Eprinomectin	71.6	6.1	7.5	86.6	11.8	12.5	74.2	6.2	8.1	2.0	1.0	10.7	19.5
Febantel	85.7	1.3	1.7	83.5	3.7	5.1	76.3	4.2	5.7	0.5	0.2	4.5	8.5
Fenbendazole	56.4	2.9	4.2	58.4	4.3	6.3	59.2	3.6	4.0	0.5	0.2	8.3	16.0
Fenoterol	59.6	1.6	1.8	58.7	2.6	2.9	64.1	2.9	3.2	0.5	0.2	4.0	7.5
Fenthion sulfoxide	74.0	2.0	3.5	78.6	5.4	8.2	72.6	2.2	2.5	0.5	0.2	10.4	20.2
Fleroxacin	71.1	2.0	2.2	73.1	2.0	2.3	71.3	1.3	1.6	0.5	0.2	5.1	9.7
Florfenicol	82.9	7.8	10.4	81.3	14.2	15.0	87.5	10.0	10.1	5.0	2.0	20.2	35.4
Flubendazole	85.7	2.0	2.6	89.3	4.7	5.7	84.6	5.5	6.8	2.0	0.2	403.6	407.2
Fluconazole	64.9	3.9	4.7	69.3	6.1	6.7	68.7	6.2	7.1	2.0	0.2	3.0	4.0
Fludrocortisone 21-acetate	75.6	9.0	9.3	72.7	10.3	10.6	72.0	7.3	8.6	5.0	2.0	16.2	27.3
Fludroxycortide	70.4	8.7	10.1	71.0	4.9	6.1	70.2	2.5	3.0	2.0	1.0	16.9	31.8
Flumequine	62.6	2.8	3.3	63.2	2.3	2.4	63.0	2.5	3.3	0.5	0.2	4.9	9.2
Flumethasone	68.7	10.5	12.7	75.1	6.2	9.6	73.4	4.9	5.9	0.5	0.2	14.2	27.9
Flumethasone pivalate	76.7	4.9	5.7	86.2	4.3	5.2	81.6	4.0	4.5	0.5	0.2	9.4	18.3

Table 1. *Cont.*

Compound Name	Fortified Concentration (n = 6)									LOQ	LOD	CCα	CCβ
	10 μg kg^{-1}			50 μg kg^{-1}			100 μg kg^{-1}						
	Recovery	RSD_r	RSD_{wR}	Recovery	RSD_r	RSD_{wR}	Recovery	RSD_r	RSD_{wR}				
Unit	%									μg kg^{-1}			
Flunixin	71.0	1.3	1.4	74.5	3.2	4.0	75.8	4.4	5.4	2.0	1.0	3.6	5.2
Fluocinolone acetonide	66.6	5.4	6.8	73.1	4.2	5.7	68.0	6.7	8.5	1.0	0.5	8.8	16.6
Fluoromethalone	73.3	2.6	4.8	71.7	8.4	12.2	73.9	4.6	7.8	2.0	1.0	5.1	8.1
Fluoxetine	86.9	2.5	3.0	86.6	2.1	2.9	89.6	1.5	2.0	0.5	0.2	9.5	18.5
Fluphenazine	78.6	4.4	4.9	80.9	2.4	3.0	80.5	3.1	4.0	0.5	0.2	11.2	21.8
Fluticasone propionate	87.0	3.7	4.9	79.4	2.4	3.5	78.1	5.2	5.7	0.5	0.2	19.4	38.3
Formoterol	75.7	1.1	1.1	79.2	3.0	3.2	78.4	1.1	1.1	0.5	0.2	9.3	18.2
Gatifloxacin	56.0	3.5	4.3	53.6	3.2	3.6	56.6	2.4	3.2	0.5	0.2	4.5	8.6
Gemifioxacin	53.4	4.7	7.8	56.1	3.8	4.0	60.4	2.9	4.7	2.0	1.0	6.0	10.0
Griseofulvin	81.6	2.8	3.0	76.3	2.2	2.4	79.1	2.2	2.5	0.5	0.2	13.9	27.3
Halcinonide	73.3	9.8	10.5	70.8	4.7	6.2	70.1	3.7	4.7	0.5	0.2	9.4	18.2
Halofuginone	64.9	3.9	4.6	64.6	3.7	4.0	69.0	1.2	1.4	0.5	0.2	10.8	21.2
Haloperidol	77.7	1.2	1.2	79.5	2.6	2.7	76.8	1.5	2.2	0.5	0.2	6.9	13.4
2-Hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI)	70.0	1.9	2.3	72.8	4.0	5.1	79.6	2.0	3.7	2.0	0.2	3.0	4.1
Hydrocortisone	72.2	4.5	5.3	71.3	3.3	3.7	71.1	3.3	3.7	2.0	1.0	7.3	12.6
Hydroxy-ipronidazole	72.6	2.5	3.5	71.5	2.2	3.4	72.2	4.8	7.4	2.0	0.2	4.9	7.7
Hydroxyzine	75.3	3.2	4.5	75.0	2.5	2.9	75.6	1.7	2.4	0.5	0.2	9.2	18.0
Imipramine	77.4	2.7	3.0	80.0	2.1	3.8	80.9	2.1	2.6	0.5	0.2	5.9	11.2
Indoprofen	54.5	2.0	2.5	58.3	8.7	10.7	57.0	2.9	3.2	0.5	0.2	10.8	21.2
Ipronidazole	73.9	2.7	2.8	77.9	2.7	3.8	78.4	1.2	1.6	0.5	0.2	10.4	20.4
Isoxsuprine	84.4	1.2	1.3	86.5	1.8	2.8	87.3	1.5	1.5	0.5	0.2	7.6	14.8
Ivermectin	80.2	9.4	10.3	88.3	5.3	6.7	85.9	2.1	2.4	0.5	0.2	22.9	45.3
Ketoconazole	56.4	2.4	3.1	55.0	5.6	6.7	58.0	4.4	6.5	2.0	0.2	4.2	6.4
Ketoprofen	65.0	6.6	7.4	65.1	2.5	3.1	65.0	3.8	4.1	0.5	0.2	9.3	18.1
Ketotifen	83.3	2.2	2.4	81.2	2.7	3.0	81.6	1.1	1.8	0.5	0.2	8.4	16.3
Labetalol	72.6	2.0	4.0	72.2	3.2	3.3	79.1	2.1	3.1	0.5	0.2	7.8	15.1
Levamisole	78.4	2.8	2.9	82.9	1.7	2.6	84.4	1.9	2.3	0.5	0.2	5.7	11.0
Lidocaine/Diocaine	77.0	1.7	1.8	78.6	1.8	2.6	81.0	1.7	2.1	0.5	0.2	5.4	10.3
Lincomycin	71.3	1.8	2.4	72.5	6.4	8.4	81.3	10.6	13.1	0.5	0.2	55.1	60.2
Invisible malachite green (LMG)	69.8	1.8	2.1	67.5	4.5	5.9	69.1	5.6	7.8	2.0	0.2	4.3	6.7
Lomefloxacin	72.0	2.9	3.1	74.8	3.2	4.1	72.4	1.6	1.6	0.5	0.2	5.3	10.0
Loratadine	68.7	1.5	2.2	66.2	1.4	3.0	64.5	4.5	4.9	0.5	0.2	10.4	20.3

Table 1. *Cont.*

Compound Name	Fortified Concentration (n = 6)									LOQ	LOD	CCα	CCβ
	10 μg kg^{-1}			50 μg kg^{-1}			100 μg kg^{-1}						
	Recovery	RSD_r	RSD_{wR}	Recovery	RSD_r	RSD_{wR}	Recovery	RSD_r	RSD_{wR}				
Unit	%									μg kg^{-1}			
Lornoxicam	55.4	4.3	4.5	58.9	7.0	7.3	57.0	4.0	5.3	2.0	0.2	5.9	9.8
Maduramycin	82.9	11.9	11.9	92.0	4.7	7.1	86.9	3.3	3.5	0.5	0.2	21.9	43.4
Marbofloxacin	63.3	2.5	3.8	64.6	4.0	5.7	70.6	3.6	5.1	0.5	0.2	11.1	21.8
Mebendazole	64.7	2.8	3.1	68.1	5.1	6.3	67.1	5.1	7.7	2.0	0.2	5.0	7.9
Mebendazole-amine (HMEB)	61.3	1.7	2.4	64.8	5.2	7.1	62.1	4.0	6.0	2.0	0.2	4.4	6.8
Mefenamic acid	54.5	1.5	1.7	57.6	5.5	8.2	54.8	3.9	4.7	1.0	0.5	9.9	18.8
Megestrol acetate	74.4	3.2	3.6	77.6	2.0	2.9	77.3	5.8	6.8	0.5	0.2	20.6	40.6
Melengestrol acetate	81.2	3.2	3.7	75.4	2.3	2.8	73.3	4.9	5.2	0.5	0.2	15.0	29.6
Melitracene	82.6	1.5	2.9	77.5	3.4	3.6	77.2	2.8	3.7	0.5	0.2	11.2	22.0
Meloxicam	68.5	2.8	3.1	68.2	4.6	7.0	69.4	3.0	5.5	2.0	0.2	5.7	9.3
Metaproterenol	71.4	7.2	8.9	74.3	2.1	3.7	81.0	2.3	2.7	0.5	0.2	17.8	35.2
Methylprednisolone	74.6	3.8	4.6	76.6	4.3	5.1	75.9	3.1	4.0	0.5	0.2	25.6	50.7
Methylprednisolone 21-acetate	94.7	5.1	5.3	93.7	6.0	6.6	84.5	5.4	7.1	0.5	0.2	6.2	11.8
Methyltestosterone	71.3	4.0	4.2	74.8	4.9	7.7	78.9	6.2	7.1	0.5	0.2	13.4	26.2
Metronidazole	64.9	2.2	2.8	66.2	2.1	2.7	65.1	6.0	9.3	0.5	0.2	16.4	32.4
Miconazole	62.9	1.1	1.2	64.8	2.8	4.2	64.4	4.1	6.3	0.5	0.2	7.5	14.5
Hydroxy metronidazole (MNZOH)	55.2	4.2	4.7	58.1	2.5	2.7	61.2	5.7	8.8	2.0	1.0	6.1	10.3
Mometasone furoate	116.1	6.9	8.2	114.7	1.8	1.9	107.7	6.7	7.1	0.5	0.2	20.3	40.1
Monensin	69.3	1.7	1.8	69.7	5.1	7.7	70.3	1.8	1.8	0.5	0.2	2.7	5.0
Moxifloxacin	61.9	1.9	4.3	54.9	2.9	3.4	53.6	1.2	1.8	2.0	0.5	13.5	25.1
Nabumetone	93.3	2.9	3.8	88.5	3.4	3.7	83.4	5.4	6.3	0.5	0.2	9.0	17.6
N-Acetyl dapson	63.8	8.9	10.7	63.0	3.5	4.8	69.7	4.9	5.4	0.5	0.2	12.4	24.2
Nadifloxacin	58.2	3.4	3.9	53.2	6.2	7.2	52.9	4.0	5.4	0.5	0.2	17.0	33.4
Nafcillin	77.3	7.2	9.4	82.5	13.1	13.5	86.3	9.9	11.2	5.0	2.0	11.3	17.5
Naftifine	59.7	6.7	9.1	61.8	11.4	13.1	66.0	5.6	7.1	2.0	1.0	13.5	25.1
Nalidixic acid	65.7	1.3	2.4	64.1	3.0	3.0	66.1	1.6	3.2	0.5	0.2	14.7	28.9
Nandrolone	78.8	3.6	3.8	75.6	4.0	4.5	76.6	4.8	5.8	0.5	0.2	11.8	23.2
Naproxen	58.1	5.7	6.5	54.0	4.1	5.2	60.8	4.7	5.2	5.0	2.0	9.2	13.4
Nequinate	55.9	2.8	3.1	56.4	4.3	6.3	56.6	3.8	4.1	0.5	0.2	21.3	42.1
Nigericin	75.8	1.8	2.1	72.6	2.2	3.0	74.5	1.8	2.3	0.5	0.2	7.2	13.9
Nimorazole	78.7	9.2	10.2	80.6	10.1	11.7	78.4	7.2	8.2	2.0	0.2	13.4	24.9
Norfloxacin	74.0	1.6	2.2	72.2	1.6	1.7	71.8	5.2	7.4	0.5	0.2	10.8	21.2
Ofloxacin	72.7	1.4	1.7	76.2	2.4	2.6	73.1	5.1	7.7	0.5	0.2	15.4	30.3

Table 1. *Cont.*

Compound Name	Fortified Concentration (n = 6)									LOQ	LOD	CCα	CCβ
	10 μg kg^{-1}			50 μg kg^{-1}			100 μg kg^{-1}						
	Recovery	RSD_r	RSD_{wR}	Recovery	RSD_r	RSD_{wR}	Recovery	RSD_r	RSD_{wR}				
Unit	%									μg kg^{-1}			
Oleandomycin	83.6	1.2	1.8	86.5	4.1	5.4	81.9	1.1	1.2	0.5	0.2	13.7	26.9
Orbifloxacin	76.9	3.0	3.1	71.1	3.8	4.4	75.1	3.1	4.1	0.5	0.2	7.1	13.8
Oxaprozin	56.2	2.7	3.3	55.4	3.1	3.1	53.2	3.9	4.8	0.5	0.2	18.6	36.7
Oxfendazole	83.8	6.4	7.2	90.1	9.0	9.6	84.4	7.1	8.6	0.5	0.2	11.9	23.3
Oxibendazole	62.6	1.6	2.5	64.2	3.4	4.0	64.1	5.1	5.7	0.5	0.2	4.3	8.1
Oxolinic acid	71.0	2.0	3.7	75.5	6.8	8.8	74.3	11.5	14.2	2.0	1.0	2.4	2.8
Oxytetracycline (OTC)	77.3	4.7	5.1	75.6	1.8	2.4	71.0	2.9	4.6	5.0	2.0	202.7	205.5
Paracetamol	85.3	2.1	2.4	88.1	2.2	2.3	81.7	1.1	1.4	0.5	0.2	5.5	10.6
Pefloxacin	72.2	2.4	2.5	73.7	5.9	7.2	79.5	5.2	7.0	0.5	0.2	16.9	33.2
Penbutolol	87.6	1.4	2.8	79.8	4.2	6.2	77.3	2.1	2.4	0.5	0.2	10.4	20.2
Phenacetin	79.8	1.5	1.8	78.5	2.3	2.5	80.3	1.9	2.0	2.0	0.2	2.5	3.0
Phenylbutazone	79.0	6.1	9.0	74.1	8.2	9.7	86.6	5.8	6.2	0.5	0.2	8.7	16.8
Phenylethanolamine A	88.2	1.6	1.9	87.5	2.0	2.6	94.5	2.1	2.2	0.5	0.2	1.9	3.3
Pipemidic acid	75.3	4.1	4.8	76.3	2.4	3.6	72.1	2.7	3.4	2.0	1.0	6.3	10.5
Pirbuterol acetate	52.7	3.3	3.8	52.5	4.9	5.2	68.6	5.1	7.7	5.0	0.2	8.8	12.6
Piroxicam	64.5	5.6	8.3	65.7	4.6	5.3	66.9	4.6	7.5	2.0	0.2	10.7	19.4
Prednicarbate	77.9	4.0	4.4	74.4	4.8	5.1	73.5	3.9	4.3	0.5	0.2	9.8	19.1
Prednisolone	62.0	1.8	2.0	57.1	3.4	4.0	59.1	4.8	5.2	0.5	0.2	15.2	30.0
Prednisone	74.2	5.8	5.9	76.6	4.8	5.5	78.9	1.8	3.8	2.0	0.5	7.0	12.0
Procainamide	58.1	1.6	2.5	53.1	6.9	10.3	63.2	1.7	1.8	0.5	0.2	7.2	13.9
Procaine/Novocaine	60.2	2.4	4.6	58.4	9.5	11.6	55.1	2.2	4.7	2.0	1.0	4.4	6.7
Procaterol	61.5	3.2	3.8	64.6	4.5	5.4	71.8	1.7	3.7	2.0	0.2	5.7	9.5
Progesterone	92.4	1.1	1.7	87.8	3.7	5.3	81.6	6.2	7.5	2.0	0.5	22.6	43.3
Promethazine	60.9	4.9	6.2	61.7	4.3	5.3	63.0	1.0	2.8	2.0	0.2	8.1	14.2
Propetamphos	58.9	10.5	11.0	63.2	9.0	10.9	52.7	9.2	9.6	5.0	0.2	14.7	24.5
Propionylpromazine	78.1	1.2	1.2	79.3	3.7	4.0	79.7	2.0	3.4	0.5	0.2	8.5	16.4
Propranolol	80.2	2.1	2.5	73.8	5.0	6.9	88.6	3.8	5.5	2.0	1.0	12.6	23.2
Propyl thiouracil	64.3	6.5	7.1	64.6	7.4	8.3	67.6	5.0	5.3	5.0	1.0	12.0	19.0
Ractopamine	76.1	2.8	3.3	79.9	2.9	3.5	82.2	1.1	1.4	0.5	0.2	22.0	43.5
Ritodrine	83.7	3.5	3.6	81.5	3.8	5.1	71.8	3.7	4.5	0.5	0.2	19.0	37.5
Robenidine	54.8	2.9	4.5	57.8	4.7	5.9	54.0	2.0	5.6	5.0	0.2	9.1	13.1
Ronidazole	75.9	1.5	2.4	73.0	3.8	4.3	72.8	1.9	3.4	2.0	0.2	3.9	5.7
Salbutamol	78.1	4.4	5.3	72.2	3.2	4.1	76.7	1.1	2.6	0.5	0.2	3.6	6.6

Table 1. *Cont.*

Compound Name	Fortified Concentration (n = 6)									LOQ	LOD	CCα	CCβ
	10 μg kg^{-1}			50 μg kg^{-1}			100 μg kg^{-1}						
	Recovery	RSD$_r$	RSD$_{wR}$	Recovery	RSD$_r$	RSD$_{wR}$	Recovery	RSD$_r$	RSD$_{wR}$				
Unit	%									μg kg^{-1}			
Salmeterol	70.9	4.4	5.2	71.2	4.2	5.6	73.2	3.0	3.0	0.5	0.2	9.8	19.1
Sarafloxacin	76.2	1.0	1.1	72.8	4.0	5.8	74.4	3.9	4.0	2.0	0.5	3.8	5.6
Secnidazole	66.3	3.6	3.7	69.3	2.2	2.9	67.0	1.8	5.1	0.5	0.2	3.6	6.7
Sineptina	73.3	3.2	3.9	79.2	6.7	7.7	85.3	4.8	5.5	2.0	1.0	6.6	11.3
Sotalol	73.4	1.6	2.8	73.4	3.3	3.3	77.3	2.2	2.6	0.5	0.2	21.8	43.1
Sparfloxacin	85.4	2.4	2.5	74.1	4.4	5.0	81.6	2.2	2.2	0.5	0.2	3.7	6.9
Sulfabenzamide	73.4	2.9	3.2	77.2	3.2	4.5	73.9	3.5	4.9	0.5	0.2	3.7	6.9
Sulfachloropyridazine	57.4	4.7	6.0	62.1	6.8	7.4	61.0	3.1	5.5	2.0	0.2	6.4	10.8
Sulfadiazine	61.7	2.8	2.9	65.4	3.8	4.5	63.4	1.1	5.5	0.5	0.2	8.5	16.4
Sulfadimethoxine	66.6	1.7	2.0	67.9	4.6	5.9	66.3	1.4	4.3	0.5	0.2	5.2	9.9
Sulfadimidine	64.3	2.7	3.6	67.1	3.8	4.0	66.3	3.4	3.4	0.5	0.2	11.2	21.9
Sulfadoxine	67.5	2.8	3.4	69.2	3.9	4.3	69.1	5.6	6.4	0.5	0.2	6.8	13.1
Sulfamerazine	60.4	3.0	3.0	63.5	3.0	3.0	64.9	5.4	5.8	0.5	0.2	6.8	13.1
Sulfameter	60.9	2.8	3.9	62.7	3.0	3.0	62.4	5.7	7.5	0.5	0.2	11.5	22.6
Sulfamethizole	57.2	4.9	6.0	61.9	4.7	5.5	62.8	3.3	5.1	2.0	0.2	7.6	13.2
Sulfamethoxazole	57.2	5.3	7.4	61.3	2.3	3.0	59.9	5.7	7.1	2.0	0.2	8.9	15.8
Sulfamethoxypyridazine	64.8	3.8	4.1	69.6	2.7	3.7	69.3	4.2	5.7	0.5	0.2	10.8	21.2
Sulfamonomethoxine	63.0	2.8	3.3	64.4	4.8	5.2	64.1	6.0	7.8	2.0	0.2	4.9	7.7
Sulfamoxol	62.3	5.3	7.1	65.1	2.1	3.2	62.5	6.8	7.4	0.5	0.2	14.1	27.7
Sulfanilamide	73.1	2.4	2.9	83.2	5.4	6.8	84.1	1.3	1.9	0.5	0.2	8.1	15.6
Sulfanitran	70.3	4.6	5.0	75.1	8.6	11.4	77.2	6.6	9.7	5.0	1.0	14.6	24.2
Sulfaphenazole	68.8	2.6	3.8	70.4	2.3	2.8	70.2	2.2	5.0	0.5	0.2	10.1	19.7
Sulfapyrazole	71.2	1.7	2.2	71.3	3.7	4.4	71.6	5.3	5.3	0.5	0.2	10.7	20.9
Sulfapyridine	61.4	2.8	3.6	63.5	3.5	4.0	63.8	5.5	7.4	0.5	0.2	9.1	17.6
Sulfaquinoxaline	56.7	6.3	6.8	63.2	6.1	7.6	60.9	5.6	5.9	0.5	0.2	13.0	25.5
Sulfathiazole	53.0	3.7	4.6	56.1	3.5	3.9	56.9	5.6	7.4	5.0	0.2	8.0	10.9
Sulfisomidine	60.9	2.8	3.3	63.5	2.5	2.6	67.5	5.0	5.6	2.0	0.5	4.8	7.7
Sulindac	70.5	4.0	4.7	72.9	4.1	5.6	73.8	1.9	2.7	2.0	0.5	16.9	31.7
Sulphacetamide	117.8	3.9	4.0	118.3	2.8	3.1	106.5	1.3	1.3	0.5	0.2	3.1	5.8
Sulpiride	72.5	1.6	3.2	77.8	4.4	6.6	83.4	5.3	6.0	0.5	0.2	9.0	17.4
Tenoxicam	62.4	2.9	3.4	61.6	3.7	4.9	65.1	4.2	5.1	2.0	0.2	5.5	8.9
Terbutaline	85.2	3.2	3.3	82.4	9.0	12.1	72.7	3.8	4.2	0.5	0.2	18.0	35.5

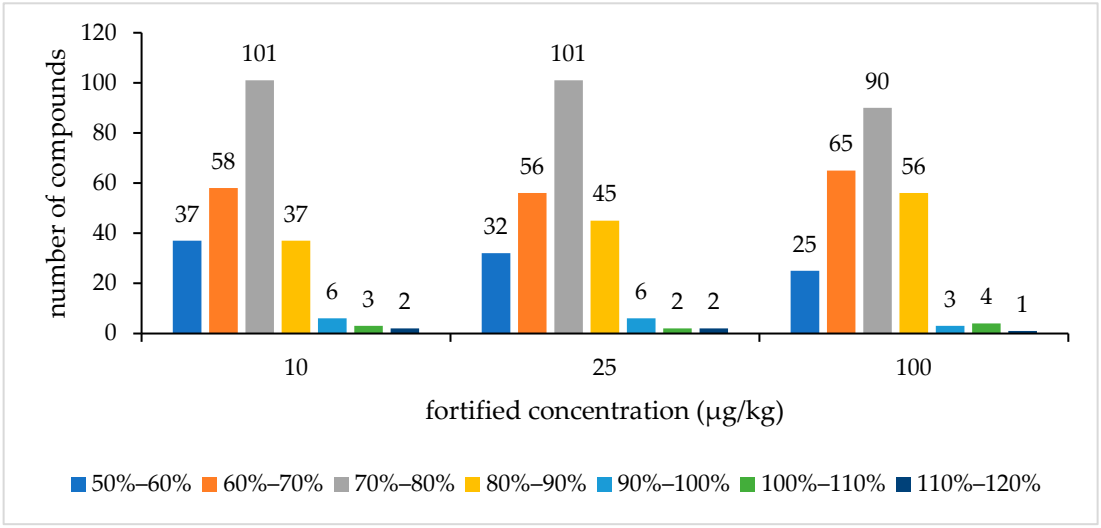
Table 1. *Cont.*

Compound Name	Fortified Concentration (n = 6)									LOQ	LOD	CCα	CCβ
	10 μg kg^{-1}			50 μg kg^{-1}			100 μg kg^{-1}						
	Recovery	RSD_r	RSD_{wR}	Recovery	RSD_r	RSD_{wR}	Recovery	RSD_r	RSD_{wR}				
Unit	%									μg kg^{-1}			
Terfenadine	84.8	1.0	3.5	84.6	3.8	5.3	83.6	2.4	3.4	0.5	0.2	6.7	13.0
Testosterone	62.9	1.1	2.3	63.3	3.4	3.7	58.0	4.5	5.3	0.5	0.2	8.1	15.7
Tetracaine	55.4	4.7	5.8	53.4	7.0	10.9	53.8	2.8	3.4	0.5	0.2	4.3	8.1
Thiabendazole	59.9	1.1	1.3	62.2	2.9	2.9	65.0	5.5	5.7	2.0	0.2	3.1	4.2
Tilmicosin	95.6	6.9	7.1	81.1	7.5	8.4	81.7	2.5	2.5	2.0	0.2	12.8	23.7
Tinidazole	63.2	2.3	2.5	65.5	3.6	3.6	66.6	4.7	5.0	0.5	0.2	5.6	10.7
Tolfenamic acid	75.7	3.8	4.2	76.9	5.1	6.5	72.2	3.1	4.0	2.0	1.0	7.3	12.5
Tolmetin	75.4	6.9	7.1	76.0	7.6	9.6	76.5	3.5	4.8	2.0	0.2	10.4	18.7
Toltrazuril	74.9	3.4	4.2	71.5	1.9	2.0	71.7	9.9	12.8	5.0	2.0	7.2	9.3
Toltrazuril sulfone	88.6	4.3	5.1	80.0	1.9	2.5	91.2	6.5	8.7	5.0	2.0	12.8	20.7
Toltrazuril-sulfoxide	73.7	5.0	5.7	76.7	3.8	5.2	77.1	6.0	6.8	2.0	1.0	10.0	18.0
Tosufloxacin	55.2	5.0	5.4	62.2	11.7	14.0	66.8	8.3	11.0	5.0	0.2	15.9	26.8
Triamcinolone acetonide	81.5	3.2	6.7	81.0	3.3	3.5	81.8	2.5	4.3	0.5	0.2	7.8	15.1
Triclabendazole	54.6	3.0	3.9	57.2	2.6	2.7	57.4	4.6	5.9	5.0	0.2	7.3	9.6
Trimethoprim	74.1	1.1	1.2	80.7	2.9	2.9	81.7	1.1	1.4	0.5	0.2	11.2	21.8
Tulobuterol	80.7	1.8	1.8	81.2	2.5	2.7	82.1	1.6	2.0	0.5	0.2	4.7	9.0
Tylosin	74.2	3.3	4.6	78.7	2.6	3.2	81.3	1.2	1.9	0.5	0.2	206.4	212.8
Valnemulin	66.9	6.1	7.3	56.3	4.8	6.0	60.8	6.4	7.9	5.0	2.0	10.1	15.1
Virginiamycin M1	62.1	3.9	4.2	68.9	3.3	3.8	66.1	1.8	3.0	0.5	0.2	11.7	22.9
Xylazine	76.1	1.5	1.7	77.3	2.9	3.5	78.1	1.2	1.4	0.5	0.2	9.2	17.9
Zolpidem	77.2	1.0	1.1	79.3	2.4	2.5	78.2	1.9	1.9	0.5	0.2	3.9	7.4

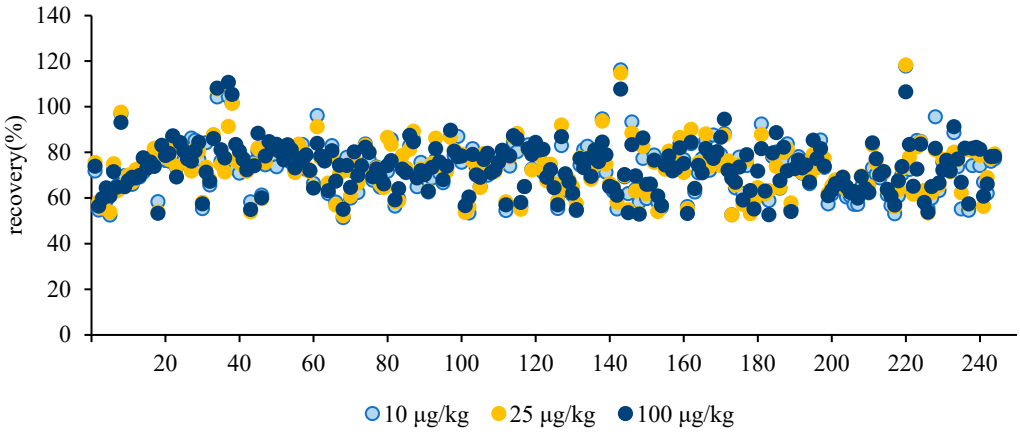
Abbreviations: RSDr: repeatability, RSDwR: reproducibility, LOD: limit of detection, LOQ: limit of quantitation, CCα: decision limit, CCβ: detection capability.

3.2.4. Accuracy and Precision

The accuracy of the experiments was reflected by the average recovery rate in three fortified concentrations with six replicates. The results of the recovery study are provided in Table 1. When the fortified concentration was 10, 50, and 100 μg kg^{-1}, the recovery rates were 51.33–117.83%, 52.29–118.28%, and 52.65–110.66%, respectively. This result is also shown in Figure 5; about 150, 60, and 30 compounds had recovery rates in the range of 70–120%, 60–70%, and 50–60% at three fortified concentrations, respectively (Figure 5a), and similar trends seemed to occur in all three spiked concentrations (Figure 5b). Above all, for those compounds with recovery rates in the range of 70–120%, this method can be directly used for quantitative calculation. Meanwhile, for those compounds with recovery rates under 70% after qualitative detection with this method, other purification methods or related internal standards should be used for more accurate quantification.



(**a**)



(**b**)

Figure 5. Distribution of recovery at the fortified concentrations of 10, 25 and 100 μg kg^{-1}. (**a**) Number of compounds in recovery ranges at the fortified concentrations of 10, 25 and 100 μg kg^{-1}; (**b**) Trends of recoveries at the fortified concentrations of 10, 25 and 100 μg kg^{-1}.

The precision of this method was determined by calculating the repeatability and within-laboratory reproducibility, which were expressed as the RSD_r and RSD_{wR} for three fortified levels (n = 6) in the egg matrix. The relevant results are presented in Table 1. In eggs, the values of RSD_r and RSD_{wR} were 1.01–14.22% and 1.08–71014.96%. It could be

observed that the RSDs were always lower than 20% for all of the chemical contaminants in eggs. The results indicated that this advanced method has excellent precision and reliability.

3.2.5. CCα and CCβ

Due to the large number of compounds, the calculation of CCα and CCβ, according to the method specified by the European Union, is time-consuming and costly. Based on this, the formula for calculating CCα and CCβ was modified [17,43,48]. Among the 244 compounds for eggs, there are only four drugs with maximum residue limits (MRLs) set by the EU, namely flubendazole, lincomycin, oxytetracycline, and tylosin; the set values are 400 μg kg^{-1}, 50 μg kg^{-1}, 200 μg kg^{-1}, and 200 μg kg^{-1}, respectively. Thus, for these four compounds, the $CC\alpha = MRL + 1.64 \times SD_{MRL}$ and $CC\beta = CC\alpha + 1.64 \times SD_{MRL}$. Most compounds do not have an MRL. The formula is $CC\alpha = LOQ + 1.64 \times SD_{LOQ}$ and $CC\beta = CC\alpha + 1.64 \times SD_{LOQ}$. The CCα and CCβ values are shown in Table 1, and the values were 1.88–40.60 μg·kg^{-1} and 2.85–407.19 μg·kg^{-1}, respectively.

3.3. Analysis of Real Egg Samples

To prove the effectiveness of the validated method, 40 eggs from different cities in China were tested using the developed method after being shelled and homogenized. The mass spectrometry conditions were checked, such as the peak of each compound ion pair, the ion ratio, and retention time deviation, which did not exceed ±0.1 min, to determine whether the samples were positive. The ion chromatograms of compounds detected in actual samples are shown in Figure 6.

The results showed that a total of six compounds were detected in 10 egg samples. In three different samples, clopidol at the concentration of 21.16 μg kg^{-1}, oblixacin at the concentration of 10.98 μg kg^{-1}, and danofloxacin at the concentration of 2.36 μg kg^{-1}, were found. Enrofloxacin was detected in two egg samples at concentrations of 3.70 μg kg^{-1} and 4.64 μg kg^{-1}, respectively. Tilmicosin was detected in two egg samples, and the residual concentrations were 19.26 μg kg^{-1} and 20.26 μg kg^{-1}, while trimethoprim had the highest detection frequencies, which was detected in three samples with values of 25.06 μg kg^{-1}, 33.80 μg kg^{-1}, and 41.78 μg kg^{-1}, respectively. The European Union has a zero-tolerance principle for enrofloxacin, danofloxacin, trimethoprim, and tilmicosin residues in eggs [49]. These results are in agreement with other studies in which enrofloxacin [50] and trimethoprim [11] were frequently detected. However, both the EU and CAC, as well as China, do not stipulate limitations for clopidol and oblixacin quantities in eggs. Its application to actual samples proved that this method has excellent practicality.

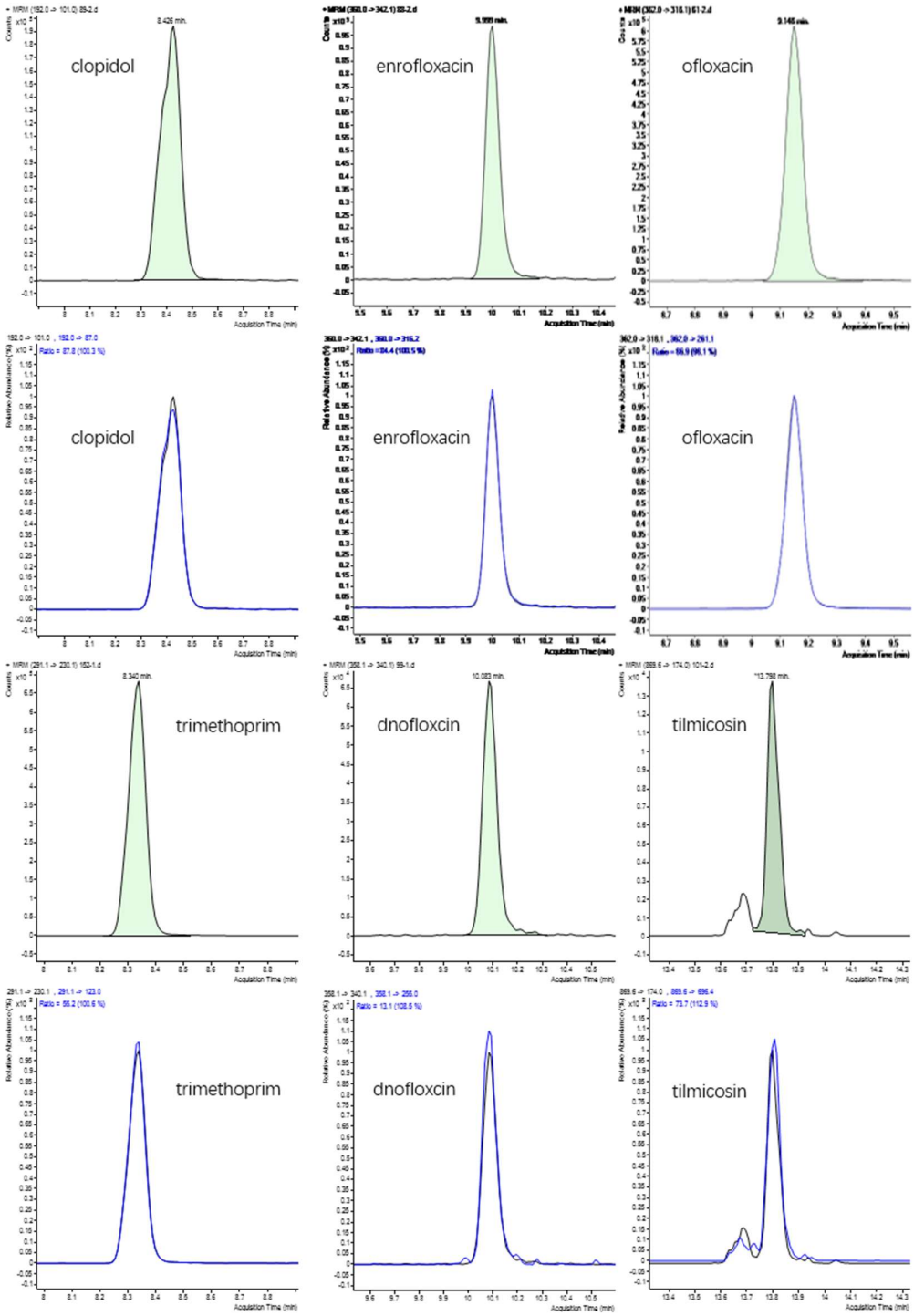


Figure 6. The ion chromatograms of the compounds detected in the actual sample.

4. Conclusions

In this study, an extraction method with 5% FA in acetonitrile (ACN) and a clean-up method with EMR-lipid material was used with LC-MS/MS to simultaneously detect a total of 244 compounds, thus significantly expanding the range of chemical contaminants monitored in eggs. The compounds included chemicals that are banned and restricted in eggs and may have residual risks following human consumption. In addition, it was shown that the method achieves good separation of the 244 compounds within 30 min. This method was validated according to the guidelines of SANTE/11813/2017 and the EU Commission Decision 2002/675/EC. The results showed that the method had good

sensitivity and accuracy. The method was successfully applied to egg samples obtained from different cities, and six compounds were detected, indicating that the method is suitable for monitoring multi-class residues in eggs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12060869/s1, Figure S1: Effect of EMR and NaCl on the number of chemical contaminants in the recovery of 50–130% and 70–120%; Table S1: Methods for the determination of veterinary drugs and pesticides in eggs [11,31,40,51–57]; Table S2: The mass spectrometry parameters of 244 chemical contaminants; Table S3: Regression Equation and correlation coefficient of 244 compounds in matrix standard curve.

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