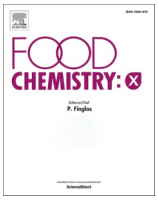
Food Chemistry: X 22 (2024) 101468

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Quantitative analysis of erythromycin, its major metabolite and clarithromycin in chicken tissues and eggs via QuEChERS extraction coupled with ultrahigh-performance liquid chromatography-tandem mass spectrometry

Bo Wang [a,b], Yali Zhu [c,d], Shuyu Liu [c,d], Han Zhang [e], Tianzhu Guan [a,b], Xuechao Xu [a,b], Xiangfeng Zheng [a,b], Zhenquan Yang [a,b,*], Tao Zhang [c,d], Genxi Zhang [c,d], Kaizhou Xie [c,d,**]

[a] College of Food Science and Engineering, Yangzhou University, Yangzhou 225127, China
[b] Yangzhou Engineering Research Center of Food Intelligent Packaging and Preservation Technology, Yangzhou University, Yangzhou 225127, China
[c] College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China
[d] Joint International Research Laboratory of Agriculture & Agri-Product Safety, Yangzhou University, Yangzhou 225009, China
[e] School of Animal Science and Technology, Jiangsu Agri-animal Husbandry Vocational College, Taizhou 225300, China

ARTICLE INFO

Keywords:
Erythromycin and its major metabolite
Clarithromycin
UHPLC-ESI–MS/MS
QuEChERS
Chicken tissues and eggs

ABSTRACT

A simple, rapid and novel method involving ultrahigh-performance liquid chromatography-electrospray ionization tandem triple quadrupole mass spectrometry (UHPLC-ESI–MS/MS) was developed to simultaneously detect erythromycin, its major metabolite and clarithromycin in chicken tissues (muscle, liver and kidney) and eggs (whole egg, albumen and yolk). Samples were extracted using acetonitrile–water (80:20, v/v), and a Cleanert MAS-Q cartridge was used to perform quick, easy, cheap, effective, rugged, and safe (QuEChERS) purification. The average recoveries were 87.78–104.22 %, and the corresponding intraday and interday relative standard deviations were less than 7.10 %. The decision limits and detection capabilities of the chicken tissues and eggs were 2.15–105.21 μg/kg and 2.26–110.42 μg/kg, respectively. For chicken tissues and eggs, the limits of detection and limits of quantification were 0.5 μg/kg and 2.0 μg/kg, respectively. The proposed method was successfully employed to analyse real samples, demonstrating its applicability.

1. Introduction

Chicken meat and eggs are popular because of their nutritional, low-fat and digestible properties, as they contain high-quality protein, vitamins, minerals, and antioxidants (Benedé & Molina, 2020; Fan et al., 2018). Due to the high-density breeding method of modern farming, chickens are usually susceptible to respiratory diseases. To maintain chicken and egg production, macrolide antibiotics (MACs) are usually used to treat chicken respiratory diseases (Wang et al., 2021). Both erythromycin A (ERY) and clarithromycin (CLA), two MACs that share comparable antibacterial processes and have an impact on both gram-positive and gram-negative bacteria, are members of the same class (Juan et al., 2010). In our previous study, the major metabolite of ERY in chicken liver microsomes was determined to be *N*-desmethyl-erythromycin A (*N*-D-ERY) (Wang et al., 2021). Reports have indicated that *N*-D-ERY does not possess antibacterial activity; however, *N*-D-ERY represents a potential risk to individuals (Lundquist et al., 2014; Sun et al., 2022). Although the use of these antibiotics reduces the mortality of poultry, some antibiotic residues, such as fluoroquinolones, tetracyclines, amphenicols, sulfonamides, MACs, lincosamides, and coccidiostats, still jeopardize the safety of poultry and their products, which needs to be addressed (Chinese Ministry of Agriculture and Rural Affairs, 2024). Consumers who are exposed to these residues experience both chronic and acute health problems, including hypersensitivity, cancer, mutagenesis, teratogenesis, disturbance of the intestinal flora, and antibiotic resistance (Beyene, 2016; Boobis et al., 2017). To ensure food safety for consumers, the European Union (EU) has set maximum residue limits (MRLs) for ERY of 200 μg/kg in animal tissues (muscle, fat,

* Corresponding author at: College of Food Science and Engineering, Yangzhou University, Yangzhou 225127, China.
** Corresponding author at: College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China.
E-mail addresses: yangzq@yzu.edu.cn (Z. Yang), yzxkz168@163.com (K. Xie).

https://doi.org/10.1016/j.fochx.2024.101468
Received 8 February 2024; Received in revised form 10 May 2024; Accepted 10 May 2024
Available online 17 May 2024

liver and kidney), 40 μg/kg in milk and 150 μg/kg in eggs (Commission Regulation (EU), 2010). In addition, many countries, such as China, the USA and Japan, have set MRLs for ERY in chicken tissues (100 μg/kg, 100 μg/kg, and 50 μg/kg for China, the USA and Japan) and eggs (50 μg/kg, 25 μg/kg, and 90 μg/kg for China, the USA and Japan) (Chinese Ministry of Agriculture and Rural Affairs, 2019; Japan Food Chemical Research Foundation, 2015; U.S. Food and Drug Administration, 2014). The risk of antibiotic and metabolite residues in animal-derived foods has attracted the attention of countries worldwide. Therefore, it is necessary to develop an efficient and fast detection method for performing trace analyses of ERY, CLA and *N*-D-ERY in chicken tissues and eggs.

Currently, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method has been used for sample preparation in antibiotic analysis since it has numerous benefits over conventional techniques, including liquid–liquid extraction (LLE) and solid-phase extraction (SPE) cartridges (Zhang et al., 2019). Although the LLE method is simple in operation, it has disadvantages, including high reagent consumption, time consumption and poor reproducibility. Therefore, the LLE method is replaced by better and more efficient sample preparation technology. The SPE method exhibits several characteristics, including astrong enrichment ability and small matrix interference, and is widely used in the extraction of antibiotic residues from animal-derived foods. However, for complex matrix samples, SPE cartridges are prone to blockage and leakage, which affects the stability and accuracy of the method. In addition, SPE cartridges are expensive, increasing the cost of detection. The principle of QuEChERS involves the adsorption of impurities through interactions between the adsorbent filler and the impurities in the matrix, thereby achieving the removal and purification of impurities (Wang et al., 2021). Compared with the LLE and SPE methods, QuEChERS is inexpensive, consumes low levels of reagents, saves time, and achieves relatively high recovery and precision (Xu et al., 2021). The combination of QuEChERS and liquid chromatography tandem mass spectrometry has been widely used in MAC residual analysis (Campanharo et al., 2023; Du et al., 2021; de Mendonça Pereira et al., 2021; Xu et al., 2021). Du et al. (2021) developed a QuEChERS method using multiwalled carbon nanotubes (MWCNTs) as the adsorbent, and 11 MACs in animal tissues and eggs were detected via ultrahigh-performance liquid chromatography tandem triple quadrupole mass spectrometry (UHPLC–MS/MS). The average recoveries of 11 MACs in animal tissues and eggs were 83.5–111.4 %, and the corresponding intraday and interday relative standard deviations (RSDs) were less than 13.6 % and 16.4 %, respectively. Campanharo et al. (2023) developed a modified QuEChERS procedure associated with the dispersive liquid–liquid microextraction (DLLME) technique for extracting ERY residues in fish filles using high-performance liquid chromatography tandem triple quadrupole mass spectrometry (HPLC–MS/MS). The QuEChERS-DLLME-HPLC–MS/MS method uses primary secondary amine (PSA) as an adsorbent and results in good recovery (103–110 %) and precision (4.5–6.3 %). Xu et al. (2021) reported a QuEChERS-HPLC–MS/MS method for the analysis of 13 MACs and 2 lincosamides in honey. The effects of different adsorbents on antibiotic recovery in honey were compared through this method, and zinc oxide (ZnO) was successfully screened as an adsorbent. The method obtained the best extraction recovery. de Mendonça Pereira et al. (2021) established a QuEChERS method combined with HPLC–MS/MS for the simultaneous determination of 5 MACs in infant formulas produced from bovine milk. Compared with the HPLC–MS/MS method, UHPLC–MS/MS has the advantages of fast analysis speed, strong separation capacity, high sensitivity, and less reagent consumption (Du et al., 2021). Therefore, the combination of QuEChERS and UHPLC–MS/MS technology was used to quickly identify antibiotic residues in animal-derived foods.

In this study, we intend to use a Cleanert MAS-Q cartridge in the QuEChERS protocol to process chicken tissue and egg samples and then analyse these targets through UHPLC–MS/MS. The developed QuEChERS-UHPLC–MS/MS method shortens the presample processing and detection times, which improves the sensitivity and accuracy of the method. Finally, the method evaluates relevant parameters through the EU (European Commission, 2021; The European Communities, 2002) and Food and Drug Administration (FDA) regulations (U.S. Department of Health and Human Services, Food and Drug Administration, 2018) and analyses 40 real samples (20 chicken muscles and 20 eggs) to verify the applicability and accuracy of the method.

2. Materials and methods

2.1. Chemicals and reagents

An ERY (98 % purity) standard was obtained from Kaishu Chemical Technology Co. (Shanghai, China). CLA (98.9 % purity) and roxithromycin (ROX, 96.5 % purity; used as an internal standard, IS) standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). An *N*-D-ERY (95 % purity) standard was acquired from Anpu Experimental Technology Co. (Shanghai, China). HPLC-grade acetonitrile (ACN) and methanol were purchased from Tedia Company, Inc. (Fairfield, OH, USA). Analytical-grade ethylenediaminetetraacetic acid (EDTA), sodium chloride (NaCl), ammonium hydroxide, anhydrous magnesium sulfate ($MgSO_4$), anhydrous sodium sulfate (Na_2SO_4), disodium hydrogen phosphate (Na_2HPO_4) and formic acid were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). ZnO (99.9 % purity) was obtained from Yuanye Bio-Technology Co. (Shanghai, China). Pesticarb (PC, size: 120–400 mesh) and Cleanert MAS-Q cartridges (PSA 50 mg + PC 8 mg + C_{18} 50 mg + $MgSO_4$ 150 mg) were purchased from Bonna-Agela Technologies (Tianjin, China). Water was obtained from a PURELAB Option-Q synthesis system (ELGA Lab Waters, High Wycombe, Bucks, UK).

2.2. Preparation of standard solutions

The standard stock solutions of ERY, CLA, *N*-D-ERY, and ROX at 1000 μg/mL in methanol were kept at −70 °C for up to three months. Standard working solutions of ERY, CLA, and *N*-D-ERY were prepared at concentrations of 100, 10 and 1 μg/mL by diluting the stock solutions with methanol and keeping them at −20 °C. A working mixed solution was prepared by dissolving appropriate individual working solutions of ERY, CLA, and *N*-D-ERY in methanol and used for spiking chicken tissue and egg samples. A 2 μg/mL ROX internal standard solution was prepared by dissolving the 10 μg/mL standard working solution in methanol.

2.3. Evaluation of the stability of standard solutions

To evaluate the stability of the standard solutions, individual standard stock solutions (1000 μg/mL) and working mixed solutions (100, 10 and 1 μg/mL) were tested on different days (15, 30, 60, 90, 120 and 180 days).

2.4. Sample information and sample preparation

The study was authorized and undertaken in accordance with the ethics requirements of the official Ethical Committee of Yangzhou University. Thirty 35-day-old Shaobo chickens (with equal proportions of males and females) (Poultry Institute, Chinese Academy of Agricultural Sciences, Yangzhou, China) used in the experiment were bred in single cages and given free access to drinking water and complete feed without antibiotics (Yangda Stall Food Factory, Yangzhou, China) once in the morning and evening for three weeks. The composition and nutrient levels of the complete feed is shown in Table S1. After three weeks, thirty Shaobo chickens were euthanized directly by cervical dislocation without anaesthesia, and blank chicken tissues (muscle, liver, and kidney) were collected. This work was carried out by professional abattoir staff. All efforts were made to minimize animal suffering.

Table 1
Gradient elution program.

Time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)	Curve
0	0.4	95.0	5.0	/
2	0.4	95.0	5.0	6
3	0.4	50.0	50.0	6
4	0.4	25.0	75.0	6
5	0.4	95.0	5.0	6

Table 2
Retention times and relevant MS parameters for the analytes.

Analyte	Retention time (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)
ERY	4.34	734.5	158.2*	95	38
			576.4	95	25
CLA	4.51	748.6	158.1*	95	35
			590.4	95	25
N-D-ERY	4.32	720.4	144.0*	95	35
			562.2	95	22
ROX	4.53	837.7	679.5*	110	28
			158.2	110	41

Note: * Quantification ion.

Antibiotic-free egg samples were obtained from a local supermarket in Yangzhou (Jiangsu Province, China). All chicken tissue (muscle, liver, and kidney) and egg (whole egg, albumen, and yolk) samples were homogenized, packed and stored at −20 °C until use.

The homogenized sample (2.0 g) was weighed into a 50-mL polypropylene centrifuge tube and spiked with 100 μL of the ROX internal standard solution (2 μg/mL). After vortexing for 1 min, 10 mL of ACN-water (80:20, v/v) was used as the extractant. The mixture was vortexed for 1 min and centrifuged on a 5810R centrifuge (Eppendorf, Hamburg, Germany) for 8 min at 10000 × g and 4 °C. One millilitre of the supernatant was transferred to a Cleanert MAS-Q cartridge, vortexed immediately for 1 min, and centrifuged on a Mini Spin Plus centrifuge (Eppendorf, Hamburg, Germany) for 8 min at 10000 × g. Finally, 0.5 mL of purified extract mixed with 0.5 mL of water was filtered through a nylon membrane filter (0.22 μm) for UHPLC–MS/MS analysis.

The sample preparation steps performed in this study were named protocol A, and other protocols (B, C and D) were revised based on these analytical methods (Lan et al., 2019; Xu et al., 2021; Zhou et al., 2017). Protocols B, C and D described in the literature were implemented and tested for their suitability for the scope of this project and are discussed in Section 3.2.2. All protocols were the same, with 2.0 g of homogenized sample combined with 100 μL of internal standard solution (2 μg/mL), and the final step was UHPLC–MS/MS analysis after filtering through a membrane filter.

2.5. UHPLC–MS/MS analysis

The UHPLC–MS/MS system consisted of a Waters Acquity UPLC™ system (Waters Corp., Milford, Massachusetts, USA), and an AB SCIEX Triple Quad™ 5500 mass spectrometer (AB SCIEX Corp., Framingham Massachusetts, USA) was used for analysis. Chromatographic separation was achieved using a Waters ACQUITY UPLC BEH C_{18} column (50 mm × 2.1 mm; i.d. 1.7 μm) protected with a guard column (Waters VanGuard™ BEH C_{18}; i.d. 1.7 μm). The mobile phase consisted of 0.1 % formic acid in water (A) and 0.1 % formic acid in ACN (B). The gradient elution program is shown in Table 1. The injection volume was 10 μL, and the column temperature was maintained at 40 °C.

Mass spectrometric analysis was carried out in multiple reaction monitoring (MRM) mode using positive electrospray ionization (ESI +). Instrument control and data processing were carried out by Analyst software (version 1.6.1). The MS/MS parameters were optimized as follows: ion spray voltage, 5.5 kV; source temperature, 550 °C; ion source gas 1 and gas 2, 50 psi; curtain gas, 25 psi; and collision gas, 8 psi. The qualitative and quantitative transitions of ERY, CLA, *N*-D-ERY and ROX and the corresponding cone voltages and collision energies are presented in Table 2.

2.6. Method validation

The optimized method was validated according to the related guidelines of the Commission Decision 2002/657/EC (The European Communities, 2002), the Commission Implementing Regulation (EU) 2021/808 (European Commission, 2021) and the FDA (U.S. Department of Health and Human Services, Food and Drug Administration, 2018) based on specificity, linearity, matrix effect (ME), limit of detection (LOD), limit of quantitation (LOQ), decision limit (CC_α), detection capability (CC_β), and recovery and precision. To evaluate the specificity of the method, interfering peaks at the retention times of ERY, CLA, and *N*-D-ERY were detected in blank chicken tissue (muscle, liver, and kidney) and egg (whole egg, albumen, and yolk) samples. The linearity and ME were evaluated by constructing matrix-matched and solvent calibration curves at six spiked concentrations of 0.2, 2, 5, 10, 20, and 30 ng/mL for each drug and 10 ng/mL for the IS. The LODs and LOQs were calculated using fortified samples, which yielded signal-to-noise (S/N) ratios of 3 and 10, respectively.

According to the Commission Implementing Regulation (EU) 2021/808 (European Commission, 2021), for antibiotics for which the MRLs are not specified, the CC_α and CC_β values for these antibiotics were calculated using blank samples spiked at the concentration of the LOQ. The CC_α and CC_β values for ERY, CLA and *N*-D-ERY were evaluated using 20 matrix samples spiked at 1 times the MRL or LOQ; the equations are as follows:

$$CC_\alpha(non-MRLsubstances) = LOQ + 2.33 \times SD_{1LOQ} \quad (1)$$

$$CC_\alpha(MRLsubstances) = MRL + 1.64 \times SD_{1MRL} \quad (2)$$

$$CC_\beta = CC_\alpha + 1.64 \times SD_{1LOQ} or SD_{1MRL} \quad (3)$$

where the MRLs of chicken tissues and eggs are 100 and 50 μg/kg, respectively.

Recovery was determined by analysing five independently (n = 5) spiked blank chicken tissue and egg samples at the LOQ, 10 × LOQ and 100 × LOQ. Precision was evaluated by intraday RSDs (repeatability) and interday RSDs (reproducibility). Intraday and interday RSDs were obtained by repeating the analysis of spiked chicken tissue and egg samples (n = 5) at three concentration levels on the same day (intraday RSDs) and three consecutive days (interday RSDs). The precision is expressed as the RSD for three concentrations of ERY, CLA and *N*-D-ERY.

2.7. Statistical analysis

All the experimental designs were completely randomized, and each treatment was conducted in triplicate. The experimental data are expressed as the mean ± standard deviation (X ± SD). Differences were considered statistically significant when $P < 0.05$ according to Duncan's multiple range test.

3. Results and discussion

3.1. Optimization of UHPLC–MS/MS analysis conditions

To select the precursor and product ions of the target analyte, 50 ng/mL individual standard working solutions were directly injected into an MS/MS system. In ESI (+) mode, nitrogen atoms found in MAC molecules can be readily protonated to create singly, doubly, or triply

charged molecular ions (Wang, 2009). Thus, mass spectrometry was performed in ESI (+) mode for full scan, and the scanning range was *m/z* 100–900. In ESI (+) mode, the precursor ions for ERY, CLA, *N*-D-ERY and ROX are protonated $[M + H]^+$ at *m/z* 734.5, 748.6, 720.4 and 837.7, respectively. The declustering potential and collision energy were optimized to select two characteristic product ions. The optimized spectrometric parameters and precursor and product ions of ERY, CLA, *N*-D-ERY and ROX are shown in Table 2. As shown in Fig. S1, the two product ions for ERY, CLA, *N*-D-ERY and ROX were observed at *m/z* 158.2 and 576.4, *m/z* 158.1 and 590.4, *m/z* 144.0 and 562.2, and *m/z* 679.5 and 158.2, respectively. These product ions are used for the quantitation of the target analyte via mass spectrometry using the MRM mode.

To obtain a good peak shape and separate target compounds, the choice of chromatography column is very important. The C_{18} column is commonly used to separate MACs in animal-derived foods and has achieved good separation effects (Du et al., 2021). Therefore, a Waters ACQUITY UPLC BEH C_{18} column (50 mm × 2.1 mm; i.d. 1.7 μm) was selected to separate target compounds in chicken tissue and egg samples. Due to the characteristics of ESI technology and the ionization state of drugs in solution, the composition of the mobile phase often significantly affects the ionization efficiency of the analytes, so this study optimized the conditions of the mobile phase and compared the effects of different mobile phase systems (water-ACN, 0.1 % formic acid in water-ACN and 0.1 % formic acid in water-0.1 % formic acid in ACN) on the response of the analytes. The results showed that 0.1 % formic acid in water-0.1 % formic acid in ACN as the mobile phase was optimal for obtaining the best response of the target compounds. The addition of 0.1 % formic acid can ensure that the target compound can form a good peak shape when separated by LC, and it can provide the H^+ ions needed for MS ionization to ensure ionization of the target compound under MS (Juan et al., 2010). In addition, we also optimized the gradient elution program to obtain good retention and separation.

Under the optimized UHPLC–MS/MS analysis conditions, blank and spiked chicken tissue and egg samples were detected to evaluate the verification parameters of the method. Taking blank chicken muscle as an example, the total ion chromatogram (TIC) and extracted ion chromatograms (XICs) of ERY, CLA, *N*-D-ERY and ROX are shown in Fig. S2. The TIC and XICs of the quantitative ions of blank chicken muscle spiked with 2 μg/kg ERY, CLA and *N*-D-ERY and 100 μg/kg ROX are shown in Fig. S3. Compared to Fig. S2, Fig. S3 shows that the four targets had sharp peaks with no tailing, and the blank chicken muscle samples did not contain artefacts of these targets.

3.2. Sample clean-up

3.2.1. Optimization of sample preparation

Chicken tissue and egg samples cannot be tested without extraction and purification steps. This was because chicken tissues and eggs contain many endogenous interfering substances, such as proteins and lipids. These interfering substances affect the separation and detection of target compounds, leading to low recovery of the samples. Because of its simplicity, time savings, efficiency, low cost, and high sensitivity, the QuEChERS method has been widely used in the analyses of MAC residues in animal-derived food (Du et al., 2021; Seyedi et al., 2022; Xu et al., 2021; Yan et al., 2017; Zhou et al., 2017). However, these methods have relatively long processing times; therefore, we developed a novel QuEChERS method using a Cleanert MAS-Q cartridge to quickly extract and purify target analytes from chicken tissue and egg samples. Moreover, this study compared the effects of ACN, 1 % ammonia in ACN, ACN-water (80:20, v/v) and 1 % formic acid in ACN on the recovery of ERY, CLA and *N*-D-ERY from chicken tissue and egg samples spiked at 50 μg/kg. Three replicates were performed. Notably, after sample processing for the QuEChERS method, the starting proportion of the organic phase of the sample volume was relatively high, which was not conducive to obtaining reproducible retention times of ERY, CLA and *N*-D-ERY in the C_{18} column. To solve this problem, 0.5 mL of purified extract was added to 0.5 mL of ultrapure water for dilution to reduce the concentration of the starting organic phase, thereby resulting in good recovery. As shown in Fig. S4, when ACN-water (80:20, v/v) was used as the extractant, the extraction recovery of the targets was significantly impacted, and the targets obtained the greatest extraction efficiency (recovery: 87.6–95.3 %, RSD: 2.4–3.5 %). Therefore, ACN-water (80:20, v/v) was selected as the extractant for the extraction of the three target compounds from chicken tissue and egg samples. In addition, the effect of ACN-water (80:20, v/v) was evaluated in various ranges (5, 10 and 20 mL). When the volume of ACN-water (80:20, v/v) was 10 mL, the method had a significant impact on the extraction recovery of the analytes, and the recovery efficiency of the analytes was satisfactory (Fig. S5). There was no significant difference in the extraction recovery of analytes between 10 mL and 20 mL, indicating that 10 mL of ACN-water (80:20, v/v) was sufficient and that further increasing the volume of ACN-water (80:20, v/v) did not improve recovery. Therefore, 10 mL of ACN-water (80:20, v/v) was used in the method.

3.2.2. Comparison of the sample preparation methods

To date, many methods have been developed for the extraction of ERY, CLA and other MACs from milk, sheep's milk, egg, fish, shrimp, honey and animal tissues, such as liquid–liquid extraction (LLE) (Jank et al., 2015; Jo et al., 2011), solid-phase extraction (SPE) (Lan et al., 2019; Wang et al., 2005), accelerated solvent extraction (ASE) (Juan et al., 2010; Tao et al., 2012), matrix solid-phase dispersion (MSPD) (García-Mayor et al., 2012) and QuEChERS extraction (Du et al., 2021; Xu et al., 2021; Zhou et al., 2017). Lan et al. (2019) established an SPE method using a PAF-6 SPE cartridge (3 mL/60 mg) combined with HPLC–MS/MS technology to detect the residues of six MACs in chicken samples. Based on previous research and a previous report (Wang et al., 2005, 2020), the Oasis PRIME HLB cartridge can extract antibiotic residues from animal-derived food. Therefore, protocol B used an Oasis PRIME HLB cartridge (6 mL/200 mg, Waters Corp) to replace the PAF-6 SPE cartridge for purifying the samples. The extract can be directly added to the Oasis PRIME HLB cartridge, so protocol B omits the steps of activating and balancing the SPE cartridge during the purification process. The additional sample preparation steps of protocols B, C and D were conducted according to previous literature (Lan et al., 2019; Xu et al., 2021; Zhou et al., 2017). In this study, we compared the effects of different protocols (A, B, C and D) on the recovery of target compounds. As shown in Fig. S6, protocols A and D were used to process chicken tissue and egg samples spiked at 50 μg/kg for ERY, CLA and *N*-D-ERY, which resulted in good recovery. The results (Fig. S6) showed that the extraction recovery of the target analytes was significantly different with protocol A. Xu et al. (2021) reported a QuEChERS method using ZnO as an adsorbent (protocol D) to extract 2 lincosamides and 13 MACs from honey samples and analysed them by HPLC–MS/MS. The results showed that when ZnO was used as an adsorbent, protocol D successfully extracted the residues of ERY, CLA and *N*-D-ERY from chicken tissues and eggs. Compared with protocol A, the sample preparation time of protocol D is relatively long (approximately 40 min for each sample). In addition, the Cleanert MAS-Q cartridge not only saves time for weighing dispersers and adsorbents but also effectively adsorbs fat and other impurities in the extraction solution, thereby increasing the recovery and precision of the method. Thus, this study selected the QuEChERS method using a Cleanert MAS-Q cartridge (protocol A) to extract three targets from chicken tissues and eggs.

3.3. Matrix effect (ME) evaluation

The matrix effect could impact the ionization efficiency of the analytes, which plays an important role in the accuracy of the analysis. The ME of the analytes was calculated with the following method: ME = slope in the matrix-matched calibration curve/slope in the standard solution curve, where an ME of < 0.8 indicates a suppression effect, and

Table 3
Regression equations, determination coefficients, decision limits (CC_α), detection capabilities (CC_β) and matrix effects (MEs) of ERY, CLA and *N*-D-ERY in chicken tissues and eggs.

Matrix	Analyte	Regression equation	Determination coefficient (R^2)	CC_α (μg/kg)	CC_β (μg/kg)	ME
Muscle	ERY	y = 0.14x − 0.0039	0.9994	105.21	110.42	0.6
	CLA	y = 0.2105x − 0.008	0.9998	2.23	2.39	0.7
	N-D-ERY	y = 0.041x − 0.0033	0.9996	2.34	2.58	0.5
Liver	ERY	y = 0.1094x + 0.0316	0.9991	103.52	107.04	0.5
	CLA	y = 0.1995x + 0.0104	0.9987	2.19	2.32	0.7
	N-D-ERY	y = 0.04x + 0.0047	0.9997	2.36	2.61	0.5
Kidney	ERY	y = 0.1195x − 0.0162	0.9997	101.68	103.36	0.5
	CLA	y = 0.2545x − 0.0418	0.9992	2.27	2.46	0.9
	N-D-ERY	y = 0.0416x − 0.0048	0.9999	2.42	2.72	0.5
Whole egg	ERY	y = 0.1559x − 0.0836	0.9979	52.45	54.90	0.7
	CLA	y = 0.2793x − 0.0114	0.9995	2.15	2.26	1.0
	N-D-ERY	y = 0.0499x − 0.0086	0.9983	2.21	2.36	0.6
Albumen	ERY	y = 0.1528x − 0.0619	0.9988	53.26	56.52	0.7
	CLA	y = 0.2327x − 0.044	0.9996	2.20	2.34	0.9
	N-D-ERY	y = 0.0508x + 0.0045	0.9961	2.25	2.43	0.7
Yolk	ERY	y = 0.1265x + 0.0192	0.9994	54.62	59.24	0.6
	CLA	y = 0.2596x −0.0302	0.9978	2.17	2.29	0.9
	N-D-ERY	y = 0.0395x + 0.0153	0.9995	2.18	2.31	0.6

an ME of > 1.2 indicates an enhancement effect. The ME can be neglected in the range of 0.8–1.2, which indicates that the matrix effect is not obvious (Matuszewski et al., 2003).

As shown in Table 3, the results showed that ERY and *N*-D-ERY had suppressive matrix effects on chicken tissue (muscle, liver and kidney) and egg (whole egg, albumen and yolk) samples. However, CLA had no obvious matrix effects on the chicken kidney or egg (whole egg, albumen or yolk) samples, except for CLA, which had suppressive matrix effects on the chicken muscle and liver samples. Through this method, the matrix effect can be effectively attenuated by optimizing sample processing and chromatographic mass spectrometry steps, reducing the injection volume, adding internal standards, and using matrix-matched calibration curves (Côté et al., 2009). Therefore, internal standard and matrix-matched calibration curves were applied in the ERY, CLA and *N*-D-ERY residue analyses to reduce the influence of the matrix on the accuracy of the experimental results.

3.4. Method validation results

Blank chicken tissue (muscle, liver and kidney) and egg (whole egg, albumen and yolk) samples were analysed to determine the specificity of the method. No signals were detected in any of the blank chicken tissue or egg samples at the corresponding retention times of ERY, CLA, *N*-D-ERY or ROX.

Blank chicken tissue (muscle, liver and kidney) and egg (whole egg, albumen and yolk) samples were extracted and purified by the QuEChERS method (Section 2.4) to obtain a blank matrix extract. Standard working solutions diluted with different blank matrix extracts at six concentrations were examined through UHPLC–MS/MS to test their linearity. The matrix-matched calibration curves were plotted according to the ratio of the peak area of the quantified ion of each analyte to the quantitative ion of the internal standard and fortified concentration. As shown in Table 3, the method showed good linearity within the concentration range of 0.2–30 ng/mL, and the determination coefficients (R^2) were all greater than 0.9961. The LODs and LOQs of ERY, CLA and *N*-D-ERY in chicken tissues and eggs were 0.5 μg/kg and 2.0 μg/kg, respectively, which are sufficiently sensitive to support surveillance monitoring. The CC_α and CC_β results are shown in Table 3. All calculated CC_α and CC_β values were experimentally verified and are feasibly detectable. The method accuracy (recovery) and precision, including the intraday RSDs and interday RSDs, which were verified at three levels in chicken tissues and eggs, were satisfactory. As shown in Tables 4 and 5, the recoveries of ERY, CLA and *N*-D-ERY in chicken tissues and eggs were 87.78–104.22 %, the RSDs were 2.50–6.83 %, the intraday RSDs were 2.24–6.90 %, and the interday RSDs were 2.88–7.10 %. Thus, the validated method fully meets the monitoring requirements for antibiotic residues in chicken tissues and eggs.

3.5. Stability of the standards in methanol

The stability of the standard in the solution is an important factor affecting the accuracy of the experimental results. These studies reported that the MAC standards are easily soluble in methanol and ACN (Tao et al., 2012; Xu et al., 2021; Zhou et al., 2017). In this study, standard stock solutions and working solutions were prepared in pure methanol. This test evaluated the stability of individual standard stock solutions stored at −70 °C for 3 months, 100 μg/mL working mixed solutions stored at −20 °C for 3 months, and 10 and 1 μg/mL working mixed solutions stored at −20 °C for 1 month. Therefore, standard stock solutions and working solutions were properly stored under these conditions.

3.6. Comparison with other methods

Various analytical methods, including HPLC–MS/MS (Campanharo et al., 2023), HPLC with UV diode array detection (DAD) (Cañadas et al., 2022), electrochemical sensors (Lai et al., 2023; Li et al., 2022), TLC densitometric methods (Sharkawi et al., 2023), and UHPLC–MS/MS (Du et al., 2021), have been used for identifying ERY, CLA and other MACs in fish, milk, honey, egg and animal tissues. In previous studies, Wang et al. (2021) developed an LC with a time-of-flight (ToF)-MS/MS method to verify whether ERY and CLA can produce *N*-desmethyl metabolites in chicken liver microsomes. The results showed that ERY produced *N*-D-ERY in chicken liver microsomes after incubation for 60 min and after an NADPH cofactor was added. Since ERY is used to treat chickens artificially infected with chronic respiratory diseases, ERY and *N*-D-ERY are likely to remain in chicken tissues and eggs. Therefore, in this study, a fast and efficient method was developed for identifying ERY, CLA and *N*-D-ERY in chicken tissues and eggs.

Campanharo et al. (2023) established a QuEChERS-DLLME-HPLC–MS/MS method for the determination of ERY in fish. The recovery, precision, LOD and LOQ of ERY in fish were 103–110 %, 4.5–6.3 %, 0.1 μg/kg and 1 μg/kg, respectively. Cañadas et al. (2022) prepared a novel nanofunctionalized molecularly imprinted membrane (MIM) for the selective binding of MACs by a UV-initiated noncovalent imprinting approach. The milk samples were extracted and purified through the MIM-SPE method and analysed by HPLC-DAD. The average recovery of ERY from the milk samples was 86.1 %, and the RSDs were less than 6.0

Table 4
Recovery and precision of ERY, CLA and *N*-D-ERY identification when added to blank chicken tissues.

Matrix	Analyte	Added level (μg/kg)	Recovery (%) (n = 5)	RSD (%) (n = 5)	Intraday RSD (%) (n = 5)	Interday RSD (%) (n = 15)
Muscle	ERY	2	90.20 ± 2.80	3.10	2.68	2.89
		20	99.60 ± 4.17	4.19	5.11	5.09
		200	97.11 ± 4.44	4.57	3.14	4.28
	CLA	2	91.40 ± 3.81	4.17	4.25	4.36
		20	94.20 ± 3.92	4.16	2.81	3.65
		200	100.97 ± 4.16	4.12	5.55	4.97
	N-D-ERY	2	92.57 ± 4.91	5.30	3.13	3.83
		20	92.83 ± 2.87	3.09	3.15	3.51
		200	95.55 ± 2.97	3.11	2.36	3.38
Liver	ERY	2	93.57 ± 2.77	2.96	2.83	2.88
		20	97.25 ± 6.23	6.41	6.52	6.48
		200	94.68 ± 3.17	3.35	4.08	3.91
	CLA	2	94.08 ± 2.35	2.50	3.94	2.91
		20	95.30 ± 5.57	5.84	6.69	5.86
		200	100.67 ± 2.83	2.81	3.16	3.60
	N-D-ERY	2	92.97 ± 3.06	3.29	3.96	3.71
		20	95.12 ± 4.69	4.93	4.78	5.42
		200	91.47 ± 2.97	3.25	3.74	3.61
Kidney	ERY	2	90.35 ± 3.65	4.04	3.82	4.21
		20	89.60 ± 2.48	2.77	3.24	3.58
		200	93.77 ± 2.82	3.01	2.25	3.27
	CLA	2	92.18 ± 3.28	3.56	4.36	4.59
		20	94.25 ± 3.05	3.24	3.97	3.77
		200	94.03 ± 2.96	3.15	3.53	3.38
	N-D-ERY	2	89.40 ± 2.51	2.81	3.69	3.78
		20	90.95 ± 3.28	3.61	4.29	4.53
		200	89.72 ± 2.87	3.20	4.48	3.83

Table 5
Recovery and precision of ERY, CLA and *N*-D-ERY identification when added to blank eggs.

Matrix	Analyte	Added level (μg/kg)	Recovery (%) (n = 5)	RSD (%) (n = 5)	Intraday RSD (%) (n = 5)	Interday RSD (%) (n = 15)
Whole egg	ERY	2	91.75 ± 3.48	3.79	4.60	4.02
		20	93.75 ± 2.66	2.84	4.07	6.02
		200	104.22 ± 4.00	3.84	4.18	5.89
	CLA	2	92.07 ± 2.92	3.17	2.24	3.64
		20	96.68 ± 3.52	3.64	4.89	5.54
		200	99.32 ± 3.52	3.54	4.64	4.11
	N-D-ERY	2	93.52 ± 3.39	3.62	2.66	4.20
		20	101.35 ± 3.83	3.78	5.01	4.89
		200	96.52 ± 3.57	3.70	4.89	5.47
Albumen	ERY	2	92.77 ± 4.80	5.17	3.56	4.17
		20	87.88 ± 4.06	4.62	3.49	5.20
		200	95.40 ± 4.98	5.22	5.34	5.48
	CLA	2	92.97 ± 5.59	6.01	4.65	5.24
		20	88.23 ± 3.72	4.21	5.35	4.04
		200	96.98 ± 5.86	6.04	6.16	6.79
	N-D-ERY	2	90.62 ± 5.06	5.58	4.69	4.97
		20	91.28 ± 3.21	3.52	3.74	4.25
		200	96.05 ± 6.56	6.83	6.90	7.10
Yolk	ERY	2	90.07 ± 3.21	3.56	4.17	3.80
		20	90.83 ± 4.16	4.58	5.09	5.79
		200	99.05 ± 6.54	6.60	5.70	6.21
	CLA	2	94.18 ± 3.06	3.25	4.02	4.18
		20	102.70 ± 3.39	3.30	4.87	6.41
		200	92.50 ± 3.86	4.17	3.03	3.16
	N-D-ERY	2	87.78 ± 3.93	4.48	4.45	4.75
		20	89.87 ± 4.30	4.78	4.52	5.91
		200	91.48 ± 3.79	4.14	5.32	4.42

%. The LOD and LOQ of this method were 0.22 μg/kg and 0.75 μg/kg in milk samples, respectively, and the detection time was 30 min. Compared to DAD detection, MS/MS has more advantages in terms of selectivity and can effectively reduce matrix interference and false positive results, thereby improving the sensitivity and accuracy of the method. Lai et al. (2023) and Li et al. (2022) used electrochemical sensor technology to determine ERY residues in honey, pork and eggs and obtained good recoveries. The disadvantages of electrochemical sensors include poor sensitivity and reproducibility and long response times. It is difficult to detect antibiotic residues for some complex matrices. In addition, the electrode preparation program of an electrochemical sensor is relatively complicated and time-consuming, and it can only detect one or more antibiotics in the target. Sharkawi et al. (2023) developed a thin-layer chromatography (TLC) densitometric method for the analysis of ERY, sulfadiazine and trimethoprim in chicken muscle and liver samples. The recovery of ERY in chicken muscle and liver samples was 96.9–103.4 %, and the LODs and LOQs were 0.16 μg/band and 0.48 μg/band, respectively. Compared with other separation technologies, TLC has the advantages of simple operation, low cost, fast separation speed, and good separation ability. However, to obtain the best separation effect, the operator also needs to have certain professional skills, especially in terms of choosing the appropriate fixed phase and solvent.

In this study, we developed a QuEChERS-UHPLC–MS/MS method for the simultaneous detection of ERY, CLA and *N*-D-ERY in chicken tissues and eggs. The recoveries of ERY, CLA and *N*-D-ERY in chicken tissues and

Table 6
Comparison of the proposed method with previously published methods.

Detection method	Sample preparation method	Animal-derived food	Analyte	Recovery (%)	LOD (μg/kg)	LOQ (μg/kg)	Detection time (min)	Ref.
HPLC–MS/MS	QuEChERS-DLLME	Fish	ERY	103–110	0.1	1	7	Campanharo et al. (2023)
HPLC-DAD	MIM-SPE	Milk	ERY	86.1	0.22	0.75	30	Cañadas et al. (2022)
Electrochemical sensor	–	Honey	ERY	96.4–104.2	2.01 nM	–	–	Lai et al. (2023)
Electrochemical sensor	LLE	Pork and eggs	ERY	95.4–103.0	2.7 nM	–	–	Li et al. (2022)
TLC densitometric	LLE	Chicken tissues	ERY	96.9–103.4	0.16 μg/band	0.48 μg/band	15	Sharkawi et al. (2023)
UHPLC–MS/MS	QuEChERS	Chicken tissues and eggs	ERY, CLA and *N*-D-ERY	87.8–104.2	0.5	2.0	5	This study

Note: "-"Not reported.

eggs were 87.78–104.22 %, the intraday RSDs and interday RSDs were less than 7.10 %, and the LOD and LOQ were 0.5 μg/kg and 2.0 μg/kg, respectively. Compared with previously published methods (Table 6), the proposed method has the advantages of a shorter sample preparation time (approximately 20 min) and detection time (5 min), less consumption of organic reagents, and high sensitivity and accuracy. Therefore, this method is suitable for the rapid determination of ERY, CLA and *N*-D-ERY in chicken tissues and eggs.

3.7. Real sample analysis

To evaluate the applicability and accuracy of the newly developed method, 20 chicken muscles and 20 eggs were purchased randomly from different local markets. Each chicken muscle and egg (whole egg) sample was homogenized, numbered and then stored at −20 °C. These actual samples were extracted and purified by the above sample preparation method (Section 2.4) and then analysed by UHPLC–MS/MS. The experiment showed that the targets could not be detected in chicken muscles, and 12.5 μg/kg ERY was detected in egg sample-05, which was obviously lower than the MRL of 50.0 μg/kg in eggs in accordance with the Chinese standard. Hence, the optimized QuEChERS-UHPLC–MS/MS method can be applied to quantify ERY, CLA and *N*-D-ERY in chicken tissue and egg samples.

4. Conclusions

In this study, we developed a rapid, efficient and sensitive method for the determination of ERY, CLA and *N*-D-ERY in chicken tissues and eggs by UHPLC–MS/MS. The QuEChERS method based on a Cleanert MAS-Q cartridge was optimized to extract and purify samples and obtain good recoveries. The combination of QuEChERS and UHPLC–MS/MS shortens the presample processing and detection times, which improves the work efficiency. The results showed that the optimized QuEChERS-UHPLC–MS/MS method validation parameters met the requirements of the EU and the FDA. Finally, the reliability and applicability of the method can be verified by actual sample analysis, and the developed method can detect the residues of ERY, CLA and *N*-D-ERY in chicken tissues and eggs.

CRediT authorship contribution statement

Bo Wang: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Yali Zhu:** Validation, Software, Methodology. **Shuyu Liu:** Validation, Software, Investigation. **Han Zhang:** Resources, Methodology, Investigation. **Tianzhu Guan:** Resources, Conceptualization. **Xuechao Xu:** Validation, Methodology. **Xiangfeng Zheng:** Software, Resources, Funding acquisition. **Zhenquan Yang:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Tao Zhang:** Investigation, Conceptualization. **Genxi Zhang:** Resources, Investigation. **Kaizhou Xie:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This research was financially supported by the Jiangsu Funding Program for Excellent Postdoctoral Talent (2023ZB044), the National Natural Science Foundation of China (NSFC) grant (No. 31901801), the Foundation of China National Key Research & Development Program (2016YFC1300201), the Jiangsu Key Research & Development Program (BE2019436-5), the Natural Science Foundation of Jiangsu Province (BK20210814), the China Agriculture Research System (CARS-41), and the Priority Academic Programme Development of Jiangsu Higher Education Institutions (PAPD).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101468.

References

Benedé, S., & Molina, E. (2020). Chicken egg proteins and derived peptides with antioxidant properties. *Foods, 9*(6), 735. https://doi.org/10.3390/foods9060735

Beyene, T. (2016). Veterinary drug residues in food-animal products: Its risk factors and potential effects on public health. *Journal of Veterinary Science & Technology, 7*(1), 1–7. https://doi.org/10.4172/2157-7579.1000285

Boobis, A., Cerniglia, C., Chicoine, A., Fattori, V., Lipp, M., Reuss, R., Verger, P., & Tritscher, A. (2017). Characterizing chronic and acute health risks of residues of veterinary drugs in food: Latest methodological developments by the joint FAO/WHO expert committee on food additives. *Critical Reviews in Toxicology, 47*(10), 889–903. https://doi.org/10.1080/10408444.2017.1340259

Campanharo, S. C., da Silva, A. F. B., Bleuzen, A., da Silva, J. J. M., de Freitas, L. V. P., Assane, I. M., Pilarski, F., & Paschoal, J. A. R. (2023). The association of modified QuEChERS and DLLME to offer high analytical detectability to assess residual

depletion profile of erythromycin in fish. *Food Chemistry, 405*, Article 134852. https://doi.org/10.1016/j.foodchem.2022.134852

Cañadas, R., Martínez, R. M. G., González, G. P., & Hernando, P. F. (2022). Development of a molecularly imprinted polymeric membrane for determination of macrolide antibiotics from cow milk. *Polymer, 249*, Article 124843. https://doi.org/10.1016/j.polymer.2022.124843

Chinese Ministry of Agriculture and Rural Affairs. (2019). GB 31650-2019 National food safety standard-Maximum residue limits for veterinary drugs in foods.

Chinese Ministry of Agriculture and Rural Affairs. (2024). Monitoring plan for veterinary drug residues in livestock and poultry products in 2024.

Commission Regulation (EU). (2010). No.37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Official Journal of European Union, 15*, 1–72.

Côté, C., Bergeron, A., Mess, J.-N., Furtado, M., & Garofolo, F. (2009). Matrix effect elimination during LC–MS/MS bioanalytical method development. *Bioanalysis, 1*(7), 1243–1257. https://doi.org/10.4155/bio.09.117

de Mendonça Pereira, B. F., Pereira, M. U., Ferreira, R. G., & Spisso, B. F. (2021). Determination of macrolide antimicrobials in infant formulas using a modified alkaline QuEChERS and high-performance liquid chromatography coupled to tandem mass spectrometry. *Food Analytical Methods, 14*, 719–733. https://doi.org/10.1007/s12161-020-01905-1

Du, J., Li, X., Tian, L., Li, J., Wang, C., Ye, D., Zhao, L., Liu, S., Xu, J., & Xia, X. (2021). Determination of macrolides in animal tissues and egg by multi-walled carbon nanotube-based dispersive solid-phase extraction and ultra-high performance liquid chromatography–tandem mass spectrometry. *Food Chemistry, 365*, Article 130502. https://doi.org/10.1016/j.foodchem.2021.130502

European Commission. (2021). 2021/808/EC Commission implementing Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC. Official Journal of the European Communities, L180, 1–31.

Fan, M., Xiao, Q., Xie, J., Cheng, J., Sun, B., Du, W., Wang, Y., & Wang, T. (2018). Aroma compounds in chicken broths of Beijing Youji and commercial broilers. *Journal of Agricultural and Food Chemistry, 66*(39), 10242–10251. https://doi.org/10.1021/acs.jafc.8b03297

García-Mayor, M., Gallego-Picó, A., Garcinuño, R., Fernández-Hernando, P., & Durand-Alegría, J. (2012). Matrix solid-phase dispersion method for the determination of macrolide antibiotics in sheep's milk. *Food Chemistry, 134*(1), 553–558. https://doi.org/10.1016/j.foodchem.2012.02.120

Jank, L., Martins, M. T., Arsand, J. B., Motta, T. M. C., Hoff, R. B., Barreto, F., & Pizzolato, T. M. (2015). High-throughput method for macrolides and lincosamides antibiotics residues analysis in milk and muscle using a simple liquid–liquid extraction technique and liquid chromatography–electrospray–tandem mass spectrometry analysis (LC–MS/MS). *Talanta, 144*, 686–695. https://doi.org/10.1016/j.talanta.2015.06.078

Japan Food Chemical Research Foundation. (2015). Maximum residue limits (MRLs) list of agricultural chemicals in foods.

Jo, M. R., Lee, H. J., Lee, T. S., Park, K., Oh, E. G., Kim, P. H., Lee, D. S., & Horie, M. (2011). Simultaneous determination of macrolide residues in fish and shrimp by liquid chromatography-tandem mass spectrometry. *Food Science and Biotechnology, 20*, 823–827. https://doi.org/10.1007/s10068-011-0114-6

Juan, C., Moltó, J. C., Mañes, J., & Font, G. (2010). Determination of macrolide and lincosamide antibiotics by pressurised liquid extraction and liquid chromatography-tandem mass spectrometry in meat and milk. *Food Control, 21*(12), 1703–1709. https://doi.org/10.1016/j.foodcont.2010.05.004

Lai, T., Shu, H., Tian, X., Ren, J., Cui, X., Bai, H., Xiao, X. C., & Wang, Y. D. (2023). Electrochemical sensor based on molecularly imprinted poly-arginine for highly sensitive and selective erythromycin determination. *Journal of Materials Science: Materials in Electronics, 34*(1), 6. https://doi.org/10.1007/s10854-022-09405-0

Lan, C., Yin, D., Yang, Z., Zhao, W., Chen, Y., Zhang, W., & Zhang, S. (2019). Determination of six macrolide antibiotics in chicken sample by liquid chromatography-tandem mass spectrometry based on solid phase extraction. *Journal of Analytical Methods in Chemistry, 2019*, 6849457. https://doi.org/10.1155/2019/6849457

Li, N., Xie, D., Zhao, H., Yu, C., Li, Z., Li, F., & Cao, Q. (2022). A novel molecularly imprinted electrode modified with carbon nanohorn and polydopamine for highly sensitive determination of erythrocin in food. *Microchemical Journal, 181*, Article 107728. https://doi.org/10.1016/j.microc.2022.107728

Lundquist, P., Englund, G., Skogastierna, C., Lööf, J., Johansson, J., Hoogstraate, J., Afzelius, L., & Andersson, T. B. (2014). Functional ATP-binding cassette drug efflux transporters in isolated human and rat hepatocytes significantly affect assessment of drug disposition. *Drug Metabolism and Disposition, 42*(3), 448–458. https://doi.org/10.1124/dmd.113.054528

Matuszewski, B. K., Constanzer, M. L., & Chavez-Eng, C. M. (2003). Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS. *Analytical Chemistry, 75*(13), 3019–3030. https://doi.org/10.1021/ac020361s

Seyedi, Z., Esmaeilipour, O., Shirani, M., Rashidi Nodeh, H., & Mazhari, M. (2022). Heterogeneous adsorbent based on CeZrO2 nanoparticles doped magnetic graphene oxide used for vortex assisted magnetic dispersive solid phase extraction of erythromycin in chicken. *Food Additives & Contaminants: Part A, 39*(9), 1521–1530. https://doi.org/10.1080/19440049.2022.2096929

Sharkawi, M. M. Z., Safwat, M. T., Abdelaleem, E. A., & Abdelwahab, N. S. (2023). TLC densitometric analysis of triple antibiotic therapy; Erythromycin, Sulfadiazine and Trimethoprim in different edible chicken tissues. *Journal of Liquid Chromatography & Related Technologies, 46*, 79–88. https://doi.org/10.1080/10826076.2023.2220126

Sun, X., Ye, Y., Sun, J., Tang, L., Yang, X., & Sun, X. (2022). Advances in the study of liver microsomes in the in vitro metabolism and toxicity evaluation of foodborne contaminants. *Critical Reviews in Food Science and Nutrition, 1–15*. https://doi.org/10.1080/10408398.2022.2131728

Tao, Y., Yu, G., Chen, D., Pan, Y., Liu, Z., Wei, H., Peng, D., Huang, L., Wang, Y., & Yuan, Z. (2012). Determination of 17 macrolide antibiotics and avermectins residues in meat with accelerated solvent extraction by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography B, 897*, 64–71. https://doi.org/10.1016/j.jchromb.2012.04.011

The European Communities. (2002). Commission decision 2002/657/EC of 12 August 2002 implementing council directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Communities, 221*, 8–36.

U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, & Center for Veterinary Medicine. (2018). Guidance for industry: Bioanalytical method validation. U.S. Department of Health and Human Services.

U.S. Food and Drug Administration (2014). CFR-Code of Federal regulations title 21 part 556 tolerances for residues of new animal drugs in food. U.S. Food and Drug Administration.

Wang, J. (2009). Analysis of macrolide antibiotics, using liquid chromatography-mass spectrometry, in food, biological and environmental matrices. *Mass Spectrometry Reviews, 28*(1), 50–92. https://doi.org/10.1002/mas.20189

Wang, J., Leung, D., & Butterworth, F. (2005). Determination of five macrolide antibiotic residues in eggs using liquid chromatography/electrospray ionization tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 53*(6), 1857–1865. https://doi.org/10.1021/jf048414p

Wang, B., Nam, S., Kim, E., Jeon, H., Lee, K., & Xie, K. (2021). Identification of erythromycin and clarithromycin metabolites formed in chicken liver microsomes using liquid chromatography–high-resolution mass spectrometry. *Foods, 10*(7), 1504. https://doi.org/10.3390/foods10071504

Wang, B., Wang, Y., Xie, X., Diao, Z., Xie, K., Zhang, G., Zhang, T., & Dai, G. (2020). Quantitative analysis of spectinomycin and lincomycin in poultry eggs by accelerated solvent extraction coupled with gas chromatography tandem mass spectrometry. *Foods, 9*(5), 651. https://doi.org/10.3390/foods9050651

Wang, B., Xie, K., & Lee, K. (2021). Veterinary drug residues in animal-derived foods: Sample preparation and analytical methods. *Foods, 10*(3), 555. https://doi.org/10.3390/foods10030555

Xu, J., Yang, M., Wang, Y., Yang, Y., Tu, F., Yi, J., Hou, J., Lu, H., Jiang, X., & Chen, D. (2021). Multiresidue analysis of 15 antibiotics in honey using modified QuEChERS and high performance liquid chromatography-tandem mass spectrometry. *Journal of Food Composition and Analysis, 103*, Article 104120. https://doi.org/10.1016/j.jfca.2021.104120

Yan, Z., Zhang, X., Li, P., Fang, Y., Yu, L., Zhang, S., Zhu, Y., & Li, Q. (2017). QuEChERS-ultra performance liquid chromatography-tandem mass spectrometry for the determination of erythromycin in fishy products. *Journal of Zhejiang University (Science Edition), 44*(3), 339–344. https://doi.org/10.3785/j.issn.1008-9497.2017.03.015

Zhang, C., Deng, Y., Zheng, J., Zhang, Y., Yang, L., Liao, C., Su, L., Zhou, Y., Gong, D., & Chen, L. (2019). The application of the QuEChERS methodology in the determination of antibiotics in food: A review. *TrAC Trends in Analytical Chemistry, 118*, 517–537. https://doi.org/10.1016/j.trac.2019.06.012

Zhou, W., Ling, Y., Liu, T., Zhang, Y., Li, J., Li, H., Wu, W., Jiang, S., Feng, F., & Yuan, F. (2017). Simultaneous determination of 16 macrolide antibiotics and 4 metabolites in milk by using Quick, Easy, Cheap, Effective, Rugged, and Safe extraction (QuEChERS) and high performance liquid chromatography tandem mass spectrometry. *Journal of Chromatography B, 1061*, 411–420. https://doi.org/10.1016/j.jchromb.2017.07.046