Comprehensive multiplexed analysis of risky drugs in eggs based on magnetic zeolitic imidazolate frameworks and UHPLC Q-Orbitrap HRMS

Follow this and additional works at: https://www.jfda-online.com/journal

Part of the Food Science Commons, Medicinal Chemistry and Pharmaceutics Commons, Pharmacology Commons, and the Toxicology Commons

This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 4.0 License.

Recommended Citation
Jia, Wei; Liu, Yuyang; Xu, Xi; Zhang, Yanxi; and Shi, Lin (2021) "Comprehensive multiplexed analysis of risky drugs in eggs based on magnetic zeolitic imidazolate frameworks and UHPLC Q-Orbitrap HRMS," Journal of Food and Drug Analysis: Vol. 29 : Iss. 3 , Article 7.
Available at: https://doi.org/10.38212/2224-6614.3360

This Original Article is brought to you for free and open access by Journal of Food and Drug Analysis. It has been accepted for inclusion in Journal of Food and Drug Analysis by an authorized editor of Journal of Food and Drug Analysis.
Comprehensive multiplexed analysis of risky drugs in eggs based on magnetic zeolitic imidazolate frameworks and UHPLC Q-Orbitrap HRMS

Wei Jia*, Yuyang Liu, Xi Xu, Yanxi Zhang, Lin Shi

Abstract

A novel magnetic adsorbent solid-phase Fe₃O₄@ZIF-8 extraction method coupled with UHPLC Q-Orbitrap high resolution mass spectrometry for the sensitive and high efficiency detection of fluoroquinolones, tetracyclines, dyes and amphenicols in eggs is described. Under optimum conditions, CCᵦ and CCᵦ of 0.62 to 26.97 µg.kg⁻¹ and 0.36 to 16.37 µg.kg⁻¹ was obtained, which is much better than that obtained by conventional methods. The accuracy, expressed as recovery, were achieved falling between 75% and 104%. The proposed strategy provides several advantages of simplicity and low energy consumption, thus retaining potential for the screening of risky drugs in various samples.

Keywords: Egg, Magnetic extraction, Matrix effect, Q-Orbitrap, Veterinary drugs

1. Introduction

Hen's eggs (Gallus gallus domesticus eggs) are widely consumed in daily diet, which contain essential and well-balanced nutrients, including lipids, proteins, minerals, vitamins, and bioactive compounds. The most effective and typical treatment and prevention of hen's common bacterial infection is adding prophylactic medication with antibiotics in the feed [1]. Several fluoroquinolones, tetracyclines and amphenicols are licensed mainly as veterinary drugs for the therapeutic treatment of bacterial infection in the EU and China [2]. At least 40 countries were involved in the fipronil contaminated poisonous egg scandals recently, which resulted in public health risks and substantial economic losses [3]. The excess intake of antibiotics can cause various human health problems like allergic reaction, diarrhea, infections of skin and bone joint etc [4]. In addition, sudan and rhodamine dyes have been banned as illegal food additives due to their carcinogenicity [5]. Several methods have already been applied on the broad scope extraction in complex matrices, such as dispersed solid phase extraction (d-SPE) approach [6], QuEChERS method [7, 8], and matrix solid phase dispersion (MSPD) [9]. It represents a crucial analytical challenge for eggs, in which the contents of ≥12% for both protein and fat are liable to cause matrix effects in extractions procedure, analyzing of multi-residues, contaminants, meanwhile affecting reliability, robustness and sensitivity [10].

Major limit of analytes extraction with different polarity can be put forward. The polarity of most characterized contaminants is stronger than that of the weak basis or acid, thus affecting the reliability. The magnetic solid-phase extraction (MSPE) has been arisen as a useful choice for complex extraction procedures with effective cleaning and high recoveries in the tested matrices recently [11-14]. Ionic liquid magnetic zeolite imidazolate framework-8 (M/ZIF-8) was employed to collect aflatoxins in milk samples with good recoveries (79.0–102.5%)
and RSD (<7.7%) [15]. Though various materials possessed satisfactory absorbability for compound, application of multiple compounds in complex matrices was rare. Zeolitic imidazolate frameworks (ZIFs) are built on tetrahedral units with 4-connected nets structures via intermolecular hydrogen bonds to ensure efficient adsorption and heterogeneous catalysis. The method had stable property in various environments and superb absorbability from low to high molecules [16]. ZIFs are a sub-family of metal organic frameworks (MOFs) which are crystalline compounds. MOFs possess unique properties like large surface area, abundant functional groups and tunable pore size, and those characteristics endow it an excellent adsorption capacity [15, 16]. At present, ZIF materials have been used widely in food analysis, among which ZIF-8 and ZIF-67 could rapidly adsorb sulfur mustard and 2-chloroethyl ethyl sulfide from aqueous solution within 1 min [17]. Simultaneously, ZIF-8 could act as a pre-concentration material to collect phthalate esters in water samples [18] and be modified with other nano-composites or metal, as chlorophenols collector from honey tea and removal pollutants [19]. To get high resolution and excellent scan sensitivity, ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry has been particularly recommended for the simultaneous analysis of multi-residue in complex matrices, such as eggs, honey, milk and fruits [20, 21]. It allows the selective and sensible identification and quantification of the trace concentration level compounds, especially in the case of co-elution or matrix interferences [22-24]. This study provides a powerful strategy for simultaneous screening of enrofloxacin, tetracycline, oxytetracycline, chlorotetracycline, doxycycline, sudan I, sudan II, sudan III, sudan IV, thiamphenicol and florfenicol in eggs based on MSPE coupled with UHPLC-Q-Orbitrap. This analytical proposal for multi-residue determination provides an effective method to ensure food safety.

2. Materials and methods

2.1. Chemicals and regents

Acetonitrile and methanol were obtained from Merck (HPLC-grade, Darmstadt, Germany). Ammonium formate and formic acid were purchased from Tian Li (HPLC-grade, Tianjin, China). Anhydrous magnesium sulfate, anhydrous sodium sulfate, anhydrous sodium acetate and sodium chloride were obtained from Shan Pu (HPLC-grade, ShangHai, China). Ethylene glycol, zinc nitrate hexahydrate, 2-methylimidazole poly (styrrenesulfonate, sodium salt) (30 wt%) and ferric chloride hexahydrate were analytical reagent and purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). PVDF syringe filters (0.22 μm) were supplied by Welch (Maryland, USA).

Tetracycline, oxytetracycline, chlortetracycline and doxycycline were purchased from Agro-Environmental Protection Institute (Tianjin, China). Sudan I, sudan II, sudan III, sudan IV, enrofloxacin, thiamphenicol and florfenicol were obtained from Dr. Ehrenstorfer (Augsburg, Germany). All standards were HPLC grade and the structures of the eleven standards are shown in Fig. S1A. Stock standard solutions (100 mg L\(^{-1}\)) were prepared by dissolving 10 mg of each compound in 100 mL methanol and working standard solutions at the concentration of 50–250 μg L\(^{-1}\) were prepared by dissolving the appropriate volume of each stock solution in methanol (consisted of 4 mmol L\(^{-1}\) ammonium formate and 0.2% formic acid). All standard solutions were stored at −20 °C in light-resistant containers.

2.2. Sample collection and preparation

ZIF-8 was fabricated by solvothermal method [16]: 2.0 g zinc nitrate hexahydrates were dissolved in 100 mL methanol as solution I, and 6.0 g 2-methylimidazoles were dissolved in 100 mL methanol as solution II. A quick stir mixt solution I with II at 40 °C water bath for 6 h, then ZIF-8 was collected after vacuum drying 10 h at 50 °C. Thus, 6.75 g ferric chloride hexahydrates were scattered in 70 mL ethylene glycol solution at 60 °C and stirred until that yellow transparent solution was generated, followed by the addition of 15.0 g anhydrous sodium acetate. The homogeneous solution was transferred to the 100 mL PTFE autoclave and reacted 10 h under 200 °C. After cooling to room temperature, the ferrosferric oxide magnetic nanoparticles were collected by using Nd-Fe-B strong magnet and dried 10 h at 80 °C. Poly (styrrenesulfonate, sodium salt) can modify the surface of ZIF-8 in solution and improve its particle stability with polydispersity index decreased. 2.0 g ferrosferric oxide magnetic nanoparticles were mixt with 70 mL 0.5% poly (styrrenesulfonate, sodium salt) and ultrasound for 20 min, while the modified magnetic nanoparticles (ferrosferric oxide) were collected after washing the mixture three times and then mixing with...
140 mL methanol, 5 g 2-methylimidazole and 2.5 g zinc nitrate hexahydrate. After swirling at room temperature for 3 min and stirring at 45 °C for 6 h, the products were washed with ultra-pure water until neutral and dried 10 h in vacuum at 50 °C then ground into fine powder (Fig. S1B).

Egg samples for analysis were collected from twelve local markets in Xi’an City (Shaanxi Province, China) and stored at 4 °C according to the standardized criteria [8]. 99 egg samples (containing 10% spike samples) were selected randomly in packs of 30 each, and each one was analyzed in sextuplicate. 1.0 g homogenized egg samples (including both white and yolk) were weighed in 50 mL polypropylene centrifuge tubes and vortexed for 5 min at room temperature to 12.5 mL with acetonitrile solution followed by 2.5 mL Na2EDTA-Mcllvaine buffer (0.1 mol L⁻¹, pH = 5.0) added and vortexed. 1.5 g sodium chloride, 1.8 g anhydrous sodium acetate and 1.0 g anhydrous magnesium sulfate were added to weaken emulsification. After centrifuged at 14000×g for 10 min, the supernatant was transferred to 50 mL polypropylene tube containing 30 mg Fe₃O₄@ZIF-8 and then vortexed for 5 min. The magnetic microspheres were collected with the help of Nd-Fe-B strong magnet and then 1.5 mL 0.1% formic acid in acetonitrile was added for the desorption with a vortex for 1 min. Finally, the supernatant of extracts was filtered by a syringe filter for the UHPLC Q-Orbitrap analysis. Eggs in which no studied compounds were detected, were used as blank according to published study [8].

2.3. Instrumentation

The experiments were performed in a Dionex Ultimate 3000 UPLC system (Thermo Fisher Scientific, USA) coupled to Quadrupole-Orbitrap HRMS (Q-Exactive, Thermo Fisher Scientific, San Jose, USA). A Thermo Hypersil GOLD aQ C18 column (150 mm × 2.1 mm, 5 μm) was used for the chromatographic separation (Thermo Fisher Scientific, San Jose, USA) at 35 °C. The injection volume was 5 μL and the flow rate was 0.3 mL min⁻¹. The mobile phase A was consisted of 4 mmol L⁻¹ ammonium formate in water with 0.2% formic acid, and mobile phase B was consisted of 4 mmol L⁻¹ ammonium formate in methanol with 0.2% formic acid. The gradient elution program was applied as follows: 0–1 min, 0% B; 1–7 min, 0–100% B; 7–15 min, 100% B; 15–17 min, 100–0% B; 17–25 min, 0% B.

The Quadrupole-Orbitrap HRMS was furnished with a heated electrospray ionization source (ESI). Parameters in positive ion mode were set as follows: vaporizer temperature (°C), 350; spray voltage (KV), 3.5; sheath gas, 4.8; auxiliary gas, 1; auxiliary gas heater temperature (°C), 350. Nitrogen was used as nebulizer and drying gas. A full MS scan (m/z 50–750) was acquired in the Orbitrap with the resolution setting of 70,000 fwhm; automatic gain control (AGC) target: 1.0E6; maximum ion injection time (ms): 50. The precursor-ion was isolated by the quadrupole and sent to the higher energy collision-induced dissociation (HCD) cell for fragmentation via the C-trap. Resolution for HCD spectra was set to 35,000 fwhm; AGC target: 2.0E5; isolation width: 1 m/z; the normalized collision energy: 30. The system was controlled by Tune 2.8 and Xcalibur 4.0. Two fragments were selected for each compound with high intensity and specificity as well as quantification and the identification (Table 1).

Magnetic property was assessed by a Lake-shoreVSM-7304 (Westerville, USA) and the Bru- nauer-Emmett-Teller (BET) test was measured via ASAP 2460 system (Micromeritics Co., Norcross, GA, USA). FT-IR spectroscopy were carried out via a Nicolet iS 5 infrared spectrometers (Thermo Fisher, USA). A JEM-2100F instrument (JEOL, Tokyo, Japan) operated with 200 kV was utilized to record transmission electron microscopy (TEM) images.

2.4. Statistical analysis and validation procedure

Statistical analyses were executed by Microsoft Excel 2016 (Microsoft Co., USA) software and OriginPro 8.0 (OriginLab Corporation, Northampton, USA), by which one-way ANOVA was accomplished and p value < 0.05 considered as significant. ChemDraw (Professional Cambridge Soft Corporation, Cambridge, MA, USA) was used to design organic structural molecule and reaction notations.

The validation of method was performed according to the procedures described in the European Commission Decision 2002/657/EC [25] and SANCO/12571/2013 [26]. The information of limit of detection (LOD), linearity, selectivity, matrix effect, decision limits (CCₐ), detection capability (CCₜ), accuracy and precision were evaluated. Precision analyses were conducted by three spiking levels, 150, 300 and 450 μg kg⁻¹ with intra-day and inter-day (n = 6) reproducibility studies. All parameters were evaluated for the acceptance of the Codex Alimentarius.
3. Results and discussion

3.1. Characterization of the Fe₃O₄@ZIF-8 composites

The morphology and structure characterization of synthetic materials are shown in Fig. 1. TEM image presents the neat crystal morphology of Fe₃O₄@ZIF-8 with consistent particle size distribution (most are 200 nm) and smooth particle surface (Fig. 1A and B). Fe₃O₄ cores and ZIF-8 shells compose magnetism Fe₃O₄@ZIF-8, as shown in Fig. 1C.

The Fe₃O₄@ZIF-8 represents a typical isotherm of type II with a small hysteresis in Fig. 1D, indicating the porous structure were developed of it [16]. When the relative pressure (P/P₀) was greater than 0.9, the adsorption amount of the material increased sharply, demonstrating that the N₂ was filled with spaces between particles and there were mesoporous structures in the material. The BET surface of Fe₃O₄@ZIF-8 was 78.951 m²/g and the BJH adsorption cumulative surface area of pores between 1.7000 nm and 300.0000 nm diameter was 4.5852 m²/g while desorption cumulative surface area was 3.9983 m²/g. Fig. 1E shows the hysteresis

Table 1. UHPLC Q-Orbitrap HRMS parameters of the eleven analytes.

| Analyte          | RT (min) | Matrix effects (%) | Molecular formula       | Exact mass (m/z) | Theoretical mass (m/z) | Experimental mass (m/z) | ΔMass (ppm) | Fragment 1 (m/z) | Fragment 2 (m/z) |
|------------------|----------|--------------------|-------------------------|------------------|------------------------|------------------------|-------------|-----------------|-----------------|
| Amphenicols      |          |                    |                         |                  |                        |                        |             |                 |                 |
| Florfenicol      | 7.4      | –4.88              | C₁₀H₁₄Cl₂FNO₅S          | 358.00774        | 358.00719              | 1.54                   | 119.03771   | 185.02669       |                 |
| Thiapenicol      | 8.2      | 13.69              | C₁₂H₁₆Cl₂N₂O₅S          | 356.01208        | 356.01196              | 0.34                   | 155.96136   | 185.02669       |                 |
| Fluoroquinolones |          |                    |                         |                  |                        |                        |             |                 |                 |
| Enroloxacin      | 8.4      | 11.25              | C₁₀H₁₄FN₂O₇             | 360.17180        | 360.17084              | 2.67                   | 245.04827   | 316.18197       |                 |
| Tetracyclines    |          |                    |                         |                  |                        |                        |             |                 |                 |
| Tetracycline     | 8.0      | 12.27              | C₁₄H₁₆Cl₂N₂O₈           | 445.16054        | 445.16045              | 0.20                   | 154.04987   | 321.07575       |                 |
| Oxytetracycline  | 8.1      | –12.50             | C₁₄H₁₆Cl₂N₂O₈           | 461.15556        | 461.15536              | 0.43                   | 201.05462   | 426.11834       |                 |
| Chlortetracycline| 8.7      | –17.23             | C₁₄H₁₆Cl₂N₂O₈           | 479.12157        | 479.12220              | 1.31                   | 154.04987   | 323.03169       |                 |
| Doxycycline      | 9.3      | –14.98             | C₁₄H₁₆Cl₂N₂O₈           | 445.16054        | 445.16042              | 0.27                   | 410.12343   | 428.13399       |                 |
| Dyes             |          |                    |                         |                  |                        |                        |             |                 |                 |
| Sudan I          | 12.1     | –12.39             | C₈H₁₀N₂O₂               | 249.10224        | 249.10188              | 1.45                   | 105.04472   | 231.09167       |                 |
| Sudan II         | 13.4     | 9.39               | C₈H₁₀N₂O₂               | 277.13354        | 277.13319              | 1.26                   | 119.06037   | 127.05423       |                 |
| Sudan III        | 15.8     | 11.50              | C₈H₁₀N₂O₂               | 353.13969        | 353.13990              | 0.59                   | 143.04914   | 180.06820       |                 |
| Sudan IV         | 17.6     | 15.69              | C₁₀H₁₂N₂O₂              | 381.17099        | 381.17069              | 0.79                   | 104.04948   | 143.04914       |                 |

Fig. 1. TEM image of Fe₃O₄@ZIF-8 at different magnification (A–C). Nitrogen adsorption-desorption isotherms (D) of Fe₃O₄@ZIF-8, magnetic hysteresis loop (E) of Fe₃O₄@ZIF-8, FT-IR spectra images (F) of adsorbent.
curve of Fe₃O₄ and Fe₃O₄@ZIF-8 in which the maximum saturation magnetization (Ms) were 117 and 102 emu g⁻¹ respectively. The Fe₃O₄@ZIF-8 could be easily dispersed evenly in aqueous solutions with the help of external magnetic field, since the higher Ms value, the stronger magnetic. Fig. 1F displays FT-IR spectra images of Fe₃O₄, ZIF-8 and Fe₃O₄@ZIF-8, in which the peaks at 3138 and 2933 cm⁻¹ were attributed to the stretching vibration of the C–H bond of methyl group and imidazole ring. The signals at 1583 and 1420 cm⁻¹ were induced by the stretching of C≡N. The bands in 600–1500 cm⁻¹ were related to the vibration of the imidazole material.

### 3.2. Optimization of the extraction procedure

Simultaneous extraction of different kinds of drugs has been plagued by their physicochemical properties, such as polarity, solubility and pKa value. Tetracyclines were chelated with metal ions that may chelate with tetracyclines, improving the recoveries. Given emulsification, magnesium sulfate was used to reduce the effect of that, which provided better results in recoveries for eggs matrix because of the change of solution polarity and moisture in the organic phase.

This proposal provided a better purification effect in the tested matrix with the usage of Fe₃O₄@ZIF-8. The recovery of studied compound was higher than 75% with relative standard deviations (RSD) ≤ 9% in most cases (Table 2) and reproducibility for 2%–8%. To obtain the best optimal extraction, the adsorption amount, adsorption time, desorption solvent and elution volume were optimized, as well as the effect of pH and salinity. The amount of magnetic adsorbent varied from 5 to 35 mg was optimized as shown in Fig. 2A. With the increasing of amount, the recovery rate of extraction efficiency also increased until 30 mg. Nevertheless, more adsorbent after 30 mg, the recoveries were tended to be stable. The better adsorption was acquired with fewer amounts because of the porous structure and large specific surface area of the synthesized material.

### Table 2. Linearity, CC, LOQ, reproducibility and recovery obtained in the validation experiments.

| Compound | Calibration | R² | LOQ (μg·kg⁻¹) | RSD | LOD* (μg·kg⁻¹) | Reproducibility (%) | RSD |
|----------|-------------|----|---------------|-----|---------------|---------------------|-----|
| Amphenicols | y = 8.1x + 2.2 | 0.9991 | 1.20 | 0.9 | 1 | 0.3 | 103 4 100 2 93 6 |
| Florfenicol | y = 18.4x + 0.7 | 0.9985 | 0.55 | 0.27 | 0.9 | 3 | 85 2 82 2 83 4 |
| Enrofloxacin | y = 11.2x + 3.2 | 0.9992 | 10.81 | 0.62 | 1.2 | 3 | 85 4 82 2 93 4 |
| Ciprofloxacin | y = 11.2x + 3.2 | 0.9996 | 14.91 | 0.12 | 0.6 | 3 | 85 2 85 1 87 3 |
| Doxycycline | y = 11.2x + 3.2 | 0.9997 | 16.37 | 0.91 | 3 | 90 1 91 3 92 1 |
| Sudan I | y = 13.2x - 10.8 | 0.9982 | 4.02 | 6.63 | 3.4 | 3 | 92 1 91 3 92 4 |
| Sudan II | y = 17.0x - 10 | 0.9982 | 2.72 | 4.49 | 2.2 | 1 | 97 3 95 1 92 4 |
| Sudan III | y = 17.0x - 10 | 0.9992 | 3.06 | 5.07 | 3.2 | 1 | 91 9 90 4 85 1 |
| Sudan IV | y = 10.8x + 1.3 | 0.9998 | 5.30 | 8.75 | 2.2 | 1 | 93 4 85 2 83 6 |
| LOD* | limit of detection; R* recovery (%) | 82.5 |

*Note: RSD = relative standard deviation; LOD = limit of detection; R* recovery (%)
Different substances were competed the adsorption of magnetic materials in the complex matrix. The adsorption time was studied as an important parameter which was ranged from 1 to 13 min (Fig. 2B). The adsorption efficiency was tended to be stable after 5 min. Shorten adsorption time led inadequate adsorption but extend adsorption time led decreased efficiency, due to the co-extraction between substances. Therefore, 5 min was employed as the appropriate adsorption time for an enough extraction.

Two extraction conditions were designed for the evaluation of the effect of salinity according to published paper [11], including (1) 2.5 mL of 0.1 M Na₂EDTA solution combined 1.5 g sodium chloride, 1.8 g anhydrous sodium acetate and 1.0 g anhydrous magnesium sulfate; (2) 2.5 mL of 0.1 M Na₂EDTA solution combined 1.5 g sodium chloride, 1.8 g anhydrous sodium acetate and 1.0 g anhydrous sodium sulfate. However, no significant differences (p = 0.395) in recoveries of targets analytes were observed. The effect of pH was tested over the range of 2-8 and the extraction efficiency of targets analytes were shown in Fig. 2C. The adsorption efficiency was improved obviously over the range of 2-5 while recoveries of some analytes tended to be decreased, might due to the inhibition of co-extraction between substances and magnetic adsorbents. Given the results, 2.5 mL of 0.1 M Na₂EDTA-Mcllvaine buffer (pH = 5.0), 1.5 g sodium chloride, 1.8 g anhydrous sodium acetate and 1.0 g anhydrous magnesium sulfate was the appropriate choice.

Five solvents were assessed for optimal extraction and recovery respectively, including methanol, acetonitrile and 0.1% formic acid in water, acetonitrile along with methanol (Fig. 2D). Comparing with methanol, acetonitrile had better recovery and could be enhanced by the addition of formic acid. The acidic solution could obtain higher extraction efficiency because of the hydrogen bonding of the acid compounds in acidic environment [18]. Fig. 2E shows the influence of elution volume in the range of 0.5–3.0 mL. The recovery of 0.5 mL was over than 60% while 1.5 mL could reach above 90%, suggesting that 1.5 mL was the optimal volume for the elution.

3.3. Interactions between Fe₃O₄@ZIF-8 and drugs

Previous studies have declared better performance of ZIF-8 in acidic drugs extraction because of its porous structure and positive Lewis acid sites [27]. It was reported that Fe₃O₄@ZIF-8 performed a good adsorption performance because its high valence metal ions and specific active sites in surface [28]. By the comprehensive analysis of the adsorption and elution time, it could be found that not only the large cages and small apertures in Fe₃O₄@ZIF-8 structure worked, but also other multiple interactions. Owing to the open metal sites of Zn (II) whose coordinated number is four, the
possible interactions between Fe₃O₄@ZIF-8 and drugs were like the coordination bonds between open metal sites and –OH or phenol group. Meanwhile, the existence of intermolecular π-π interaction was associated with the respective presence of imidazole ring and phenyl ring in adsorbent microspheres as well as drugs. Hydrophobic imidazole rings structure was also aroused the hydrophobic interactions between adsorbent microspheres and drugs. Furthermore, the electrostatic attraction availed extraction since the hydrogen-bond interactions occurred between ionizable compounds of some hydroxyl groups in drugs and Fe₃O₄@ZIF-8 during adsorption. Drugs can provide H-bond donors and Fe₃O₄@ZIF-8 has H-bond acceptors [27]. Additionally, the van der Waals interactions also occurred between these small molecule drugs and adsorbent. All these multiple interactions and prominent surface properties inspired the adsorption of analytes in molecular state, making Fe₃O₄@ZIF-8 an efficient adsorbent and showing the excellent extraction performance. After absorption, the adsorbents were recovered easily with 0.1% formic acid in acetonitrile during the desorption process, which certified the existent of both physisorption and chemisorption as illustrated in the study [28].

3.4. Validation of the proposed method

The matrix effect (ME) is important in the method validation because the ion suppression or enhancement may affect the quantification of the multiple classes compounds [29]. The egg extracts contained plenty of matrix components such as protein, lipid, and carbohydrate, leading the interference of coeluting matrix components in the ionization of the target compounds. Strong MEs were observed for oxytetracycline (−78.11%), chlortetracycline (−59.73%) and florfenicol (65.13%) and the ME (%) was evaluated by the following equation.

\[
\text{ME} \, (\%) = \left( \frac{\text{peak area of standard in matrix}}{\text{peak area in solvent}} - 1 \right) \times 100
\]

There was no matrix effect when the value of ME (%) was from −20 to 20% while the value was greater than 20% considered as enhancing effect or less than −20% as suppressing effect. As shown in Table 1, low MEs (from −17.23 to −4.88%) were obtained for three drugs after using Fe₃O₄@ZIF-8. Acceptable ME values (from −17.23% to +15.69%) were observed for eleven drugs, which indicated that the MSPE procedure could successfully extract...
multiple classes of risky substances from egg samples and eliminate most of the matrices. The matrix-matched calibration was utilized to recede the matrix effect and acquire accurate quantitative results. The matrix-matched calibration curves were evaluated from 150 to 750 μg.kg⁻¹ with five levels, in sextuplicate for individual compounds. Taking enrofloxacin, tetracycline, sudan I and thiamphenicol as examples, Fig. 3 shows their standard and matrix-matched curves.

CC₁ and CC₂ were ruled by the following equations (Eqs. (2) and (3)), which were evaluated as described in European Commission Decision 2002/657/EC. Maximum residue limit (MRL) values were in accordance with 2002/657/EC and the VL (validation level) was chose for analytes without established MRL. μMRL was the mean measured concentration of blanks spiked at the MRL while σMRL was the standard deviation of within-day repeatability. The calculations of CC₁ and CC₂ were based on the minimum required performance level, and they showed highly satisfactory results with the suitable extraction method (Table 2).

$$CC_1 = \mu\text{MRL} + 1.64 \times \sigma\text{MRL}$$ (2)

$$CC_2 = CC_1 + 1.64 \times \sigma\text{MRL}$$ (3)

The CC₁ values of fluoroquinolones, tetracyclines, dyes and amphenicols were varied from 0.36 to 16.37 μg.kg⁻¹, while the CC₂ values were ranged from 0.62 to 26.97 μg.kg⁻¹. The linearity was evaluated by the standard solutions of eleven drugs spiked in the blank samples (n = 6). The R² values of linear evaluation were greater than 0.99, indicating that the established model was suitable for all the studied compounds.

Because the residue limits of fluoroquinolones and amphenicols in eggs and all studied dyes are uncertain, their appearance means disqualification of sample. The accuracy and precision of the method were assessed by recovery while RSD were obtained under repeatability (intraday precision) and precision (interday precision) respectively. The

Table 3. Detection results of real-samples from twelve local markets (in sextuplicate).

| Analyte          | Positive Samples (concentration, μg.kg⁻¹)                  |
|------------------|------------------------------------------------------------|
| Fluoroquinolones | Sample 1 (149.2 ± 0.5*), Sample 18 (220.5 ± 0.9*)        |
| Enrofloxacin     | Sample 9 (236.2 ± 1.2*), Sample 18 (117.7 ± 1.3*), Sample 32 (327.3 ± 1.3**) |
| Tetracyclines    | Sample 2 (225.1 ± 0.9*), Sample 9 (161.2 ± 1.3*), Sample 32 (189.8 ± 1.1*) |
| Oxytetracycline  | Sample 1 (390.4 ± 1.6**), Sample 2 (296.0 ± 1.4**), Sample 9 (231.1 ± 1.0*), Sample 32 (242.3 ± 1.1**) |
| Amphenicols      | Sample 6 (323.1 ± 0.6**), Sample 7 (217.8 ± 0.8*), Sample 10 (296.6 ± 0.2**) |

*p < 0.05.

**p < 0.01.
homogeneous samples were analyzed in short time intervals under the same conditions (n = 6) and the results as % RSD of peak areas were ranged from 1–9 with good precision. Individual average recovery values were in range of 75% to 104%. Both recovery levels and RSD were within the limits set by European Regulations, showing the acceptable accuracy and precision of this method.

3.5. Sample analysis

The established method was applied to the analysis of egg samples which were collected from twelve local markets (n = 6) and derived from different origins in Xi’an City (Shaanxi Province, China). 99 egg samples were analyzed by the established method under the optimum experimental conditions and the extracted ion chromatogram and spectra from a full MS/dd-MS2 experiment using UHPLC-Q-Orbitrap in spiked sample (100 µg.kg⁻¹) are shown in Fig. 4. To further evaluate the established method, a comparison between the present work and other previous publications was performed. Both instrumentals errors and confidence range were discussed, including recovery, linearity, LOD and RSD (%). In view of the pretreatment method in Table S1, various materials utilized to enrich contaminants in eggs were compared. MSPE was a novel option as the convenient pretreatment to collect multi pollutants from eggs while most of the pretreatment was QuEChERS method. Although magnetic materials in food analysis have a wide range of applications with simple and easy operation, the use of Fe₃O₄@ZIF-8 is rare to extract multi residues from egg matrix. Nine samples were found positive for different compounds related to fluoroquinolones, tetracyclines and amphenicols, and all results were presented in Table 3. The positive responses were obtained including enrofloxacin (two samples) ranged from 148.7 to 221.4 µg.kg⁻¹, tetracycline (three samples) from 116.4 to 328.8 µg.kg⁻¹, oxytetracycline (three samples) from 159.9 to 226.0 µg.kg⁻¹, thiamphenicol (three sample) from 217.0 to 323.7 µg.kg⁻¹ and florfenicol (four samples) from 230.1 to 392.0 µg.kg⁻¹ (Fig. S2). The method was proved to be accurate with recoveries from 81.6% to 103.9% for eleven compounds.

4. Conclusions

A MSPE method combined with UHPLC Q-Orbitrap for the analysis of eleven risky drugs in egg was developed. The MSPE procedure includes: extraction with a mixture containing acidified acetonitrile, Na₂EDTA McIlvaine buffer and anhydrous magnesium sulfate; Fe₃O₄@ZIF-8 as an adsorbent for enrichment; elution to collect the residue. Lowest values achieved for CCβ and CCα were, respectively, of 0.62 to 26.97 µg.kg⁻¹ and 0.55 to 16.37 µg.kg⁻¹ for multi-residues. Satisfactory recoveries were achieved falling between 81.6% and 103.9%. The application of the proposed method in egg samples was provided satisfying results with the modified MSPE and the detection of fluoroquinolones, tetracyclines and amphenicols were present in the samples at concentrations of 117–390 µg.kg⁻¹, highlighted the frequent appearance of veterinary drugs in some eggs. The acceptable results show that the established method has gained good results in the detection of multiple classes risky substances in eggs. The proposed strategy is expected to ensure the safety and public health of eggs as well as their products, revealing good potential for monitoring various types contaminants in other animal origin matrices.

Conflict of interest

The authors declare no conflicting financial interest.

Acknowledgments

The research was financially supported by the National Natural Science Foundation of China (No. 31801643), Scientific Research Program Funded by Shaanxi Province Education Department, China (No. 2019C004), Science and Technology Project of Weiyang District, Xi’an City (No. 201936) and Key Research and Development Program of Shaanxi (No. 2019NY-117).
Appendix

A

Oxytetracycline  Tetracycline  Chlortetracycline  Doxycycline

Sudan I  Sudan II  Sudan III  Sudan IV

Enrofloxacin  Florfenicol  Thiamphenicol

B

FeCl₃·6H₂O  (CHOH)₂, CH₃COONa  200 °C, 10 h  Fe₃O₄

Zn(NO₃)₂·6H₂O + C₄H₆N₂  CH₃OH  200 °C, 10 h  ZIF-8

Fe₃O₄ + ZIF-8  Poly (styrenesulfonate, sodium salt)  45 °C, 6 h  Fe₃O₄@ZIF-8

Fig. S1. Molecular structures of eleven standards (A) and preparation of the Fe₃O₄@ZIF-8 (B). Tetracycline, oxytetracycline, chlortetracycline and doxycycline are tetracyclines, while Sudan I, II, III and IV are dyes. Enrofloxacin belongs to fluoroquinolones while thiamphenicol and florfenicol belong to amphenicols. The structure and functional groups of these drugs are related to the adsorption efficiency.

Fig. S2. Positive egg sample containing florfenicol (390.4 μg.kg⁻¹) and enrofloxacin (149.2 μg.kg⁻¹).
Table S1. Compared developed method with other methods.

| Pretreatment methods | Adsorbent                   | Detecting instruments      | Recovery (%) | Linearity     | Precision (RSD%) | LOD (mg.g⁻¹) | Ref            |
|----------------------|-----------------------------|----------------------------|--------------|---------------|------------------|--------------|----------------|
| *                    | -                           | Raman spectrometry         | 81.7-152.4   | ≥0.9979       | 0.3200           |              | [5]            |
| MSPD                 | C18                         | GC-MS                      | 65.0-95.0    | ≥0.9928       | <18.00          | 0.0000-0.0150 | [9]            |
| QuEChERS            | d-SPE kit (P/N 5982-5158)   | GC-MS                      | 72.0-119.0   | ≥0.9979       | 12.70            | 0.0000-0.0050 | [7]            |
| QuEChERS            | d-SPE kit (P/N 5982-5158)   | LC-MS/MS                   | 71.0-108.0   | >0.9950       | 13.38            | 0.0009-0.0024 | [10]           |
| QuEChERS            | PSA and C18,                | HPLC-QTOF-MS               | 54.0-126.0   | -             | <19.20          | 0.0145-0.4820 | [6]            |
| MFF                 | PSA, C18, and magnesium sulphate | UHPLC-MS/MS              | 63.0-110.8   | -             | <15.30          | 0.1000-1.0000 | [8]            |
| MSPE                | Fe₃O₄-MWCNTs                | UHPLC-MS/MS                | 60.5-114.6   | >0.9910       | <20.00          | 0.0300-5.1900 | [11]           |
| MSPE                | Fe₃O₄@ZIF-8                 | UHPLC-MS/MS                | 81.6-103.9   | >0.9991       | <9.92           | 0.0001-0.0153 | This work     |

*no date.

References

[1] Rizzetti TM, de Souza MP, Prestes OD, Adaime MB, Zanella R. Optimization of sample preparation by central composite design for multi-class determination of veterinary drugs in bovine muscle, kidney and liver by ultra-high-performance liquid chromatography-tandem mass spectrometry. Food Chem 2018;246:404–13.

[2] Ali AH, Zou X, Lu J, Abed SM, Wang X. Identification of phospholipids and molecular species in different types of egg yolk by using UPLC-Q-TOF-MS. Food Chem Chem J 2013;110:395-402.

[3] Tu Q, Hickey ME, Yang T, Gao S, Zhang Q, Qu Y, et al. A simple and rapid method for detecting the pesticide fipronil on egg shells and in liquid eggs by Raman microscopy. Food Control 2019;96:16–21.

[4] Piatkowska M, Jedziniak P, Zmudzki J. Multiresidue method for the simultaneous determination of veterinary medicinal products, feed additives and illegal dyes in eggs using liquid chromatography-tandem mass spectrometry. Food Chem 2016;221:58–66.

[5] Piatkowska M, Olejnik M, Jedziniak P, Zmudzki J. The transfer of a genotoxic and carcinogenic azo-dye Sudan I to eggs after feeding of laying hens with contaminated feed. Toxicol Lett 2015;236:578.

[6] Hou X, Xu X, Xu X, Han M, Qiu S. Application of a multiclass screening method for veterinary drugs and pesticides using HPLC-QTOF-MS in egg samples. Food Chem 2020;309:12574-5.

[7] Song N-E, Lee JY, Mansur AR, Jang HW, Lim M-C, Lee Y, et al. Determination of 60 pesticides in hen eggs using the QuEChERS procedure followed by LC-MS/MS and GC-MS/MS. Food Chem 2019;298:125050.

[8] Zhang X, Song Y, Jia Q, Zhang L, Zhang W, Mu P, et al. Simultaneous determination of 58 pesticides and relevant metabolites in eggs with a multi-functional filter by ultra-high performance liquid chromatography-tandem mass spectrometry. J Chromatography A 2019;1593:81-90.

[9] Souza MRdR, Moreira CO, de Lima TG, Aquino A, Dórea HS. Validation of a matrix solid phase dispersion (MSPD) technique for determination of pesticides in lyophilized eggs of the chicken Gallus gallus domesticus. Microchem J 2013;110:395-401.

[10] Choi S, Kim S, Shin JY, Kim M, Kim J-H. Development and verification for analysis of pesticides in eggs and egg products using QuEChERS and LC-MS/MS. Food Chem 2015;173:1236–42.

[11] Xu X, Xu X, Han M, Qiu S, Hou X. Development of a modified QuEChERS method based on magnetic multiwalled carbon nanotubes for the simultaneous determination of veterinary drugs, pesticides and mycotoxins in eggs by UPLC-MS/MS. Food Chem 2019;276:419–26.

[12] Zheng X, He L, Duan Y, Jang X, Xiang G, Zhao W, et al. Poly(ionic liquid) immobilized magnetic nanoparticles as new adsorbent for extraction and enrichment of organophosphorus pesticides from tea drinks. J Chromatography A 2014;1359:39–45.

[13] Nasir ANM, Yahaya N, Zain NNM, Lim V, Kamaruzaman S, Saad B, et al. Thiol-functionalized magnetic carbon nanotubes for magnetic micro-solid phase extraction of sulfonamide antibiotics from milks and commercial chicken meat products. Food Chem 2019;276:458–66.

[14] Xu Y, Ding J, Chen H, Zhao Q, Hou J, Yan J, et al. Fast determination of sulfonamides from egg samples using magnetic multiwalled carbon nanotubes as adsorbents followed by liquid chromatography-tandem mass spectrometry. Food Chem 2013;140:83–90.

[15] Gao S, Wu Y, Xie S, Shao Z, Bao X, Yan Y, et al. Determination of aflatoxins in milk sample with ionic liquid modified magnetic zeolitic imidazolate frameworks. J Chromatography B 2019;1128:121778.

[16] Lu G, Li S, Guo Z, Farha OK, Hauser BG, Qi X, et al. Imparting functionality to a metal-organic framework material by controlled nanoparticle encapsulation. Nat Chem 2012;4:310–316.

[17] Son Y-R, Ryu SG, Kim HS. Rapid adsorption and removal of sulfur mustard with zeolitic imidazolate frameworks ZIF-8 and ZIF-67. Microporous Mesoporous Mat 2020;293:109819.

[18] Maddah B, Shamsi J. Extraction and preconcentration of trace amounts of diazinon and fenithrothion from environmental water by magnetic octadecylsila nanoparticles. J Chromatography A 2012;1256:40–91.

[19] Li M, Wang J, Jiao C, Wang C, Wu Q, Wang Z. Magnetic porous carbon derived from a Zn/Co bimetallic metal-organic framework as an adsorbent for the extraction of chlorophenols from water and honey tea samples. J Sep Sci 2016;39:1894–91.

[20] De Paep E, Wauters J, Van Der Borght M, Claes J, Huysman S, Croubels S, et al. Ultra-high-performance liquid chromatography coupled to quadrupole orbitrap high-resolution mass spectrometry for multi-residue screening of pesticides, (veterinary) drugs and mycotoxins in edible insects. Food Chem 2019;293:187–96.

[21] Wang J, Leung D, Chow W, Chang J, Wong JW. Target screening of 105 veterinary drug residues in milk using UHPLC/ESI Q-Orbitrap multiplexing data independent acquisition. Analytical Bioanalytical Chem 2018;410:5373–89.

[22] Li Q, Liang X, Zhao L, Zhang Z, Xue X, Wang K, et al. UPLC-Q-Exact Orbitrap/MS-Based Lipidomics Approach To Characterize Lipid Extracts from Bee Pollen and Their In Vitro Anti-Inflammatory Properties. J Agri Food Chem 2017;65:6848–60.

[23] Masia A, Suarez-Varela MM, Llopis-Gonzalez A, Picó Y. Determination of pesticides and veterinary drug residues in food by liquid chromatography-mass spectrometry: A review. Analystica Chimica Acta 2017;936:40–51.

[24] Berendsen BJA, Meijer T, Mol HJG, van Ginkel L, Nienol MWF. A global inter-laboratory study to assess acquisition modes for multi-compound confirmatory
analysis of veterinary drugs using liquid chromatography coupled to triple quadrupole, time of flight and orbitrap mass spectrometry. Analytica Chimica Acta 2017;962:60–72.

[25] European Communities. Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Commission Decision 2002/657/EC Off J Europ Comm 2002. No. L221/8.

[26] Document SANCO/12571/2013. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. http://www.eurl-pesticides.eu/library/docs/allcrl/AqcGuidance_Sanco_2013_12571.pdf.

[27] Wu Y, Li B, Wang X, Yu S, Pang H, Liu Y, et al. Magnetic metal-organic frameworks (Fe₃O₄@ZIF-8) composites for U(VI) and Eu(III) elimination: simultaneously achieve favorable stability and functionality. Chem Eng J 2019;378:122105.

[28] Liu Y-D, Xin G-Z, Li W, Liu F-J, Yao Z-P, Di X. A novel liquid-liquid-solid microextraction strategy for bio-sample preparation by in situ self-assembly of zeolitic imidazolate framework 8 on hollow fiber membrane. Analytica Chimica Acta 2020;1095:118–28.

[29] Moreno-González D, Alcántara-Durán J, Gilbert-López B, García-Reyes JJ, Molina-Díaz A. Matrix-effect free quantitative Liquid Chromatography Mass Spectrometry analysis in complex matrices using nanoflow LC with integrated emitter tip and high dilution factors. J Chromatography A 2017. S0021967317313092.