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

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Abstract

A novel magnetic adsorbent solid-phase $\text{Fe}_3\text{O}_4@\text{ZIF-8}$ 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, $\text{CC}\beta$ and $\text{CC}\alpha$ of 0.62 to 26.97 $\mu\text{g}\cdot\text{kg}^{-1}$ and 0.36 to 16.37 $\mu\text{g}\cdot\text{kg}^{-1}$ 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 $\geq 12\%$ 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%)

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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, chlortetracycline, 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 reagents

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 (styrenesulfonate, 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 $\mu\text{g}\cdot\text{L}^{-1}$ 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 ferrosiferic oxide magnetic nanoparticles were collected by using Nd-Fe-B strong magnet and dried 10 h at 80 °C. Poly (styrenesulfonate, sodium salt) can modify the surface of ZIF-8 in solution and improve its particle stability with polydispersity index decreased. 2.0 g ferrosiferic oxide magnetic nanoparticles were mixt with 70 mL 0.5% poly (styrenesulfonate, sodium salt) and ultrasound for 20 min, while the modified magnetic nanoparticles (ferrosiferic 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-McIlvaine 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

(HESI). 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 Lakeshore VSM-7304 (Westerville, USA) and the Bruhauer-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 (CC_α), detection capability (CC_β), 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.

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

Analyte	RT (min)	Matrix effects (%)	Molecular formula	Exact mass	Theoretical mass (m/z)	Experimental mass (m/z)	Δ Mass (ppm)	Fragment 1 (m/z)	Fragment 2 (m/z)
Amphenicols									
Florfenicol	7.4	−4.88	C ₁₂ H ₁₄ Cl ₂ FNO ₄ S	[M+H] ⁺	358.00774	358.00719	1.54	119.03771	185.02669
Thiamphenicol	8.2	13.69	C ₁₂ H ₁₅ Cl ₂ NO ₅ S	[M+H] ⁺	356.01208	356.01196	0.34	155.96136	185.02669
Fluoroquinolones									
Enrofloxacin	8.4	11.25	C ₁₉ H ₂₂ FN ₃ O ₃	[M+H] ⁺	360.17180	360.17084	2.67	245.04827	316.18197
Tetracyclines									
Tetracycline	8.0	12.27	C ₂₂ H ₂₅ ClN ₂ O ₈	[M+H] ⁺	445.16054	445.16045	0.20	154.04987	321.07575
Oxytetracycline	8.1	−12.50	C ₂₂ H ₂₄ N ₂ O ₉	[M+H] ⁺	461.15556	461.15536	0.43	201.05462	426.11834
Chlortetracycline	8.7	−17.23	C ₂₂ H ₂₄ Cl ₂ N ₂ O ₈	[M+H] ⁺	479.12157	479.12220	1.31	154.04987	323.03169
Doxycycline	9.3	−14.98	C ₂₂ H ₂₄ N ₂ O ₈	[M+H] ⁺	445.16054	445.16042	0.27	410.12343	428.13399
Dyes									
Sudan I	12.1	−12.39	C ₁₆ H ₁₂ N ₂ O	[M+H] ⁺	249.10224	249.10188	1.45	105.04472	231.09167
Sudan II	13.4	9.39	C ₁₈ H ₁₆ N ₂ O	[M+H] ⁺	277.13354	277.13319	1.26	119.06037	127.05423
Sudan III	15.8	11.50	C ₂₂ H ₁₆ N ₄ O	[M+H] ⁺	353.13969	353.13990	0.59	143.04914	180.06820
Sudan IV	17.6	15.69	C ₂₄ H ₂₀ N ₄ O	[M+H] ⁺	381.17099	381.17069	0.79	104.04948	143.04914

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/P_0) 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^{−1} and the BJH adsorption cumulative surface area of pores between 1.7000 nm and 300.0000 nm diameter was 4.5852 m² g^{−1} while desorption cumulative surface area was 3.9983 m² g^{−1}. Fig. 1E shows the hysteresis

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 Fe_3O_4 and $\text{Fe}_3\text{O}_4@\text{ZIF-8}$ in which the maximum saturation magnetization (Ms) were 117 and 102 emu g^{-1} respectively. The $\text{Fe}_3\text{O}_4@\text{ZIF-8}$ 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 Fe_3O_4 , ZIF-8 and $\text{Fe}_3\text{O}_4@\text{ZIF-8}$, in which the peaks of 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 ring while the peak at 1310 cm^{-1} was associated to Zn–N stretch. Moreover, the band in 580 cm^{-1} was ascribed to Fe–O–Fe vibration of magnetite. The absence of N–H···N hydrogen bond (2600 cm^{-1}) and N–H bond (1843 cm^{-1}) indicated that 2-methyl imidazole could be completely deprotonated and $\text{Fe}_3\text{O}_4@\text{ZIF-8}$ was generated with pure ZIF-8.

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 in samples when 0.1 mol L^{-1} Na_2EDTA -Mcllvaine was added, since it provides bonding sites for the 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 $\text{Fe}_3\text{O}_4@\text{ZIF-8}$. The recovery of studied compound was higher than 75% with relative standard deviations (RSD) $\leq 9\%$ 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, $\text{CC}\alpha$, $\text{CC}\beta$, reproducibility and recovery obtained in the validation experiments.

Compound	Calibration curve	R^2	$\text{CC}\alpha$ ($\mu\text{g}\cdot\text{kg}^{-1}$)	$\text{CC}\beta$ ($\mu\text{g}\cdot\text{kg}^{-1}$)	LOD* ($\mu\text{g}\cdot\text{kg}^{-1}$)	Intraday precision (%), n = 6						Interday precision (%), n = 6						Reproducibility (%), n = 6					
						150	300	450	150	300	450	150	300	450	150	300	450	150	300	450	150	300	450
						R*	RSD	R	RSD	R	RSD	R	RSD	R	RSD	R	RSD	R	RSD	R	RSD	R	RSD
Amphenicols																							
Flufenicol	$y = 8.1x + 2.2$	0.9991	1.20	1.99	0.90	103	4	103	5	97	1	99	4	104	5	96	6	93	6	100	2	93	6
Thiamphenicol	$y = 18.4x + 0.7$	0.9985	0.55	0.94	0.27	87	3	89	3	82	3	85	5	89	2	82	3	82	2	83	1	75	3
Fluoroquinolones																							
Enrofloxacin	$y = 12.3x + 1.9$	0.999	0.36	0.62	0.12	85	4	82	2	82	4	83	3	91	2	88	3	81	4	88	1	82	6
Tetracyclines																							
Tetracycline	$y = 11.2x - 8.3$	0.9992	10.81	17.76	9.06	93	1	89	2	99	3	87	3	94	3	101	6	80	4	92	2	98	3
Oxytetracycline	$y = 9.2x - 11.2$	0.9996	14.19	23.29	15.32	95	3	95	5	93	3	90	1	89	4	95	4	87	1	85	4	91	3
Chlortetracycline	$y = 3.2x - 4.7$	0.9991	15.35	25.32	13.90	84	1	88	1	87	1	87	2	91	3	85	5	81	3	84	8	78	7
Doxycycline	$y = 8.1x - 2.8$	0.9997	16.37	26.97	12.03	86	3	85	3	83	3	90	1	92	1	84	5	84	5	89	1	81	9
Dyes																							
Sudan I	$y = 13.2x - 10.8$	0.999	4.02	6.63	3.24	97	3	95	2	91	3	98	3	101	1	90	3	91	5	98	6	88	4
Sudan II	$y = 11.2x - 2.3$	0.9982	2.72	4.49	2.20	100	4	97	1	96	3	91	4	95	2	94	2	85	4	93	8	89	7
Sudan III	$y = 17.0x + 0.1$	0.9992	3.06	5.07	3.21	95	1	98	3	96	3	95	2	95	1	100	5	92	9	90	8	95	3
Sudan IV	$y = 10.8x - 4.3$	0.9998	5.30	8.75	5.20	93	4	89	2	90	1	88	3	83	5	84	5	82	5	77	7	82	5

LOD* limit of detection; R* recovery (%).

Fig. 2. Effect of the adsorbent amount (A), adsorption time (B) and the pH (C) on extraction efficiency, desorption solvent (D) and elution volume (E) on extraction efficiency. The concentration of mixed working solution used in the optimization procedure was $100 \mu\text{g.L}^{-1}$.

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 Na_2EDTA 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 Na_2EDTA 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 Na_2EDTA -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 $\text{Fe}_3\text{O}_4@\text{ZIF-8}$ 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 $\text{Fe}_3\text{O}_4@\text{ZIF-8}$ 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 $\text{Fe}_3\text{O}_4@\text{ZIF-8}$ structure worked, but also other multiple interactions. Owing to the open metal sites of Zn (II) whose coordinated number is four, the

Fig. 3. The standard and matrix-matched curves of enrofloxacin, tetracycline, sudan I and thiamphenicol.

possible interactions between $\text{Fe}_3\text{O}_4@\text{ZIF-8}$ and drugs were like the coordination bonds between open metal sites and $-\text{OH}$ or phenol group. Meanwhile, the existence of intermolecular $\pi-\pi$ 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 $\text{Fe}_3\text{O}_4@\text{ZIF-8}$ during adsorption. Drugs can provide H-bond donors and $\text{Fe}_3\text{O}_4@\text{ZIF-8}$ 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 $\text{Fe}_3\text{O}_4@\text{ZIF-8}$ 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.

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

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 $\text{Fe}_3\text{O}_4@\text{ZIF-8}$. Acceptable ME values (from −17.23% to +15.69%) were observed for eleven drugs, which indicated that the MSPE procedure could successfully extract

Fig. 4. Extracted ion chromatogram and spectra from a full MS/dd-MS2 experiment using UHPLC-Q-Orbitrap in spiked sample ($100 \mu\text{g.kg}^{-1}$).

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 $750 \mu\text{g.kg}^{-1}$ 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.

$CC\alpha$ and $CC\beta$ 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 $CC\alpha$ and $CC\beta$ were based on the minimum required performance level, and they showed highly satisfactory results with the suitable extraction method (Table 2).

$$CC\alpha = \mu\text{MRL} + 1.64 \times \sigma\text{MRL} \quad (2)$$

$$CC\beta = CC\alpha + 1.64 \times \sigma\text{MRL} \quad (3)$$

The $CC\alpha$ values of fluoroquinolones, tetracyclines, dyes and amphenicols were varied from 0.36 to $16.37 \mu\text{g.kg}^{-1}$, while the $CC\beta$ values were ranged from 0.62 to $26.97 \mu\text{g.kg}^{-1}$. The linearity was evaluated by the standard solutions of eleven drugs spiked in the blank samples ($n = 6$). The R^2 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, $\mu\text{g.kg}^{-1}$)
Fluoroquinolones	
Enrofloxacin	Sample 1 ($149.2 \pm 0.5^*$), Sample 18 ($220.5 \pm 0.9^*$)
Tetracyclines	
Tetracycline	Sample 9 ($236.2 \pm 1.2^*$), Sample 18 ($117.7 \pm 1.3^*$), Sample 32 ($327.3 \pm 1.5^{**}$)
Oxytetracycline	Sample 2 ($225.1 \pm 0.9^*$), Sample 9 ($161.2 \pm 1.3^*$), Sample 52 ($189.8 \pm 1.1^*$)
Amphenicols	
Florfenicol	Sample 1 ($390.4 \pm 1.6^{**}$), Sample 2 ($296.0 \pm 1.4^{**}$), Sample 9 ($231.1 \pm 1.0^*$), Sample 32 ($242.3 \pm 1.1^{**}$)
Thiamphenicol	Sample 6 ($323.1 \pm 0.6^{**}$), Sample 7 ($217.8 \pm 0.8^*$), Sample 10 ($296.6 \pm 0.2^{**}$)

* $p < 0.05$.

** $p < 0.01$.

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 ($100 \mu\text{g.kg}^{-1}$) 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 $\text{Fe}_3\text{O}_4\text{@ZIF-8}$ 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 $221.4 \mu\text{g.kg}^{-1}$, tetracycline (three samples) from 116.4 to $328.8 \mu\text{g.kg}^{-1}$, oxytetracycline (three samples) from 159.9 to $226.0 \mu\text{g.kg}^{-1}$, thiamphenicol (three sample) from 217.0 to $323.7 \mu\text{g.kg}^{-1}$ and florfenicol (four samples) from 230.1 to $392.0 \mu\text{g.kg}^{-1}$ (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, Na_2EDTA McIlvaine buffer and anhydrous magnesium sulfate; $\text{Fe}_3\text{O}_4\text{@ZIF-8}$ as an adsorbent for enrichment; elution to collect the residue. Lowest values achieved for CC β and CC α were, respectively, of 0.62 to $26.97 \mu\text{g.kg}^{-1}$ and 0.55 to $16.37 \mu\text{g.kg}^{-1}$ 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 $\mu\text{g.kg}^{-1}$, 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.

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Appendix

A **B**

Fig. S1. Molecular structures of eleven standards (A) and preparation of the $\text{Fe}_3\text{O}_4@\text{ZIF-8}$ (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 ($390.4\text{ }\mu\text{g.kg}^{-1}$) and enrofloxacin ($149.2\text{ }\mu\text{g.kg}^{-1}$).

Table S1. Compared developed method with other methods.

Pretreatment methods	Adsorbent	Detecting instruments	Recovery (%)	Linearity	Precision (RSD%)	LOD ($\mu\text{g}\cdot\text{g}^{-1}$)	Ref
–*	–	Raman spectrometry	81.7–152.4	≥ 0.9970	–	0.3200	[3]
MSPD	C18	GC-MS	65.0–95.0	> 0.9928	< 18.00	0.0030–0.0150	[9]
QuEChERS	d-SPE kit (P/N 5982-5158)	GC-MS	72.0–119.0	> 0.9979	< 12.70	0.0010–0.0050	[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	Fe_3O_4 -MWCNTs	UHPLC-MS/MS	60.5–114.6	–	< 20.00	0.0300–5.1900	[11]
MSPE	Fe_3O_4 @ZIF-8	UHPLC-MS/MS	81.6–103.9	> 0.9910	< 9.92	0.0001–0.0153	This work

*no date.

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