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Membrane–protected magnetic covalent organic framework for efficient extraction of estrogens in dairy products

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ABSTRACT

The presence of estrogens residues in dairy products is a growing concern due to their potential health risk. Herein, in this study, we have developed a membrane–protected magnetic solid–phase extraction (MP–MSPE) method that utilized a magnetic adsorbent (Fe_3O_4@COF–LZU1) with *in–situ* growth for the efficient extraction of estrone (E1), 17β–estradiol (E2), and estriol (E3). When combined with HPLC–FLD, this method allows for the efficient detection of estrogens in dairy products. The stability of the MP–MSPE was improved by the presence of a dialysis membrane, which remained a high extraction efficiency (90 %) even after ten reuse cycles. The hydrogen bonding, π–π interactions and pore size effect contribute to the excellent adsorption of three estrogens onto Fe_3O_4@COF–LZU1. Under optimal conditions, the method exhibits a low detection limit (0.01–0.15 μg L^{-1}), wide linear range (0.1–800 μg L^{-1}), and favorable recoveries (77.3 %–109.4 %) at three concentration levels (10, 50 and 100 μg L^{-1}). This proposed method is characterized by its simplicity, high efficiency and eco–friendliness, making it a promising approach for extracting estrogens from dairy products.

1. Introduction

The three typical endogenous hormones, namely estrone (E1), 17β–estradiol (E2), and estriol (E3) are mainly produced by the ovary and placenta (Chen, Wang, Lu, Chen, & Xie, 2018). These estrogens play an important role in the development of secondary sexuality and the maturation of sexual organs (Gunatilake, Munasinghe, Ranaweera, Mlsna, & Xia, 2016). They have been widely used in the livestock industry to boost milk production (Xiong, Wu, & Lu, 2018). However, excessive consumption of dairy products containing estrogens may harm human health, especially affecting the reproductive and endocrine systems. The European Union has banned the use of estrogens as a growth promoter in animal feed (Janssens, Mangelinckx, Courtheyn, Kimpe, Matthijs, & Bizec, 2015). Estrogens (E1, E2 and E3) have been classified as contaminants of emerging concern by the United States Environmental Protection Agency (Chen, Zhang, Fu, Li, & Lin, 2018). Therefore, it is of great importance to monitor the estrogen residues in dairy products.

Nowadays, various detection techniques have been employed for the determination of E1, E2 and E3, including immunoassays (Zhao, Lin, Li, & Ying, 2006), fluorescence sensing (Zhang, Cui, Yang, Fang, Liu, & Wang, 2021), and electrochemical method (Wei et al., 2022), gas or liquid chromatography coupled to mass spectrometry (GC–MS or LC–MS) (Davoodi, Scopus preview – Nabizadeh Nodehi, Rastkari, Zinatizadeh, Mahvi, & Fattahi, 2020; Stella & Dey, 2021) and high–performance liquid chromatography (HPLC). Among these methods, HPLC is the conventional approach for detecting estrogens EEs. However, a complex pretreatment process is often required due to the low concentration of estrogens and the complexity of the dairy products.

Magnetic solid–phase extraction (MSPE), a significant subset of solid–phase extraction (SPE), has received increasing attention recently, as it notably simplifies the extraction process (Du, Yan, Chen, Wu, Qiu, Li, & Wu, 2022). In MSPE, the magnetic adsorbents are dispersed in the extraction solution to extract and separate analytes with the assistance of an external magnet, avoiding the need for centrifugation or filtration (Li et al., 2021). This approach quickly achieves high extraction efficiency (Lin, Wang, Wang, Yuan, Di, Wang, & Ding, 2020). Meanwhile, it helps to address issues related to the large consumption of organic

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solvents, high back pressures, and column blockage (Chen, He, Lei, Gao, Xie, Tong, & Lin, 2018). To improve the analytical performance of MSPE, extensive efforts have been devoted to exploring more effective adsorption nanomaterials (Er, Bozyiğit, Büyükpınar, & Bakırdere, 2022). At present, various magnetic sorbents were used for MSPE, such as magnetic metal–organic frameworks (Wang, Liu, & Yan, 2021), magnetic ordered porous carbon (Xia, Cai, Dong, Wang, Wang, Kang, & Chen, 2018), polydopamine–coated magnetic composite nanoparticles (Zhang, Wang, Yang, Deng, Li, Su, & Yang, 2018), molecularly imprinted magnetic nanoparticles (Negar Sadegh, 2021), and magnetic zeolitic imidazolate framework/graphene (Huang, Liu, Liu, Li, Xu, Zheng, & Gao, 2018). However, their applications are limited by issues such as poor structural stability, tedious synthesis procedures, or low selectivity.

Covalent organic frameworks (COF), which are a type of porous crystalline materials (Ding, Gao, Li, Huang, Shi, & Feng, 2011), have attracted intensive interest from researchers since their first report in 2005 (Cao, Li, Zhu, & Pang, 2019; Goda, Hong, Pandit, Rehman, Singu, Akhtar, Sohail, El–Bahy, Gamal, & Yoon. 2023). COFs are composed of organic monomers connected by strong covalent bonds, offering advantages such as large surface areas, tunable pore size, and good stability. They exhibits great potential in the various fields including separation, sensing, optoelectronics device,and catalysis (Li, Jiang, Geng, Wang, Gao, Tang, & Kong, 2020; Li, Yang, & Yan, 2017; Abu Elella, Abdallah, Gamal, Moustafa, & Goda, 2022). Magnetization has effectively solved the issue of low density and challenging recovery in COFs. Recently, magnetic nanomaterials based on COFs have been rapidly developed and applied in MSPE to extract the environmental pollutants, pesticides and biotoxins from the environmental, food and biological samples (Ding, Zhao, Liu, & Zhang, 2020; Jiang, Xue, Sun, Lin, Zhang, Wang, & Zhao, 2021).

Dialysis membranes are typically made of cellulose and available in various molecular weight cut–offs (Ding, Gao, Wang, Zhang, Song, Su, & Wang, 2011). They have been widely utilized in different field such as hemodialysis, protein separation and purification, and desalination (Chaikhan, Udnan, Ampiah–Bonney, & Chaiyasith, 2023). Given that dairy products containproteins and other macromolecular substances with molecular weight ranging from 10 kDa to 100 kDa (Lopes, Morés, da Silva, Schneider, Merib, & Carasek, 2022), selecting a dialysis membrane with a low molecular weight cutoff of 10 kDa is advantageous. Such choice prevents direct contact between the adsorbent and sample matrix, allowing macromolecular substances to remain outside while estrogens can enter under the osmotic pressure.

The objective of this work is to magnetize COF materials to increase their density and facilitate recycling. By combining with membrane protection, we aim to provide an effective method for extracting estrogens from dairy products. In this study, COF–LZU1, synthesized by p–phenylenediamine (PDA) and triformylbenzene (TFB) under the catalysis of acetic acid, served as starting material. The magnetic sorbent material (Fe_3O_4@COF–LZU1) was fabricated by grafting COF–LZU1 onto the surface–modified Fe_3O_4 nanoparticles and filling them into a hollow dialysis membrane to develop a membrane–protected MSPE (MP–MSPE) method. This method enables rapid and efficient extraction of three estrogens (E1, E2 and E3). Combined with HPLC–FLD, the developed method was successfully applied to extract and quanfity these estrogens in dairy products including pasteurized milk, raw milk, UHT milk, and skimmed milk.

2. Materials and methods

2.1. Reagent and materials

Chromatographic grade acetonitrile, methanol and n–hexane were obtained from Thermo Fisher Scientific Co., Ltd. (Shanghai, China). 1,3,5–trialdehyde benzene (TFB), 1,4–diaminobenzene (PDA), 17beta–estradiol (E2), estriol (E3) and estrone (E1) were obtained from Aladdin Chemistry Co., Ltd. (Shanghai, China). Ultrapure water was purchased from Wahaha Foods Co., Ltd. (Hangzhou, China). Glacial acetic acid, ethanol, 1,4–dioxane, N, N–dimethylformamide (DMF), tetrahydrofuran (THF), iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ethylene glycol (EG), ethylenediamine and anhydrous sodium acetate (CH_3COONa) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). A dialysis membrane with a molecular weight cutoff (MWCO) of 7000 Da was supplied by Yibo Biological Co., Ltd. (Beijing, China). 0.22 μm nylon filter membranes were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Instrumentation

The X–ray diffraction spectrometry (XRD) patterns were recorded on a D2 PHASER (BRUKER AXS GMBH, Germany). Fourier–transform infrared (FT–IR) spectra were operated on an IS10 FT–IR spectrophotometer (Nicolet, USA). Transmission electron microscopy (TEM) images were obtained by using an FEI Tecnai G2 F30 transmission electron microscope (FEI, USA). The elemental mapping patterns were contained using an XM–2 EDX (EDAX, USA) equipped with a Tecnai G2 F30 S–TWIN field emission transmission electron microscope (FEI, USA).

2.3. Preparation of Fe_3O_4@COF–LZU1 and dialysis membrane

The amino–functionalized Fe_3O_4 was synthesized by the solvothermal method according to the literature (Guan, Wu, Yang, Wang, & Wu, 2020) and details of the synthetic process are described in **Section S1**.

Fe_3O_4@COF–LZU1 composites were fabricated according to the method described in **Section S2**.

The dialysis membrane was prepared as described in **Section S3**.

2.4. MP–MSPE procedure

The extraction experiments were conducted in a 50 mL centrifuge tube. 3 mg Fe_3O_4@COF–LZU1 was dispersed in the dialysis membrane with 5 mL deionized water and sealed at both ends of the dialysis membrane. Then, it was put into a 20 mL extraction solution and extracted at 50 ℃ for 10 min. After the extraction, the solution and Fe_3O_4@COF–LZU1 in the dialysis membrane all were transferred to a 10 mL centrifuge tube and separated with an external magnet. Afterward, the analytes were desorbed from the Fe_3O_4@COF–LZU1 with 2 mL of CAN (elute two times and 1 mL each time), and the eluate was collected. Finally, the eluate was filtered with a 0.22 μm nylon filter membrane and analyzed by HPLC–FLD.

2.5. Collection and preparation of real sample

Dairy products (pasteurized milk, raw milk, UHT milk, and skimmed milk) were purchased from the local supermarket (China, Wuxi). Before the experiment, the dairy products were pretreated. 8 mL of dairy products sample was measured into a 50 mL polypropylene centrifuge tube, added three estrogens working solutions of different concentrations (10, 50 and 100 μg L^{-1}) and 16 mL ultrapure water. The mixture was ultrasonically dispersed for 10 min and then centrifuged for 10 min at 9000 rpm. The 20 mL middle layer was transferred to a 40 mL centrifuge tube with a stopper and stored at 4 °C for the membrane separation MSPE process.

3. Results and discussion

3.1. The design of magnetic COF for MP–MSPE of estrogens

The molecular size of estrogens (E1, E2, and E3) is lower than 12.4 Å, which are notably inferior to the pore dimensions of COF–LZU1(20.8 Å), thus suggesting the compound can enter the pore structure of COF–LZU1 (**Fig. S1**). COF–LZU1 is a porous material with a benzene ring and a 2D

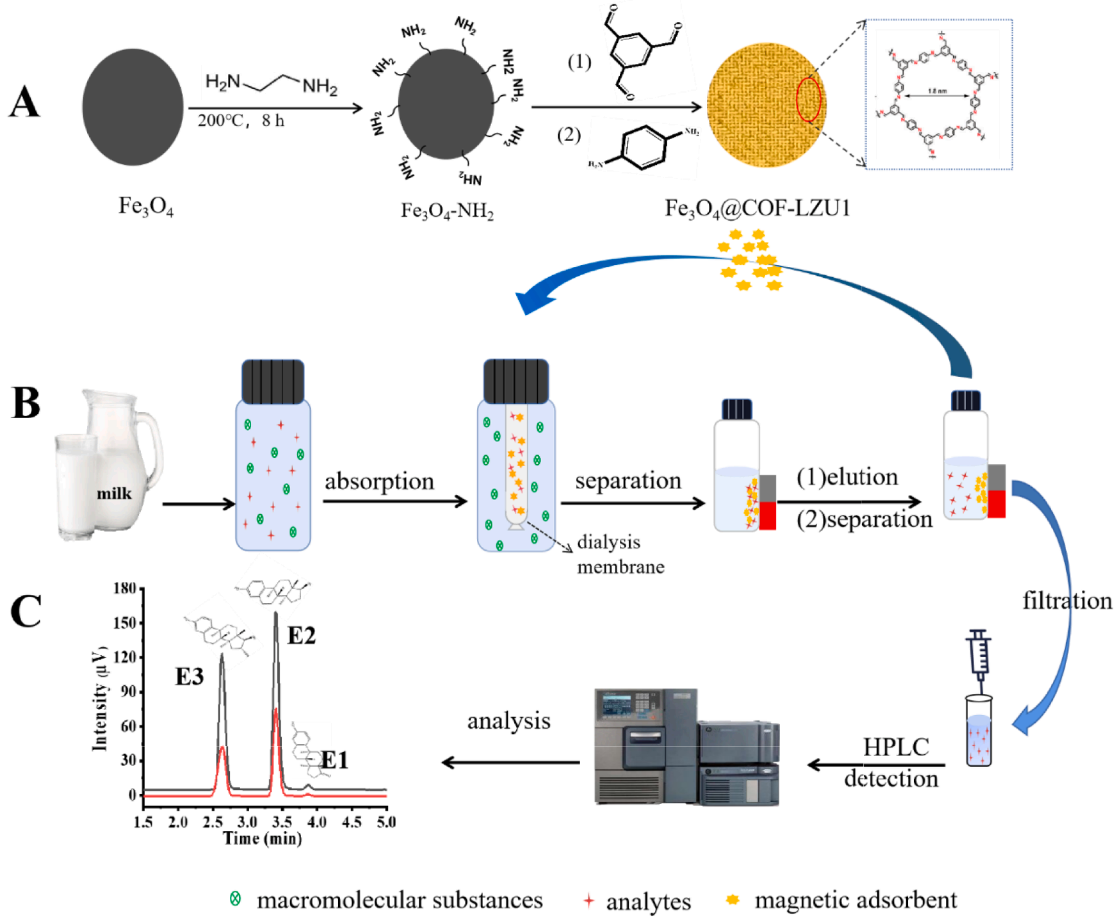

Fig. 1. Schematic diagram of (A) preparation of Fe_3O_4@COF–LZU1, (B) membrane separation MSPE process, and (C) HPLC–FLD detection of estrogens.

network structure with a pore size of about 18.0 Å. COF–LZU1 is expected to achieve efficient adsorption of estrogens through the synergistic effects of pore size and π–π interactions. After grafting COF–LZU1 onto the surface–modified Fe_3O_4 nanoparticles (Fig. 1**A**), the magnetization of COF can achieve rapid extraction.

Dairy products are rich in protein and other macromolecular substances, which often necessitate the employment of organic solvents and/or the complex filtration process during sample preparation. The dialysis membrane allows macromolecular substances stay outside while estrogens are adsorbed. Consequently, this approach reduces pollution and improve the enrichment effect (Fig. 1**B**). When combined with HPLC–FLD, it enables the sensitive detection of E1, E2, and E3 in dairy products (Fig. 1**C**).

3.2. *Characterization of Fe_3O_4@COF–LZU1*

The chemical composition and structure information of Fe_3O_4, COF–LZU1, and Fe_3O_4@COF–LZU1 were characterized by FT–IR (Fig. 2**A**). For Fe_3O_4@COF–LZU1, the absorption peak at 591 cm^{-1} was attributed to the Fe–O stretching vibration of Fe_3O_4, while the characteristic peaks at 1570, 1150 and 1618 cm^{-1} were caused by the C═C, C—N and C═N stretching vibration of COF–LZU1, respectively. The crystalline structure of Fe_3O_4, COF–LZU1 and Fe_3O_4@COF–LZU1 were characterized by XRD (Fig. 2**B**). The diffraction peak observed at 5.0° in Fe_3O_4@COF–LZU1 was ascribed to COF–LZU1, while the additional diffraction peaks at 30.1°, 35.5°, 43.2°, and 56.9° were found to correspond to the crystal structure of Fe_3O_4. This suggests that Fe_3O_4@COF–LZU1 exhibits not only the characteristic peaks of both Fe_3O_4 and COF–LZU1, but also possesses a high degree of crystallinity.

In addition, the TEM image showed the Fe_3O_4@COF–LZU1 was a spherical and uniform morphology with an average diameter of 10 nm (Fig. S2**A**) and the surface of Fe_3O_4 wrapped with COF–LZU1 (Fe_3O_4@COF–LZU1) was semi–transparent and fuzzy (Fig. S2**B**). According to HAADF–STEM and EDX elemental mapping data (Fig. 2**C**), it could be seen that the elements C, N, O of COF–LZU1 and Fe, O of Fe_3O_4 were evenly distributed, indicating that COF–LZU1 grew uniformly on the surface of Fe_3O_4.

The vibrating sample magnetometer (VSM) test was performed, and the result (Fig. 2**D**) showed that the saturation magnetic intensity of Fe_3O_4@COF–LZU1 (48.1 emu g^{-1}) decreased slightly rather than that of Fe_3O_4 (67.2 emu g^{-1}), but it had been satisfied the requirements of rapid separation (about 30 s) from solution by an external magnetic field. Based on the Brunauer Emmett Teller (BET) theory, the specific surface area of Fe_3O_4 is determined to be 37.03 m^2 g^{-1}. The specific surface area was 83.27 m^2 g^{-1} and the pore width distribution was 2.08 nm of Fe_3O_4@COF–LZU1 by N_2 adsorption–desorption isotherms (Fig. 2**E**). All these results demonstrates the successful preparation of Fe_3O_4@COF–LZU1.

3.3. *Optimization of MP–MSPE conditions*

Extraction temperature, adsorbent dosage, desorption solvent and volume were optimized by analyzing 20 mL of the standard solution (250 μg L^{-1}) with an HPLC–FLD detector to obtain the optimal extraction efficiency.

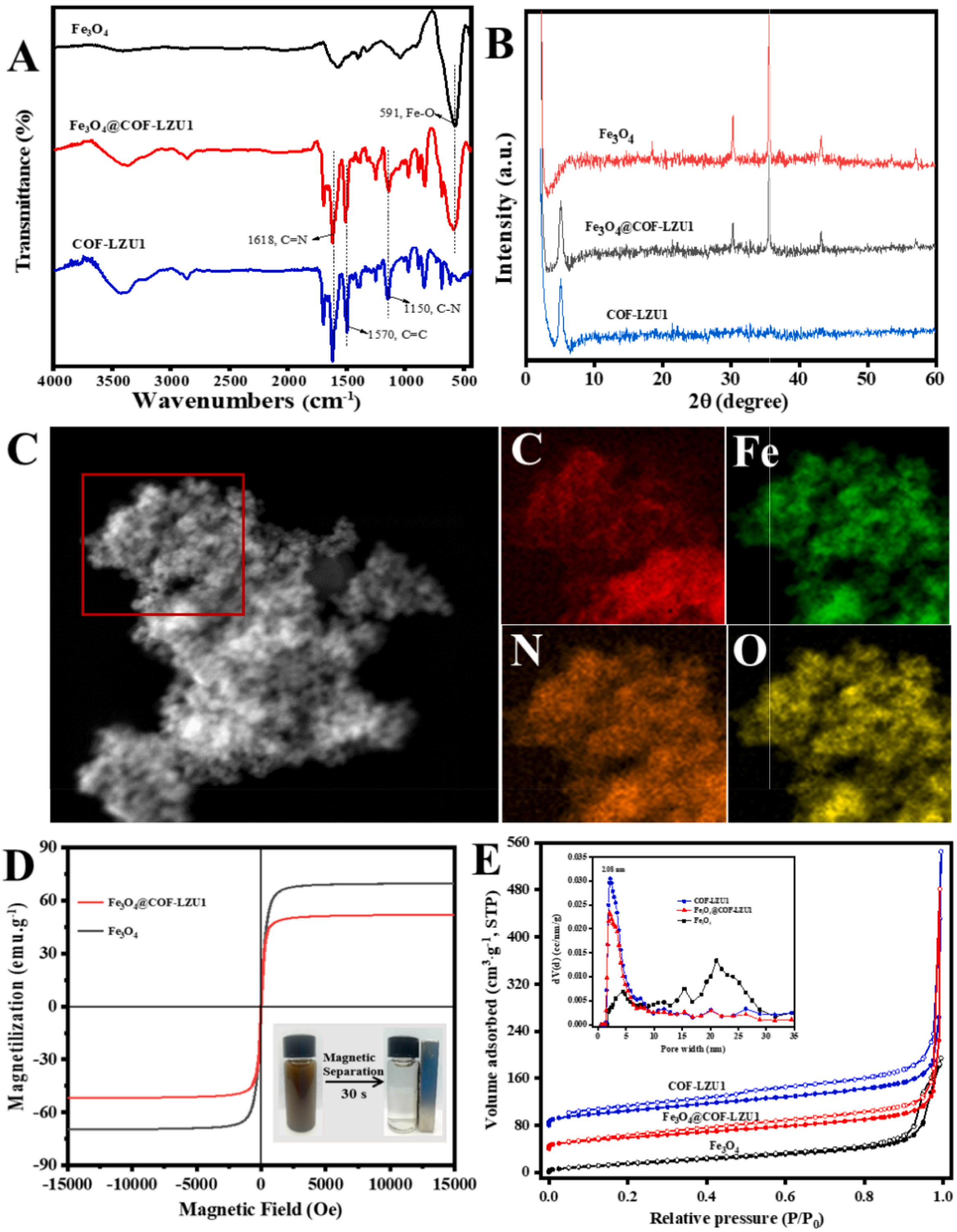

Fig. 2. (A) FT–IR characterization of Fe_3O_4, COF–LZU1 and Fe_3O_4@COF–LZU1; (B) XRD images of Fe_3O_4, COF–LZU1 and Fe_3O_4@COF–LZU1; (C) HAADF–STEM image and EDX elemental mapping of Fe_3O_4@ COF–LZU1; (D) hysteresis loops (the inset shows magnetic separation behavior of the Fe_3O_4@COF–LZU1 nanocomposites); (E) nitrogen adsorption–desorption isotherms of COF–LZU1, Fe_3O_4@COF–LZU1 and Fe_3O_4 (the inset shows the pore size distribution of COF–LZU1, Fe_3O_4@COF–LZU1 and Fe_3O_4).

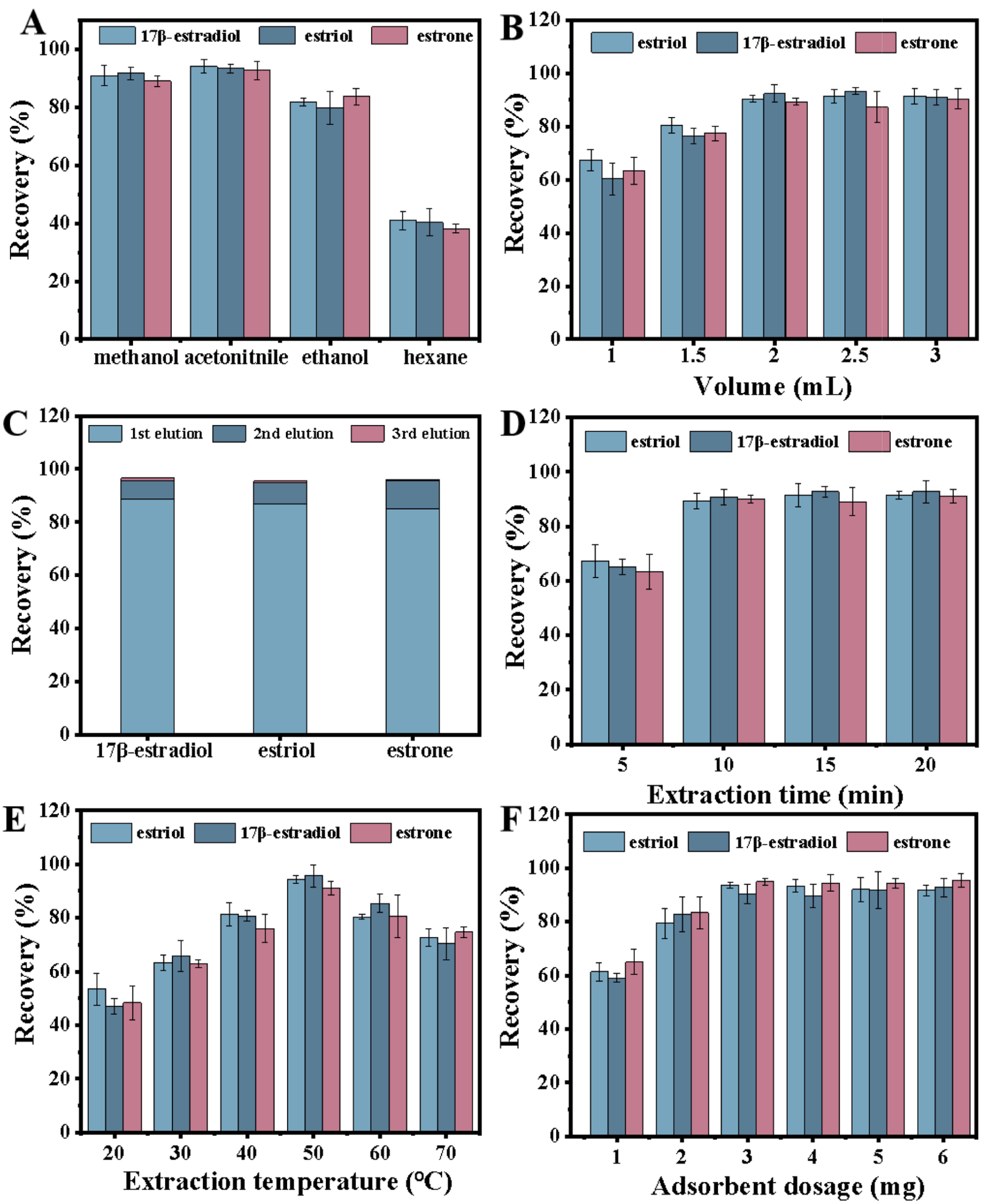

Fig. 3. Effect of (A) desorption solvent, (B) desorption solvent volume, (C) elution times, (D) extraction time, (E) extraction temperature, and (F) adsorbent dosage on extraction efficiency of estrogen.

3.3.1. Effect of desorption solvent and its volume and elution times

Four different organic solvents including methanol, acetonitrile, ethanol and n–hexane were compared for its efficiency of extracting the three estrogens. The results were shown in Fig. 3**A**, acetonitrile showed the highest recovery rate (92.7–94.0 %) for all three estrogens, making it the preferred choice for further study.

The volume of the desorption solvent was another important factor affecting recoveries. Hence, different volumes of acetonitrile (1.0, 1.5, 2.0, 2.5, and 3.0 mL) were tested after the extraction process (Fig. 3**B**). The recoveries of three estrogens showed a growing trend with the rise in acetonitrile volume, eventually plateauing at the 2 mL level with the recoveries ranging from 91.3 % to 94.5 %. No further improvement of the recovery was observed with addition acetonitrile. As a result, 2 mL was selected as the optimal volume of acetonitrile.

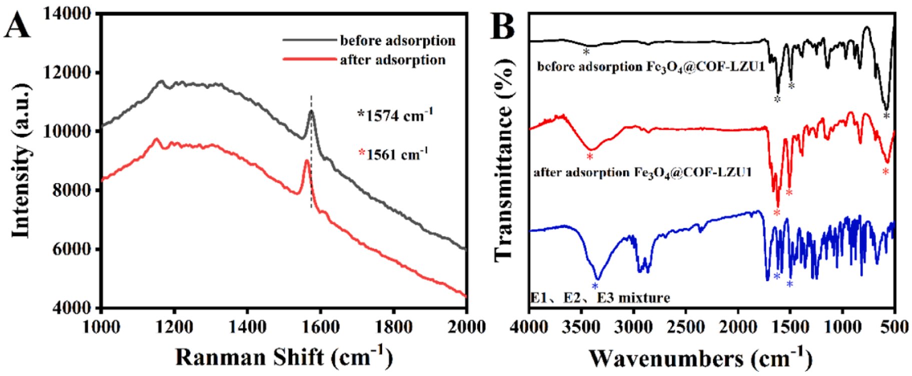

Fig. 4. (A) Raman spectra and (B) FT–IR before and after Fe_3O_4@COF–LZU1 adsorbed three estrogens.

In addition, the effect of solvent elution times was investigated. The result was shown in Fig. 3**C**, the recoveries of three estrogens reached to 95.0 %–96.8 % after twice elution with acetonitrile (1 mL each time), while the recovery of three estrogens after a third elution failed to rise further. Hence, two elution times was enough to eluate all three estrogens maximumly.

3.3.2. *Effect of extraction time*

The extraction time affects the achieving of absorption equilibrium for three estrogens on the Fe_3O_4@COF–LZU1, in order to maximize adsorption without unnecessary time consumption, the extraction time including 5, 10, 15 and 20 min were tested and optimized. As shown in Fig. 3**D**, the recoveries of three estrogens exhibited a significant upward trend within the first 5 to 10 min, with a slight additional increase at 15 min, and followed by a subsequent decrease (the highest recoveries of three estrogens reached 95.0–96.8 %). Consequently, 15 min was determined to be the optimal extraction time.

3.3.3. *Effect of extraction temperature*

Appropriate extraction temperature can accelerate the mass transfer of the analytes into the membrane and promote the adsorption of Fe_3O_4@COF–LZU1 (Huang et al., 2020). Therefore, different extraction temperatures (20, 30, 40, 50 and 60 °C) were investigated (Fig. 3**E**). The recoveries of the analytes exhibited a gradual upward trend in the range of 20–50 °C (the highest recoveries of three estrogens reached at 95.0–96.8 %). However, the recovery of analytes was reduced when the extraction temperature exceeded 50 °C, possibly due to the exothermic process of the analytes adsorbing onto the Fe_3O_4@COF–LZU1.Excessive temperature was found to be unfavorable for adsorption (Yue, Huang, Shen, Yang, & Pang, 2020). Therefore, the optimal extraction temperature was chosen at 50 °C.

3.3.4. *Effect of the adsorbent dosage*

Various dosages of sorbent (1, 2, 3, 4, 5 and 6 mg) were investigated to determine the most profitable adsorption for the analytes (Fig. 3**F**). The recovery rate remained stable when the adsorbent exceeded 3 mg. A higher adsorbent dosage would lead to a prolonged separation time and greater use of desorption solvent, therefore, we selected 3 mg as the optimal adsorbent dosage condition. This proves that a higher sorbent dosage is advantageous since it provids accessible bonding sites and a larger surface area for the adsorption of the three estrogens (Gopal, Al deeb, Raaov, Suah, Samad, Yahaya, & Zain, 2020). However, increasing the adsorbent dosage would result in longer separation time and a higher consumption of the desorption solvent. Hence, the optimal adsorbent dosage was 3 mg.

3.4. *Extraction kinetics*

Fig. S3**A** was showed the extraction of E1, E2 and E3 in the initial concentration of 250 μg L^{-1} as functions of time. Due to the abundantly available sites in the initial stage, three estrogens could be extracted rapidly in the first 6 min. Then the extraction rate decreased gradually and reached the extraction equilibrium in 10 min. To further assess three estrogens adsorption behavior of Fe_3O_4@COF–LZU1, the pseudo–first–order and the pseudo–second–order model were used to examine the adsorption kinetic. The models were expressed respectively by Eq. (1) and Eq. (2) listed as follows.

Pseudo–first–order: $\ln(q_e - q_t) = lnq_e - K_1 t$ (1) .

pseudo–second–order: $\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e}$ (2) .

Where, q_e and q_t (mg/g) respectively delegated the adsorption capacity at equilibrium and at a certain time, K_1 and K_2 respectively corresponded to pseudo–first–order and pseudo–second–order rate constants. The results were shown in **Fig. S3 (B, C)** and **Table S1**, the experimental data was well fitted to a pseudo–second–order kinetic model with a good fit coefficient ($R^2 = 0.9961$), which meant that the extraction process was mainly chemical adsorption rather than diffusion (Feng, Xu, Liu, Xue, & Zhang, 2020).

3.5. *The adsorption force*

According to the Raman spectrum (Fig. 4**A**), Fe_3O_4@COF–LZU1 showed a G band at 1574 cm^{-1}, attributed to the decrease in the size of the in–plane sp^2 domains. The analytes acting as electron–acceptors or donors can affect the electronic structure of adsorbents with conjugated systems through the π–π interaction (Wang, Zhang, & Wei, 2019). The electron–acceptors could make the absorption band shift to high frequency, while the electron–donors do the opposite. After the adsorption of three estrogens, the G band shifted to 1561 cm^{-1}, showing an electron–donating effect (Deng et al., 2019). Therefore, it indicated that there was a π–π interaction between the Fe_3O_4@COF–LZU1 and three estrogens.

According to the FT–IR spectrum (Fig. 4**B**), there were some groups of O—H and C═O at the surface of Fe_3O_4@COF–LZU1, which made it possible to have the hydrogen–bond interaction between the groups of Fe_3O_4@COF–LZU1 surface and O—H in three estrogens may be present (Wang et al., 2023). After adsorption, the O—H and C═O stretching vibration in Fe_3O_4@COF–LZU1 (Fig. 4**B, black curve**) was increased (Fig. 4**B, red curve**), and the O—H stretching vibration at 3347 cm^{-1} (Fig. 4**B, blue curve**) in three estrogens was shifted to 3429 cm^{-1} (Fig. 4**B, red curve**). These results were all evidence of hydrogen–bond interaction (Geng, Miao, Jin, & Yang, 2015).

According to the nitrogen adsorption (Fig. 2**E**), COF–LZU1 had a pore width distribution of 20.8 Å, which was suitable for the E1, E2 and E3 with a molecular size between 9.0 Å and 12.0 Å.

Hence, due to the synergy of the hydrogen bonding, π–π interactions and pore size effect, the Fe_3O_4@COF–LZU1 showed good adsorption performance towards three estrogens.

Table 1
Determination of 17β–estradiol, estriol and estrone in dairy products ($n = 3$).

Dairy products samples	Analytes	Found	Spiked recovery (RSD, %, $n = 3$)		
		μg kg^{-1}	10 μg kg^{-1}	50 μg kg^{-1}	100 μg kg^{-1}
Pasteurized milk	17β–estradiol	ND	89.63 (1.5)	90.06 (5.4)	88.86 (1.7)
	estriol	ND	91.49 (4.1)	99.65 (7.4)	93.22 (9.0)
	estrone	ND	91.25 (6.5)	95.90 (1.5)	77.25 (6.2)
Raw milk	17β–estradiol	ND	90.49 (2.0)	108.08 (7.7)	87.16 (3.3)
	estriol	0.53	82.10 (9.0)	91.88 (5.4)	109.40 (8.2)
	estrone	ND	89.06 (1.6)	87.25 (6.2)	83.32 (6.4)
UHT milk	17β–estradiol	ND	90.72 (1.7)	91.21 (5.4)	106.39 (3.3)
	estriol	ND	109.17 (3.4)	80.84 (8.9)	97.34 (2.9)
	estrone	ND	77.99 (6.5)	78.14 (3.8)	86.45 (5.9)
Skimmed milk	17β–estradiol	ND	80.08 (7.4)	98.84 (6.9)	86.11 (3.4)
	estriol	ND	94.54 (5.4)	79.77 (3.7)	79.30 (8.0)
	estrone	1.04	81.98 (8.2)	84.29 (3.4)	92.37 (1.5)

ND: not detect.

3.6. *Reused Fe_3O_4@COF–LZU1 nanocomposites*

Reusability was an important factor in presenting the excellent property and improving the application potential of Fe_3O_4@COF–LZU1. The used same batch of Fe_3O_4@COF–LZU1 was retrieved and eluted again with 2 mL acetonitrile. The eluting solution was analyzed by HPLC–FLD to prove that there were no residual estrogens on the sorbent, and the sorbent was dried in an oven at 60 °C. Then, the used Fe_3O_4@COF–LZU1 was reused for the extraction experiment of membrane separation MSPE under optimized conditions (Fig. S4 and Table S2). The recovery of three estrogens had hardly changed (no more than 4.01 %) after the sorbent had been used 10 times.

3.7. *Method validation*

To validate the developed MP–MSPE method, various analytical parameters were assessed under optimal conditions as shown in Table S3. It was found that good linearities of E1, E2 and E3 were obtained in the range of 0.1–800 μg L^{-1} ($R^2 > 0.996$). The limit of detection (LOD, S/N = 3) and limit of quantitation (LOQ, S/N = 10) of three estrogens were in the range of 0.01–0.15 μg L^{-1} and 0.06–0.80 μg L^{-1}, respectively. Six replicate samples at three concentration levels (50, 100, 200 μg L^{-1}) were analyzed to verify method stability. The results showed that the inter–day and intra–day RSDs were in the range of 3.7–7.3 % and 4.7–7.1 %, respectively, indicating acceptable repeatability and reproducibility. In addition, the batch–to–batch RSDs were less than 9.1 %, implying the good synthetic reproducibility of the Fe_3O_4@COF–LZU1.

3.8. *Real sample analysis*

The dairy products including pasteurized milk, raw milk, UHT milk, and skimmed milk were used to evaluate the applicability and reliability of the proposed method. The results are shown in Table 1, among the analyzed samples, the E3 was found in raw dairy products at the concentration of 0.53 μg L^{-1}. In addition, the accuracy of the method was verified by analyzing spiked dairy product samples at three concentrations of 10, 50 and 100 μg L^{-1}. The recoveries of three estrogens ranged from 77.3 to 109.4 % with RSD < 9.0 % for seven dairy product samples. These results indicated that the developed MP–MSPE coupled with the HPLC–FLD method had satisfied recovery and precision and was suitable for the extracting and analyzing estrogens in dairy products.

3.9. *Comparison with other references*

The MP–MSPE method was also compared with other methods reported previously for the determination of estrogens in dairy products (Table 2). Compared with other methods, MP–MSPE method employs less quantity (3 mg) of adsorbent, and less organic solvent during the sample preparation. Furthermore, this new method can be applied for a wider linearity range (0.1 to 800 μg L^{-1}) with comparable or even better LODs and LOQs than other previously reported methods.

4. Conclusions

In this study, the membrane–protected magnetic composite material (Fe_3O_4@COF–LZU1) was developed to obtain fast separation and effective enrichment of estrogens (E1, E2, E3). When combined with HPLC–FLD, this method enables a sensitive detection of three estrogens in dairy products. In addition, the developed MP–MSPE showed good stability as the extraction efficiency remaining above 90 % even after ten reuse cycles. The use of dialysis membranes in dairy products helps to reduce pollution and avoid the use of organic solvents. This developed method is simple, highly efficient and eco–friendly, which shows good application in the extraction and analysis of estrogens from dairy sample matrix.

CRediT authorship contribution statement

Cheng Yang: Validation, Investigation, Data curation. **Zheng–Lian Mo:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Qiu–Fang Zhang:** Validation, Investigation, Methodology. **Jin–Jie Xu:** Writing – original draft, Writing – review & editing. **Xiao–Fang Shen:** Conceptualization, Resources, Writing – review & editing, Project administration. **Yue–Hong Pang:** Methodology,

Table 2
Comparison with other methods for the determination of estrogens in dairy products.

Methods	Adsorbent (mg)	Instrument	Organic solvent (mL)	Analytes	LODs (μg L^{-1})	LOQs (μg L^{-1})	Linear range (μg L^{-1})	Ref.
DSPE	17β–E2–RAM–MIP (20)	RP–HPLC–UV	Methanol (3) Acetic acid	E2	2.08	9.29	10–500	(Wang et al., 2020)
TFME	—	HPLC–FLD	—	E3, E2, E1, 4–OP, 4–NP	0.2–6.1	0.5–20.0	0.5–250	(Lopes et al., 2019)
MSPE	rGO/$ZnFe_2O_4$ (10)	HPLC–DAD	Methanol (10.5)	E1, E2, EE2,	0.01–0.02	0.05	0.05–500	(Li et al., 2020)
MSPE	MMIPs (200)	HPLC–UV	Acetonitrile (40)	E1, E2, E3	0.03–0.08	0.11–0.27	—	(Chen et al., 2018)
MP–MSPE	Fe_3O_4@COF–LZU1 (3)	HPLC–FLD	—	E1, E2, E3	0.01–0.15	0.06–0.80	0.1–800	This work

Formal analysis, Validation, Investigation, Project administration, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.137984.

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