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Analytical Methods

Magnetic nanoparticles based dispersive micro-solid-phase extraction as a novel technique for the determination of estrogens in pork samples

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ABSTRACT

A simple and rapid magnetic nanoparticles (MNPs) based dispersive micro-solid-phase extraction (D-μ-SPE) method coupled with HPLC-DAD has been proposed for simultaneous determination of three estrogens (17β-estradiol (E2), estrone (E1) and diethylstilbestrol (DES)) in pork samples. In this paper, the synthesis of cetyltrimethyl ammonium bromide (CTAB)-coated Fe_3O_4@caprylic acid NPs as an efficient sorbent for its high surface area, excellent adsorption capacity, good dispersion ability and high superparamagnetic property was successfully applied to adsorb estrogens. Vortex was used to enhance mass transfer rate as it provided mild and effective mixing of sample solution and increased the contact between analytes and MNPs. The parameters affecting the extraction efficiency were investigated in detail. The dosages of sorbent and eluate are 100 μL and 500 μL, respectively. The extraction equilibrium was achieved within 2 min and the MNPs can be reused. The proposed technique provided high recoveries (93.3–106.7%), good linearity (0.9993–0.9999), low LODs (0.021–0.033 ng mL^{-1}) and repeatability (RSD% = 1.87–2.92).

1. Introduction

Steroid estrogens are a large group of lipophilic, low-molecular weight, high estrogenic active compounds, and they were roughly classified as natural and synthetic estrogens (Wang et al., 2011). Natural estrogens (also called endogenous estrogens) include estradiol (E2) and its most common metabolites or precursors: estrone (E1) and estriol (E3). These estrogens can promote growth of animals and improve the conversion efficiency of feeds, so they were extensively used in animal husbandry (Lammers, Heinrichs, & Kensinger, 1999). Also, it has been reported that estrogens existed in aquatic environments (Hecker, Tyler, Hoffmann, Maddix, & Karbe, 2002; Johnson, & Sumpter, 2001) and caused the feminization of male fish at much lower concentrations (1 pg mL^{-1}) in aquatic environment (Hansen et al., 1998). In consideration of the possible harmful effects on public health (Fuh, Huang, & Lin, 2004), the use of estrogens in food producing animals has been prohibited in European Community and China (Xu et al., 2013). Due to their potential carcinogenic properties and other adverse effects in human health, considerable interest was focused on developing cost-effective analytical methods for determining these compounds in samples at low concentration level (Wang et al., 2011). Therefore, developing a selective, accurate and sensitive analytical method for detecting estrogens in meat samples is crucial for the investigation of potential use of estrogens in food-safety area.

Up to now, several analytical methods have been described for estrogens analysis including high performance liquid chromatography (HPLC–DAD), liquid chromatography with mass spectrometry (LC-MS) (Iparraguirre et al., 2014), gas chromatography (GC) and gas chromatography with mass spectrometry (GC–MS) (Hansen et al., 2011), as well as micellar electrokinetic chromatography (MEKC) (Wen et al., 2013). These chromatographic methods involve traditional sample pretreatment procedures, such as liquid–liquid extraction (LLE) (Fernandez, Ikonomou, & Buchanan, 2007), liquid phase microextraction (LPME) and dynamic liquid–liquid–solid microextraction (DLLSME) (Zhong, Hu, Hu, & Li, 2012), ultrasound-assisted surfactant-enhanced emulsification microextraction (UASEME) (Zou et al., 2012), solid phase extraction (SPE) (Zhang, You, Ning, Song, & Suo, 2013), solid phase micoextraction (SPME) (Lan et al., 2014), stir bar sorptive extraction (SBSE) (Hu, Zheng, Zhu, & Li, 2007) and micro-solid-phase extraction (μ-SPE) (Wang et al., 2013).

SPE is one of the most popular sample pretreatment methods and has many significant advantages, such as improvements in

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automation, reproducibility and high-throughput capability (Li, & Lee, 2001; Mitani, Fujioka, & Kataoka, 2005). Dispersive micro-solid phase extraction (D-μ-SPE), which is categorized as a SPE technique, has many advantages compared to the traditional SPE such as short time requirement and reduced solvent consumption (Basheer, Alnedhary, Rao, & Lee, 2009; Basheer, Chong, Hii, & Lee, 2007), economic and easy to perform and convenience for efficiency of recovery. Magnetic carrier technology (MCT), based on magnetic nanoparticles (MNPs) for their high surface area-to-volume ratio and super-paramagnetism property, was first reported by Robinson (Robinson, Dunnill, & Lilly, 1973) and became popular as an analysis tool in analysis area. Naked Fe_3O_4 NPs, which have large surface area, were easy to cause particles agglomeration and form large clusters. Therefore, it is necessary to engineer the surface of MNPs to minimize their agglomeration problem through coating/modifying processes (Beiraghi, Pourghazi, & Amoli-Diva, 2014). Besides, the sensitivity and selectivity of MNPs were obtained by the modification of surface with functionalities (Moller, Kobler, & Bein, 2007; Tahmasebi, Yamini, Seidib, & Rezazadeh, 2013).

The purpose of this work is to develop a MNPs based D-μ-SPE method combining with HPLC–DAD for the preconcentration and determination of estrogens in pork samples. CTAB-coated Fe_3O_4@caprylic acid was used as sorbent and vortex was utilized as an assisted approach to accelerate the mass transfer. In addition, the sorbent was separated from the aqueous samples by an external magnet. E2, E1, and DES were selected as model compounds for examine the feasibility of method. Affecting factors of the CTAB-coated Fe_3O_4@caprylic acid based D-μ-SPE of three target estrogens (sorbent type, sorbent dosage, CTAB amount, sample pH, extraction time, and salt concentration) were investigated and optimized and the proposed method was successfully applied to the extraction and preconcentration of estrogens in pork samples.

2. Experimental

2.1. Chemicals and materials

Standards of 17β-estradiol (E2, 99.5%), estrone (E1, 99.0%), and diethylstilbestrol (DES, 99.7%) were supplied by Sigma (Sigma, USA). Ferric chloride ($FeCl_3$), ammonium ferrosulfate ($(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$), ammonium hydroxide (28%, w/v), hexanoic acid (HA), caprylic acid (CA), decanoic acid (DA) and cetyltrimethyl ammonium bromide (CTAB) were all purchased from Aladdin Chemistry (Shanghai, China). Methanol and acetonitrile (HPLC grade) was obtained from Merck (Darmstadt, Germany). Ultra-pure water was produced by a milli-Q system (Bedford, MA, USA). All reagents were at or above the analytical reagent grade.

2.2. Apparatus

An Agilent 1200 Series HPLC system (Agilent Technologies, Calif., USA) was used for Chromatographic separation and evaluation. This HPLC system includes an auto sampler, vacuum degasser, quatpump, and diode array detector, which is equipped with a reversed-phase C18 analytical column of 150 × 4.6 mm (Agilent TC-C18). Empowered software was employed to acquire and analyze chromatographic data.

Fourier transform infrared spectra (FTIR) were recorded on a TENSOR27 infrared scanner (Bruker, Germany) with a resolution of 2 cm^{-1} and a spectral range of 4000–400 cm^{-1}. Other instruments were used in the procedure, including a CS-400 transmission electron microscopy (TEM) (No. 45 Research Institute of CETC, China), a D8-advance X-ray diffraction (XRD) (Bruker, Germany), a sample bead homogeneous instrument (Pingli foreign trade and economic Ltd., Beijing, China), a vortex agitator (Kylin-Bell Lab Instruments Co. Ltd., Jiangsu, China), an ultrasonic cleaner (Kunshan ultra-sonic instrument plant, Jiangsu, China), a water bath (Shanghai happy instrument equipment Co. Ltd., Shanghai, China), a mechanical stirrer (Huanglong experiment instrument plant, Jiangsu, China) and a vacuum oven (Shanghai Yuezhong instrument equipment Co. Ltd., Shanghai, China). A strong Nd-Fe-B magnet (New magnetic factory, Guangzhou, China) was used for sorbent collection and magnetic decantation.

2.3. Chromatographic conditions

Acetonitrile and water were used as mobile phases and the gradient program contained the following steps: linear gradient of 35% acetonitrile for 0–4.5 min, from 35% to 55% acetonitrile for 6.0–14 min, from 55% to 35% acetonitrile for 14–16 min. The flow rate was set at 1.0 mL min^{-1}, the injection volume was 10 μL and the column temperature was maintained at 25 °C. The detection wavelength was at 280 nm.

2.4. Preparation of standard solutions and real samples

Stock standard solution of estrogens (1000 μg mL^{-1}) was prepared in methanol and stored in dark at 4 °C, which can be used for 2 months. Standard working solutions were prepared by dilution of the stock solution in methanol before use.

The fresh pork samples were purchased from supermarkets in Kunming, China. These pork samples were stored at 4 °C until analysis. A piece of fresh pork sample (about 2.0 g) was crushed to homogenate by using a sample bead homogeneous instrument, followed by adding 2 mL of methanol and sonicating for 20 min at room temperature. The organic phase was diluted to 5 mL with ultra-pure water for MNPs based D-μ-SPE procedure.

2.5. Synthesis of the MNPs

2.5.1. Synthesis of Fe_3O_4@caprylic acid NPs

Analogously to the synthesis method of Fe_3O_4@caprylic acid NPs which was studied by Asgharinezhad, Mollazadeh, Ebrahimzadeh, Mirbabaei, & Shekari (2014). Briefly, 2.05 g of ammonium ferrosulfate and 1.41 g of ferric chloride were dissolved under N_2 atmosphere in 40 mL of ultra-pure water with constant stirring at 1500 rpm using a mechanical stirrer. 6 mL of $NH_3{\cdot}H_2O$ and 2 mL of caprylic acid were added when the solution was heated to 80 °C. The crystal growth was allowed to proceed in water bath at 80 °C for 30 min under vigorous stirring under N_2 atmosphere. Then the water-based suspension was cooled down to room temperature slowly. After that the suspension was precipitated with ethanol and ultra-pure water, and the precipitates were isolated from the supernatant solution by magnetic decantation. This washing decantation procedure was repeated six times to remove the excess of caprylic acid. At last, the obtained Fe_3O_4@caprylic acid NPs were vacuum-dried at 55 °C for 10 h. Then the synthesized Fe_3O_4@caprylic acid NPs were stored at 4 °C.

2.5.2. Synthesis of other functionalized NPs

The synthesis of Fe_3O_4@HA NPs and Fe_3O_4@DA NPs was similar with the above synthesis of Fe_3O_4@caprylic acid NPs.

The synthesis of Fe_3O_4 NPs: 2.05 g of ammonium ferrosulfate and 1.41 g of ferric chloride were dissolved under N_2 atmosphere in 40 mL of ultrapure water with constant stirring at 1500 rpm using a mechanical stirrer. 6 mL of $NH_3{\cdot}H_2O$ was added when the solution was heated to 80 °C. The crystal growth was allowed to proceed in water bath at 80 °C for 30 min under vigorous stirring under N_2 atmosphere. Then the water-based suspension was cooled down to room temperature slowly. After that the