Journal of Chromatography A, 1331 (2014) 10–18

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

An automated solid-phase microextraction method based on magnetic molecularly imprinted polymer as fiber coating for detection of trace estrogens in milk powder

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ARTICLE INFO

Article history:
Received 1 October 2013
Received in revised form 5 January 2014
Accepted 10 January 2014
Available online 15 January 2014

Keywords:
Magnetic molecularly imprinted polymer
Solid-phase microextraction
Automation
Estrogens
High performance liquid chromatography

ABSTRACT

A new automated solid-phase micro extraction (SPME) sampling method was developed for quantitative enrichment of estrogens (ES) from milk powder, using magnetic molecularly imprinted polymer (MMIP) as fiber coating. The method (MMIP-SPME) was built with several electromagnetic stainless steel fibers, placed in parallel for simultaneously extraction. The MMIP was synthesized using core–shell Fe_3O_4@SiO_2 nanoparticles (NPs) as magnetic support. Estradiol (E2) was employed as the template molecule, acrylamide (AA) as functional monomer, and ethylene glycol dimethacrylate (EGDMA) as cross-linker. MMIP can be easily absorbed or desorbed from fibers when the current is turned on or off, creating magnetism. Compared to traditional MIP-SPME, the prepared procedure of MMIP-SPME is time-saving and organic solvent-free. The proposed device significantly improved the efficiency of separation and enrichment of estrogens from complex matrices thereby and facilitating the pretreatment steps by electromagnetically controlled extraction fibers to achieve full automation. Several experimental parameters were studied, including extraction and desorption kinetics, solution pH, desorption solution, ratio, and shuttle rate. The newly developed MMIP-SPME showed good sensitivity and high binding capacity, fast adsorption kinetics and desorption kinetics for estrone (E1), estradiol (E2), estriol (E3) and diethylstilbestrol (DES) under optimized conditions. The detection limits for the four estrogens were 1.5–5.5 ng g^{-1} with excellent reproducibility (RSD values less than 7.1%) when milk powder samples spiked with analytes at 20, 100 and 250 ng g^{-1} were studied.

1. Introduction

Estrogens are a group of steroidal hormones, both of natural and synthetic origin, among which the prominent compounds are naturally occurring 17β-estradiol (E2), estrone (E1), estriol (E3) and synthetic diethylstilbestrol (DES). Widespread evidence has shown that estrogens can adversely affect humans and animals at low-ng/L concentrations due to their high estrogenic activities [1–5]. Furthermore, new findings indicated that some of these estrogens or their metabolites play a role in human carcinogenesis, such as in breast, prostate, and ovarian cancers [6–10]. Estrogens have been identified in environmental waters, sediments, and some aquatic product matrices [11–16]. However, only few studies have been reported for milk powder matrix in this regard [17–19]. In addition to this, illegal application of estrogens to promote growth rate of animals became a main hazard to human health, as they are transported in blood and then synthesized by the mammary glands and finally excreted in the milk [19]. So it is understood that milk is an important source of estrogens. It has been reported that approximately 60–80% of estrogens come from milk and other dairy products in Western diets [20]. Therefore, there is an urgent need to develop a fast and sensitive analytical method for determining ultra-trace levels of estrogens in milk powder samples, for protecting the health of humans.

Preparation of sample is a crucial step in the analysis of targets in actual specimens: as it is a tedious process and any imprecision or inaccuracy in the process can confound the whole analysis [21]. Such sample preparation methods like liquid–liquid extraction (LLE) [22] and solid-phase extraction (SPE) [23] have been usually applied to determine the level estrogens. However, these methods are time-consuming and require large volumes of organic solvent. Recently, solid-phase microextraction (SPME) has been reported for monitoring estrogens due to its convenience, low solvent consumption and ease to couple with chromatographic instruments [24]. However, various materials, such as polyacrylate (PA) [25],

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http://dx.doi.org/10.1016/j.chroma.2014.01.016

bis(trimethylsilyl) trifluoroacetamide (BSTFA) [26], polydimethylsiloxane/divinylbenzene (PDMS/DVB) [27] and poly(AA-VP-Bis) monolith [28] that are used for coating, are insufficient for selectivity adsorption of ES. Molecularly imprinted polymer (MIP) is acclaimed to be artificial antibodies, owing to their unique characteristics such as predetermined selective recognition for target, high chemical and physical stabilities, good preparation convenience and long lifetime [29]. Till date, MIP has been widely employed in many fields for the analysis of estrogens, such as solid phase extraction (SPE) [30,31], dispersive solid phase extraction (DSPE) [32,33] and dynamic liquid–liquid–solid microextraction (DLLSME) [34], whereas its application in SPME for analysis of estrogens has been seldom reported [16]. As we know, for application of MIP in SPME, the MIP was prepared by different techniques such as free-radical polymerization (FRP) and controlled/living radical polymerizations (CRP). However, preparation of MIP with above methods presents some problems such as complex chemical bonding and multiple co-polymerization strategy [35]. Using a time saving and solvent free preparation is a possible alternative to overcome the above troubles. Magnetic molecular imprinted polymer (MMIP), which can selective recognize of targets and readily be isolated from the complex matrix by the application of an external magnetic field, have recently attracted many researchers' attention [36–38]. In addition, the electronically controlled stainless steel fibers can create magnetic field and be used as substrate for SPME for bonding with MMIP to form a MMIP-SPME fiber. The MMIP can be easily and stably coated on the fiber by adequate magnetic force by increasing the current level to the fiber. The binding force of the magnetic adsorption is strong. Moreover, the film can be easily renewed when the magnetic field is removed.

Automation is very important in practical application of sample pretreatment, which can perform the extraction and solvent desorption steps of multiple samples in parallel and presents a time-efficient method [39]. This is particularly important to analytical laboratories where sample throughput is high and sample turnaround time should be as short as possible. Therefore, the automation of SPME has risen dramatically in recent years [39–42]. Significantly, Pawliszyn and his group are currently working on automation of SPME in a commercially 96-well plate format, which involved the construction of a multi-fiber SPME top plate. The array of SPME devices is expected to perform the extraction and solvent desorption steps in parallel presenting a time-efficient method for the evaluation of multiple samples [39]. It provided us a useful guidance for adoption of multiple fibers (24 stainless steel fibers coated by MMIP layer) in MMIP-SPME to make the sample pretreatment automated.

In this study, MMIP was prepared with E2 as template and easily coated on the surface of stainless steel fibers through electromagnetic bonding strategy, which can facilitate the preparation of SPME. The characterization of MMIP was investigated and the preparation conditions of MMIP coating were also studied. We also fabricated an automatic MMIP-SPME apparatus for sample preparation, which contained an array of 24 electromagnetic fibers and was engineered for simultaneous treatment of several samples and to determine the amounts of four estrogens in milk powder when coupled with high performance liquid chromatography (HPLC).

2. Experimental

2.1. Chemicals and reagents

Ethyleneglycol dimethacrylate (EGDMA), γ-methacryloxypropyl trimethoxysilane (MPS), acrylamide (AA), methacrylic acid (MAA), azo(bis)-isobutyronitrile (AIBN), estrone (E1), estradiol (E2), estriol (E3) and diethylstilbestrol (DES), were obtained from Aladdin Chemistry (Shanghai, China). Tetraethoxysilane (TEOS) was purchased from Sigma–Aldrich (St. Louis, MO, USA). The HPLC-grade acetonitrile was acquired from CNW Technologies (Dusseldorf, Germany). The electromagnetic control system was designed and produced by GENE INN technology (Ningbo, China). All other reagents were of analytical grade. All solutions used for HPLC mobile phase were filtered through a nylon 0.22 μm filter prior to use. The commercial SPME fibers with PA, PDMS and PDMS/DVB coatings were purchased from Supelco (Bellefonte, PA, USA).

HPLC was performed with a Shimadzu (Japan) system consisted of a LC-20AT pump and a SPD-20A UV-detector. All separations were carried out on a C_{18} column (GL Sciences, 5 μm, 150 × 4.6 mm) with a flow rate of 1.0 mL min^{-1} at room temperature. The UV–vis detector was operated at 280 nm.

The particle size and structure of the nanocomposites were observed by using a JEOL 2100 transmission electron microscope (TEM). The Fourier transform infrared (FT-IR) spectra of the obtained nanocomposites were taken in KBr pressed pellets on a NEXUS 670 infrared Fourier transform spectrometer (Nicolet Thermo, Waltham, MA). The magnetic properties were conducted using a Quantum Design Physical Property Measurement System (PPMS-9, Quantum Design, CA). The XRD characterization was performed using X-ray diffraction (Bruker, D8 Focus) with Cu Kα radiation at room temperature.

2.2. Preparation of MMIP

The whole protocol for synthesis of MMIP is shown in Fig. 1S in the supplemental material. At first Fe_3O_4@SiO_2-MPS microspheres were prepared. The Fe_3O_4 particles were synthesized using a solvothermal method according to the work of Xuan et al. [43]. Typically, 2.70 g of $FeCl_3{\cdot}6H_2O$ was first dissolved in 80 mL ethylene glycol to form a yellow solution, 7.20 g of sodium acetate was then added to the above solution, stirred for 30 min, and the resultant solution was transferred to a stainless-steel autoclave (100 mL) lined with Teflon, sealed and heated at 200 °C for 8 h, and then cooled to room temperature. The obtained black magnetite particles were collected, washed several times with ethanol and dried under vacuum at 50 °C for 12 h. Then the core–shell Fe_3O_4@SiO_2 particles were prepared through a sol–gel approach according to Deng et al. [44]. The resultant Fe_3O_4@SiO_2 microspheres were separated by an external magnetic field, washed with ethanol and purified water, then dried in vacuum at 50 °C. Then MPS was modified on the surface of the obtained Fe_3O_4@SiO_2 spheres through a surface modification approach with MPS as a polymerizable silane coupling agent [45].

Four types of MMIP and their corresponding magnetic non-imprinted polymer (MNIP) were prepared by surface-imprinted polymerization method through E2 as template. Different polymerization mixtures used were listed in Table 1. The polymerization solution was prepared by transferring 0.1 g MPS modified Fe_3O_4@SiO_2, together with 0.11 mmol template (E2) and 0.44 mmol functional monomer to a 250 mL round-bottom flask containing 60 mL solvent. The mixture was stirred for 1 h at room temperature until the template-monomer complex was formed by non-covalent interactions, then 1.76 mmol EGDMA and 50 mg AIBN were added. The resulting solution was placed under nitrogen and submitted to 5 min ultra-sonication in an ice-bath. The mixture was heated at 50 °C for 6 h, 65 °C for 24 h and 85 °C for 8 h. After polymerization, the resulting MMIP was separated by an external magnet and rinsed with purified water and ethanol. Finally, the obtained MMIP beads were eluted by 15 mL methanol/acetic acid (4/1, v/v) solution for 90 min (with shaking). After eluted for 5 times, the template E2 could not detected by HPLC. The final