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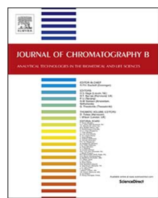

Development of immunoaffinity solid phase microextraction rods for analysis of three estrogens in environmental water samples

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A B S T R A C T

In this study, immunoaffinity solid phase microextraction (SPME) rods were developed for the analysis of diethylstilbestrol (DES), hexestrol (HES) and dienestrol (DIS) followed by ultra high-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS). This immunoaffinity SPME device was built with three stainless steel rods bundled together as one and modified with porous silicate particles. As an extraction sorbent, antibody immobilization approach was employed based on the covalent attachment of the anti-diethylstilbestrol monoclonal antibody (mAb) onto the rods. The immunoaffinity SPME rod was characterized and confirmed by scanning electron microscopy and X-ray photoelectron spectroscopy analysis. The porous network showed a very large active surface area and significantly increased the adsorption capacity which can reach 49.6 pmol/cm^2. Moreover, the immunoaffinity sorbent showed good sturdiness at least 10 times with stable extraction performance. Once the main experimental parameters were optimized, the method was used to detect DES, HES and DIS in environmental water samples. The limits of detection for the three estrogens were 0.05–0.15 ng/mL, and the limits of quantification was 0.5 ng/mL. The average recoveries ranged from 34.2 to 62.7% were achieved with good intra-day and inter-day precision ranging from 7.8 to 12.7% and from 8.2 to 13.5% respectively. The newly developed immunoaffinity SPME showed high adsorption capacity, good sensitivity and reproducibility and was successfully applied to the analysis of DES, HES and DIS in environmental water samples.

1. Introduction

Diethylstilbestrol (DES), hexestrol (HES) and dienestrol (DIS) as typical synthetic non-steroid estrogens, are commonly used for treating estrogen deficiency and infertility in food animals. In addition, the above estrogens can improve the feed conversion rate and have been used widely as a typical growth promoter in animal husbandry [1]. However, it could enter human body through food chain and interfere with the normal functions of the endocrine systems, even cause breast cancer and prostate cancer [2].

Till now, it is of great importance while still a big challenge to develop a selective, simple, fast, sensitive and environmental-friendly method for detecting estrogens in the environmental water and food samples. As we all know, sample preparation is very important to increase the sensitivity and selectivity of the method by concentrating the analytes and decreasing the interference of matrix. However, conventional sample preparation methods including liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are expensive and time-consuming also require lots of toxic organic solvents which are harmful both to the laboratory staff and to the environment. Therefore, extensive efforts have been made in developing novel sensitive, simple and solvent-minimum sample preparation methods nowadays. Such as ionic liquid-dispersive liquid–liquid microextraction (IL-DLLME) [3], micro-dispersive solid phase extraction (m-μSPE) which used the magnetic nanoparticles as sorbent [4], magnetic molecularly imprinted polymer (MMIP) [5], stir bar sorptive extraction (SBSE) [2,6–8], hollow fiber liquid–liquid microextraction (HF-LLME) [9] and solid phase microextraction (SPME) [10]. For SBSE, the main drawback is the friction between the stir bar and container which shorten the lifespan of the coating obviously. HF-LLME is low cost, easy to operate and has less solvent consumption but low reproducibility [11].

Compared with other sample preparation methods, SPME has a great advantage of simplicity by integrating sampling, extraction, concentration and sample introduction into a single step [12]. Also it has been reported for monitoring estrogens due to its convenience, simplicity, low solvent consumption and easy to couple with various chromatographic instruments (LC,GC) [13]. Several literature applied SPME in sample preparation for analysis of estrogens, Mitani et al. [14]

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Table 1
The basic properties, column capacities and cross-reactivity of target estrogens.

Compounds	Molecular formula	Molecular mass	Chemical structures	Column capacities (pmol/cm^2)	Cross reactivity (%)
DES	$C_{18}H_{20}O_2$	268.35	HO, OH	49.6	100
HES	$C_{18}H_{22}O_2$	270.37	HO, OH	44.6	108
DIS	$C_{18}H_{18}O_2$	266.33	HO, OH	23.8	64

report the application of in-tube SPME coupled with liquid chromatography–tandem mass spectrometry (LC/MS/MS) for the determination of five estrogens several years before. Recently, Mei, Meng et al. [15] developed a novel method for simultaneous monitoring of six estrogen mimics by coupling multiple monolithic fiber solid-phase microextraction (MMF-SPME) to high performance liquid chromatography with diode array detection. In the present study, SPME was combined with immunoaffinity enrichment to create antibody-linked immunoaffinity SPME sorbents capable of identifying 3 stilbenes, which can hinder the effects of co-extraction from interferents owing to their unique merit of extraordinary specific recognition ability towards target analytes.

Immunoaffinity SPME is a new form of immunoaffinity chromatography (IAC) in which highly selective antibodies are immobilized on solid supports such as fused silica fiber and fused silica capillary. SPME rods with antibodies specific for the analytes of interest have been investigated previously to overcome some difficulties like low selectivity observed with the use of conventional adsorptive phases for analysis of drugs in biological samples[16]such as 7-aminoflunitrazepam [16], benzodiazepines [17], fluoxetine [18], quinolones [19] with mAb immobilized on the silica rods or capillaries and achieved good results. Liu et al. [20] introduced another method for antibody immobilization by using porous silica particles entrapped in a network of polymerized silicate on stainless steel fiber to identify protein PBP2a. This silica-based coating was prepared primarily by Francois Breton and his coworkers, and showed very good ruggedness and robustness [21].

To the best of our knowledge, there is no previous publication nor report about immunoaffinity SPME fabricated in the way mentioned above for determine estrogens in complex samples. In the present study, we prepared immunoaffinity SPME silica-based coatings on stainless steel rods for the determination of 3 estrogens in environmental water samples prior to ultra high-performance liquid chromatography-mass spectrometry analysis.

2. Materials and methods

2.1. Chemicals and materials

Standard of diethylstilbestrol, hexestrol and dienestrol were purchased from Dr. Ehrenstorfer GmbH (Germany). Methanol and acetonitrile (HPLC grade) were obtained from Merck (Darmstadt, Germany). Ultrapure water was produced by a milli-Q system (Bedford, MA, USA). Sodium chloride, potassium chloride, potassium phosphate monobasic and sodium phosphate dibasic were purchased from Tianjin Chemical Reagent No. 3 Plant (Tianjin, China). Absolute ethanol was purchased from Tianjin Jinke Fine Chemical Research Institute (Tianjin, China). (3-aminopropyl)-triethoxysilane (APTES), glutaraldehyde grade II (25% aqueous solution) and ethanolamine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Potassium silicate (Kasil 1, SiO_2:K_2O mole ratio, 3.92; weight ratio, 2.50) was obtained from PQ Corporation (Valley Forge, PA, USA). Ascentis porous silica particles (underivatized), 5 µm in diameter, were provided by Supelco (Bellafonte, PA, USA). Hydrochloric acid and nitric acid were provided by Tianjin agricultural university. Stainless steel wires, grade 304 were purchased from Xinde stainless steel plant (Tianjin, China). The anti-diethylstilbestrol monoclonal antibody (mAb) was produced by our laboratory and showed cross-reactivity with DES, HES and DIS in the range of 64%-108% (Table 1). All reagents were at or above the analytical reagent grade. Samples of the Haihe river were collected from Tianjin, China.

Stock standard solution of DES (100 µg/mL), HES and DIS (10 µg/mL) were prepared by dissolving appropriate of each standard compound in methanol and stored in the refrigerator at 4 °C. Working standard solutions were prepared by daily diluting the stock solution with PBS. Prior to HPLC analysis, all obtained samples were filtered through the 0.22 µm filter of nylon.

2.2. Preparation of immunoaffinity SPME (IA-SPME)rods

One end of stainless steel rods (diameter of 2 mm, length ~10 cm), measuring 1.8 cm in height, were coated with porous silica particles using a procedure previously described in the literature [21]. Then antibodies were immobilized to the resulting silica network according to Liu et al. [20] and Lord et al. [17] with some modifications. Briefly, one end of the stainless steel rods were etched with hydrochloric acid for 30 min then the rods were rinsed with deionized water copiously and dried in an oven at 130 °C for 1 h. The dried and cooled rods were dipped from the etched end (length: 2 cm) in potassium silicate solution, and then gently rolled over 5 µm porous silica particles. The coating was exposed to fumes of concentrated nitric acid for 15 s and allowed to dry at ambient temperature for 15 h. Then the coated rods were heated in a programmed oven to ensure thorough drying. After heating, the rods were immersed into ethanolic APTES solution (5 mL APTES, 5 mL water and 90 mL absolute ethanol) overnight with shaking (60 rpm) at room temperature. The bottle of APTES was layered with nitrogen after each use. After rinsing with H_2O and ethanol, the rods were dried in a vacuum oven at 80 °C and flushed with N_2 for 15 h. The rods were then functionalized with carbonyl groups

Table 2
LC–MS/MS acquisition parameters.

compounds	Parent ion (*m/z*)	daughters ion (*m/z*)	Cone (V)	Collision (V)
DES	267.1	237.1[a]	35	29
		251.1	35	25
HES	269.1	119.0	25	40
		134.0[a]	25	16
DIS	265.1	93.0[a]	35	25
		171.0	35	25

[a] Quantificative ion.

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