Journal of Pharmaceutical and Biomedical Analysis 94 (2014) 118–124

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Preparation of a stir bar coated with molecularly imprinted polymer and its application in analysis of dopamine in urine

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ARTICLE INFO

Article history:
Received 21 October 2013
Received in revised form 23 January 2014
Accepted 26 January 2014
Available online 2 February 2014

Keywords:
Molecularly imprinted polymer
Stir bar sorptive extraction
Dopamine
Human urine

ABSTRACT

A molecularly imprinted polymer coated stir bar (MIP-SB) using dopamine as a template was fabricated. The adsorptive capacity of the MIP-SB was almost 4 times over that of non-imprinted stir bar. The MIP-SB showed good extracting selectivity for dopamine when used to adsorb dopamine and its analogs. An analytical method to determine dopamine in urine sample was established by combining MIP-SB sorptive extraction with HPLC-fluorescence detector. The linear range of dopamine concentration was 0.378–18.9 ng/ml with correlation coefficient of 0.9990, and the limit of quantification was about 0.0945 ng/ml (S/N = 10). The recoveries of dopamine with spiked urine samples at three different levels were between 92.3 and 106.9%, and the relative standard deviations were within 13.2% (n = 3). The method is simple and suitable for the determination of dopamine in human urine for clinical application.

1. Introduction

Dopamine (DA) is a well known neurotransmitter in the central nervous system. The dopaminergic system affects a wide range of behaviors and functions, including cognition, motor activity, emotion, memory, neuroendocrine regulation, and selective attention [1,2]. Deficiency in DA is associated with movement and neuropsychiatric disorder, such as Parkinson's disease, Alzheimer's disease and depression [3,4]. On the other hand, excess of DA in human body can cause a state of excitement, which may relate with anxiety disorder and attention-deficit hyperactivity disorder [1]. So the quantification of DA in biological samples occupies an important position in clinical diagnosis and pathological mechanism research of neuropsychiatric diseases.

Capillary electrophoresis and liquid chromatography equipped with fluorescence detector (FLD) [5,6], electrochemical detectors [7], mass spectrometry [8,9], or ultraviolet detector [10] have been developed to detect DA in biological fluids. But these instrumental analyses could not avoid the complicated procedures of sample preparation. Traditional sample preparations such as liquid-liquid extraction, solid phase extraction (SPE), etc., are time-consuming, organic solvent-consuming and tough operating. In recent years, many new methods have been developed to overcome the defects of the sample preparation described above [11–13], and stir bar sorptive extraction (SBSE) has obtained especial attention [14,15].

SBSE technique has been highly noted due to its advantages of much higher capacity and no additional stirring compared with SPE [16]. Up to now, there are three types of commercially available stir bars, which are polydimethylsiloxane (PDMS) Twister, ethylene glycol-silicone Twister and polyacrylate Twister [17,18]. However, the three kinds of Twisters lack extraction selectivity because they extract target compounds together with those having similar polarity, which would bring troubles for further quantitative analysis. In recent years, many efforts have been made in developing various new SB coatings to expand applications of SB and improve its extraction selectivity. For example, Hu et al. prepared a SB with PDMS/β-cyclodextrin as the coating and applied it in the extraction separation of polar compounds [19]. Huang et al. introduced a monolithic material obtained by the copolymerization of octyl methacrylate and ethylene dimethacrylate for the SB coating and used it to analyses polycyclic aromatic hydrocarbons [20]. Molecularly imprinted polymers (MIPs) with their unique characteristics of high selectivity for the template and simple preparation have proved to be powerful materials as the coatings of SB [21]. Zhan et al. prepared a highly selective SB coated with dummy MIP for trace analysis of bisphenol A in milk [22], Li et al. prepared MIP-SBs and applied them in the trace analysis of ractopamine and trimethoprim in complex samples, respectively [23,24]. Prasad et al. [25] also prepared a MIP-SB using DA as a template. The

Abbreviations: DA, dopamine; SPE, solid phase extraction; SBSE, stir bar sorptive extraction; MIP-SB, molecularly imprinted polymer coated stir bar; PDMS, polydimethysiloxane; E, L-epinephrin; NE, L-norepinephrine; 5-HT, 5-hydroxytryptamine; EGDMA, ethylene glycol dimethacrylate; TRIM, trimethylolpropan trimethacrylate; γ-MAPS, 3-(trimethoxysilyl) propyl methacrylate; 4-VPY, 4-vinylpyridine; MAA, methacrylic acid; ACM, acrylamide; AIBN, 2,2′-azobis-(isobutyronitrile).

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http://dx.doi.org/10.1016/j.jpba.2014.01.041

silane coupling agent and functional monomer used in their MIP-SB preparation are not available, and need to be synthesized. The synthesis of MIP-SB for DA is multistep and complex.

In the present study, a simple synthetic method for preparing the MIP-SB using DA as the template molecule was introduced. The synthetic process of the MIP-SB is very simple and all the reagents used in the synthesis are commercially available. The coating of the MIP-SB was characterized by scanning electron microscope (SEM) and Fourier-transform infrared spectrometer (FT-IR). The capacity and selectivity of the MIP-SB for extracting DA were also investigated. The extraction conditions were optimized in aspects of solvent, time and pH. The MIP-SBSE was successfully applied in the determination of trace DA in human urine samples. The urine samples were simply stirred with the MIP-SB; no protein precipitation and organic solvents were needed.

2. Experimental

2.1. Materials and chemicals

Dopamine hydrochloride (DA, 98%), L-epinephrine (E, 99.0%), L-norepinephrine (NE, 98%), 5-hydroxytryptamine (5-HT, 98%), ethylene glycol dimethacrylate (EGDMA), trimethylolpropan trimethacrylate (TRIM), and 3-(trimethoxysilyl) propyl methacrylate (γ-MAPS, 97%) were purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). 4-Vinylpyridine (4-VPY, 98%) was purchased from Sigma–Aldrich (St. Louis). Methacrylic acid (MAA, 98%), sodium phosphate dibasic dodecahydrate and sodium phosphate monobasic dihydrate were purchased from Lingfeng Chemical Reagent Co. Ltd. (Shanghai, China). Acrylamide (ACM, 98%) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). 2,2′-Azobis-(isobutyronitrile) (AIBN, recrystallized before use) was purchased from Shanghai Jingchun Chemiscal Reagent Co. Ltd. Ultrapure water was obtained from a PURELAB Classic water purification system (PALL, Washington, NY, USA). Methanol was of HPLC-reagent grade and other chemicals were analytical pure.

A commercial stir bar (Twister; Gerstel; Germany) coated with 10 mm in length and 0.5 mm film thickness of PDMS (PDMS-SB) was used to compare with MIP-SB.

2.2. Instruments

A S-3400N II scanning electron microscope (Hitachi, Japan) was used to investigate the surface and thickness of the coating. FT-IR spectra were recorded on a TENSOR 27 spectrometer (Bruker, Germany).

The method validation and urine sample analysis were conducted on a HPLC-FLD system equipped with a LC-20A pump (Shimadzu, Japan), a RF-10Axl fluorescence detector (Shimadzu, Japan), and 7725i manual injector (Waters, Oasis, USA) with 20 μl loop. The optimization of the experimental conditions, such as selection of functional monomer, cross-linker, porogen, extraction and desorption solvent was performed with HPLC-UV detection system, which included a Waters 515 pump, a water 2487 Dual λ absorbance detector (Waters, USA) and a 7725i manual injector (USA) with 20 μl loop. Agilent 6410B Triple Quard LC-MS-MS with ESI source was used to identify DA in urine sample. The capillary voltage was set at 4000 V with a cone voltage of 15 V for DA. The desolvation temperature was set at 340 °C. The desolvation gas flow was set at 9 l/min.

2.3. Preparation of MIP-SB

The bare glass bars were made by putting an iron wire into a glass tube (3 cm × 5.0 mm id), and then the two sides of each tube were sealed by heat. Before polymerization, the glass bars were treated as follows. First, the bars were immerged in 1.0 M sodium hydroxide for 8 h, rinsed with water, and then neutralized with 0.1 M hydrochloric acid for 8 h. Secondly, the activated bars were washed again with water and dried in an oven at 120 °C for 2–3 h, then silylated for 3 h in acetone containing 10% (v/v) γ-MAPS, and finally washed with methanol and dried under a stream of nitrogen.

The reactant solution for synthesis of molecular imprinted polymer was prepared by mixing 189 mg of DA, 0.34 ml of MAA, 1.95 ml of EGDMA, 0.05 ml of TRIM, 35 mg of AIBN, 2.8 ml of methanol and 0.7 ml of water. Then the mixture was stirred thoroughly and degassed for 20 min in an ultrasonic bath. Subsequently, 2 ml of the reactant solution was poured into a small glass tube, deoxygenized with a nitrogen stream for 5 min. The pretreated bars above were vertically inserted into the reactant solution and the polymerization was sustained at 60 °C under nitrogen for 24 h. The New MIP-SB was aged at 100 °C in vacuum drying oven, and then eluted with methanol-acetic acid (99:1, v/v) until no DA was detected in the soaking solution by HPLC. The non-imprinted polymer coated stir bar (NIP-SB) was prepared with the same procedure except the addition of DA.

2.4. Characterization of MIP coating of the prepared stir bar

FT-IR spectrometry was performed to characterize the coatings of MIP-SB and NIP-SB. The scanning electron microscope (SEM) was also employed to investigate the morphology and thickness of the MIP-SB coating.

2.5. Chromatographic conditions

A Phenomenex C18 column (5 μm, 250 mm × 4.6 mm id, Guangzhou, China) was used for chromatographic experiments. The wavelength for the UV detector was set at 280 nm. The excitation and emission wavelengths for the FLD were 290 nm and 325 nm, respectively. 10 mM phosphate buffer solution with pH 5.8 (A) and methanol (B) were used as the elutions (A:B = 98:2, v/v) for the two chromatographic systems, and the both flow rates were 1 ml/min at 30 °C.

2.6. Optimization of MIP-SBSE procedure

The conditions of MIP-SBSE procedure were optimized in order to obtain satisfactory recovery of DA. Methanol, ultrapure water, ethanol, acetonitrile and acetone were tested to investigate the extraction effect of MIP-SBSE for DA. 10 ml of each solvent containing 18.9 ng/ml DA was extracted with a MIP-SB in a round bottom flask. After extraction, the MIP-SBs were taken out from the solutions, gently dried with tissue paper, and immersed separately in 1.0 ml of methanol-acetic acid (99:1, v/v) for ultrasonic vibration to release the extracted analyte. The solvents were evaporated under a stream of nitrogen and the residues were redissolved with 150 μl of ultrapure water for HPLC-UV analysis. The optimized extraction solvent was chosen based on the recovery of DA.

10 ml of the above optimized extraction solvent containing 18.9 ng/ml DA was added into a round bottom flask in six copies, and six MIP-SBs were separately put into the six flasks. After extraction, the six MIP-SBs were separately immersed in 1.0 ml of methanol, ultrapure water, acetonitrile, methanol-water (9:1, v/v), methanol-acetic acid (9:1, v/v) and methanol-acetic acid (99:1, v/v) for ultrasonic vibration. The solvents were evaporated under a stream of nitrogen and the residues were redissolved with 150 μl of ultrapure water for HPLC-UV analysis. The optimized desorption solvent was chosen based on the recovery of DA.

Similarly, the extraction time, desorption time and effect of pH on the extraction recovery of DA were investigated by HPLC-UV