Journal of Chromatography A, 1342 (2014) 37–43

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Online micro-solid-phase extraction based on boronate affinity monolithic column coupled with high-performance liquid chromatography for the determination of monoamine neurotransmitters in human urine

Xiaoting Yang, Yufei Hu*, Gongke Li*

School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou 510275, China

ARTICLE INFO

Article history:
Received 5 February 2014
Received in revised form 13 March 2014
Accepted 14 March 2014
Available online 21 March 2014

Keywords:
Online micro-solid-phase extraction
Boronate affinity monolithic column
Monoamine neurotransmitters
High-performance liquid chromatography
Urine.

ABSTRACT

Quantification of monoamine neurotransmitters is very important in diagnosing and monitoring of patients with neurological disorders. We developed an online analytical method to selectively determine urinary monoamine neurotransmitters, which coupled the boronate affinity monolithic column micro-solid-phase extraction with high-performance liquid chromatography (HPLC). The boronate affinity monolithic column was prepared by in situ polymerization of vinylphenylboronic acid (VPBA) and *N*,*N*′-methylenebisacrylamide (MBAA) in a stainless capillary column. The prepared monolithic column showed good permeability, high extraction selectivity and capacity. The column-to-column reproducibility was satisfactory and the enrichment factors were 17–243 for four monoamine neurotransmitters. Parameters that influence the online extraction efficiency, including pH of sample solution, flow rate of extraction and desorption, extraction volume and desorption volume were investigated. Under the optimized conditions, the developed method exhibited low limit of detection (0.06–0.80 μg/L), good linearity (with R^2 between 0.9979 and 0.9993). The recoveries in urine samples were 81.0–105.5% for four monoamine neurotransmitters with intra- and inter-day RSDs of 2.1–8.2% and 3.7–10.6%, respectively. The online analytical method was sensitive, accurate, selective, reliable and applicable to analysis of trace monoamine neurotransmitters in human urine sample.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Monoamine neurotransmitters (MNTs) play significant roles in the control and regulation of principal functions and behaviors of humans. It has been demonstrated that the monoamine neurotransmitters, including 5-hydroxytryptamine (5-HT), norepinephrine (NE), epinephrine (E), and dopamine (DA), were implicated in many brain processes, such as mood, cognition, reward, sleep, and motor activity in humans [1]. These monoamine neurotransmitters were also highlighted as molecular targets involved in the treatments of multiple neuropsychiatric disorders, such as Parkinson's and Alzheimer's disease [2–4]. Thus, the simultaneous determination of the monoamine neurotransmitters in biological fluid has unique importance in the diagnosing and monitoring of these serious diseases.

The determination of MNTs in biological fluid requires high sensitive and selective analytical methods. They are usually measured by coupling reverse phase high-performance liquid chromatography with online detectors, such as electrochemical (ECD) [5,6], mass spectrometry (MS) [7,8], or fluorescence (FD) [9,10]. However, the sensitivity and selectivity of these methods were limited by the matrix interference, low abundance and potential instability of monoamine neurotransmitters [8,11]. Therefore, various sample pretreatment methods, including liquid–liquid extraction (LLE) [9], derivatization procedures [12,13], solid-phase extraction (SPE) [14,15], and solid-phase microextraction (SPME) [16,17], have been introduced for the clean-up of the complex biological samples before instrumental analysis as well as for the enrichment of trace amount of MNTs. These methods were generally achieved in offline mode, consequently, recognized as tedious, labour-intensive, and becoming the time-limiting step in the analytical process. Online sample preparation techniques have gained great interest in recent years, and miniaturization and automation have been carried out to decrease the cost and time required for sample preparation in environmental, food, and biological analyses [18,19]. Moreover, it is

* Corresponding authors. Tel.: +86 20 84110922; fax: +86 20 84115107.
E-mail addresses: huyufei@mail.sysu.edu.cn (Y. Hu), cesgkl@mail.sysu.edu.cn (G. Li).

http://dx.doi.org/10.1016/j.chroma.2014.03.041
0021-9673/© 2014 Elsevier B.V. All rights reserved.

possible that extracted analytes are effectively introduced into the analytical system in the online process, which can decrease human errors caused by handling during sample preparation.

Organic polymer monolith is the material that can be possibly used as sorbent in the online sample preparation techniques. Organic polymer monolith prepared by sol–gel polymerization technology does not require frits at column extremities, which often remains the main source of endogenous material adsorption. Depending on its structure, organic polymer monolith has high permeability. And the extraction of biological samples can be performed with a high flow rate without generating high backpressure [20]. Organic polymer monolith also offers chemical stability in a wide pH range [21]. Due to its unique properties, organic polymer monolith is very suitable for the analysis of biochemical samples. The first synthesis of boronate affinity monolith in a single step was reported in 2009 by Liu et al. [22]. Boronate affinity material is well known as a powerful sorbent for the selective isolation and enrichment of cis-diol-containing compounds, such as nucleosides, glycoproteins, glycopeptides, and catechols [23–29]. The primary mechanism is that boronate groups can form covalent bonds with cis-diol-containing compounds to generate five- or six-membered cyclic esters in a basic aqueous medium, while the complexation reversibly releases boronic acids and the cis-diols in an acidic condition [30]. Therefore, boronate affinity monolith is an ideal extraction phase to capture MNTs through 1,2-*cis*-diol groups in the online sample preparation techniques.

The aim of this work was to develop a new online sample preparation method based on a well-designed monolith column for determination of MNTs in complicated samples. For this purpose, a boronate affinity monolithic column was prepared by one step in situ polymerization as the micro-solid-phase extraction sorbent, and coupled to HPLC for online analysis of four MNTs. The extraction selectivity, reproducibility and linear range were investigated. The experimental results indicated that the developed method could simultaneously determine four trace MNTs in urine samples.

2. Experimental

2.1. Chemicals and materials

Dopamine hydrochloride and norepinephrine bitartrate were obtained from Aladdin Industrial Corporation (Shanghai, China). Epinephrine hydrochloride and serotonin hydrochloride were purchased from J&K Chemical Ltd. (Shanghai, China). The purity of these standards was higher than 98%. VPBA, MBAA, and 1-heptanesulfonate were provided by Aladdin Industrial Corporation (Shanghai, China). Azobisisobutyronitrile (AIBN) was obtained from Damao Reagent Plant (Tianjin, China). Acetonitrile and methanol of HPLC grade were purchased from Dikma (Beijing, China).Water used was obtained from Millipore water purification system. All other reagents were at least of analytical grade.

2.2. Preparation of poly (VPBA-co-MBAA) monolithic column

Stainless steel capillary columns (6 cm × 0.1 mm i.d.) were filled with 1 mol/L HCl, and then sonicated for 30 min to generate a rough surface. After rinsed with purified water, they were dried at 100 °C in oven for 1 h. The polymer monolithic column was synthesized according to a method reported previously with a slight modification [31]. Briefly, the binary porogenic solvent solution was prepared by mixing 175 μL *n*-propanol with 435 μL 1,4-butanediol. Then 20 mg VPBA, 20 mg MBAA and 2.5 mg AIBN were added to the porogenic solution. After sonicated for 20 min and degassed with nitrogen for 10 min, the solution was filled into the treated stainless steel capillary column. The column was immediately sealed by silicone rubbers at each end to perform the polymerization at 60 °C for 18 h. The in situ polymerization reaction was illustrated in Fig. 1a. Before extraction, the new poly (VPBA-*co*-MBAA) monolithic column was eluted by methanol formic acid (90:1, v/v) to remove the unreacted reagents and porogens.

2.3. Online extraction procedures

The poly (VPBA-*co*-MBAA) monolithic column was online coupled to HPLC. And the online conjunction system was illustrated in Fig. 1b. Briefly, the online extraction includes several steps. (a) Sample loading and precondition. Valve 1 and valve 2 were set to load position. The sample solution was loaded into the sample loop. At the same time, the carrier solution (water) and mobile phase were separately driven by HPLC pump A and pump B to flow through the monolith and the analytical column for precondition. (b) Extraction. Valve 2 was switched to inject position and valve 1 was kept in load position. The sample solution was driven by pump A to flow through the monolith at a flow rate of 0.4 mL/min for extraction. (c) Cleaning-up. Valve 1 and valve 2 were set to load position. The monolith was cleaned up by water at 0.4 mL/min for 1 min to eliminate the residual sample solution. (d) Desorption. Pump B was temporarily stopped and then valve 1 and valve 2 were switched to inject position. The desorption solution was loaded into the sample loop, and then was driven by pump A at a flow rate of 0.05 mL/min to desorb the extracted analytes to analytical column. (e) HPLC Analysis. Pump B was started again, and valve 1 and valve 2 were switched to load injection.

2.4. Chromatographic conditions

All chromatographic measurement were performed using a Shimadzu LC-10AT (Shimadzu, Japan), which consists of three pumps (LC-10ATvp), an injection valve (Rheodyne model 7725i) equipped with a 20 μL injection loop, a fluorescence detector (RF-10A XL), a system controller (SCL-10Avp), and an acquisition data software Class-VP.

After optimization of chromatographic conditions, separation was carried out on a Waters Atlantis® T3 column (150 mm × 4.6 mm i.d., 5 μm particle size). The mobile phase consisted of acetonitrile and water containing 1 mmol/L heptanesulfonate, 5 mmol/L ammonium formate and 0.5% formic acid (pH 3.0). The mobile phases were eluted at 1.0 mL/min at room temperature following the gradient as follows: 5% acetonitrile and maintained for 1 min, increased to 70% within 15 min and held for 5 min. Fluorescence was monitored at an excitation wavelength of 280 nm and an emission wavelength of 330 nm.

2.5. Sample pretreatment

The standard stock solution of NE, E, DA, and 5-HT (1 mg/mL) were prepared at 0.1 mol/L HCl, and preserved in dark condition (−20 °C) to reduce possible oxidation. The working standard solution was freshly prepared by appropriate dilution of the stock standard solution with 0.05 mol/L phosphate buffer solution (pH 8.5) to obtain a desired concentration. Urine samples were collected from healthy volunteers. In order to avoid catechol group oxidation, 0.5 mL 1 mol/L HCl was added to 10 mL urine sample and stored at −20 °C. The pH of urine samples was adjusted to 7.0 with 1 mol/L NaOH before analysis and then diluted 5 folds with 0.05 mol/L phosphate buffer solution (pH 8.5). After being filtered through 0.45 μm membrane filter, the samples were used for online analysis.

Download English Version:

https://daneshyari.com/en/article/1203218

Download Persian Version:

https://daneshyari.com/article/1203218

Daneshyari.com