Journal of Chromatography A, 1409 (2015) 125–131

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Selective extraction and analysis of catecholamines in rat blood microdialysate by polymeric ionic liquid-diphenylboric acid-packed capillary column and fast separation in high-performance liquid chromatography-electrochemical detector

Xinguang Zhou, Anwei Zhu, Guoyue Shi *

School of Chemistry and Molecular Engineering, East China Normal University, 500 Dongchuan Road, Shanghai 200241, China

ARTICLE INFO

Article history:
Received 10 March 2015
Received in revised form 24 June 2015
Accepted 9 July 2015
Available online 14 July 2015

Keywords:
PIL-DPBA-packed capillary column
Catecholamines
Solid phase extraction
Rat blood microdialysate

ABSTRACT

Concentration of blood catecholamines (CAs) is linked to a host of cardiovascular diseases, including hypertension and stenocardia. The matrix interferences and low concentration require tedious sample pretreatment methods before quantitative analysis by the gold standard method of high-performance liquid chromatography-electrochemical detector (HPLC-ECD). Solid phase extraction (SPE) has been widely used as the pretreatment technique. Here, a facile polymeric ionic liquid (PIL)-diphenylboric acid (DPBA)-packed capillary column was prepared to selectively extract dopamine (DA), noradrenaline (NE) and epinephrine (E) prior to their quantitative analysis by a fast separation in HPLC-ECD method, while microdialysis sampling method was applied to get the analysis sample. Parameters that influenced desorption efficiency, such as pH, salt concentration, acetonitrile content and wash time, were examined and optimized. The proposed method, combining microdialysis sampling technique, SPE and HPLC-ECD system, was successfully applied to detect CAs in rat blood microdialysate with high sensitivity and selectivity in small sample volumes (5–40 μl) and a short analysis time (8 min), yielding good reproducibility (RSD 6.5–7.7%) and spiked recovery (91–104.4%).

1. Introduction

Catecholamines (CAs), including dopamine (DA), epinephrine (E) and norepinephrine (NE), play important roles in the function of the central nervous, cardiovascular, renal and hormonal systems [1,2]. Disordered CA concentrations *in vivo* can indicate that diseases such as schizophrenia, Parkinson's disease or Huntington's disease have developed. The sensitive and selective determination of CA concentrations is therefore critical to identifying endocrine disorders and other physiological and pathological abnormalities *in vivo* so that patients can take preventative measures [3]. HPLC-ECD is regarded as the gold standard method for quantitative determination of CAs [4]. However, there are two major problems encountering detecting CAs in blood. One is the low circulating concentration of CAs with matrix interferences; the other is difficult to get and store the precious biological sample without the potential instability of CAs. So an effective sample preparation method is needed to solve these problems.

Several reliable methods have been developed for the extraction of CAs from complex biological matrices [5–7], including liquid–liquid extraction (LLE) [8,9], addition of a diphenyl borate reagent [10] and solid phase microextraction (SPME) [11,12]. SPE has been rapidly developed for pretreatment and extraction because it is more sensitive, environmentally friendly, faster and conserves sample [13]. Several different SPE sorbents have been reported to extract CAs from biological samples, including crown ether-modified polymers [12], calixarene [14], boronate compounds [15,16], ion exchange resin [17,18], C18 matrix [19] and alumina [20,21]. An advanced 96-well microplate extraction method using packed activated aluminum oxide was reported to decrease the plasma catecholamine analysis time and offer higher analytical specificity. However, the activated alumina lacked the ability to extract CAs selectively, and the minimum analysis volume was 50 μl [21]. Furthermore, extraction of plasma requires a tedious sample pretreatment procedure prior to analysis. This process involves anticoagulation, centrifugation, protein precipitation, which inevitablely result the oxidation and loss of CAs. So

* Corresponding author. Tel.: +86 21 54340043; fax: +86 21 54340043.
E-mail addresses: gyshi@chem.ecnu.edu.cn, iamshgy@hotmail.com (G. Shi).

http://dx.doi.org/10.1016/j.chroma.2015.07.040
0021-9673/

new materials and devices to determinate CAs by SPE are therefore needed to reduce sample consumption and eliminate interferences.

Microdialysis is a powerful sampling technique *in vitro* and *in vivo* based on diffusion of molecules across a size-controllable membrane. The semipermeable membrane only permits the small molecules to diffuse into the perfusate which can remove the interferences from proteins and cells in blood [22]. And the rapid sampling technique avoids the loss of neurotransmitters. This sampling method has been widely applied to human and animal studies [23,24].

Ionic liquids (ILs) composed of cations and anions are frequently used in analytical chemistry due to their selectivity and sensitivity in sample pretreatment [25–27], such as single-drop extraction [28,29], liquid-phase microextraction (LPME) [30–32], and solid phase extraction (SPE) fiber coatings [33,34]. However, the loss of the ILs during the desorption step decreases their service life and reproducibility. Polymeric ionic liquids (PILs) not only possess the advantages of ILs but also have exceptional thermal stability, highly reproducible extraction efficiencies and long lifetimes [35,36]. ILs can also be coordinately polymerized with cross-linker compounds to prepare cross-linked polymeric ionic liquids [37–39], overcoming the issue of linear PILs swelling and dissolving after exposure to organic solvents with good extraction performance [40].

In the present work, the PIL was copolymerized by 1-vinyl-3-propylphenyl imidazolium chloride ([VPPIM][Cl]) with divinylbenzene (DB) as the monomer and dimethyl sulfoxide as the porogen. [VPPIM][Cl] was selected because of the π–π interactions of the benzene and imidazole rings in the cation and the high hydrogen bond basicity of the $[Cl]^-$ anion, which will facilitate combination with the DPBA-ethanolamine ester in the steady state [36]. As shown in Fig. 1, the DPBA-ethanolamine ester could be dissociated into ethanol-amine and DPBA, which could specifically integrate with the CAs. Construction of the PIL-DPBA adsorbents made this SPE method specifically suitable for CAs extraction. CAs in blood microdialysate were cleaned up and enriched on a PIL-DPBA-packed capillary SPE column prior to HPLC-ECD. This selective and rapid method was successfully applied to the determination of CAs in rat blood with good accuracy and reproducibility.

2. Experimental

2.1. Chemicals

1-Vinylimidazole ($C_5H_6N_2$) was purchased from the Adamas Reagent Co. Ltd. (Shanghai, China). Dimethyl sulfoxide (DMSO), azodiisobutyronitrile (AIBN), silica gel and sodium silicate (Na_2SiO_3) were purchased from the Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). A 0.9–1.1 mm glass column was purchased from the Shanghai Great Wall science instrument shop (Shanghai, China). 1-Chloro-3-phenylpropane, 2-propanol, divinylbenzene and HPLC-grade methanol were all from the Sigma Company (St. Louis, MO, USA). The HPLC mobile phase was prepared by mixing sodium phosphate monobasic dehydrate (75 mM), 1-octanesulfonic acid sodium salt (1.7 mM), 100 μl/L triethylamine (TEA), 25 μM disodium ethylenediaminetetraacetic acid (EDTA), 10% acetonitrile, and KCl (2 mM) in ultrapure water (18.2 MΩ/cm). And 90% (v/v) phosphoric acid solution was used to adjust the HPLC mobile phase to pH 3. The mobile phase chemicals and all neurotransmitter standards, including dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT), epinephrine (E), noradrenaline (NE), 4-hydroxy-3-methoxymandelic acid (VMA), 5-hydroxyindole acetic acid (5-HIAA), and 4-hydroxy-3-methoxyphenylaceticacid (HVA), were all from the Sigma Company (St. Louis, MO, USA). 0.2% DPBA-ethanolamine ester and 5 g/L EDTA were prepared in 2 M NH_4Cl–NH_4OH buffer and adjusted to pH 8.5. Artificial cerebrospinal fluid (aCSF) solution was prepared by mixing NaCl (126 mM), KCl (2.4 mM), KH_2PO_4 (0.5 mM), $MgCl_2$ (0.85 mM), $NaHCO_3$ (27.5 mM), Na_2SO_4 (0.5 mM), and $CaCl_2$ (1.1 mM) in ultrapure water, and the solution pH was adjusted to 7.4. The purity of these chemicals was analytical grade or greater.

2.2. Instrumentation and conditions

The PIL was characterized on a HITACHIS-4800 scanning electron microscope (SEM) (Hitachi Co. Ltd., Tokyo, Japan). The surface area of the PIL was measured by Specific Surface Area (BELSORP-max, BEL Inc., Japan). Elemental analysis (EA) was carried out on an elemental analyzer (Vario-EL III, Elementar, Germany). FT-IR was performed on an iS50 FT-IR (Thermo Nicolet, Madison, WI, USA). A microinjection pump (Baoding Longer Precision Pump, China) was used to operate the syringe.

The sample was analyzed by HPLC (LC-30A, Shimadzu, Japan) with an electrochemical detector (DECADEII, Antec, Netherlands) using a VT-03 flow cell with a 25-μm spacer. The column was a shim-pack XR-ODS III (1.6 μm) from Shimadzu. 1.0 μl of the sample was injected by the autosampler and analyzed rapidly. Data processing was carried out on a Lenovo computer running the Lab solutions software developed by Shimadzu.

2.3. Synthesis of the PIL and fabrication of the capillary column

Figure S3 shows the chemical structure of the PIL. The ionic liquid monomer [VPPIM][Cl] was first synthesized by reacting vinyl imidazole with 1-chloro-3-phenylpropane at 70 °C in isopropanol at a molar ratio of 1:1.1 for 72 h [36]. The IL monomer was characterized using ^{1}H NMR and ^{13}C NMR. ^{1}H NMR (500 MHz, d_6-DMSO): δ 9.46 (s, 1H, —N—CH—N—), 8.17 (s, 1H, —N—*CH*—CH—N—), 7.93 (s, 1H, —N—CH—*CH*—N—), 7.32–7.19 (m, 6H, $-C_6H_5$; C=CH—N—), 5.94 (dd, 1H, CH_2=), 5.43 (dd, 1H, CH_2=), 4.23 (t, 2H, $-CH_2-C-C-$), 2.65 (t, 2H, $-C-CH_2-C-$), 2.18 (p, 2H, $-C-C-CH_2-$). ^{13}C NMR (126 MHz, d_6-DMSO): 140.92, 128.73, 128.88, 126.57 ($-C_6$); 135.88, 123.68, 119.6 (—CHNCHNCH—); 109.08, 129.35 ($-CH_2CHN$); 49.5, 30.94, 32.16 ($NCH_2CH_2CH_2-$). The IL was then mixed with DB at a mass ratio of 1:2, and 10 ml of DMSO was added as the porogen and solution. AIBN was added as the polymerization initiator at 1% (w/w) of the total mixture. The reactant solution was purged with nitrogen for 15 min and kept at 65 °C for 12 h. The white precipitate was collected under vacuum filtration, washed with methanol to remove the monomers and dried at 40 °C until a constant weight was obtained. The PIL was then ground into a powder. Each PIL was synthesized by different proportions of reactants using the same procedure to optimize extraction performance. By calculating the decreased peak area of CAs before and after adsorption, PIL 8 (Table 1) owns the highest adsorption capacity.

The fabrication of the PIL-DPBA-packed capillary column involved three main steps: (1) the sodium silicate and silica gel mixture was absorbed at one end of a capillary column (i.d. 0.9–1.1 mm, o.d. 1.0–1.25 mm) by capillarity, and the end was then heated by a nichrome wire for a few seconds to form a stable frit; (2) the PIL was dispersed in methanol, and this solution was perfused into the above-mentioned capillary column until the length of PIL material was 2 cm in the column; (3) a 0.2% (w/w) DPBA solution was perfused into the capillary column and absorbed by the PIL. This packed capillary column had greater recovery and stability than a polymer monolithic column.

2.4. Linearity of the PIL column extraction

CAs are adsorbed and subsequently released during extraction. The standard sample was injected into the PIL-DBPA-packed