Selective solid-phase extraction of catecholamines by the chemically modified polymeric adsorbents with crown ether

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Received 2 June 2006; received in revised form 13 June 2007; accepted 19 June 2007
Available online 26 June 2007

Abstract

A simple and selective one-step solid-phase extraction procedure using chemically modified polymer resin (Amberlite XAD-4) with crown ether was investigated for the measurement of urinary catecholamines. After loading the urine samples (adjusted to pH 4) on the synthesized adsorbent cartridge, the column was washed with methanol followed by water and then the adsorbed catecholamines were eluted by 1.0 mL of 6.0 M acetic acid. The effectiveness of sample clean-up method was demonstrated by reversed-phase ion-pair high-performance liquid chromatography with electrochemical detection. Under optimal condition, the recoveries of epinephrine, norepinephrine, and dopamine from spiked urine sample were >86% for all catecholamines. The detection limits (n = 5) for epinephrine, norepinephrine, and dopamine were 37, 52, and 46 nmol/L, respectively.

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Keywords: Catecholamine; Crown ether; Chemically modified polymer resin; Solid-phase extraction

1. Introduction

Catecholamines represents a group of biogenic amines among which dopamine (DA), epinephrine (E), and norepinephrine (NE) are very well known as adrenal hormones and neurotransmitters in the human body [1]. The concentrations of catecholamines in urine reflect the activity of the sympathetic-adrenal system [2]. Thus the determination of urinary free catecholamines is very important for diagnosis of tumors such as neuroblastic tumors and pheochromocytomas [3–6].

Measurement of catecholamines in complex matrices, such as urine samples is usually performed by HPLC with electrochemical detection (ED), which requires a preliminary sample preparation step to remove potentially interfering compounds and enrich the analytes [7,8]. Several methods have been proposed for the extraction of catecholamines. Liquid–liquid extraction and off-line solid-phase extraction (SPE) are the two most frequently used techniques for the extraction and pre-concentration of catecholamines from clinical samples. The solid-phase extraction is preferred over liquid–liquid extraction, because it does not require not only large volume of toxic organic solvent but also labor intensive and time-consuming double extraction procedure. Classical adsorbents for the extraction of catecholamines from clinical samples were Sephadex G10 [9], weak or strong cation exchange resin [10–13], C18 matrix [14], boronate gels [10,11], and alumina [9,15].

Several groups [16–18] described a rapid, one-step liquid–liquid procedure for the extraction of catecholamines from urine employing diphenyl boronic acid (DPBA) ethanolamine ester and a C18 SPE sorbent. The diphenyl boronate forms a stable negatively charged complex with cis-hydroxyl groups of catecholamines, which is strongly retained on a C18 extraction sorbent when operating in alkali media [17]. This allows column washing with methanol-buffer solutions to remove interfering compounds without the loss of the catecholamines which are eluted by disrupting the complex under acid conditions. However, this method also requires control of samples to alkaline pH and it may cause decomposition of catecholamines by oxidation during pretreatment of samples.

The adsorbents based on chemically modified polymer resin were also applied to enhance selectivity and recovery. In recent years, Hyun et al. described the applications of synthesized
stationary phases to chiral separation of amino acids based on crown ether immobilized onto silica gel through a peptide bond [19,20]. Our group also reported the selective solid-phase extraction of various phenols and PAHs with the chemically modified resins with porphyrins onto Amberite XAD resins, which are commercially available non-polar styrene-divinyl benzene copolymers, developed for selective extraction pre-concentration and clean up of many organics from aqueous media [21–23].

The main objective of this study is to simplify sample treatment procedure for determining catecholamines in urinary sample by applying the synthesized adsorbents to solid-phase extraction without any pretreatments and we could also improve selectivity for catecholamines with crown ether on resin and enhance cleaning effect of coexisting organics with XAD base.

2. Experimental

2.1. Chemicals and reagents

4′-Carboxybenzo-18-crown-6 (CBCE), 3,4-dihydroxybenzylamine hydrobromide (DHBA), 1-octanesulfonic acid sodium (OSA) were purchased from Fluka (Buchs, Switzerland). Dopamine (DA), (−)-norepinephrine (NE), (−)-epinephrine (E), EDTA disodium salt, dihydrogen phosphate, hydrochloric acid, ortho-phosphoric acid 85%, and sodium hydroxide pellets were purchased from Sigma–Aldrich (Louis, MO, USA). Amberlite XAD resins were supplied from Rohm and Haas (Philadephia, PA, USA). HPLC-grade methanol, acetonitrile, and glacial acetic acid were purchased from Baker (Philipsburg, NJ, USA). All other reagents were of analytical grade, and reverse osmosis Milli-Q water (18 MΩ cm) (Millipore, Billerica, MA, USA) was used. The standard solutions of catecholamines and internal standards (DHBA) were 20 μmol/L which was prepared by dissolving appropriate amounts of this chemical in 0.1 mol/L HCl and stored in the dark place at 4 °C.

2.2. Apparatus and chromatographic conditions

The HPLC system used throughout this study is consisted of a Dynamax SD-200 pump (Rainin, Emeryville, CA, USA), a Model 7125 injector (Rheodyne, Cotati, CA, USA) with a 20 μL loop, and a model ECD-200 electrochemical detector (Lab Alliance). System control and data analysis were carried out with an Autochro data module (Young Lin, Seoul, South Korea). The HPLC column was used was a Spherisorb (5 μm particle size, 250 mm × 4.6 mm i.d.), obtained from Waters (Bedford, MA, USA).

The isocratic mobile phase used for ion-pair, reversed-phase HPLC is consisted of 30 mmol/L potassium dihydrogen phosphate buffer, 25 mL/L methanol, 15 mL/L acetonitrile, 60 mg/L EDTA, and 20 mg/L OSA (counter ion). The pH of the mobile phase was adjusted to 3.0 with 6.0 mol/L phosphoric acid, filtered through a 0.45 μm membrane. The flow rate of mobile phase was 1.00 mL/min and the injection volume was 20 μL. ED was set up to 0.8 V for cell potential and 20 nA/V for sensitivity level.

2.3. Preparation of the chemically modified polymeric adsorbents

Chemically modified adsorbents were prepared from XAD resins and 4′-carboxybenzo-18-crown-6 through the ketone linkage according to Rollman’s method [24]. In brief, 0.134 mol of thionyl chloride was added to 0.018 mol of crown ether, and heated after addition of a portion of XAD resin (10 g) and 30 mL of 1,1,2,2-tetrachloroethane. After cooled to room temperature, aluminum chloride was added and the solution was kept at 10 °C for 16 h. The slurry was filtered and washed with 1,1,2,2-tetrachloroethane, methanol, and basic aqueous solution. Synthetic scheme for the preparation of adsorbent is given in Fig. 1. The resulting chemically modified polymeric adsorbents were stored in a vacuum desiccator.

2.4. Adsorption experiments

Adsorption and desorption (Kd) experiments were carried out in a batch mode. A fixed amount of the adsorbent (10 mg) and a known concentration of adsorbate solution were placed in a glass vial and shaken at 2000 rpm for 1 h at 25 °C using a Fisher Vortex-Genie 2 Mixer (Fisher Scientific, Pittsburgh, PA, USA). After mixing, solution was filtered through a 0.2 μm PTFE syringe filter (Whatman, Maldstone, UK) and injected into the HPLC to determine the amount of adsorption of analyte on the adsorbent.

The solid-phase extraction [25] cartridge was prepared by the following method: the adsorbent was packed in a polyether ether ketone (PEEK) cartridge (50 mm × 2 mm i.d.), and titanium frits (2 μm in pore size) were located above and below each adsorbent bed. Sample adsorption and elution was carried out with a Minipuls 3 peristaltic pump (Gillon, Villiers-le-bel, France). For the measurements of the breakthrough volume, the solid-phase extraction cartridge was conditioned by flushing with methanol and then water at a flow rate of 0.80 mL/min until the detector response reached stability. Breakthrough curves were obtained by eluting 10 μg/mL of catecholamines into the cartridge directly connected to the detector at a flow rate of 0.80 mL/min. Breakthrough volumes were calculated at 1% of the maximum adsorbance of the adsorbates.

The solid-phase extraction was performed as follows: solid-phase extraction cartridge was conditioned by 3.0 mL of methanol followed by 1 mL of water at a flow rate of 0.50 mL/min. The sample was loaded by elution of 1.0 mL sample solution to cartridge at a flow rate of 0.80 mL/min. The adsorbed catecholamines were eluted by 1 mL of 6.0 M acetic acid at a flow rate of 0.20 mL/min.

2.5. Determination of catecholamines

The concentration of standard catecholamines (norepinephrine, epinephrine, and dopamine) for calibration curve were 50, 100, 200, 500, 1000, 1500, 2000, 2500, and 3000 nmol/L, respectively, and all standard solutions contained internal standard DHBA, at a concentration of 500 nmol/L.
For the recovery test, artificial urine was prepared. The artificial urine consisted of 19.4 g/L of urea, 8.0 g/L of NaCl, 1.1 g/L of magnesium sulfate heptahydrate, and 0.6 g/L of CaCl₂ and the solution was adjusted at pH 4 with 6 M HCl. The collected human urine samples were centrifuged at 10,770 \( g \) for 10 min and all experiments were performed within 2 h for the stability of catecholamines.

3. Results and discussion

3.1. Adsorption and desorption characteristics of the adsorbents to catecholamines

The crown ether modified XAD-4 resin was used as an adsorbent for solid-phase extraction material. The structure of crown ether has the correct geometry for binding catecholamines by forming hydrogen bonds and thus showed selective binding of catecholamines over other interfering compounds contained in urine samples. Protonation of the amino group of catecholamines enhances the complexation of the ammonium ion inside the cavity of the crown ether ring. For optimal interaction between catecholamines and crown ether, very careful adjustment of the sample pH was required.

For finding optimal pH condition, adsorption and desorption (\( K_d \)) experiments were carried out in a batch mode varying the pH of sample solution between 2 and 6 (data not shown). When the catecholamines dissolved in water, the \( K_d \) value showed maximum at pH 6. However, in case of urine sample, selectivity decreased by interfering materials in sample matrix over pH 4. For desorption of the adsorbed catecholamines, acidic solution below pH 2, was required.

Using the SPE cartridge, adsorption of capacity of the chemically modified adsorbent was also investigated with changing pH from 2 to 6. The concentration of catecholamines (10 mg/L) and loading volume (10 mL) was used for this study. At the same time, the adsorbent amount was 70 mg. We found that the recovery percentage values were almost constant over the pH 4 and approximately 100% for all catecholamines. According to our results, pH value of 4 was selected as a sample loading and 6.0 M acetic acid was employed for elution of the adsorbed catecholamines.

3.2. Solid-phase extraction by the crown ether modified adsorbent

The calibration curves of catecholamines were obtained using internal standard method, showed a good linear relationship up to 3000 nmol/L with >0.999 of correlation coefficients (\( R^2 \)). For calculation of breakthrough volume of the SPE column (50 mm X 2 mm i.d.), the cartridge was equilibrated by methanol followed by water, and then catecholamine solutions (norepinephrine, epinephrine, and dopamine), were eluted through the cartridge at a rate of 0.8 mL/min, respectively. The breakthrough volumes were calculated by the 1% of the maximum UV absorbance of the eluting solutions. Breakthrough volume of catecholamines (norepinephrine, epinephrine, and dopamine) are 6.75 ± 0.99, 16.73 ± 3.55, and 28.04 ± 3.90 mL, and adsorption capacity of catecholamines (norepinephrine, epinephrine, and dopamine) are 0.96 ± 0.14, 2.39 ± 0.51, and 4.01 ± 0.56 µg/mg, respectively were found in our study.

For measuring the recoveries of catecholamines, the standard catecholamines were added to the artificial urine as concentration of 200, 500, 1000, 2000, and 3000 nmol/L, respectively (Table 1). Up to 3000 nmol/L, the mean recovery of each catecholamine was high and reproducible. The recovery values of norepinephrine were somewhat lower than those of the other catecholamines (epinephrine and dopamine). Raggi et al. [26] described about the different cartridges, viz., Varian BondElut...
Table 1

Recovery (%) of catecholamines in artificial urine ($n=3$)

<table>
<thead>
<tr>
<th>Concentration (nmol/L)</th>
<th>Epinephrine Recovery (%)</th>
<th>RSD (%)</th>
<th>Norepinephrine Recovery (%)</th>
<th>RSD (%)</th>
<th>Dopamine Recovery (%)</th>
<th>RSD (%)</th>
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<td>93.2</td>
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<td>81.2</td>
<td>10.4</td>
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<tr>
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<td>79.1</td>
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<td>4.7</td>
</tr>
<tr>
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<td>95.1</td>
<td>2.6</td>
<td>85.8</td>
<td>7.9</td>
<td>94.0</td>
<td>4.7</td>
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<tr>
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<tr>
<td>3000</td>
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<td>85.2</td>
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</table>

Composition of artificial urine is 19.4 g/L of urea, 8.0 g/L of sodium chloride, 1.1 g/L of magnesium sulfate heptahydrate, and 0.60 g/L of calcium chloride and its pH is 4.

C$_8$, Step Bio Isolute C$_8$, and Waters Oasis HLB. According to their results, only Waters Oasis HLB cartridge has shown good and consistent results of percent recovery; but the other cartridges used by them did not show consistent percent recovery values. Whereas, the percent recovery values of E (90–95%), NE (79–86%) and DA (92–94%) have shown almost consistent results in our case. In brief, the percent recovery values of E, NE and DA in our cartridge are in good agreement with Waters Oasis HLB. For the practical application of the system, 1000 nmol/L of catecholamines were added to real urine sample. The limit of detection ($n=5$) were 37 nmol/L for epinephrine, 52 nmol/L for norepinephrine, and 46 nmol/L for dopamine. The repeatability of extraction with this method is very good as RSD of less than 10%.

3.3. Chromatography with electrochemical detection

Fig. 2 illustrates the chromatographic profiles of catecholamines by ion-pair reversed-phase chromatography with electrochemical detection. No interfering peaks were observed in the chromatogram, in spite of only one-step SPE purification of urine sample. The results indicate that the crown ether modified adsorbent is very powerful for purification of catecholamines. Under the optimized mobile phase condition, norepinephrine, epinephrine, internal standard, and dopamine peaks were easily separated with capacity factor ($k'$) of 1.0, 1.8, 2.1, and 3.6, respectively. The concentration of the ion-pair (1-octanesulfonic acid) and organic modifier, combination of acetonitrile and methanol, in the mobile phase had a major effect on the peak shape and resolution of catecholamine peaks. For the stability of ED response, EDTA was added to the mobile phase. The pH of the mobile phase was adjusted to 3.0 with phosphoric acid for obtaining the optimal analysis time.

4. Conclusion

Generally, sample clean-up procedure is considerably labor intensive, expensive, and time-consuming. The main objective of this study was to develop a rapid, simple, selective and reproducible clean-up method for determination of catecholamines for routine assessment of physiological or various pathological processes. For the optimization of the extraction of catecholamines, we investigated the effect of analytical variables on the efficiency of the extraction procedure, such as pH of loading and desorption solution, flow rate and washing buffer. The crown ether modified XAD-4 resin exhibited >86% recoveries for all catecholamines without any interfering compounds. The applicability of this method will be expanded towards simultaneous assessment of a number of other amine containing biologically important molecules.

References