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High-throughput and selective solid-phase extraction of urinary catecholamines by crown ether-modified resin composite fiber

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ABSTRACT

In the present study, we developed a simple and high-throughput solid phase extraction (SPE) procedure for selective extraction of catecholamines (CAs) in urine samples. The SPE adsorbents were electrospun composite fibers functionalized with 4-carboxybenzo-18-crown-6 ether modified XAD resin and polystyrene, which were packed into 96-well columns and used for high-throughput selective extraction of CAs in healthy human urine samples. Moreover, the extraction efficiency of packed-fiber SPE (PFSPE) was examined by high performance liquid chromatography coupled with fluorescence detector. The parameters affecting the extraction efficiency and impurity removal efficiency were optimized, and good linearity ranging from 0.5 to 400 ng/mL was obtained with a low limit of detection (LOD, 0.2–0.5 ng/mL) and a good repeatability (2.7%–3.7%, n = 6). The extraction recoveries of three CAs ranged from 70.5% to 119.5%. Furthermore, stable and reliable results obtained by the fluorescence detector were superior to those obtained by the electrochemical detector. Collectively, PFSPE coupled with 96-well columns was a simple, rapid, selective, high-throughput and cost-efficient method, and the proposed method could be applied in clinical chemistry.

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1. Introduction

Crown ether is a host molecule with binding sites for guest molecules, which has been mainly investigated in supramolecular chemistry. A great deal of attention has been paid to crown ether due to its ability to selectively interact with cations since it has been discovered by Pedersen [1]. It is known that discrete crown ether compounds can form complexes with metal ions. Meanwhile, discrete crown ether compounds are used to separate metal ions and act as chromogenic reagents for photometric analysis of metal ions [2,3]. However, discrete crown compounds are chemically unstable and can be dissolved in most organic and inorganic solvents, leading to their complicated separation and recovery. Nevertheless, crown ether moieties chemically anchored to a polymer backbone can solve these problems [4,5]. After incorporating crown ether units into the polymer backbones, the mobility of the polymer structure is restricted, and the charge density along the polymer chain is increased, resulting in enhanced complexing properties. Moreover, immobilization of crown ethers onto polymers allows easy handling, regeneration, easy purification, low toxicity and adaptation to continuous processes for this important set of complexants. High polymer crown ether (PCE) compounds are easy to spin fiber and make into film, which are suitable for various applications.

In the late 1970s, Cram and co-workers have reported the first example of chiral crown ethers attached to a polymer resin [6]. This work has led to numerous examples of crown ethers incorporated as a stationary phase in chromatography. Hyun et al. have demonstrated that enantiomeric compounds possessing a primary amino group can be separated by crown ether-based separation [7]. Lee and co-workers have reported that the crown ether-modified polymer resin can be used as solid phase extraction (SPE) adsorbents for the purification of catecholamines (CAs) in urine. Since then, SPE by crown ether resin has been applied in pretreatment process [5]. Moreover, more attention has been paid to the supramolecular interaction between PCE and RNH_3^+ [8,9].

Currently, crown ether-grafted polymers are obtained by immobilizing crown ethers on organic supports (such as polystyrene beads) [10] or inorganic supports (such as magnetic-multiwalled carbon nanotubes) [4]. Lee and co-workers have prepared PCE via crown ether monomers grafted with macroporous resin promising in SPE of CAs [5]. It is an improved idea to selectively extract CAs using PCE material. However, it is still a traditional method of SPE. Moreover, condensation of the target remains difficult, since Lee's method provides a limit of quantity at 40 ng/mL and it is

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unable to realize the actual analysis of CAs in urine (actual urine E: 8.7–18.3 ng/mL, NE: 23.9–56.6 ng/mL, DA: 70–120 ng/mL [11]). Chen et al. have reported two types of PCE composite nanofibers in enrichment and purification of urinary CAs and plasma CAs [12–14]. However, the adsorption of CAs varies with different PCE structures and biological matrices. Therefore, the studies on PCE composite fibers with different functional groups are necessary to find out the proper PCE composite fibers for pretreatment of different biological matrices and optimize the extraction and purification conditions.

Compared with the conventional particle-packed SPE cartridge, the fibers prepared by electrospinning can be good adsorbents of SPE, offering a reduced volume of both the solid phase and desorption solvent [15,16]. Moreover, fiber-packed SPE can exert sufficient binding sites of the functional groups and reduce the production cost at the same time, conferring an efficient and inexpensive adsorbent. In the present study, 4-carboxybenzo-18-crown-6 ether modified XAD resin (4CB18C6-XAD)/polystyrene (PS) composite fibers were prepared by electrospinning. We assessed the adsorption properties of 4CB18C6-XAD/PS composite fibers in selective separation of urinary CAs. Furthermore, a comparative study was performed to characterize selective adsorption properties of 4CB18C6-XAD/PS composite fibers and 4CB18C6-XAD resin material. In combination with 96-well columns packing composite fibers, a high-throughput and efficient kit was developed for extraction of urinary CAs, which could solve the bottleneck problem of sample pretreatment [17]. In addition, we confirmed that packed-fiber SPE (PFSPE) was superior to conventional particle-packed SPE cartridge. Therefore, the detectable amount of CAs could be directly adsorbed from urine samples by using PFSPE, presenting a broad application prospect. Moreover, the high performance liquid chromatography coupled with fluorescence detector (HPLC-FD) was successfully applied to determine urinary CAs, and the results seemed to be satisfactory. HPLC coupled with electrochemical detector (HPLC-ECD) is regarded as golden standard for determination of urinary CAs [18,19]. However, we found that HPLC-FD was more stable and applicable in this study. Therefore, the method established in this study might be useful in clinical laboratories.

2. Experimental

2.1. Reagents

Epinephrine hydrochloride (E), norepinephrine bitartrate (NE), dopamine hydrochloride (DA), 3,4-dihydroxybenzylamine hydrobromide (DHBA) and diphenylborinic acid 2-aminoethyl ester (DPBA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were obtained from Tianjin Chemical Reagent Company (Tianjin, China). All other reagents were of analytical grade unless otherwise indicated. PS (Mw = 185,000) was provided by Shanghai Chemical Agents Institute (Shanghai, China). 4CB18C6-XAD was synthesized in the laboratory of Tianjin Medical University.

Solutions of CAs (1 mg/mL) and internal standard (DHBA) were prepared by dissolving appropriate amounts of respective chemicals in water and stored in the dark at 4 °C. DPBA solution (0.2 mg/mL) was prepared as above-mentioned and used as a complexing reagent [the reason for reducing the DPBA dosage was shown in Fig. S1 in Electronic Supplementary information (ESI)].

2.2. Apparatus and chromatographic conditions

HPLC-FD was carried out on a Waters-1225 HPLC connected to a Waters 2487 fluorescence detector (Waters, USA). The wavelengths of excitation and emission were 286 and 318 nm, respectively. HPLC-ECD was performed on a Waters 1225 HPLC connected to a Waters 2465 electrochemical detector with a glassy carbon working electrode (set at 0.7 V for determination). A 50-μL volume was injected into a YMC-Pack ODS-A column (100 × 4.0 mm, 3-μm particle size) via a Waters 2707 autosampler. A HPLC software package (Waters, USA) was used for the data analysis. The mobile phase consisted of 5.5% acetronitrile, 45 mM sodium dihydrogen phosphate, 35 mM citric acid, 2 mM sodium heptanesulfonate and 0.25 mM EDTA, the pH was adjusted to 4.2 by 2 M NaOH, and the flow rate was set at 0.5 mL/min. The temperature of the column oven was set to 30 °C.

2.3. Preparation of crown ether-modified XAD resin

4CB18C6 was synthesized according to a previously reported method [20]. 4CB18C6-XAD was synthesized via immobilizing 4CB18C6 on macroporous adsorption resin support based on a method reported by Lee and co-workers [5]. 1H and 13C NMR spectra (Figs. S2–S3 in ESI) were recorded with a Bruker Avance III spectrometer (Bruker, German) using DMSO-*d*6 as the solvent. EI-LCMS spectra (Fig. S4 in ESI) were obtained at 70 eV on an Agilent 6890N mass spectrometer (Agilent, America). Fourier transform infrared (FTIR) spectra (Fig. S5 in ESI) were recorded with a Bruker Tensor 27 spectrometer (Bruker, German).

2.4. Electrospinning

4CB18C6-XAD/PS composite fibers were fabricated by electrospinning. Briefly, 5% 4CB18C6-XAD solution (w/v) was prepared by dissolving an appropriate amount of 4CB18C6-XAD in dimethylsulfoxide. Next, 10% (w/v) PS solution was prepared by dissolving an appropriate amount of PS in a mixture of dimethylformamide and tetrahydrofuran (4:6, v/v). The 4CB18C6-XAD solution was slowly dropped into the PS solution (2:5, v/v), and the mixture was blended until it became homogeneous.

4CB18C6-XAD/PS solution was loaded into a 10-mL glass syringe through a stainless steel needle with a diameter of 0.5 mm and a flat tip, which was connected to the anode. The aluminum foil collecting equipment was connected to the cathode. The distance between the needle tip and the collector was 15 cm. A voltage of 20 kV was supplied by a Dongwen high-voltage generator (model DW-P403-1AC, Tianjin, China). The feed rate of the precursor solution was fixed at 2.0 mL/h. The fibers were examined using a Nanosem 430 scanning electron microscopy (FEI, America) and an infrared (IR) spectroscopy (Fig. S5 in ESI).

2.5. Preparation of column for SPE

In this experiment, 1-mL microcolumn adapted for the 96-well microplate device was used as SPE column. The PFSPE columns were prepared as described in a previous paper [12]. Filter support was not necessary for packing fiber (3 mg) into 1-mL microcolumn. However, two cylindrical filter supports were inserted at the bottom and upper side of the microcolumn for particle-packed SPE, and then 4CB18C6-XAD material (4 mg) was packed in the middle. Fig. S6B in ESI illustrates that 1-mL microcolumn adapted for the 96-well microplate device was packed with the 4CB18C6-XAD/PS composite fiber, and Fig. S6C in ESI shows the 96-well microplate device loaded with 1-mL microcolumns.

2.6. Extraction of CAs

The SPE was performed on the MULTI-SPE M96 (positive pressure solid phase extraction device). Briefly, the microcolumn was firstly installed onto the 96-well microplate device (Fig. S6C in ESI) and placed in a fixed position of the MULTI-SPE M96 (Fig. S6A in

ESI). Then, 100 μL of methanol and water were loaded onto the microcolumns and pushed through the sorbent by nitrogen positive pressure. Next, the samples were loaded onto the microcolumns and pushed through the sorbents. Subsequently, the adsorbed target compounds were eluted with 1 M acetic acid solution (HAc, 50 μL), and water (50 μL) was added to the eluent (the solvent effect was reduced by diluting eluate). Finally, 50 μL eluate mixture was injected onto the HPLC column via an autosampler.

2.7. Sample preparation

Human urine samples were donated by 21 drug-free and healthy volunteers. The study protocol was approved by the Ethics Committee of Tianjin Medical University, and all experimental procedures were conducted in accordance with the Declaration of Helsinki. All participants provided their written informed consents prior to the study. Briefly, 0.5 mL human urine sample was filtered through a 0.22-μm membrane filter, and 200 μL filtered urine sample was mixed with 200 μL phosphate buffer (pH 7.8). Subsequently, 50 μL DHBA (internal standard, 200 ng/mL) and 50 μL DPBA (complexing reagent, 0.2 mg/mL) were added into the urine samples. The extraction of urine samples was then performed as described in Section 2.6. A washing procedure of 50 μL 10% methanol and 50 μL water was carried out for urine samples (the specific explanation was given in Section 3.4). Finally, 50 μL eluate mixture was injected onto the HPLC column via an autosampler.

3. Results and discussion

3.1. Morphology of 4CB18C6-XAD/PS composite fibers

Morphologies of the 4CB18C6-XAD/PS composite fibers were characterized by SEM, and the obtained micrographs were shown in Fig. 1. It was clear that the surface morphology of the composite fibers was rough, displaying a broad fiber diameter distribution, ranging from 500 to 3500 nm. The poor solubility of 4CB18C6-XAD might induce the broader fiber diameter distribution. However, the process of blended spinning was smoother, and the precipitation in spinning solution or blocking of pipeline needles was not found.

3.2. The optimal operating conditions of the MULTI-SPE M96 for extraction of CAs

The MULTI-SPE M96 was used for high-throughput extraction of CAs in this study. Fig. S6 in ESI shows that the 4CB18C6-XAD/PS composite fibers were packed into 1-mL microcolumns adapted for the 96-well microplate device, which could provide a high-throughput pretreatment of CAs by one-step operation. Once the parameter of nitrogen flow could be optimized, no other parameters needed to be adjusted. Moreover, 96 samples could be simultaneously filtered by the MULTI-SPE M96. Table 1 lists the optimal operating conditions of the MULTI-SPE M96 for extraction of CAs. The pressure intensity was increased from 15 Kpa to 50 Kpa, and the duration of procedure ranged from 60 min to 2 min accordingly. However, no big difference was observed in the extraction efficiency of CAs (90.6–100.0%). Therefore, 40 Kpa was selected for further analysis because of the best efficiency and suitable operation duration.

In the development history of the PFSPE, no easy-to-use SPE apparatus has been developed to match the PFSPE, leading to limited use efficiency of the PFSPE [21–23]. The MULTI-SPE M96 can simultaneously pretreat 96 samples, and thus it can be applied to large quantities of sample testing.

3.3. The influence of elution solvent

In our previous studies, the optimal elution solvent of CAs is 12 M HAc. However, high concentration of HAc can result in chromatographic peak broadening and shift of CAs (Fig. 2). The chromatographic peak of CAs eluted by 12 M HAc is wider and lower compared with 1 M HAc. The histogram data in Fig. 2 showed that the extraction efficiency of CAs was slightly decreased when the HAc concentration was reduced. However, the chromatographic peak of CAs eluted by 1 M HAc was high and narrow, which was more conducive to separate CAs from the surrounding impurity peaks and improve the selectivity and sensitivity detection of CAs. Therefore, 1 M HAc was selected as the optimal elution solvent in this study.

3.4. The influence of washing agent

The impurities in urine can block ODS column, weakening chromatographic column efficiency and ECD signal. Therefore, it is necessary to pretreat the urine samples before loading onto column. In this study, the urine samples were firstly filtered through a 0.22-μm membrane, and the granule impurities were removed. However, the yellow pigment can go through the membrane. After loading the urine samples into the PFSPE, a yellow layer was adsorbed on the white fiber in the column. Water and methanol have been considered to be great washing reagents. The yellow impurities cannot be washed by water. However, the yellow pigment can be eluted by low-concentration methanol. Therefore, methanol can be used as a candidate washing reagent. In the present study, 50 μL 10% methanol and 50 μL water were selected for pretreatment of urine samples based on the preliminary experiment. After PFSPE was treated as shown in Fig. 3, the chromatographic peak of unwashed urinary CAs coexisted with a quantity of impurities, especially in E and DA positions. In contrast, the coexisting impurities were significantly reduced after washing procedure using 50 μL 10% methanol and 50 μL water. Therefore, 50 μL 10% methanol and 50 μL water could ensure accurate determination of the target materials.

The loss of CAs was observed after washing procedure (as shown in the top right histogram in Fig. 3). Fortunately, the relative high concentration of CAs in urine offered us the opportunity to obtain satisfactory results. On the contrary, washing procedure not only guaranteed the efficiency and life span of chromatographic column, but also avoided interference of impurities in urine samples. Finally, 50 μL 10% methanol and 50 μL water were regarded as the optimal washing agents in this study.

3.5. The influence of extraction pH

In our previous studies for CAs, extraction pH is a key issue in PFSPE, and the optimal condition is pH 7.2–7.5. For actual clinical tests, precision control of each person's urine pH not only increases the test difficulty, but also decreases the reproducibility. In this case, it is difficult to reproduce this experiment by other labs. It is found that the pH of most normal human urine samples is approximately 6.0. The pH of 200 μL urine samples can be adjusted to around 7.2 by adding 200 μL phosphate buffer (pH 7.8). Fig. 4 shows the effect of different extraction pH values (pH 6.0, pH 7.2 and pH 7.5) on chromatograms. The chromatogram of unadjusted urine samples (pH 6.0) revealed low extraction efficiency of CAs and impurity interference. In contrast, the chromatograms of pH-adjusted urine samples (pH 7.2 or pH 7.5) displayed great extraction efficiency of CAs. Therefore, the extraction efficiency of CAs could be improved by adjusting the pH of urine samples with phosphate buffer (pH 7.8) in this PFSPE experiment.

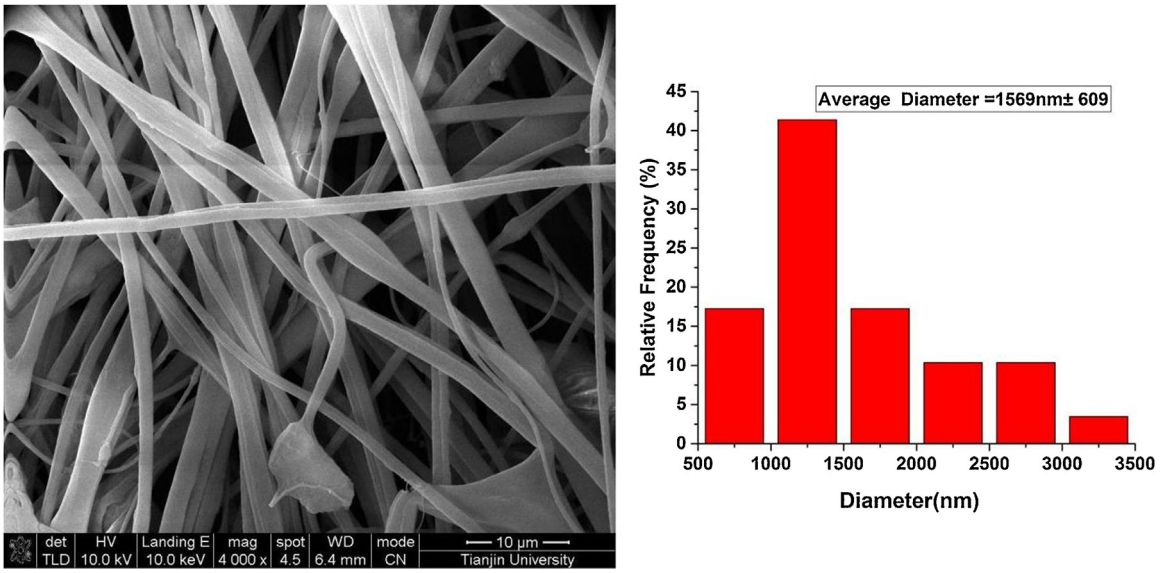


Fig. 1. SEM images of 4CB18C6-XAD/PS composite fibers.

Table 1
The optimal operating conditions of the MULTI-SPE M96 for CAs extraction.

High flow pressure (Kpa)		15	20	25	30	35	40	50
Extraction efficiency (%)	NE	98.4	97.4	90.6	93.3	98.2	109.2	100.7
	E	97.5	96.0	92.1	94.5	99.4	107.1	98.1
	IS	100.9	100.0	99.8	100.3	104.8	112.8	104.7
	DA	96.3	93.3	92.3	92.5	101.5	107.0	101.9
Time of procedure (min)		60	10	8	5	4	3	2

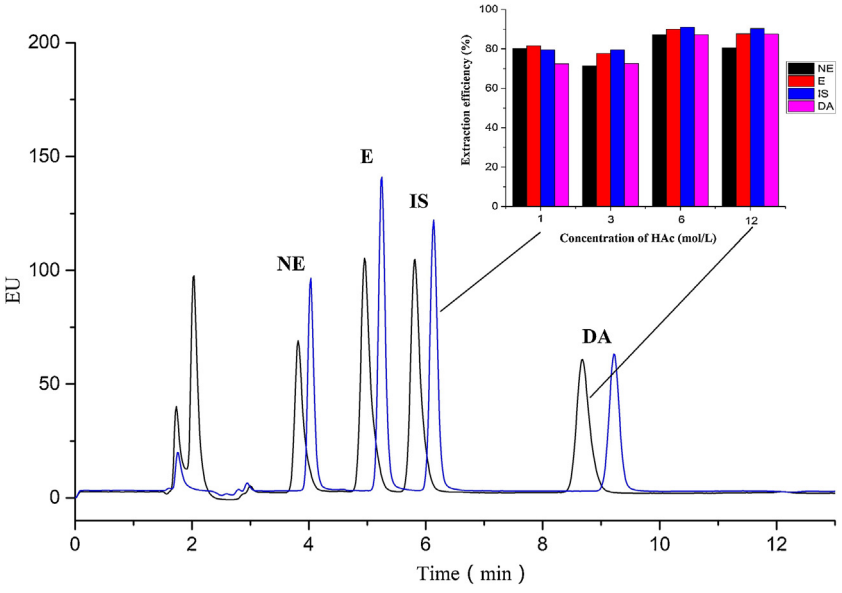


Fig. 2. HPLC-FD chromatograms of CAs eluted by 1 M HAc and 12 M HAc.
The top right histogram showed the extraction efficiency of CAs by different concentrations of HAc.

3.6. The contrast of 4CB18C6-XAD/PS composite fiber and 4CB18C6-XAD resin

Generally, the packing quantity of traditional particle-packed SPE column is about 50 mg or even more. Lee and co-workers have adopted traditional particle-packed SPE for determination of urinary CAs, and they have difficulty in CAs enrichment. Therefore, they have failed to complete the actual analysis of CAs in urine. In the present study, we conducted a comparative experiment between particle-packed SPE and PFSPE. For the convenience of expression, PFSPE (4CB18C6-XAD/PS composite fiber as adsorbent) was represented with A, while particle-packed SPE (4CB18C6-XAD resin as adsorbent) was represented with B. The standard solution of CAs was loaded onto the columns A and B under the same condition, and the waste and eluate of each column were collected for chromatographic analysis. The extraction efficiency was calculated,

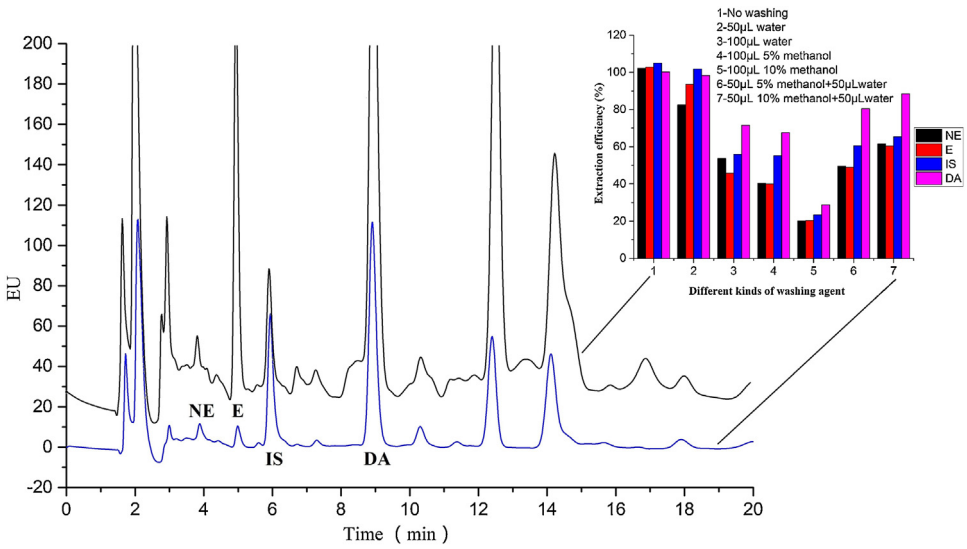


Fig. 3. HPLC-FD chromatograms of urine sample before and after washing procedure. The top right histogram showed the extraction efficiency of CAs by different types of washing agents.

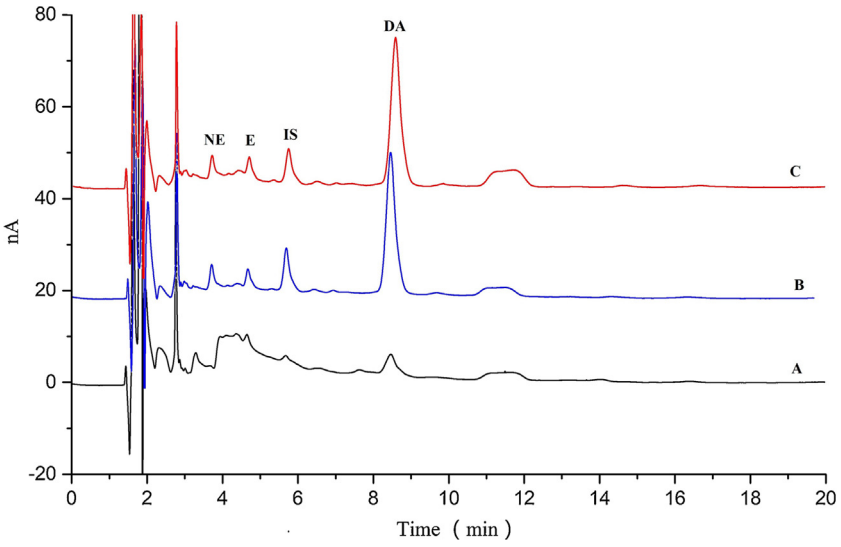


Fig. 4. HPLC-ECD chromatograms of urine sample under extraction pH 6 (A), pH 7.2 (B) and pH 7.5 (C).

Table 2
The extraction efficiency of CAs by fiber-packed SPE and particle-packed SPE.

CAs	Direct extraction				Extraction with adding DPBA			
	A (%)		B (%)		A (%)		B (%)	
	Waste	Eluate	Waste	Eluate	Waste	Eluate	Waste	Eluate
NE	–	64.0	66.9	16.8	–	92.3	1.4	95.6
E	–	93.9	50.8	46.2	–	92.5	–	94.8
IS	–	88.5	40.6	53.1	–	96.5	5.0	98.3
DA	–	91.6	27.3	72.8	–	90.1	3.6	98.5

and the results were shown in Table 2. The experimental results indicated that B was superior to A in direct extraction of CAs. The CAs were not detected in waste, suggesting an adsorption efficiency of 100.0% by B. In contrast, the adsorption and elution efficiencies of A were both poor in direct extraction of CAs. After adding the DPBA, the adsorption efficiency of A was dramatically improved, and the elution efficiency of A became superior to B. Moreover, the co-existing impurities were greatly eliminated by A compared with B.

Fig. 5 shows the chromatogram of human urine samples, which were pretreated by columns A and B accordingly. The elution of column B had more impurities, and the interferential chromatographic peak in position E was abnormally high. The impurities and CAs were extracted by B at the same time. Moreover, the washing procedure could not completely remove these impurities. However, the elution of A presented perfect detection line, indicating excellent impurity removal efficiency of column A and high extraction efficiency of CAs. Furthermore, PFSPE greatly reduced the dosage and

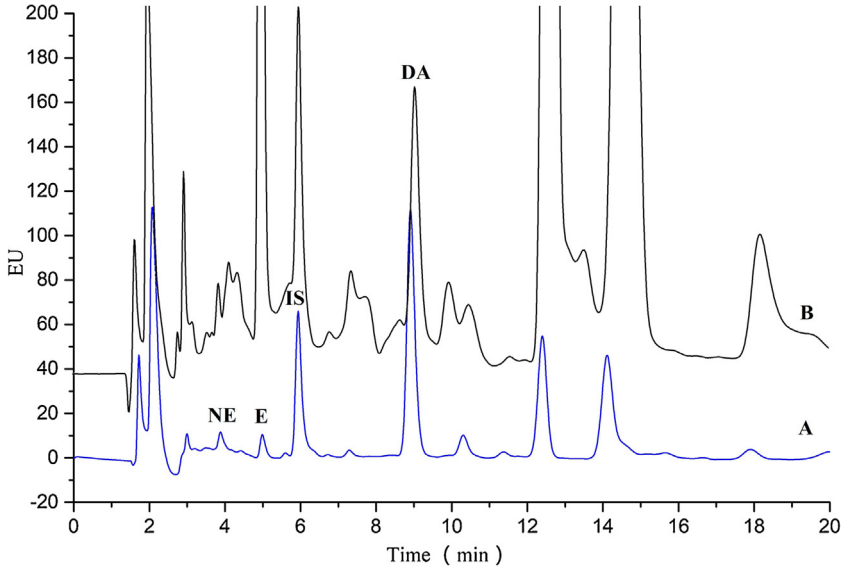


Fig. 5. HPLC-FD chromatograms of urine sample pretreated by PFSPE (A) and particle-packed SPE (B).

cost of solid adsorbent by blending 4CB18C6-XAD resin and PS (2:5, v/v). Therefore, this PFSPE coupled with the MULTI-SPE M96 could provide a high-throughput pretreatment method for the extraction of urinary CAs, which was suitable for large-scale sample analysis.

3.7. The contrast of two types of composite fibers

In our previous studies, a PS/poly (dibenzo-18-crown-6) composite fiber (PS/PDB18C6) has been developed for the selective adsorption of plasma CAs [14]. Here, the two types of composite fibers doped with different crown ethers were compared for pretreatment of urinary CAs. Fig. 6 shows that the chromatograms of human urine samples treated by two types of composite fibers were very similar, except for the determination of E. The extraction efficiency of E by PS/PDB18C6 composite fiber was relative lower than that by 4CB18C6-XAD/PS composite fiber (80.0%–100.0% shown in Figs. 3 and 6). When the same washing procedure was applied, the extraction efficiency of E by two types of composite fibers was 20.0% and 60.0%, respectively. Therefore, PS/PDB18C6 composite fiber was impossible to provide a higher extraction efficiency of E in the same urine sample compared with 4CB18C6-XAD/PS composite fiber. Moreover, a big difference was found in the parallel urine samples treated by PS/PDB18C6 composite fiber. However, the result for 4CB18C6-XAD/PS composite fiber was satisfactory (Fig. S7 in ESI). Therefore, compared with PS/PDB18C6 composite fiber, 4CB18C6-XAD/PS composite fiber was more suitable for pretreatment of urinary CAs.

3.8. Linearity and recovery of the procedure

Quantification was performed using the internal standard method. Spiked standard solution containing CAs (NE, E and DA) were prepared at various concentrations (0.5, 1.0, 5.0, 20.0, 100.0, 200.0 and 400.0 ng/mL), and then 200 μL of serial spiked standard solutions was respectively mixed with 50 μL of DHBA solution (internal standard, 200.0 ng/mL) and 50 μL of DPBA solution (complexing reagent, 0.2 mg/mL). The extraction process of mixed samples by PFSPE was then performed as described above. The calibration curves were constructed by plotting the peak-area ratio of CAs-to-IS against the concentrations of CAs. The linearity was ideal within the range of 0.5 ng/mL–400.0 ng/mL for NE and E, which was 1.0 ng/mL–400.0 ng/mL for DA. The limit of detection (LOD) for NE and E was 0.2 ng/mL (S/N = 3), and it was 0.5 ng/mL for DA (S/N = 3). The precision (n = 6) was less than 5%, which could meet the testing requirements of biological samples. Recoveries were determined as percentages of the measured concentrations (calculated from the standard curve equation) against the spiked concentrations. Moreover, the recoveries were 70.5%, 102.8% and 119.6% for NE, E and DA, respectively. Fig. S7 in ESI shows the results of the precision and the recovery.

Table 3
The actual urine determination results from 22 healthy persons.

Samples	NE (ng/mL)	E (ng/mL)	DA (ng/mL)	Gender	Age
1#	36.4	11.9	223.5	male	24
2#	16.8	24.6	297.6	male	24
3#	15.5	9.1	336.0	male	23
4#	78.9	18.4	381.8	male	24
5#	22.3	23.1	108.0	male	23
6#	18.7	16.9	117.7	male	24
7#	18.4	9.0	100.9	male	25
8#	16.5	22.3	353.5	male	22
9#	14.1	17.2	199.6	male	24
10#	56.2	7.9	122.1	male	23
11#	32.8	19.8	104.8	male	24
12#	41.3	11.7	118.7	male	24
13#	15.6	8.1	168.1	female	23
14#	51.0	15.2	92.9	female	23
15#	32.0	7.5	219.4	female	24
16#	30.8	3.7	162.0	female	23
17#	20.9	7.7	224.5	female	23
18#	15.7	9.7	162.8	male	38
19#	35.6	3.5	206.7	female	36
20#	47.7	10.2	132.4	female	36
21#	19.6	14.8	194.5	male	9
22#	16.5	13.6	98.5	male	9
Mean ± SD	29.7 ± 16.9	13.0 ± 6.1	187.5 ± 87.2		24 ± 6

3.9. Application of the method to human urine samples

In the present study, first morning urine was collected for easy compliance with elimination of medications and food. Free CAs were measured in morning urine samples from 22 individuals (7 females and 15 males, 9–38 years old with a median age of 24) using our newly developed method. Fig. S8 in ESI shows the stable and reliable results obtained by the FD compared with the ECD. Therefore, the FD was finally selected in this experiment. Table 3 reveals

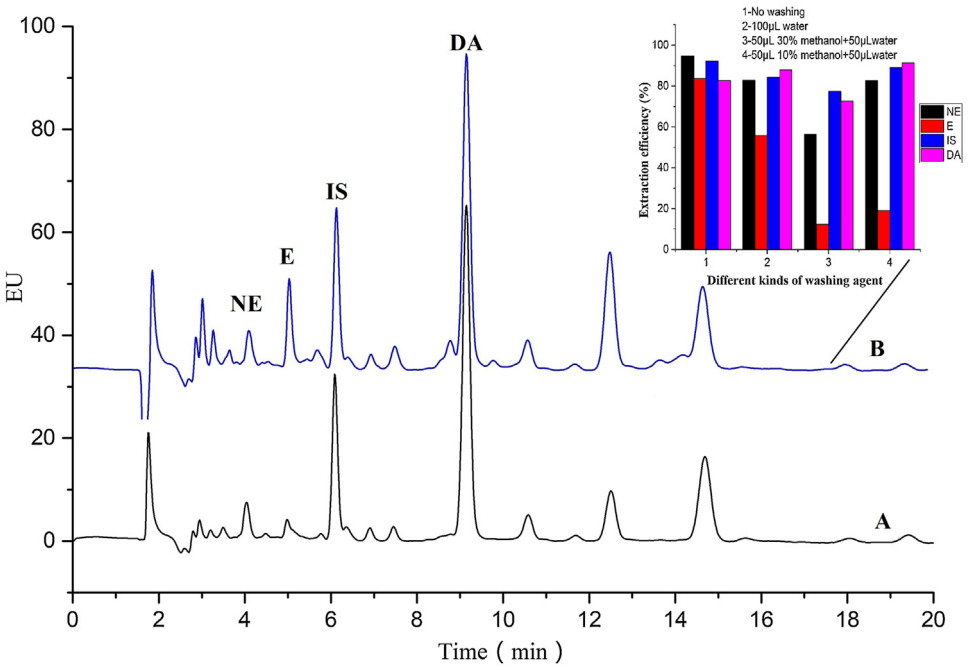


Fig. 6. HPLC-FD chromatograms of urine sample pretreated by 4CB18C6-XAD/PS composite fiber (A) and PS/PDB18C6 composite fiber (B). The top right histogram showed the extraction efficiency of CAs by different types of washing agents using PS/PDB18C6 composite fiber.

that NE ranged from 14.1 to 78.9 ng/mL with a mean of 29.7 ng/mL, E ranged from 3.5 to 24.6 ng/mL with a mean of 13.0 ng/mL, and DA ranged from 92.9 to 381.8 ng/mL with a mean of 187.5 ng/mL. The determined values were consistent with previously reported data [18,24–26].

4. Conclusions

In the present study, we described a simple, cost-effective and high-throughput pretreatment and analytical method for the simultaneous determination of urinary CAs using PFSPE packed with 4CB18C6-XAD/PS composite fibers coupled with HPLC-FD. The analysis cost was considerably reduced due to decreased adsorbent, sample volume, solvent and prevalent testing instrument. The excellent validation data in combination with successful application in real urine samples suggested that the proposed method was sensitive, reliable and high-throughput for routine testing of urinary CAs. Therefore, the proposed method could be useful in clinical chemistry.

Ethical standards

The data presented in the paper were prior testing data stored anonymously.

Conflict of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.chroma.2018.05.041.

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