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Simultaneous determination of 5-hydroxyindoles and catechols from urine using polymer monolith microextraction coupled to high-performance liquid chromatography with fluorescence detection

Yu-Bo Wu[a,b], Jian-Hong Wu[a,b], Zhi-Guo Shi[a,b], Yu-Qi Feng[a,b,*]

[a] Department of Chemistry, Wuhan University, Wuhan 430072, China
[b] Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Wuhan University, Wuhan 430072, China

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ABSTRACT

To make analytes amenable for fluorescence (FL) detection, polymer monolith microextraction (PMME) coupled to high-performance liquid chromatography with FL detection was developed for the simultaneous determination of catechols and 5-hydroxyindoleamines (5-HIAs) from urine samples. In this method, a two-step pre-column derivatization method was employed to derivatize the analytes and a poly(methacrylic acid-co-ethylene glycol dimethacrylate) monolithic capillary column was used as the extraction medium for PMME. The conditions for the derivatization and subsequent extraction of 5-HIAs and catechols derivatives were optimized. Using our optimum conditions, the detection limit of the target analytes were 0.11–21 nM. Reproducibility of the method was obtained with intra-day and inter-day relative standard deviations less than 12% and a recovery of higher than 82%. In this study, we show how our proposed method can be used as a rapid sensitive technique for the determination of catechols and 5-HIAs from urine samples.

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1. Introduction

Catechols and 5-hydroxyindoleamines (5-HIAs) are widely found in biological systems and play important physiological roles in modulating human behavior, sleep, and feeding [1]. These monoamines are also responsible for some neuronal disorders such as depression [2–4], anxiety [5,6], schizophrenia, Parkinson's disease [7–9], as well as other diseases such as hypertension [10], carcinomas and diseases related to the cardiovascular system [11] and adrenal function [12]. Therefore, it is important to determine the quantity and content of monoamines in biological samples.

Up to now, various methods for the determination of monoamines, such as 5-hydroxytryptamine (5-HT), noradrenaline (NE), dopamine (DA) and their metabolites have been described. Among the most commonly used methods are high-performance liquid chromatography (HPLC) coupled with electrochemical (EC) [13–15] or fluorescence (FL) [16,17] detectors, while gas chromatography–mass spectrometry (GC–MS) [18,19] have also been adopted for the determination of derivatized amines. LC–EC offers a simple and sensitive analytical method, and can be broadly applied for the detection and analysis of 5-HT, NE, DA and their metabolites from biological samples. However, for routine analysis, the EC detector has various problems such as high noise and instrument instability.

The LC-FL method with direct excitation of native monoamines offers a simple analytical system but provides relatively low sensitivity, especially for catechols. To improve FL sensitivity, derivatization of the sample has been employed. Benzylamine (BA) and 1,2-diphenylethylenediamine (DPE) in the presence of potassium hexacyanoferrate (III) under weakly alkaline conditions, have been demonstrated to be highly selective FL reagents in forming benzoxazole derivatives with 5-HIAs and catechols, respectively [20–24]. For the simultaneous determination of 5-HT, NE, DA and metabolites from brain extracts and microdialysis samples, a two-step derivatization with BA and DPE strategy has been proposed [25].

However, in urine samples, the sensitivity of HPLC-FL analysis after derivatization is comparably low [26–28], due to the very low concentrations of most monoaminergic neurotransmitters in human urine and the presence of many native interfering substances in the biological sample. Therefore, it is necessary to subject the sample to pretreatment procedures such as sample clean-up and enrichment prior to analysis. Traditional extraction strategies, such as liquid–liquid extraction (LLE) [26] and solid-phase extraction (SPE) [27,28] have been employed for this purpose. Miniaturization has been a key factor in achieving economy, time efficiency and solvent saving costs. Solid-phase microextraction

* Corresponding author at: Department of Chemistry, Wuhan University, Wuhan 430072, China. Tel.: +86 27 68755595; fax: +86 27 68755595.
E-mail address: yqfeng@whu.edu.cn (Y.-Q. Feng).

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(SPME) is the most popular microextraction method and can be performed in various configurations, such as coated-silica fiber, in-tube microextraction or by extraction tips [29–35].

Our group has made significant contributions to the polymer monolith microextraction (PMME) method [36]. The monolithic capillary column by *in situ* polymerization [37–39] has several attractive features including frit-free construction, easy preparation with good control of porosity and diverse surface chemistry. Compared to other in-tube SPME systems [40,41], this technique offers convenient operation and less dependence on instrumentation.

In our previous research, PMME based on a capillary monolithic column of poly(methacrylic acid-co-ethylene glycol dimethacrylate) (*p*(MAA-co-EGDMA)) has been successfully used for the determination of basic drugs and angiotensin II receptor antagonists in biological matrices [42–44]. The monolithic material exhibited a high extraction capacity as well as benign biocompatibility [45,46].

In this study, a PMME technique using the same monolithic column in combination with a BA and DPE two-step derivatization process was developed for the extraction of monoaminergic neurotransmitters in human urine followed by HPLC-FL detection. The derivatization and PMME conditions were investigated in detail and our present method enables highly sensitive and selective determination of 5-HIAs and catechols from urine samples.

2. Experimental

2.1. Chemicals and materials

Deionized distilled water was purified with a Milli-Q apparatus (Millipore, Bedford, MA, USA) system and was used for all aqueous solutions. Four 5-HIAs (5-hydroxytryptamine hydrochloride (5-HT), DL-5-hydroxytryptophan (DL-5-HTP), 5-hydroxyindole-3-acetic acid (5-HIAA), *N*-acetyl-5-hydroxytryptamine (*N*-Ac-5-HT)), six catechols (DL-noradrenaline hydrochloride (NE), L-epinephrine hydrochloride (E), dopamine hydrochloride (DA), 3,4-dihydroxy-L-phenylalanine (L-DOPA), 3,4-dihydroxyphenylacetic acid (DOPAC), DL-3,4-dihydroxymandelic acid (DOMA)) and DPE were obtained from Sigma (St. Louis, MO, USA). 3-Cyclohexylaminopropanesulfonic acid (CAPS) and glycine were purchased from Tianyuan Chemicals (Wuhan, China). Analytical reagent grade potassium hexacyanoferrate (III) and 1-octanesulfonic acid sodium salt were purchased from Shanghai Chemical Co. Ltd. (Shanghai, China). BA hydrochloride was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) and used after purification by recrystallization in absolute ethanol.

Stock solutions (5 mM) of 5-HIAs and catechols were prepared in water and stored at −20 °C. BA hydrochloride (0.3 M) and potassium hexacyanoferrate (III) (20 mM) solutions were all prepared in water. CAPS buffer (0.1 M, pH 11.0) was prepared in a mixture of methanol and water (1/5, v/v). DPE (50 mM) and glycine (50 mM) solutions were prepared in 0.1 M hydrochloric acid and water, respectively. The BA derivatization reagent solution was a mixture containing BA–CAPS–potassium hexacyanoferrate (III) solution–methanol (10/11/22/23, v/v/v/v). The DPE derivatization reagent solution was a mixture of DPE–glycine solutions (19/10, v/v). All reagent solutions were stable for at least 2 weeks at room temperature. The sample solutions were diluted further with water to the desired concentrations before use. For chromatographic quantification, *N*-Ac-5-HT (100 nM) was used as an internal standard (I.S.).

The extraction device for PMME with poly(MAA-co-EDGMA) monolith in fused silica capillary (3 cm × 530 μm i.d.) was purchased from Micromole Separation and Testing Technology (Beijing, China). The extraction device is composed of an extraction pinhead and syringe barrel, with the original metallic needle of the pinhead replaced by a 3 cm-long monolithic capillary (Fig. 1) [36].

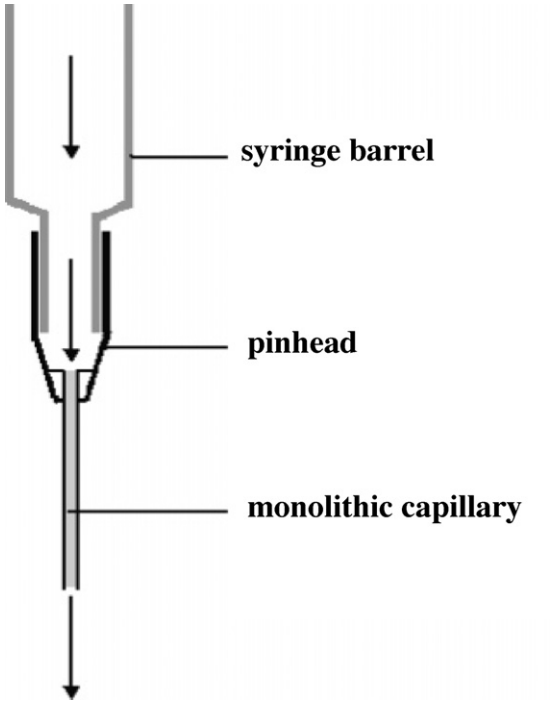

Fig. 1. Scheme for the PMME device [36].

2.2. Sample preparation

Urine samples were collected by single-morning urination from healthy volunteers and patients of Zhongnan Hospital (Wuhan, China). The urine samples were stored at −20 °C until analysis. The urine samples were diluted with water to the desired concentration, centrifuged for 5 min at 12,000 rpm (4 °C), and passed through a disposable filter (0.45 μm, 13 mm i.d.)

2.3. Derivatization-PMME procedure

To a 100 μL portion of an aqueous test solution containing 5-HIAs and catechols (or a diluted urine sample), 100 μL of the BA derivatization solution was added. After 5 min, 100 μL of the DPE derivatization solution was also added. The tube was covered and heated at 25 °C for 20 min in a water bath and then allowed to cool. A 20-μL aliquot of the final mixture was injected into the HPLC system for analysis.

A TCI-II syringe infusion pump (Silugao High-Technology Development, Beijing, China) was used to provide the driving force for extraction. The whole extraction process included pretreatment, adsorption, clean-up and desorption, which were all carried out at a velocity of 0.15 mL/min. Firstly, 0.3 mL methanol and 0.3 mL water were driven via the monolithic capillary in sequence to equilibrate the monolith. After derivatization, 0.5 mL of the sample solution was injected through the column. After the column was washed with 0.2 mL water, the residual water in the monolith was driven out by air. Subsequently, 50 μL acetonitrile/5% acetic acid aqueous solution (60/40, v/v) was used for the desorption of the analytes from the monolith. The eluate was collected into a vial and was used for HPLC analysis.

2.4. Chromatographic apparatus and operating conditions

An Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) with FL detector was used in our experiments. The FL detection wavelengths were set to $\lambda_{ex}/\lambda_{em}$ = 345/480 nm.

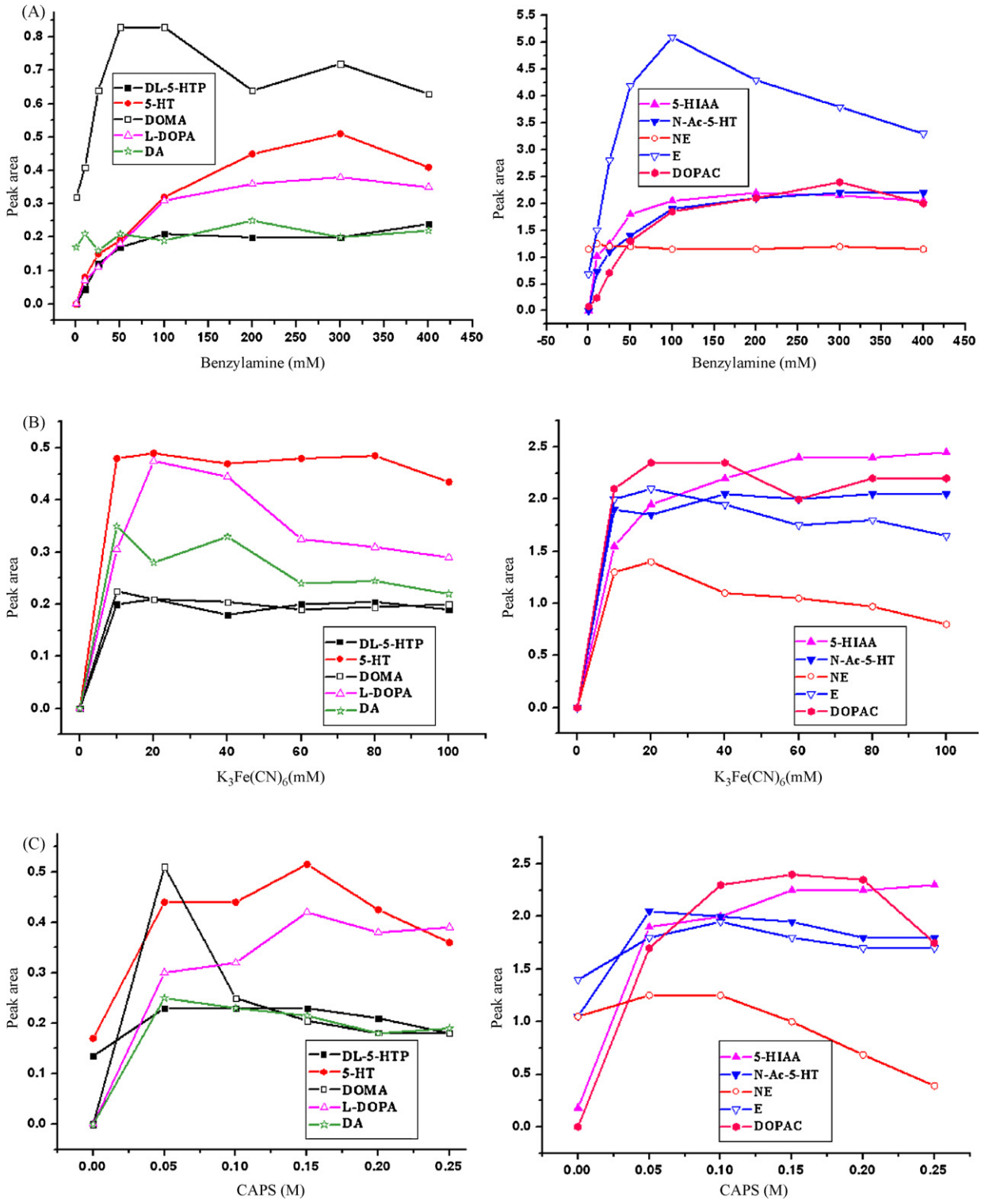

Fig. 2. Effects of benzylamine (A), potassium hexacyanoferrate (III) (B) and CAPS buffer concentrations (C) on the peak areas of 5-HIAs and catechols.

The XDB-C_8 column (150 mm × 4.6 mm i.d., 5 μm) was purchased from Agilent Technologies. The mobile phase consisted of acetonitrile–15 mM acetate buffer (pH 4.5) containing 1 mM octanesulfonic acid sodium salt. Separation of the 5-HIAs and catechols derivatives was achieved with gradient elution (0–13 min, ACN: 25.0–32.0%; 13–25 min, ACN: 33.5–40%; 25–27 min, ACN: 49–50%). The flow-rate was set at 1 mL/min and the column temperature at 30 °C.

3. Results and discussion

3.1. Optimization of derivatization conditions

The derivatization reaction conditions were examined using a standard solution containing NE, E and DOPAC, 50 nM each; DL-5-HTP, 5-HT, 5-HIAA and N-Ac-5-HT, 100 nM each; DOMA, L-DOPA and DA, 1 μM each.

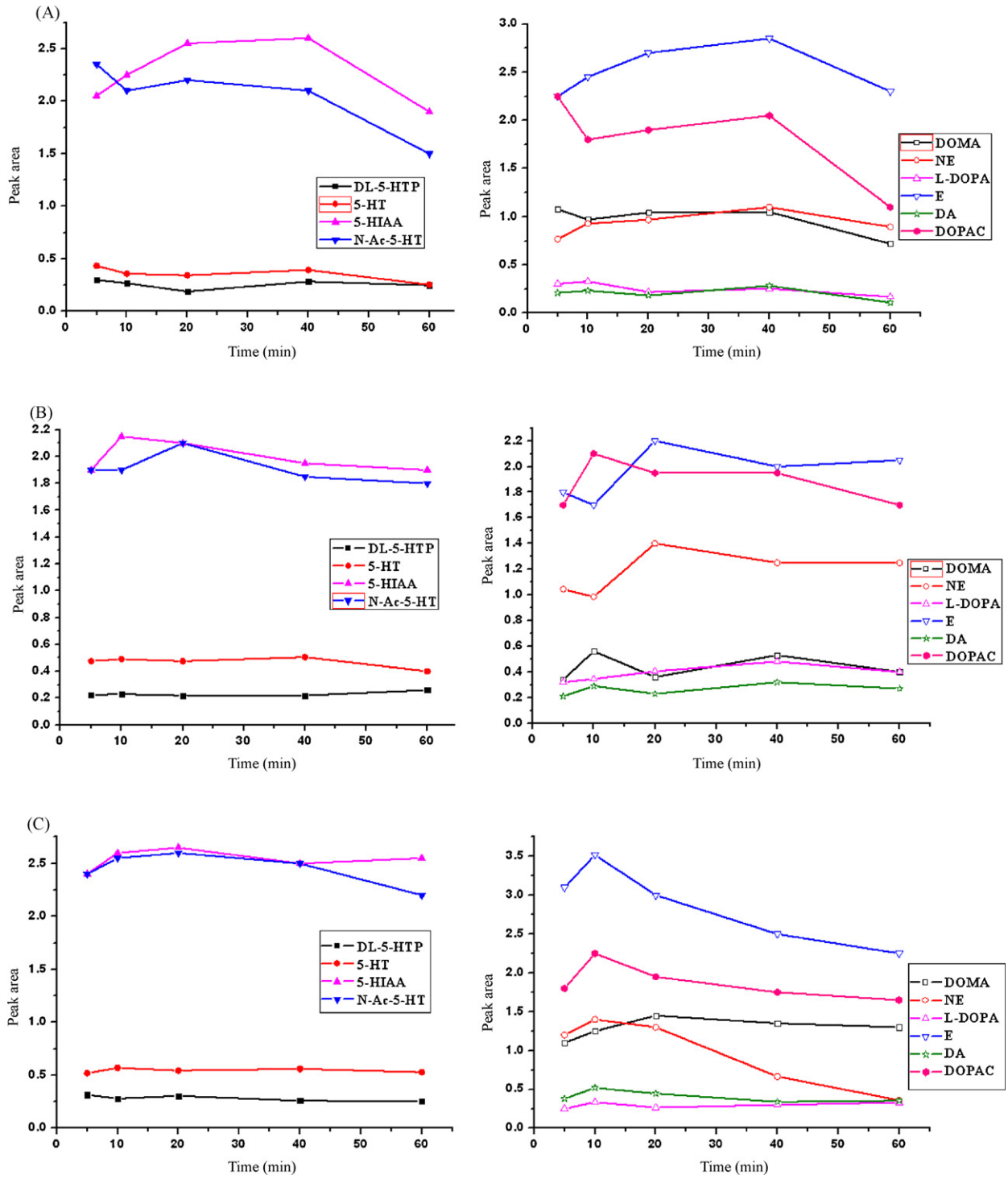

Fig. 3. Effects of reaction time and temperature on the second derivation step with DPE on the peak areas of 5-HIAs and catechols. (A) 25 °C, (B) 50 °C and (C) 80 °C. A standard solution containing NE, E and DOPAC, 50 nM each; DL-5-HTP, 5-HT, 5-HIAA and N-Ac-5-HT, 100 nM each; DOMA, L-DOPA and DA, 1 μM each was used.

In other studies it has been reported that reaction between 5-HIAs and BA occurred rapidly within 2–5 min at room temperature [20–22,47], and therefore chose 5 min at room temperature for our first step derivatization.

The effects of BA, potassium hexacyanoferrate (III), CAPS buffer and methanol concentrations on the FL intensity (peak areas) have been studied (Fig. 2A–C). BA concentrations greater than 0.1 M in solution gave relatively stable peak areas for all derivatives except for E and DOMA (Fig. 2A). Maximum peak areas were attained at 0.3 M BA concentration for most monoaminergic neurotransmitters, and a 0.3 M solution was adopted in subsequent procedures.

Potassium hexacyanoferrate (III) was used as an accelerator to promote the reaction between 5-HIAs and BA. The influence of the concentration of potassium hexacyanoferrate (III) was investigated (Fig. 2B). We found that the yield of the reaction was almost the same when the concentration of potassium hexacyanoferrate (III) was 10–40 mM. 20 mM potassium hexacyanoferrate (III) was chosen for our procedures.

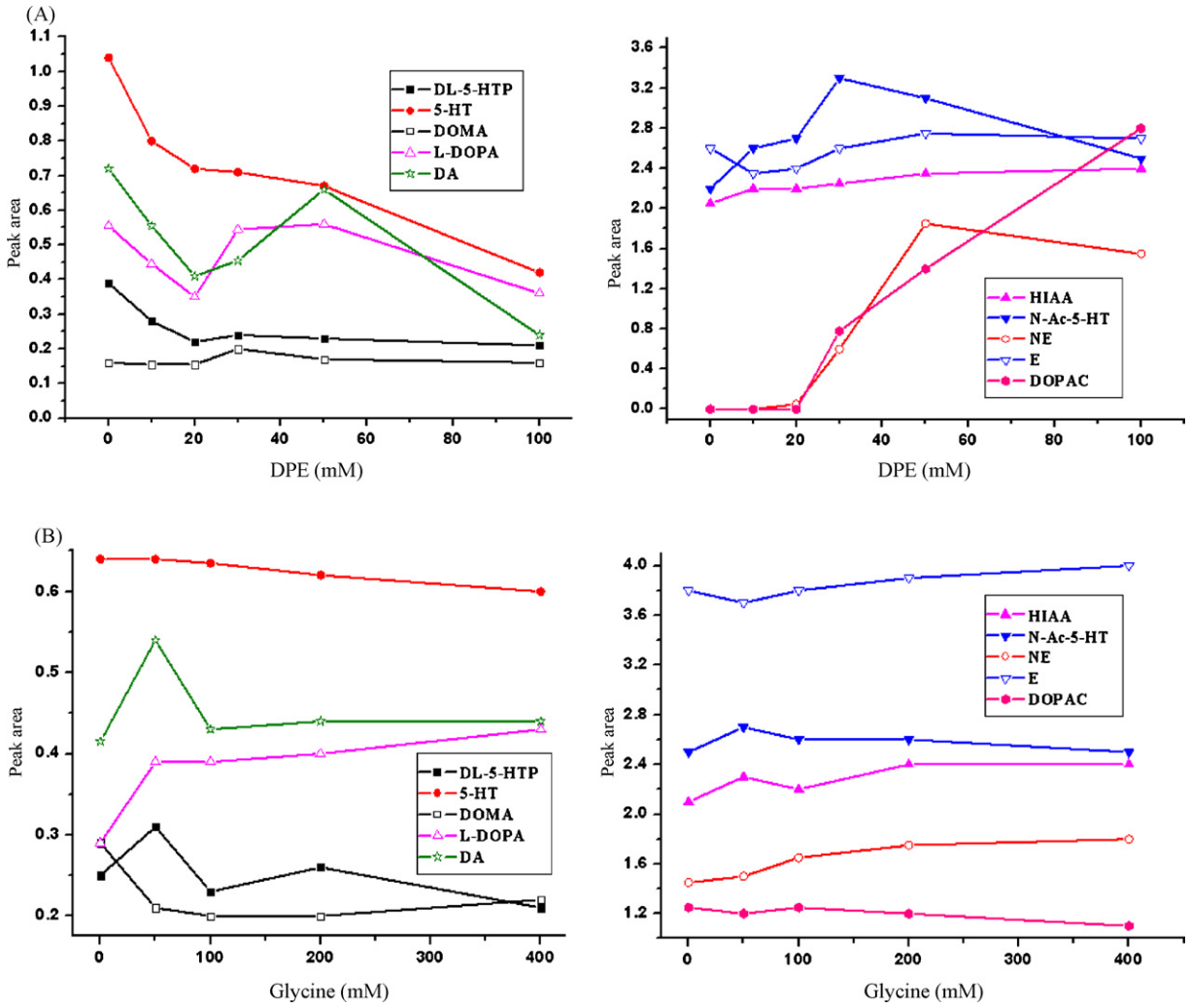

Fig. 4. Effects of DPE (A), glycine concentration (B) on the peak areas of 5-HIAs and catechols.

Based on the previous work by Fujino et al. [24], CAPS buffer at pH 11.0 was suitable for our derivatization procedure. The pH effect on FL intensity was not examined in our current study, however, the influence of the CAPS buffer concentration on the FL intensity was examined from 0 to 0.25 M (Fig. 2C). As the CAPS concentration was increased from 0 to 0.1 M, most of the responding signals showed a corresponding increase, which remained constant from 0.1 to 0.5 M. Furthermore, maximum peak areas for most monoaminergic neurotransmitters were attained at a CAPS concentration of 0.1 M. Therefore, 0.1 M CAPS at pH 11.0 buffer solution was taken as the optimal buffer for the derivatization process.

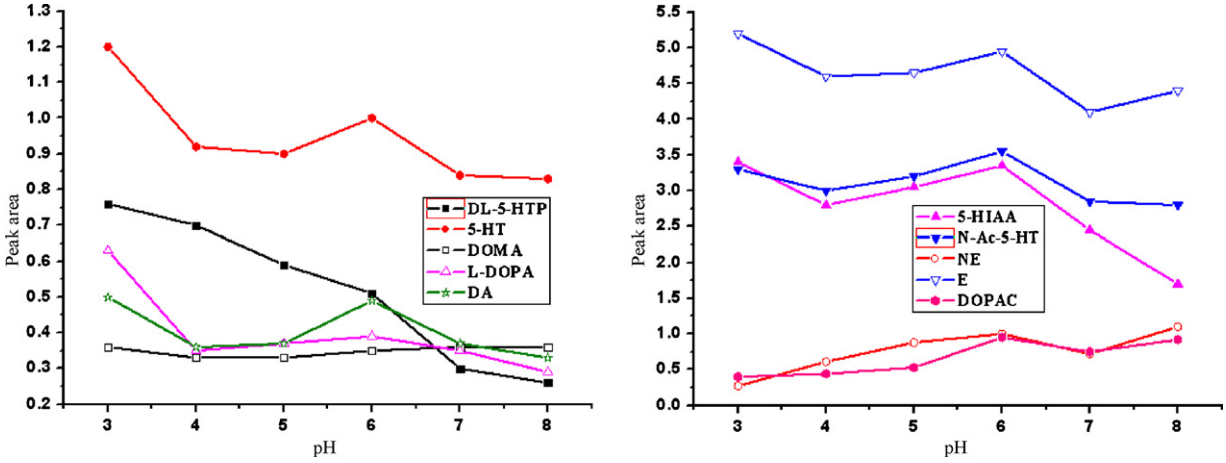

Fig. 5. Effects of solution pH on the peak areas of 5-HIAs and catechols.

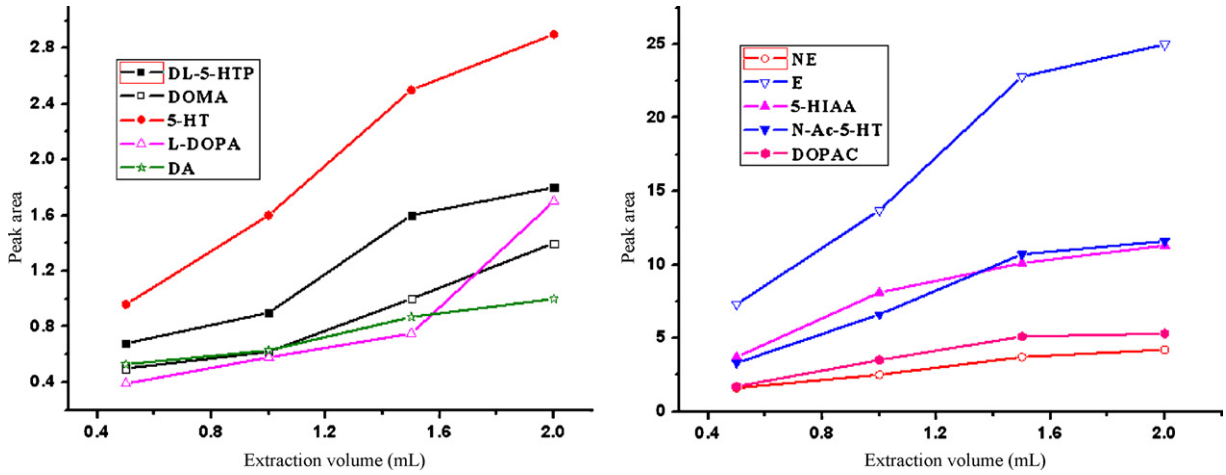

Fig. 6. Effects of extraction volume on the peak areas of 5-HIAs and catechols.

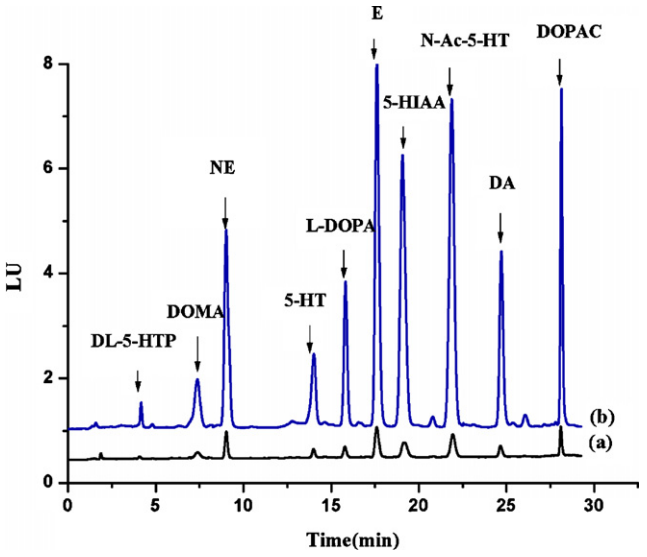

Fig. 7. Chromatograms of standard solution obtained by (a) direct HPLC and (b) PMME–HPLC analysis. NE, E and DOPAC were 50 nM each; DL-5-HTP, 5-HT, 5-HIAA and N-Ac-5-HT were 100 nM each; DOMA, L-DOPA and DA were 1 μM each.

Water-soluble organic solvents such as methanol or acetonitrile typically accelerate the FL reaction and increases overall yield [22,24]. It was found that the maximal FL signal occurred at 40–60% (v/v) MeOH in aqueous buffer solution [24]. Considering the adsorption of the target analytes is dependent mainly on hydrophobic interaction, we chose minimum organic solvent content (40% MeOH, v/v) for the derivatization process in our current study.

The effects of reaction time and temperature in the second derivatization step were investigated. The reaction products were monitored by HPLC-FL (Fig. 3A–C). The reaction between catechols and DPE was found to be the slowest among our investigated reactions. The maximum peak areas of most derivatives was achieved by 10 min at 80 °C or 20 min at 25 or 50 °C. For operational convenience, we chose 25 °C and 20 min as the optimal reaction condition in our study.

Although DPE–glycine solution was demonstrated to be effective for the derivatization of catechols [24], its concentration was unexpectedly found to affect the derivatization reactions of both catechols and 5-HIAs in our experiments. The influence of DPE concentration was studied using 100 mM glycine concentration (Fig. 4A). 50 mM of DPE was used as the recommended concentration as the maximum peak areas were obtained for low concentrations of DA, E and NE. Glycine was used as an accelerator for the derivatization reaction between catechols and DPE [48]. 50 mM glycine, which provided almost maximum peak areas for all substances, was selected for our procedures (Fig. 4B).

3.2. Optimization of PMME conditions

In order to obtain maximum PMME efficiency, several parameters affecting the extraction efficiency, the pH and extraction equilibrium profiles were optimized. The extraction conditions were examined using a standard solution containing NE, E and DOPAC (50 nM each); DL-5-HTP, 5-HT, 5-HIAA and N-Ac-5-HT (100 nM each); DOMA, L-DOPA and DA (1 μM each).

The effect of sample pH on the extraction efficiency was conducted in 20 mM phosphate matrix solution over the pH 3–8 range, in which most 5-HIAs and catechols derivatives were protonated. As shown in Fig. 5, the responding signals of most derivatives (except

Table 1
Regression equations of 5-HIAs and catechols in standard solutions.

Compound	Linear equation	*R*	Linear range (nM)	LODs (nM)	LOQs (nM)
DL-5-HTP	$Y = 7.30E{-}4X - 0.0306$	0.9951	10–3000	1.2	4.2
DOMA	$Y = 5.92E{-}5X - 0.00286$	0.9943	100–30,000	8.3	28
NE	$Y = 1.02E{-}2X - 0.0655$	0.9973	5–1500	0.15	0.50
5-HT	$Y = 3.22E{-}3X - 0.128$	0.9980	10–3000	1.5	5.0
L-DOPA	$Y = 5.10E{-}5X - 0.0376$	0.9955	100–30,000	21	71
E	$Y = 3.14E{-}2X - 1.18$	0.9976	5–1500	0.11	0.36
5-HIAA	$Y = 7.22E{-}3X + 0.316$	0.9971	10–3000	0.75	2.5
DA	$Y = 2.06E{-}4X + 0.318$	0.9962	100–30,000	5.2	17
DOPAC	$Y = 5.46E{-}3X + 0.0651$	0.9946	5–1500	0.14	0.46

Under the optimized derivatization and PMME conditions, details were outlined in Sections 3.1 and 3.2. Extraction column: a 3-cm poly(MAA-co-EDGMA) monolith in fused silica capillary. Extraction volume was 0.5 mL and the derivative solutions were adjusted to pH 6.0 in the microextraction process.

Table 2
Precisions of 5-HIAs and catechols in standard solutions.

Compound	Precisions (RSDs, %)					
	Intra-day ($n=4$)			Inter-day ($n=6$)		
	Low	Medium	High	Low	Medium	High
DL-5-HTP	9.2	6.9	6.0	12	10	8.1
DOMA	7.0	6.0	6.9	14	12	8.3
NE	5.0	6.8	8.7	10	8.3	7.0
5-HT	5.9	5.0	3.3	6.7	6.0	6.0
L-DOPA	6.0	9.0	4.0	15	10	6.6
E	3.8	5.6	6.6	9.0	11	6.9
5-HIAA	3.0	2.8	3.4	5.4	3.3	5.0
DA	9.3	8.9	5.8	6.4	5.1	6.6
DOPAC	7.8	4.0	6.0	10	8.0	7.0

The precisions were established by repeating analysis of different concentrations under the optimized derivatization and PMME conditions. Low: DL-5-HTP, 5-HT, 5-HIAA: 100 nM; NE, E, DOPAC: 50 nM; DOMA, DA, L-DOPA: 1 μM. Medium = low × 10; high = low × 20.

Table 3
Comparison of the LOD between different methods for 5-HIAs and catechols.

Determination method	Analytes	LOD (nM)	Reference
PMME–HPLC-FL HPLC-FL	Four 5-hydroxyindoles and six catechols	0.11–21	This work
HPLC-FL	Five 5-hydroxyindoles and seven catechols	2.5–43	[24]
HPLC-FL	Two 5-hydroxyindoles and four catechols	4–28	[25]
HPLC-FL	5-HT, 5-HIAA	5–7	[22]
Photocatalytic oxidation LC-FL	Three 5-hydroxyindoles	2–8	[51]

NE and DOPAC) reached maximums at pH 3, which may be ascribed to the strong hydrophobic interactions between the sample and the monolithic column. With increasing pH, the ionization degree of the polymer monolith increased accordingly, leading to decreased hydrophobic interactions for the sample. While the extraction efficiency showed small decreases for most analytes, the decrease was negligible (Fig. 5). At pH 6.0, the extraction efficiency for NE and DA was relatively higher than at other pHs (Fig. 5). While these two samples are present in human urine at extremely low concentrations, the derivative solutions were adjusted to pH 6.0 in the microextraction process.

To investigate the extraction capacity of the monolithic capillary, a sample solution of 0.5–2.0 mL was passed through the column at an extraction flow-rate of 0.15 mL/min. As shown in Fig. 6, the extraction amounts of the 10 derivatives (presented as peak areas) increased with increasing sample loading volume. The extraction equilibrium was not obtained even after 2.0 mL of the entire sample solution was fed, indicating that the monolithic capillary has remarkable extraction capacity for analytes. While further increasing the sample loading volume would prove beneficial to the following analysis, the time cost and high sensitivity of modern instrumentation, meant 0.5 mL of sample loading volume was suitable for our studies and this was selected for subsequent analysis.

Table 5
Regression equations of 5-HIAs and catechols in human urine.

Compound	Linear equation	R	Linear range (nM)
NE	$Y=3.60E-3X+0.0731$	0.9954	1–100
5-HT	$Y=1.46E-3X+0.0888$	0.9946	5–500
E	$Y=7.03E-3X+0.287$	0.9933	1–100
DA	$Y=5.48E-4X+0.190$	0.9937	20–2000
DOPAC	$Y=2.98E-3X+0.141$	0.9945	1–100

In this section, the PMME–HPLC-FL method was used. The single-morning urine samples were diluted with 20 mM pH 6.0 phosphate buffer solution by five times with the sample preparation (details can be seen in Section 2.2). The linearity of the proposed method was investigated using urine samples spiked with the standard solution.

Fig. 7 represents a typical chromatogram of samples after direct injection and PMME extraction using a standard solution containing four 5-HIAs and six catechols. The mobile phase was acetonitrile–15 mM acetate buffer (pH 4.5) containing 1 mM octanesulfonic acid sodium salt. The HPLC conditions are described in Section 2.4. After extraction, all of the samples were concentrated, for standard solutions, a 10-fold preconcentration could be obtained by this method with an extraction efficiency of over 95%, thus providing a promising method for trace analysis of these samples.

3.3. Validation of the method

All the tested analytes exhibited good linearity with good squared regression coefficients (Table 1). Squared regression coefficients were all >0.9943. The limits of detection (LODs) and quantification (LOQs) were calculated at a signal-to-noise ratio of 3 and 10, respectively. The LODs and LOQs are listed in Table 1. The precision was established by repeated analysis at different concentrations (Table 2). The RSDs did not exceed 15% for all catechols and 5-HIAs.

A comparative study of our developed method with other published work is presented in Table 3. It can be seen that the developed method is more sensitive, making this method attractive for the trace determination of 5-HIAs and catechols from different biological samples.

3.4. Analysis of monoamines in urine sample matrix

It has been reported that 5-HIAA, NE, E, DOPAC, 5-HT and DA are the main metabolic products in urine [49]. Therefore the analysis of monoamines in urine samples is of importance.

5-HIAA is the most abundant end product of both central and peripheral 5-HT metabolism and is found at high concentrations in urine sample, therefore direct HPLC-FL detection is applicable for this analyte. In the present study, the urine samples were diluted 50-fold with water prior to analysis. The detailed sample preparation method is described in Section 2.2.

The calibration curve of 5-HIAA in urine samples was constructed by the standard addition method, and plotting the ratios of peak areas against the I.S. peak areas. The regression equation and linear correlation coefficient of the calibration graph was

Table 4
Precisions and recoveries of 5-HIAA in human urine.

Compound	Added concentration (μM)	Precisions (RSDs, %)		Recoveries (%)	RSDs (%) ($n=5$)
		Intra-day ($n=5$)	Inter-day ($n=6$)		
5-HIAA	1	2.6	4.2	84	5.0
	5	2.1	3.1	95	3.9
	25	1.6	3.5	90	4.6

The analysis of 5-HIAA is direct HPLC-FL detection without PMME. The urine samples were diluted with water by 50 times, and the sample preparation can be seen in Section 2.2. The calibration curve of 5-HIAA in urine sample was constructed by the standard addition method. The RSDs and recoveries of 5-HIAA derivatives were studied by spiking with low, medium and high 5-HIAA concentrations into urine samples.

Table 6
Precisions and recoveries of 5-HIAs and catechols in human urine.

Compound	Added concentration (nM)	Precisions (RSDs, %)		Recoveries (%)	RSD (%) (n = 4)
		Intra-day (n = 4)	Inter-day (n = 6)		
5-HT	25	8.4	9.8	90.2	7.8
	250	8.0	10.5	94.9	6.7
	400	6.2	8.6	98.3	7.2
NE	5	8.6	11.4	86.8	9.7
	25	8.5	10.3	88.3	6.8
	75	7.9	9.0	82.6	5.9
E	5	6.0	8.2	89.9	5.7
	25	7.3	9.4	97.6	6.4
	75	7.5	8.9	97.3	4.6
DA	100	8.4	9.7	85.2	8.6
	500	6.5	8.3	95.4	4.5
	1500	5.3	7.9	96.8	5.6
DOPAC	5	7.2	9.4	97.2	5.1
	25	6.4	8.7	105	5.3
	75	5.1	8.1	99.3	4.9

The RSDs and recoveries of 5-HT, NE, E, DA and DOPAC were studied by spiking with low, medium and high concentrations standard solutions into urine samples. The derivatization and separation conditions can be seen in Sections 2.3 and 2.4.

Table 7
Urinary excretion (single-morning urination) of monoamine transmitters from volunteers.

Sample no.	Age	Sex	5-HT (nM)	5-HIAA (μM)	NE (nM)	E (nM)	DA (nM)	DOPAC (nM)
1	22	Male	23.9 ± 2.1	24.8 ± 1.8	17.4 ± 1.8	27.8 ± 3.0	212 ± 18	320 ± 23
2	23	Female	16.5 ± 1.2	24.1 ± 2.2	22.1 ± 1.5	36.4 ± 3.3	156 ± 14	448 ± 35
3	24	Female	26.2 ± 2.4	25.2 ± 2.0	28.5 ± 2.4	33.2 ± 2.9	144 ± 15	356 ± 21
4	24	Female	31.9 ± 3.2	32.4 ± 2.9	16.3 ± 1.3	33.6 ± 4.0	229 ± 19	468 ± 39
5	54	Male	83.6 ± 6.5	78.3 ± 7.3	28.7 ± 2.5	32.8 ± 3.0	204 ± 19	496 ± 45
6	45	Male	21.5 ± 2.3	52 ± 4.9	42.1 ± 3.8	38.4 ± 3.5	365 ± 32	309 ± 26
7	55	Male	41.4 ± 4.0	19.9 ± 2.1	26.5 ± 2.4	35.2 ± 4.0	336 ± 25	290 ± 23
8	41	Male	37.8 ± 3.6	12.3 ± 1.3	32.3 ± 2.7	37.6 ± 3.3	429 ± 39	162 ± 13

The proposed HPLC-FL/PMME–HPLC-FL method was applied to analysis monoamines in urine samples of different people (n = 3). Samples from nos. 1 to 4 were obtained from healthy volunteers; samples from nos. 5 to 8 were obtained from patients of Zhongnan Hospital in Wuhan, China.

$Y = 0.3218X + 1.4538$ and 0.9955, respectively. The RSDs did not exceed 3.0% for intra-day determinations (n = 5) and 4.2% for inter-day determinations (n = 6). The recoveries of derivatives from the healthy urine samples spiked with low, medium and high 5-HIAA concentrations were studied by comparing the actual amounts of 5-HIAA added to those obtained by calculation from the standards, respectively. The recoveries were in the range 84–95% (Table 4).

In order to detect other 5-HIAs and catechols (such as 5-HT, E, NE, DA, and DOPAC) at low concentrations, the PMME–HPLC-FL method was adopted. The single-morning urine samples were initially diluted 5-fold with 20 mM pH 6.0 phosphate buffer solution. Additional necessary preparation steps are the same as before as described in Section 2.2. The linearity of the proposed method was investigated using urine samples spiked with standard solutions. Good linearity can be observed with correlation coefficients above 0.9933 for all substances tested. Details of the linear equation, linear range, and correlation coefficient (R) are listed in Table 5. The recoveries of 5-HIAs and catechols spiked with low, medium and high concentrations in the urine sample were also calculated. The recovery of the sample ranged from 82 to 105% (Table 6). The intra-day and inter-day reproducibility of the developed method were illustrated as relative standard deviations (RSDs) of four and six measurements. The intra-day and inter-day RSDs were less than 8.6 and 11.4%, respectively. Full RSD details are shown in Table 6.

Fig. 8(a) and (b) illustrates chromatograms for a urine sample taken from a healthy volunteer by direct HPLC analysis and PMME-HPLC analysis, respectively. Fig. 8(c) demonstrates the chromatograms for the urine sample spiked with standard analytes: NE, E and DOPAC (50 nM each); 5-HT 100 nM and DA (1 μM). It can be found that the recoveries (n = 3) were 87.2%, 96.3%, 99.7%, 92.8% and 96.7%, respectively. After extraction, all of the samples can be concentrated 7–9-fold with extraction efficiencies above 80%. The sample matrix was shown to have little influence on the extraction and detection of these analytes.

The proposed HPLC-FL/PMME–HPLC-FL method was applied to analysis of eight urine samples. Samples 1–4 were obtained from

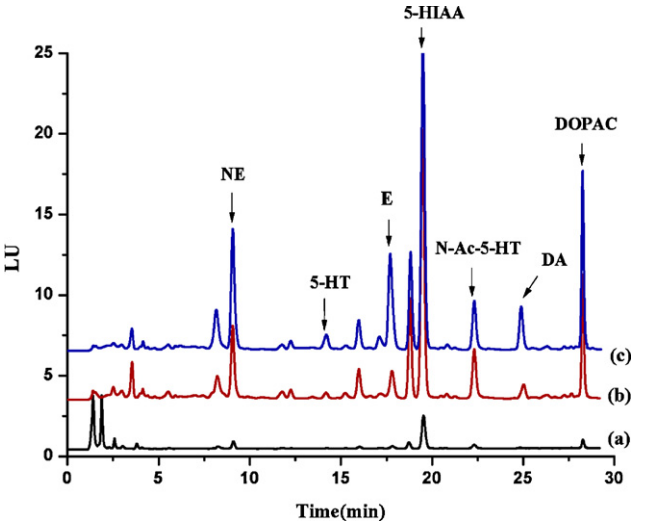

Fig. 8. Chromatograms of urine samples taken from a healthy volunteer were analyzed by direct HPLC analysis (a) and PMME–HPLC analysis (b), chromatogram (c) of a urine sample spiked with standard analytes: NE, E and DOPAC (50 nM each) and 5-HT 100 nM and DA (1 μM each).

healthy volunteers and samples 5–8 were obtained from patients from Zhongnan Hospital in Wuhan, China. Using our proposed method, the concentrations of monoamines were measured in urine samples of different people ($n = 3$) (Table 7). Our 5-HIAA values are similar to those obtained previously by other researchers (2–50 μM) [50,51] and our mean values for 5-HT, NE and E from urine samples from healthy volunteers are in good agreement with those obtained by other studies [52,53].

4. Conclusion

In this study, we investigated how the HPLC coupled to PMME with pre-column derivatization can be used to determine 5-hydroxyindoleamines and catechols in urine samples and show it to be a simple, sensitive, and reliable technique. This is the first report where PMME coupled to HPLC-FL detection has been used for the analysis of monoamine compounds from biological samples. PMME is not only able to enrich the analytes, but is also able to purify the sample and reduce the effect of the sample matrix. Its high sensitivity makes this method attractive for the trace determination of 5-hydroxyindoleamines and catechols from different biological samples, such as vitreous bodies, cerebrospinal fluid and plasma.

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References

[1] M. Some, A. Helander, Life Sci. 71 (2002) 2341.
[2] T.A. Ban, J. Neural, Transmission 108 (2001) 707.
[3] G. Laux, H.P. Volz, H.J. Moller, CNS Drugs 3 (1995) 145.
[4] M.E. Thase, M.H. Trivedi, A.J. Rush, Neuropsychopharmacology 12 (1995) 185.
[5] M. Catalano, Am. J. Hum. Genet. 65 (1999) 606.
[6] M.B. Parent, M.K. Habib, G.B. Baker, Biochem. Pharmacol. 59 (2000) 1253.
[7] S.L. Ho, A.L. Kapadi, D.B. Ramsden, A.C. Williams, Ann. Neurol. 37 (1995) 403.
[8] J.H. Kurth, M.C. Kurth, S.E. Poduslo, J.D. Schwankhaus, Ann. Neurol. 33 (1993) 368.
[9] M. Ebadi, S. Sharma, S. Shavali, H.E.L. Refaey, J. Neurosci. Res. 67 (2002) 285.
[10] Y.L. Wang, J.W. Wei, A.Y. Sun, Neurochem. Res. 18 (1993) 1293.
[11] P. Chaiyakul, D. Reidman, L. Pilipovic, T. Maher, A. Ally, Brain Res. 900 (2001) 186.
[12] J. Lesage, F. Bernet, V. Montel, J.P. Dupouy, Neurochem. Res. 21 (1996) 87.
[13] N.C. Parker, C.B. Levtzow, P.W. Wright, L.L. Woodard, J.F. Chapman, Clin. Chem. 32 (1986) 1473.
[14] F. Mashige, Y. Matsushima, C. Miyata, R. Yamada, H. Kanazawa, I. Sakuma, N. Takai, N. Shinozuka, A. Ohkubo, K. Nakahara, Biomed. Chromatogr. 9 (1995) 221.
[15] T.H. Tsai, C.F. Chen, J. Chromatogr. A 730 (1996) 121.
[16] M.H. Mills, D.C. Finlay, P.R. Haddad, J. Chromatogr. 564 (1991) 93.
[17] A.T. Wood, M.R. Hall, J. Chromatogr. B 744 (2000) 221.
[18] J.W. Commissiong, J. Neurochem. 41 (1983) 1313.
[19] L.U. Sneddon, A.C. Taylor, F.A. Huntingford, D.G. Watson, J. Exp. Biol. 203 (2000) 537.
[20] J. Ishida, M. Yamaguchi, M. Nakamura, Analyst 116 (1991) 301.
[21] J. Ishida, T. Yoshitake, K. Fujino, K. Kawano, J. Kehr, M. Yamaguchi, Anal. Chim. Acta 365 (1998) 227.
[22] T. Yoshitake, R. Iizuka, J. Kehr, H. Nohta, J. Ishida, M. Yamaguchi, J. Neurosci. Methods 109 (2001) 91.
[23] T. Yoshitake, K. Fujino, J. Kehr, J. Ishida, R. Iizuka, H. Nohta, M. Yamaguchi, Anal. Biochem. 312 (2003) 125.
[24] K. Fujino, T. Yoshitake, J. Kehr, H. Nohta, M. Yamaguchi, J. Chromatogr. A 1012 (2003) 169.
[25] T. Yoshitake, J. Kehr, S. Yoshitake, K. Fujino, H. Nohta, M. Yamaguchi, J. Chromatogr. B 807 (2004) 177.
[26] A. Gironi, G. Seghieri, M. Niccolai, P. Mammini, Clin. Chem. 34 (1988) 2504.
[27] J. Jouve, J. Martineau, N. Mariotte, C. Barthelemy, J.P. Muh, G. Lelord, J. Chromatogr. 378 (1986) 437.
[28] E. Pussard, N. Guigueno, O. Adam, J.F. Gindicelli, Clin. Chem. 42 (1996) 1086.
[29] R.J. Flanagan, P.E. Morgan, E.P. Spencer, R. Whelpton, Biomed. Chromatogr. 20 (2006) 530.
[30] L.G. Blomberg, Anal. Bioanal. Chem. 393 (2009) 797.
[31] S.S. Liang, S.H. Chen, J. Chromatogr. A 1216 (2009) 2282.
[32] L. Hou, H.K. Lee, Anal. Chem. 75 (2003) 2784.
[33] C. Basheer, V. Suresh, R. Renu, H.K. Lee, J. Chromatogr. A 1033 (2004) 213.
[34] X. Wen, C. Tu, H.K. Lee, Anal. Chem. 76 (2004) 228.
[35] X. Jiang, H.K. Lee, Anal. Chem. 76 (2004) 5591.
[36] M. Zhang, W. Fang, Y.F. Zhang, J. Nie, Y.Q. Feng, J. Chromatogr. A 1102 (2006) 294.
[37] E.F. Hilder, F. Svec, J.M.J. Frechet, J. Chromatogr. A 1044 (2004) 3.
[38] T. Ikegami, N. Tanaka, Curr. Opin. Chem. Biol. 8 (2004) 527.
[39] Y. Shintani, X. Zhou, M. Furuno, H. Minakuchi, K. Nakanishi, J. Chromatogr. A 985 (2003) 351.
[40] Y. Fan, Y.Q. Feng, S.L. Da, Z.G. Shi, Anal. Chim. Acta 523 (2004) 251.
[41] Y. Saito, M. Kawazoe, M. Hayashida, K. Jinno, Analyst 125 (2000) 807.
[42] H.J. Zhang, J.F. Huang, H. Wang, Y.Q. Feng, Anal. Chim. Acta 565 (2006) 129.
[43] H.J. Zhang, J.S. Li, H. Wang, Y.Q. Feng, Anal. Bioanal. Chem. 386 (2006) 2035.
[44] J.F. Huang, H.J. Zhang, B. Lin, Q.W. Yu, Y.Q. Feng, Rapid Commun. Mass Spectrom. 21 (2007) 2895.
[45] J. Nie, Q. Zhao, J.F. Huang, B.R. Xiang, Y.Q. Feng, J. Sep. Sci. 29 (2006) 650.
[46] Y. Wen, M. Zhang, Q. Zhao, Y.Q. Feng, J. Agric. Food Chem. 53 (2005) 8468.
[47] K. Fujino, T. Yoshitake, O. Inoue, N. Ibii, J. Kehr, J. Ishida, H. Nohta, M. Yamaguchi, Neurosci. Lett. 320 (2002) 91.
[48] H. Nohta, M.K. Lee, Y. Ohkura, Anal. Chim. Acta 267 (1992) 137.
[49] T. Yoshitake, J. Kehr, K. Todoroki, H. Nohta, M. Yamaguchi, Biomed. Chromatogr. 20 (2006) 267.
[50] I.P. Kema, E.G. De Vries, F.A. Muskiet, J. Chromatogr. B 747 (2000) 33.
[51] K. Todoroki, Y. Nakashima, H. Yoshida, H. Nohta, M. Yamaguchi, Anal. Chim. Acta 555 (2006) 14.
[52] Y. Umegae, H. Nohta, M.K. Lee, Y. Ohkura, Chem. Pharm. Bull. 38 (1990) 2293.
[53] M. Yamaguchi, J. Ishida, M. Yoshimura, Analyst 123 (1998) 307.