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Selective liquid-chromatographic determination of native fluorescent biogenic amines in human urine based on fluorous derivatization

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ABSTRACT

A liquid chromatographic (LC) derivatization method for simple and selective determination of catecholamines and indoleamines in human urine has been developed. This method uses "fluorous interaction" in which perfluoroalkyl compounds show affinity with each other. The amino groups of native fluorescent analytes are precolumn derivatized with a non-fluorescent fluorous isocyanate, 2-(perfluorooctyl)ethyl isocyanate, and the fluorous-labeled analytes are retained in the fluorous LC column, whereas underivatized substances are not. Only the retained fluorous-fluorescent analytes are detected fluorometrically at appropriate retention times, and retained amines without fluorophores are not detected. In this study, 3,4-dihydroxyphenylalanine, dopamine, norepinephrine, epinephrine, and metanephrine were used as the representative of catecholamines. Tryptophan, 5-hydroxytryptophan, and 5-hydroxytryptamine were used as the representative indoleamines. This method was applied to determine eight biogenic amines in urine from healthy humans. The fluorous-labeled amines could be separated by fluorous LC column under conditions of isocratic elution within 35 min and simultaneously determined without interference from contaminants in biological samples. The detection limits for eight biogenic amines were 31–640 fmol on column. Calibration curves of them were linear over the range of at least 10–100 nmol/mL urine ($r^2 > 0.9989$) with good repeatability.

1. Introduction

Biogenic amines are mainly nitrogenous, low molecular weight compounds with biological activity that are formed or metabolized in the cells of living organisms. Biogenic amines are derived mainly from amino acids through substrate-specific decarboxylase enzymes [1]. For example, catecholamines such as dopamine (DA), norepinephrine (NE), and epinephrine (E), are produced from tyrosine; indoleamines such as 5-hydroxytryptamine (5-HT) are produced from tryptophan. These amines play physiologically important roles, are related to psychic and nervous function in the body, and are related to various diseases [1–4]. Thus, a simple method for the simultaneous determination of biogenic amines is needed.

Several methods have been reported for the measurement of these biogenic amines. The most widely used methods are based on liquid chromatography (LC) using native fluorescent detection [5,6], ultraviolet detection [7], electrochemical detection (ECD) [8,9], and mass spectrometry (MS) [10,11]. Native fluorescent detection provides reproducible results and requires simpler analytical systems. However, there are many fluorescent compounds, which create peaks that interfere with the chromatogram in biological samples. Therefore, highly sophisticated LC separation conditions and/or troublesome sample clean-up procedures are required to eliminate the interferences from other, co-existing compounds. Only a few fluorogenic derivatization methods have been reported for amino moieties [12–14]. These methods, however, are also not selective for indoleamines and catecholamines. On the other hand, highly selective LC methods based on specific fluorogenic derivatization methods [15–17], and derivatization in which fluorescent resonance energy transfer occurs between analytes and a labeled fluorescent moiety [18–20], have been developed. These approaches are sensitive to, and specific to, their respective target amines.

Recently, we developed a simple LC determination method for native fluorescent carboxylic acids based on "fluorous derivatization" [21], in which "fluorous" means that which is related to the interaction between highly fluorinated compounds and highly fluorinated compounds that show the special property of being fluorophilic [22–25]. The principle behind the reported fluorous derivatization method is the following: native fluorescent

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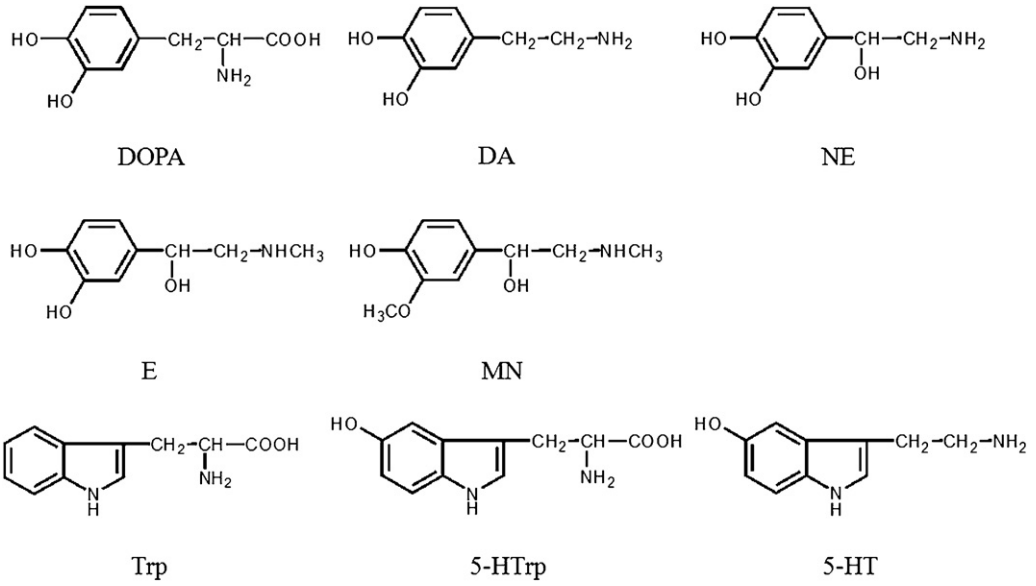

Fig. 1. The structure of biogenic amines examined.

carboxylic acids are precolumn derivatized with a non-fluorescent fluorous amine, thereafter the fluorous-labeled carboxylic acids are retained in the fluorous-phase column. Neither non-fluorescent carboxylic acids nor underivatized native fluorescent compounds interfere with the detection. Until now, fluorous derivatization followed by the fluorescence detection method was successfully applied to the determination of pharmaceutical agents, which create carboxylic groups in human plasma after administration [26]. Furthermore, this method was carried out for the specific measurement of not only native fluorescent carboxylic acids but also sialic acids using MS detection [27].

In the present paper, we describe a fluorous derivatization for native fluorescent biogenic amines [catecholamines; 3,4-dihydroxy-L-phenylalanine (DOPA), DA, NE, E, and metanephrine (MN): indoleamines; tryptophan (Trp), 5-hydroxytryptophan (5-HTrp), and 5-HT] (Fig. 1). In this method, 2-(perfluorooctyl)ethyl isocyanate (PFOEI) was used as a fluorous derivatization reagent for amino groups with a fluorous structure. The amino groups of the analytes were converted to the corresponding fluorous-labeled derivatives by PFOEI (Fig. 2). It has been said that there are many contaminants in urine; thus, it is too difficult to separate biogenic amines from these contaminants in the LC column. However the present method allows simultaneous determination of eight biogenic amines in human urine, and is highly sensitive and selective in its measurement of them. This process occurs with simple pretreatment and without interference from contaminants in the urine sample.

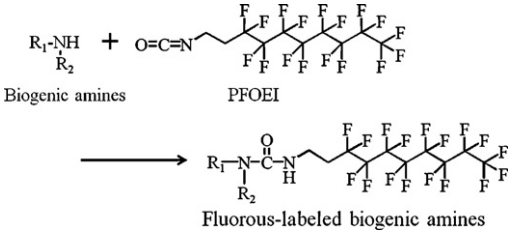

Fig. 2. Fluorous derivatization reaction for biogenic amines with PFOEI.

2. Experimental

2.1. Reagents and solutions

Unless stated otherwise, all chemicals mentioned below were of the highest purity available and were used as received. DOPA, DA hydrochloride, NE bitartrate, E, and 5-HT hydrochloride were purchased from Wako Pure Chemicals (Osaka, Japan), MN hydrochloride was from Sigma (St. Louis, MO, USA), Trp was from Kishida (Osaka, Japan), 5-HTrp hydrate was from Nacalai Tesque (Kyoto, Japan), and PFOEI was from Fluka (Buchs, Switzerland). All organic solvents were of LC grade. Ultrapure water, purified using a Milli-Q gradient system (Millipore, Billerica, MA, USA), was used to produce all aqueous solutions.

Stock solutions (1.0 μmol/mL) of the analytes were prepared in water and stored at room temperature. They were stable for at least 1 week and were diluted further with tetrahydrofuran (THF) and water to the required concentrations before use. A solution of 100 mM PFOEI in THF was usable for at least 1 week when stored at room temperature in a light resistant test tube.

2.2. Derivatization procedure

To the sample solution (40 μL) placed in a 1.5-mL vial, 40 μL of 100 mM PFOEI was added. The vial was tightly sealed and heated at 60 °C for 10 min. After derivatization, 80 μL of 1 M aqueous ammonia was added to the vial, and the entire reaction solution was placed in the autosampler of an LC system. A reagent blank was prepared by the same procedure, using a 40-μL aliquot of 90% (v/v) THF instead of the sample solution.

2.3. Urine sample and pretreatment

Urine samples were obtained from healthy male volunteers (23–25 years old, $n = 7$) in our laboratory. They understood the purpose and significance of the experiment and donated urine after signing an agreement. The obtained urine samples were immediately pretreated.

To 10 μL of urine sample placed in a 1.5-mL polypropylene tube, 90 μL of THF was added. After vortex-mixing for several seconds, the mixture was immediately centrifuged at 9000 × *g* for 5 min

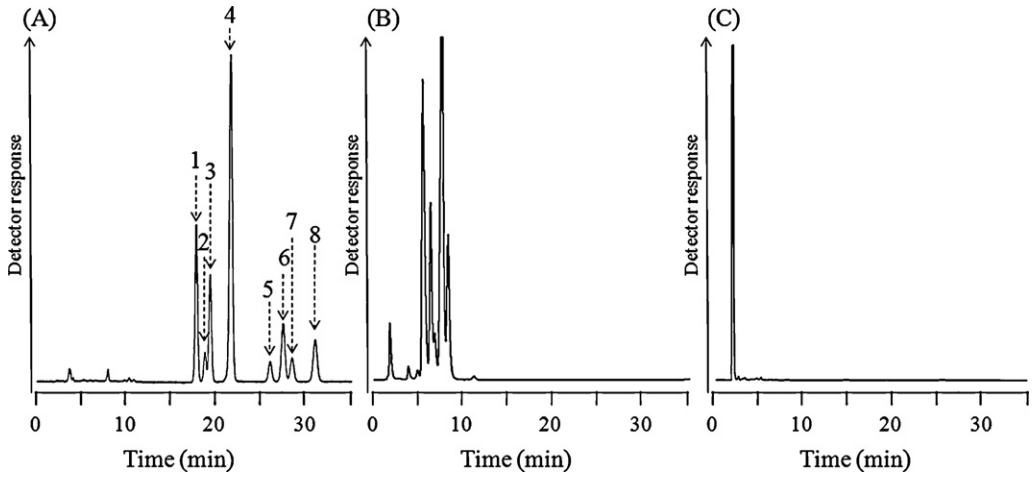

Fig. 3. Chromatograms obtained with eight standard biogenic amines (A) with fluorous derivatization, (B) with dodecyl isocyanate derivatization, and (C) without derivatization using fluorous LC separation. LC conditions: Column, Fluofix-II 120E column (250 mm × 4.6 mm i.d.); mobile phase, acetonitrile–water–trifluoroacetic acid (60:40:0.05, v/v); flow rate, 1.0 mL/min; column temperature, 30 °C. Peaks and amounts (pmol on column): 1, DOPA (50); 2, NE (5); 3, 5-HTrp (5); 4, E (5); 5, DA (5); 6, 5-HT (5); 7, MN (5); 8, Trp (5).

at 4 °C, and the supernatant was passed through a disposable filter (0.20 μm, i.d. 13 mm, polytetrafluoroethylene; Advantec Toyo, Tokyo, Japan). Thereafter, a portion (40 μL) of the filtrate was subjected to derivatization.

2.4. LC system and conditions

We used an isocratic LC system consisting of an LC-10AD liquid chromatography pump, an SIL-10A autoinjector, a DGU-12A online degasser, a CTO-10A column oven, a separation column, an RF-10AXL spectrofluorometer equipped with a 12-μL flow cell, and a CBM-20A controller. With the exception of the separation column, all components of the LC system were manufactured by Shimadzu (Kyoto, Japan). Injection of each 20-μL sample into the system was carried out automatically. The flow rate of the mobile phase was set at 1.0 mL/min, and the column temperature was 30 °C. Both of the monochromators in the fluorescence detector had a slit width of 15 nm.

A Wakopak Fluofix-II 120E column (250 mm × 4.6 mm i.d., 5 μm; Wako Pure Chemicals) was used as an analytical column, and a mixture of acetonitrile, water, and trifluoroacetic acid (60:40:0.05, v/v) was used as a mobile phase. The fluorescence detector was operated at excitation and emission wavelengths of 280 nm and 320 nm, respectively.

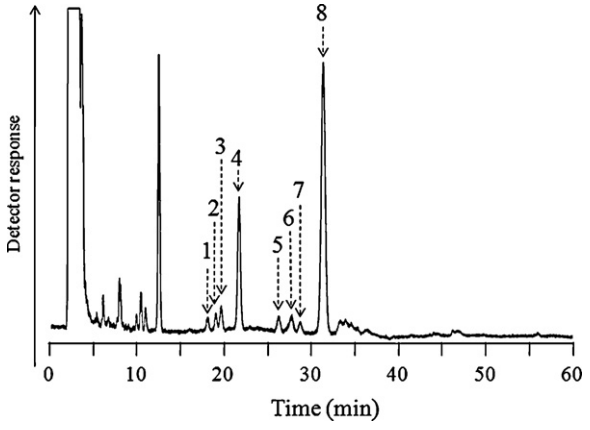

Fig. 4. Chromatogram obtained from urine sample with PFOEI derivatization using fluorous LC separation. LC conditions: see Fig. 3. Peaks and concentrations (nmol/mL urine): 1, DOPA (6.4); 2, NE (4.3); 3, 5-HTrp (1.6); 4, E (1.7); 5, DA (5.2); 6, 5-HT (2.6); 7, MN (3.4); 8, Trp (26).

2.5. Examination for comparative study

2.5.1. Comparison between fluorous reagent and non-fluorous reagent

The derivatization procedure was the same, except for the kind of derivatization reagent used. Dodecyl isocyanate, the straight chain alkyl isocyanate, which has a carbon number two higher than PFOEI, was used.

2.5.2. Native fluorescent detection for biogenic amines

A reversed-phase LC with linear gradient elution was used. The LC condition was the same as previous method [5], except for the separation column. An XBridge™ C18 column (150 mm × 4.6 mm i.d., 5 μm; Waters, Milford, MA, USA) was used as a separation column. Mixtures of methanol, water, and trifluoroacetic acid (2.5:97.5:0.05, v/v) and (60:40:0.05, v/v) were used as mobile phases A and B, respectively. A gradient elution was as follows: 0 min, 100% A; 1 min, 100% A; 16 min, 50% A and 50% B (linear gradient from 1 to 16 min); 16.05 min, 100% A to return the column to initial conditions by 20 min.

2.6. Method validation

In order to obtain the validation parameters, peak heights [estimated with the baseline-to-baseline method using a software tool by Shimadzu software (LC solution, ver. 1.23)] were used for the quantification.

2.6.1. Calibration curves

For quantitative analysis, the calibration curves were compared between standard and spiked urine samples. Standard solutions (n = 3 each) with concentration ranges from 1 to 100 nmol/mL (1, 5, 10, 50, and 100 nmol/mL) for DOPA; from 0.5 to 25 nmol/mL (0.5, 1, 5, 10, and 25 nmol/mL) for DA, NE, and MN; and from 0.1 to 10 nmol/mL (0.1, 0.5, 1, 5, and 10 nmol/mL) for E, Trp, 5-HTrp, and 5-HT were prepared by diluting the stock solutions. For the urine samples, calibration spiked solutions (n = 3 each) with a concentration range of 10–1000 nmol/mL urine (10, 50, 100, 500, and 1000 nmol/mL urine) for DOPA; of 5–250 nmol/mL urine (5, 10, 50, 100, and 250 nmol/mL urine) for DA, NE, and MN; and of

Table 1
Validation data for standard samples.

	Calibration curve ($n=3$)[a]						Repeatability (RSD) ($n=6$)[d]		Detection limit[e] [fmol (pmol/mL)]
	r^2	Slope	Intercept	Slope S.E.[b]	Intercept S.E.[b]	F-Value[c]	Intraday (%)	Interday (%)	
DOPA	0.9989	27,491	−8084	331	842	3.1×10^9	2.9	5.8	640(130)
DA	0.9989	57,247	−7893	540	1139	3.5×10^8	3.2	5.6	470(94)
NE	0.9994	88,282	−10,856	781	557	9.9×10^8	1.3	5.7	330(66)
E	0.9999	1,022,186	4110	8544	156	2.9×10^{10}	2.1	4.3	31(6.2)
MN	0.9999	71,669	−5899	1003	1716	2.4×10^8	2.7	5.8	370(74)
Trp	0.9992	113,289	−4848	1270	106	4.9×10^8	0.7	2.9	170(34)
5-HTrp	0.9999	250,081	7887	1715	544	1.7×10^9	2.3	6.6	92(18)
5-HT	0.9998	153,131	1658	1003	1716	3.4×10^8	3.1	5.2	130(24)

[a] Correlation curve in the concentration range of 1–100 nmol/mL (DOPA; 5–500 pmol per 20 μL injection volume), 0.5–25 nmol/mL (DA, NE, and MN; 2.5–125 pmol per 20 μL injection volume), and 0.1–10 nmol/mL (E, Trp, 5-HTrp, and 5-HT; 0.5–50 pmol per 20 μL injection volume).
[b] Standard error.
[c] F-Value = S_{high}/S_{low}.
[d] Relative standard deviation of peak height of 10 nmol/mL for DOPA and 1 nmol/mL for other amines.
[e] Defined as the amount per 20 μL injection volume and the concentration of analyte yielding a signal-to-noise ratio of 3.

1–100 nmol/mL urine (1, 5, 10, 50, and 100 nmol/mL urine) for E, Trp, 5-HTrp, and 5-HT were prepared by spiking the stock solutions diluted with human urine, then the 10-times diluted urine was used for derivatization. The equations of the calibration curves were determined using a least-squares linear prediction. The experimental F-value is expressed as the ratio between the variances obtained at the lowest (S_{low}) and at the highest (S_{high}) concentration level of the working range, and the table F-value is obtained from the F-table at the confidence level of 99% for $f_1 = f_2 = (n-1)$ degrees of freedom.

2.6.2. Repeatability

The precision (intraday and interday) of peak height in the present method was estimated during the analytical procedures (sample dilution, pretreatment, derivatization, and LC separation) using the standard solutions (DOPA, 10 nmol/mL; other amines, 1 nmol/mL) and spiked homogeneous urine (DOPA, 100 nmol/mL urine; other amines, 10 nmol/mL urine). The intraday and interday precision values were assessed by performing an analysis six times on the same day and by conducting the analysis on six different days in a month, respectively.

2.6.3. Recovery

To demonstrate applicability to real samples, the recovery using a urine sample spiked with standards was evaluated. For this purpose, standard solution (DOPA, 100 nmol/mL urine; other amines, 10 nmol/mL urine) was added to the urine sample, and the recovery was calculated on the basis of the results obtained by the proposed method. The recovery (R) was calculated as $R = 100 \times \{(C_{spike} - C_{sample})/C_{standard}\}$, where C_{spike} is the concentration in the spiked urine sample, C_{sample} is the concentration in the urine sample without spiking, and $C_{standard}$ is the added concentration ($n=3$ each).

2.6.4. Detection and quantitation limits

The detection limits were determined as the lowest amounts and concentrations in standard solution for which the signal-to-noise ratio was 3. The quantitation limits were determined as the lowest concentrations in urine for which the signal-to-noise ratio was 10.

3. Results and discussion

3.1. Fluorous LC separation

The Fluofix-II 120E column (250 mm × 4.6 mm i.d.), which is a fluorous column with C_6F_{13}-branched [4,4-bis(trifluoromethyl)-5,5,6,6,7,7,7-heptafluoroheptyl] chemically modified silica type stationary phase, effectively separated the fluorous-labeled eight biogenic amines within 35 min. For the separation, a mixture of acetonitrile, water, and trifluoroacetic acid (60:40:0.05, v/v) was used as the mobile phase with isocratic elution. Fig. 3A shows a typical chromatogram obtained with fluorous-labeled amines. In contrast, Fig. 3B and C shows the chromatograms obtained with dodecyl isocyanate (the straight chain alkyl isocyanate that has two larger carbon number than PFOEI) labeled biogenic amines and unlabeled amines, respectively. These chromatograms indicate that only fluorous-labeled biogenic amines were retained in the fluorous column but that non-fluorous compounds including unlabeled amines were not. Thus the fluorous column can selectively retain fluorous compounds, such as fluorous-labeled analytes, on the basis of fluorous separation as opposed to hydrophobicity, and PFOEI derivatives had a good fluorophilicity to be retained among other compounds, if optimal column temperature and mobile phase are chosen.

3.2. Derivatization conditions

Optimization studies for the derivatization were carried out to maximize the fluorescence peak height using eight biogenic amines (DOPA, 10 nmol/mL; other amines, 1 nmol/mL). The derivatization reaction proceeded in the presence of organic solvents: methanol, ethanol, acetonitrile, dimethylsulfoxide, THF, or *N*,*N*-dimethylformamide. The best results were obtained when THF was used for the preparation of the PFOEI solution. When the concentration of PFOEI in the reagent solution was more than 1 mM, the peak heights of all the biogenic amines were maxima. Therefore, a 100-mM PFOEI solution was chosen as the optimal concentration and was used in all further analyses. The derivatization reaction proceeded more rapidly with increasing reaction temperature in the range of 20–80 °C, but a temperature higher than 80 °C caused a decrease in the peak height of fluorous-labeled biogenic amines. The optimal conditions were selected at 60 °C and for a reaction time of 10 min.

After the derivatization procedure, 1 M aqueous ammonia was added to stop derivatization. The fluorescence intensities of fluorous-labeled biogenic amines were constant, even after the mixture was left to stand for at least 1 week in the dark at room temperature.

3.3. Analysis of standards

Validation data for the standard samples are presented in Table 1. The relationship between the amounts of biogenic amines

Table 2
Validation data for urine samples.

	Calibration curve[a]		Repeatability (RSD) ($n = 6$)[b]		Recovery (%)[c] (mean ± SD)	Quantitation limit (nmol/mL)[d]
	r^2	Slope	Intraday (%)	Interday (%)		
DOPA	0.9999	2665	1.6	3.7	96.9 ± 1.0	4.2
DA	0.9998	5416	2.3	6.1	99.7 ± 1.5	3.2
NE	0.9995	8632	2.7	4.3	103.3 ± 1.2	2.2
E	0.9998	98,269	5.1	5.4	99.3 ± 2.7	0.21
MN	0.9999	7007	2.2	4.3	97.3 ± 1.3	2.5
Trp	0.9999	10,279	2.7	5.8	98.7 ± 2.1	1.2
5-HTrp	0.9997	23,807	2.4	3.6	103.0 ± 1.9	0.61
5-HT	0.9994	14,292	1.9	4.0	100.3 ± 1.3	0.87

[a] Correlation curve in the concentration range of 10–1000 nmol/mL urine (DOPA), 5–250 nmol/mL urine (DA, NE, and MN), and 1–100 nmol/mL urine (E, Trp, 5-HTrp, and 5-HT).
[b] Relative standard deviation of peak height for spiked 100 nmol/mL urine (DOPA) and spiked 10 nmol/mL urine (other amines).
[c] The recovery (R) was calculated as $R = 100 \times \{(C_{spike} - C_{sample})/C_{standard}\}$, where C_{spike} is the concentration in the spiked urine sample, C_{sample} is the concentration in the urine sample without spiking, and $C_{standard}$ is added concentration ($n = 3$ each).
[d] Defined as the concentration in urine yielding a signal-to-noise ratio of 10.

examined and the peak heights were linear over the concentration range of 1–100 nmol/mL (DOPA; 5–500 pmol per 20 μL injection volume), 0.5–25 nmol/mL (DA, NE, and MN; 2.5–125 pmol per 20 μL injection volume), and 0.1–10 nmol/mL (E, Trp, 5-HTrp, and 5-HT; 0.5–50 pmol per 20 μL injection volume) in the standard solution. The determination coefficient values of eight biogenic amines were 0.9989–0.9999 ($n = 3$). The F-value revealed a significant difference between the variances by comparing to the F-table [F-table (2, 2, 0.99) = 19] (Table 1). The intraday and interday precisions of peak height were estimated during the entire process by repeated determination ($n = 6$ in each case) using mixtures of the standard compounds (DOPA, 10 nmol/mL; other amines, 1 nmol/mL); the relative standard deviations from intraday samples were within 3.2% and those from interday samples were within 6.6% (Table 1). The detection limits (signal-to-noise ratio = 3) that were dependent on the fluorescence intensities of the eight biogenic amines were less than 640 fmol per 20-μL injection volume, which corresponded to 130 pmol/mL (Table 1). The fluorescent intensities of fluorous-labeled amines were relatively the same as those of unlabeled amines (data not shown).

3.4. Examination using urine sample

To investigate the applicability of the fluorous derivatization method to biomedical analysis, we used this method for the determination of biogenic amines in human urine. Urine was diluted 10-fold with THF to deproteinize the sample and optimize the reaction solvent. Fig. 4 shows a typical chromatogram obtained from a derivatized urine sample. No peaks due to urine components were observed at the retention times near to and beyond the objective peaks, as the native fluorescent compounds without amino group in the urine were not derivatized, and thus, were not retained in the fluorous column. Furthermore, non-fluorescent amines were not detected, even though they were retained. The components of peaks 1–8 in Fig. 4 were identified as fluorous-labeled DOPA, NE, 5-HTrp, E, DA, 5-HT, MN, and Trp, respectively, both by comparing their retention times with those shown in Fig. 3 and by co-chromatography using various mobile phases. The peak components observed at retention times of approximately 10 min might be attributed to the fluorous-labeled endogenous native fluorescent amines without analytes in this study. When PFOEI solution was replaced by a solvent, it was found that all compounds were eluted in the solvent front (data not shown). Thus, there are few compounds retained in the fluorous column in human urine under the selected LC conditions.

A comparison between the fluorous separation and the reversed-phase separation [5] indicated that the proposed method is advantageous for biological analysis. Fig. 5A shows chromatograms obtained with the same urine sample as those used in Fig. 4 without fluorous derivatization and the special pretreatment method described in previous work [5]. It was difficult to determine the biogenic amines in urine samples as many disturbance peaks appeared at the retention times of the eight amines. These results support the claim that the combination of fluorous derivatization and LC separation with a fluorous stationary phase yields a simple and effective method for determining native fluorescent amines in human urine.

Validation data for the spiked-urine samples are presented in Table 2. The calibration graphs of the eight biogenic amines in urine were linear ($r^2 = 0.9994$–0.9999) in the concentration range corresponding to 10–1000 nmol/mL urine (DOPA), 5–250 nmol/mL urine (DA, NE, and MN), and 1–100 nmol/mL urine (E, Trp, 5-HTrp, and 5-HT). No significant changes in the slopes of the graphs were observed between standards and spiked urine samples (Tables 1 and 2). The recoveries of the eight biogenic amines in urine samples spiked with amines were evaluated. For this

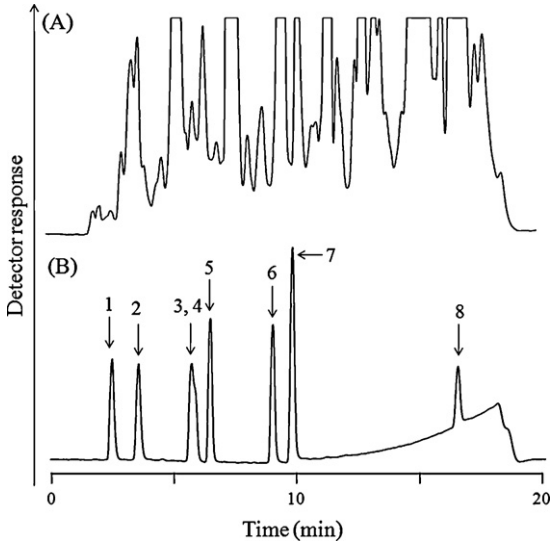

Fig. 5. Chromatograms obtained from (A) urine sample and (B) eight standard biogenic amines without derivatization using reversed-phase LC separation [5]. LC conditions: column, XBridge™ C18 column (150 mm × 4.6 mm i.d.); Mobile phases, methanol–water–trifluoroacetic acid (A: 2.5:97.5:0.05, v/v), (B: 60:40:0.05, v/v). Gradient elution, see Section 2; flow rate, 1.0 mL/min; column temperature, 30 °C. Peaks and amounts in chromatogram (B) (pmol on column): 1, NE (20); 2, E (20); 3, DA (20); 4, 5-HTrp (20); 5, DOPA (20); 6, MN (20); 7, 5-HT (20); 8, Trp (20).

purpose, spiked urine samples were prepared at 100 nmol/mL urine (DOPA) and 10 nmol/mL urine (other amines) concentration levels. The examined biogenic amines were recovered from the urine in the range of 96.9–103.3% (Table 2). Thus, the slope of the calibration curves and the recoveries demonstrate that this method allows simultaneous determination of eight biogenic amines without interference from contaminants in human urine. The intraday and interday precisions of peak height were established by repeated determination ($n = 6$) of eight biogenic amines in urine (DOPA, 100 nmol/mL urine; other amines, 10 nmol/mL urine); the relative standard deviations of intraday samples were within 5.1% and those of interday samples were within 6.1% (Table 2). The quantitation limits (signal-to-noise ratio = 10) were less than 4.2 nmol/mL urine (Table 2).

3.5. Determination of biogenic amines in human urine

The concentrations of biogenic amines in healthy human urine samples ($n = 7$) were determined by the proposed method. The values (mean ± SD) of DOPA, DA, NE, E, MN, Trp, 5-HTrp, and 5-HT were 5.4 ± 2.4, 5.0 ± 1.6, 4.4 ± 1.6, 1.9 ± 1.1, 3.6 ± 1.0, 32 ± 6.5, 1.4 ± 0.69, and 2.6 ± 0.81 nmol/mL urine, respectively, all of which are in good agreement with previously reported data [6,7,9,11,18,28].

4. Conclusion

This paper reports the development and application of the determination of biogenic amines using fluorous derivatization and fluorous-phase separation. This method is the most selective for native fluorescent amines presently available. Thus, biogenic amines can be analyzed by simple pretreatment and derivatization, and detected with high selectivity, without interference from contaminants in the biological sample. This method was successfully applied to the simultaneous determination of eight biogenic amines in human urine.

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