Analysis of neurotransmitter catecholamines and related amines in human urine and serum by chromatography and capillary electrophoresis with 1, 3, 5, 7-tetramethyl-8-(N-hydroxysuccinimidyl propionic ester)-difluoro-boradiaza-s-indacene

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ABSTRACT

Two sensitive and effective methods were developed for the detection of catecholamines and related biogenic amines (dopamine, epinephrine, norepinephrine, serotonin, levodopa and tyramine) using high performance liquid chromatography with fluorescence detection and capillary electrophoresis with laser-induced fluorescence detection. A BODIPY fluorescent dye, 1, 3, 5, 7-tetramethyl-8-(N-hydroxysuccinimidyl propionic ester)-difluoroboradiaza-s-indacene was used as pre-column derivatization reagent. The separation and derivatization conditions were optimized in detail. In high performance liquid chromatography with fluorescence detection method, the derivatization reaction was completed at 35°C for 20 min. At the wavelength of $\lambda_{ex}/\lambda_{em} = 493\text{ nm}/513\text{ nm}$, dopamine, epinephrine, norepinephrine, and levodopa derivatives achieved baseline separation within 15 min. The limits of detection ($S/N = 3$) were 1.0, 2.0, 5.0, and 0.5 nmol/L, respectively. In capillary electrophoresis with laser-induced fluorescence detection method, the derivatization reaction was completed at 25°C for 20 min. Serotonin, tyramine and dopamine derivatives reached baseline separation within 10 min at the wavelength of $\lambda_{ex} = 473\text{ nm}$. The limits of detection ($S/N = 3$) for serotonin, tyramine, and dopamine were 0.3, 0.02, and 0.2 nmol/L, respectively. The amino compounds in human serum and urine samples were detected successfully, and the recoveries were 93.3%–106.7% and 91.0%–103.1%, respectively.

KEYWORDS
catecholamines (CAs), 1, 3, 5, 7-tetramethyl-8-(N-hydroxysuccinimidyl propionic ester)-difluoroboradiaza-s-indacene (TMB-NHS), derivatization, capillary electrophoresis with laser-induced fluorescence detection (CE-LIF), high performance liquid chromatography with fluorescence detection (HPLC-FLD)

INTRODUCTION

Catecholamines (CAs), usually including dopamine (DA), epinephrine (E), norepinephrine (NE), are a class of biogenic amines which play a major role as important diagnostic markers and neurotransmitters in central and peripheral nervous systems [1]. For example, DA has been reported as a major biomarker of Parkinson’s and Huntington’s disease [2]. Besides, there are also many other significant catecholamine-related amines. For instance, tyramine (Tyr) was reported to induce the release of CAs, in addition to its classical role as a neurotransmitter [3]. Levodopa (L-DOPA), a prodrug of DA, has no pharmacological
activity, but it could enter the central nervous system and be converted into DA [4]. As for serotonin (5-hydroxy-tryptamine, 5-HT), which is a classical neurotransmitter, it also takes part in the control of numerous physiological functions of nervous systems, such as sleep, thermoregulation, food intake and sexual activity [1]. Recently, the CAs and related amines have attracted special attentions [5–8]. It was reported that CAs in excess concentrations cause dysregulation of physiological cascades. This dysregulation subsequently leads to various conditions, many of which are risk factors closely linked to adverse outcomes in patients with COVID-19 [5]. Branco et al. reported that 5-HT could play a positive role in the regulation of systemic inflammation and participate in a coadjuvant therapy to attenuate neurological complications of COVID-19 [6]. Therefore, it is of great significance to establish a rapid, accurate and sensitive analytical method for CAs and related amines, which is helpful to the discovery, treatment of biomarkers for Parkinson’s and other related disease, and exploring the role of those compounds in the pathophysiology of COVID-19.

Sensitive and rapid analysis of CAs and related amines remains an analytical challenge because of their low concentrations in samples, the oxidation-prone catechol moiety, potential interferences, and poor fragmentation characteristics in the mass spectrometer [9]. To date, several methods have been proposed for the determination of CAs in various biological samples [10–13]. Among them, chromatography and capillary electrophoresis (CE) are important due to its characteristics such as strong separation ability, good repeatability, low sample volume, high selectivity, and satisfactory sensitivity for clinical purposes, especially suitable for complex samples. Combined with ultraviolet (UV) [10], electrochemical (ECD) [11], and mass spectrometry (MS) detection [12, 13], high performance liquid chromatography (HPLC) and CE have been widely used for the analysis of biogenic amines. Moreover, if HPLC and CE are equipped with fluorescence detection (FLD) [14], especially laser-induced fluorescence (LIF) [15], the limits of detection can reach 10⁻⁸ mol/L to 10⁻¹² mol/L [16]. However, there are only several commercial laser detectors available, for example, He-Cd laser (325 nm) and Ar ion laser (488 nm).

Since most biogenic amines do not possess native fluorescence properties, chemical derivatization with a fluorescent label is required. Pre-column derivatization was often used to determine amines. The reagents commonly used for the derivatization of amines are 1, 2-diphenylethlenedi-amine (DPE) [17], dansyl chloride (Dns-Cl) [18], 9-fluorenyl-methoxy-carbonyl chloride (FMOC-Cl) [19], 4-chloro -7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) [20], 1, 3, 5, 7-tetramethyl-8-(N-hydroxy-succinimidyl butyric ester)-difluoroboradiazia-s-indacene (TMBB-Su) [21], and so on. However, these probes present various disadvantages, such as low sensitivity and poor stability. A BODIPY fluorescent dye, 1, 3, 5, 7-tetramethyl-8-(N-hydroxy-succinimidyl propionic ester)-difluoroboradiazia-s-indacene (TMB-NHS) can be used to label proteins and amino-containing molecules. Its fluorescent wavelength in visible region matches well with the commercial LIF detectors. Although TMB-NHS is generally used for the staining of cells or tissues and exhibits good performance in biological samples [22], there is no report on the use of biological small molecules for derivatization to date.

Herein, we present chromatography and CE methods combined with sensitive fluorescent detections to analysis CAs and related biogenic amines, employing TMB-NHS as the derivatization reagent to obtain sensitive results. As HPLC and CE were two different methods with different separation mechanism, HPLC-FLD and CE-LIF methods were both developed, validated and compared. Three CAs (namely DA, E, NE) and another DA-related compound, L-DOPA, were derivatized, separated, and detected by the method of HPLC-FLD. In CE-LIF method, the detection of DA, 5-HT and Tyr were investigated. The developed methods were also applied to different biologic samples.

**EXPERIMENTAL**

**Apparatus**

Chromatography determination was performed using Agilent 1260 HPLC system comprising G1321C fluorescence detector (Agilent Technologies, Inc.) and a reverse-phase column (ZORBAX Eclipse XDB-C18, 5 μm, 4.6 × 250 mm, Agilent Technologies, Inc.). CE was performed using a laboratory-built system. A high voltage power supply (0–30 kV, Dongwen High Voltage power supply Co., Ltd, Tianjin, China) was employed to drive the electrophoresis. The CE system was equipped with a laser-induced fluorescence detector (Dalian Institute of Chemical physics, Dalian, China). A laser-diode double-pumped solid-state (LD-DPSS) laser emitting at 473 nm (5 mW, New technology, Inc., Changchun, China) was utilized as an excitation source. Uncoated fused-silica capillaries (52 cm (40 cm to the detector) × 75 μm i.d.) (Yongnian Optic Fiber Inc., Hebei, China) were used for the separation at 25°C.

**Chemicals and reagents**

Standard E, DA, NE, Tyr, L-DOPA and 5-HT were obtained from Aladdin Reagent (Shanghai, China), and the stock solutions were prepared at a concentration of 5 × 10⁻³ mol/L (stored at −4°C) using ultrapure water as the solvent. TMB-NHS was provided by Life Technologies (New York, USA), and a 2.4 × 10⁻³ mol/L solution was prepared in pretreated dimethyl sulfoxide (stored at −20°C). HPLC-grade acetonitrile was from MREDA (MREDA Technology Inc., USA). HPLC-grade dimethyl sulfoxide was purchased from Sigma-Aldrich (St. Louis, MO, USA). All aqueous solutions were prepared from ultrapure water.

**Derivatization procedure**

To a certain amount of biogenic amines mixture in a vial, 0.05 mol/L borax buffer, a certain volume of 2.4 × 10⁻³ mol/L
TMB-NHS solution, and 100 µL acetonitrile were added, respectively. The solution was kept in a hot-water-bath to react at certain temperature for certain time after being diluted to 1.0 mL with ultrapure water. The mixture was filtered through 0.45 µm membrane filter before the analysis of HPLC and CE.

Liquid chromatography and capillary electrophoresis

Before analysis, the C_{18} column was activated with the organic phase for 40 min then eluted with the transitional mobile phase for 20 min, equilibrated for 30 min with the initial mobile phase finally. The mobile phase consisted of acetonitrile, water, sodium citrate buffer. 20 µL of sample solution was injected into the C_{18} column and the derivatives were eluted at a flow rate of 0.9 mL/min with gradient elution at room temperature. The detection wavelengths: λ_{ex} = 493 nm, λ_{em} = 513 nm.

New capillary was activated with ultrapure water, 1.0 mol/L NaOH, ultrapure water, 1.0 mol/L HCl, ultrapure water, 0.1 mol/L NaOH, ultrapure water, 0.1 mol/L HCl for 30 min, respectively. Before continuous detection every day, the capillary was rinsed with ultrapure water, 0.1 mol/L NaOH, and ultrapure water for 20 min individually. In order to ensure the reproducibility of the experiment, 0.1 mol/L NaOH, ultrapure water, and buffer solution were washed for 5 min between two runs. The injection time was 15 s.

Method validation of chromatography and CE

To construct the calibration curves for the methods, five standard concentrations of biogenic amines mixture were prepared and derivatized as the procedure mentioned above (L-DOPA: 0.025–6.0 µmol/L, DA: 0.05–500 µmol/L, E: 0.1–24.0 µmol/L, NE: 0.01–10.0 µmol/L, 5-HT: 0.5–500 µmol/L, Tyr: 0.05–50 µmol/L). The LODs were determined as the lowest concentration level resulting in a peak height three times the baseline noise (S/N = 3). Reproducibility was evaluated by repeating five consecutive runs of the derived standard solutions for the peak area and retention time/migration time.

Sample preparation

Fresh blood from healthy volunteer was stored in vacuum blood collection tube with EDTA-K_{2} as anticoagulant. The sample was centrifuged at 8,000 × g for 20 min, and then 3 mL methanol was added to deproteinize the supernatant liquid. After centrifugation at 8,500 × g for 15 min, the upper liquid was collected, blown by nitrogen until the volume is constant, and filtered through a 0.45 µm membrane filter. The resultant solution was used for the derivatization as described above.

Fig. 1. Effect of running buffer concentration (A), the buffer pH (B) and separation voltage (C) on the migration time. Peaks: (■) 5-HT; (●) Tyr; (▲) DA
Fresh urine samples were from volunteers. A certain amount of urine sample was centrifuged at 8,000 \( \times g \) for 15 min. And then, the supernatant liquid was filtered through a 0.45 \( \mu \text{m} \) membrane filter.

**RESULTS AND DISCUSSION**

**Optimization of HPLC separation conditions**

A C\(_{18}\) column was used for the separation. At first, the separation of the TMB-NHS derivatives was attempted in isocratic elution mode. It was found that the peaks of the derivatives were wide and could not be separated on baseline using methanol and sodium citrate buffer as mobile phase when methanol volume ratio was in the range of 40\%–70\%. To detect the biogenic amines rapidly and sensitively, gradient program should be adopted. When methanol-water-sodium citrate buffer solution (10 mmol/L, pH 4.6) was selected as the mobile phase in gradient program, the peak of L-DOPA overlapped with the derivatization reagent peak. No obvious improvement could be found after adjusting the proportion of three components. Fortunately, all derivative peaks were well separated when methanol was replaced by acetonitrile. The optimized gradient elution program was as follows. Initial mobile phase was composed of acetonitrile-water-sodium citrate (40:50:10, V/V/V). Next, the volume fraction of acetonitrile in the mobile phase was increased to 42\% and maintained 5 min. In the following time, the volume fraction of acetonitrile was raised to 58\%. The volume fraction of sodium citrate solution is fixed at 10\%.

![Fig. 2. Effect of buffer pH (A), reagent concentration (B), reaction temperature (C) and reaction time (D) on the derivatization efficiency.](image)

(V) DA; (■) L-DOPA; (●) NE; (★) E

![Fig. 3. Typical chromatogram of derivatives of NE (2 \( \times 10^{-6}\) mol/L), E (4 \( \times 10^{-6}\) mol/L), DA and L-DOPA (1 \( \times 10^{-6}\) mol/L). Peaks: 1, L-DOPA; 2, NE; 3, E; 4, DA. Chromatographic conditions: column, Agilent C\(_{18}\) column; fluorescence detection, \( \lambda_{ex}/\lambda_{em} = 493 \text{ nm}/513 \text{ nm} \); mobile phase, acetonitrile-water-10 mmol/L sodium citrate buffer (pH 4.6); injection volume, 20 \( \mu\text{L} \); flow rate, 0.9 \( \text{mL/min} \); room temperature](image)
Optimization of CE separation conditions

The selection of an effective and simple background electrolyte was important for routine and fast analysis. Borate, sodium citrate and phosphate buffer were used and compared as the background electrolyte, respectively. When borate was used, the peaks height was short and the TMB-NHS peak overlapped with 5-HT derivatives. There is still no improvement of the separation when phosphate was adopted. However, while sodium citrate buffer was used, migration time and peak shape were both satisfactory. Thus, sodium citrate buffer was chosen as the background electrolyte. The effect of sodium citrate concentration was investigated in the range of 5–40 mmol/L (Fig. 1A). It was found that the concentration of sodium citrate solution has no obvious effect on the separation. Nevertheless, with the increase of buffer concentration, the migration time of derivatives become longer. Therefore, the concentration of sodium citrate buffer was selected as 30 mmol/L.

The pH value of the sodium citrate buffer can influence the Zeta potential and the charge of the solute, resulting in the difference of separation effect. Different pH values (7.8, 8.5, 9.1, 9.6, 10.0, 10.6) of sodium citrate buffer were evaluated in detail (Fig. 1B). The results showed that the separation was very sensitive to the change of pH value. The derivative peaks could be separated on the baseline when pH 9.1 was adopted as the pH value in the analysis. Finally, pH 9.1 was used in the subsequent work.

The separating voltage was further investigated between 8.5–110 kV (Fig. 1C). Increasing voltage could speed up electrophoretic velocity at a cost of separation selectivity. To achieve the best compromise between complete separation and quick analysis speed, 10.0 kV was performed for the separation.

Optimization of derivatization conditions

Although TMB-NHS has been applied to the determination of protein molecules, there are no relevant reports on the derivatization of small molecules. Therefore, we discussed the derivatization reaction conditions of this reagent with amines in detail. In general, there are many factors that affect the derivatization efficiency of amines, such as the type of buffer solution, pH value, reaction temperature, reaction time, and the reagent concentration. Therefore, this experiment optimized the derivatization conditions described above.

When HPLC-FLD was used, the results shown in Fig. 2. Firstly, the effects of Na2B4O7, NaH2PO4 and NaHCO3 buffer solutions with the same concentration of 50 mmol/L at pH 8.0 were studied. The results showed that the peak areas of derivatives were the largest in the H3BO3-Na2B4O7 buffer solution. The influence of various pH values (7.2, 7.6, 8.0, 8.4, 8.6) on the peak areas was also studied. At pH 8.0, the peak areas of the derivatives reached the largest. Thus, pH 8.0 was selected. The reactive group of the TMB-NHS is an N-hydroxysuccinimide ester, which is easily hydrolyzed in water. Therefore, in order to ensure the maximum efficiency of the derivatization reaction, an excess of TMB-NHS is needed. The effect of the TMB-NHS amount on the derivatization was explored in the range of 0.6×10⁻⁵–2.1×10⁻⁵ mol/L. The optimal

| Table 1. Linear range, regression equations, correlation coefficients and detection limits |
|-----------------------------------------------|---------------|----------------|----------|--------|
| Analyte | Linear range (μmol/L) | Regression equation | Correlation Coefficient, R | RSD (%) | LOD (nmol/L) |
|         |                           |                           |                        | PA, RT/MT |                  |
| HPLC-FLD |                              | 0.025–6.0                | Y = 0.0309x + 8.352   | 0.9973  | 3.3, 0.2        | 5.0   |
|         |                              | 0.05–6.0                 | Y = 0.0156x + 9.069   | 0.9968  | 3.6, 0.1        | 0.5   |
|         |                              | 0.1–24.0                 | Y = 0.0056x + 9.107   | 0.9971  | 2.8, 0.3        | 2.0   |
|         |                              | 0.01–10.0                | Y = 0.0045x + 4.486   | 0.9959  | 2.8, 0.3        | 1.0   |
| CE-LIF  |                              | 0.5–500                  | Y = 207.47x + 716.38  | 0.9959  | 4.5, 3.4        | 0.3   |
|         |                              | 0.05–50                  | Y = 121.60x + 670.79  | 0.9976  | 2.3, 1.6        | 0.02  |
|         |                              | 0.5–500                  | Y = 165.59x + 1,019.4 | 0.9960  | 4.8, 3.7        | 0.2   |

a) Y: peak area; X: analyte concentration (nmol/L).
b) PA, peak area; RT, retention time; MT, migration time.
c) LOD: the limits of detection.
concentration was chosen as $4.8 \times 10^{-5}$ mol/L. The efficiency of different reaction temperatures from 25°C to 45°C was also studied. The peak areas of 1-DOPA, NE, and E reached a maximum at 35°C. When the derivatization temperature exceeded 35°C, the peak area of the derivatives decreased. The peak area of DA derivatives reached a maximum at 40°C, then have no change obviously. It might be due to the fact that high temperature accelerates the hydrolysis of TMB-NHS or initiates other side reactions. So, 35°C is set as the reaction temperature. The influence of derivatization time was then investigated in the range of 5–50 min, keeping the temperature at 35°C. With the increasing of time, the derivatization reaction proceeded gradually. After 20 min, the peak area of derivatives did not change significantly. Thus, 20 min was employed for derivatization reaction in subsequent experiments.

When CE-LIF was used, the optimized derivatization conditions were also optimized. The reaction conditions were similar to those of the HPLC method. In summary, the derivatization reaction of $2.4 \times 10^{-6}$ mol/L TMB-NHS with the analytes were performed at 25°C for 20 min in 50 mmol L$^{-1}$ H$_3$BO$_3$-Na$_2$B$_4$O$_7$ (pH 7.5) buffer solution.

In general, the reaction of TMB-NHS with biogenic amines was under mild conditions and possessed desirable labeling chemistry.
Analytical characterization of chromatography and CE

Under the optimal conditions of separation and derivatization, typical chromatogram (Fig. 3) and electropherogram (Fig. 4) were obtained. The linear range of the method was estimated by analyzing standard solutions containing different concentrations of biogenic amines. The LODs were determined as the amounts of biogenic amines that resulted in a peak height three times larger than that of the baseline noise ($S/N = 3$). All the dates are listed in Table 1. Good linearity was obtained with the correlation coefficients for these amines ranging from 0.9959 to 0.9976. The LODs for the derivatized biogenic amines ranged from 0.5 to 5.0 nmol/L with HPLC-FLD and were as low as 0.02 nmol/L for the CE-LIF method.

Sample analysis

In order to verify the feasibility of the established methods, the experiments were performed on spiked recovery tests in human serum and urine. Fig. 5 and Fig. 6 show the chromatograms and electropherograms of serum and urine samples, respectively. As can be seen from Table 2 and Table 3, both human serum and urine contained trace amounts of amines. In the HPLC method, four analytes could be detected in urine samples, whereas in serum
Table 2. Recoveries and RSDs in human urine and serum samples by HPLC-FLD

| Samples | Analytes | Added (nmol/L) | Found (nmol/L) | Recovery (%) | RSD (% n = 5) | RT | PA |
|---------|----------|----------------|----------------|--------------|--------------|-----|----|
|         |          |                |                |              |              |     |    |
| Human   | L-DOPA   | 0              | 0.09           | 0.2          | 1.4          |     |    |
| Urine   | 0.30     | 0.41           | 106.7          | 0.8          | 2.1          |     |    |
|         | 1.50     | 1.58           | 99.3           | 0.7          | 1.6          |     |    |
| NE      | 0        | 0.06           | 0.67           | 0.4          | 1.9          |     |    |
|         | 0.60     | 0.67           | 101.7          | 0.6          | 1.8          |     |    |
|         | 3.00     | 3.25           | 106.3          | 0.1          | 3.9          |     |    |
| E       | 0        | 0.07           | 1.25           | 0.4          | 1.5          |     |    |
|         | 1.20     | 1.25           | 98.3           | 0.5          | 2.0          |     |    |
|         | 6.00     | 6.11           | 100.7          | 0.1          | 2.7          |     |    |
| DA      | 0        | 0.02           | 0.31           | 0.4          | 2.0          |     |    |
|         | 0.30     | 0.31           | 96.7           | 0.4          | 3.3          |     |    |
|         | 1.50     | 1.54           | 101.3          | 0.3          | 3.0          |     |    |
| Human   | L-DOPA   | 0              | 0.10           | 0.1          | 1.9          |     |    |
| Urine   | 0.30     | 0.41           | 103.3          | 0.7          | 1.7          |     |    |
|         | 1.50     | 1.60           | 100.0          | 0.1          | 3.0          |     |    |
| NE      | 0        | 0              | 0.58           | 0.3          | 3.1          |     |    |
|         | 0.60     | 0.58           | 96.7           | 0.3          | 3.1          |     |    |
|         | 3.00     | 3.07           | 102.3          | 0.2          | 4.3          |     |    |
| E       | 0        | 0.04           | 1.31           | 0.5          | 2.3          |     |    |
|         | 1.20     | 1.31           | 105.8          | 0.7          | 2.0          |     |    |
|         | 6.00     | 6.12           | 101.3          | 0.1          | 3.3          |     |    |
| DA      | 0        | 0.07           | 1.58           | 0.4          | 1.8          |     |    |
|         | 0.30     | 0.39           | 106.7          | 0.5          | 2.1          |     |    |
|         | 1.50     | 1.58           | 100.7          | 0.1          | 3.9          |     |    |
| Human   | L-DOPA   | 0              | 0.08           | 0.3          | 1.5          |     |    |
| Serum   | 0.30     | 0.36           | 93.3           | 0.3          | 2.3          |     |    |
| 1.50     | 1.60     | 101.3          | 0.4           | 3.7          |     |    |
| NE      | 0        | 0              | 0.60           | 0.5          | 3.9          |     |    |
|         | 0.60     | 0.59           | 95.0           | 0.7          | 1.8          |     |    |
|         | 3.00     | 3.01           | 99.7           | 0.3          | 2.7          |     |    |
| E       | 0        | 0              | 1.20           | 1.2          | 1.8          |     |    |
|         | 1.20     | 1.18           | 98.3           | 0.8          | 2.4          |     |    |
|         | 6.00     | 6.01           | 100.2          | 0.4          | 3.7          |     |    |
| 0.30     | 0.36     | 103.3          | 0.6           | 1.3          |     |    |
|         | 1.50     | 1.57           | 101.3          | 0.1          | 2.0          |     |    |
| NE      | 0        | 0              | 0.60           | 0.6          | 3.1          |     |    |
|         | 0.60     | 0.61           | 101.7          | 0.6          | 2.2          |     |    |
|         | 3.00     | 3.10           | 103.3          | 0.1          | 3.9          |     |    |
| E       | 0        | 0              | 1.20           | 1.2          | 1.5          |     |    |
|         | 1.20     | 1.24           | 103.3          | 0.6          | 1.5          |     |    |
|         | 6.00     | 5.97           | 99.5           | 0.1          | 2.1          |     |    |
| 0.30     | 0.32     | 96.7           | 0.6           | 2.4          |     |    |
|         | 1.50     | 1.57           | 102.7          | 0.1          | 3.3          |     |    |
### Table 3. Recoveries and RSDs in human urine and serum samples by CE-LIF

| Samples   | Analytes | Added (nmol/L) | Found (nmol/L) | Recovery (%) | RSD (% n = 5) | Samples   | Analytes | Added (nmol/L) | Found (nmol/L) | Recovery (%) | RSD (% n = 5) |
|-----------|----------|----------------|----------------|--------------|---------------|-----------|----------|----------------|----------------|--------------|---------------|
| Human     | 5-HT     | 0              | 12.5           | 0.8          | 1.6           | Human     | 5-HT     | 0              | 17.9           | 0.3          | 1.8           |
| Urine 1   | 100      | 111.3          | 98.8           | 0.5          | 2.8           | Serum 1   | 100.0   | 115.8          | 97.9           | 0.5          | 4.0           |
|           | 400      | 402.7          | 97.6           | 0.4          | 1.0           |           | 400.0   | 416.4          | 99.6           | 0.4          | 1.5           |
|           | Tyr      | 0              | 5.1            | 0.3          | 1.7           | Tyr       | 0       | 4.5            | 0.7            | 2.5          |
|           |          | 10             | 15.3           | 0.2          | 4.3           |           | 10.0    | 13.9           | 0.3            | 3.2          |
|           |          | 40             | 43.8           | 0.5          | 3.1           |           | 40.0    | 45.7           | 103.0          | 0.3         | 0.8           |
|           | DA       | 0              | 24.1           | 0.3          | 1.7           | DA        | 0       | 19.8           | 1.1            | 1.6          |
|           |          | 100            | 119.4          | 0.4          | 1.1           |           | 100.0   | 122.3          | 102.5          | 0.4         | 4.1           |
|           |          | 400            | 426.5          | 0.3          | 2.1           |           | 400.0   | 413.1          | 98.3           | 0.3         | 3.8           |
| Human     | 5-HT     | 0              | 19.8           | 0.8          | 3.2           | Human     | 5-HT     | 0              | 22.1           | 1.1          | 2.4           |
| Urine 2   | 100      | 118.3          | 98.5           | 0.3          | 1.5           | Serum 2   | 100.0   | 119.7          | 97.6           | 0.2         | 1.9           |
|           | 400      | 417.5          | 99.4           | 0.2          | 1.4           |           | 400.0   | 423.2          | 100.3          | 0.6         | 1.6           |
|           | Tyr      | 0              | 11.3           | 1.2          | 4.4           | Tyr       | 0       | 5.3            | 0.8            | 1.4          |
|           |          | 10             | 20.6           | 0.5          | 1.4           |           | 10.0    | 14.4           | 91.0           | 0.5         | 2.9           |
|           |          | 40             | 51.1           | 0.4          | 0.7           |           | 40.0    | 42.7           | 93.5           | 0.3         | 1.0           |
|           | DA       | 0              | 35.7           | 0.5          | 2.1           | DA        | 0       | 26.5           | 0.3            | 1.6          |
|           |          | 100            | 137.1          | 1.1          | 4.1           |           | 100.0   | 128.7          | 102.2          | 0.2         | 2.6           |
|           |          | 400            | 429.4          | 0.7          | 1.6           |           | 400.0   | 425.1          | 99.7           | 1.2         | 1.0           |
| Human     | 5-HT     | 0              | 15.7           | 0.4          | 3.1           | Human     | 5-HT     | 0              | 18.4           | 1.0          | 0.9           |
| Urine 3   | 100      | 116.9          | 101.2          | 0.7          | 1.5           | Serum 3   | 100.0   | 116.7          | 98.3           | 0.2         | 3.7           |
|           | 400      | 410.2          | 98.6           | 0.4          | 2.2           |           | 400.0   | 413.9          | 98.9           | 0.3         | 1.9           |
|           | Tyr      | 0              | 8.4            | 1.7          | 1.9           | Tyr       | 0       | 7.2            | 0.2            | 2.4          |
|           |          | 10             | 18.7           | 0.4          | 3.2           |           | 10.0    | 16.5           | 93.0           | 0.5         | 3.7           |
|           |          | 40             | 45.8           | 0.7          | 2.0           |           | 40.0    | 48.3           | 102.8          | 1.0         | 2.1           |
|           | DA       | 0              | 26.6           | 2.0          | 2.4           | DA        | 0       | 31.4           | 1.0            | 1.7          |
|           |          | 100            | 129.4          | 0.5          | 1.2           |           | 100.0   | 134.5          | 103.1          | 0.5         | 3.9           |
|           |          | 400            | 425.1          | 0.4          | 3.5           |           | 400.0   | 430.7          | 99.8           | 0.9         | 1.6           |
sample L-DOPA and DA could be detected. In the CE method, both human urine and serum samples contained trace amounts of 5-HT, Tyr, and DA with satisfactory RSDs for migration time and peak area. The recoveries of the samples were 93.3%–106.7% for HPLC-FLD and 91.0%–103.1% for CE-LIF, respectively. The background substrate of sample had no interference on the detection of biogenic amines.

Comparison with other chromatography and CE methods

A comparison of the present methods with other chromatography and CE methods in terms of detection wavelength, derivatization conditions and detection limits were listed in Table 4 [17, 21, 23–29]. As for the HPLC-FLD method, firstly, the detection wavelength of TMB-NHS is in the visible light, which means less interfering fluorescence from biological samples. Secondly, the derivatization conditions of TMB-NHS are mild and almost reacted at room temperature, which is superior to TMBB-Su and FMOC-Cl reagents in this respect. Finally, the LODs of the present methods were lower than those listed in the table when other reagents such as TMBB-Su, BA&DPE, and FMOC-Cl were adopted. In the CE-LIF method, TMB-NHS can derivatize with CAs and related amines at room temperature, and the LODs are superior to those by FITC, NBD-Cl and CBQCA.

The comparison of the proposed HPLC-FLD with CE-LIF methods was investigated too. It could be concluded that the detection sensitivity of CE-LIF was advantageous to that of HPLC-FLD. This might be attributed to the excellent sensitivity of LIF detection device. Nevertheless, in the respect of reproducibility, HPLC-FLD shows certain advantages, resulting in better RSDs in sample analysis.

In summary, TMB-NHS can react rapidly with CAs and related amines under mild conditions. The methods are simple, convenient, sensitive and selective.

CONCLUSIONS

Two effective and validated methods (CE-LIF and HPLC-FLD) were successfully applied to analyze CAs and related amines by derivatization with TMB-NHS in human serum and urine samples. Under the optimum conditions, it was demonstrated that baseline separation of analytes could be achieved within 15 min in HPLC-FLD and 10 min in CE-LIF, respectively. The detection limits for the derivatized amines were as low as 0.5 nmol/L with HPLC-FLD and 0.02 nmol/L with CE-LIF method, respectively. Compared to the HPLC-FLD method, the CE-LIF method is more sensitive, and efficient. The two techniques have characteristics of simple operation, satisfactory sensitivity and high reproducibility, which have prospect in exploring the role of those compounds in the pathophysiology of COVID-19, Parkinson’s and other related disease.

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REFERENCES

1. MacDonald, S. W.; Nyberg, L.; Backman, L. Trends Neurosci. 2006, 29, 474–80.
2. Yildirim, A.; Bayindir, M. Anal. Chem. 2014, 86, 5508–12.
3. Branicky, R.; Schafer, W. R. Neuron. 2009, 62, 458–60.
4. Wang, Y.; Yao, L.; Gao, S. S.; Zhang, G. J.; Zhang, Q. C.; Liu, W. Y.; Zhou, Y. Q.; Sun, Y. N.; Liu, J. Brain Res. 2021, 1754, 147266.
5. Gubbi, S.; Nazari, M. A.; Pacak, K. Lancet Diabetes & Endo. 2020, 8, 978–86.
6. Costa, L. H. A.; Santos, B. M.; Branco, L. G. S. Eur. J. Pharmacol. 2020, 889, 173629.
7. Serrano, M. M.; Pérez-Sánchez, J. R.; Sánchez, S. P.; Casa-Fages, B. D. L.; Jimeno, V. M.; Tamayo, I. P.; Grandas, F. J. Neurol. Sci. 2020, 415, 116944.
8. Attademo, L.; Bernardini, F. Eur. J. Psychiat. 2021, 35, 62–3.
9. Faassen, M. V.; Bischoff, R.; Eijkelenkamp, K.; Jong, W. H. A.; Ley, C. P.; Kema, I. P. Anal. Chem. 2020, 92, 9072–8.
10. Liu, S. J.; Xu, J. J.; Ma, C. L.; Guo, C. F. Food Chem. 2018, 266, 275–83.
11. Adimucar, V.; Öztokin, N.; Erım, F. B. Food Anal. Method. 2018, 11, 1374–9.
12. Ochi, N. J. Chromatogr. A. 2019, 1601, 115–20.
13. Hook, V.; Kind, T.; Podvin, S.; Palazoglu, M.; Tran, C.; Toneff, T.; Samra, S.; Lietz, C.; Fiehn, O. ACS Chem. Neurosci. 2019, 10, 1369–79.
14. Baranowska, I.; Plonka. J. Food Anal. Method. 2015, 8, 963–72.
15. Zhang, Y.; Zhang, Y.; Wang, G.; Chen, W. J.; Li, Y.; Zhang, Y. T.; He, P. G.; Wang, Q. J. J. Chromatogr. B. 2016, 1025, 33–9.
16. Cao, L. W.; Wei, T.; Shi, Y. H.; Tan, X. F.; Meng, J. X. J. Liq. Chromatogr. R. T. 2018, 41, 58–65.
17. Liu, L.; Li, Q.; Li, N.; Ling, J.; Bi, K. J. Sep. Sci. 2011, 34, 1198–204.
18. Anguloa, M. F.; Floresa, M.; Arandab, M.; Henriquez-Aedo, K. Food Chem. 2020, 309, 125699.
19. Smith, L.; Francis, K. A.; Johnson, J. T.; Gaskill, C. L. Food Chem. 2017, 234, 174–9.
20. Deng, W. F.; Cheng, C.; Yang, H.; Wang, H.; Tan, Y. M.; Xie, Q. J.; Ma, M.; Yao, S. Z. Talanta. 2019, 202, 244–50.
21. Huang, X.; Guo, X. F.; Wang, H.; Zhang, H. S. Arab. J. Chem. 2019, 12, 1159–67.
22. Keliher, E. J.; Reiner, T.; Earley, S.; Klubnick, J.; Tassa, C.; Lee, A. J.; Ramaswamy, S.; Bardeesy, N.; Hanahan, D.; DePinho, R. A. Neoplasia. 2013, 15, 684–93.
23. Yoshitake, T.; Kehr, J.; Todoroki, K.; Nohta, H.; Yamaguchi, M. Biomed. Chromatogr. 2006, 20, 267–81.
24. Chan, E. C. Y.; Wee, P. Y.; Ho, P. Y.; Ho, P. C. J. Chromatogr. B. 2000, 749, 179–89.
25. Wang, X. N.; Liang, Y. W.; Wang, Y. Q.; Fan, M. Q.; Sun, Y. N.; Liu, J. L.; Zhang, N. Biomed. Chromatogr. 2018, 32, e4211.
26. Cao, L. W. Biomed. Chromatogr. 2007, 21, 708–15.
27. Zhang, Y. X.; Zhang, Z. J.; Yang, F. Chin. J. Chem. 2008, 26, 489–94.
28. Lei, Z.; Wang, W. P.; Wang, S. M.; Hui, Y.; Luo, Z.; Hu, Z. D. Anal. Chim. Acta. 2008, 611, 212–9.
29. Xiong, S.; Han, H.; Zhao, R.; Yi, C.; Liu, G. Biomed. Chromatogr. 2001, 15, 83–8.