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Determination of primary and secondary aliphatic amines with high performance liquid chromatography based on the derivatization using 1,3,5,7-tetramethyl-8-(N-hydroxysuccinimidyl butyric ester)-difluoroboradiaza-*s*-indacene

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ABSTRACT

In this article, the simultaneous determination of primary and secondary aliphatic amines including dimethylamine (DMA), diethylamine and eleven primary aliphatic amines by high performance liquid chromatography (HPLC) with fluorescence detection has been achieved using a BODIPY-based fluorescent derivatization reagent, 1,3,5,7-tetramethyl-8-(N-hydroxysuccinimidyl butyric ester)-difluoroboradiaza-*s*-indacene (TMBB-Su). The derivatization reaction of TMBB-Su with aliphatic amines was optimized with orthogonal design experiment and the derivatization reaction proceeded at 15 °C for 25 min. The baseline separation of these derivatives was carried out on a C_8 column with methanol–tetrahydrofuran-50 mM pH 6.50 HAc–NaAc buffer (55/5/40, v/v/v) as a mobile phase. Detected at the excitation and emission of 490 and 510 nm, respectively, the detection limits were obtained in the range of 0.01–0.04 nM (signal-to-noise ratio = 3). The proposed method has been applied to the determination of trace aliphatic amines in viscera samples from mice without complex pretreatment or enrichment method. The recoveries ranged from 95.1% to 106.8%, depending on the samples investigated.

1. Introduction

As is well known, most aliphatic amines including dimethylamine co-exist in biological body from biodegradation products of organic matter such as proteins, amino acids, and other nitrogen containing organic compounds [1–3]. Of all the aliphatic amines, secondary amines have been reported to have unspecified toxic effects on biological tissues for they are precursors of carcinogenic N-nitrosamines, a class of chemical compounds which exhibit a high carcinogenic activity in a wide variety of animal species [4–6]. Exogenous N-nitrosamines mostly come from the ingestion of food (e.g., beer and cured meat) and the use of rubber articles (e.g., teats) [7–10]. Endogenous ones can readily form directly in various organisms as a result of nitrosation compared with other classes of carcinogens because their precursors are widespread amines and nitrogen-containing substances [11,12]. Since the content of exogenous N-nitrosamines are much lower, endogenous formation of N-nitrosamine is being taken into account more seriously. At the same time, there is significant difference in the nitrosation rate of secondary amines, depending upon the alkaline of secondary amines and the nitrosation reaction of dimethylamine most readily happens in secondary amines [13–15]. Therefore, with respect to the potential pathophysiological significance of dimethylamine and other aliphatic amines, it is of great interest to develop sensitive and selective analytical methods for the simultaneous determination of a variety of aliphatic amines in the biological samples targeted in the field of biological and medical sciences.

Many analytical methods have been developed for the separation and determination of aliphatic amines, such as isotachophoresis [16], ion chromatography [17], thin-layer chromatography [18], gas chromatography [19,20], high-performance liquid chromatography (HPLC) [2,21] and capillary electrophoresis (CE) [22]. However, it is very difficult to detect aliphatic amines at trace level directly in complex matrices due to the lack of intrinsic chromophores or fluorophores as well as their volatility and activity. To overcome these problems, gas chromatography and high-performance liquid chromatography (HPLC) with pre-column or post-column chemical derivatization have been employed for the determination of aliphatic amines [23,24], and pre-column fluorescence derivatization in conjunction with HPLC is one of the commonly used methods. The detection sensitivity and detection wavelength of fluorescence derivatization-based HPLC method

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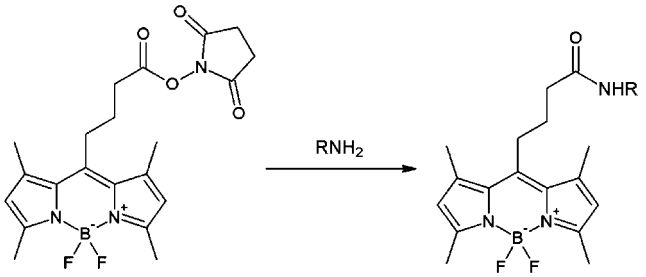

Fig. 1. The derivatization reaction of TMBB-Su with aliphatic amines.

are mostly dependent on the fluorophore of the derivatization reagent besides the reactive functional group. Difluoroboradiaza-*s*-indacene (boron-dipyrromethene, BODIPY) has been attracting increasing interest because of its high fluorescence quantum yield, long emission wavelength, good photostabilization and relative independence on changes in the local environment [25]. As a result, several BODIPY-based fluorescent derivatization reagents have been developed in our lab, such as 8-phenyl-(4-oxy-acetic acid *N*-hydroxysuccinimidyl ester)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (TMPAB-OSu) for amines [26], 1,3,5,7-tetramethyl-8-(3′,4′-diaminophenyl) difluoroboradiaza-*s*-indacene for NO [27], 1,3,5,7-tetramethyl-8-phenyl-(4-iodoacetamido)-difluoroboradiaza-*s*-indacene for thiols [28] and 1,3,5,7-tetramethyl-8-aminozide-difluoroboradiaza-*s*-indacene for aldehyde [29]. Since BODIPY dyes with an alkyl on 8-position exhibit considerably higher fluorescence compared to those with a phenyl [30], a new BODIPY-activated esters, 1,3,5,7-tetramethyl-8-(N-hydroxysuccinimidyl butyric ester)-difluoroboradiaza-*s*-indacene (TMBB-Su) (Fig. 1) has been designed and synthesized in our group. Experiments shows that the fluorescence quantum yield of TMBB-Su derivatives is about 0.94 which is far greater than most of other reagents. In this work, TMBB-Su has been used for the labeling of thirteen aliphatic amines including dimethylamine and diethylamine and the simultaneous determination of primary and secondary aliphatic amines has been achieved with HPLC-fluorescence detection. The detection limits are in the range 0.01–0.04 nM with the signal-to-noise ratio of 3. The proposed method has been applied to the direct determination of aliphatic amines in the heart, liver and kidney samples of mice with recoveries of 95.1–106.8%. Our studies demonstrate that TMBB-Su has a good reactivity with dimethylamine and a lowest detection limits are obtained compared with the existing HPLC methods.

2. Experimental

2.1. Apparatus

An LC-20A HPLC system (Shimadzu, Tokyo, Japan) with RF-10Axl fluorescence detector (Shimadzu, Tokyo, Japan) and LabSolutions/LCsolution Lite chromatography chemstation (Shimadzu, Tokyo, Japan) were used in the experiments. Sample injection volume was 20 μL. The separation was performed on a C_8 column (5 μm, 250 mm × 4.6 mm i.d., Kromasil, Bohus, Sweden).

2.2. Chemicals and reagents

Unless otherwise specified, all reagents used were of analytical grade. Aliphatic amines standards were purchased from Sigma (St. Louis, MO, USA). TMBB-Su (Fig. 1) was synthesized in our lab and its synthesis is to be published elsewhere. Methanol and tetrahydrofuran (THF) of HPLC grade were purchased from Shanghai Chemicals Company (Shanghai, China). Water used for preparing solutions was purified by a Milli-Q ultrapure system (Millipore, Bedford, MA, USA).

The TMBB-Su stock solution was prepared by dissolving TMBB-Su in acetonitrile to give a concentration of 1.0×10^{-3} M. The stock solutions of the aliphatic amines (1.0×10^{-3} M) were prepared by dissolving appropriate aliphatic amines in acetonitrile, and if necessary, THF was added until the compound dissolved. Dilution of these stock solutions to appropriate concentrations with acetonitrile was performed immediately before use. H_3BO_3–$Na_2B_4O_7$ buffer was prepared by mixing 0.05 M $Na_2B_4O_7$ solution with 0.2 M H_3BO_3 solution to the required pH value. HAc–NaAc buffer was prepared by mixing 0.1 M HAc solution with 0.1 M NaAc solution to the required pH value. When not in use, all standards were stored at 4 °C in a refrigerator.

2.3. Derivatization procedure and analysis

To a 0.5 mL vial containing appropriate amount of mixed amines and 50 μL of H_3BO_3–$Na_2B_4O_7$ buffer (pH 7.20), 30 μL 1×10^{-3} M TMBB-Su was added. The whole solution was diluted to the mark with acetonitrile and was kept at 15 °C for 25 min.

An aliquot (20 μL) of the reaction mixture was diluted and injected into the chromatographic system. The reagent blanks without aliphatic amines were also treated in the same way. The derivatization reaction was optimized in Section 3.3.

2.4. Chromatographic separation

The HPLC separations of TMBB-Su and aliphatic amines derivatives were performed on a Kromasil C_8 column with a binary gradient. Eluent A was methanol-THF (11:1, v/v) and eluent B was 50 mM HAc–NaAc buffer solution (pH 6.50). The gradient elution condition began with a isocratic elution of (A:B) 60:40, v/v for 15 min, followed by a gradual linear increase of A to (A:B) 90:10, v/v until 40 min. Finally, the mobile phase was reset (A:B) 60:40, v/v at 45 min and stayed for 5 min to equilibrate for the next injection. The flow rate was set at a flow rate of 1.0 mL/min and the column temperature was kept at 25 °C. The fluorescence emission wavelength was set at 510 nm (excitation at 490 nm). Before the analysis, the C_8 column was pre-equilibrated for 30 min with the mobile phase composition was 60% A and 40% B.

2.5. Sample preparation

Kunming mice (Hubei Sanitation and Anti-epidemic Station, Wuhan, China) were anesthetized with aether and sacrificed by decollation. The heart, liver, and kidney tissues were collected and frozen immediately. A portion of the tissue sample (about 20 mg) was immediately cut into pieces as small as possible with scissors, and then ground with 0.1 M HCl solution (5 mL) on ice using a tissue grinder. The homogenate was sonicated for 5 min and centrifuged (5000 rpm for 15 min) at 4 °C. The supernatant was transferred into another 1.5 mL vial and further deproteinized by mixing it with acetonitrile at the volume ratio of 1:10 (v/v). The solution was left on ice for 1 h and then centrifuged at 5000 rpm for 15 min at 4 °C. The supernatant was collected and derivatized directly with TMBB-Su as described above. The samples were stored at −40 °C when they were not used.

3. Results and discussion

3.1. Optimization of separation conditions

The parameters affecting separation were optimized.

At first, the separation of the TMBB-Su derivatives was studied using isocratic elution mode owing to its simpleness. When

methanol content was above 60%, the peaks of dimethylamine and ethylamine overlapped. If the methanol content was lower than 50%, longer analysis time was needed. When methanol content was in the range of 52–55%, the peaks could be well separated on a baseline. Thus, 55% was the optimum methanol volume fraction in the mobile phase. In order to improve the chromatographic separation and the peak shape, tetrahydrofuran (THF) was added to the mobile phase as an organic additive. When the THF content was less than 4%, the improvement was unconspicuous, whereas when it was more than 6%, the derivatives of dimethylamine and ethylamine were coeluted. Consequently, 5% THF was used in the mobile phase. Based on the preliminary optimizations, a baseline separation could be obtained within 22 min for the derivatives of TMBB-Su with methylamine, dimethylamine, ethylamine and *n*-propylamine. However, the long retention times and broaden peaks were observed for the derivatives of long-chain amines, which would make the detection limits high and analysis time-consuming. In order to determine more aliphatic amines sensitively and rapidly, gradient program were used instead of isocratic elution, which allowed complete separation of early eluted derivatives and short retention time of the last one.

The effect of buffers on the separation was investigated by using phosphate and acetate buffers. With gradient elution, when the methanol concentration in the mobile was increased to 85%, the use of phosphate concentrations higher than 0.05 M occasionally gave rise to crystallization and precipitation problems (most likely disodium hydrogen phosphate), and HAc–NaAc buffer was chosen to control pH value. In this experiment, the retention time as well as peak area of each derivative had no obvious change as the pH value varied from 3.50 to 7.50, which have been usually used in the mobile phase of HPLC. This phenomenon indicated also that the derivatives of aliphatic amines with TMBB-Su were pH-insensitive and stable. In our study, pH 6.50 was employed.

The effect of the buffer concentration in the mobile phase was also examined. In the range of 10–50 mM, all the derivatives were completely separated in shorter time. Thus, 20 mM of HAc–NaAc buffer was chosen in the following studies.

The optimized gradient elution conditions are summarized in Section 2.4. Under the optimum conditions, the separation of thirteen derivatives of aliphatic amines including methylamine, dimethylamine, ethylamine, *n*-propylamine, *n*-butylamine, diethylamine, *n*-amylamine, *n*-hexylamine, *n*-heptylamine, *n*-octylamine, *n*-nonylamine, *n*-decylamine and *n*-dodecylamine with TMBB-Su was accomplished within 40 min at 25 °C, as is shown in Fig. 2.

3.2. The capability of TMBB-Su for the labeling of secondary aliphatic amines

Most of the activated esters could not react with trace secondary aliphatic amines in the mild derivatization conditions [24]. It was found that TMBB-Su could easily react with dimethylamine at trace level ($<10^{-7}$ M) in the aqueous solution. However, the reaction of TMBB-Su with other secondary amines in the same conditions was hard to proceed. For an example, the reaction concentration of diethylamine was higher than that of dimethylamine for one magnitude. The reaction between activated esters and aliphatic amines is a SN2 nucleophilic substitution. Steric hindrance of substituent group may be the significant influence factors. Compared with other activated esters, the steric hindrance is smaller because the carbon chain between succinimide and fluorophore is longer in TMBB-Su. On the other hand, due to steric hindrance, with the increasing bulk of substituent group on the nitrogen atom, as the nucleophilic reagent, the reaction activity of secondary amines will be decrease [31]. Therefore, the reactivity between dimethylamine and TMBB-Su is the best in the common secondary aliphatic amines.

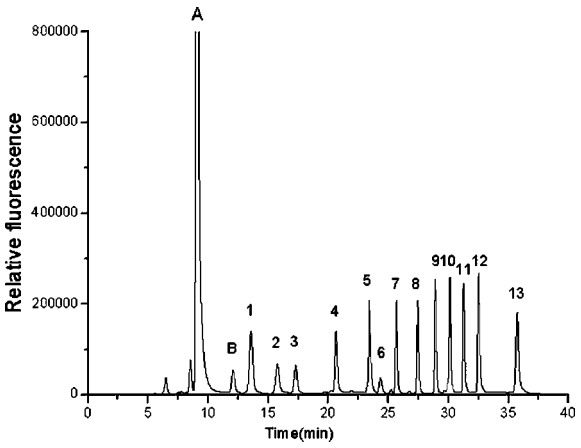

Fig. 2. Separation of TMBB-Su and amine derivatives on column C_8. Mobile phase: as gradient program described in Section 2.4, containing 20 mM HAc–NaAc buffer solution (pH = 6.50). Detection: fluorescence (490/510 nm). Flow rate: 1 mL/min. Injection volume: 20 μL. Standard aliphatic amines concentration: 0.05 μM (diethylamine concentration: 1 μM). Peaks: (A) TMBB, (B) ammonia, (1) methylamine, (2) dimethylamine, (3) ethylamine, (4) *n*-propylamine, (5) *n*-butylamine, (6) diethylamine, (7) *n*-amylamine, (8) *n*-hexylamine, (9) *n*-heptylamine, (10) *n*-octylamine, (11) *n*-nonylamine, (12) *n*-decylamine, and (13) *n*-dodecylamine.

3.3. Orthogonal design experiment for the optimization of derivatization conditions [32]

In pre-column derivatization strategy, the sensitivity and detection limits were partly determined by the derivatization efficiency. For succinimidyl ester labeling, the reaction is usually performed in aqueous media. A competition reaction takes place between the labeling and the hydrolysis, and excess-labeling reagents and alkaline buffer should be employed. As a result, the derivatization efficiency depends upon the amount of labeling reagent, the pH value of buffer, the concentration of buffer, reaction time and temperature. Therefore, these five important factors affecting the derivatization of amines with TMBB-Su were investigated using orthogonal design experiment. A $L_{25}(5^6)$ orthogonal form with a blank column was employed, and the assignments of factors and levels are shown in Table 1. The levels were chosen by referring to the known optimum conditions used in similar derivatization reactions of N-hydroxysuccinimidyl ester labeling reagents with amines in our previous works [2,24,26]. Because the reactivity of diethylamine with TMBB-Su is much less than those of dimethylamine and primary aliphatic amine, diethylamine is excluded in the following optimization of derivatization conditions. All the concentrations of other twelve amines were 0.1 μM. The peak areas of the twelve target analytes under all conditions were considered as the indexes reflecting the derivatization efficiency. The orthogonal design was performed by SPSS software, and all of the data were processed with variance analysis and Duncan multiple test by SPSS software.

In all the target analytes, dimethylamine is more important than other primary aliphatic amines in views of detection interest and derivatization chemistry, its experimental data is shown in Table 1. Accordingly, dimethylamine was taken as the representative of the target analytes for data analysis, and the results of variance analysis were obtained firstly. The significance values of factor A (TMBB-Su concentration), factor B (the pH value of buffer solution), factor C (buffer solution concentration), factor D (reaction temperature) and factor E (reaction time) are 0.037, 0.347, 0.330, 0.041 and 0.549, respectively. The results indicate that the influences of factor A and D on the dimethylamine derivatization reaction are significant (significance value < 0.05), and the others

Table 1
$L_{25}(5^6)$ orthogonal array and the experimental results of DMA.

No.	Factors					Experimental results of DMA
	TMBB-Su concentration (μM)	pH	Buffer solution concentration (mM)	Reaction temperature (°C)	Reaction time (min)	
1	20	7.2	10	15	5	462,852
2	20	7.6	20	20	10	320,517
3	20	8.0	30	25	15	274,804
4	20	8.4	40	30	20	159,478
5	20	8.8	50	35	25	189,607
6	30	7.2	20	25	20	351,686
7	30	7.6	30	30	25	249,350
8	30	8.0	40	35	5	273,244
9	30	8.6	50	15	10	309,103
10	30	8.8	10	20	15	304,413
11	40	7.2	30	35	10	357,921
12	40	7.6	40	15	15	287,268
13	40	8.0	50	20	20	331,427
14	40	8.4	10	25	25	407,269
15	40	8.8	20	30	5	223,376
16	50	7.2	40	20	25	478,436
17	50	7.6	50	25	5	368,830
18	50	8.0	10	30	10	263,374
19	50	8.4	20	35	15	365,710
20	50	8.8	30	15	20	471,686
21	60	7.2	50	30	15	327,272
22	60	7.6	10	35	20	460,257
23	60	8.0	20	15	25	517,920
24	60	8.4	30	20	5	442,592
25	60	8.8	40	25	10	425,943

Table 2
Liner calibration range, regression equation and detection limits of derivatives.

TMBB-amine derivative	Calibration range (μM)	Regression equation[a]	γ	RSD (%) $n=6$		Detection limit[b] (nM)
				Within-day	Between-day	
Methylamine	0.002–0.2	$Y=8154.4+3.044\times10^{12}X$	0.9998	3.9	4.3	0.02
Dimethyl amine	0.004–0.2	$Y=223126.7+2.054\times10^{12}X$	0.9911	2.7	3.1	0.04
Ethylamine	0.004–0.2	$Y=216860.2+2.378\times10^{12}X$	0.9910	1.7	3.5	0.04
n-Propylamine	0.001–0.2	$Y=128191.3+4.972\times10^{12}X$	0.9977	1.3	3.2	0.01
n-Butylamine	0.002–0.2	$Y=11337.6+4.634\times10^{12}X$	0.9984	2.8	3.2	0.02
Diethylamine	0.05–5	$Y=291668.3+4.716\times10^{11}X$	0.9954	1.4	1.8	10
n-Amylamine	0.001–0.2	$Y=71216.7+5.415\times10^{12}X$	0.9992	1.6	3.9	0.01
n-Hexylamine	0.001–0.2	$Y=39950.1+5.903\times10^{12}X$	0.9978	3.5	3.8	0.01
n-Heptylamine	0.001–0.2	$Y=20271.5+6.394\times10^{12}X$	0.9982	1.7	2.8	0.01
n-Octylamine	0.001–0.2	$Y=6116.6+5.590\times10^{12}X$	0.9982	1.5	3.4	0.01
n-Nonylamine	0.001–0.2	$Y=30052.0+6.036\times10^{12}X$	0.9983	2.1	3.7	0.01
n-Decylamine	0.001–0.2	$Y=35790.1+5.974\times10^{12}X$	0.9986	1.7	2.8	0.01
n-Dodecylamine	0.002–0.2	$Y=28531.9+5.959\times10^{12}X$	0.9980	1.2	2.3	0.02

[a] X: concentration of amine (M); Y: peak area of amine derivatives.
[b] $S/N=3$, per 20 μL injection volume.

have no significant effect. The influence order of each factor on the derivatization of dimethylamine is A > D > C > B > E. The variance analysis was performed with the other eleven primary aliphatic amines. Similar results are obtained for *n*-propylamine in terms of significant factor (A and D). For *n*-amylamine, *n*-hexylamine, *n*-heptylamine, *n*-octylamine and *n*-nonylamine, factor A is significant, and there is no significant factor in the rest of aliphatic amines. For eleven primary aliphatic amines, the influence order of each factor is A > D > B > E > C.

Duncan's multiple range test was performed for evaluating the effect of every factor on the derivatization of twelve target analytes with TMBB-Su, and the results for dimethylamine as a model were interpreted. For TMBB-Su concentration, from 20 μM to 60 μM, their peak areas are 54125.4, 57223.0, 61817.8, 74924.6, 83616.0, and 60 μM is better than other levels. The other factors could be dealt in the same way. For the pH value of buffer solution, buffer solution concentration, reaction temperature and reaction time, pH 7.20, 10 mM H_3BO_3–$Na_2B_4O_7$ buffer solution, 15 °C and

Table 3
Comparison of the derivatization condition and detection limit of the reagents reported for dimethylamine.

Reagent	Ex (nm)/Em (nm)	Derivatization time (min)	Derivatization temperature	Detection limit (nM)	Ref.
FMOC	264/313	30	Room temperature	110	[33]
	265/310	2	Room temperature	45	[34]
FITC	488/520	Overnight	21 °C	1	[35]
NPA-OSu	353/422	20	Room temperature	0.8	[2]
TMBB-Su	490/510	25	15 °C	0.04	This work

25 min are better, respectively. It was the same for methylamine. As for the rest of primary aliphatic amines, the only difference from dimethylamine was buffer solution concentration, which was not a significant (significance value > 0.05) factor. Consequently, the optimized derivatization reaction was performed at 15 °C for 25 min using 60 μM TMBB-Su and 10 mM pH 7.20 H_3BO_3–$Na_2B_4O_7$ buffer solution.

3.4. Interference

Ammonia derivative with TMBB-Su was investigated under the chosen chromatographic conditions. It could be well separated from the reagent hydrolysates and other aliphatic amines derivatives. In the biological samples, the potential interferences come from amino acids which readily react with succinimidyl ester. The chromatographic behaviors of the TMBB-Su derivatives with amino acids (alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, serine, threonine, lysine, cystine, cysteine, tryptophan, glycylglycine and glutathione) were carefully examined. Under the chosen chromatographic conditions, the retention times of amino acid derivatives are much shorter than those of aliphatic amines derivatives and amino acids cannot become interferences in the analysis of aliphatic amines. In addition, aromatic amines and alcohols cannot make trouble in the detection of aliphatic amines because TMBB-Su does not react with them under the chosen optimal reaction conditions.

3.5. Stabilities of TMBB-Su and its derivatives

Stability study was carried out. As observed, after an anhydrous acetonitrile solution of TMBB-Su has been stored at 4 °C in darkness for two weeks, the derivatization yields of aliphatic amines with TMBB-Su barely change. If the formed derivatives are stored at 4 °C or room temperature in darkness, they are stable with the variation of peak areas within 2.0% (n = 6) for one week.

3.6. Analytical calibration

Under the optimized derivatization and separation conditions, a test mixture of thirteen aliphatic amines with different concentrations (0.001–0.2 μM) was analyzed for the quantification. The linear calibration ranges, regression equations, and detection limits of these aliphatic amines were calculated. The results are listed in Table 2. The correlation coefficients are in the range of 0.9910–0.9998, and the relative standard deviations (RSDs) are from 1.2% to 3.9% for within-day determination (n = 6) and from 2.3% to 4.3% for between-day determination (n = 6). The detection limits (S/N = 3) for the labeled aliphatic amines range from 0.01 nM to 0.04 nM, which are sufficiently sensitive for the determination of trace amount aliphatic amines in biological samples and better than those obtained by the existing HPLC methods based on aliphatic amines labeling [2,33–35], as shown in Table 3.

3.7. Sample analysis

As an application of the developed method, the determination of thirteen aliphatic amines in the mice tissues (heart, liver and kidney) has been performed. Since nitrosodimethylamine has extensive liver and kidney damage, they were chosen as the targets for the determination of dimethylamine. The spiking experiments were employed to evaluate the accuracy of this proposed method. The chromatograms of the samples unspiked and spiked with the standard solutions are shown in Fig. 3. The analytical results are summarized in Table 4. From the results, four short-chain aliphatic amines (methylamine, dimethylamine, ethylamine and n-propylamine) were found in the samples. The contents of

Table 4
Analytical results for the mice organ samples.

Samples	Heart tissue				Liver tissue				Kidney tissue			
	Added (μg/L)	Found (μg/L)	RSD (%, n = 6)	Recovery (%)	Added (μg/L)	Found (μg/L)	RSD (%, n = 6)	Recovery (%)	Added (μg/L)	Found (μg/L)	RSD (%, n = 6)	Recovery (%)
Methylamine	0	0.21	1.2		0	0.20	3.4		0	0.71	2.8	
	3.1	3.51	2.4	106.5	3.1	3.40	2.8	103.2	3.1	3.78	2.3	98.9
Dimethylamine	0	0.87	0.9		0	2.16	1.9		0	3.08	0.7	
	4.5	5.29	2.8	98.2	4.5	6.44	2.1	95.1	4.5	7.66	2.9	101.7
Ethylamine	0	0.77	1.3		0	1.08	0.7		0	0.95	1.8	
	4.5	5.31	3.1	100.8	4.5	5.42	2.8	96.5	4.5	5.40	2.3	99.0
n-Propylamine	0	0.57	2.3		0	0.68	2.1		0	0.81	2.6	
	5.9	6.21	0.7	95.5	5.9	6.49	0.9	98.5	5.9	6.76	0.5	100.9
n-Butylamine	0	0			0	0			0	0		
	7.3	7.5	2.6	102.7	7.3	7.8	2.8	106.8	7.3	7.2	2.3	98.6
n-Amylamine	0	0			0	0			0	0		
	8.7	8.3	1.8	95.4	8.7	8.5	1.2	97.7	8.7	8.8	2.5	101.1
n-Hexylamine	0	0			0	0			0	0		
	10.1	9.7	1.4	96.0	10.1	10.2	1.6	101.0	10.1	9.99	1.9	98.9
n-Heptylamine	0	0			0	0			0	0		
	11.5	11.3	1.1	98.3	11.5	11.3	0.9	98.3	11.5	11.6	1.4	100.9
n-Octylamine	0	0			0	0			0	0		
	12.9	12.8	1.6	99.2	12.9	13.3	1.9	103.1	12.9	12.3	2.0	95.3
n-Nonylamine	0	0			0	0			0	0		
	14.3	14.2	0.5	99.3	14.3	14.4	0.9	100.7	14.3	14.1	0.7	98.6
n-Decylamine	0	0			0	0			0	0		
	15.7	15.3	1.8	97.5	15.7	15.5	2.5	98.7	15.7	16.0	2.2	101.9
n-Dodecylamine	0	0			0	0			0	0		
	18.5	18.1	2.6	97.8	18.5	18.2	1.3	98.4	18.5	17.7	1.7	95.7

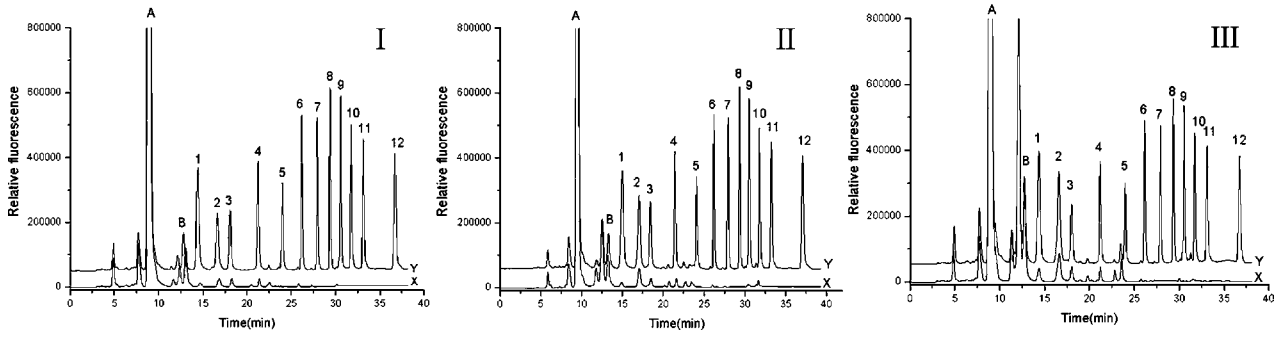

Fig. 3. Chromatograms obtained from samples.
Chromatographic conditions. Peaks: (A) TMBB, (B) ammonia, (1) methylamine, (2) dimethylamine, (3) ethylamine, (4) *n*-propylamine, (5) *n*-butylamine, (6) *n*-amylamine, (7) *n*-hexylamine, (8) *n*-heptylamine, (9) *n*-octylamine, (10) *n*-nonylamine, (11) *n*-decylamine, and (12) *n*-dodecylamine. (I) Chromatograms obtained from (X) heart, and (Y) the same sample spiked with 0.1 μM of standard amines, (II) chromatograms obtained from (X) liver, and (Y) the same sample spiked with 0.1 μM of standard amines, and (III) chromatograms obtained from (X) kidney, and (Y) the same sample spiked with 0.1 μM of standard amines.

dimethylamine in the liver and kidney are obviously higher than those in the heart. The recoveries range from 95.1% to 106.8% and the RSDs vary from 0.5% to 3.4%.

4. Conclusions

This work provides a simple and sensitive method for the determination of trace aliphatic amines in biological samples based on the derivatization using TMBB-Su, which has been synthesized in our lab. Under the optimum derivatization and separation conditions, the detection limits are in the range of 0.01–0.04 nM (signal-to-noise ratio = 3), which are better than the reported HPLC methods for the analysis of aliphatic amines. Moreover, the simultaneous determination of primary and secondary aliphatic amines has been achieved and the results indicate that the proposed method has a hopeful potential in the detection of a variety of aliphatic amines in biological samples.

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