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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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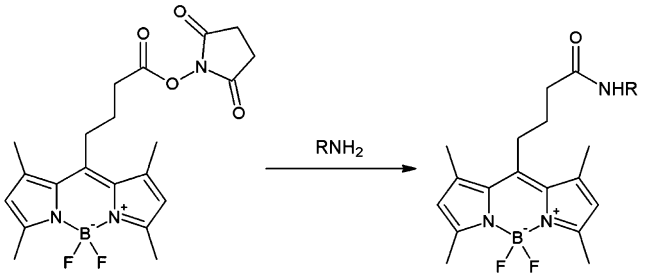

Fig. 1. The derivatization reaction of TMBB-Su with aliphatic amines.

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