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Determination of aliphatic amines by high-performance liquid chromatography-amperometric detection after derivatization with naphthalene-2,3-dicarboxaldehyde

Sushma Lamba, Arti Pandit, Sunil Kumar Sanghi, V. Sorna Gowri, Ankita Tiwari, Vishal Kumar Baderia, Deepesh Kumar Singh, Preeti Nigam*

Microfluidics and MEMS Centre, Advanced Materials and Processes Research Institute, Bhopal 462026, India

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ABSTRACT

A simple and sensitive liquid chromatographic method has been developed for the determination of low molecular weight aliphatic amines after their pre-column derivatization with naphthalene-2,3-dicarboxaldehyde (NDA). Derivatization conditions, including the NDA concentration, reaction pH and reaction time have been investigated for method optimization. The chromatographic separation of five amines was performed on ABZ PLUS column using mobile phase of methanol–water (80:20, v/v) at a flow rate of 0.2 mL min^{-1}. The detection was carried out with a 6 mm glassy carbon electrode at the applied potential of 0.7 V *versus* Ag/AgCl reference electrode. The detection limits were between 23.3 and 34.4 nmol L^{-1} of amines with a sample injection volume of 2 μL. The present method was applied for the determination of aliphatic amines in lake water. The recovery ranged 52.2–127.9%. The RSD in analytes retention time was less than 0.3% and 2.4% for intra- and inter-day analyses, respectively. The RSD in peak area was below 5.8% for both intra-day and inter-day analyses. The total analysis was completed within 20 min.

1. Introduction

Low molecular weight (LMW) aliphatic amines are an important group of compounds that are derived in nature through degradation of organic matter such as proteins, amino acids and other nitrogen containing compounds. Animal wastes and microbial activities are also assumed to contribute towards the aliphatic amine concentration in aqueous bodies [1]. They are emitted in air through vehicle exhaust and tobacco smoke. Amines are used as intermediates in manufacturing of a wide range of industrial chemicals [2], pharmaceuticals, polymers, pesticides, dyestuffs and corrosion inhibitors [3]. Most of the aliphatic amines have unpleasant smell and are toxic and irritant to skin, mucous membranes and respiratory tract. They react with nitrozating agents to form carcinogenic *N*-nitrosamines [4–6]. Therefore, monitoring of LMW amines is important to protect human health and the environment. However, their high polarity, volatility, basicity and solubility in water make them stubborn candidates for detection at low concentration [2].

There are several methods available for the determination of LMW aliphatic amines in environmental samples mostly involving GC and mass spectrometric [7–9] or flame ionization detection [10–12]. However, direct GC analysis of amines

* *Corresponding author*. Tel.: +91 755 2489402; fax: +91 755 2488323.
E-mail address: sanghisk@gmail.com (S.K. Sanghi).
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is difficult due to their highly polar nature that gives rise to tailing peaks, ghosting phenomenon and decreased sensitivity [6,13,14]. Alternative methods have been developed using thin-layer chromatography [15,16], spectrofluorimetry [17], high-performance liquid chromatography (HPLC) [18–21], capillary electrophoresis [22] and capillary electrochromatography [23]. However the overall methods for quantification of these compounds at trace level are complex. Multiple extraction and sample enrichment are required to achieve good analyte recoveries. In addition, aliphatic amines lack native sensitivity to common LC detectors such as UV, fluorescence and electrochemical [24,25].

For these reasons, chemical derivatization or labeling becomes a necessary procedure to transform the analytes into derivatives that can be more easily isolated, separated and detected [26]. Thus for sensitive determination of primary aliphatic amines, many derivatization agents have been developed including o-phthalaldehyde (OPA) [27,28], dansyl chloride (Dns-Cl) [29], 9-fluorenylmethyl chloroformate (FMOC) [30], fluorescamine (fluram) [31], *N*-hydroxysuccinimidyl fluorescein-O-acetate (SIFA) [32], 1-naphthylisothiocynate [33], 4-fluoro-7-nitrobeno-2-oxa-1,3-diazole (NBD-F) [34] and phenylisothiocynate [26].

Electrochemical detection techniques such as amperometry and potentiometry are gaining importance due to their low cost and simple instrumentation required and the sensitivity that could be attained in the analysis of electroactive compounds such as catecholamines [35] and aromatic amines [36]. The use of metallic copper electrode as potentiometric detector has been successfully shown for the analysis of aliphatic amines after LC [37] and flow injection analysis [38]. A few labeling reagents have been explored for amperometric detection of amines. Santagati et al. [39] investigated the reaction of aliphatic amines with 2,5-dihydroxybenzaldehyde to give Schiff base derivatives, which were electroactive. Amperometric detection has also been done after derivatization of amines with phenylisothiocynate [40] and salicylic acid chloride [41]. These methods suffer from the limitation of prolonged reaction time.

Naphthalene-2,3-dicarboxaldehyde (NDA) has been known to react with primary amines in presence of cyanide to produce *N*-substituted 1-cynobenz[*f*]isoindole (CBI) derivatives which are both fluorescent [42,43] and electroactive [44]. Conventional microchip and CE measurements of amino acids following their NDA derivatization have also been reported [45,46]. The electroactive properties of NDA have also been utilized for the determination of aliphatic diamines by capillary electrophoresis [47].

In the present work, we proposed a simple, sensitive and selective HPLC method for the determination of five aliphatic amines by their pre-column derivatization with NDA and amperometric detection. The derivatization process is fast and requires minimal consumption of solvents. The detection limit is in low nmol range which is comparable or better than that reported by existing electrochemical detection methods. The proposed method was applied to the determination of aliphatic amines in lake water. To the best of our knowledge there is no method available describing the electrochemical detection of aliphatic amine with NDA derivatization.

2. Materials and methods

2.1. Instrumentation

High-performance liquid chromatography was carried on a system equipped with a Hitachi L-7110 pump (Merck, Darmstadt, Germany), a Rheodyne 9725 injector and a Supelcosil ABZ PLUS (100 mm × 2.1 mm; particle size 5 μm) column (Supelco, Bellefonte, USA). A Unijet Radial Cell (Bioanalytical Systems, West Lafayette, IN, USA) was used for electrochemical detection in conjunction with LC-3D potentiostat (Bioanalytical Systems). Electrochemical cell consisted of a three-electrode system of 6 mm glassy carbon working electrode and Ag/AgCl pseudo-reference electrode housed in a peek block; the stainless steel half of the electrode served as a counter electrode. Amperometric detection was performed at a constant potential of 0.7 V *versus* Ag/AgCl reference electrode. The acquisition and analysis of chromatographic data were performed using DAx version 7.1 data acquisition software (Prince Technologies, CD-Emmen, The Netherlands).

2.2. Chemicals and reagents

1-Propylamine and 1-pentylamine were obtained from Fluka (Buchs, Switzerland), 1-hexylamine, 1-heptylamine and 1-octylamine from Acros Organics (Springfield, NJ, USA), and they were used as such. Their stock solutions, 10 mmol L^{-1}, were prepared in HPLC grade methanol (Ranbaxy, Ropar, India). Working standards were prepared by mixing aliquots of the stock solutions and diluting with methanol. The stock and working standards were stored in dark at 4 °C when not in use. Naphthalene-2,3-dicarboxaldehyde (NDA) was obtained from Fluka and its solution was prepared in methanol, 20 mmol L^{-1}, on weekly basis and refrigerated when not in use. Phosphate buffer, pH 7, was prepared by adding 7.7 mL of 100 mmol L^{-1} di-sodium hydrogen phosphate (E. Merck, Mumbai, India) and 12.26 mL of 100 mmol L^{-1} sodium di-hydrogen phosphate (E. Merck) and made up to 100 mL with Milli-Q water (Millipore, Bedford, MA, USA). Mobile phase was prepared by mixing HPLC grade methanol (Ranbaxy) and Milli-Q water in 80:20 (v/v) ratios. A 4 mL portion of 100 mmol L^{-1} potassium chloride (E. Merck) was included in the aqueous phase of the mobile phase so the final KCl concentration was 4 mmol L^{-1}. The mobile phase was prepared daily and filtered through a 0.45 μm nylon filter (Filtech Pharma Lab, Mumbai, India) prior to use. All other chemicals used were analytical reagent grade. Other solutions were prepared in doubly distilled water and filtered through a 0.45 μm nylon membrane filter.

2.3. Derivatization procedure

A 30 μL portion of mixed amines solution containing 10^{-4} mol L^{-1} each of propylamine, pentylamine, hexylamine, heptylamine and octylamine, 10 μL of 20 mmol L^{-1} phosphate buffer (pH 7), 10 μL of 10 mmol L^{-1} cyanide and 20 μL of 20 mmol L^{-1} NDA solution in methanol were added in sequence and mixed thoroughly. The derivatization mixture was allowed to stand for 5 min at ambient temperature prior to the injection. The order of addition of reagents, as mentioned,