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Vortex-assisted liquid–liquid microextraction coupled with derivatization for the fluorometric determination of aliphatic amines

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ABSTRACT

A new one-step derivatization and microextraction technique was developed for the fluorometric determination of C_1–C_8 linear aliphatic primary amines in complex sample solutions containing high levels of amino acids. In this method, amines were derivatized with o-phthalaldehyde (OPA) and 2-mercaptoethanol (2-ME) in aqueous solution and extracted simultaneously by vortex-assisted liquid–liquid microextraction (VALLME). Parameters affecting the extraction efficiency were investigated in detail. The optimum conditions were as follows: 50 μL of isooctane as the extractant phase; 2.0 mL aqueous donor samples with 12 mM OPA, 24 mM 2-ME, and 0.1 M borate buffer at pH 10; 1 min vortex extraction time; centrifugation for 4 min at 6000 rpm. After centrifugation, the enriched analytes in the floated extractant phase were determined by HPLC–FL in less than 14 min. Under the optimum conditions, the limits of detection were of the order of 0.09–0.31 nM. The calibration curves showed good linearity over the investigated concentration range between 0.4 and 40 nM. The proposed method has been applied to the determination of aliphatic amines in acidophilus milk, beer, and Cu(II)/amino acid solution.

1. Introduction

Low molecular weight aliphatic primary amines have received much attention as pollutants and as reaction intermediates in the environment [1–9]. They are ubiquitous degradation products from natural and industrial sources such as amino acids, proteins, and other nitrogen-containing organic compounds. The presence of amines in food and beverages also deserves attention. Quantification of some amines has been used in the quality control of food and beverages as important indicators of unhygienic production conditions [10–14]. On the contrary, aliphatic primary amines of two to five carbons found in tea, wine, vegetables, fruits, and bacteria are gaining importance due to their proven immunity benefits [15–17]. Therefore, the analysis of aliphatic amines is an important task to protect human health and the environment.

The direct determination of aliphatic amines at trace level in complex matrices is difficult due to their high volatility and polarity, chemical instability, and the lack of intrinsic chromophores. To overcome these problems, several methods coupled with chemical derivatization have been developed [2–9,18]. High-performance liquid chromatography (HPLC) with o-phthalaldehyde and 2-mercaptoethanol (OPA/2-ME) derivatization is one of the most popular and sensitive methods to separate and determine amino acids and amines [18], which can quickly form strong fluorescent isoindoles at room temperature. However, the reaction products are very unstable [19]. The reactions of the OPA derivatives of amines with four different thiol-group containing additives (2-ME, 3-mercaptopropionic acid, N-acetyl-L-cysteine, and ethanethiol) have been studied and discussed in detail [20,21]. Several new reagents with different fluorophores or reactive functional groups for the derivatization of amines have been designed and compared with the established OPA method [3,7].

Amino acids and amines are co-existing compounds in several biological and food matrices in overwhelming concentration ratios. Although several HPLC methods for the simultaneous analysis of amino acids and amines without any preliminary extraction have been reported since 1978, it is always a challenge to find the optimum derivatization and chromatographic conditions for amino acids and amines in complex matrices [12,22–24]. Moreover, each single analytical run takes at least 1 h.

Dispersive liquid–liquid microextraction (DLLME) has drawn great attention since its introduction in 2006 [25–27]. This method is based on a ternary component solvent system in which the extraction solvent and disperser solvent are quickly injected into an aqueous sample matrix to form an emulsified solution. Since the extractant is highly dispersed in the aqueous phase, extraction equilibrium can be quickly achieved within a few seconds. DLLME has been successfully applied in extraction and concentration of

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polycyclic aromatic hydrocarbons (PAHs) [25,28], organophosphorus pesticides (OPPs) [29], chlorophenols (CPs) [30], chlorophenoxy acid herbicides [31], and even metal ions [32], mainly from water samples. However, the selection of extraction and disperser solvents is limited and highly depends on analytes. The use of a disperser solvent as the third component usually decreases the partition coefficient of analytes into the extraction solvent. The use of ultrasound as a means for improving liquid–liquid extraction has been reported to speed up the emulsification step [33–35]. However, its main drawbacks include the fast decline of power, the lack of uniformity in the transmission, and the potential for analyte degradation. Vortex mixing as a mild emulsification step has been used as an alternative to disperse the extraction solvent into the aqueous matrix. A new and fast equilibrium-based solvent microextraction technique termed vortex-assisted liquid–liquid microextraction (VALLME) has recently been developed and used for the trace analysis of alkylphenols [36].

The combination of derivatization and extraction in one step can simplify the procedure and reduce the analysis time. Although simultaneous derivatization and extraction of primary short-chain aliphatic amines in water samples has been reported using DLLME based on solidification of floating organic droplet, dynamic hollow fiber liquid-phase microextraction, or an ultrasonic-mixed water-ionic liquid two-phase system [9,37,38], it has not been used in the analysis of amines in complex solutions containing high levels of amino acids. In this work, a new one-step method based on VALLME and OPA/2-ME derivatization was developed. This method has two major advantages: the excellent stabilization of OPA derivatives of amines and the elimination of amino acid interference. Factors affecting the extraction efficiency were investigated and optimized. The method was applied to the analysis of amines in beverage samples and amine photoproducts in Cu(II)/amino acid complex systems where photoproducts of ammonia and aldehydes were investigated in previous studies [39,40].

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical or reagent grade, or the highest purity available from several suppliers and were used as received. Methylamine hydrochloride, n-decane, n-tetradecane, 1-decanol were purchased from Alfa Aesar (Lancashire, UK). Ethylamine hydrochloride was purchased from Chemservice (West Chester, PA). 1-Propylamine, 1-butylamine, 1-pentylamine, 1-hexylamine, 1-heptylamine, and 1-octylamine were from TCI (Tokyo). Sodium chloride (>99.8%), sodium dihydrogen phosphate 2-hydrate, and potassium chloride (>99.5%) were obtained from Riedel-deHaën (Seelze, Germany). Acetate acid (>99%), 2-mercaptoethanol (99.0%), o-phthalaldehyde (>99%), boric acid (>99.8%), and chloroform were purchased from Merck (Darmstadt, Germany). Sodium tetraborate (>99%) and sodium acetate (>99%) were from Sigma–Aldrich (St. Louis, MO). Sodium hydroxide, hydrochloric acid, n-hexane, 1-octanol, methanol (>99.5%), and acetonitrile (>99.9%) were from J. T. Baker (Phillipsburg, NJ). 20 common amino acids in L-form (>99.0%) and β-alanine were purchased from Fluka (Buchs, SG, Switzerland). Various brands of beverage samples were purchased from local stores.

2.2. Sample preparation

Stock solutions of C_1–C_8 linear aliphatic primary amines were prepared in methanol and deionized water, each in 5 μM. The stock solutions were stored at 4 °C. The standard solutions of amines were diluted to proper concentrations (each 0.4, 5.0, 10, 20, 30, 40 nM). Low-level standards were used immediately after preparation. Doubly deionized water prepared with a Milli-Q system (Millipore, Bedford, MA, USA) was used exclusively for all solutions (≥18.2 MΩ-cm resistivity). *o*-Phthalaldehyde (OPA) derivatizing reagent was prepared by dissolving 0.0536 g of OPA in 0.4 mL of methanol, then added 56 μL of 2-mercaptoethanol (2-ME). The solution was filtered through a conditioned C_{18} extraction cartridge (Oasis, Waters, USA) to remove impurities. While OPA and 2-ME stayed in the cartridge, borate buffer (0.1 M, pH 10) was used to elute them out to 4.0 mL. The final concentrations of OPA and 2-ME in derivatizing reagent were 100 and 200 mM, respectively. The reagent was stored in the dark (in a brown bottle and wrapped with aluminum foil) in the refrigerator and was stable for several weeks. 0.025 M of sodium acetate buffer was prepared by dissolving 0.92044 g of NaOAc in ca. 450 mL water, then 69 μL of 99% CH_3COOH was added and graduated to 500.0 mL. The final pH value of the solution was 5.7. The solution was stored in the refrigerator and filtered through a 0.45 μm membrane to remove impurities before use. The pH of sample solutions was adjusted by adding aliquots of 1 M or 0.1 M NaOH to the desired pH. The pH of the buffer was checked periodically and readjusted when necessary.

Real samples were diluted tenfold with deionized water. After centrifuging at 6000 rpm for 10 min, the supernatant was collected and filtered through a 0.45-μm membrane. 250 μL of the filtered solution was carefully withdrawn and injected in 1.51 mL of 0.1 M borate buffer at pH 10. Aliquots of 1.76 mL of the solution were prepared for further VALLME and derivatization.

2.3. Apparatus

The binary gradient HPLC system consisted of the following components connected in series: two microvolume double-plunger pumps (LC-10AD and LC-10ATvp, Shimatzu, Kyoto, Japan), a Rheodyne Model 7125 (Cotati, CA, USA) injector, a guard column and a Xterra ODS C18 column (150 mm × 4.6 mm; 5 μm particle size, Waters), and a variable-wavelength fluorescence detector (FL) equipped with a xenon lamp (RF-10AXL, Shimatzu). The data were collected and processed by a chromatographic data station software (SISC 32.3.1, Taiwan).

Ultraviolet–visible absorbance measurements were made with a Varian Cary 50 spectrophotometer and a custom-built constant-temperature (25 °C, BL-20, TIT recirculator) variable-path-length aluminum cuvette holder (black-anodized). Spectral characteristics of the OPA derivatives of amines were made with a Varian Cary Eclipse fluorescence spectrophotometer. Solution pH was measured with a Radiometer analytical Ioncheck 45 meter and combination glass electrode (Mettler Toledo Inlab 439/120).

2.4. VALLME and derivatization procedure

The experimental setup of the one-step derivatization and microextraction is illustrated in Fig. 1. In the simultaneous VALLME and derivatization, 1.76 mL of sample solution containing target amines and amino acids in 0.1 M borate buffer at pH 10 was placed in a 2.5 mL conical bottomed tube and 50 μL of isooctane was then slowly added to the sample solution. After that, 0.24 mL of derivatizing reagent prepared with 100 mM OPA and 200 mM 2-ME was injected rapidly, then the tube was sealed by a snap cap with Teflon-faced silicone septum and shaken by hand for 10 s. The mixture was then vigorously shaken using a vortex agitator (Thermolyne Maxi Mix II 37600 Mixer) at 3000 rpm for 1 min. As a result, a cloudy solution was formed. In this step, only amines derivatized with OPA/2-ME were extracted into the fine isooctane droplets. Then, the organic solvent droplets were separated and floated on the surface of the aqueous solution by centrifugation at 6000 rpm for 4 min (Beckman Coulter Microfuge 18 Centrifuge). The water