Journal of Chromatography A, 1282 (2013) 1–10

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

An *in situ* benzoylation-dispersive liquid–liquid microextraction method based on solidification of floating organic droplets for determination of biogenic amines by liquid chromatography–ultraviolet analysis

Shaodong Jia [b], Yeonsuk Ryu [a], Sung Won Kwon [b,*], Jeongmi Lee [a,**]

[a] *School of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi-do 440-746, Republic of Korea*
[b] *College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea*

ARTICLE INFO

Article history:
Received 4 October 2012
Received in revised form
24 December 2012
Accepted 8 January 2013
Available online 16 January 2013

Keywords:
Biogenic amine
In situ derivatization
Benzoylation
Dispersive liquid–liquid microextraction
Solidification of floating organic droplets
Alcoholic beverage

ABSTRACT

A novel analytical method consisting of *in situ* derivatization combined with liquid phase microextraction followed by liquid chromatography–ultraviolet detection (LC–UV) was developed to determine the biogenic amines (BAs) of alcoholic beverages. Nine BAs (putrescine, cadaverine, 1,3-diaminopropane, tryptamine, phenylethylamine, spermidine, spermine, histamine, and tyramine) were derivatized *in situ* with benzoyl chloride, extracted by dispersive liquid–liquid microextraction based on solidification of floating organic droplets (DLLME-SFO), and then chromatographed by LC–UV. Factors influencing the derivatization and extraction efficiency were optimized, including the reaction buffer pH and concentration, amount of derivatization reagent, reaction time, types and volumes of extraction and dispersive solvents, and extraction time. Under the optimized conditions, the method was linear over 0.05–8.0 μg mL^{-1} with an $r^2 \geq 0.992$ and exhibited intra- and inter-day precision less than 8.8% and 11.5%, respectively. The limit of detection ranged between 0.005 and 0.01 μg mL^{-1}. The developed method using a basic LC–UV system is sensitive, rapid, convenient, green, and cost-effective. Moreover, it is versatile and practical for the analysis of BAs, as demonstrated by the successful application in four different types of popular alcoholic beverages (white wine, red wine, rice wine, and beer).

1. Introduction

Biogenic amines (BAs), small organic compounds with aliphatic, aromatic, or heterocyclic structures, are found in many foods and alcoholic beverages [1], and are mainly produced by decarboxylation of amino acids and transamination of aldehydes and ketones by microorganisms in foods [2]. BAs can be markers for levels of microbiological food contamination as their concentrations increase during fermentation or spoilage [3]. Although BAs play an essential role in physiology, including normal cell growth and development [4], they may cause harmful biological reactions by directly or indirectly affecting the vascular and nervous system [5]. Moreover, some BAs can be precursors to carcinogenic nitrosamines [1]. The activities of some enzymes specifically devoted to converting BAs to non-toxic products could be inhibited by ethanol [6]. Hence, it is important to monitor BA levels in foods and alcoholic beverages [1,7].

In order to monitor BA levels, various analytical methods have been developed including thin layer chromatography (TLC) [8], gas chromatography (GC) [9], capillary electrophoresis (CE) [10], and liquid chromatography (LC) [5]. Among these methods, LC has been considered the most reliable due to its convenience, high sensitivity, and applicability [5]. As many BAs are polar basic compounds lacking chromophores, derivatization is usually required for LC analysis. Several derivatization reagents including dansyl chloride, dabsyl chloride, benzoyl chloride, and *o*-phthaldialdehyde have been used [11].

Derivatization of BAs usually requires a clean-up step such as extraction prior to chromatographic separation, depending on the derivatization method used. Liquid–liquid extraction (LLE) is the most common extraction method [9,12–14] although this method is labor-intensive, requires large volumes of organic solvents, and generally takes a long time. Other extraction procedures including solid-phase extraction (SPE) [5] and cloud point extraction (CPE) [15] have also been reported as efficient clean-up procedures. Recently, microextraction, a miniaturized extraction method, has been extensively investigated because the very small amounts of solvent and sample required make it a more eco-friendly analytical method [16]. A solvent-free method known as solid phase microextraction (SPME) is a reliable sample preparation method

* Corresponding author. Tel.: +82 2 880 7844; fax: +82 2 886 7877.
** Corresponding author. Tel.: +82 31 290 7784; fax: +82 31 290 7723.
E-mail addresses: swkwon@snu.ac.kr (S.W. Kwon), jlee0610@skku.edu, jlee0610@gmail.com (J. Lee).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.01.041

for BA analysis [4]. However, it has some drawbacks such as high cost, short life-span for its extraction fiber, and issues with sample carry-over [17].

A number of other methods are classified as solvent-minimized extraction or liquid phase microextraction (LPME). Dispersive liquid–liquid microextraction (DLLME), which employs a ternary solvent system consisting of an aqueous sample, extractant, and disperser, is a recent LPME method [18]. It has been extensively explored during the past few years because of its simplicity, rapidity, convenience, and low cost [19]. A modified version of DLLME, DLLME-SFO, based on the solidification of floating organic droplets [20–24], has recently gained popularity as it uses low-density, less toxic organic solvents and provides more precise and accurate extraction.

More recently, there has been a growing interest in integrating the two steps of LPME and derivatization, followed by chromatographic separation by either LC or GC for the analysis of different kinds of compounds [22,25–30]. These methods not only simplified the experimental procedures, but also decreased sample loss and increased method sensitivities. There have been only a limited number of reports using integrative approaches in BA analysis. The Huang group has developed both ultrasonic-assisted DLLME and ionic liquid-based ultrasonic-assisted LLME method, in combination with 2,6-dimethyl-4-quinolinecarboxylic acid N-hydroxysuccinimide ester (DMQC-OSu) derivatization for LC-fluorescence (FL) detection [26,29]. These methods required a relatively long derivatization time (~40 min), the number of determined BAs was quite small (three BAs), and most importantly, the derivatizing agent is not commercially available.

In this study, benzoylation was employed as the derivatization method because it allows *in situ* derivatization in an aqueous solution in a relatively short time and it utilizes benzoyl chloride, an inexpensive and readily available reagent [13]. LC–UV analysis of BAs based on benzoylation normally requires LLE for workup and subsequent evaporation to dryness and re-dissolution before analysis, and these steps are tedious, labor-intensive, and time-consuming. In the present study, experimental conditions allowing the *in situ* benzoylation of BAs combined with the DLLME-SFO technique were optimized. Factors influencing the derivatization efficiency, including reaction buffer pH and concentration, amount of derivatization reagent, and reaction time, were optimized. The benzoylated products were extracted and chromatographed by DLLME-SFO and LC–UV, respectively. Factors affecting the extraction efficiency, including types and volumes of extraction and dispersive solvents, and extraction time, were optimized. The developed method was then validated in terms of linearity, precision, accuracy, and limit of detection (LOD). The method was applied to real samples, *i.e.*, four popular types of alcoholic beverages (white wine, red wine, rice wine, and beer). To the best of our knowledge, this is the first report of *in situ* benzoylation combined with a DLLME technique for analysis of a relatively large number of BAs in alcoholic beverages.

2. Experimental

2.1. Chemicals, standard solutions, and samples

Putrescine dihydrochloride (PUT), cadaverine dihydrochloride (CAD), 1,3-diaminopropane dihydrochloride (DAP), tryptamine hydrochloride (TRP), 2-phenylethylamine hydrochloride (PEA), spermidine trihydrochloride (SPD), norspermidine (internal standard, IS), histamine hydrochloride (HIS), tyramine hydrochloride (TYR), and all other reagents including boric acid, sodium hydroxide, and benzoyl chloride were purchased from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise noted. Spermine tetrahydrochloride (SPM), 1-undecanol, and 2-dodecanol were from TCI (Tokyo, Japan). 1-Dodecanol and sodium chloride were obtained from Daejung Chemical (Siheung, Korea) and Merck (Darmstadt, Germany), respectively. Acetone and acetonitrile were purchased from Duksan (Ansan, Korea). LC grade methanol and water were from J.T. Baker (Philipsburg, NJ, USA).

Standard stock solutions were prepared by dissolving each standard in ultra-pure water to obtain the desired concentration. Standard working solutions were prepared from the stock solutions by serial dilution with ultra-pure water. All alcoholic beverage products were purchased from various local markets in Korea. Each product was diluted to 1:10–1:20 (v/v) in ultra-pure water, followed by filtration through a 0.2 μm PTFE membrane (Sartorius Stedim Biotech GmbH, Goettingen, Germany). After the internal standard was added, the diluted solution was used directly for further derivatization and extraction.

2.2. Benzoylation of BAs and extraction of the derivatives

A 2-mL volume of standard solution or diluted sample was placed in a 12 mL glass test tube and 1 mL of 0.5 M borate buffer (pH = 10.0) was added, followed by the addition of 125 μL of 3% benzoyl chloride in acetonitrile. After a brief vortexing, the mixture was ultra-sonicated for 30 min at 30 °C. Benzoylated BAs were extracted by the DLLME-SFO method, which was operated as follows: a mixture of 450 μL of methanol (dispersive solvent) and 50 μL of 1-dodecanol (extraction solvent) was rapidly injected into the sample solution with a 1 mL Hamilton syringe. A cloudy solution was formed and subject to vortexing for 1 min. After centrifugation for 5 min at 3600 rpm, the glass test tube was placed in an ice bath for 10 min. The solidified extraction solvent floating on the solution surface was collected into a 1.5 mL Eppendorf tube using a customized scoop. The obtained droplet was then thawed and centrifuged at 12,000 rpm for 2 min, and 30 μL of the upper layer solution was diluted to 90 μL with methanol for direct LC injection.

2.3. Instrumentation

The LC–UV analysis was performed using a PerkinElmer HPLC system (CT, USA) equipped with a PerkinElmer Flexar pump, a column oven, an auto-sampler, and a photodiode array detector. Chromera software was used for the LC–UV system operation and data analysis. Mass analysis, which was used to confirm the identity of each derivatized compound, was performed using a Micromass Quattro Micro triple quadrupole mass spectrometer (MS; Waters Corporation, MA, USA) in the positive electrospray ionization (ESI) mode, and chromatograms in the total ion current (TIC) mode were obtained. The MS settings were as follows: capillary voltage, 3.0 kV; cone voltage, 60 V; source temperature, 130 °C; desolvation temperature, 400 °C; desolvation gas flow, 700 L/h. Data collection and LC–MS system operation were achieved by the Masslynx software.

2.4. Chromatographic conditions

Complex mobile phases have been used to chromatograph benzoylated BAs in a number of reports [12,13,31–35]. After elaborate optimization in the current study, the determined chromatographic method utilized a simple gradient elution system consisting of water (Eluent A) and methanol (Eluent B). The benzoylated products were separated on a Gemini C18 column (5 μm, 150 mm × 4.6 mm) from Phenomenex (CA, USA) at 30 °C (Fig. 1a). The linear gradient program was as follows: 0 min, 38% B; 0–5 min, 38–52% B; 5–10 min, 52–58% B; 10–15 min, 58–67%