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Simultaneous determination of aliphatic, aromatic and heterocyclic biogenic amines without derivatization by capillary electrophoresis and application in beer analysis

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ABSTRACT

Biogenic amines (BAs) play significant roles in indicating human health or food quality. Aiming to simultaneously determine three structures (aliphatic, aromatic and heterocyclic) of underivatized BAs, we explored a simple and rapid capillary electrophoresis (CE) method only coupled with conventional UV detector for the separation of thirteen key BAs. The strategy is to choose a UV absorbing probe as co-ion in the background electrolyte (BGE), and different BAs could be characterized by positive or negative peaks according to the fact that their UV absorptivity coefficients at a certain wavelength are better or worse than that of the UV absorbing probe. After the detailed investigation of critical parameters as pH, the concentration of Imidazole (Im) and α-cyclodextrin (α-CD), the optimized BGE consisted of 12.0 mmol/L Im as the UV probe and 10.0 mmol/L α-CD as the additive (at pH 4.50 adjusted with acetic acid). With such condition, the targets of thirteen BAs were baseline separated in 9.0 min and appeared at nine positive peaks and four negative peaks at 200 nm. The obtained LODs and LOQs (S/N = 3 or 10) were in the range of 0.36–3.67 and 1.2–12.2 μmol/L, respectively. The interday RSDs of migration time and peak area were less than 0.7% and 4.7% (n = 6), respectively. To the best of our knowledge, this is the first report on separating diverse structures of BAs by using Im as UV absorbing probe. The thirteen BAs were simultaneously detected by direct and indirect UV detection in a CE process. To verify the applicability, this method was used to analyze BAs in commercial beer samples. The recoveries of all BAs except carnosine (not identified by the interference) ranged from 70.4 to 119.6%, and four aliphatic and aromatic amines were satisfactorily identified and quantified.

1. Introduction

Biogenic amines (BAs) are low-molecular-mass organic bases with aliphatic, aromatic or heterocyclic structures, and widely exist in living organisms and foods [1–3]. Due to their biological activities, the abnormal level of certain BAs, or their metabolites, in human biological samples has been reported to be correlated with diseases [4,5]. For example, the concentration of catecholamine and their metabolites in urine or plasma specimens have been clinically used to diagnose pheochromocytoma [6]. In addition, BAs are present in a variety of foods, primarily as a consequence of microbial amino acid decarboxylation. The critical BAs as histamine (HA), cadaverine (Cad), putrescine (Put), spermidine (Spd) and tyramine (TA) are considered as indicators of freshness of foods. Excessive intake of these amines in food can cause various physiological symptoms and even severe toxicological effects, e.g. nausea, respiratory distress, headache and heart palpitations. Therefore, identification of BAs is much concerned in the related fields. The most widely used analytical technique for BAs determination is high performance liquid chromatography. As a liquid phase separation tool, capillary electrophoresis (CE) has already become a powerful alternative tool for BAs determination due to its primary advantages of simple instrumentation, low sample consumption, short analysis time and high resolution [7,8]. Although there are numerous articles related to the analysis of BAs, developing new methods or improving the current methods for separation and quantification of BAs is still a focused area.

The UV detector is the most universal detection approach coupled with commercial CE, but always complained of its limitations (major detecting for UV absorbing analytes) and low sensitivity.

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For the aromatic and heterocyclic BAs owning native UV absorption, the LODs are at μmol/L level by CE with direct UV detection [9]. In our previous study, ten BAs were simultaneously separated and three of them were identified in urine sample by CE-UV with the LODs in the range of 0.2–1.2 μmol/L [10]. The way to improve the sensitivity of CE relies on the advanced detectors and stacking techniques. The LODs of BAs could be improved to nmol/L level by sweeping with laser-induced native fluorescence detection [11], or dynamic pH junction with amperometric detection [12]. When the BAs exhibit weak or no native UV absorption, such as the aliphatic types, the laser-induced fluorescence (LIF) detection [13,14], electrochemical detection (ECD) [15], or mass spectrometry (MS) detection [16] could be applied to address this issue. Unfortunately, these detection techniques are relatively limited in routine analysis due to the indispensable derivatization process for LIF detection, expensive costs for MS, or poor reproducibility and limited conditions for ECD. As an alternative approach, the indirect UV detection has already been proved very effective in detection of these no UV absorbing substances. Many theoretical and experimental works including quantitative analysis, effects of additives in the background electrolyte (BGE), and system peaks in the indirect UV detection were studied [17–22]. So the amines without UV absorption could be indirectly detected by dissolving proper UV absorbing probes as copper sulphate [23,24], imidazole (Im) [25], quinine sulphate [26,27] or 4-methylbenzylamine [28] in BGE. Furthermore, these BAs could be sensitively detected by indirect laser-induced fluorescence (ILIF) [29–31], which was first reported by Kuhr by using dansyl-amino acids in a phosphate buffer. The LODs could be improved to sub μmol/L by ILIF detection using cresyl violet as a probe [31].

So far, few works are related in simultaneous detection of aliphatic, aromatic and heterocyclic BAs without derivatization. There was just one report that mentioned some positive peaks also appeared beside the negative peaks when using copper sulphate in BGE for indirect UV detection of amines in the synthetic sample, but lack of further discussion and detail investigation [32]. Herein, we propose a novel thought to achieve the aim of detecting diverse BAs with or without UV absorption at the same time. What we need to do is to find an appropriate UV absorbing probe that dissolved in the BGE as the co-ion. The analytes that possess higher UV absorptivity coefficient than the probe will appear as positive peaks, otherwise, the negative peaks will be detected. In this work, the UV absorbing probe, the pH of BGE, and the additives have been studied to achieve our purpose. Under the optimal conditions, thirteen BAs consisted of aliphatic, aromatic and heterocyclic series without derivatization were well separated in 9.0 min. The developed method was successfully employed to detect BAs in beer samples.

2. Experimental

2.1. Apparatus

All experiments were conducted on a Beckman P/ACE^{TM} MDQ CE system (Beckman Coulter, USA) equipped with a photo-diode array (PDA) detector, with which the samples can be measured at different wavelengths ranging from 190 to 600 nm. The polyimide-coated fused-silica capillary was from Ruifeng Chromatographic Co., Ltd. (Hebei, China) of 75-μm i.d. with a total length of 60 cm, and 50 cm to the detector, and thermostated at 25 °C with coolant liquid. Purified water used in the preparation of all solutions was delivered by a Milli-Q Labsystem (Millipore, Germany). The UV absorption spectra (obtained from 190 to 600 nm) of BAs and Im was investigated by using an UV spectrophotometer (Techcomp, China), and the molar absorptivity (ε) was calculated according to Lambert-Beer's Law. The determination of pH was obtained by a pH meter from Mittler-Toledo Instrument Co., Ltd. (Shanghai, China).

2.2. Chemicals

All chemicals were of analytical grade or better and used without purification. Thirteen BAs of phenethylamine (PEA), HA, Tryptamine (Try), TA, 5-hydroxytryptamine (5-HT), octopamine (OA), dopamine (DA), norepinephrine (NE), epinephrine (E), carnosine (CAR), Put, Spd, Cad, and α-cyclodextrin (α-CD) were all purchased from Sigma-Aldrich (MO, USA or Wuxi, China). Im and acetic acid (HAc) were from J&K Chemical Ltd. (Shanghai, China) and TCI Chemical Ltd. (Tokyo, Japan), respectively. The chloroform and *n*-butanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.3. CE conditions

New capillaries were conditioned with 0.1 mol/L NaOH, Milli-Q water, and BGE for 10 min each prior to be used. Between separations, the capillary was conditioned with Milli-Q water and BGE for 2 and 3 min, respectively. The sample injection was achieved by the pressure of 0.5 psi for 5.0 s. A constant voltage (+15 kV) was applied as a positive potential to the inlet vial for sample separation. The BAs were respectively dissolved in ultrapure water (Spd, Put, HA, Cad, PEA, TA, DA, 5-HT, OA and CAR) or in 0.1 mol/L HCl (Try, NE and E) as the stock solution of 1.0 mmol/L. These stock solutions were stored at 4.0 °C in darkness. Standard solutions of analytes were freshly prepared by dilution of stock solutions in water to a certain concentration prior to analysis. The final optimized BGE was composed of 12.0 mmol/L Im and 10.0 mmol/L α-CD (pH of 4.50 adjusted with 1.0 mol/L HAc) and filtered through 0.22 μm filter membrane before use. All LODs given hereafter were estimated according to three times the S/N ratio which was defined as maximum peak height divided by the background noise.

2.4. Treatment of beer sample

The BAs in three kinds of well-known band beer were identified and quantified to verify the developed method. To avoid matrix interference, the BAs in the beer were extracted by liquid-liquid extraction (LLE) method and eventually transferred into the aqueous phase for CE analysis as a process introduced in the previous report [33]. A 5.0 mL beer sample was first degassed, saturated by solid NaCl, adjusted the pH to 12.0 by adding 0.1 mol/L NaOH, and extracted three times by adding 5.0 mL organic solvent (volume ratio of *n*-butanol vs. chloroform at 1:1). The solid NaCl was used to accelerate the sedimentation, and NaOH was for improving the dispersive of organic phase. The process was the sedimentation accelerated by inorganic salt dispersive LLE, which was good for improving the extraction efficiency. For the extraction, the mixture was homogenized by vortex agitation for 5 min and then centrifuged at 3600 rpm for 10 min. All supernatants (around 15 mL) were harvested, acidified by adding 0.5 mL HCl at 1.0 mol/L, and evaporated at 40 °C under the protection of nitrogen. Finally, the residue was dissolved in 1.0 mL HCl (0.01 mol/L), and appropriately diluted with pure water to the proper concentrations prior to CE analysis. The concentration of BAs was eventually converted into that in the original beer sample.