Ology Science

Research Article

Journal of Research Analytica

Determination of Biogenic Amines in Wine Using Micellar Electrokinetic Chromatography

Abstract

Production on a large-scale of fermented alcoholic drinks made from different kinds of fruits is carried out in homes. Although wine laws regulating home production exist, they do not include upper concentration limits of biogenic amines. Therefore, a quick and inexpensive way to determine BAs in wine is crucial. A new analytical method based on micellar electrokinetic chromatography has been developed for the separation of the four most frequently occurring biogenic amines (histamine, tryptamine, tyramine and 2-phenylethylamine). An electrophoresis buffer of 20 mM borate (pH 9.3) containing 30 mM SDS and 5% (v/v) methanol was found to provide the optimum separation with respect to resolution and migration time. Finally this method was applied to the determination of biogenic amines in home-made red wines.

Keywords: Biogenic amines; Micellar electrokinetic chromatography; Home-made wine; Analytical separation

Justyna Płotka–Wasylka[1], Jacek Namieśnik[1] and Ewa Kłodzińska[2]*

[1]Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, Poland

[2]Instiute of Sport - National Research Institute, Department of Anti-Doping Research, Poland

***Corresponding author:** Ewa Kłodzińska

E-mail: Ewa.Klodzinska@impib.pl **(E.K)**

Introduction

Biogenic amines (BAs), naturally present in wine, arise from two sources: raw materials and fermentation process. Although BAs at low concentrations are essential for many physiological functions, their pharmacological activity in large quantities may become toxic. For instance, BAs cause headache, nausea, and cardiac palpitations in sensitive individuals especially when alcohol is involved. The level of BAs in wine have received much attention due to their inhibiting effect on enzymes responsible for detoxification [1]. Moreover, consumer demand for high-quality foods has increased interest in biogenic amines, given their importance to human health and food safety. For this reason, many importers in the EU require analysis of wine samples in order to determine BAs [2] even though official regulation about their maximum content does not exist. Furthermore, production on a large scale of fermented alcoholic drinks from different fruits is carried out in homes, for trading as a regional product [3]. Although laws regulating home production exist (e.g. in Poland [4]), they do not include upper concentration limits of BAs. This leads to the conclusion that not only industrial wine production should be controlled, but also home-made wines produced as regional products. Therefore, a quick and inexpensive way to determine BAs in wine is crucial.

Different analytical methods have been developed to determine BAs in wine samples based on gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE). GC methods are not often used as it needs a chemical derivatization step to increase the volatility by decreasing the polarity of BAs. However, two GC-MS methods were recently developed for the determination of BAs in wine using isobutyl chloroformate as a derivatizing reagent. In the first method, derivatization was simultaneously performed with a dispersive liquid-liquid microextraction technique [3]. In the second method, derivatization was carried out in a two-phase reaction system [5]. Both methods afford accurate identification and rapid quantification of a high number of BAs at high efficiency and reproducibility.

Due to its high sensitivity, adequate resolution and analytical versatility, high performance LC with detection by UV absorbance, fluorescence emission, or mass spectrometry (MS) is the most extensively used technique for the determination of BAs in wine samples. Prior sample preparation is often required in order to remove other compounds that may interfere with the chromatographic analysis, or to concentrate the trace analytes of interest. In addition, the determination of BAs by HPLC usually requires a derivatization process, because these compounds do not have adequate light absorption or fluorescence emission properties. Moreover, derivatization reduces the polarity of BAs, thus improving their separation by reverse phase HPLC on a C18 column, and making them more sensitive towards MS detection than the underivatized amines. The most common derivatizing agents are ophthalaldeyde, benzoyl or dansyl chloride, and 1,2-naphthoquinone-4-sulphonate [5-9]. Liquid chromatography coupled with a tandem mass spectrometer and ultra-performance LC coupled to a quadrupole-time of flight mass spectrometer have been shown to be very powerful techniques for BAs determination, also without derivatization [8].

Electrophoresis has become a very useful technique for analytical separations. The main advantages of switching from HPLC to electrophoresis include better resolution, higher effectiveness due to automation, and very low cost of chemicals consumption. Determination of BAs using capillary electrophoresis (CE) is not reported numerously in the literature. The three established approaches are: (a) aromatic or heterocyclic amines can be separated in selected buffers without derivatization, (b) polyamines can be separated after derivatization, and (c) indirect detection of amine compounds. In 1998, a micellar electrokinetic chromatography (MEKC) method for the determination of BAs in food products in 30 minutes was proposed [10]. A flow-injection manifold was used for automating the determination of BAs in wine samples by CE with indirect UV detection; however this method involved clean-up by solid phase extraction (SPE) [11]. Very interesting CE applications were reported on the determination of BAs in mammalian decomposition fluids [12] and in breast cancer cells [13]. In another approach, MEKC was applied with specific derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate [14] or with 1,2-naphthoquinone-4-sulfonate [15]. A very good way to obtain satisfied separation results is connected with sample preconcentration and computer simulation of the experimental data [16].

In the present study, MEKC is demonstrated to be a very fast and useful technique for the determination and separation of selected BAs in home-made wine without any derivatization procedure. For MEKC analysis, the most important and the most frequently occurring in food biogenic amines were selected: histamine, tryptamine, tyramine and 2-phenylethylamine.

Experimental

Reagents and materials

The chemicals used in the investigation were purchased from the following suppliers: the amine standards (histamine, tryptamine, tyramine, and 2-phenylethylamine) were obtained, mostly as hydrochloride salts, from Sigma Aldrich (Steinheim, Germany); borate buffers (pH 4, 7, 9) and boric acid were obtained from Chempur (Torun, Poland). Sodium dodecyl sulfate (SDS) and methanol were purchased from Merc (Darmstadt, Germany). DI water was produced in our laboratory using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Stock solution: Stock solutions (1.0 mg/mL) were prepared by weighing each free compound and dissolving it in deionized water. The solutions were stored at room temperature in silanized screw-capped vials with solid PTFE-lined caps (Supelco, Bellefonte, PA). Standard solutions were prepared by dilution and mixing of stock solutions with deionized water.

Instrumentation: The analyses were performed using the HP3DCE system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector. Experimental conditions: uncoated fused silica capillary: L=64.5 cm , 50 μm ID (purchased from Composite Metal Services, Worcester, UK) , borate buffer (20 mM) with SDS (30 mM) and methanol (5% by volume),

λ=200 nm, U=20 kV, injection: 10 mbar × 20 s. The wine sample was directly injected into the CE system.

Results and Discussion

Proper selection and optimization of experimental parameters is not a trivial task, due to their complex interrelation and combined effects on the separation efficiency and peak resolution. The most labor-intensive and time-consuming task was the selection of appropriate buffer, surfactant concentration, applied voltage, and hydrodynamic injection. After performing a series of analyses using standard mixtures of biogenic amines in several buffer solutions, the most suitable buffer concentration

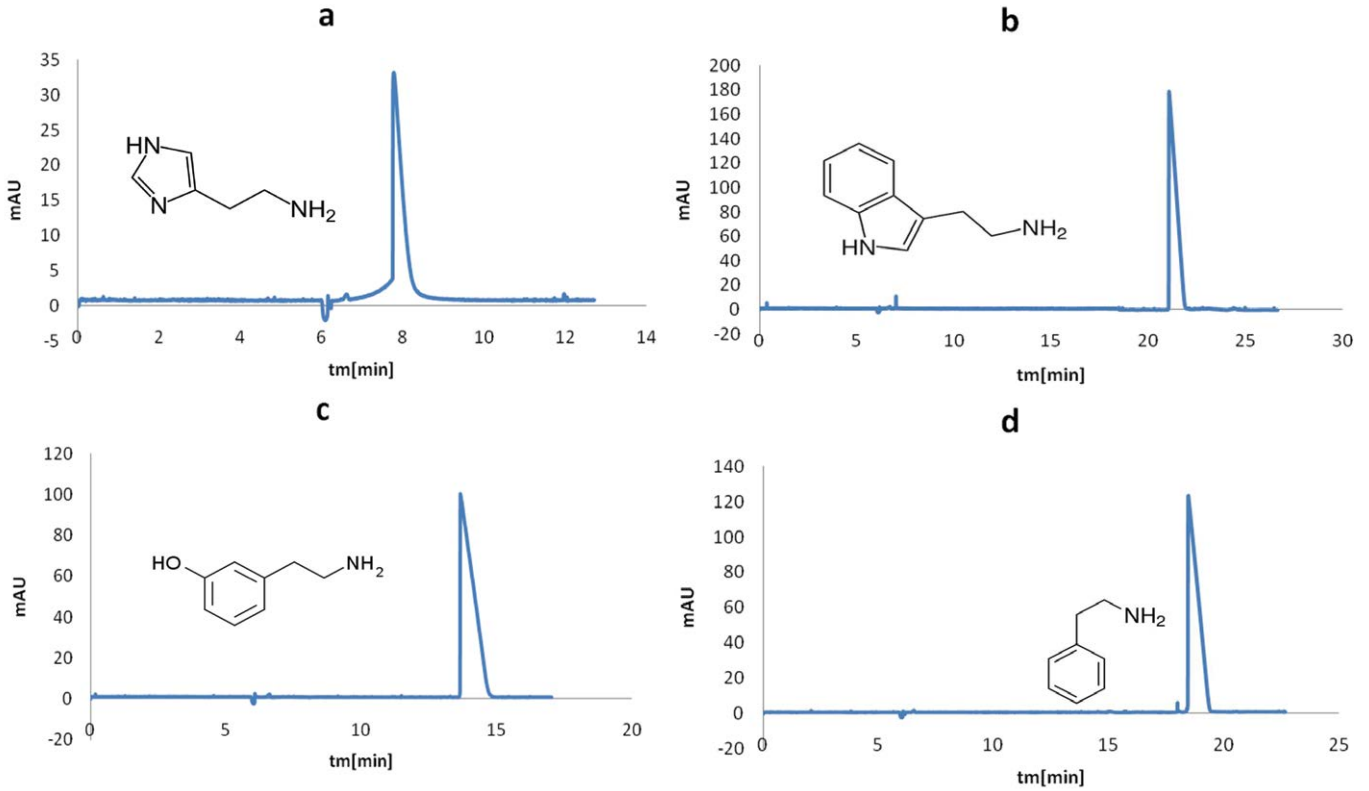

Figure 1: Determination of BAs by MEKC. Experimental conditions: uncoated fused silica capillary: L=64.5 cm, 50 µm ID, borate buffer (20 mM) with SDS (30 mM) and methanol (5%), λ=200 nm, U=20 kV, injection: 10 mbar × 20 s.

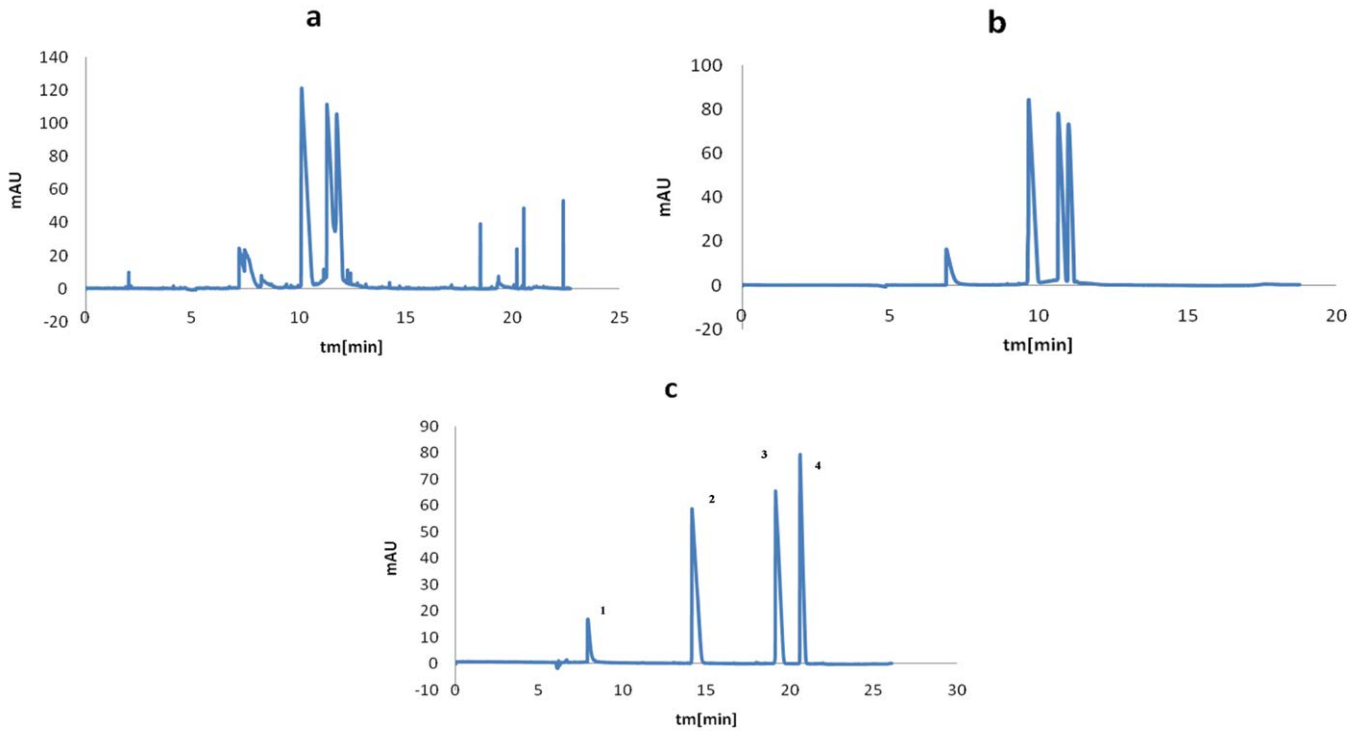

Figure 2: Separation of four BAs (a) without SDS addition to the buffer, (b) with addition of SDS to the running buffer but without methanol, and (c) with SDS (30 mM) and methanol (5%). 1: histamine, 2: tyramine, 3: 2-phenylethylamine, 4: tryptamine.

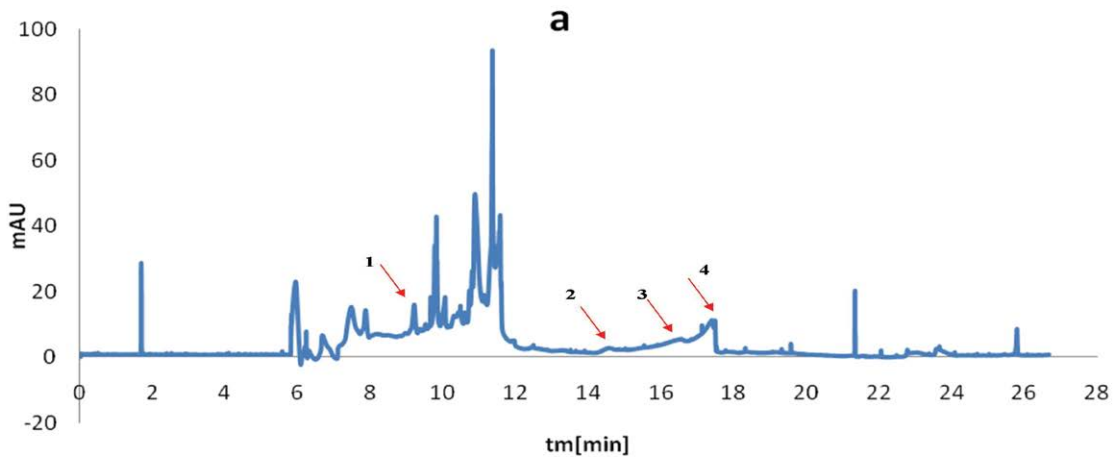

Figure 3: Electropherogram obtained by MEKC for a sample of red wine. Experimental conditions as in Figure 1. Histamine, tyramine, 2-phenylethylamine, and tryptamine are indicated by four red arrows.

and pH, were selected. Determination of histamine, tryptamine, chemical derivatization. Migration times obtained for each biogenic amine were considerably different leading to very good separation results as shown in Figure 1.

MEKC separation of a mixture of BAs were performed next. From Figure 2a it is evident that without SDS addition to the buffer, adequate separation of the four amines was not attained. A lack of suitable filtration and degassing of running buffer before the electrophoretic analysis resulted in the acquisition of spurious peaks. Addition of methanol (5% by volume) was actually necessary to obtain the satisfactory resolution shown in Figure 2c. Without methanol, the 2-phenylethylamine and tryptamine peaks were not well separated, as illustrated in Figure 2b.

Last, the separation of BAs in a wine sample was performed. The wine matrix was composed of a nonvolatile fraction (including polyphenolic compounds, proteins, and carbohydrates) and a volatile fraction (incorporating flavor and aroma compounds). The complex matrix composition made the electrophoretic analysis much more difficult due to overlapping of the many unknown component peaks in Figure 3. Some components were adsorbed irreversibly on the fused-silica surface inside the capillary to jeopardize the MEKC analysis.

To summarize, the proper choice and optimization of all experimental parameters is key to the good separation of BAs for accurate determination in a complex wine matrix. Here only a preliminary determination of BAs is presented to demonstrate the feasibility. The MEKC method needs further development before accurate determination of BAs in alcoholic products can be succeeded without matrix interference. For now, it can be concluded that the determination of standard BAs by MEKC is easy in regards to sample preparation. The method provides complete separation of the four important BAs within 25 minutes with good efficiency.

Acknowledgements

The research is funded in the framework of program of Polish Ministry of Science and Higher Education, "Iuventus Plus" in the years 2015–17 (Project no: IP2014 037573).

References

1. Pozo-Bayón MÁ, Monagas M, Bartolomé B, Moreno-Arribas MV. Wine features related to safety and consumer health: an integrated perspective. Crit Rev Food Sci. 2012; 52(1):31-54.
2. Lehtonen P. Determination of amines and amino acids in wine-a review. Am J Enol Vitic. 1996; 47:127-33.
3. Płotka-Wasylka J, Simeonov V, Namieśnik J. An *in situ* derivatization-dispersive liquid-liquid microextractioncombined with gas-chromatography-mass spectrometry fordetermining biogenic amines in home-made fermented alcoholicdrinks. J Chromatogr A. 2016; 1453:10-8.
4. Act of Polish law: Art. 1 i art. 16–art. 25 ustawy z dnia 22 stycznia 2004 r. owyrobie i rozlewie wyrobów winiarskich, obrocie tymi wyrobami iorganizacji rynku wina (Dz. U. z 2004 r., Nr 34, poz. 292 ze zm.
5. Cunha SC, Faria MA, Ferdandes IO. Gas chromatography-mass spectrometry assessment of amines in port wine and grape juice after fast chloroformate extraction/derivatization. J Agric Food Chem. 2011; 59(16):8742-53.
6. Arrieta MP, Prats-Moya MS. Free amino acids and biogenic amines in Alicante Monastrell wines. Food Chem. 2012; 135(3):1511-9.
7. Özdestan Ö, Üren A. A method for benzoyl chloride derivatization of biogenic amines for high performance liquid chromatography. Talanta. 2009; 78(4):1321-6.
8. Hlabangana L, Hernández-Cassou S, Saurina J. Determination of biogenic amines in wines by ion-pair liquid chromatography and post-column derivatization with 1,2-naphthoquinone-4-sulphonate. J Chromaogr A. 2006; 1130(1):130-6.
9. Millán S, Sampedro MC, Unceta N, Goicolea MA, Barrio RJ. Simple and rapid determination of biogenic amines in wine by liquid chromatographyelectrospray ionization ion trap mass spectrometry. Anal Chim Acta. 2007; 584(1):145-52.
10. Ginterová P, Marák J, Staňová A, Maier V, Ševčík J, Kaniansky D. Determination of selected biogenic amines in red wines by automated

on-line combination of capillary isotachophoresis-capillary zone electrophoresis. J Chromatogr B. 2012; 904:135-9.

11. Arce L, Ríos A, Valcárcel M. Direct determination of biogenic amines in wine by integrating continuous flow clean-up and capillary electrophoresis with indirect UV detection. J Chromatogr A. 1998; 803(1):249-60.
12. Swanna LM, Forbes SL, Lewis SW. A capillary electrophoresis method for the determination of selected biogenic amines and amino acids in mammalian decomposition fluid. Talanta. 2010; 81(4):1697-702.
13. Kao YY, Liu KT, Huang MF, Chiu TC, Chang HT. Analysis of amino acids and biogenic amines in breast cancer cells by capillary electrophoresis using polymer solutions containing sodium dodecyl sulfate. J Chromatogr A. 2010; 1217(4):582-7.
14. Kovács A, Simon-Sarkadi L, Ganzler K. Determination of biogenic amines by capillary electrophoresis. J Chromatogr A. 1999; 836(2):305-13.
15. García-Villar N, Saurina J, Hernández-Cassou S. Capillary electrophoresis determination of biogenic amines by field-amplified sample stacking and in-capillary derivatization. Electrophoresis. 2006; 27(2):474-83.
16. Ciura K, Kowalski P, Nowakowska J, Markuszewski M, Baczek T, Dziomba S. Sweeping of hydrophobic amines under inhomogeneous electric field and low surfactant concentration in micellar electrokinetic chromatography. Electrophoresis. 2016; 37:1161-5.