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Simultaneous determination of tyramine and tryptamine and their precursor amino acids by micellar liquid chromatography and pulsed amperometric detection in wines

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Abstract

Two biogenic amines, tryptamine and tyramine, and their precursors, tryptophan and tyrosine, were determined by a liquid chromatographic procedure. A hybrid micellar mobile phase of sodium dodecyl sulphate (SDS) and 1-propanol, a C18 column and electrochemical detection were used. A pH study in the range of 3–9 was performed and pH 3 was finally selected in accordance with resolution and analysis time. Oxidation potential was also checked in the range 0.6–0.9 V: the maximum area obtained in all those potentials was at 0.8 V, which was selected to carry out the analysis using a sequence of pulsed amperometric detection waveform. The four compounds were resolved using a mobile phase of 0.15 M SDS–5% 1-propanol with an analysis time of 16 min. Repeatabilities and intermediate precision were evaluated at three different concentrations for each compound with RSD values lower than 2.6 and 4.8%, respectively. Limits of detection and quantification were also obtained within the 10–40 and 33–135 ng/ml ranges, respectively. Finally, the applicability of the procedure was tested in several types of wine and no matrix effect was observed. The possibility of direct sample introduction simplifies and greatly expedites the treatments with reduced cost, improving the accuracy of the procedures.

Keywords: Micellar liquid chromatography; Pulsed amperometric detection; Biogenic amines; Wine samples; Direct injection

1. Introduction

Biogenic amines (BAs), low molecular weight organic bases, can be formed and degraded as a result of the normal metabolic activity in animals, plants and micro-organisms. Amino acids are naturally present in grapes. Lactic acid bacteria are responsible for the transformation of amino acids into BAs. They can grow in acidic fruit juice and wine. The two BAs studied in this work, tyramine (TyrA) and tryptamine (TryptA), are formed from the decarboxilation of tyrosine (TyrO) and tryptophan (TryptO), respectively [1].

Increased levels of BAs in foods are of interest from several points of view. Once BAs are formed, they are difficult to be destroyed by pasteurization or cooking. For this reason, it is important to control the manufacture process to reduce the concentration of BAs as much as possible. They can cause direct or indirect toxicity when their concentration levels are high [2]. For this reason, some countries have established regulations regarding either their intake content in various kinds of foods or their maximum limit requirements. There may be three possible origins of BAs in wines: they are already present in the mustiness, they are formed by yeast during alcoholic fermentation, and/or they are formed in wine by the action of bacteria involved in the malolactic fermentation [3].

TyrA has one of the best known psychoactive and/or vasoactive effects. Despite this, scarcely any information is available on the health effects of TryptA. Some general diseases of intoxication from aromatic amines have been associated with migraines and hypertension [4].

In general, the most widely used analytical methods to identify BAs in wines and foods are high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). Most HPLC methods make use of derivatizating agents [5–7]. Information about the determination of BAs in HPLC applied to foods was also reviewed [8]. Finally, CE is also an important technique to determine these compounds as it involves a wide range of detection systems, such as conductimetric detection, mass-spectrometry, pulsed amperometric detection, UV, and chemiluminiscence [9–13].

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Micellar liquid chromatography (MLC) is an alternative to conventional HPLC since it uses a surfactant solution instead of aqueous–organic solvents. It is interesting to remark that one of the goals of MLC is the possibility of injecting the sample into the chromatographic system without the need of a previous extraction procedure. Micellar medium has been used in the determination of some BAs (agmatine, cadaverine, histamine, phenylethylamine, putrescine, spermidine, spermine, tryptamine and tyramine) in various food substrates to enhance the resolution of the peaks, quantification and sensitization of the benzene ring absorption after solid phase extraction and derivatization on-line with HPLC [14]. MLC has been used in the field of food analysis, and in the determination of food preservatives [15], cholesterol [16] and phenolic antioxidants [17].

The aim of this work is to determine the BAs, TyrA and TryptA, and their precursors, TyrO and TryptO, in wines by using a MLC technique with a mobile phase of SDS–propanol, a C18 column and pulsed amperometric detection.

2. Materials and methods

2.1. Chemicals and reagents

The reagents used in the mobile phases were the surfactant SDS (99% purity, Merck, Darmstadt, Germany), 1-propanol, 1-butanol (Scharlab, Barcelona, Spain), the buffer salt sodium dihydrogenphosphate (Panreac, Barcelona), citric acid and boric acid (Merck), and NaOH and HCl (Panreac).

The two BAs and their precursors (Fig. 1) were TyrA from Sigma (St. Louis, MO, USA), TryptA from Fluka Chemie (Buchs, Germany), and TyrO and TryptO from Merck. The analyzed wines were purchased from a local supermarket.

2.2. Instrumentation and chromatographic conditions

The analytical balance used was a Mettler-Toledo AX105 Delta-Range (Greifensee, Switzerland). A Crison potentiometer (Model micropH 2001, Barcelona) equipped with a combined Ag/AgCl/glass electrode was used to measure the pH. An ultrasonic bath was used to dissolve the standards (model Ultrasons-H, Selecta, Abrera, Spain).

An Agilent Technologies Series 1100 chromatograph (Palo Alto, CA, USA), equipped with an isocratic pump, an autosampler and a Rheodyne valve (Cotati, CA, USA) was used.

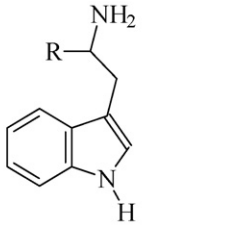
An electrochemical detector (Agilent Technologies Series 1049) was employed to monitor the BAs.

The micellar mobile phase recommended for the analysis is 0.15 M SDS–5% (v/v) 1-propanol buffered at pH 3 with sodium dihydrogenphosphate. The injection volume and flow-rate were 20 μl and 1.0 ml/min, respectively. Analyses were performed in a Kromasil column (Scharlab, 5 μm particle size, 120 mm × 4.6 mm I.D.). An Ag/AgCl electrode served as a reference in the electrochemical detector while Au was the working electrode. Electrochemical detection was set at 0.8 V and sensitivity was maintained at 500 nA full scale deflection. The dead time was determined as the mean value of the first significant deviation from the base-line in the chromatograms of the compounds. The signal was acquired and treated by a PC connected to the chromatograph through an HP Chemstation (Rev. A.10.01). Microsoft® Excel 2002 (Microsoft Inc., USA) was also used in calculations.

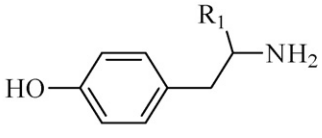
2.3. Preparation of solutions

Micellar mobile phases were prepared by weighing amounts of SDS and selected buffer powder and dissolving them in water, adjusting to the desired pH with NaOH or HCl and, finally, a suitable volume of alcohol, depending on the percentage, was added.

Stock solutions containing 100 μg/ml of each compound were prepared by dissolving the analyte in a few millilitres of methanol, and were then filled with 0.05 M SDS at pH 3 up to the flask-mark. Stock solutions were stored at 4 °C. The working standards (0.1–10 μg/ml) were freshly prepared from stock



Tryptamine	R = H	pK_a = 10.2 [a]
$C_{10}H_{12}N_2$		log $P_{o/w}$ = 1.55 [b]
Tryptophan	R = COOH	pK_a = 2.46; 9.41 [a]
$C_{11}H_{12}N_2O_2$		log $P_{o/w}$ = -1.05 [b]



Tyramine	R_1 = H	pK_a = 9.74; 10.52 [a]
$C_8H_{11}NO$		log $P_{o/w}$ = 0.86 [b]
Tyrosine	R_1 = COOH	pK_a = 2.20; 9.21; 10.46 [a]
$C_9H_{11}NO_3$		log $P_{o/w}$ = -2.26 [b]

[a] Dissociation constants of organic acids and bases http://www.zirchrom.com/organic.htm

[b] from ref. [18]

Fig. 1. Structure of studied compounds [18].