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Determination of biogenic amines in alcoholic beverages by ion chromatography with suppressed conductivity detection and integrated pulsed amperometric detection

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Abstract

The determination of biogenic amines in alcoholic beverages is important to assess the potential risks associated with the consumption of high concentrations of these compounds. In addition, product storage conditions and the length of storage can cause the formation of biogenic amines that reduce product quality. We report a new method using cation-exchange chromatography with either suppressed conductivity, integrated pulsed amperometry, UV, or a combination of these detection techniques to determine biogenic amines in alcoholic beverages. The main objective was to provide a direct comparison between IPAD and suppressed conductivity detection for determining biogenic amines in alcoholic beverages. Suppressed conductivity is the simplest detection approach for determining putrescine, cadaverine, histamine, agmatine, phenylethylamine, spermidine, and spermine with good sensitivity (0.004–0.08 mg/l) and was used to evaluate the influence of storage time and conditions on the evolution of biogenic amines in alcoholic beverages. Integrated pulsed amperometric detection (IPAD) detects more biogenic amines than suppressed conductivity detection, enabling the detection of dopamine, tyramine, and serotonin. Tyramine was simultaneously determined by UV detection and IPAD to provide confirmation and ensure the accuracy of the analytical results. The linearity of biogenic amine responses was within 0.1–20 mg/l and peak area precisions were 0.24–4.97% for IPAD, suppressed conductivity-IPAD, and UV detection. The sensitivity for the 10 biogenic amines using the 3 detection techniques varied considerably from 0.004–1.1 mg/l and recoveries were within 85–122%.

Keywords: Cation-exchange chromatography; Biogenic amines; Integrated pulsed amperometric detection; IPAD; Electrochemical detection; Alcoholic beverages

1. Introduction

Biogenic amines are widespread in plants and animals where they have important metabolic and physiological roles, such as the regulation of growth (putrescine, spermidine, spermine), control of blood pressure (indoleamines and histamine), and neural transmission (catacholamines and serotonin) [1,2]. In foods and beverages, biogenic amines can be formed by the decarboxylation of amino acids from microbial activity [3]. Their presence in food is not only important from a toxicological view, but can also be used as an indicator of spoilage [4]. Some biogenic amines, such as histamine, may be present before foods appear spoiled or have an unacceptable appearance [5]. The intake of dietary biogenic amines in a normal diet is not considered harmful because healthy individuals can readily metabolize the amines by acetylation and oxidation reactions mediated by the enzymes monoamine oxidase, diamine oxidase, and polyamine oxidase [6]. However, the consumption of an excess amount of these amines can induce severe toxicological effects and produce various physiological symptoms, such as nausea, respiratory distress, headache, sweating, heart palpitations, and hyper- or hypotension [7].

The determination of the toxicity threshold of biogenic amines is a complex and difficult process because the toxic dose is strongly dependent on the efficiency of the detoxification mechanism of each individual [4]. Toxicity levels can also depend on the amount of biogenic amines in the food consumed and the presence of other amines [7]. For example, the presence of putrescine and cadaverine can have a synergistic effect by increasing the toxicity of histamine due to reduced histamine oxidation [1]. In addition, patients prescribed monoamine oxidase inhibitor drugs, such as antidepressants or anti-Parkinsonian agents, are particularly at risk of experiencing symptoms from the consumption of food containing high con-

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centrations of biogenic amines [8]. The consumption of alcohol is also known to inhibit monoamine oxidase and therefore the presence of even low concentrations of biogenic amines in alcoholic beverages can induce a toxicological response [9].

The presence of biogenic amines in wines has been associated with malolactic fermentation or the action of yeasts in primary fermentation [2]. Common biogenic amines in wines include tyramine, putrescine, cadaverine, histamine, and phenylethylamine [9]. Histamine can cause headaches, flushing of the face and neck, and hypotension, whereas some aromatic amines, such as tyramine and phenylethylamine, can produce migraines and hypertension [1]. The concentration and content of biogenic amines in wines can vary extensively depending on the storage time and conditions, quality of raw materials, and possible microbial contamination during the winemaking process [10]. Putrescine, agmatine, spermidine, and spermine are considered natural beer constituents that primarily originate from malt. However, tyramine, cadaverine, and histamine in beer have been associated with the activities of contaminating lactic acid bacteria during brewing [11].

The determination of biogenic amines presents a challenging analytical problem due to their structures and low concentrations in complex matrices. Reversed-phase HPLC is commonly used to determine biogenic amines in alcoholic beverages. HPLC requires either pre- or postcolumn chemical derivatization prior to UV or fluorescence detection to achieve the required sensitivity. *o*-Phthalaldehyde (OPA) in combination with a thiol compound, such as 2-mercaptoethanol (MCE), is a common derivatizing agent for determining biogenic amines in wine [2,10,12–14] and beer [15,16]. This derivatization is usually performed postcolumn, as OPA derivatives are unstable [17]. In general, derivatization adds complexity to the analysis, requires additional skilled labor, and can sometimes produce by-product interferences.

Ion chromatography (IC) coupled with pulsed amperometric detection (PAD) or integrated pulsed amperometric detection (IPAD) after postcolumn base addition has also been reported for the determination of biogenic amines [18–20]. These methods provide good sensitivity and selectivity without derivatization for many biogenic amines of interest in foods and beverages. However, high acid or salt concentrations combined with an organic solvent were required to separate the strongly retained amines, such as spermidine and spermine [20]. The use of organic solvents, such as acetonitrile, with amperometric detection can produce undesirable decomposition by-products resulting in potential interferences [21].

IC has not widely been reported as a technique used for the determination of biogenic amines. This is at least partially due to the strong hydrophobic interactions between the protonated amines and typical cation-exchange stationary phases resulting in long retention times and poor peak shapes. In addition, eluents required to separate the amines are often not compatible with suppressed conductivity detection, which can provide one of the simplest approaches for detecting some of the major biogenic amines. The development of a weak carboxylic acid functionalized cation-exchange column that reduced the hydrophobic interactions of hydrophobic analytes [22] allowed the use of suppressed conductivity detection and was successfully applied to the determination of biogenic amines in fish [23] and meat [24] samples.

A new weak carboxylic acid functionalized cation-exchange column specifically designed for the determination of polar amines has a slightly higher hydrophobicity than the column previously described [22], and therefore improves the separation of closely eluting peak pairs, such putrescine and cadaverine. We used this column with IPAD to determine biogenic amines in beer and wine samples purchased from a local market. Because relatively little information exists on the evolution of biogenic amines in alcoholic beverages during storage, we examined this effect with suppressed conductivity detection coupled to IPAD. Tyramine cannot be detected by suppressed conductivity detection due to the loss of a proton upon suppression. However, UV detection can provide selectivity for certain classes of compounds and therefore was used to confirm the presence of tyramine in some alcoholic beverages. The primary objective was to compare suppressed conductivity detection to IPAD in terms of linearity, detection limits, precision, and recovery of biogenic amines spiked in beer and wine samples.

2. Experimental

2.1. Materials and chemicals

All standards and samples were prepared with 18 MΩ cm or better (Labconco, Kansas City, MO, USA) deionized (DI) water. Methanesulfonic acid (MSA) eluent was generated online with an EG-3000 eluent generator (Dionex Corporation, Sunnyvale, CA, USA) equipped with an EGC II MSA cartridge. Sodium hydroxide (NaOH), 50% (w/w) was purchased from Fisher Scientific (Hampton, NH, USA). Dopamine hydrochloride, serotonin hydrochloride ≥98%, tyramine 99%, putrescine dihydrochloride ≥98%, cadaverine dihydrochloride >98%, histamine ∼97%, and agmatine sulfate 97% were purchased from Sigma–Aldrich (St. Louis, MO, USA). Spermidine trihydrochloride >98% and spermine tetrahydrochloride ≥99% were purchased from Calbiochem (San Diego, CA, USA).

2.2. Chromatography

The chromatography system consisted of a Dionex ICS-3000 Reagent-Free™ Ion Chromatograph (Dionex Corporation, Sunnyvale, CA, USA) with a DP-3000 dual gradient pump, a DC-3000 detector compartment with a conductivity cell and an electrochemical cell, an EG-3000 eluent generator with an EluGen® EGC II MSA cartridge, and an AS autosampler. Biogenic amines were separated with an IonPac® CS18 (250 mm × 2 mm I.D., Dionex Corporation) analytical column and its respective guard column, CG18 (50 mm × 2 mm I.D.) with a flow rate of 0.30 ml/min and a thermostatted temperature of 40 °C. A CSRS ULTRA II (2 mm) self-regenerating suppressor operating at 40 mA in the external water mode was used for suppressed conductivity detection. A 5 μl sample injection