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

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Available online 4 February 2007

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-
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-FreeTM 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 x 2 mm I.D., Dionex Corporation) analytical column and its respective guard column, CG18 (50 mm x 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...
volume was used throughout. The gradient elution conditions consisted of: 3 mM MSA from 0 to 6 min, 3 to 10 mM from 6 to 10 min, 10 to 15 mM from 10–22 min, 15 mM from 22 to 28 min, 15 to 30 mM from 28 to 35 min, 30 to 45 mM from 35.1 to 45 min. A postcolumn addition of 0.1 M NaOH, to increase the effluent pH for detection by IPAD, was delivered to a mixing tee at a flow rate of 0.24 ml/min using the DP-3000. A 125 μl knitted reaction coil was installed between the mixing tee and electrochemical cell. The electrochemical cell consisted of a conventional Au working electrode, a titanium counter electrode, and a pH-Ag/AgCl combination reference electrode. The electrochemical waveform was +0.13 V from 0.000 to 0.040 s, +0.33 V from 0.500 to 0.210 s, +0.55 V from 0.220 to 0.460 s, +0.33 V from 0.470 to 0.536 s, −1.67 V from 0.546 to 0.576 s, +0.93 V from 0.586 to 0.626 s, and +0.13 V at 0.636 s, using the pH reference electrode mode with current integrated between 0.210 and 0.536 s for detection. To remove oxygen generated by the EGC II MSA cartridge, the EG degas device was continuously supplied with external DI water from a helium pressurized bottle flowing through the Regen channel. For detection using IPAD, the Regen flow rate was −0.5–0.6 ml/min and was increased to −1–3 ml/min for combined suppressed conductivity and IPAD. An AD25 UV–vis detector ( Dionex Corporation) with a combined deuterium/tungsten lamp and 10-mm cell was installed between the column outlet and electrochemical cell and was used at a wavelength of 276 nm to confirm the presence of tyramine in the effluent between the column outlet and electrochemical cell and was used at a wavelength of 276 nm to confirm the presence of tyramine in the effluent. Most alcoholic beverages were diluted two to five times with DI water before analysis. However, due to the formation of sediments in the California Cabernet Sauvignon red and rosé wine samples, centrifugation (6000 rpm, 4 °C, 30 min) was required. The California red wine was then diluted 1:5 with DI water and the rosé wine was injected directly without further preparation.

3. Results and discussion

3.1. Separation of biogenic amines

Previous studies have demonstrated the use of cation-exchange chromatography for the separation of biogenic amines. However, due to their strong hydrophobic interactions with the chromatographic stationary phase, separations required the use of a high concentration acid or salt gradient in the eluent phase that is often modified with an organic solvent to elute stronger retained amines [18–20]. To simplify the separation of biogenic amines by IC, a weak acid cation-exchange column, the IonPac CS17, was introduced [22]. This column consists of a very low hydrophobic stationary phase with a moderate capacity (363 μeq/column, 250 mm × 2 mm) that allows the use of low concentrations of an acidic eluent with no solvent required to separate hydrophobic amines. While biogenic amines would not have been amenable to suppressed conductivity detection using the eluents previously described, this detection technique was now possible due to the simple acidic eluent used with the CS17.

In this study, a new cation-exchange column, the IonPac CS18, was used for the separation of biogenic amines. This column is more hydrophobic and has a slightly lower capacity (290 μeq/column, 250 mm × 2 mm) than the CS17. The column’s increased hydrophobicity allows better resolution of closely eluting peak pairs, such as putrescine/cadaverine. Fig. 1 shows the separation of biogenic amines using the CS18 with suppressed conductivity, integrated pulsed amperometry, and UV detections (not connected in series). The gradient was optimized using an electrolytically generated MSA eluent to resolve the target biogenic amines in 40 min. While a relatively low MSA concentration (~10–15 mM) could be used to resolve most amines, a gradient up to 40 mM was required to separate strongly retained amines, such as spermidine and spermine. The use of a modest MSA concentration with no organic solvent allowed detection by suppressed conductivity.

3.2. Linearity and limits of detection

The linear ranges for suppressed conductivity, IPAD, and UV detections of biogenic amines were evaluated by tabulating peak area versus concentration. Calibration curves were prepared for each biogenic amine in 3 mM MSA using five increasing concentrations in the range of 0.10–5.0 mg/l for dopamine, cadaverine, histamine, serotonin, spermidine, and spermine. For tyramine, putrescine, and agmatine the linearity was determined in the 0.20–10 mg/l range. Phenylethylamine was linear in the range of 1–20 mg/l. The increase in baseline noise upon placing the electrochemical cell after the suppressor resulted in an increase in the lower linear range limit for some biogenic amines. The correlation coefficients using a least squares linear regression fit were between 0.997 and 0.999. The limits of detection (LODs) were determined based on the slopes of the calibration curves using three times the average baseline noise (S/N = 3). The LODs using suppressed conductivity, IPAD, and IPAD after suppression were in the range of 0.004–0.08, 0.021–0.39, and 0.090–1.1 mg/l, respectively. For amines that could be detected by both methods, suppressed conductivity detection was about 2–20 times more sensitive than IPAD and the LODs increased by a factor of two when IPAD was placed after the suppressor. All detection limits were confirmed by analyzing a standard solution.
of each amine at the corresponding LODs. The calibration data and LODs for the three detection configurations are summarized in Table 1.

### 3.3. Retention time and peak area precisions

The peak area and retention time RSDs were determined for replicate injections of a standard biogenic amine solution containing 5 mg/l tyramine, putrescine, and agmatine and 1 mg/l of dopamine, cadaverine, histamine, serotonin, spermidine, and spermine. Intraday precision was evaluated for the three detectors by performing 10 consecutive injections of the standard amine solution. Retention time RSDs for the consecutive injections ranged 0.010–0.17% and the peak area RSDs were 0.24–4.97%. In general, the peak area precisions were lower using suppressed conductivity detection and the highest RSDs were observed for IPAD after suppression. The higher RSDs for IPAD in this configuration are expected due to the increase in IPAD baseline noise caused by the suppressor.

The interday precision for suppressed conductivity detection was determined over 12 days. The peak area RSDs over the elapsed time ranged 1.0–4.6% ($n=44$) for putrescine, cadaverine, histamine, agmatine, spermidine, and spermine that included a total of 190 injections of samples or standards. The lowest and highest RSD values were observed for agmatine and histamine, respectively. The change in peak area response over the first 5 consecutive days for IPAD after suppression was within ±5% with the exception of histamine which resulted in a change in peak area of −16.6%. The interday peak area precision was in the range of 4.9–6.2% during this same time period except for histamine (9.1%).

The interday retention time precision was also evaluated for IPAD and suppressed conductivity-IPAD over 15 and 12 consecutive days, respectively. For IPAD, the retention time precision was in the range of 0.12–1.0% over the specified time. The percent change in retention time from the beginning to the end of this time period was in the range of −0.3 to −4.3%. The interday retention time precision for suppressed conductivity-IPAD was in the range of 0.04–0.39% with a decrease in retention time of <0.3% over 12 consecutive days.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>IPAD</th>
<th>Suppressed conductivity detection</th>
<th>IPAD (post-suppression)</th>
<th>UV detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range (mg/l)</td>
<td>Linearity ($r^2$)</td>
<td>LOD (mg/l)</td>
<td>Range (mg/l)</td>
</tr>
<tr>
<td>Dopamine</td>
<td>0.1–5</td>
<td>0.9999</td>
<td>0.021</td>
<td>N/A*</td>
</tr>
<tr>
<td>Tyramine</td>
<td>0.2–10</td>
<td>0.9999</td>
<td>0.073</td>
<td>N/A</td>
</tr>
<tr>
<td>Putrescine</td>
<td>0.2–10</td>
<td>0.9979</td>
<td>0.039</td>
<td>0.2–10</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>0.1–5</td>
<td>0.9999</td>
<td>0.069</td>
<td>0.1–5</td>
</tr>
<tr>
<td>Histamine</td>
<td>0.1–5</td>
<td>0.9999</td>
<td>0.028</td>
<td>0.1–5</td>
</tr>
<tr>
<td>Serotonin</td>
<td>0.1–5</td>
<td>0.9998</td>
<td>0.054</td>
<td>N/A</td>
</tr>
<tr>
<td>Agmatine</td>
<td>0.2–10</td>
<td>0.9998</td>
<td>0.17</td>
<td>0.2–10</td>
</tr>
<tr>
<td>Spermidine</td>
<td>1–20</td>
<td>0.9999</td>
<td>0.39</td>
<td>1–20</td>
</tr>
<tr>
<td>Spermine</td>
<td>0.1–5</td>
<td>0.9999</td>
<td>0.062</td>
<td>0.1–5</td>
</tr>
</tbody>
</table>
| *N/A = not applicable.
3.4. Determination of biogenic amines in alcoholic beverages with IPAD

The analysis of alcoholic beverages by IPAD can produce complex chromatograms with several unidentified peaks with similar retention times that correspond to the analytes of interest. The analytes with the highest probability of interfering with the determination of biogenic amines using electrochemical detection include free amino acids, aliphatic amines, and aromatic amines. The presence of an abundance of unknowns can complicate the correct identification of the analytes of interest. The separation of several amino acid precursors, such as hydroxytryptophan, tryptophan, ornithine, and histidine interfered with the determination of dopamine using the IonPac CS18 column with the gradient conditions previously described. In addition, arginine eluted within 0.3 min of tyramine and therefore would interfere with its determination in samples containing arginine. Further optimization of the gradient conditions does yield a satisfactory arginine/tyramine resolution (Fig. 2). However, the change in gradient conditions did not resolve all interferences in all samples and did not fully resolve several other target biogenic amines. Therefore, confirmation of tyramine in samples that produced a positive identification by IPAD was verified by UV detection using the gradient conditions described in Section 2. The amino acids described above were the only known interferences to occur in this study.

Tyramine was initially detected in all alcoholic beverages examined by IPAD. These samples were considered suspect for tyramine due to the known interference with arginine and were therefore also analyzed by UV absorbance detection. IPAD detected 5.6 mg/l tyramine in the California Cabernet Sauvignon (red wine) sample, however, UV detected only 2.6 mg/l tyramine with a spike recovery of 95% for 2.2 mg/l added to the sample (Fig. 3). This suggests that tyramine is present in the sample that also contains an IPAD active interferent. Both UV detection and a change in gradient conditions confirmed that tyramine was not present in the Pinot Grigio wine sample. Tyramine in the Australian Cabernet Sauvignon and rosé wine samples could not be confirmed by UV due to a large broad (∼4 min wide) peak that eluted within the retention time window of tyramine.

IPAD identified tyramine in the beer samples within the same concentration range (10–17 mg/l) reported in the literature. Tyramine has been identified as a major biogenic amine in Belgian beer samples with concentrations of 28.7 ± 17.3 mg/l [16]. In our study, all beer samples were spiked with known tyramine concentrations, resulting in calculated recoveries in the range of 86–109%. Further investigation of these samples by UV detection revealed an absence of tyramine. The acceptable spiked recoveries calculated from these samples indicate that the unknown peak produces a similar electrochemical response as tyramine, further complicating an accurate identification. This
demonstrates the benefit of using multiple detectors for peak identification in complex matrices.

The total biogenic amine concentrations varied considerably from 1.6–25.7 mg/l between the four wines. The California red wine contained the highest total biogenic amine concentration while the white and rosé wines had almost equally low biogenic amine concentrations of 2 and 1.6 mg/l, respectively. Red wines commonly contain higher concentrations of amines as a result of the malolactic fermentation (MLF) process [25]. The recoveries of the biogenic amines were determined by spiking known concentrations of the target biogenic amines in the wine samples resulting in calculated recoveries within 83–104% using cation-exchange chromatography coupled with IPAD.

Table 2 summarizes the results obtained for biogenic amines in alcoholic beverages using IPAD. Putrescine was the only biogenic amine detected in all wine samples, but the concentration varied considerably from 0.4–16 mg/l with higher concentrations detected in the red wines compared to the rosé and white wine samples. The highest concentration of histamine (4.9 mg/l) was detected in the California red wine and nearly an order of magnitude lower concentration was detected in the Australian red wine. No histamine was found in either the white or rosé wine samples. Similar results were reported for putrescine in Spanish and Portuguese red wines [13,14]. Putrescine and histamine are generally found in higher concentrations in red wine where MLF occurs compared to white or rosé wines where MLF does not naturally occur or takes place to a lower extent [12]. Histamine has also been found at higher concentrations in red wines with a lower total sulfur dioxide level [26]. Currently, there are no legal maximum tolerable limits for biogenic amines in wine. Although 2 mg/l histamine in wine has been suggested as a permissible limit [3], many European countries have recommended limits in the range of 3–10 mg/l [10]. The histamine concentration found in the California red wine in this study was still significantly less than the 20 mg/l concentration described as producing physiological effects in humans [12].

Cadaverine was detected at <1 mg/l in the red and white wine samples. Agmatine was only detected in the rosé wine at a concentration of 1.2 mg/l and spermidine was found in the California red wine with a concentration of 1.7 mg/l. Cadaverine can originate from the decarboxylation of lysine and has also been associated with Enterobacteriaceae in meat [4]. However, there is unlikely any direct correlation between this strain of bacteria and cadaverine in wine samples as enterobacteria is not normally found during the vinification process. Spermidine is a ubiquitous polyamine that is involved in a number of physiological processes, such as cell division, fruit development, and response to stress [4]. The occurrence of spermidine in wine may be derived from grapes or yeast lysis while the variability in different wines could be related to harvest conditions, such as temperature, rain, and soil nutrients, etc. [14]. Agmatine appears to be more common in beer than wine and relatively little information is known about its significance during the wine making process. However, agmatine is an intermediate of putrescine formation from arginine.

The analysis of three different bottled beers revealed the presence of putrescine, histamine, and agmatine in all samples. The
Biogenic amine concentrations were determined in most of the samples reported in Table 2. Table 3 summarizes these results after sample storage at 4 °C for up to 3 weeks. Amine concentrations increased in nearly all samples after storage. However, cadaverine was no longer detected in the white wine after storage for 1 week. The most interesting results were the detection of spermidine and spermine that were not previously observed in the alcoholic beverages prior to storage, with the exception of spermidine in the California red wine. The detection of these amines is at least partially due to the better sensitivity of suppressed conductivity detection. Analytes: 1-putrescine (6.6 mg/l), 2-cadaverine (0.67 mg/l), 3-histamine (0.60 mg/l), 4-agmatine (7.7 mg/l), 5-spermidine (1.2 mg/l), 6-spermine (0.73 mg/l).

Concentration ranges detected in the beer samples were 2–4 mg/l putrescine, 0.2–0.4 mg/l histamine, and 6–14 mg/l agmatine. Putrescine, agmatine, spermidine, and spermine are considered natural beer constituents that are present in malt and yeast at higher concentrations than in hops [11]. The putrescine concentrations in our beer samples were within the normal range of 0.2–8.0 mg/l reported for European beers [27]. Relatively little variability was observed for the histamine concentrations between beer samples. The presence of histamine has previously been used as an indication of containing lactic acid bacteria during the brewing process [11]. The histamine concentrations found in our samples are significantly lower relative to the other amines present and are not considered to represent any toxicological hazard. The total biogenic amine concentration of each beer was similar, in the range 10–17 mg/l. Loret et al. proposed a beer biogenic amine index (BAI) to assess the quality of the production process [16]. The BAI is calculated by taking the ratio of the biogenic amines of bacterial origin (i.e., tyramine, putrescine, cadaverine, histamine, phenylethylamine, and tryptamine) to the natural biogenic amine found in malt (agmatine). The BAIs for our beer samples were all <1, suggesting that they were produced by a non-contaminated fermentation process (high microbiological quality). Overall, recoveries for the spiked beer samples were 87–104% using IPAD.

3.5. Determination of changes in biogenic amine concentrations in alcoholic beverages during storage at 4 °C with detection by suppressed conductivity-IPAD

The biogenic amine concentrations in alcoholic beverages during storage at 4 °C with detection by suppressed conductivity-IPAD were 0–65% for putrescine, 67–184% for histamine, and 26% (wheat beer #2) for agmatine. Fig. 4 shows a separation of biogenic amines determined in wheat beer #2 after 2 weeks storage using suppressed conductivity detection. No significant change in the agmatine concentration was observed between the lager and wheat beer #1. The increase in the histamine concentration for wheat beer #1 (+184%) was the most significant observed for the biogenic amines previously detected by IPAD. Kalac et al. also observed a significant histamine increase in one of the
Biogenic amine concentrations in alcoholic beverages detected by suppressed conductivity and IPAD

<table>
<thead>
<tr>
<th>Sample</th>
<th>Suppressed conductivity detection</th>
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<tbody>
<tr>
<td></td>
<td>Putrescine</td>
</tr>
<tr>
<td></td>
<td>Amount found (mg/l)</td>
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<tr>
<td></td>
<td>Recov. (%)</td>
</tr>
<tr>
<td>Wheat beer #1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.4 ± 0.0</td>
</tr>
<tr>
<td>Wheat beer #2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.6 ± 0.0</td>
</tr>
<tr>
<td>Lager beer&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.0 ± 0.0</td>
</tr>
<tr>
<td>California Cabernet</td>
<td>19.4 ± 0.1</td>
</tr>
<tr>
<td>Sauvignon&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Australian Cabernet</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td>Sauvignon&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Pinot Grigio&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.7 ± 0.0</td>
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<td></td>
<td></td>
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<tr>
<td>IPAD (post-suppression)</td>
<td></td>
</tr>
<tr>
<td>Wheat beer #1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>Wheat beer #2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>Lager beer&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.0 ± 0.0</td>
</tr>
<tr>
<td>California Cabernet</td>
<td>22.1 ± 0.4</td>
</tr>
<tr>
<td>Sauvignon&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Australian Cabernet</td>
<td>6.9 ± 0.3</td>
</tr>
<tr>
<td>Sauvignon&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Pinot Grigio&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.5 ± 0.1</td>
</tr>
</tbody>
</table>

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<sup>a</sup> Stored at 4°C for 3 weeks.
<sup>b</sup> Stored at 4°C for 1 week.
<sup>c</sup> Stored at 4°C for 2 weeks.
<sup>d</sup> <DL, less than the detection limit.
beer samples studied when stored in bottles at 21 °C for 8 days [29]. The presence of lactic acid bacteria, primarily lactobacilli, has been demonstrated to be the primary cause for histamine increase in bottled beers over time [29]. Recalculating the BAI for all beers after storage at 4 °C results in an index value of <1 for wheat beer #1 and the lager beer and a BAI of 1.0 for wheat beer #2. According to the authors, a BAI between 1.0 and 10.0 would indicate that the beer had been produced by fermentation procedures that could be moderately contaminated by decarboxylating bacteria (intermediate level of microbiological quality) [16]. The average amine recoveries for the spiked beer samples were in the range 88–118%. The calculated concentrations by IPAD in the suppressed conductivity-IPAD configuration were within ±12% of the concentrations determined by suppressed conductivity.

4. Conclusion

The described IC method successfully determined the target biogenic amines in alcoholic beverages. The eluent conditions enable the use of suppressed conductivity, IPAD, and UV detections to allow the identification and confirmation of biogenic amines in chemically complex alcoholic beverages. Suppressed conductivity detection demonstrated good precision and recovery for many of the biogenic amines and superior sensitivity compared to previously reported methods in the literature. IPAD provides a wider selectivity than suppressed conductivity and good sensitivity for many of the biogenic amines of interest. The use of UV detection adds confidence to the analytical results by providing confirmation of tyramine in the alcoholic beverages.

References