Determination of Biogenic Amines in Foods and Alcoholic Beverages Using Ion Chromatography with Suppressed Conductivity and Integrated Pulsed Amperometric Detections

Brian M. De Borba and Jeffrey S. Rohrer, Dionex Corporation, Sunnyvale, CA, USA

ABSTRACT

The determination of biogenic amines in foods and alcoholic beverages is important due to their physiological and toxicological effects. Amine formation in food and beverages occurs by the microbial decarboxylation of amino acids. At high concentrations, these compounds can lead to serious health effects. Therefore, the accurate determination of biogenic amines and knowledge regarding their accumulation under different storage conditions is critical in the food and beverage industry. Ion chromatography has not commonly been used for this determination primarily due to the strong hydrophobic interactions between two or more protonated amino groups and the stationary phase that result in long retention times and poor peak symmetries. In this paper, we describe the use of a recently developed cation-exchange column combined with suppressed conductivity detection, integrated pulsed amperometric detection (IPAD), and UV detection for the determination of common biogenic amines in fermented and non-fermented food products and alcoholic beverages. The linearity, precision, and recoveries are compared between the different detection techniques.

INTRODUCTION

Biogenic amines are low molecular weight organic bases that possess biological activity. The requirements for the formation of biogenic amines in foods and beverages includes:

- Availability of free amino acids
- The presence of decarboxylase-positive microorganisms
- Conditions that enable bacterial growth

In general, the consumption of biogenic amines does not present a health hazard to individuals unless large amounts are consumed or the natural mechanism for the breakdown of amines is inhibited. The most well known intoxications of biogenic amines are scombroid poisoning and the "cheese" reaction, which occur from consuming food products containing high concentrations of histamine or tyramine, respectively. Currently, there are no government regulations for most biogenic amines in food and alcoholic beverages, with the exception of histamine. The U.S. FDA has established a defect action level of 50 mg/kg histamine and a toxicity level of 500 mg/kg histamine in fish.

EXPERIMENTAL

Method Conditions

Columns:	IonPac® CG18, CS18, 2 mm
Eluent:	3 mM MSA from 0–6 min, 3–10 mM from 6–10 min, 10–15 mM from 10–22 min, 15 mM from 22–28 min, 15–30 mM from 28–35 min, 30–45 mM from 35.1–45 min
Eluent Source:	EGC II MSA
Flow rate:	0.30 mL/min
Temperature:	40 °C
Injection Vol.:	5 µL
Detection:	Suppressed conductivity,CSRS® ULTRA II, 2 mm, AutoSuppression® external water mode and/or UV-Vis detection set at 276 nm
Postcolumn Addition:	100 mM NaOH
Postcolumn Reagent Flow:	0.24 mL/min
Detection:	Integrated pulsed amperometry, conventional Au electrode

Figure 1 illustrates the instrument configurations used to determine biogenic amines in foods and alcoholic beverages. As shown, three separate instrument configurations were used in these experiments that included IPAD only, suppressed conductivity-IPAD, and UV-IPAD. A wider range of selectivity is observed with IPAD, which enables it to detect all of the target biogenic amines. Suppressed conductivity detection cannot detect dopamine, tyramine, or serotonin, but has better sensitivity for the remaining amines than IPAD. Absorbance detection is more selective toward compounds that have aromatic character, such as tyramine, and therefore was used to confirm or refute the presence of tyramine in selected samples.

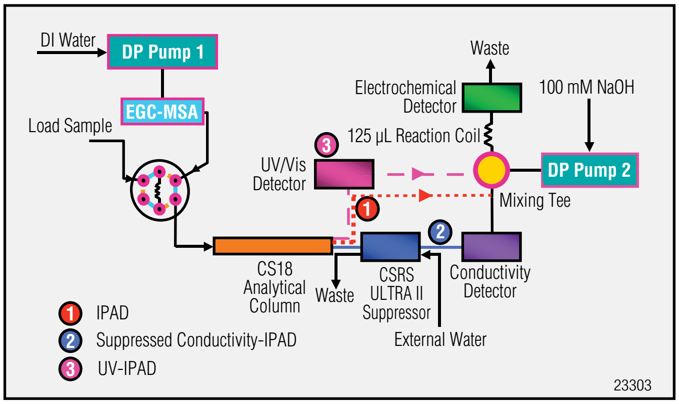
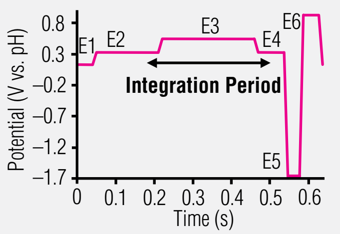


Figure 1. Schematic diagram of the system configurations used for the determination of biogenic amines.

Figure 2 illustrates a modified IPAD waveform that is typically used for amino acid analysis. However, the waveform can also be used to detect amines and some sulfur compounds.



E1 – adsorption potential
E2 – delay potential before integration begins
E3 – oxidation potential (Au → AuO)
E4 – second integration potential
E5 – cathodic cleaning
E6 – activation potential

Time (s)	E (V vs. pH)	Integration
0.000	0.13	
0.040	0.13	
0.050	0.33	
0.210	0.33	On
0.220	0.55	
0.460	0.55	
0.470	0.33	
0.536	0.33	Off
0.546	–1.67	
0.576	–1.67	
0.586	0.93	
0.626	0.93	
0.636	0.13	

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Figure 2. IPAD waveform for biogenic amine detection.

RESULTS AND DISCUSSION

Method Performance

Table 1 summarizes the linearity and limits of detection (LODs) for biogenic amines detected by IPAD, suppressed conductivity detection, and UV detection. As shown, the LODs were significantly better for most of the biogenic amines detected by suppressed conductivity compared to IPAD. The peak area precisions for 10 replicate injections of a biogenic amine standard was <3%, <1.5%, and <1.5% (tyramine only) for IPAD, suppressed conductivity detection, and UV detection, respectively (data not shown).

Figure 3 demonstrates the selectivity of the CS18 column for the separation of biogenic amines using IPAD (chromatogram A), suppressed conductivity detection (chromatogram B), or UV detection (chromatogram C). Notice that dopamine, tyramine, and serotonin (peaks 1, 2, and 6) cannot be detected by suppressed conductivity due to their minimal dissociation upon suppression.

Biogenic Amines in Alcoholic Beverages

A variety of beer and wine samples were analyzed in this study for the presence of biogenic amines. In beer, putrescine, spermidine, and spermine are considered natural constituents that primarily originate from malt. However, the presence of tyramine, cadaverine, and histamine has been associated with the activities of contaminating lactic acid bacteria during the brewing process. In wine samples, malolactic fermentation or the action of yeasts in primary fermentation can produce tyramine, putrescine, cadaverine, histamine, and phenylethylamine.

Figure 4 demonstrates the separation of biogenic amines in wheat beer using suppressed conductivity detection. Agmatine, which is routinely found in beer samples because it originates from yeast and malt, was the highest concentration detected.

Table 1. Linearity and Limits of Detection of Biogenic Amines

	IPAD Only			Suppressed Conductivity		UV	
Analyte	Range (mg/L)	Linearity (r^2)	LOD[a] (µg/L)	Linearity (r^2)	LOD (µg/L)	Linearity (r^2)	LOD (µg/L)
Dopamine	0.1–5	0.9999	20	—	—	—	—
Tyramine	0.2–10	0.9999	80	—	—	0.9997	110
Putrescine	0.2–10	0.9979	50	0.9986	3.5	—	—
Cadaverine	0.1–5	0.9999	70	0.9997	5.3	—	—
Histamine	0.1–5	0.9999	40	0.9998	18	—	—
Serotonin	0.1–5	0.9998	70	—	—	—	—
Agmatine	0.2–10	0.9998	170	0.9998	9.0	—	—
Phenylethylamine	1–20	0.9999	400	0.9999	81	—	—
Spermidine	0.1–5	0.9999	80	0.9993	4.0	—	—
Spermine	0.1–5	0.9996	50	0.9990	9.0	—	—

[a]LOD = limit of detection = 3 × S/N

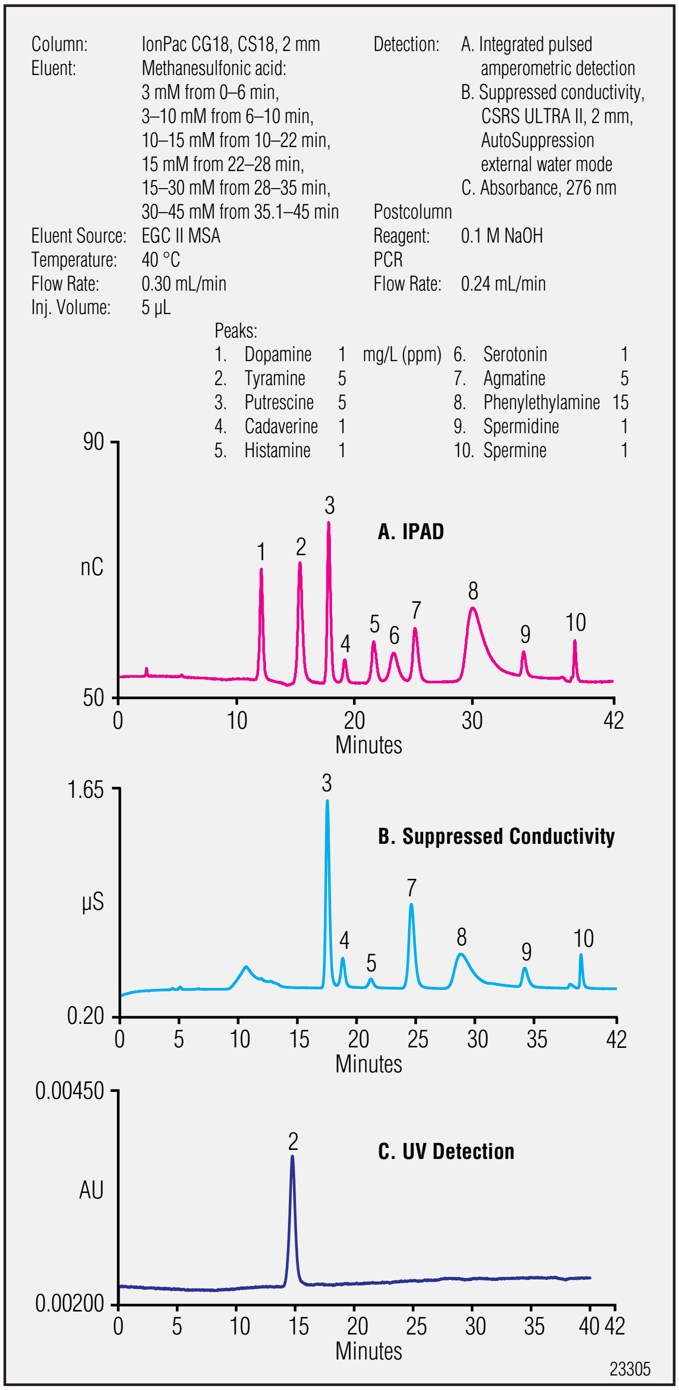

Figure 3. Separation of a biogenic amine standard on the IonPac CS18 column.

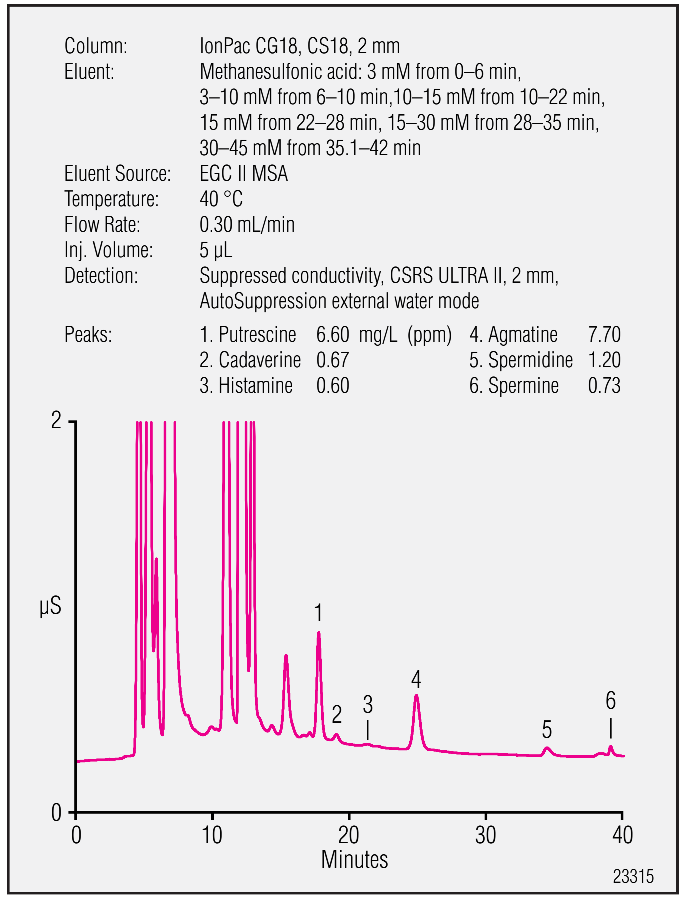

Figure 4. Determination of biogenic amines in wheat beer using suppressed conductivity detection.

Figure 5 shows a separation of biogenic amines in California red wine using IPAD and UV detection (tyramine only). Among the wine samples investigated in this study, the California red wine contained the highest total biogenic amine concentration, including the highest concentration of histamine for all the alcoholic beverages studied.

Each sample was spiked with known concentrations of biogenic amines, which resulted in calculated recoveries within 83–104% and 88–122% for IPAD and suppressed conductivity detection, respectively.

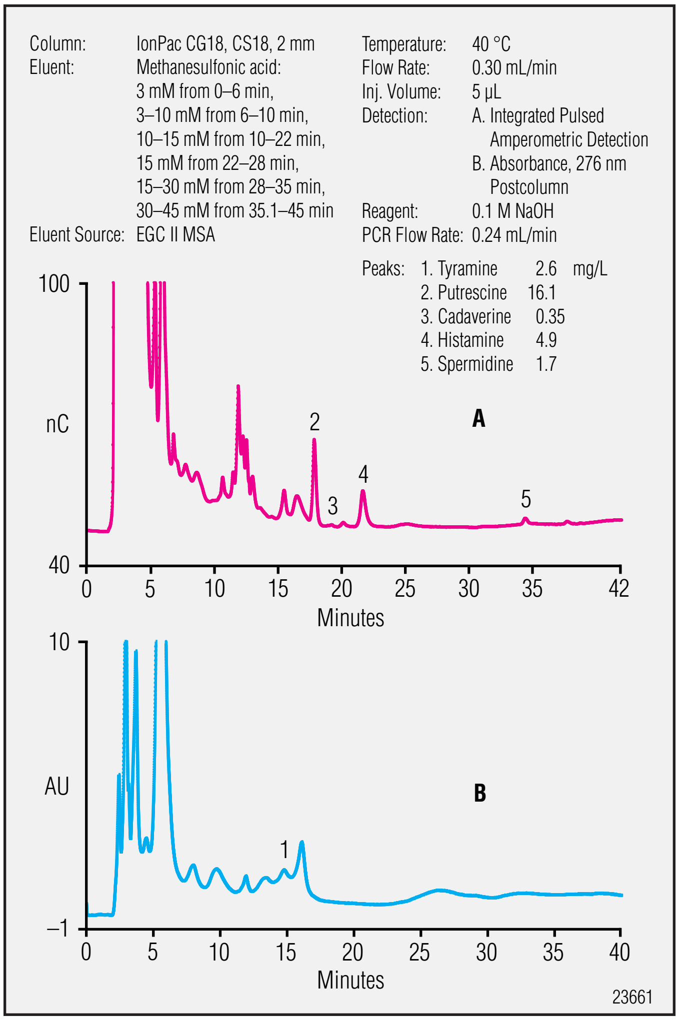

Figure 5. Determination of biogenic amines in California Cabernet Sauvignon using IPAD and UV detection.

Biogenic Amines in Food Products

A variety of fermented and non-fermented, fresh and spoiled food products were assayed for the presence of biogenic amines by IPAD. In addition, the effect of storage time and temperature on biogenic amine concentrations in selected food products was examined using IPAD or suppressed conductivity detection. Figure 6 demonstrates the determination of biogenic amines in fresh spinach leaves by IPAD. The primary biogenic amines detected in the sample were histamine and spermidine. The histamine concentration was the highest detected relative to other investigated food samples.

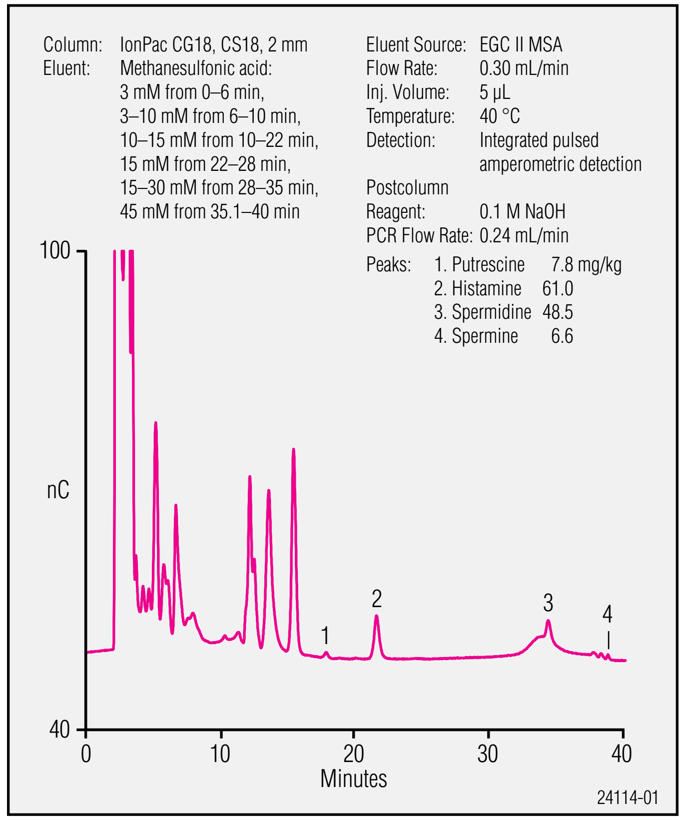

Figure 6. Determination of biogenic amines in spinach leaves using IPAD.

Increased concentrations of biogenic amines can be used as an indication of spoilage. To investigate this effect, several food products were allow to spoil by storage at room temperature (25 °C) from one to two weeks. Figure 7 demonstrates the effect of spoilage on the concentration of biogenic amines. For some samples, biogenic amines only appeared after spoilage. For example, putrescine was only detected in tuna and cheddar cheese after spoilage. The presence of putrescine and cadaverine has been reported as an indication of seafood decomposition and can enhance the toxicity of histamine, if present in spoiled fish. For the cheese samples, the tyramine concentration increased significantly upon spoilage. For the spoiled Swiss cheese sample, the tyramine concentration exceeded the 1100 mg/kg concentration known to induce toxicological effects.

Spiked recoveries for the investigated fresh and spoiled samples were within 85-110% and 88–106% for IPAD and suppressed conductivity detection, respectively.

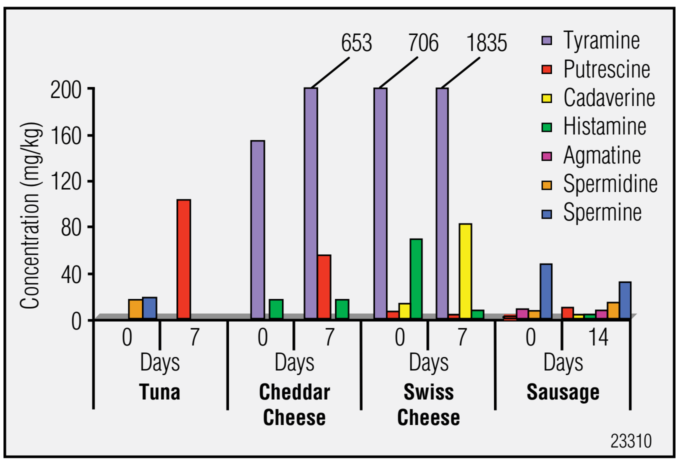

Figure 7. Effect of storage at 25 °C on the concentration of biogenic amines in food products.

CONCLUSIONS

- Biogenic amines can be determined in a wide range of fermented and non-fermented food products and beverages by suppressed conductivity or IPAD.
- Suppressed conductivity detection demonstrated the lowest detection limits and good selectivity for biogenic amines with no interferences from common cations.
- IPAD demonstrated the widest selectivity for biogenic amines enabling the detection of dopamine, tyramine, and serotonin.
- Multiple detectors can be configured easily for confirmation of suspect analyte(s) in complex matrices.

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