

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

INTRODUCTION

Biogenic amines are common 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 (catecholamines 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 Biogenic amines, such as histamine, may be present before foods appear spoiled or have an unacceptable appearance.⁵ The normal dietary intake of biogenic amines 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 The consumption of an excess amount of these amines, however, can induce severe toxicological effects and

produce various physiological symptoms, such as nausea, respiratory distress, headache, sweating, heart palpitations, and hyper- or hypotension.⁷

Malolactic fermentation or the action of yeasts in primary fermentation has been associated with the production of biogenic amines such as tyramine, putrescine, cadaverine, histamine, and phenylethylamine in wine samples.^{2,8} Histamine can produce headaches, flushing of the face and neck, and hypotension, whereas some aromatic amines, such as tyramine and phenylethylamine, can cause migraines and hypertension.¹ The concentration and content of biogenic amines in wines are variable depending on the storage time and conditions, quality of raw materials, and possible microbial contamination during the winemaking process.⁹ Putrescine, agmatine, spermidine, and spermine are considered natural beer constituents that primarily originate from malt. The presence of tyramine, cadaverine, and histamine, however, has been associated with the activities of contaminating lactic acid bacteria during the brewing process.¹⁰

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The determination of biogenic amines presents a challenging analytical problem because they are usually hydrophobic, are poor chromophores, and often occur in low concentrations in complex matrices. Reversed-phase high-performance liquid chromatography (HPLC) combined with pre- or postcolumn chemical derivatization and UV or fluorescence detection is commonly used for determining biogenic amines in alcoholic beverages. *o*-Phthalaldehyde (OPA) combined with a thiol compound, such as 2-mercaptoethanol (MCE), is the most frequently reported derivatizing agent used to determine biogenic amines in wine^{2,9,11–13} and beer^{14,15} samples. Because OPA derivatives have limited stability, however, OPA-MCE postcolumn derivatization procedures are generally preferred over precolumn procedures.¹⁶ Unfortunately, this chemical derivatization adds complexity to the analysis, requires additional skilled labor, and can sometimes produce by-product interferences.

Ion chromatography (IC) coupled to pulsed amperometric detection (PAD) or integrated pulsed amperometric detection (IPAD) after postcolumn base addition has been used for the determination of underivatized biogenic amines.^{17–19} These procedures require high acid or salt gradients combined with an organic solvent to separate strongly retained amines, such as spermidine and spermine.¹⁹ Organic solvents, however, such as acetonitrile, can produce undesirable decomposition by-products with amperometric detection, resulting in potential interferences.²⁰

Consequently, the use of IC for the determination of biogenic amines has not been widely reported. This is at least partially due to the strong hydrophobic interactions between the protonated amines and stationary phases, resulting in long retention times and poor peak shapes. In addition, eluents required to separate these amines are often not compatible with suppressed conductivity, the simplest detection method for some of the major biogenic amines. The development of the IonPac[®] CS17, a weak carboxylic acid functionalized cation-exchange column that reduces the interactions of hydrophobic analytes,²¹ allows the use of suppressed conductivity detection. This combination of column and detector was successfully applied to the determination of biogenic amines in fish²² and meat²³ samples.

A newer cation-exchange column, the IonPac CS18, was specifically designed for the determination of small polar amines. This column has a slightly higher hydrophobicity than the CS17 and therefore improves the separation of close-eluting peak pairs, such as putrescine and cadaverine.

Suppressed conductivity detection is one of the simplest detection configurations, allowing the detection of most target biogenic amines. IPAD provides a broader selectivity, enabling the detection of all biogenic amines of interest. UV detection can provide selectivity towards aromatic compounds. Therefore all three detectors were employed and compared in this Application Note. The IonPac CS18 column was coupled to IPAD to detect biogenic amines in beer and wine samples prior to storage. Because relatively little information exists on the accumulation of biogenic amines in alcoholic beverages during storage, refrigerated samples were analyzed using suppressed conductivity detection coupled to IPAD. UV detection was used to confirm the presence of tyramine in some alcoholic beverages. Suppressed conductivity and IPAD were also compared in terms of linearity, detection limits, precision, and recovery of biogenic amines spiked in beer and wine samples.

EQUIPMENT

Dionex ICS-3000 system consisting of:

DP Dual Pump with in-line degas option

DC Detector/Chromatography module with conductivity and electrochemical cells

Electrochemical cell consisting of a pH/Ag/AgCl reference electrode and a conventional Au electrode (PN 063722)

EG Eluent Generator module

EluGen[®] EGC II MSA cartridge (P/N 058902)

AD25 UV/Vis Absorbance Detector with 10-mm cell

Mixing Tee, 3-way, 1.5 mm i.d. (P/N 024314)

Knitted Reaction Coil, 125 μ L (P/N 053640)

Two 4-L plastic bottle assemblies for external water mode of operation

Chromeleon[®] 6.7 Chromatography Management software
Centrifuge (Beckman Coulter, Brea, CA)

REAGENTS AND STANDARDS

Reagents

Deionized water, Type I reagent grade, 18 M Ω -cm resistivity or better

Sodium hydroxide, 50% (w/w) (Fisher Scientific, SS254-1)

Methanesulfonic acid, 99% (Dionex Corporation, P/N 033478)

Standards

Dopamine hydrochloride (Sigma Chemical Co., H8502)

Serotonin hydrochloride, \geq 98% (Sigma Chemical Co., H9523)

Tyramine, 99% (Aldrich Chemical Co., T90344)

Putrescine dihydrochloride, \geq 98% (Sigma Chemical Co., P7505)

Cadaverine dihydrochloride, $>$ 98% (Sigma Chemical Co., C8561)

Histamine, \sim 97% (Sigma Chemical Co., H7125)

Agmatine sulfate, 97% (Aldrich Chemical Co., 101443)

β -Phenylethylamine, 99% (Aldrich Chemical Co., 128945)

Spermidine trihydrochloride, $>$ 98% (Calbiochem, 56766)

Spermine tetrahydrochloride, \geq 99% (Calbiochem, 5677)

CONDITIONS

Columns: IonPac CS18 Analytical, 2 \times 250 mm (P/N 062878)

IonPac CG18 Guard, 2 \times 50 mm (P/N 062880)

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 35–45 min

Flow Rate: 0.30 mL/min

Temperature: 40 $^{\circ}$ C (lower compartment)
30 $^{\circ}$ C (upper compartment)

Inj. Volume: 5 μ L

Detection**: Suppressed conductivity, CSRS[®] ULTRA II (2 mm), AutoSuppression[®] device, external water mode, power set at 40 mA and/or UV-Vis detection set at 276 nm

Background

Conductance: 0.4–0.5 μ S

Conductance

Noise: 0.2–0.3 nS

System

Backpressure: \sim 2500 psi

Postcolumn Addition

Detection: Integrated pulsed amperometry, conventional Au electrode

Postcolumn

Reagent Flow: 100 mM NaOH at 0.24 mL/min

IPAD

Background: 40–50 nC

IPAD Noise: 60–70 pC (without suppressor installed)
 \sim 210 pC (with suppressor installed)

* The column was equilibrated at 3 mM MSA for 5 min prior to injection.

** This application note discusses three separate detection configurations: IPAD, suppressed conductivity-IPAD, and UV-IPAD.

Waveform

Time(s)	Potential vs pH (V)	Gain Region	Ramp	Integration
0.000	+0.13	Off	On	Off
0.040	+0.13	Off	On	Off
0.050	+0.33	Off	On	Off
0.210	+0.33	On	On	On
0.220	+0.55	On	On	On
0.460	+0.55	On	On	On
0.470	+0.33	On	On	On
0.536	+0.33	Off	On	Off
0.546	-1.67	Off	On	Off
0.576	-1.67	Off	On	Off
0.586	+0.93	Off	On	Off
0.626	+0.93	Off	On	Off
0.636	+0.13	Off	On	Off

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solution

Generate the methanesulfonic acid (MSA) eluent on-line by pumping high quality deionized water (18 M Ω -cm resistivity or better) through the EGC II MSA cartridge. Chromeleon software will track the amount of MSA used and calculate the remaining lifetime.

Alternatively, prepare 10 mM MSA by carefully adding 0.961 g of concentrated MSA to a 1-L volumetric flask containing about 500 mL of deionized water. Dilute to the mark and mix thoroughly. Prepare 100 mM MSA by carefully adding 9.61 g of concentrated MSA to a 1-L volumetric flask containing about 500 mL of deionized water. Dilute to the mark and mix thoroughly. Degas the eluents and store in plastic labware. The 3 mM MSA eluent is then proportioned between 10 mM MSA and high quality deionized water. The gradient is proportioned between the 100 mM MSA solution and deionized water.

Postcolumn Base Addition Solution for IPAD

100 mM Sodium Hydroxide

Prepare 100 mM sodium hydroxide solution by adding 8 g of 50% w/w NaOH to ~800 mL of degassed deionized water in a 1000 mL volumetric flask and then dilute to volume. *Sodium hydroxide pellets, which are coated with a thin layer of sodium carbonate, must not be used to prepare this solution. The 100 mM NaOH solution should be stored under helium in a pressurized container at all times.*

STANDARD PREPARATION

Prepare biogenic amine stock standard solutions at 1000 mg/L each by dissolving 123.8 mg of dopamine hydrochloride, 100 mg of tyramine, 182.7 mg of putrescine dihydrochloride, 171.4 mg of cadaverine dihydrochloride, 96 mg of histamine, 120.7 mg of serotonin hydrochloride, 172.7 mg of agmatine sulfate, 100 mg of phenylethylamine, 175.3 mg of spermidine trihydrochloride, and 172.1 mg of spermine tetrahydrochloride in separate 100 mL volumetric flasks. Bring to volume with deionized water. Stock solutions should be stored at 4 °C and protected from light. Prepare working standard solutions for generating calibration curves with an appropriate dilution of the stock solutions in 3 mM MSA. These solutions should be prepared fresh weekly and stored at 4 °C when not in use.

SYSTEM PREPARATION AND SETUP

Integrated Pulsed Amperometric Detection

Do not use a continuously regenerated cation trap column (CR-CTC) with IPAD. Install the EGC II MSA cartridge in the EG-3000 and configure the setup of the cartridge with the Chromeleon server configuration. Connect the cartridge to the EG degas assembly and install sufficient backpressure tubing (~91.4 cm of 0.003" i.d.) in place of the column set to produce a system pressure of ~2000 psi at 1 mL/min. Condition the cartridge with 50 mM MSA for 30 min at 1 mL/min. Remove the backpressure tubing temporarily installed in place of the column set and install a 2 x 50 mm CG18 and a 2 x 250 mm CS18 column. Make the sure the backpressure is at an optimal pressure of ~2300 psi when 45 mM MSA is delivered at 0.30 mL/min. Install additional backpressure tubing between the EG degas and injection valve as necessary to achieve an optimal pressure reading. Connect the external water source outlet to the Regen In port of the EG degas and adjust the head pressure on the reservoir to deliver a flow rate of 0.5-1 mL/min (~10-15 psi for a 4 L bottle). Divert the column effluent to waste until the electrochemical cell is properly installed and ready for use. *It is important to verify the external water flow through the degas Regen channel to effectively remove gases generated by the MSA cartridge. Failure to properly remove oxygen from the EG will result in a significant decline in the electrochemical background signal.*

Calibrate the pH electrode according to the instructions provided by the Chromeleon software. Install the Au working electrode in the electrochemical cell and then install a short piece (~25 cm) of black tubing (0.010" i.d.) on the cell outlet. For delivery of the 100 mM NaOH postcolumn reagent, we highly recommend using the DP-3000 to have an accurate and consistent flow rate throughout the analyses. Alternatively, a pressurized reservoir may be used to deliver NaOH to the mixing tee. A comparison between the pump and reservoir resulted in nearly equivalent baseline noise, but the pump was found to deliver a more consistent flow, particularly at the low flow rate described in this application note.

Install sufficient backpressure tubing on the pump used for post column addition to achieve a system pressure of approximately 2000 psi when 100 mM NaOH is delivered at 0.24 mL/min. Connect the outlet of this pump to the mixing tee and install a 125 µL knitted reaction coil between the mixing tee and cell inlet. Set the flow rate at 0.24 mL/min for the postcolumn base addition and turn the pump on with the third port of the mixing tee plugged with a 1/4-28" fitting. Allow the NaOH to flow through the cell for about 10 min and then connect the column outlet to the third port of the mixing tee (previously plugged) while the analytical pump is still running. *Be sure to wear gloves to avoid exposure to MSA solution from the column outlet.*

Program the waveform in the Chromeleon software. Set the waveform mode and reference electrode to IntAmp and pH, respectively. After selecting the waveform, set the cell voltage to the ON position. *Make sure that flow is passing through the cell before turning the voltage to the ON position.* The pH recorded by the reference electrode in the electrochemical cell should be within 12.05–12.40 for the gradient described in this application. A significant deviation from this range may be an indication of excessive reference electrode wear (if addition of the NaOH has been verified), and therefore may require replacement (routinely every 6–12 months for the ICS-3000 cell). However, variations in the pH reading may occur depending on the accuracy of the NaOH concentration. The background should remain within the range 30–70 nC for the conditions described in this application document. Significantly higher or lower values may be an indication of electrode malfunction or contamination within the system.

When turning the system off be sure to disconnect

the column outlet from the mixing tee while the pump is still running to prevent backflow of NaOH into the analytical column. Do not allow NaOH to enter the column as this can result in permanent damage.

Suppressed Conductivity–Integrated Pulsed Amperometric Detection

Suppressed conductivity detection can precede IPAD to obtain a dual determination of biogenic amines. Suppressed conductivity detection can also be used independently. Neither of these configurations, however, will allow the detection of dopamine, tyramine, or serotonin, which can be detected by using IPAD independently, or by using UV detection. Prepare the CSRS ULTRA II suppressor by hydrating the membranes with a disposable plastic syringe and push 3 mL of degassed deionized water through the Eluent Out port and 5 mL of degassed deionized water through the Regen In port. Allow the suppressor to stand for approximately 20 min to fully hydrate the suppressor screens and membranes. Install the CSRS ULTRA II suppressor for use in the external water mode by connecting the Regen Out of the suppressor to the Regen In of the EG degas and the Regen In of the suppressor to the external water source. Adjust the head pressure on the reservoir to deliver a flow rate of 1–3 mL/min (20–25 psi for a 4 L bottle). If IPAD is connected in series with the conductivity detector then install a short piece of 0.01" i.d. black tubing (5–6") on the cell outlet. *Do not install red tubing (0.005" i.d.) on the cell outlet because the combined pressure of the electrochemical cell and conductivity cell outlet tubing will result in backflow of NaOH through the suppressor and column. Backflow of NaOH can permanently damage the analytical column.* Connect the black tubing from the cell outlet to the mixing tee while flow is still on for both the postcolumn reagent and analytical column. Follow the setup instructions for the EG, column, and IPAD as previously described.

UV Absorbance–Integrated Pulsed Amperometric Detection

The UV absorbance detector was coupled to IPAD to gain further information on the presence of tyramine. Install the EG, column, and IPAD as previously described. Connect the column outlet to the UV detector cell inlet and the detector outlet to the mixing tee. Set the wavelength to 276 nm. Alternatively, UV can be used in-line with suppressed conductivity detection to

determine whether tyramine is present in the samples. In this configuration, the UV detector must be installed before the suppressor.

SAMPLE PREPARATION

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.

RESULTS AND DISCUSSION

Separation of Biogenic Amines

Figure 1 shows the separation of a standard mixture of biogenic amines with the column coupled directly to IPAD, suppressed conductivity, or UV detection using the gradient conditions described earlier. The separation was optimized to improve the resolution between histamine, serotonin, and agmatine. Dopamine, tyramine, and serotonin cannot be detected by suppressed conductivity detection because they are uncharged following suppression. Although dopamine, tyramine, and serotonin absorb at 276 nm, only tyramine was monitored by UV detection to confirm its presence in samples that had previously been identified with tyramine by IPAD.

Method Performance

The linearity, limits of detection, and precision of the method using suppressed conductivity detection, IPAD, and UV detection were examined. Dopamine, cadaverine, histamine, serotonin, spermidine, and spermine exhibited a linear peak area response in the range 0.10–5.0 mg/L. The linear range was 0.20–10 mg/L for tyramine, putrescine, and agmatine and 1–20 mg/L for phenylethylamine. The lower linear range limits for IPAD placed after the suppressor was slightly higher due to increased baseline noise. Calibration curves based on peak area response produced correlation coefficients between 0.997–0.999. The detection limits of the bio-

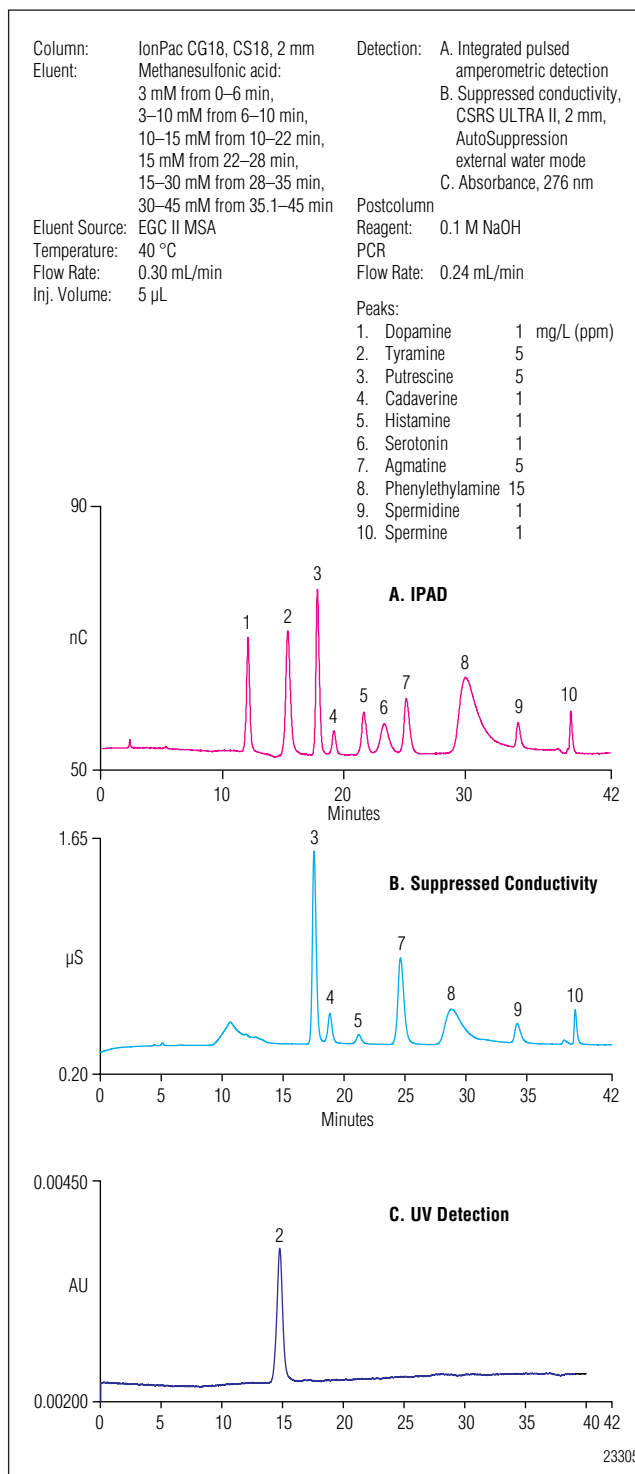


Figure 1. Separation of biogenic amines with (A) IPAD, or (B) suppressed conductivity detection. (C) Tyramine determined by UV detection.

Table 1. Linearity and Limits of Detection of Biogenic Amines

Analyte	IPAD Only			Suppressed Conductivity Detection			IPAD (post-suppression)			UV		
	Range (mg/L)	Linearity (r ²)	LOD (µg/L)	Range (mg/L)	Linearity (r ²)	LOD (µg/L)	Range (mg/L)	Linearity (r ²)	LOD (µg/L)	Range (mg/L)	Linearity (r ²)	LOD (µg/L)
Dopamine	0.1–5	0.9999	20	—	—	—	—	—	—	—	—	—
Tyramine	0.2–10	0.9999	80	—	—	—	—	—	—	0.2–10	0.9997	110
Putrescine	0.2–10	0.9979	50	0.2–10	0.9986	3.5	0.2–10	0.9974	97	—	—	—
Cadaverine	0.1–5	0.9999	70	0.1–5	0.9997	5.3	0.25–5	0.9997	160	—	—	—
Histamine	0.1–5	0.9999	40	0.1–5	0.9998	18	0.1–5	0.9998	88	—	—	—
Serotonin	0.1–5	0.9998	70	—	—	—	—	—	—	—	—	—
Agmatine	0.2–10	0.9998	170	0.2–10	0.9999	9.0	0.5–10	0.9999	290	—	—	—
Phenylethylamine	1–20	0.9999	400	1–20	0.9999	81	5–20	0.9999	1090	—	—	—
Spermidine	0.1–5	0.9999	80	0.1–5	0.9993	4.0	0.25–5	0.9996	140	—	—	—
Spermine	0.1–5	0.9996	50	0.1–5	0.9990	9.0	0.1–5	0.9998	90	—	—	—

genic amines were determined by using a signal-to-noise ratio of 3. Table 1 summarizes the linearity and limits of detection (LOD) for the biogenic amines detected by IPAD, suppressed conductivity detection, IPAD (post-suppression), and UV detection. As shown, the LODs were significantly better for most of the biogenic amines detected by suppressed conductivity compared to IPAD. In addition, suppressed conductivity detection produced nearly an order of magnitude lower LODs than HPLC with fluorescence detection, while IPAD was comparable.^{2,11} The improvement in sensitivity by suppressed conductivity detection is mainly due to the exceptionally low baseline noise of 0.2–0.3 nS and minimal baseline drift as result of electrolytically generating the MSA eluent online.

The peak area and retention time precisions for the biogenic amines were determined for the different detection configurations (IPAD, suppressed conductivity-IPAD, UV). A standard of biogenic amines containing 5 mg/L each of tyramine, putrescine, and agmatine and 1 mg/L dopamine, cadaverine, histamine, serotonin, spermidine, and spermine was used to determine precision. Replicate injections (n = 10) were performed and the retention time and peak area RSDs were calculated for each amine. Cation-exchange chromatography coupled to IPAD produced retention time and peak area precisions for 10 biogenic amines in the range 0.01–0.07% and 0.79–2.87%, respectively. For suppressed conductivity

detection, retention time and peak area precisions for seven biogenic amines were in the range 0.01–0.04% and 0.24–1.29%, respectively. IPAD placed after the suppressor resulted in higher retention time and peak area precisions of 0–0.14% and 1.22–4.97%, respectively due to the increased baseline noise. The retention time and peak area precisions for tyramine detected by UV were 0.17% and 1.28%, respectively.

Determination of Biogenic Amines in Alcoholic Beverages with IPAD

Beer and wine samples can generate complex chromatograms with several unknown peaks that correspond to, or overlap with, the target biogenic amines. For alcoholic beverages, some of these unknowns may include free amino acids, aliphatic amines, aromatic amines, or possibly other components with similar functional groups that are detected electrochemically. The presence of an abundance of unknowns can often complicate the correct identification of the analytes of interest. The separation of the amino acid precursors to the biogenic amines of interest revealed several interferences for the determination of dopamine using the IonPac CS18 column. Therefore, the determination of dopamine by this method was not feasible. In addition, arginine interfered with tyramine, with only a 0.3 min difference in retention times. Further optimization of the gradient conditions does yield a satisfactory arginine/tyramine

resolution (6 mM MSA from 0–3.5 min, 6–27 mM from 3.5–11 min, 27 mM from 11–18 min, 27–45 mM from 18–35 min), but other biogenic amines of interest were not fully resolved by this method. Furthermore, changing the parameters for one sample type may not produce acceptable results for another sample type. Therefore, the presence or absence of tyramine producing a positive identification by IPAD was confirmed by UV detection.

Tyramine was initially detected in all alcoholic beverages by IPAD. These samples were considered suspect for tyramine due to the known interference with arginine and were therefore confirmed by UV absorbance detection. Confirmation of tyramine in the California Cabernet Sauvignon red wine sample by UV detection produced a positive identification for tyramine with a concentration of 2.6 mg/L and spiked recovery of 95% (Figure 2). The result from the same sample using IPAD was 5.6 mg/L, further indicating a high probability of an interferent in the sample. The eluent gradient conditions for the Pinot Grigio wine sample were altered to verify the detection of tyramine. The change in gradient conditions and a secondary confirmation by UV detection indicated that tyramine was not present. The Australian Cabernet Sauvignon red wine and rosé wine samples could not be confirmed by UV due to a very broad (~4 min) unknown interferent that eluted within the same retention time window as tyramine.

Tyramine has been reported as a major biogenic amine in Belgian beer samples, with concentrations of 28.7 ± 17.3 mg/L.¹⁵ Tyramine levels detected in the beer samples by IPAD were within the concentration range of 10–17 mg/L. However, further investigation of these samples by UV detection revealed that no tyramine was present. In our study, all beer samples were also tested spiked with known tyramine concentrations, resulting in calculated recoveries in the range of 86–109%. The acceptable spiked recoveries calculated from these samples indicate that the unknown peak produces a similar electrochemical response to tyramine, further complicating the identification process. This demonstrates the benefit of using multiple detection systems for peak identification in complex matrices.

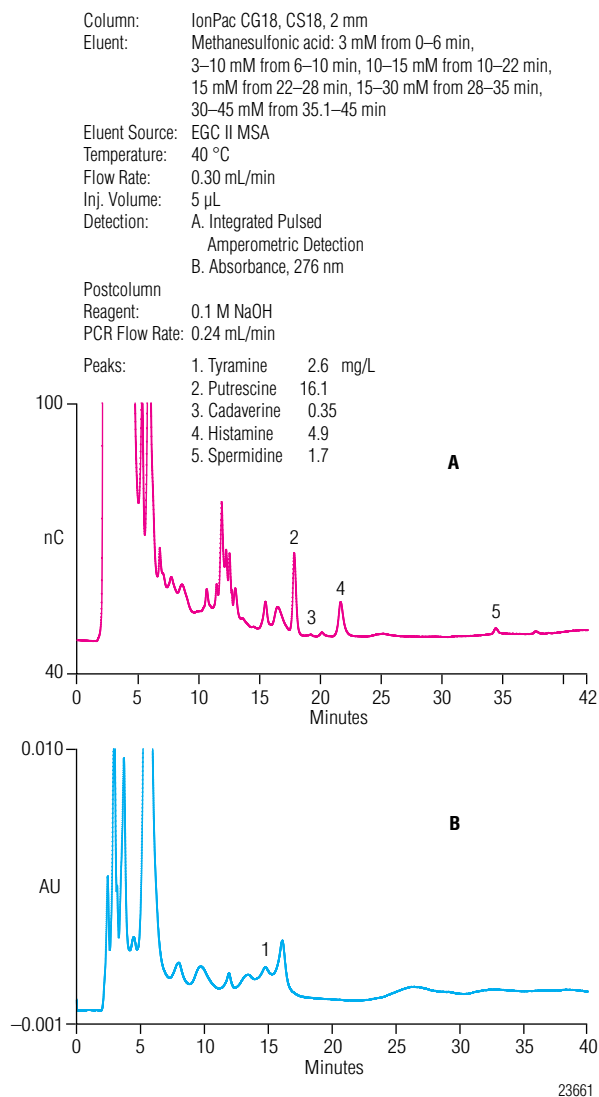


Figure 2. Determination of biogenic amines in a California Cabernet Sauvignon by (A) IPAD and (B) UV absorbance detection.

Table 2. Biogenic Amine Concentrations in Alcoholic Beverages Determined by IPAD^a

Sample	Tyramine		Putrescine		Cadaverine		Histamine		Serotonin		Agmatine		Spermidine		Spermine	
	Amount Found ^b (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)
Wheat Beer #1	— ^c	—	6.2±0.2	87.1	<DL	—	0.19±0.01	99.9	<DL	—	8.7±0.2	96.8	<DL	—	<DL	—
Wheat Beer #2	<DL ^d	—	4.0±0.1	88.6	<DL	—	0.36±0.02	102.1	<DL	—	6.1±0.1	91.7	<DL	—	<DL	—
Lager Beer	<DL	—	1.9±0.1	90.1	<DL	—	0.39±0.02	104.0	<DL	—	14.4±0.3	95.6	<DL	—	<DL	—
California Cabernet Sauvignon	2.6±0.1 ^e	94.7	16.1±0.0	85.3	0.35±0.05	92.9	4.9±0.1	90.6	<DL	—	<DL	—	1.7±0.1	104.1	<DL	—
Australian Cabernet Sauvignon	—	—	5.2±0.1	90.6	0.35±0.02	83.3	0.45±0.02	96.9	<DL	—	<DL	—	<DL	—	<DL	—
Rosé Wine	Int ^f	—	0.36±0.01	84.6	<DL	—	<DL	—	<DL	—	1.2±0.0	100.4	<DL	—	<DL	—
Pinot Grigio	<DL	—	1.3±0.0	97.0	0.68±0.01	94.4	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—

^aTyramine was determined by UV absorbance detection.

^bAverage concentration based on triplicate injections.

^cUnconfirmed.

^d<DL = less than the detection limit.

^eConcentration determined after 6 weeks storage at 4 °C.

^fInt = Chromatographic interference observed in UV detector.

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.^{12, 13} Putrescine and histamine are generally found in higher concentrations in red wine where malolactic fermentation (MLF) occurs, compared to white or rosé wines where MLF does not naturally occur or takes place to a lower extent.¹¹ Histamine has also been found at higher concentrations in red wines with a lower total sulfur dioxide level.²⁴ 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.⁹ 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.¹¹

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. 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 different quantities in different wines could be related to the harvest conditions, such as temperature, rain, and soil nutrients, among other possibilities.¹³

The total biogenic amine concentrations varied considerably among the four wines from 1.6–25.7 mg/L. 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 mg/L and 1.6 mg/L, respectively. Red wines commonly contain higher concentrations of amines as a result of the MLF process.²⁵ The recoveries of the biogenic amines were determined by spiking known concentrations of the target biogenic amines in the wine samples that resulted in calculated recoveries within 83–104% using the IonPac CS18 column coupled to IPAD.

The analysis of three different bottled beers revealed the presence of putrescine, histamine, and agmatine in all samples. The 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.¹⁰ The putrescine concentrations in our beer samples were within the normal range of 0.2–8.0 mg/L reported for European beers.²⁶ Relatively little variability was observed for the histamine concentration between beer samples. The presence of histamine has previously been used as an indication of lactic acid bacteria contamination during the brewing process.¹⁰ The histamine concentrations found in our samples were significantly lower relative to the other amines present and are not considered to represent any toxicological hazard. The total biogenic concentrations for each beer were not significantly different and were within the range 10–17 mg/L. Loret et. al. proposed a beer biogenic amine index (BAI) to assess the quality of the production process.¹⁵ 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 calculated for our beer samples were <1, which indicates a non-contaminated fermentation process (high microbiological quality). Overall, recoveries for the spiked beer samples were within 87–104% using IPAD.

Determination of Changes in Biogenic Amine Concentrations in Alcoholic Beverages during Storage at 4 °C Using Suppressed Conductivity-IPAD

Table 3 summarizes the results obtained by suppressed conductivity-IPAD for most alcoholic beverages previously analyzed. The biogenic amine concentrations were determined after sample storage at 4 °C for up to three weeks. Nearly all amine concentrations increased after storage. Cadaverine, however, was not detected in the white wine after storage for one week. The most interesting result was the detection of agmatine and spermine that were not previously observed before storage. The detection of these amines was at least partially due to the improvement in sensitivity by suppressed conductivity resulting in about 5–10 times lower LODs for agmatine and spermine.

In the Australian red wine, spermidine increased from 0 to 1.4 mg/L after two weeks storage at 4 °C compared to no change in spermidine for the California red wine after three weeks storage. The putrescine concentration increased 20 to 36% for the three wine samples. Cadaverine increased 50 to 125% in the red wine samples, but completely diminished in the white wine sample. For histamine, the concentration increased 12% for the California red wine and 87% for the Australian red wine. The observed increases in putrescine, cadaverine, and histamine concentrations upon storage in our study were in agreement with previous findings for bottled wine samples stored at 4 °C, with the exception of the disappearance of cadaverine in our white wine sample.²⁷

There are numerous variables that can affect the formation or degradation of biogenic amines by bacteria in wines. Therefore, it is difficult to determine the exact cause of the observed changes among the same or different types of wines. However, the increase in histamine at the beginning of the storage period is speculated to occur as a result of MLF and the decarboxylase activity of the microorganisms that remain in the wine.²⁷ Wine samples spiked with known quantities of the target biogenic amines produced recoveries within 88–122% using suppressed conductivity detection.

A direct comparison could not be made between the biogenic amines detected by suppressed conductivity and the amines detected by IPAD after suppression due to significant differences in the LODs. In terms of the biogenic amines that were detected in the wine samples by IPAD in this configuration, the concentration ranges were 1.5–22.1 mg/L putrescine, 0.67–5.4 mg/L histamine, and 1.5–2.0 mg/L spermidine. For suppressed conductivity detection, the concentrations were in the range 1.7–19.4 mg/L putrescine, 0.84–5.5 mg/L histamine, and 1.4–1.9 mg/L spermidine. Therefore, no significant differences in concentrations were observed between the two detectors in series. Configuring the electrochemical cell after the suppressor can be advantageous for monitoring method performance for biogenic amines detected at higher concentrations and can also be used to evaluate the performance of the Au electrode over time by comparing the concentrations determined by the two detectors in series.

Table 3. Biogenic Amine Concentrations in Stored Alcoholic Beverages Determined by Suppressed Conductivity Detection and IPAD

Suppressed Conductivity Detection												
Sample	Putrescine		Cadaverine		Histamine		Agmatine		Spermidine		Spermine	
	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)
Wheat Beer #1 ^a	6.4±0.0	96.0	0.28±0.02	91.4	0.54±0.02	95.4	9.1±0.0	102.3	0.45±0.01	101.0	0.47±0.02	113.0
Wheat Beer #2 ^b	6.6±0.0	95.8	0.67±0.00	88.5	0.60±0.01	99.0	7.7±0.0	102.4	1.2±0.0	104.0	0.73±0.01	117.5
Lager Beer ^c	3.0±0.0	101.2	<DL ^d	—	0.72±0.03	98.2	14.9±0.1	104.8	0.14±0.01	104.3	0.33±0.02	—
California Cabernet Sauvignon ^a	19.4±0.1	97.6	0.79±0.00	103.1	5.51±0.06	103.7	0.37±0.00	89.3	1.9±0.0	101.6	0.19±0.01	121.9
Australian Cabernet Sauvignon ^b	7.1±0.1	95.8	0.53±0.01	88.5	0.84±0.03	99.0	0.23±0.02	95.8	1.4±0.0	104.0	0.21±0.02	100.0
Pinot Grigio ^c	1.7±0.0	103.5	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—
IPAD (post-suppression)												
Sample	Putrescine		Cadaverine		Histamine		Agmatine		Spermidine		Spermine	
	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)	Amount Found (mg/L)	Recov. (%)
Wheat Beer #1	6.2±0.1	96.0	<DL	—	<DL	—	8.7±0.2	94.0	0.42±0.00	100.6	0.48±0.01	97.3
Wheat Beer #2	5.8±0.1	95.6	<DL	—	<DL	—	7.2±0.1	87.2	1.2±0.0	95.4	0.67±0.03	110.8
Lager Beer	3.0±0.0	94.6	<DL	—	<DL	—	14.5±0.1	93.7	<DL	—	<DL	—
California Cabernet Sauvignon	22.1±0.4	106.5	<DL	—	5.4±0.2	99.3	<DL	—	2.0±0.1	100.6	<DL	—
Australian Cabernet Sauvignon	6.9±0.3	103.6	<DL	—	0.67±0.04	98.1	<DL	—	1.5±0.0	104.9	<DL	—
Pinot Grigio	1.5±0.1	100.7	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—

Stored at 4 °C for ^a3 weeks, ^b1 week, ^c2 weeks.
^d<DL = less than the detection limit.

The biogenic amines in the beer samples were also determined by suppressed conductivity-IPAD after storage at 4 °C. The most significant changes in the biogenic amine concentrations after one to three weeks storage were the detection of cadaverine in the wheat beer and spermidine and spermine in all beer samples that were not detected prior to storage. As previously discussed,

the detection of these amines is at least partially due to the improvement in sensitivity by suppressed conductivity detection. The wheat beer #2 produced the largest evolution of biogenic amines with increases in putrescine, histamine, and agmatine of 65%, 67%, and 26%, respectively. The range of concentration increase for all beer samples during storage was 0–65% for pu-

trescine, 67-184% for histamine, and 26% for agmatine. Figure 3 shows a separation of biogenic amines determined in wheat beer #2 using suppressed conductivity detection. Wheat beer #1 produced the most significant increase in histamine (184%) relative to the other beer samples. This observation is in agreement with a previous study that demonstrated a significant increase of histamine for a bottle beer sample stored at 21 °C for eight days.²⁸ The presence of lactic acid bacteria, primarily lactobacilli, has been demonstrated to be the primary cause for histamine increase in bottled beers over time.²⁸ 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 value 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).¹⁵ The average amine recoveries for the spiked beer samples were in the range 88–118%. The calculated concentrations by IPAD in series with the suppressor were within $\pm 12\%$ of the concentrations determined by suppressed conductivity.

CONCLUSION

The described method demonstrates the use of the IonPac CS18 column for the separation of several target biogenic amines in alcoholic beverages. Simple MSA gradient conditions provide suitable compatibility for use with suppressed conductivity detection, IPAD, and UV detection to further examine and characterize the presence of biogenic amines in alcoholic beverages. Suppressed conductivity detection demonstrates good precision and recovery for many of the biogenic amines and superior sensitivity compared to previously reported methods in the literature. In addition, this detection technique provides the simplest approach for determining biogenic amines compared to methods requiring complex derivatizing procedures that are often prone

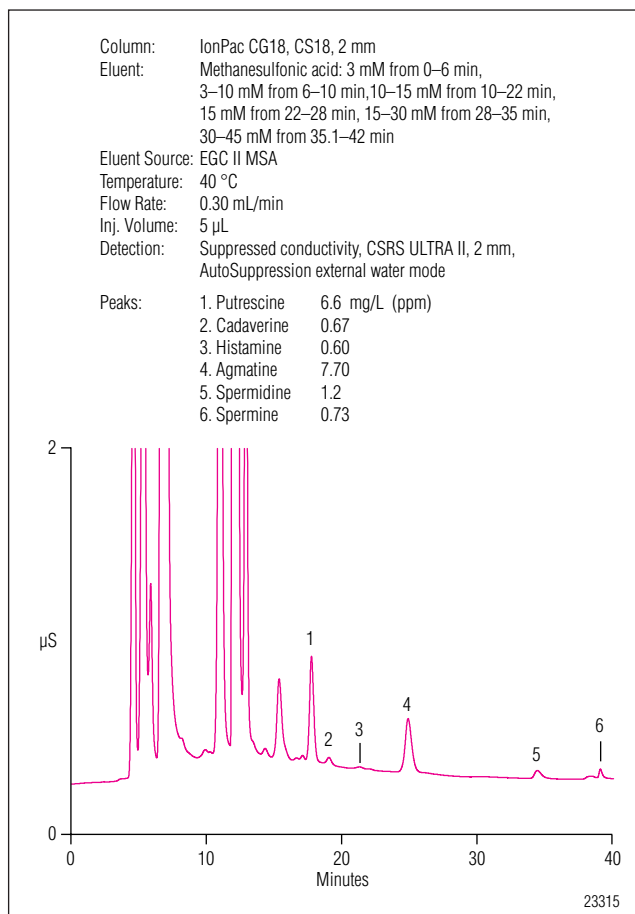


Figure 3. Determination of biogenic amines in wheat beer #2 using suppressed conductivity detection.

to errors. IPAD provides a wider selectivity than suppressed conductivity and good sensitivity for many of the biogenic amines of interest. The addition of UV detection adds confidence to the analytical results by confirming the presence or absence of tyramine in the alcoholic beverages. The combination of three detection configurations described demonstrates the versatility and potential of cation-exchange chromatography for determining hydrophobic amines in complex matrices.

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