DIONEX

Application Note 183

Determination of Biogenic Amines in Fermented and Non-Fermented Foods Using Ion Chromatography with Suppressed Conductivity and Integrated Pulsed Amperometric Detections

INTRODUCTION

Biogenic amines are biologically active compounds with aliphatic (putrescine, spermidine, spermine), aromatic (dopamine, tyramine, phenylethylamine), or heterocyclic (histamine, serotonin) structures. Several biogenic amines have critical roles in human and animal physiological functions,[1] such as regulation of body temperature, stomach volume, stomach pH, and brain activity.[2] Polyamines such as putrescine, spermidine, and spermine are important in the synthesis of proteins, RNA, and DNA, and are therefore essential for cell proliferation and growth.[3,4] Several studies have revealed higher concentrations of biogenic amines in cancer patients compared to healthy individuals.[5] The inhibition of the biosynthesis of these amines in tumor-bearing patients is a major area of cancer therapy research. [3-5]

The formation of biogenic amines requires 1) free amino acids or proteins, 2) microorganisms that can decarboxylate amino acids, and 3) conditions that promote microbial activity.[6] Biogenic amines in food and food products are related to food spoilage and safety.[1] Consumption of low concentrations of biogenic amines in the average diet is not dangerous, but high concentrations can result in hypotension (histamine, putrescine, cadaverine), hypertension (tyramine), migraines (tyramine, phenylethylamine), nausea, rash, dizziness, increased cardiac output, and increased respiration.[6,7] Biogenic amines are known to occur in a wide variety of fermented and non-fermented foods, such as fish, meat, dairy, fruits, vegetables, and chocolate.[4] The determination of biogenic amines in food products is critical to assess potential health risks before consumption.

In the past three decades, several analytical methods have been introduced for the determination of biogenic amines in a wide variety of food and beverage matrices. These determinations are often accomplished by reversed-phase HPLC followed by UV or fluorescence detection. Because most biogenic amines lack a suitable chromophoric or fluorophoric group, however, either pre- or postcolumn chemical derivatization is required for detection. The most common derivatizing agents are dansyl chloride,[7,8-10] benzoyl chloride,[11-14] and o-phthalaldehyde (OPA).[8,15] These derivatization procedures are time-consuming, laborious, can produce potential by-product interferences, and sometimes under- or over-estimate the amount of amines.[9,16]

Pulsed amperometric detection (PAD) with a multipotential waveform has demonstrated good sensitivity for the detection of underivatized biogenic amines. A conventional three-step waveform provides analyte detection by forming a gold oxide (AuO) layer, cleaning the electrode through exhaustive reduction of the electrode surface, reactivating the oxidative surface, and reducing the AuO back to Au.[17] The current generated from the initial oxidation of the gold surface contributes to the background, baseline noise, and baseline instability. To minimize this effect and enhance the amine signal on the gold oxide surface, integrated pulsed amperometric detection (IPAD) was introduced.[18] The advantage of using IPAD is that the current is continuously integrated during working electrode oxidation and during removal of the oxide surface, thus minimizing baseline disturbances.[18,19] IPAD has recently been used to detect biogenic amines in chocolate.[20]

Ion chromatography (IC) has not commonly been used for the determination of biogenic amines. This is due to the strong hydrophobic interactions between protonated amino groups and the stationary phase of many columns, resulting in long retention times and poor peak symmetry. To alleviate this problem, either high concentrations of an acidic eluent or an organic solvent are required to elute the amines from the column. Unfortunately, these eluents preclude the use of suppressed conductivity detection, the most common detection technique associated with IC. The introduction of a weak cation-exchange column, the IonPac® CS17, specifically designed for the separation of hydrophobic amines using a simple acidic eluent and no organic solvent, allows the determination of biogenic amines by suppressed conductivity detection.[21] The IonPac CS17 combined with suppressed conductivity detection has been used for the successful determination of biogenic amines in fish tissue[22] and meat[23] samples.

The stationary phase of the IonPac CS18 column is slightly more hydrophobic than that of the CS17 and therefore provides better resolution between close eluting peaks, such as putrescine and cadaverine. This column was used with suppressed conductivity and IPAD, configured separately and in tandem, for the determination of biogenic amines in selected food products. Because absorbance detection can provide selective detection of certain compounds that have aromatic character, such as tyramine, UV in combination with IPAD was also used to confirm or refute the presence of tyramine in suspect samples. The linear ranges, limits of detection, precisions, and recoveries of biogenic amines spiked in fermented (dairy, meat) and non-fermented (fish) products were analyzed and compared using suppressed conductivity detection and IPAD.

EQUIPMENT

Dionex ICS-3000 system consisting of:

- DP Dual Pump with in-line degas option
- DC Detector/Chromatography module (dual temperature zones) with conductivity and electrochemical cells
- The electrochemical cell consisted 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 µL (P/N 053640)
- Two 4-L plastic bottle assemblies for external water mode of operation

Chromeleon® 6.7 Chromatography Management Software

Blender (household or industrial strength type)

Centrifuge (Beckman Coulter, Brea, CA)

Vortex mixer (Fisher Scientific)

REAGENTS AND STANDARDS

Reagents

Deionized water, type I reagent grade, 18 MΩ-cm resistivity or better

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

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

Trichloroacetic acid, ≥99.5% (Fluka Chemical Co., Sigma-Aldrich P/N 91228)

Standards

Dopamine hydrochloride (Sigma Chemical Co., H8502)

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

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

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

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

Histamine, ~97% (Sigma Chemical Co., H7125)

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

ß-phenylethylamine, 99% (Aldrich Chemical Co., 128945)

Spermidine trihydrochloride, >98% (Calbiochem, 56766)

Spermine tetrahydrochloride, ≥99% (Calbiochem, 5677)

CONDITIONS

Columns:	IonPac CS18 Analytical, 2 x 250 mm (P/N 062878)
	IonPac CG18 Guard, 2 x 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 35.1–45 min
Eluent Source:	EG Eluent Generation module
Flow Rate:	0.30 mL/min
Temperature:	40 ºC (lower compartment)
	30 ºC (upper compartment)
Injection Volume:	5 µL
Detection:**	Suppressed conductivity, CSRS® ULTRA II (2 mm), AutoSuppression® external water mode, power setting–40 mA and/or UV-Vis detection set at 276 nm
Background Conductance:	0.4–0.5 µS
Conductance Noise:	0.2–0.3 nS
System Backpressure:	~2500 psi

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

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

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)
	~210 pC (with suppressor installed)

Waveform

Time (s)	Potential (V vs. pH)	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 methanesulfonic acid (MSA) online by pumping high quality deionized water (18 MΩ-cm resistivity or better) through the EGC II MSA cartridge. Chromeleon software will track the amount of MSA used and calculate the remaining lifetime.

Alternately, prepare 10 mM MSA by adding 0.961 g of concentrated MSA to a 1-L volumetric flask containing approximately 500 mL of deionized water. Bring to volume and mix thoroughly. Prepare 100 mM MSA by adding 9.61 g of concentrated MSA to a 1-L volumetric flask containing approximately 500 mL of deionized water. Bring to volume and mix thoroughly. Degas the eluents and store in plastic labware. The 3 mM MSA eluent is produced by proportioning between 10 mM MSA and 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 approximately 800 mL of degassed deionized water in a 1-L volumetric flask and bring 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.

Acid Extraction Solutions

100 mM Methanesulfonic Acid

Add 4.81 g of MSA to a 500-mL volumetric flask containing approximately 300 mL of deionized water. Bring to volume and mix thoroughly. Store solution in plastic labware.

5% and 1.5% Trichloroacetic Acid

Prepare 5% trichloroacetic acid (TCA) by adding 25 g of trichloroacetic acid to a 500-mL volumetric flask containing about 300 mL of deionized water. Bring to volume and mix thoroughly. Store the solution in plastic labware. Prepare 1.5% TCA by adding 30 mL of the 5% trichloroacetic acid solution to a 100-mL volumetric flask containing approximately 50 mL deionized water. Bring to volume and mix thoroughly. Store solution in plastic labware.

STANDARD AND SAMPLE PREPARATION

Standards

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 each to volume with deionized water. Store stock solutions at 4 °C and protected from light. Prepare working standard solutions for generating the calibration curve 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.

Samples

Previous studies have found that TCA is a good acid for extracting fish and meat samples because it is highly effective for precipitating proteins.[9] The canned tuna and sausage samples were prepared by adding 5 g of ground sample to separate 50-mL centrifuge tubes, followed by 20 mL of 1.5% (sausage) or 5% (tuna) TCA. The mixtures were homogenized on a vortex mixer for 1 min and centrifuged at 6000 rpm for 20 min at 4 °C. The supernatants were decanted and filtered with a 0.2-µm filter into separate 50-mL volumetric flasks. An additional 20 mL aliquot of TCA was added to each tube and the extraction procedure was repeated. The supernatants were again filtered into their respective flasks, and each flask was brought to volume with deionized water. The canned tuna extract was further diluted 1:5 with deionized water before analysis.

The cheddar and Swiss cheese extracts were prepared as described above, except 100 mM MSA was used in place of TCA. Each extract was diluted 1:1 with deionized water before analysis.

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 Eluent Generator module and configure the setup of the cartridge with the Chromeleon server configuration. Connect the cartridge to the EG degas assembly. In place of the column set, install sufficient backpressure tubing (~91.4 cm of 0.003 in. i.d.) 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 and install a 2 x 50 mm CG18 and 2 x 250 mm CS18 column. Confirm the backpressure is ~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 this pressure. Connect the external water source outlet to the Regen In 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 significant noise 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 ~25 cm of black tubing (0.010 in. i.d.) on the cell outlet. For delivery of the 100 mM NaOH postcolumn reagent, we recommend using the DP Dual Pump to maintain an accurate and consistent flow rate. 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 low flow rates.

Install sufficient backpressure tubing on the pump used for postcolumn 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. Plug the third port of the mixing tee with a 1/4-28 in. fitting. Set the flow rate at 0.24 mL/min for the postcolumn base addition and turn the pump on. 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.*

Using 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. *Confirm that eluent is flowing 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. If the pH deviates significantly from this range, first verify NaOH addition by testing the column effluent with pH indicating paper. Next check the accuracy of the NaOH concentration. Deviations may indicate excessive reference electrode wear and may require reference electrode replacement (routinely required every 6–12 months for the ICS-3000 cell). The background should remain within the range of 30–70 nC for the conditions described in this application document. Significantly higher or lower values may indicate 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 for a dual detection method to determine biogenic amines. Alternatively, suppressed conductivity detection can be used independently from IPAD. Neither configuration, however, will allow the detection of dopamine, tyramine, or serotonin.

Prepare the CSRS ULTRA II suppressor by hydrating its membranes. Fill a disposable plastic syringe with degassed deionized water. Push 3 mL of the deionized water through the Eluent Out port and 5 mL 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 5–6 in. of 0.01 in. i.d. black tubing on the cell outlet. Do not install red tubing (0.005 in. 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. CAUTION: 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 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 cell outlet to the mixing tee. Set the wavelength to 276 nm. Alternatively, UV can be used in-line with suppressed conductivity detection to determine if tyramine is present in the samples. In this configuration, the UV detector must be installed before the suppressor.

RESULTS AND DISCUSSION

Separation and Detection of Biogenic Amines

The IonPac CS18 cation-exchange column is more hydrophobic and has a slightly lower exchange capacity (290 µequiv/column, 250 x 2 mm) than the IonPac CS17. This increased hydrophobicity enables better resolution of close eluting peaks, such as putrescine/cadaverine. Figure 1 shows the separation of biogenic amines with suppressed conductivity, integrated pulsed amperometric, and UV detections (not connected in series). Dopamine, tyramine, and serotonin cannot be detected by suppressed conductivity because they lack a positive charge after suppression. Therefore, IPAD was required to detect all 10 biogenic amines. Although dopamine, tyramine, and serotonin all absorb at 276 nm, only tyramine was monitored by UV detection to confirm its presence in samples that had previously been identified as containing tyramine by IPAD.

Electrolytically generated MSA eluent was used to simplify the method and streamline the process of developing an optimum gradient for the separation of the target biogenic amines. An electrolytically generated eluent has not been used with IPAD in previous studies due to the production of oxygen during generation of the MSA eluent. Dissolved oxygen in the eluent stream can result in significant changes in the background signal and therefore should be removed. Oxygen was removed by passing the eluent stream through the eluent channel and external water through the Regen channel of the EG degas device. This appeared to remove the oxygen created by the EG as no erratic changes in the background were observed. The EG simplified the method development by requiring only the addition of DI water, thus avoiding potential errors and inconsistencies that can occur when manually preparing eluents off-line.

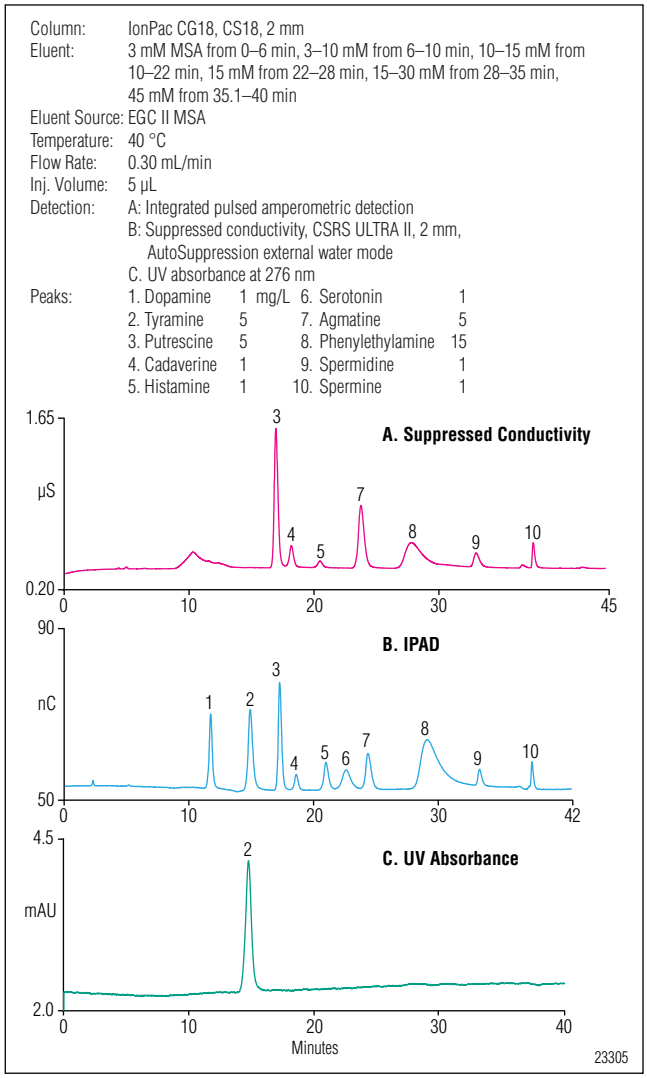

Figure 1. Separation of biogenic amines and detection by (A) suppressed conductivity, (B) IPAD, and (C) tyramine by UV detection.

Table 1. Linearity and Limits of Detection of Biogenic Amines

	IPAD			Suppressed Conductivity Detection			IPAD (post-suppression)			UV		
Analyte	**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	0.1-5	—	—	—	—	—	—	—	—
Tyramine	0.2-10	0.9999	80	0.2-10	—	—	—	—	—	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	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	81.0	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	0.15	0.9996	50	0.1-5	0.9990	9.0	0.1-5	0.9998	90	—	—	—

Table 2. Intraday Retention Time and Peak Area Precisions of Biogenic Amines

	IPAD		Suppressed Conductivity Detection		IPAD (post-suppression)		UV	
Analyte	**Retention time (RSD[a])**	**Peak Area (RSD)**	**Retention time (RSD)**	**Peak Area (RSD)**	**Retention time (RSD)**	**Peak Area (RSD)**	**Retention time (RSD[a])**	**Peak Area (RSD)**
Dopamine	0.03	1.18	—	—	—	—	—	—
Tyramine	0.03	1.53	—	—	—	—	0.17	1.28
Putrescine	0.03	0.79	0.01	0.24	0.02	1.22	—	—
Cadaverine	0.04	2.86	0.01	1.29	0.06	4.97	—	—
Histamine	0.03	1.88	0.01	0.95	0.04	4.80	—	—
Serotonin	0.07	1.92	—	—	—	—	—	—
Agmatine	0.04	1.61	0.01	0.50	0.04	2.67	—	—
Phenylethylamine	0.07	1.94	0.04	0.29	0.14	1.83	—	—
Spermidine	0.03	2.87	0.01	0.53	0.04	3.97	—	—
Spermine	0.01	2.48	0.01	0.56	0.00	2.82	—	—

[a]RSD = relative standard deviation where n = 10.

System Performance

The detection limits, linearity, reproducibility, and precision were measured over the course of 15 days to determine the robustness of this method. The calibration data and LODs for the three detection configurations are summarized in Table 1. The peak area and retention time relative standard deviations were determined for replicate injections of a standard biogenic amine solution containing 5 mg/L each of tyramine, putrescine, and agmatine and 1 mg/L each of dopamine, cadaverine, histamine, serotonin, spermidine, and spermine. Intraday precision was evaluated by performing 10 consecutive injections of the standard amine solution (Table 2). For a detailed description of these experiments and their results, please see Appendix A.

Determination of Biogenic Amines in Food Products with IPAD

The acid extraction of food samples not only removes biogenic amines but also extracts free amino acids, non-biogenic aliphatic and aromatic amines, and other electrochemically active components. This can produce complex chromatograms with a significant number of unknown peaks, some with retention times that overlap with or match the analytes of interest.

Most amino acids are weakly retained on the IonPac CS18 column and therefore do not interfere with many of the biogenic amines. Dopamine and tyramine, however, are also weakly retained. The determination of dopamine in food products was not feasible by this method due to several amino acids coeluting with

dopamine. Arginine, a direct amino acid precursor to agmatine, interfered with tyramine using the gradient conditions previously described. Several attempts were made to optimize the chromatographic conditions to resolve arginine and tyramine, but this decreased the resolution of other biogenic amines of interest. In addition, changing the parameters for one sample type may not yield satisfactory results for another sample type. Therefore, we recommend optimizing the gradient conditions for a particular sample type or combining two detectors in series, such as UV and IPAD. Because this document describes the determination of biogenic amines in a wide range of different food products, the use of UV was the most feasible approach for verifying the presence or absence of tyramine in samples that IPAD suggested contained tyramine.

A variety of fermented and non-fermented fresh and spoiled food products were assayed for the presence of biogenic amines by IPAD (Table 3). Tyramine, spermidine, and spermine were detected at concentrations of 33.4, 16.6, and 17.9 mg/kg, respectively, in unstored canned albacore tuna. After six weeks storage of the canned tuna at 4 °C, IPAD detected a tyramine concentration of 58.4 mg/kg compared to 33.4 mg/kg detected by UV. The higher concentration observed with the electrochemical detector was suspected to be due to an interferent. Analysis of this same sample by suppressed conductivity detection revealed an unknown peak within the same retention time window as tyramine. Because tyramine, like arginine, cannot be detected by suppressed conductivity, this confirmed the presence of an interferent. The unknown peak was detected by IPAD and suppressed conductivity, but did not absorb at 276 nm, so it could be an aliphatic amine.

Although histamine is the most critical biogenic amine implicated in food poisoning from fish, no histamine was detected in our sample using suppressed conductivity detection. According to the FDA Office of Regulatory Affairs, a "decomposed product (determined organoleptically) does not always produce histamine…"[25] To determine the potential for further increase of biogenic amines in the canned tuna, the product was stored at room temperature (~25 °C) for one week to allow spoilage. The spoiled tuna contained several chromatographic interferents for histamine, spermidine, and spermine and therefore these analytes could not be determined. However, putrescine could be determined and increased significantly from 0 to 102.9 mg/kg. Putrescine and cadaverine have been reported as indicators of seafood decomposition[25] and can enhance the toxicity of histamine by inhibiting the histamine metabolizing enzyme.[14]

Significant differences in biogenic amine concentrations were observed between the cheddar and Swiss cheese samples. Tyramine and histamine were detected in cheddar cheese at concentrations of 154.1 and 12.5 mg/kg, respectively. In Swiss cheese, tyramine, putrescine, cadaverine, and histamine were detected at concentrations of 706, 5.7, 13.6, and 68.5 mg/kg, respectively. Upon spoilage after one week of storage at 25 °C, tyramine increased by approximately 320% in the cheddar cheese and 160% in the Swiss cheese. In the

Table 3. Biogenic Amine Concentrations in Food Products Determined by IPAD[a]

	Tyramine		Putrescine		Cadaverine		Histamine		Serotonin		Agmatine		Spermidine		Spermine	
Sample	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**
Canned Tuna	33.4±1.3[b]	95.3	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—	16.6±0.6	98.0	17.9±1.0	92.5
Spoiled Canned Tuna[c]	<DL[d]	—	103±1	109.7	<DL	—	int	—	<DL	—	<DL	—	<DL	—	<DL	—
Cheddar Cheese	154±4[e]	100.6	<DL	—	<DL	—	12.5±0.4	92.3	<DL	—	<DL	—	<DL	—	<DL	—
Spoiled Cheddar Cheese[c]	653±4[e]	94	55.2±0.7	107.5	<DL	—	15.2±0.3	100.2	<DL	—	<DL	—	<DL	—	<DL	—
Swiss Cheese	706±4[e]	85.2	5.7±0.3	96.7	13.6±0.4	97.8	68.5±3.3	91.8	<DL	—	<DL	—	<DL	—	<DL	—
Spoiled Swiss Cheese[c]	1835±19[e]	102.6	3.4±0.2	105.9	82.2±2.5	101.4	7.2±0.3	100.2	<DL	—	<DL	—	<DL	—	<DL	—
Smoked Sausage	<DL	—	int[f]	—	<DL	—	<DL	—	<DL	—	8.9±0.5	103.2	8.9±0.4	99.9	58.1±0.7	95.7

[a]Tyramine determined by either UV or IPAD as noted.

[b]UV absorbance value determined after 6 weeks of storage at 4 °C.

[c]Stored at room temperature for one week.

[d]<DL = less than the detection limit.

[e]confirmed UV absorbance, value shown was determined by IPAD.

[f]int = chromatographic interference.

cheddar cheese, putrescine increased from undetected to 55 mg/kg, but no significant change in histamine was observed. For the spoiled Swiss cheese, putrescine decreased slightly from 5.7 to 3.4 mg/kg, histamine decreased from 68.5 to 7.2 mg/kg, and cadaverine increased from 13.6 to 82.2 mg/kg.

The accuracy of the tyramine results reported by IPAD was confirmed by reanalyzing the cheese samples (after one month storage at 4 °C) by combining UV and IPAD. Due to the increased storage time, microbial activity was observed in both cheese samples. For the refrigerated cheddar cheese sample, the calculated tyramine concentrations were 312 and 349 mg/kg for UV and IPAD, respectively. Tyramine concentrations determined in Swiss cheese by UV and IPAD were 716 and 747 mg/kg, respectively. The concentration differences of approximately ±10% suggested that the original determinations by IPAD were accurate. The microbial activities in the cheese samples were increased by storage at room temperature for one week to verify the absence of any potential tyramine interferents. Comparing UV to IPAD for the spoiled cheddar cheese sample, the tyramine concentrations were 1061 and 1063 mg/kg, respectively. For the spoiled Swiss cheese, the concentrations were 1139 and 1157 mg/kg, respectively for UV and IPAD. This provides further confirmation that the results originally reported using only IPAD for the previously spoiled cheeses were accurate. Figure 2 shows the confirmation of tyramine in spoiled Swiss cheese by IPAD and UV detection.

The polyamines spermidine and spermine, and to a lesser extent putrescine, are the primary amines reported in sausages.[26] In the smoked sausage sample, spermidine was the most abundant biogenic amine detected, followed by equal concentrations of agmatine and spermine. Agmatine is an intermediate product to putrescine from arginine and therefore trace amounts of putrescine may be expected in the sample. An initial analysis of the sausage extract by IPAD revealed significantly high concentrations of putrescine (>3000 mg/kg). Because most data in the literature report relatively trace concentrations of putrescine in sausages, further confirmation was required. Analysis by suppressed conductivity showed no interferent, indicating that an electrochemically active compound, one not detected by suppressed conductivity, has the same retention time, or coelutes with, putrescine. This was the only sample where an unknown peak was shown to interfere with the determination of putrescine.

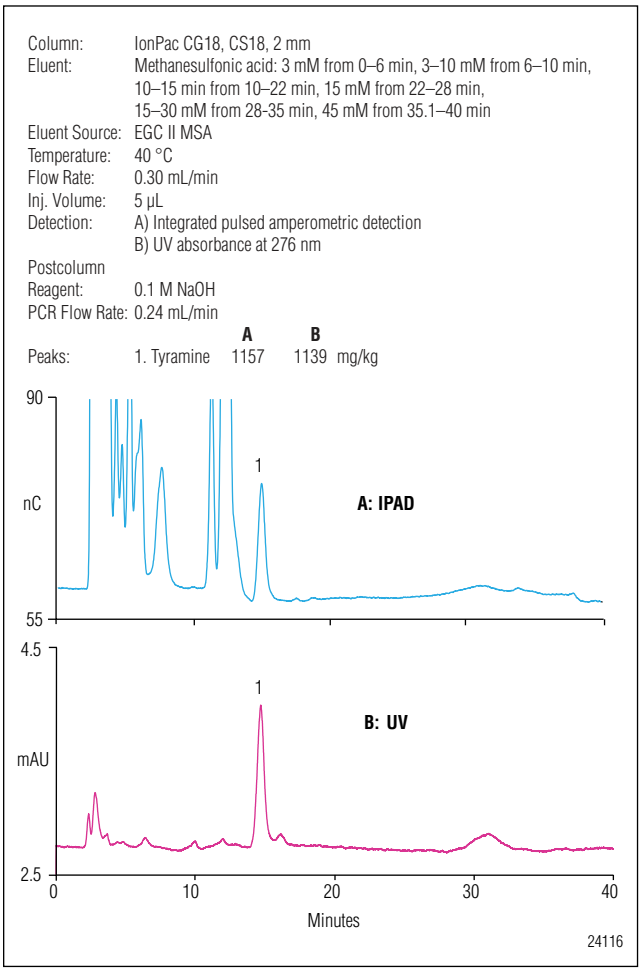

Figure 2. Determination of biogenic amines in spoiled Swiss cheese by (A) IPAD and (B) UV detection.

Changes in Biogenic Amine Concentrations in Food Products during Storage at 4 and 25 °C Detected Using Suppressed Conductivity-IPAD

A selection of food products was used for the determination of biogenic amines by suppressed conductivity-IPAD (Table 4). Samples were stored at 4 °C for one to three weeks prior to analysis, with the exception of the spoiled canned tuna and spoiled sausage samples, which were stored at 25 °C for two weeks to allow spoilage. For the canned tuna stored at 4 °C, the spermidine concentration increased 73% compared to only a marginal change in the spermine concentration. Spoilage of the canned tuna resulted in the detection of putrescine, cadaverine, agmatine, and spermidine. An interferent precluded the detection of spermine. Putrescine and spermidine produced the most significant increases in concentration, from 0 to 162.7 mg/kg and 9.6 to 49.9 mg/kg, respectively.

Table 4. Biogenic Amine Concentrations in Food Products Determined by Suppressed Conductivity and IPAD

Suppressed Conductivity Detection

	Putrescine		Cadaverine		Histamine		Agmatine		Spermidine		Spermine	
Sample	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**
Canned Tuna[a]	<DL[b]	—	<DL	—	<DL	—	<DL	—	9.6±0.3	96.0	20.1±0.9	104.5
Spoiled Canned Tuna[c]	162.7±0.4	100.5	6.2±0.2	94.1	<DL	101.4	11.5±0.0	100.2	49.9±0.2	94.4	int[e]	—
Smoked Sausage[d]	0.65±0.0	100.0	<DL	—	<DL	—	8.2±0.0	101.4	7.6±0.1	101.4	46.6±0.1	99.6
Spoiled Sausage[c]	9.5±0.1	101.8	3.1±0.1	88.2	1.6±0.1	89.7	6.9±0.1	96.8	14.3±0.2	103.2	32.1±0.6	105.1

IPAD (Post-Suppression)

Sample	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**	**Amount Found (mg/kg)**	**Recovery (%)**
Canned Tuna[a]	<DL	—	<DL	—	<DL	—	<DL	—	<DL	—	22.1±0.6	100.8
Spoiled Canned Tuna[c]	148.7±0.6	93.0	<DL	—	<DL	—	<DL	—	49.1.±2.8	90.3	int	—
Smoked Sausage[d]	<DL	—	<DL	—	<DL	—	<DL	—	7.1±0.2	106.4	46.1±0.6	104.0
Spoiled Sausage[c]	8.9±0.4	96.2	<DL	—	<DL	—	<DL	—	13.5±0.8	102.9	33.5±1.5	100.3

[a]Stored at 4 °C for three weeks.
[b]<DL = less than the detection limit.
[c]Stored at 25 °C for two weeks.
[d]Stored at 4 °C for two weeks.
[e]int = chromatographic interference.

Cadaverine and agmatine were not previously detected in spoiled tuna using IPAD alone. It is unclear whether these biogenic amines were detected because of the improved sensitivity of suppressed conductivity or if they were generated during the additional week of storage at 25 °C compared to the initial study.

Storage of the smoked sausage at 4 °C for two weeks resulted in decreases in the concentrations of agmatine, spermine, and spermidine. Similar concentrations for the physiological polyamines putrescine, spermidine, and spermine have been reported for fermented sausages.[27] Storage at 25 °C for two weeks produced significant changes in the content and concentrations of the biogenic amines. Figure 3 shows a separation of biogenic amines in spoiled sausage using suppressed conductivity detection. Cadaverine and histamine were not observed in the sausage stored at 4 °C, but evolved during storage at 25 °C. Vidal-Carou demonstrated that the histamine concentration in meat products increases more rapidly at room temperature than refrigerated temperature during the same time period.[28] Putrescine and spermidine increased significantly at room temperature whereas agmatine and spermine decreased. Similar trends for putrescine and spermine were observed by Bover-Cid et. al. in fermented

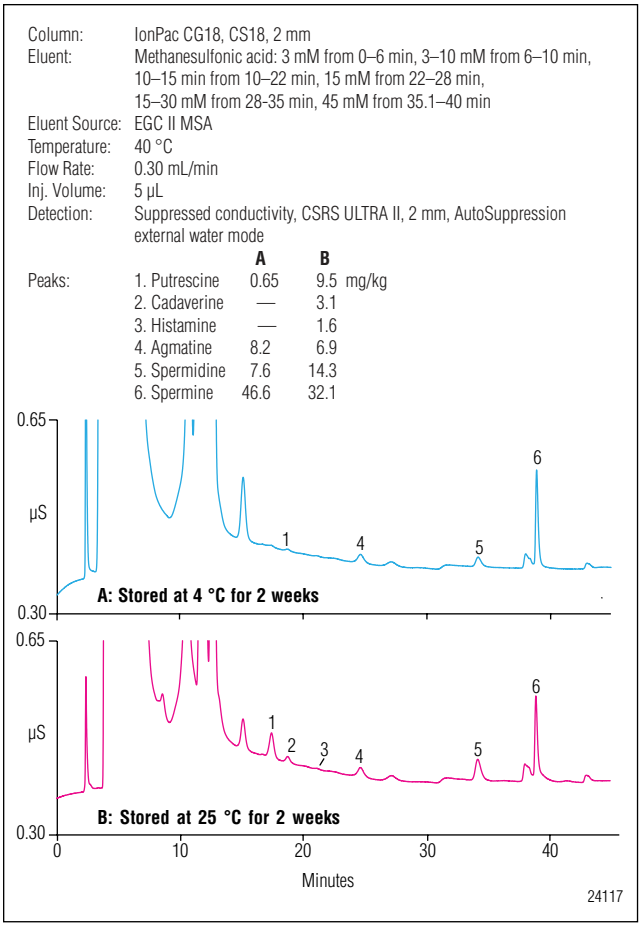

Figure 3. Determination of biogenic amines in spoiled sausage by IC with suppressed conductivity detection.

sausages stored at 19 °C.[27] The presence of putrescine and cadaverine in meat products has been related to lysine and ornithine decarboxylase activity in Enterobacteriaceae. The concentrations of putrescine, spermidine, and spermine determined by IPAD following suppression were within the standard deviations of the concentrations determined by suppressed conductivity.

CONCLUSION

The IonPac CS18, a polymeric weak acid cation-exchange column, was used to separate biogenic amines in a variety of fermented and non-fermented food samples, with detection by IPAD, suppressed conductivity, and UV. The described method uses a simple electrolytically generated MSA eluent without requiring the use of solvents or aggressive eluent systems that have previously been reported. In addition, the method results in good precision and recovery over a wide range of sample matrices and avoids the need for complex and long derivatization procedures. The use of three different detection configurations provides additional information and confirms the identification of tyramine to increase confidence in the analytical results. Suppressed conductivity had exceptionally low LODs for the main biogenic amines of interest without chromatographic interferences from common cations and amines present in many of the food samples. In addition to the amines detected by conductivity, IPAD allows the detection of dopamine, serotonin, and tyramine, which can be confirmed with a UV detector.

REFERENCES

1. Halász, A.; Baráth, Á.; Simon-Sarkadi, L.; Holzapfel, W. Biogenic Amines and their Production by Microorganisms in Food. *Trends Food Sci. Technol.* **1994**, *5*, 42-49.
2. ten Brink, B.; Damink, C.; Joosten, H. M. L. J.; Huis in 't Veld, J. H. J. Occurrence and Formation of Biologically Active Amines in Foods. *Int. J. Food Microbiol.* **1990,** *11,* 73-84.
3. Bardócz, S. Polyamines in Food and their Consequences for Food Quality and Human Health. *Trends Food Sci. Technol.* **1995,** *6,* 341-346.
4. Santos, M. H. S. Biogenic Amines: their Importance in Foods. *Int. J. Food Microbiol.* **1996,** *29*, 213-231.
5. Sun, X.; Yang, X.; Wang, E. Determination of Biogenic Amines by Capillary Electrophoresis with Pulsed Amperometric Detection. *J. Chromatogr., A* **2003,** *1005,* 189-195.
6. Shalaby, A. R. Significance of Biogenic Amines to Food Safety and Human Health. *Food Res. Int.* **1996,** *29,* 675-690.
7. Chiacchierini, E.; Restuccia, D.; Vinci, G. Evaluation of Two Different Extraction Methods for Chromatographic Determination of Bioactive Amines in Tomato Products. *Talanta* **2005,** *69* (3), 548-555.
8. Moret, S.; Smela, D.; Populin, T.; Conte, L. S. A Survey on Free Biogenic Amine Content of Fresh and Preserved Vegetables. *Food Chem.* **2005,** *89,* 355-361.
9. Moret, L. S.; Conte, S. High Performance Liquid Chromatograhic Evaluation of Biogenic Amines in Foods: An Analysis of Different Methods of Sample Preparation in Relation to Food Characteristics. *J. Chromatogr., A* **1996,** *729,* 363-369.
10. Moret, S.; Bortolomeazzi, R.; Lercker, G. Improvement of Extraction Procedure for Biogenic Amines in Foods and their High-Performance Liquid Chromatographic Determination. *J. Chromatogr., A* **1992,** *591,* 175-180.
11. Kalac, P.; Švecová, S.; Pelikánová, T. Levels of Biogenic Amines in Typical Vegetable Products. *Food Chem.* **2002,** *77,* 349-351.
12. Tsai, Y. H.; Kung, H. F.; Lin, Q. L.; Hwang, J. H.; Cheng, S. H.; Wei, C. I.; Hwang, D. F. Occurrence of Histamine and Histamine-Forming Bacteria in Kimchi Products in Taiwan. *Food Chem.* **2005,** *90,* 635-641.
13. Tsai, Y. H.; Kung, H. F.; Lee, T. M.; Chen, H. C.; Chou, S. S.; Wei, C. I.; Hwang, D. F. Determination of Histamine in Canned Mackerel Implicated in a Food Borne Poisoning. *Food Contr.* **2005,** *16,* 579-585.
14. Su, S. C.; Chou, S. S.; Chang, P. C.; Hwang, D. F. Determination of Biogenic Amines in Fish Implicated in Food Poisoning by Micellar Electrokinetic Capillary Chromatography. *J. Chromatogr., B: Biomed.* **2000,** *749,* 163-169.

15. Suzuki, S.; Kobayashi, K.; Noda, J.; Suzuki, T.; Takama, K. Simultaneous Determination of Biogenic Amines by Reversed-Phase High-Performance Liquid Chromatography. *J. Chromatogr., A* **1990,** *508,* 225-228.
16. Bouchereau, A.; Guénot, P.; Larher, F. Analysis of Amines in Plant Materials. *J. Chromatogr., B: Biomed.* **2000,** *747,* 49-67.
17. Hoekstra, J.C.; Johnson, D.C. Comparison of Potential-Time Waveforms for the Detection of Biogenic Amines in Complex Mixtures Following Their Separation by Liquid Chromatography. *Anal. Chem.* **1998,** *70,* 83-88.
18. Clarke, A.P.; Jandik, P.; Rocklin, R.D.; Liu, Y.; Avdalovic, N. An Integrated Amperometry Waveform for the Direct, Sensitive Detection of Amino Acids and Amino Sugars Following Anion-Exchange Chromatography. *Anal. Chem.* **1999,** *71,* 2774-2781.
19. Draisci, R.; Giannetti, L.; Boria, P.; Lucentini, L.; Palleschi, L.; Cavalli, S. Improved Ion Chromatography-Integrated Pulsed Amperometric Detection Method for the Evaluation of Biogenic Amines in Food of Vegetable or Animal Origin and in Fermented Foods. *J. Chromatogr., A* **1998,** *798,* 109-116.
20. Pastore, P.; Favaro, G.; Badocco, D.; Tapparo, A.; Cavalli, S.; Saccani, G. Determination of Biogenic Amines in Chocolate by Ion Chromatographic Separation and Pulsed Integrated Amperometric Detection with Implemented Wave-Form at Au Disposable Electrode. *J. Chromatogr., A* **2005,** *1098,* 111-115.
21. Rey, M.; Pohl, C. Novel Cation-Exchange Column For the Separation of Hydrophobic and/or Polyvalent Amines. *J. Chromatogr., A* **2003,** *997,* 199-206.
22. Cinquina, A. L.; Calí, A.; Longo, F.; De Santis, L.; Severoni, A.; Abballe, F. Determination of Biogenic Amines in Fish Tissues by Ion-Exchange Chromatography with Conductivity Detection. *J. Chromatogr., A* **2004,** *1032,* 73-77.
23. Saccani, G.; Tanzi, E.; Pastore, P.; Cavalli, S.; Rey, M. Determination of Biogenic Amines in Fresh and Processed Meat by Suppressed Ion Chromatography-Mass Spectrometry Using a Cation-Exchange Column. *J. Chromatogr., A* **2005,** *1082,* 43-50.
24. Rohrer, J. S.; Thayer, J.; Weitzhandler, M.; Avdalovic, N. Analysis of the N-Acetylneuraminic Acid and N-Glycolylneuraminic Acid Contents of Glycoproteins by High-pH Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC/PAD). *Glycobiology* **1998,** *8,* 35-43.
25. U.S. Food and Drug Administration, *ORA Laboratory Manual: Seafood Chemistry,* Vol. 4, Sect. 9-Seafood Chemistry, 2004.
26. Suzzi, G.; Gardini, F. Biogenic Amines in Dry Fermented Sausages: A Review. *Int. J. Food Microbiol.* **2003,** *88,* 41-54.
27. Bover-Cid, S.; Izuierdo-Pulido, M.; Vidal-Carou, M. C. Changes in Biogenic Amine and Polyamine Contents in Slightly Fermented Sausages Manufactured with and without Sugar. *Meat Science* **2001,** *57,* 215-221.
28. Vidal-Carou, M. C.; Izquierdo-Pulido, M.L.; Martín-Morro, M. C.; Font, M. Histamine and Tyramine in Meat Products: Relationship with Meat Spoilage. *Food Chem.* **1990,** *37,* 239-249.

APPENDIX A: LINEARITY, PRECISION, AND REPRODUCIBILITY

Linear Ranges and Limits of Detection

The linear ranges for suppressed conductivity, IPAD, and UV detection were evaluated by tabulating peak area versus concentration. Calibration curves were prepared for each biogenic amine in 3 mM MSA using five increasing concentrations. Dopamine, cadaverine, histamine, serotonin, spermidine, and spermine were tested in the range of 0.10–5.0 mg/L. For tyramine, putrescine, and agmatine the linearity was determined in the 0.20–10 mg/L range. Phenylethylamine's linearity was determined 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 limits 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 ranges of 3.5–81 µg/L, 20–400 µg/L, and 88–1090 µg/L, respectively. Suppressed conductivity detection was approximately 2–20 times more sensitive than IPAD and the LODs increased by a factor of two when IPAD was placed after the suppressor. The calibration data and LODs for the three detection configurations are summarized in Table 1.

Precision and Reproducibility

The peak area and retention time relative standard deviations (RSDs) were determined for replicate injections of a standard biogenic amine solution containing 5 mg/L each of tyramine, putrescine, and agmatine and 1 mg/L each of dopamine, cadaverine, histamine, serotonin, spermidine, and spermine. Intraday precision was evaluated by performing 10 consecutive injections of the standard amine solution (Table 2). Retention time RSDs were 0.01–0.17% and the peak area precisions were 0.24–4.97%. In general, the peak area precisions were better using suppressed conductivity detection and the highest RSDs were observed for IPAD installed after the suppressor. The higher RSDs for IPAD in this configuration are expected due to the increase in baseline noise caused by the suppressor.

The between-day precision was determined for the conventional gold electrode using IPAD coupled directly to the analytical column. Because a significant loss in peak area can indicate a reduction in the gold layer of the working electrode, the standard solution was injected intermittently over 15 days of continuous sample analysis to monitor the electrode response over time. Figure 4 shows the peak area trend for biogenic amines over 15 consecutive days. Dopamine, cadaverine, serotonin, spermidine, and spermine exhibited the least change in peak area responses for the first five consecutive days of analysis (71 total injections) with percent changes in the range of 0% to -6.5% (n = 25). The between-day precision for these biogenic amines during the same time period ranged from 2.0 to 6.7%. For tyramine, putrescine, and agmatine the changes in peak area response during the first five days were higher than the other amines, resulting in percent changes ranging from -9.3% to -14.8%.

The most significant decreases in peak area occurred after 96 h of continuous operation, with the exceptions of putrescine and histamine. These changed mainly during the first 24 h, with putrescine decreasing by 10% and histamine decreasing by 18%. Histamine had the most significant five day decrease in peak area response compared to all other biogenic amines, with a percent change of -38%.

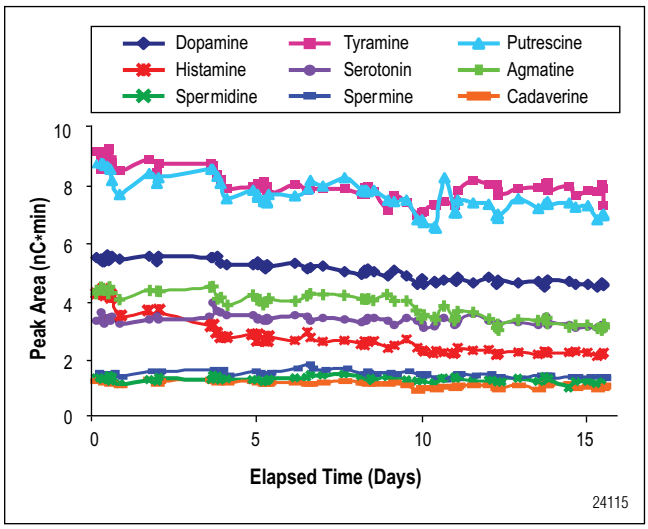

Figure 4. Peak area reproducibility of biogenic amines detected by IPAD over 15 days of continuous analysis.

Histamine's peak area decreases were less significant during the second week, with a percent change of -13%. Spermidine and spermine had the lowest change in response during the second week with a peak area standard deviation of ±2%. The peak area RSDs for these biogenic amines during this time span were 5.1% and 7.2%, respectively. The changes in peak area were greater for dopamine, cadaverine, and serotonin during the second week with a percent change in the range of -7.9% to -16.1%. The change in response for the third week was within ±5% for all biogenic amines except agmatine, which experienced a change in peak area response of -11.1%.

These results indicate that with continuous use of the gold working electrode, the recession of the gold layer will decrease over time, thereby reducing the loss in peak area response. In addition, previous studies have shown that a working electrode with significant gold recession can still generate accurate analytical results despite loss in sensitivity and changes in the slopes of the calibration curves.[24] It is unclear why histamine experienced a significantly greater loss in peak area response compared to other biogenic amines. To assure the integrity of the analytical results, a standard biogenic amine solution was injected before and after the sample replicates and the recoveries were calculated. A decrease in peak area response by 10–15% necessitated recalibration of the system before continuing with the analysis. Based on the results previously discussed, the system required calibration at least weekly. However, no samples required a repeat analysis due to a loss in peak area response.

The between-day precision for suppressed conductivity detection was determined over 12 days. The peak area RSDs over the 190 injections of samples or standards performed during that time ranged from 1.0 to 4.6% (n=44) for putrescine, cadaverine, histamine, agmatine, spermidine, and spermine. The lowest and highest RSD values were observed for agmatine and histamine, respectively. The Au working electrode was also monitored with the suppressor inline after previously being used continuously for three weeks. Because there was already a significant loss of gold, a smaller decrease in peak area was expected. The change in peak area response over the first five consecutive days for IPAD after suppression ranged from -5% to +5% with the exception of histamine peak area which decreased 16.6%. This is a significant improvement compared to earlier results due to prior use of the working electrode. The between-day peak area precision was in the range of 4.9% to 6.2% during this same time period for all of the biogenic amines except histamine (9.1%).

The between-day 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 to 1.0% over the specified time. The percent change in retention time from the beginning to the end of this time period ranged from -0.3% to -4.3%. The between-day retention time precision for suppressed conductivity-IPAD was in the range of 0.04 to 0.39% with a decrease in retention time of <0.3% over 12 consecutive days.

LPN 1889-02 PDF 09/16