Food Safety and Testing Application Note Compendium: Beer

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Application Note: 42170

Determination of Nitrogen/Protein in Brewing Industry Products with the Thermo Scientific FLASH 4000 Elemental Analyzer

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Key Words

- Combustion
- Malt and barley
- Nitrogen
- Wort

Introduction

The brewing industry needs to perform routine analytical testing to ensure the quality of products and raw materials. Nitrogen/protein determination is a key process and enables manufacturers to monitor the content and stability of a product.

Control and measurement of protein throughout the brewing process is important in order to ensure the survival, growth and productivity of the yeast utilized to convert sugars to ethanol and carbon dioxide. The yeast organisms depend on a variety of conditions, including the availability of amino groups derived from enzymatic hydrolysis of protein during the brewing process. While in the past methods have traditionally focused on investigating foam and haze-forming properties of protein in beer, there is now an increasing demand for techniques to accurately measure and quantify protein concentration.

The traditional method for determining protein content is the Kjeldahl method through the determination of nitrogen (N x 6.25 = protein concentration). An advanced analytical technique based on the Dumas combustion method has been developed to offer an alternative to the classical Kjeldahl method. The method is approved by a broad range of recognized associations (AOAC®, AACC®, ISO® and IFFO®), including the American Society of Brewery Chemists (ASBC®).

The Thermo Scientific FLASH 4000 Nitrogen/Protein Analyzer (Figure 1) utilizes the Dumas combustion method and requires no sample digestion or toxic chemicals. Due to the automated and accessible nature of the instrument, it provides significant advantages for the quantitative determination of nitrogen/protein, including reproducibility, high accuracy, minimization of human error and high sample throughput.

Experimental data compared to results obtained by spectrophotometric, near infrared spectroscopy (NIR) and Kjeldahl methods as well as correlation with Round Robin Tests values, demonstrate the validity of the new instrument as an alternative to traditional wet chemistry procedures.

Figure 1: FLASH 4000 Nitrogen/Protein analyzer

Methods

FLASH 4000 Method (in accordance with the European Brewery Convention Method, EBC 4.9.3 Soluble Nitrogen of Dumas Combustion Method)

The sample is weighed in a tin capsule and introduced into the combustion reactor via the Thermo Scientific MAS 4000 Autosampler together with a controlled amount of oxygen using the Thermo Scientific OxyTune function, ensuring a complete combustion of the sample. Malt and barley samples are homogenized and weighed directly in the tin capsule in the range of 700 – 1000 mg. A 2 ml sample of wort is pipetted onto filter paper in the tin capsule, then dried in an oven at 105 °C for 90 minutes. The sample is not dried completely as the filter still has to be moist to be able to close the capsule for analysis (Figure 2).

After combustion, the resulting gases are carried by a helium flow to a second reactor filled with copper. The water is trapped through a Peltier system while the CO_2 is adsorbed by the NoStop Twin Traps. The nitrogen is then passed through a GC column and finally detected by a thermal conductivity detector (Figure 3). A complete report is automatically generated by the dedicated Thermo Scientific Eager Xperience data handling software.

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Tools: tin disk,capsulator, filter paper, wort sample.

The tin disk is rested on the capsulator.

The tin disk is pressed through a cylindrical tool.

Filter paper is placed in the tin capsule.

The wort sample is adsorbed by the filter paper.

The sample is dried in an oven at 105°C for 90 min.

The tin disk is closed by hand using the capsulator.

Using the cylindrical tool, the disk is pressed into the cavity.

The top of the device is pressed downwards to release the capsule.

Figure 2: Wort sample weighing technique

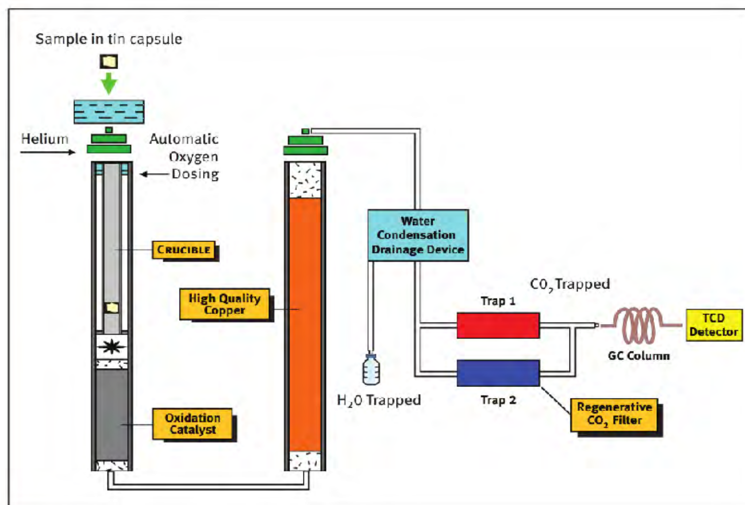

Figure 3: FLASH 4000 Nitrogen/Protein configuration

Analytical conditions:

Oxidation reactor (Left furnace): 950 °C
Reduction reactor (Right furnace): 840 °C
Oven Temperature: 50 °C
Carrier Flow: 300 ml/min
Reference Flow: 300 ml/min

Note: The Oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune® function present in the Eager Xperience software.

Spectrophotometric Method (in accordance with the European Brewery Convention Method, EBC 4.9.2 Soluble Nitrogen of Malt)

The soluble nitrogen in wort is determined using a spectrophotometric method, from absorbance measurements at 215 nm and 225 nm. The sample is diluted 1/100 with 5 g/l sodium chloride solution and the absorbance of the diluted wort is measured at both 215 and 225 nm with the 5 g/l sodium chloride solution set at zero. The soluble protein content is calculated based on the difference in absorbance between 215 and 225 nm. A wort with a known content of soluble protein is used for calibration of the linear equation. A Skalar continuous flow analyzer was used for the analysis.

Results

The samples analyzed were chosen on the basis of their varying nature and different content of nitrogen. These differences meant that the combustion and the amount of oxygen required were completely different. The data obtained demonstrated no-matrix effects in the determination of nitrogen, indicating complete combustion for all types of samples.

For malt and barley samples, calibration was performed using Thermo Scientific Pasta Reference Material on the FLASH 4000 Analyzer. A K factor calibration method was used.

For wort samples, a Boortmalt Wort Reference Material (0.075 N %) and a glycine solution were used for calibration. The protein factor used to calculate the protein content via the conversion of nitrogen value was 6.25.

The data obtained were compared with the spectrophotometric and NIR methods. In addition, the quality of the measurements obtained by the FLASH 4000 Analyzer were evaluated through participation in International Round Robin Tests.

Table 1 shows the reproducibility and comparison of total nitrogen/protein data obtained from the malt and barley samples with the FLASH 4000 Analyzer.

Sample	FLASH 4000 Analyzer		
	Nitrogen %	Protein %	RSD %
Malt 1	1.748 - 1.756	10.923 - 10.975	0.336
Malt 2	1.723 - 1.716	10.768 - 10.727	0.269
Barley 1	1.622 - 1.638	10.140 - 10.237	0.673
Barley 2	1.646 - 1.635	10.285 - 10.222	0.434

Table 1: Reproducibility of Nitrogen/Protein data of malt and barley samples

Table 2 and Table 3 present the Total Protein and Soluble Protein data obtained during a Circuit of Interlaboratories Malt Test and iFBM Ring Test (Institute Francais des Boissons, de la Brasserie et de la Malterie). In each round, two malt A & B samples were analyzed using the FLASH 4000 Analyzer. The protein data obtained were within the range of nitrogen concentration approved by the iFBM statistic studies (minimum and maximum values accepted).

Month /	Sample	FLASH 4000	iFBM Ring Test		
Year	Code	Total Protein %	Total Protein %	Minimum %	Maximum %
			Assigned value		
8/2010	A	9.2	9.36	9.00	9.75
	B	9.1	9.32	9.05	9.65
9/2010	A	9.9	9.74	9.40	10.10
	B	9.8	9.69	9.30	10.00
10/2010	A	9	9.27	8.85	9.67
	B	8.9	9.27	8.90	9.64
11/2010	A	9.00	9.73	9.00	10.37
	B	9.80	9.74	9.20	10.54

Table 2: iFBM Total Protein data 4.3.1 / 4.3.2 EBC Method (percentage dry matter)

Month /	Sample	Spectrophometric method	FLASH 4000	iFBM Ring Test		
Year	Code	Soluble Protein %	Soluble Protein %	Soluble Protein%	Minimum %	Maximum %
				Assigned value		
8/2010	A	4.11	3.98	3.89	3.67	4.11
	B	4.02	4.10	3.86	3.56	4.16
9/2010	A	4.21	3.90	3.86	3.60	4.10
	B	4.15	4.17	3.90	3.64	4.15
10/2010	A	4.22	4.22	4.10	3.84	4.25
	B	4.30	4.33	4.13	4.00	4.40
11/2010	A	4.20	3.93	3.86	3.57	4.20
	B	4.20	3.88	3.86	3.50	4.26

Table 3: iFBM Soluble Protein data 4.9.1 / 4.9.2 EBC Protein (percentage dry matter).

Tables 4, 5 and 6 show Total Nitrogen and Soluble Nitrogen data of malt, barley and wort obtained during a MAPS Ring Test (Malt Analytes Proficiency Testing Scheme, LGC Standards Proficiency Testing, UK). In each cycle, a malt or barley sample was analyzed with the FLASH 4000 Analyzer. The nitrogen values obtained were within the range of nitrogen concentration approved by the MAPS statistic studies. The data were comparable with the Kjeldahl, combustion, NIR and spectrophotometric methods.

Round	FLASH 4000	MAPS Ring Test					
No.	Analyzer	Kjeldahl		Combustion		NIR	
	Total N %	Total N %	Range %	Total N %	Range %	Total N %	Range %
163	1.78	1.76	1.70-1.82	1.79	1.73-1.85	1.79	1.71-1.87
164	1.52	1.54	1.48-1.60	1.55	1.49-1.61	1.55	1.47-1.63
165	1.38	1.39	1.31-1.49	1.40	1.35-1.44	1.40	1.32-1.48
166	1.63	1.65	1.59-1.71	1.67	1.61-1.73	1.67	1.59-1.75
167	1.39	1.36	1.27-1.41	1.35	1.25-1.48	1.41	1.33-1.49
169	1.89	1.83	1.77-1.89	1.86	1.86-1.92	1.86	1.78-1.94

Table 4: MAPS Total Nitrogen data of malt samples.

Round	FLASH 4000	NIR	MAPS Ring Test					
No.	Analyzer	Analyzer	Kjeldahl		Combustion		NIR	
	Total N %	Total N%	Total N %	Range %	Total N %	Range %	Total N %	Range %
163	1.57	1.63	1.55	1.48-1.60	1.55	1.49-1.61	1.55	1.49-1.61
166	1.71	1.74	1.74	1.68-1.80	1.77	1.71-1.83	1.77	1.71-1.83
167	1.47	1.49	1.46	1.40-1.52	1.49	1.43-1.55	1.49	1.43-1.55
168	1.76	1.73	1.71	1.65-1.77	1.77	1.71-1.83	1.74	1.68-1.80
169	1.55	1.60	1.55	1.49-1.61	1.57	1.51-1.63	1.57	1.51-1.63

Table 5: MAPS Total Nitrogen data of barley samples.

Round	FLASH 4000	Spectrophotometric	MAPS Ring Test			
No.	Analyzer	method	Kjeldahl		Combustion	
	Soluble N %	Soluble N %	Soluble N %	Range %	Soluble N %	Range %
164	0.59	0.60	0.57	0.51-0.63	0.58	0.54-0.62
165	0.66	0.68	0.66	0.60-0.72	0.66	0.60-0.72
166	0.85	0.85	0.82	0.76-0.88	0.84	0.78-0.90
167	0.62	0.62	0.60	0.54-0.66	0.60	0.54-0.66
168	0.69	0.67	0.66	0.60-0.72	0.66	0.60-0.72
169	0.82	0.81	0.81	0.75-0.87	0.82	0.76-0.88

Table 6: MAPS Soluble Nitrogen data of wort samples.

Conclusion

The results demonstrate that the FLASH 4000 Analyzer offers excellent reproducibility, with no memory effect observed when using large sample weights. The system's ability to accurately analyze nitrogen in a wide range from low to high content without matrix effects and without the use of sample digestion or toxic chemicals is also demonstrated. The data obtained through the use of the instrument were within the range accepted for the Kjeldahl, Combustion and NIR methods included in the iFBM and MAPS Ring Tests, indicating the high performance of the system. In addition, the FLASH 4000 results are comparable to those obtained using the spectrophotometric and NIR methods, demonstrating the validity of the instrument as an alternative to traditional wet chemistry procedures.

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DIONEX

Application Note 182

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]

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 fish[22] and meat[23] 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 μL (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 MΩ-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, ≥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 × 250 mm (P/N 062878) IonPac CG18 Guard, 2 × 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 °C (lower compartment) 30 °C (upper compartment)
Inj. Volume:	5 µL
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 µS
Conductance Noise:	0.2–0.3 nS
System Backpressure:	~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) ~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 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.

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

Prapare 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 × 50 mm CG18 and a 2 × 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-

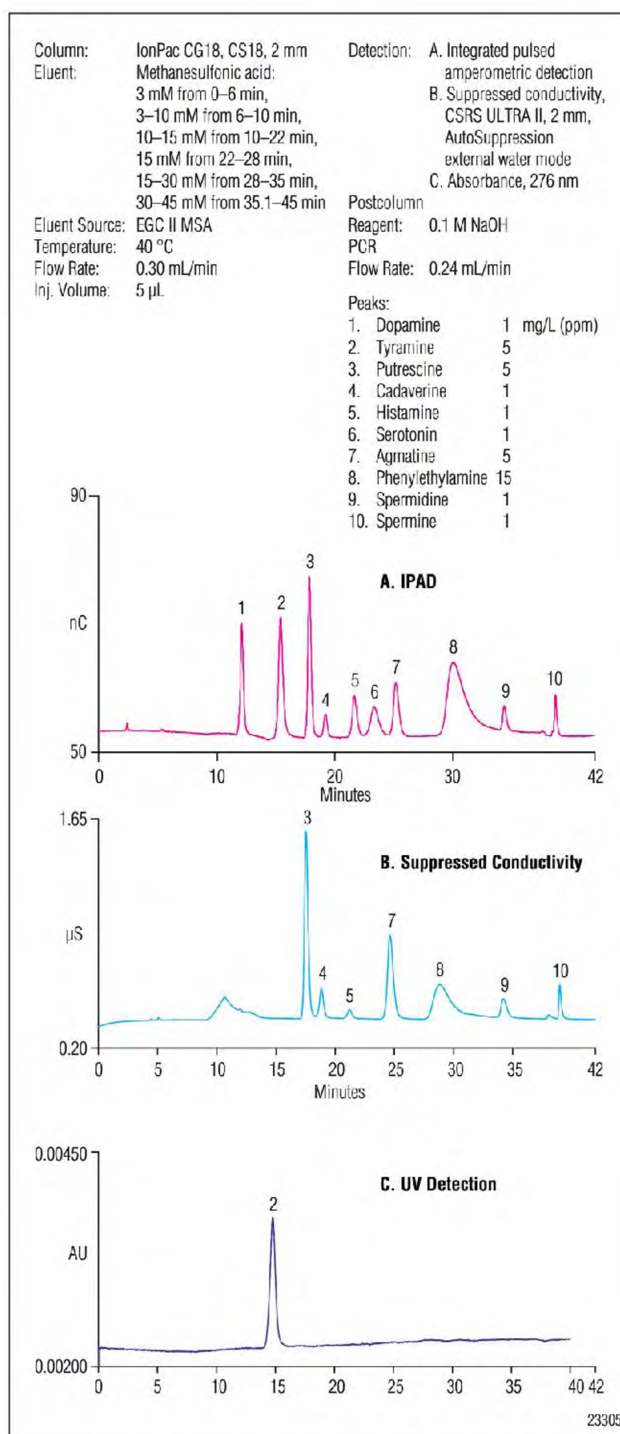

Figure 1. Separation of biogenic amines with (A) IPAD, or (B) suppressed conductivity detection. (C) Tyramine determined by UV detection.

Table 1. Linearity and Limits of Detection of Biogenic Amines												
	IPAD Only			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	—	—	—	—	—	—	—	—	—
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