

Determination of Biogenic Amines in Foods Using Ion

● Chromatography with Suppressed Conductivity and Integrated Pulsed Amperometric Detections

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Outline

- Introduction
- Traditional methods for biogenic amines analysis
- Ion chromatography of biogenic amines
- Applications in food & beverages
- Summary

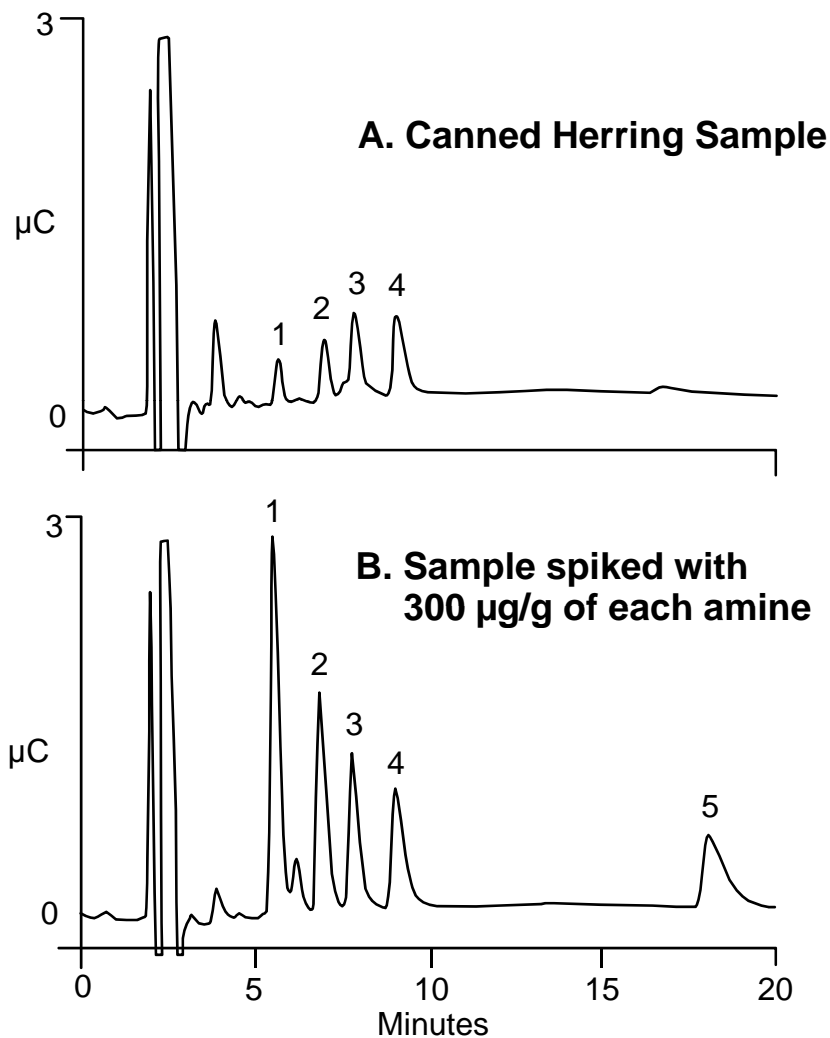
Relevance of Biogenic Amines

- Aliphatic (putrescine, spermidine, spermine), aromatic (dopamine, tyramine, phenylethylamine) and heterocyclic (histamine, serotonin) structures
- Biologically active compounds with important metabolic and physiological roles in humans and animals
 - Growth regulation
 - Control of blood pressure
 - Neural transmission
- Occur in a wide variety of foods such as fish, meat, dairy, vegetables, fruits, and chocolate and indicate spoilage
- Consumption of high concentrations can result in hypotension, hypertension, migraines, dizziness, increased respiration, etc.

Traditional Methods for the Analysis of Biogenic Amines

- Reversed-phase liquid chromatography with UV or fluorescence detection
- Pre- or post-column derivatization required due to non-chromophoric and non-fluorophoric nature of many biogenic amines
- Common derivatization reagents include dansyl chloride, benzoyl chloride, and *o*-phthaldialdehyde in combination with 2-mercapto-ethanol
- **Ion chromatography not commonly been used in the past because of strong hydrophobic interaction resulting in long retention times and poor peak symmetry**

Biogenic Amines in Spoiled Canned Herrings



Column: IonPac CS10 with guard
 Eluent: A. MeCN-water (90:10, v/v)
 B. 0.5 mol/L perchloric acid
 C. 1 mol/L sodium perchlorate
 D. Water

Gradient:	Time [min]	%A	%B	%C	%D
	0.0	5	10	60	25
	1.0	5	10	60	25
	10.0	5	10	85	0
	25.0	5	10	85	0
	26.0	5	10	60	25

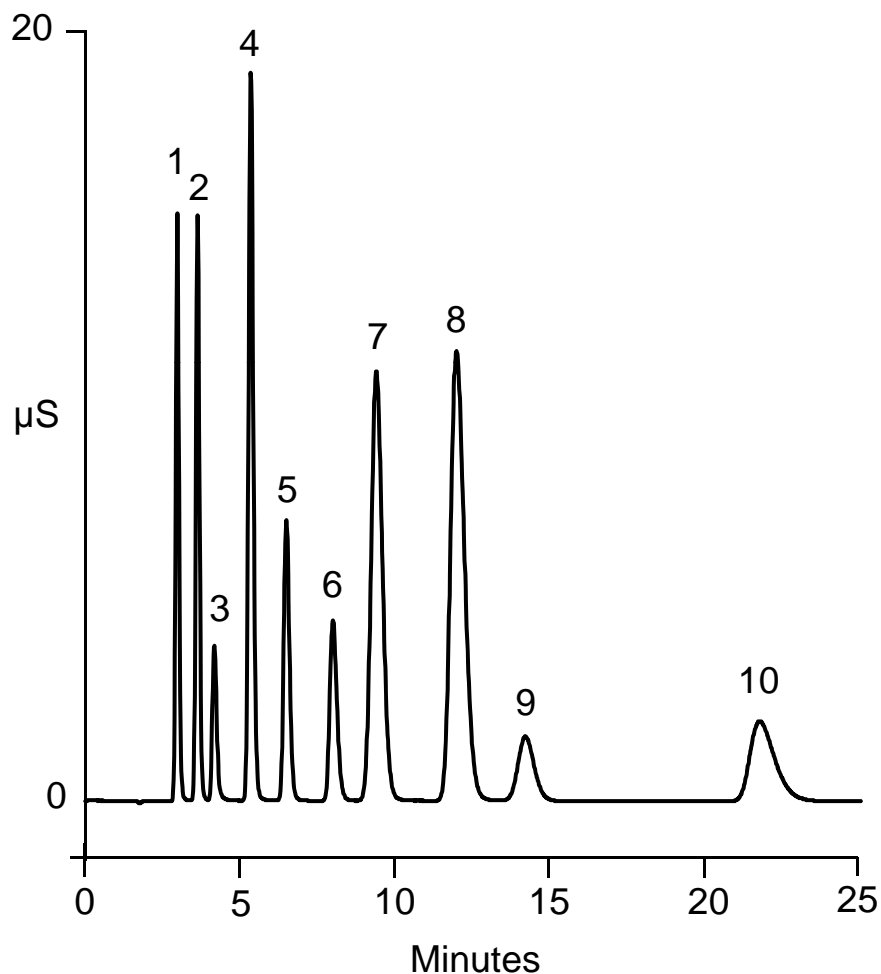
Flow rate: 1.0 mL/min
 Detection: Integrated pulsed amperometry
 Sample: Canned herring

Peaks:	1. Putrescine	16	µg/g
	2. Histidine	103	
	3. Cadaverine	187	
	4. Histamine	172	
	5. Spermidine	—	

Modern Biogenic Amines Analysis

- Cation exchange chromatography utilizing grafted surface-functionalized cation exchangers
- Integrated pulsed amperometric detection

Separation of Inorganic Cations on a Grafted Surface Functionalized Weak Acid Cation Exchanger



Column: IonPac CS12A
Eluent: 18 mmol/L MSA
Flow rate: 1 mL/min
Inj. volume: 25 μL
Detection: Suppressed conductivity,
Recycle Mode

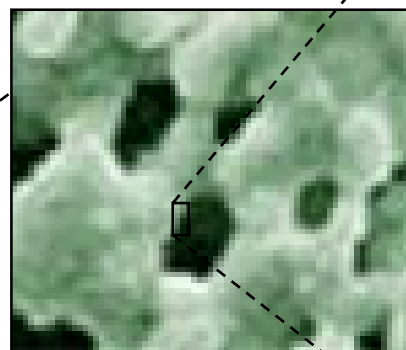
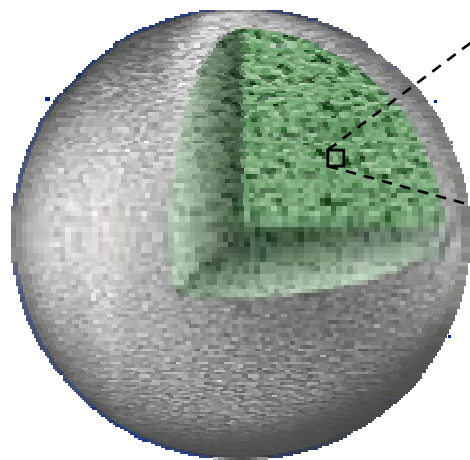
Peaks:

1. Lithium	1 mg/L
2. Sodium	4
3. Ammonium	5
4. Potassium	10
5. Rubidium	10
6. Cesium	10
7. Magnesium	5
8. Calcium	10
9. Strontium	10
10. Barium	10

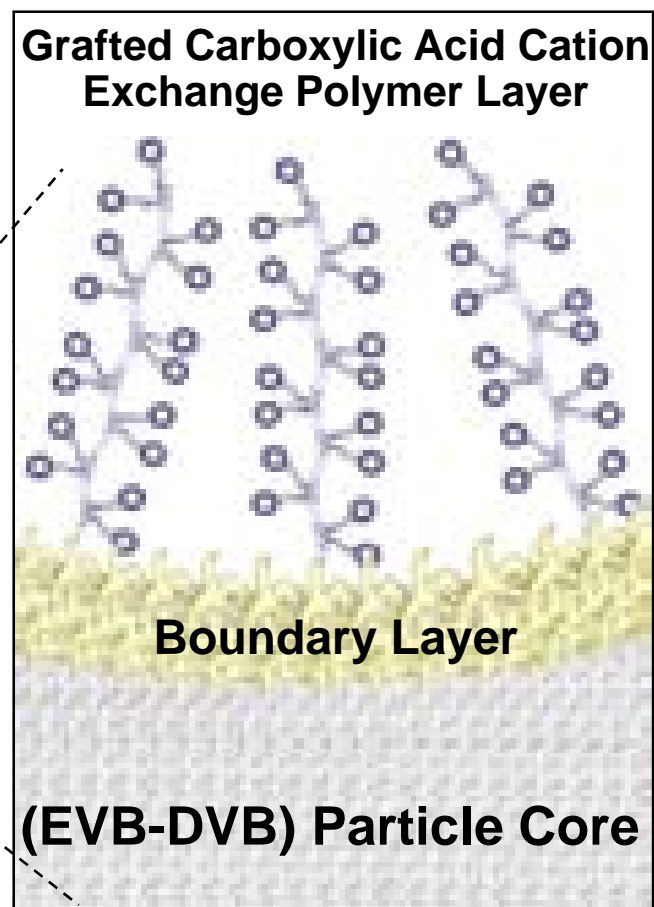
Schematics of a Cation Exchanger for Hydrophobic and Polyvalent Amines

7- μm macroporous
EVB/DVB particle with
55% crosslinking

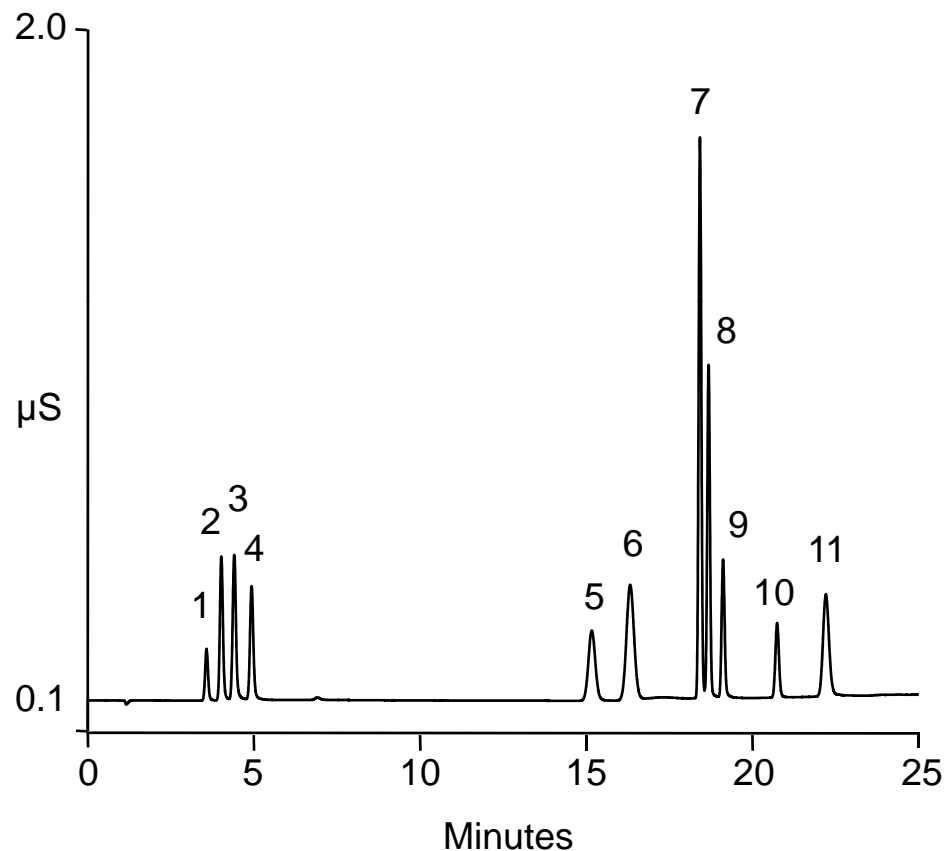
Surface area: 450 m^2/g



Pore Structure



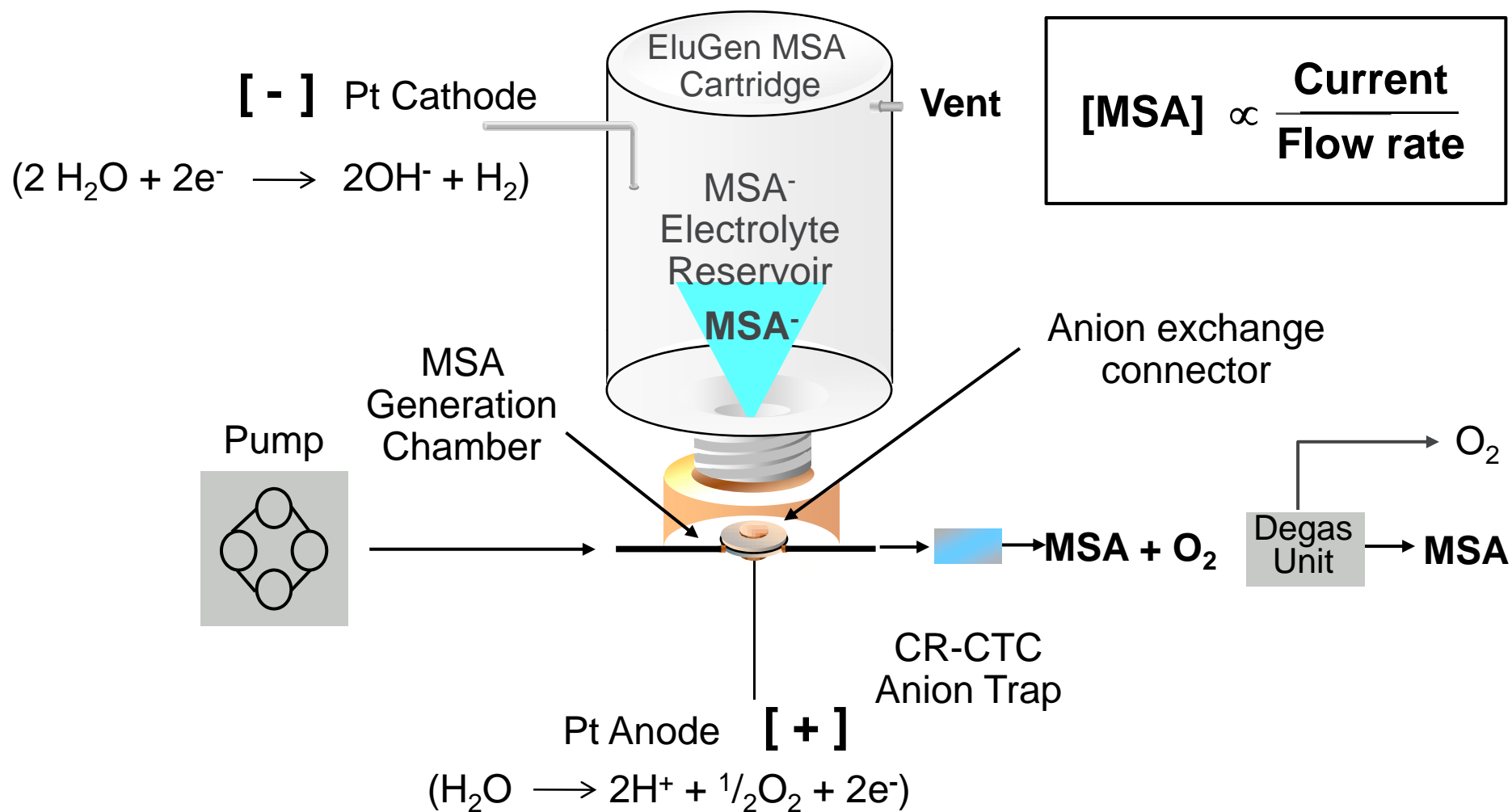
Separation of Biogenic Amines



Column: IonPac CS17 with guard (2 mm)
Temperature: 40°C
Eluent: MSA (EG)
Gradient: 3 mmol/L isocratic until 3.5 min;
3-6 mmol/L gradient in 8.5 min;
isocratic until 15 min; gradient to 40
mmol/L in 5 min
Flow rate: 0.4 mL/min
Inj. volume: 25 µL
Detection: Suppressed conductivity,
AutoSuppression, Recycle Mode

Peaks:		
1. Lithium	10	µg/L
2. Sodium	40	
3. Ammonium	50	
4. Potassium	100	
5. Magnesium	50	
6. Calcium	100	
7. Putrescine	1000	
8. Cadaverine	600	
9. Histamine	600	
10. Spermidine	200	
11. Spermine	400	

MSA Eluent Generation for Cation/Amine Analysis



Detection of Biogenic Amines via Pulsed Amperometry

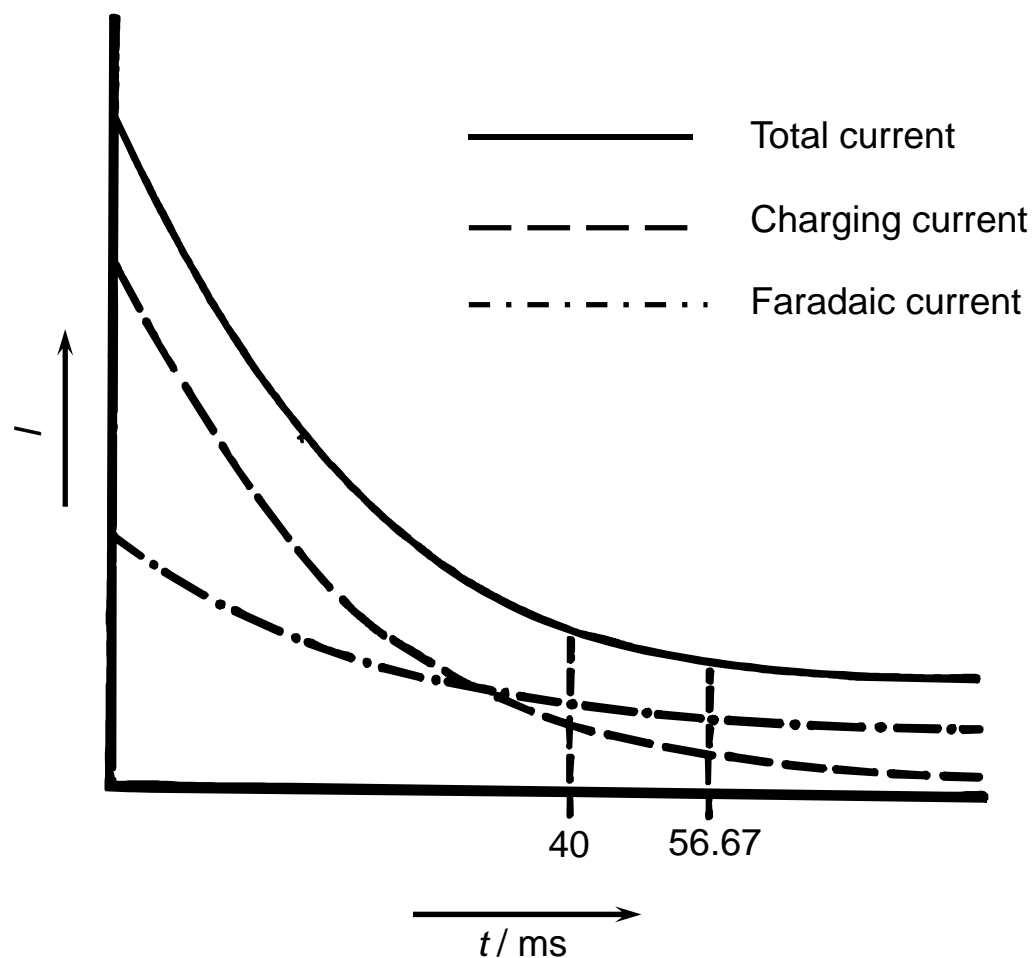
The potential is applied in form of pulses and is changed stepwise after every pulse. The resulting voltammogram does not differ from that in hydrodynamic voltammetry.

Immediately after a pulse in the diffusion controlled plateau, the measured current is very high, because the molecules A in the vicinity of the electrode are oxidized. More distant molecules are transported to the electrode surface by diffusion and are then oxidized, so that the current decreases accordingly.

During the oxidation of A to B two different currents are generated:

- Charging current
Charging the layer between electrode and solution which acts as a capacitor
- Faradaic current
Electron transfer during the oxidation from A to B

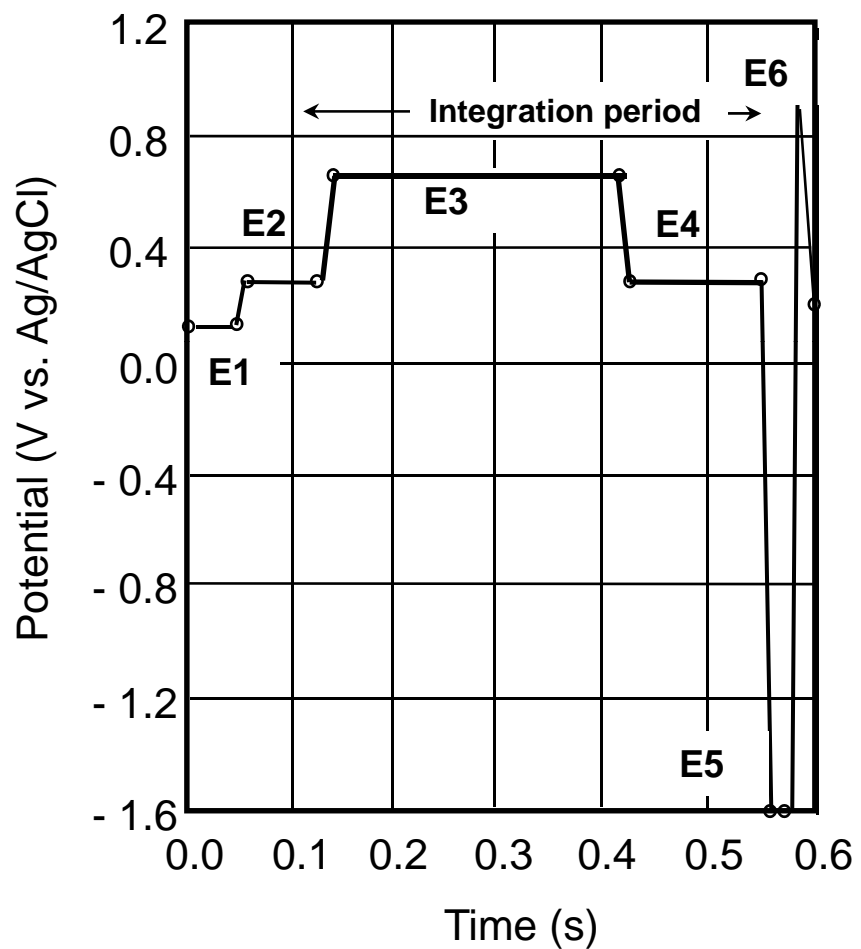
Decay of Total Current, Faradaic Current, and Charging Current after Applying a Pulse



Integrated Pulsed Amperometry

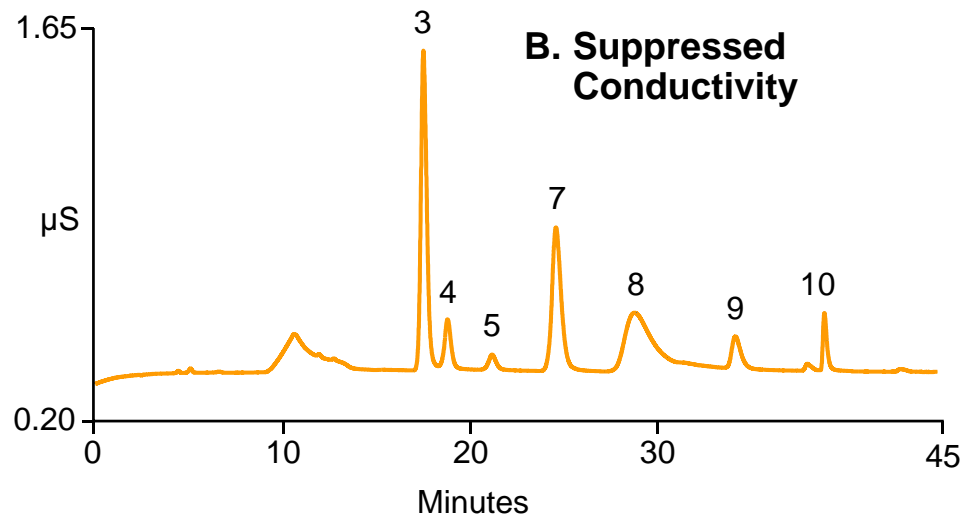
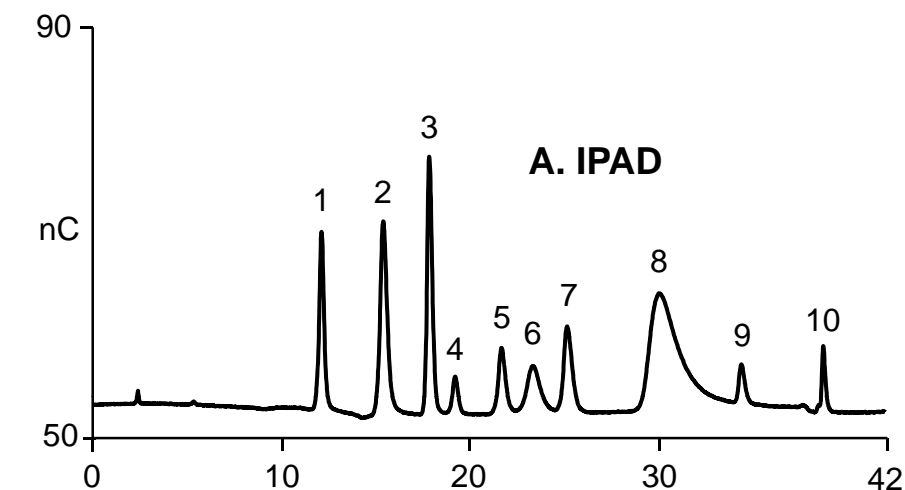
- Variant of pulsed amperometry.
- Applicable for the analysis of amino acids, amines, and organic sulfur compounds.
- Baseline disturbances due to pH gradients, solvent gradients, changes in ionic strength or metal oxide formation are minimized.
- Potential E_1 is varied in a cycle between a high and a low value.
- Analyte and metal oxidation take place at the same time at the higher potential.
- The metal oxide formed at that potential is immediately reduced at the lower potential.
- Since the oxidation of the electrode surface is reversible, and the oxidation of the analyte is not, the resulting signal is characterized predominantly by the contribution of the analyte oxidation.
- By integrating the current yield during this cycle the net signal for the respective analyte is resulting.

Waveform for Biogenic Amines Analysis



Time [s]	Pot. [V]	Integ.
0.00	0.13	
0.04	0.13	
0.05	0.33	
0.21	0.33	Begin
0.22	0.55	
0.46	0.55	
0.47	0.33	
0.536	0.33	End
0.546	-1.67	
0.576	-1.67	
0.586	0.93	
0.626	0.93	
0.636	0.13	

Separation of Biogenic Amines on IonPac CS18



Column: IonPac CS18 with guard, 2 mm
 Eluent: MSA (EG)
 Gradient: 3 mmol/L from 0-6 min, 3-10 mmol/L from 6-10 min, 10-15 mmol/L from 10-22 min, 15 mmol/L from 22-28 min, 15-30 mmol/L from 28-35 min, 45 mmol/L from 35.1-40 min

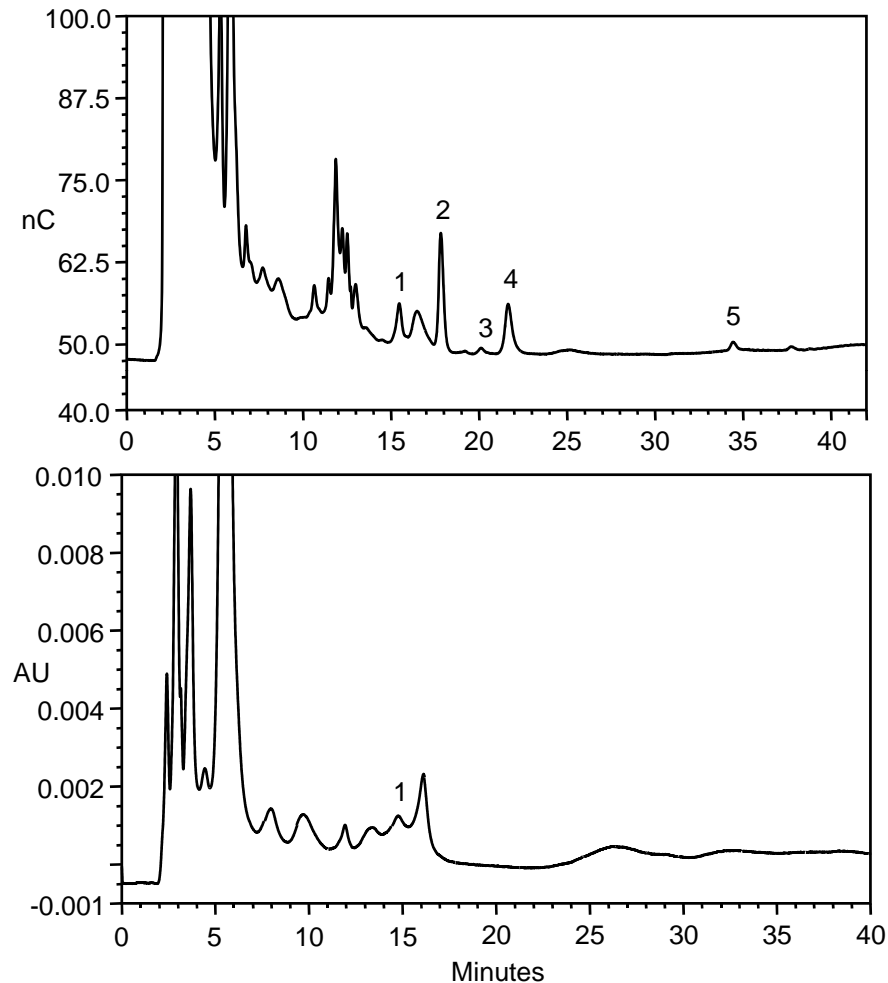
Temperature: 40 °C
 Flow rate: 0.3 mL/min
 Inj. volume: 5 µL
 Detection: A. Integrated pulsed amperometry

B. Suppressed conductivity,
 AutoSuppression,
 External water mode

Post-column reagent: 0.1 mol/L NaOH
 PCR flow rate: 0.24 mL/min

Peaks:	1. Dopamine	1	mg/L
	2. Tyramine	5	
	3. Putrescine	5	
	4. Cadaverine	1	
	5. Histamine	1	
	6. Serotonin	1	
	7. Agmatine	5	
	8. Phenylethylamine	15	
	9. Spermidine	1	
	10. Spermine	1	

Determination of Biogenic Amines in a Californian Red Wine

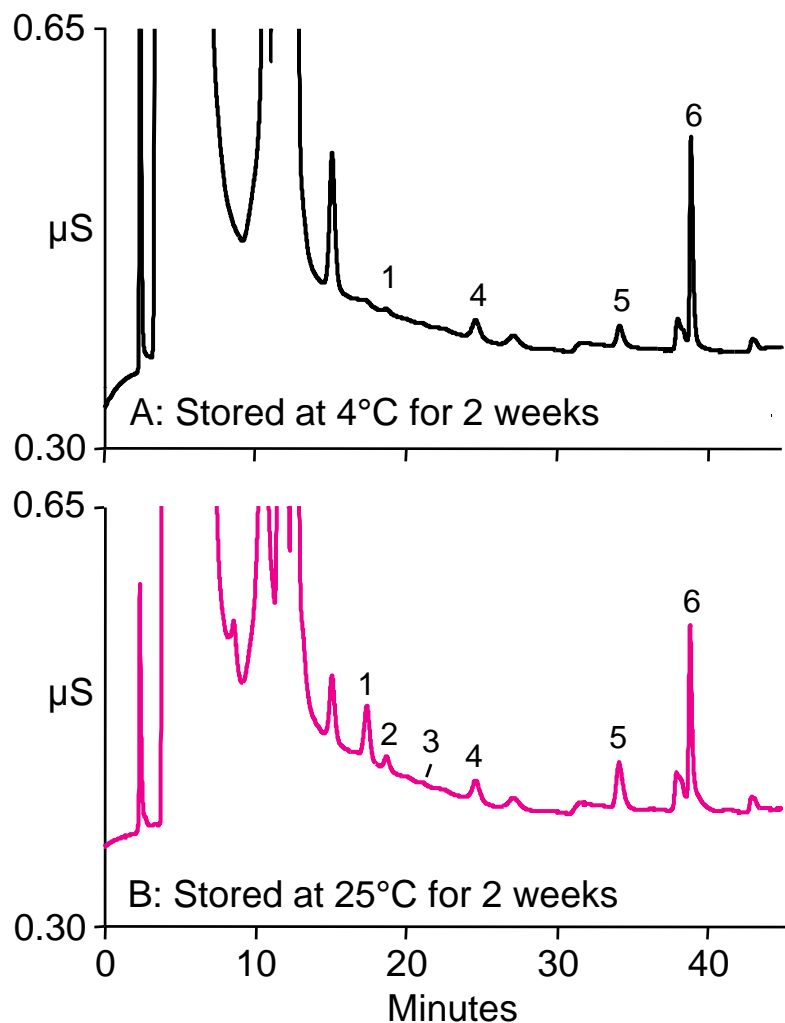


Column: IonPac CS18 with guard, 2 mm
Eluent: MSA (EG)
Gradient: 3 mmol/L from 0-6 min, 3-10 mmol/L from 6-10 min, 10-15 mmol/L from 10-22 min, 15 mmol/L from 22-28 min, 15-30 mmol/L from 28-35 min, 45 mmol/L from 35.1-40 min
Temperature: 40 °C
Flow rate: 0.30 mL/min
Inj. volume: 5 µL
Detection: A. Suppressed conductivity, AutoSuppression, external water mode
B. UV, 276 nm
Post-column reagent: 0.1 mol/L NaOH
PCR flow rate: 0.24 mL/min
Sample: BV Cabernet Sauvignon, 1:5 diluted

Peaks:

1. Tyramine	2.6	mg/L
2. Putrescine	16.1	
3. Cadaverine	0.35	
4. Histamine	4.9	
5. Spermidine	1.7	

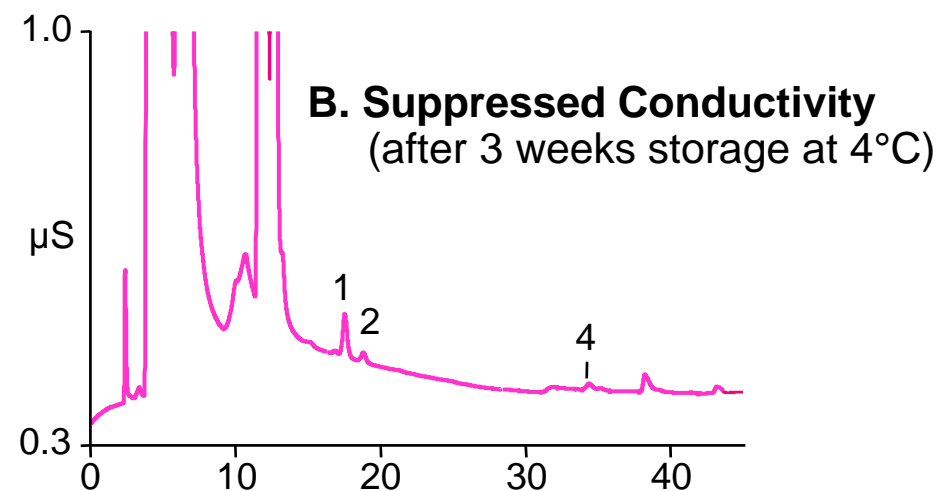
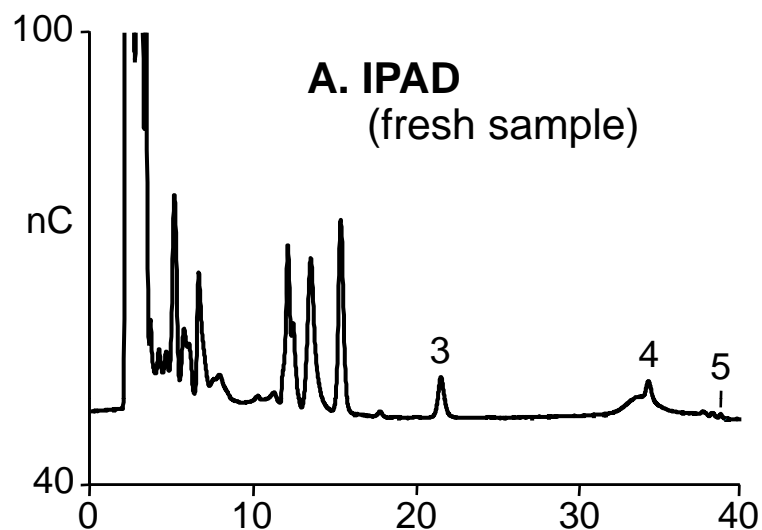
Determination of Biogenic Amines in Spoiled Sausage



Column: IonPac CS18 with guard, 2 mm
 Eluent: MSA
 Gradient: 3 mmol/L from 0-6 min, 3-10 mmol/L from 6-10 min, 10-15 min from 10-22 min, 15 mmol/L from 22-28 min, 15-30 mmol/L from 28-35 min, 45 mmol/L from 35.1-40 min
 Temperature: 40 °C
 Flow rate: 0.30 mL/min
 Inj. volume: 5 μL
 Detection: Suppressed conductivity, AutoSuppression

Peaks:	A	B
1. Putrescine	0.65	9.5 mg/kg
2. Cadaverine	—	3.1
3. Histamine	—	1.6
4. Agmatine	8.2	6.9
5. Spermidine	7.6	14.3
6. Spermine	46.6	32.1

Separation of Biogenic Amines in Spinach by (A) IPAD and (B) Suppressed Conductivity



Column: IonPac CS18 with guard, 2 mm
 Eluent: MSA (EG)
 Gradient: 3 mmol/L from 0-6 min, 3-10 mmol/L from 6-10 min, 10-15 mmol/L from 10-22 min, 15 mmol/L from 22-28 min, 15-30 mmol/L from 28-35 min, 45 mmol/L from 35.1-40 min

Temperature: 40 °C
 Flow rate: 0.3 mL/min
 Inj. volume: 5 µL
 Detection: A) Integrated pulsed amperometry
 B) Suppressed conductivity, AutoSuppression, External water mode

Post-column reagent: A) 0.1 mol/L NaOH
 PCR flow rate: A) 0.24 mL/min

Peaks:	A	B	mg/L
1. Putrescine	7.8	13.1	
2. Cadaverine	4.4	—	
3. Histamine	61	—	
4. Spermidine	48.5	3.3	
5. Spermine	6.5	—	

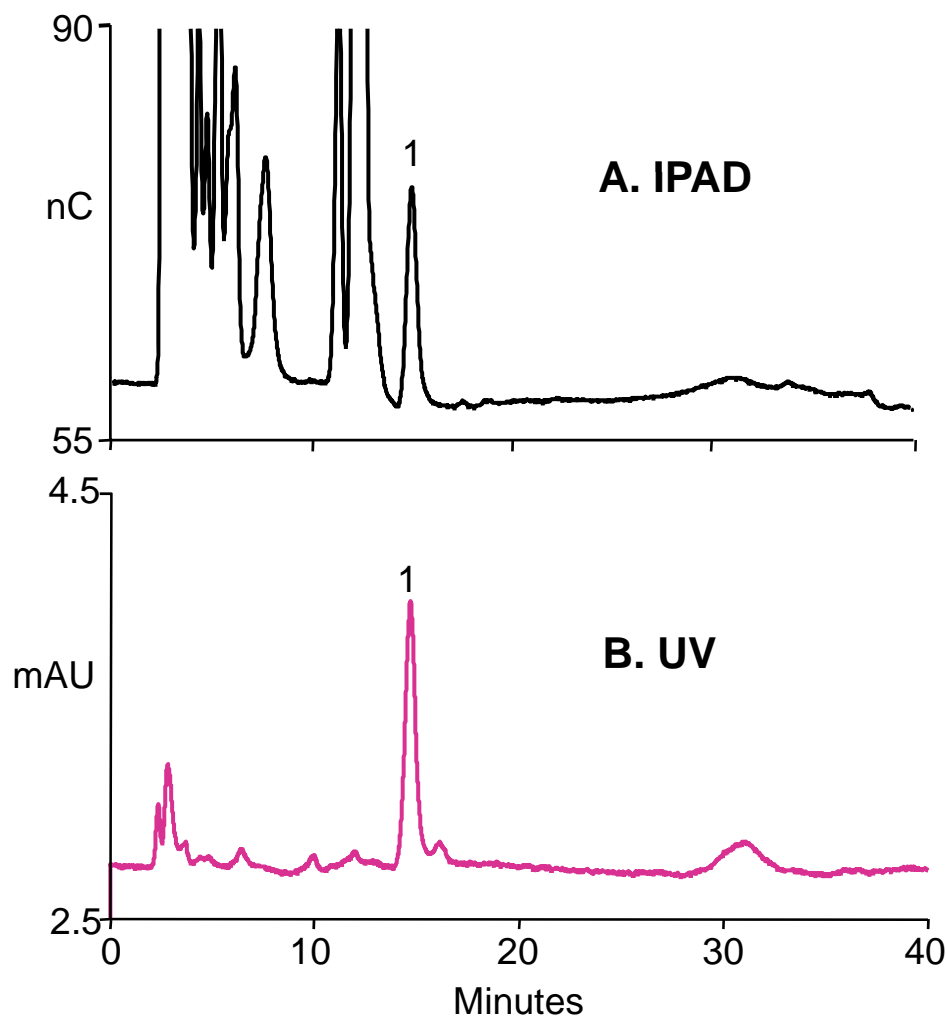
Sample Preparation Example

- Add 5 g of ground sample to a 50 mL centrifuge tube
- Add 20 mL of MSA ($c = 100 \text{ mmol/L}$)
- Homogenize on a vortex mixer for 1 min and centrifuge at 6000 rpm for 20 min at 4°C
- Filter the supernatant with 0.2 μm into a 50 mL volumetric flask
- Add another 20 mL MSA to the centrifuge tube and repeat procedure
- Bring up the combined extracts up to volume and dilute 1:1 with DI water

Linearity and Limits of Detection

Analyte	IPAD			Suppressed Conductivity			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]
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	—	—	—
Cadaverine	0.1-5	0.9999	70	0.1-5	0.9997	5.3	—	—	—
Histamine	0.1-5	0.9999	40	0.1-5	0.9998	18	—	—	—
Serotonin	0.1-5	0.9998	70	—	—	—	—	—	—
Agmatine	0.2-10	0.9998	170	0.2-10	0.9999	9.0	—	—	—
Phenylethyl-amine	1-20	0.9999	80	1-20	0.9999	81	—	—	—
Spermidine	0.1-5	0.9999	80	0.1-5	0.9993	4.0	—	—	—
Spermine	0.1-5	0.9996	50	0.1-5	0.9990	9.0	—	—	—

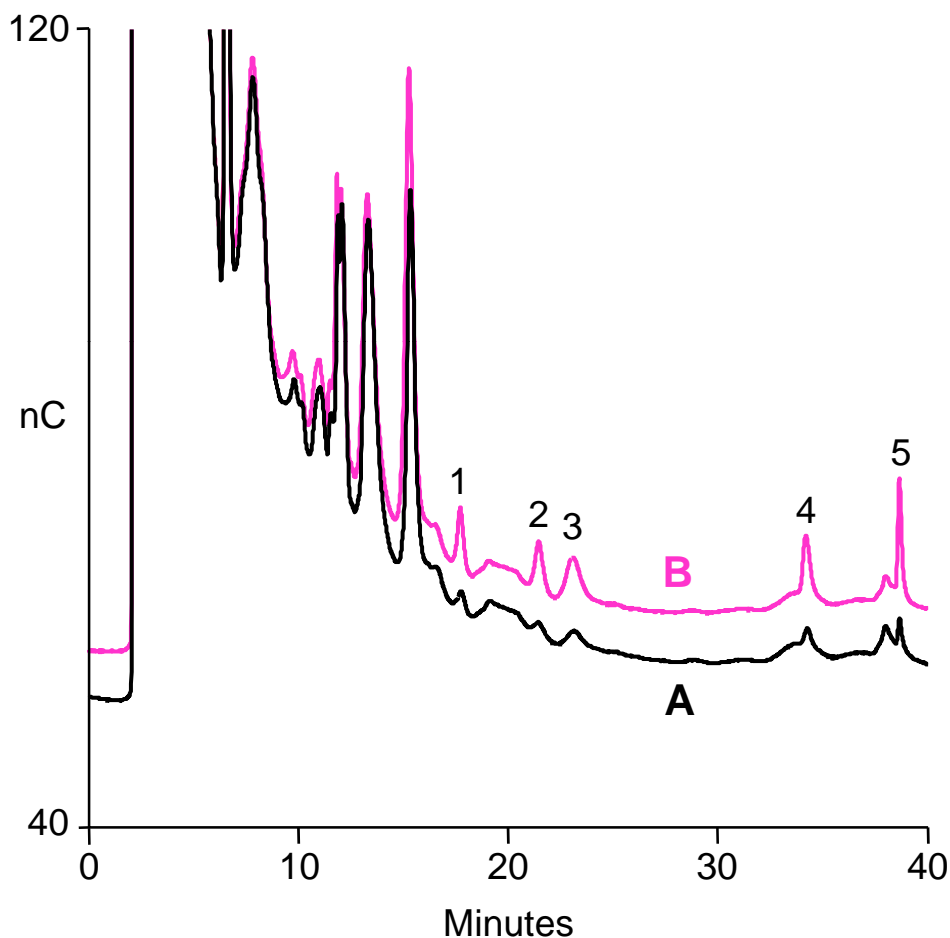
Determination of Biogenic Amines in Spoiled Swiss Cheese by (A) IPAD and (B) UV Detection



Column: IonPac CS18 with guard, 2 mm
Eluent: MSA (EG)
Gradient: 3 mmol/L from 0-6 min, 3-10 mmol/L from 6-10 min, 10-15 mmol/L from 10-22 min, 15 mmol/L from 22-28 min, 15-30 mmol/L from 28-35 min, 45 mmol/L from 35.1-40 min
Temperature: 40 °C
Flow rate: 0.30 mL/min
Inj. volume: 5 µL
Detection: A) Integrated pulsed amperometry
B) UV, 276 nm
Post-column reagent: 0.1 mol/L NaOH
PCR flow rate: 0.24 mL/min
Peaks: 1. Tyramine

	A	B
1. Tyramine	1157	1139 mg/kg

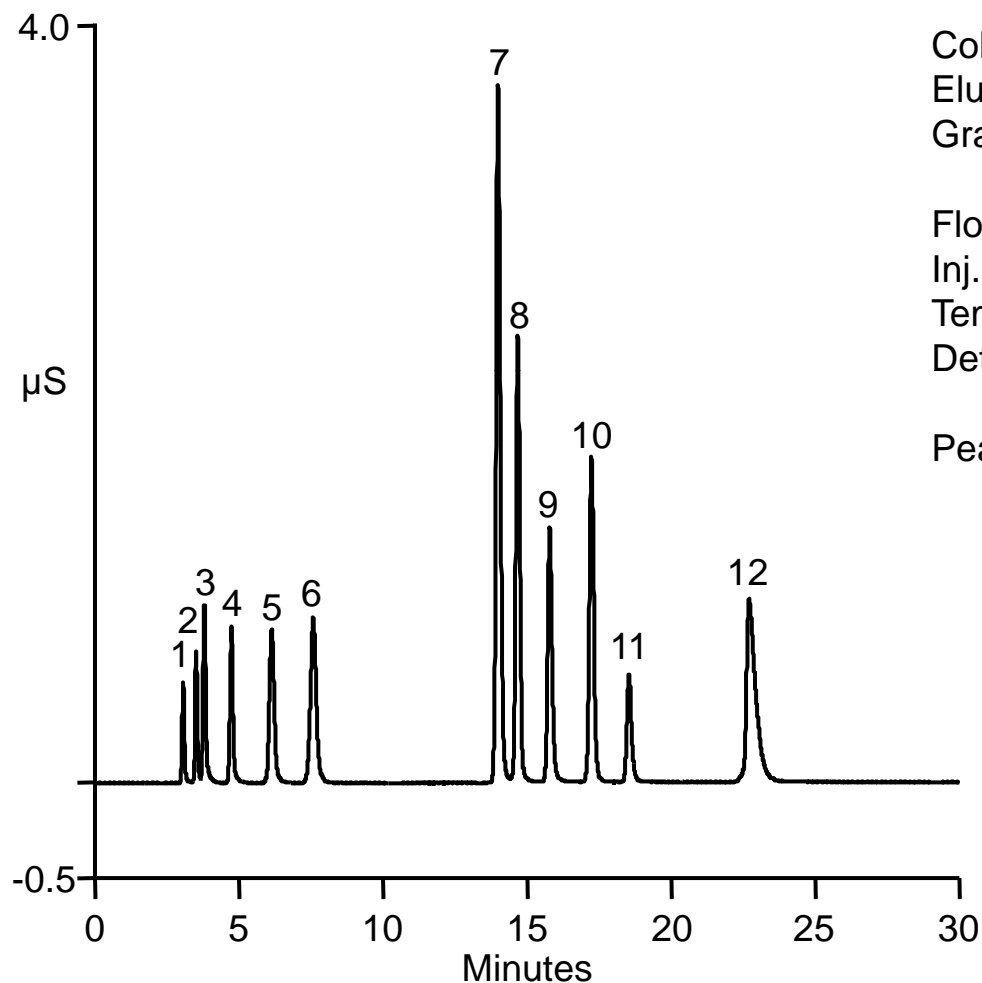
Detection of Biogenic Amines in Chocolate Containing 70% Cocoa by IPAD



Column: IonPac CS18 with guard, 2 mm
 Eluent: MSA (EG)
 Gradient: 3 mmol/L from 0-6 min, 3-10 mmol/L from 6-10 min, 10-15 mmol/L from 10-22 min, 15 mmol/L from 22-28 min, 15-30 mmol/L from 28-35 min, 45 mmol/L from 35.1-40 min
 Temperature: 40 °C
 Flow rate: 0.3 mL/min
 Inj. volume: 5 µL
 Detection: Integrated pulsed amperometry
 Post-column reagent: 0.1 mol/L NaOH
 PCR flow rate: 0.24 mL/min
 Sample: A. unspiked sample, B. spiked sample

Peaks:	A	B	mg/L
1. Putrescine	7.8	26.0	
2. Histamine	3.3	16.8	
3. Serotonin	7.3	21.7	
4. Spermidine	9.8	35.6	
5. Spermine	9.8	35.4	

Separation of Biogenic Amines on IonPac CS19



Column: IonPac CS19 (250 mm × 2 mm ID)
Eluent: MSA (EG)
Gradient: 10 mmol/L for 7 min, 10-40 mmol/L over 6 min, 40-60 mmol/L over 7 min
Flow rate: 0.3 mL/min
Inj. volume: 0.4 µL
Temperature: 30 °C
Detection: Suppressed conductivity

Peaks:		
1.	Lithium	0.1 mg/L
2.	Sodium	0.1
3.	Ammonium	0.1
4.	Potassium	0.1
5.	Magnesium	0.1
6.	Calcium	0.1
7.	Putrescine	15
8.	Cadaverine	9
9.	Histamine	13
10.	Agmatine	10
11.	Spermidine	3
12.	Spermine	6

Conclusions

- Biogenic amines can be separated on a weak acid cation exchanger in a variety of food samples, with detection by suppressed conductivity, IPAD, and UV.
- The use of three different detection methods provides additional information and confirms the identification of tyramine.
- Suppressed conductivity detection has exceptionally low LODs for the main biogenic amines without interferences from common inorganic cations.
- IPAD allows the detection of dopamine, serotonin, and tyramine, which can be confirmed by UV.

Thank you for your kind attention!