Analyze Biogenic Amines in Salmon and Shrimp by Capillary Electrophoresis-Tandem MS

Application Note

Food Testing and Agriculture

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Abstract

Biogenic amines are important indicators of freshness and quality in food. This application note presents the determination of 11 biogenic amines (spermine, spermidine, putrescine, histamine, cadaverine, agmatine, 2-phenyl-ethylamine, tyramine, tryptamine, urocanic acid, and serotonin) by capillary electrophoresis-tandem mass spectrometry (CE-MS/MS) in commercial samples of salmon and shrimp using a polyvinyl alcohol-coated (PVA) capillary, which increases the separation efficiency. The correlation coefficients of the calibration curves in the range of 0.02 to 100 µg/mL were greater than 0.998, and the limits of detection (LODs) and limits of quantification (LOQs) were in the range of 1.1–2.7 ng/mL and 3.6–8.9 ng/mL, respectively. Precision and accuracy were verified through recovery for spiked samples at three concentration levels (1, 2, and 5 mg/kg), in triplicate measurements. The recovery values ranged between 86 to 113% with RSD lower than 5.5%.

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Introduction

Biogenic amines (BAs) are vasoactive compounds present in living organisms at very low concentrations, and are essential for many physiological functions. The pharmacological activity of biogenic amines may become toxic if large amounts of these substances are consumed, causing adverse effects such as headache, nausea, renal intoxication and, in severe cases, intracerebral hemorrhage and death [1].

The determination of biogenic amines is an important and difficult task in the analysis of food samples. Various methods of separation, identification, and determination of BAs have been described [2,3]. However, BAs are extremely polar compounds, and sometimes do not contain chromophore groups. Therefore, derivatization is required for their determination by reversed-phase chromatography with ultraviolet (UV), fluorescence (FL), or mass spectrometry (MS) detection. Free zone capillary electrophoresis (CE) is an alternative technique because it uses the ionic nature of these compounds to retain them. When coupled to an MS detector, CE becomes the derivatization procedure, making chromophore reagents unnecessary. We have demonstrated the advantage of using capillary electrophoresis for the analysis of polar pesticides, and how this approach couples easily with a triple quadrupole mass spectrometer (MS/MS) [4-6]. Biogenic amines are another class of compounds that can be addressed equally successfully by capillary electrophoresis-tandem mass spectrometry (CE-MS/MS).

Currently, histamine is the only BA having official limits in fish products. Histamine is regulated as 50 mg/kg by the United States Food and Drug Administration (FDA) [7], and at 100 mg/kg by the European Union [8]. There are no Maximum Residue Limits (MRLs) established for other BAs, because the amounts naturally present vary over a wide range depending on the type of food.

We describe a CE-MS/MS method for the simultaneous determination of 11 biogenic amines in commercial samples of salmon and shrimp. The method is sensitive, fast, and produces a low amount of waste, making this an environmentally friendly, or "greener", technique.

Experimental

CE conditions

Instrument	Agilent 7100 CE system
Background electrolyte	0.5 M acetic acid
Applied voltage	25 kV
Fused-silica capillary	PVA CE-MS, 50 µm id × 65 cm total length (p/n G1600-67219)
Injection	10 s at 50 mbar
Temperature	25 °C

MS conditions

Instrument	Agilent 6430 Triple Quadrupole LC/MS
Ion mode	ESI, positive ionization
Sheath liquid	5 mM acetic acid:methanol (50:50 v/v).
Flow rate	6.0 µL/min
Capillary voltage	4,500 V
Drying gas flow (N_2)	6 L/min
Drying gas temperature	150 °C
Nebulizer pressure	12 psi

Figure 1 shows molecular structures and pKas values of the biogenic amines we analyzed. All separations were performed at 25 °C using 0.5 M acetic acid, pH 2.5, as the background electrolyte (BGE). New PVA capillaries were preconditioned by flushing with Milli-Q water for 3 minutes, followed by BGE for 5 minutes. Samples were introduced hydrodynamically in 10 seconds at 50 mbar, and analyzed with an applied voltage of 25 kV. The mass spectrometer was operated in positive multiple reaction monitoring (MRM) mode using two transitions for each biogenic amine. The most intense transition was used for quantification, and the other was used as a qualifying ion. Table 1 lists the monitored ions and other MS/MS acquisition parameters.

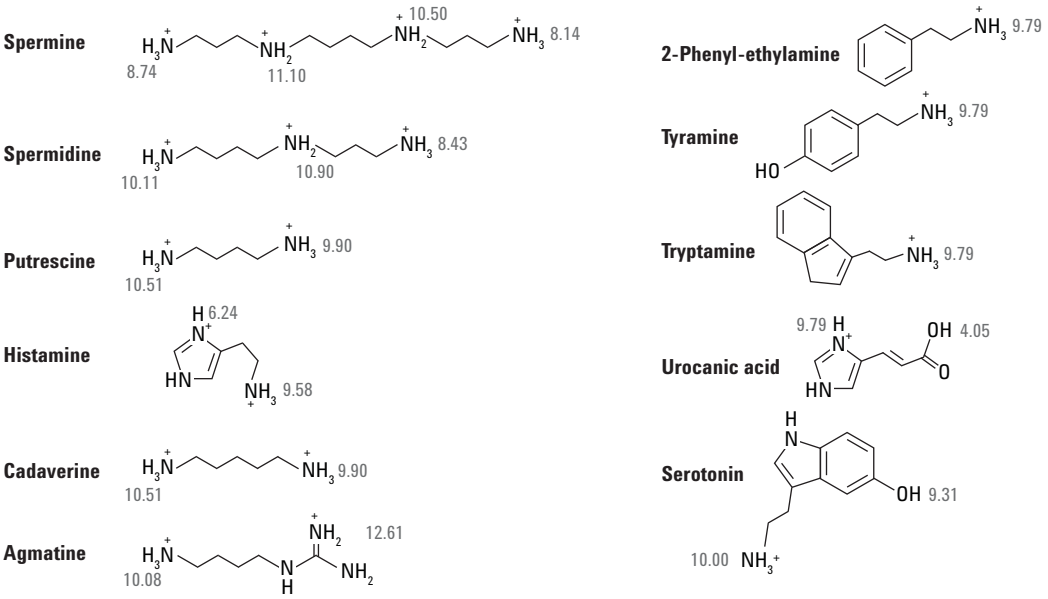

Figure 1. Chemical structures and pKa values calculated at www.chemicalize.org (accessed July 2015).

Table 1. Migration time (tM) and MS/MS acquisition parameters used for the identification and quantification of biogenic amines.

Biogenic amines	t_M (min)	Precursor ion (*m/z*)	Fragment ions (*m/z*)	Collision energy (V)	Fragmentor energy (V)
Spermine	3.71	203.2	112.3* 129.3	16 16	45
Spermidine	3.83	146.2	72.3* 112.1	16 4	35
Putrescine	4.06	89.1	72.1* 30.1	8 20	30
Histamine	4.11	112.1	95.1* 41.1	16 24	45
Cadaverine	4.30	103.1	86.2* 41.1	8 20	35
Agmatine	4.48	131.1	72.2* 60.2	8 12	35
1,7 Diaminoheptane (IS)	4.70	131.2	114.2* 41.1	8 12	45
2-Phenyl-ethylamine	6.68	122.1	105.2* 51.3	8 56	35
Tyramine	7.10	138.1	121.3* 77.1	4 32	40
Tryptamine	7.14	161.1	144.1* 117.1	8 28	45
Urocanic acid	7.31	139.0	93.2* 39.0	24 48	40
Serotonin	7.51	177.1	160.1* 115.1	8 28	81

* Transitions used for quantification.

Sample preparation

Samples of salmon and shrimp were purchased from a local street market in São Paulo, Brazil, and transported to the laboratory in separate insulated polystyrene boxes with ice. The samples were homogenized in a cryogenic mill (Spex 6750, Metuchen, NJ, USA), and stored at –20 °C until analysis. A 5 g aliquot of homogenized sample was:

1. Placed into a 15 mL centrifuge tube
2. Extracted using 10 mL of 10% trichloroacetic acid
3. Shaken in vortex for 3 minutes
4. Centrifuged for 5 minutes at 5,000 rpm

The supernatant was then filtered through a 0.45 µm regenerated cellulose membrane (p/n 5190-5284), and directly analyzed after spiking with 1,7-diaminoheptane, used as internal standard.

The recovery tests were carried out by spiking the samples before the treatment using a trichloroacetic acid step with a known amount of the analyte, resulting in three different levels of biogenic amines (0.1, 0.2, and 0.5 µg/mL) in the samples of salmon and shrimp.

Results and Discussion

A capillary coated with polyvinyl alcohol (PVA) (p/n G1600-67219) was used to achieve a good compromise between analysis time and peak resolution by reducing the electro-osmotic flow (EOF). It also prevented the interaction between highly polar compounds and the surface of the capillary, thereby avoiding peak tailing. Figure 2 shows an MRM electropherogram of a mixture of the 11 biogenic amines at 10 µg/mL each, and 1,7-diaminoheptane at 5 µg/mL used as an internal standard in BGE through a PVA-coated capillary.

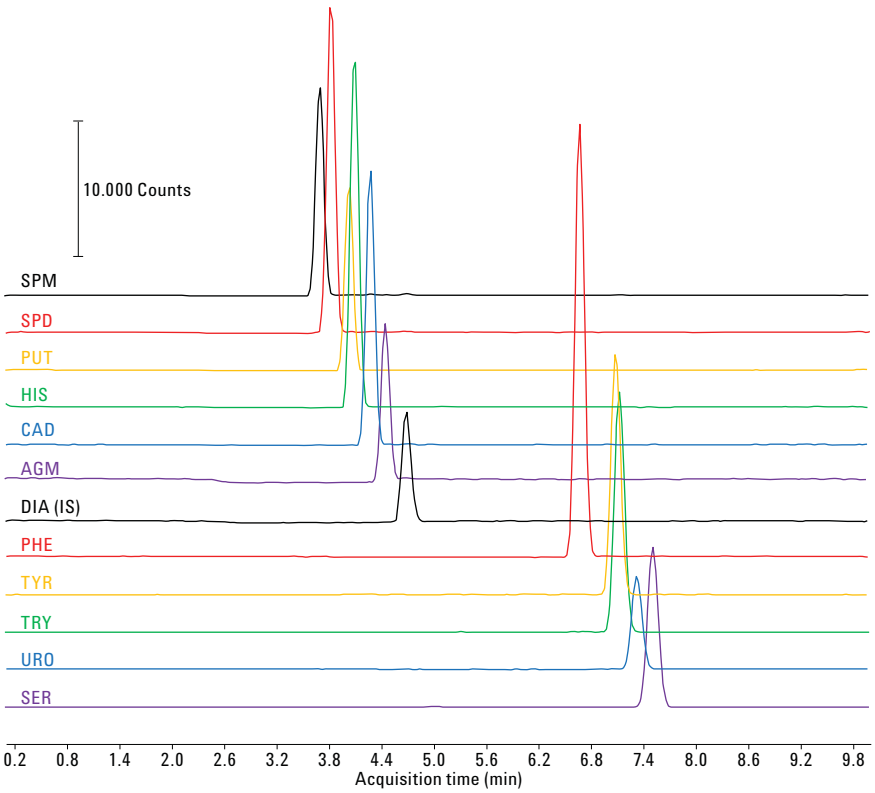

Figure 2. CE-MS/MS electropherogram of a mix of biogenic amines at 10 µg/mL each in background electrolyte. Spermine (SPM), spermidine (SPD), putrescine (PUT), histamine (HIS), cadaverine (CAD), agmatine (AGM), 1,7-diaminoheptano (DIA) at 5 µg/mL (internal standard), phenylethylamine (PHE), tyramine (TYR), tryptamine (TRY), urocanic acid (URO), serotonin (SER).

The linearity of the analytical curve was studied in BGE at 12 different levels of concentrations ranging from 0.02 to 100 µg/mL, using Agilent MassHunter Workstation Quantitative Analysis Software, as shown in Figure 3.

For all calibration curves, the correlation coefficients (R^2) presented values greater than 0.998. The limit of detection (LOD) and limit of quantification (LOQ) were determined using three times the baseline noise and 10 times the baseline noise, respectively, in a time close to the migration time of each biogenic amine. Table 2 summarizes these results.

Table 2. Important parameters of the proposed method for biogenic amine determination

Biogenic amine	y = a ± bx	R^2	LOD (ng/mL)	LOQ (ng/mL)
Spermine	y = 0.0056 + 0.1177x	0.999	1.9	6.3
Spermidine	y = 0.0144 + 0.1927x	0.999	2.6	8.6
Putrescine	y = 0.0112 + 0.0826x	0.999	2.5	8.3
Histamine	y = 0.0330 + 0.2254x	0.999	1.1	3.6
Cadaverine	y = 0.0164 + 0.1503x	0.999	1.6	5.3
Agmatine	y = 0.0240 + 0.0931x	0.999	2.6	8.6
2-Phenyl-ethylamine	y = 0.0044 + 0.3064x	0.998	1.2	4.0
Tyramine	y = 0.0223 + 0.1820x	0.999	1.6	5.3
Tryptamine	y = 0.0078 + 0.1863x	0.998	2.6	8.6
Urocanic acid	y = 0.0135 + 0.0743x	0.999	2.5	8.3
Serotonin	y = 0.0330 + 0.1340x	0.999	2.7	8.9

a = Intercept; b = Slope; R^2 = Determination coefficient;
LOD = Limit of detection; LOQ = Limit of quantitation.

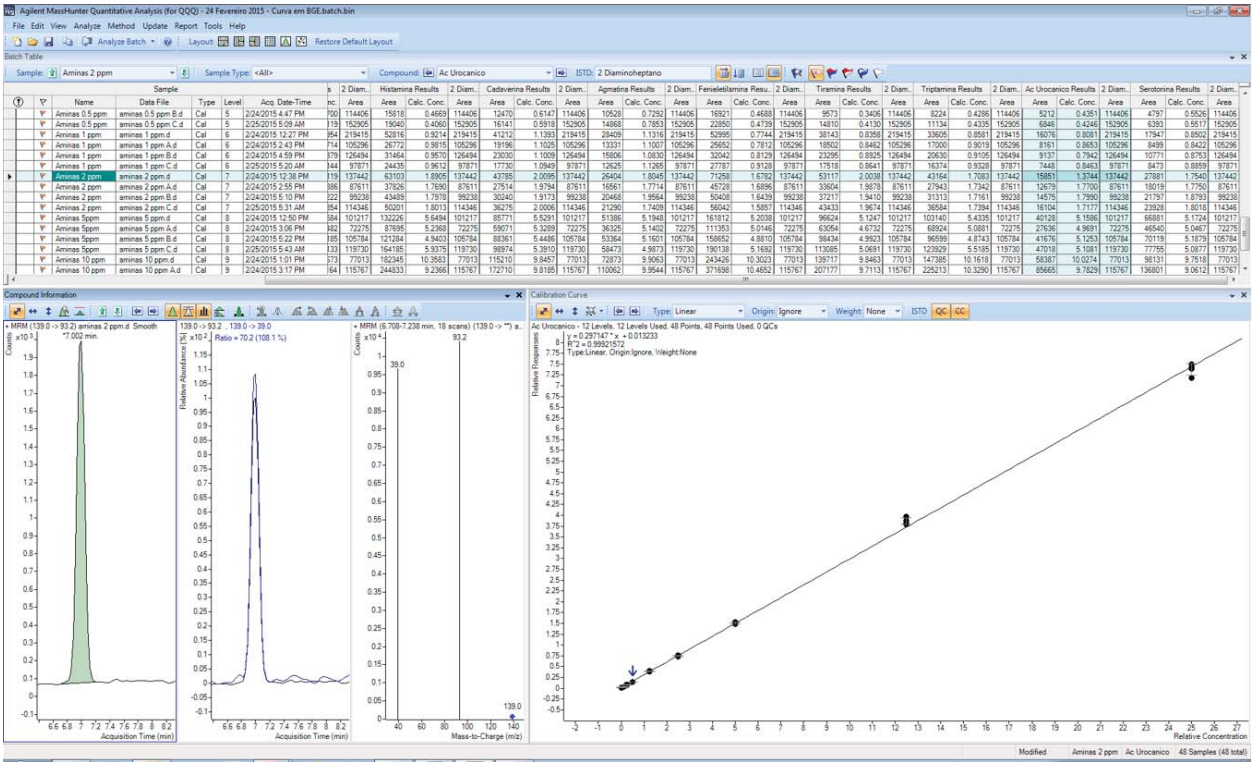

Figure 3. A screenshot from Agilent MassHunter WorkStation Quantitative Analysis Software reveals the curve linearity obtained when analyzing biogenic amines.

Precision and accuracy, expressed in terms of recovery from shrimp and salmon samples, were studied by analyzing spiked samples at three different concentrations levels in triplicate. Table 3 shows these results and the biogenic amine values found in the samples by the standard addition method.

Table 3. Concentration (mg/kg) of biogenic amines in shrimp and salmon, recovery tests (n = 3) and RSD% values.

Analyte		Shrimp	Salmon
Spermine	**Conc. found**	0.16 (1.4%)	4.50 (4.5%)
	Spiking		
	0.10	0.10 (104%)	0.09 (95%)
	0.20	0.19 (97%)	0.22 (108%)
	0.50	0.53 (106%)	0.52 (104%)
Spermidine	**Conc. found**	0.14 (1.6%)	0.54 (5.2%)
	Spiking		
	0.10	0.09 (92%)	0.11 (107%)
	0.20	0.18 (88%)	0.20 (99%)
	0.50	0.49 (98%)	0.47 (95%)
Putrescine	**Conc. found**	0.72 (2.1%)	4.20 (4.7%)
	Spiking		
	0.10	0.09 (91%)	0.10 (104%)
	0.20	0.20 (102%)	0.21 (105%)
	0.50	0.53 (106%)	0.51 (102%)
Histamine	**Conc. found**	ND	1.56 (5.5%)
	Spiking		
	0.10	0.10 (99%)	0.10 (105%)
	0.20	0.17(86%)	0.21 (104%)
	0.50	0.47 (94%)	0.51 (103%)
Cadaverine	**Conc. found**	1.02 (4.3%)	1.78 (1.5%)
	Spiking		
	0.10	0.10 (98%)	0.10 (104%)
	0.20	0.19 (97%)	0.20 (98%)
	0.50	0.50 (100%)	0.47 (95%)
Agmatine	**Conc. found**	5.92 (2.4%)	4.10 (0.3%)
	Spiking		
	0.10	0.10 (102%)	0.10 (101%)
	0.20	0.21(105%)	0.20 (100%)
	0.50	0.47 (93%)	0.48 (97%)
2-Phenyl-ethylamine	**Conc. found**	0.14 (2.8%)	0.06 (1.1%)
	Spiking		
	0.10	0.09 (94%)	0.10 (100%)
	0.20	0.21 (103%)	0.20 (100%)
	0.50	0.49 (98%)	0.50 (99%)
Tyramine	**Conc. found**	10.56 (0.6%)	1.44 (5.3%)
	Spiking		
	0.10	0.11 (113%)	0.10 (97%)
	0.20	0.20 (103%)	0.20 (99%)
	0.50	0.52 (105%)	0.49 (99%)
Tryptamine	**Conc. found**	0.18 (2.0%)	0.34 (4.3%)
	Spiking		
	0.10	0.11 (106%)	0.11 (106%)
	0.20	0.21 (104%)	0.21 (105%)
	0.50	0.51 (102%)	0.50 (100%)
Urocanic acid	**Conc. found**	0.26 (2.1%)	ND
	Spiking		
	0.10	0.09 (91%)	0.11 (106%)
	0.20	0.20 (102%)	0.20 (99%)
	0.50	0.49 (99%)	0.48 (97%)
Serotonin	**Conc. found**	ND	0.18 (3.1%)
	Spiking		
	0.10	0.10 (104%)	0.10 (105%)
	0.20	0.18 (91%)	0.20 (101%)
	0.50	0.50 (100%)	0.50 (100%)

ND = Not detected.

Conclusion

We showed that CE-MS/MS is suited for monitoring biogenic amines in food. The sensitivity and specificity of the method demonstrates its potential for successful application in other food products. The use of a PVA-coated silica capillary provided EOF suppression and increased separation efficiency with no peak tailing effects. Because low LODs were acquired, we could detect biogenic amines in a foodstuff in their initial degradation, once the recoveries were satisfactory for low levels of biogenic amine employed for assays in salmon and shrimp matrices. The proposed method is simple, uses a small amount of sample with low reagent consumption, and has good sensitivity and precision. Moreover, due to the short migration time for all biogenic amines (< 10 minutes), the method may be described as fast, with low waste generation.

References

1. Loukou, Z.; Zotou, A. *J. Chromatog. A.* **2003**, *996*, 103-113.
2. Önal, A.; Tekkeli, S. E. K.; Önal, C. *Anal. Methods* **2013**, *138*, 509-515.
3. Önal, A. *Food Chem*. **2007**, *103*, 1475-1486.
4. Vidal, D. T. R.; do Lago, C. L.; Daniel, D. *Development of an Analytical Method for the Determination of Glufosinate, Glyphosate, and AMPA in Soybean Milk by CE-MS/MS*; Application Note, Agilent Technologies, Inc. Publication number 5991-2888EN, **2013**.
5. Daniel, D.; dos Santos, V. B.; Vidal, D. T. R.; do Lago, C. L. *Food Chem*. **2015**, *175*, 82-84.
6. Vidal, D. T. R.; do Lago, C. L.; Daniel, D. *Determination of Halosulfuron-Methyl in Bell Peppers Using QuEChERS and CE/MS/MS*; Application Note, Agilent Technologies, Inc. Publication number 5991-5858EN, **2015**.
7. Anon. *Scombrotoxin (Histamine) Formation; Chapter 7*, Guidance for the Industry: Fish and Fishery Products Hazards and Controls Guidance, Fourth Edition, April 2011. U.S. Department of Health and Human Services, Food and Drug Administration, Washington, DC. http://www.fda.gov/downloads/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Seafood/UCM251970.pdf
8. Anon. *EU Directive, Regulation (EC) No 1441/2007 of 5 December 2007*. Official Journal of European Union, The Publications Office of the European Union, Luxembourg.

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