

DIRECT ANALYSIS OF BIOGENIC AMINES IN FOOD BY HILIC-MS

Biogenic amines are present in protein-rich food such as fish, meat or milk. Their concentration increases during food decomposition. The reasons to monitor biogenic amines in food products are to determine that the food is suitable for consumption and to establish appropriate storage conditions. This application note demonstrates the analysis of six biogenic amines in 15 minutes under HILIC conditions using a TSKgel Amide-80 column without the need for a complex and time-consuming derivatization procedure.

INTRODUCTION

Biogenic amines, such as histamine or tyramine are low molecular weight organic bases generated through decarboxylation of free amino acids by microorganisms. They are present in a wide range of foods products, including fish, meat, cheese, wine, beer, vegetables, fruits, and nuts. In fermented foods their presence is a result of the fermentation process.

SAMPLE PRETREATMENT PROCEDURE OF LC-MS/MS METHOD

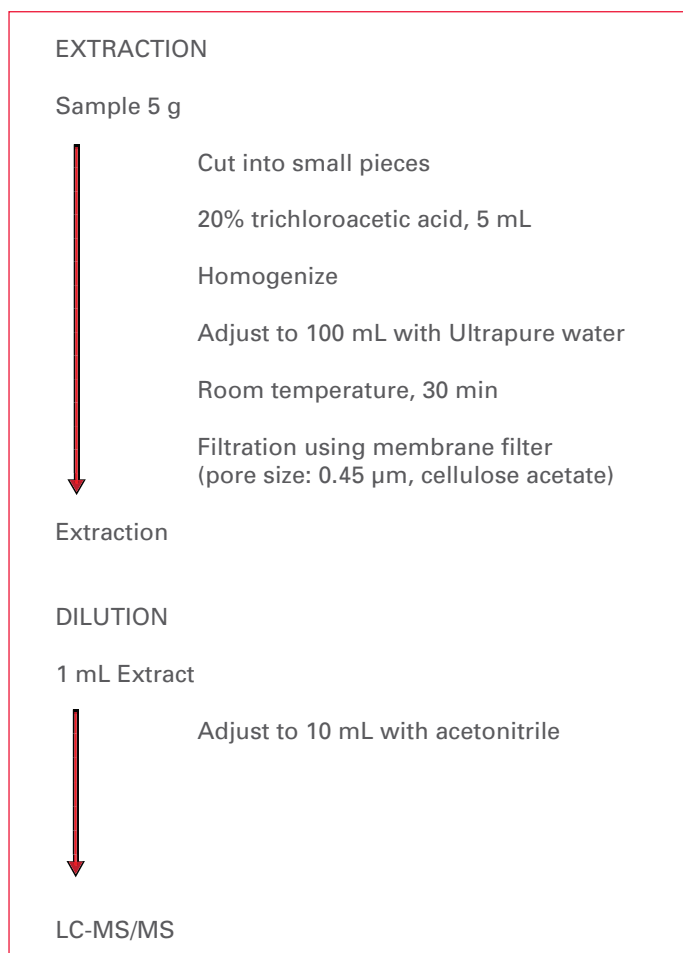


Figure 1

In non-fermented foods, the presence of biogenic amines indicates undesired microbial activity. Therefore, the amine level could be used as an indicator of microbial spoilage. Spoiled mackerels and tuna can show extremely high concentrations of histamine. Pseudo-allergic reactions such as skin irritations, headache, sickness, and diarrhea can appear as symptoms of poisoning directly after consumption of spoiled products. Histamine-rich fresh fishes should not contain more than 200 mg histamine per kilo (EU threshold for fish products), mature fish products (e.g. matie herring) not more than 400 mg/kg. In 2007 extremely high histamine values (> 6,600 mg histamine/kg) were detected in tuna samples, which were not stored correctly.

Analysis methods for biogenic amines include reversed phase HPLC with fluorescence detection using derivatization with dansyl chloride or o-phthalaldehyde (OPA). The drawbacks of these methods are a 40 minute analysis time, derivatization, and liquid phase extraction.

EXTRACTED ION CHROMATOGRAMS

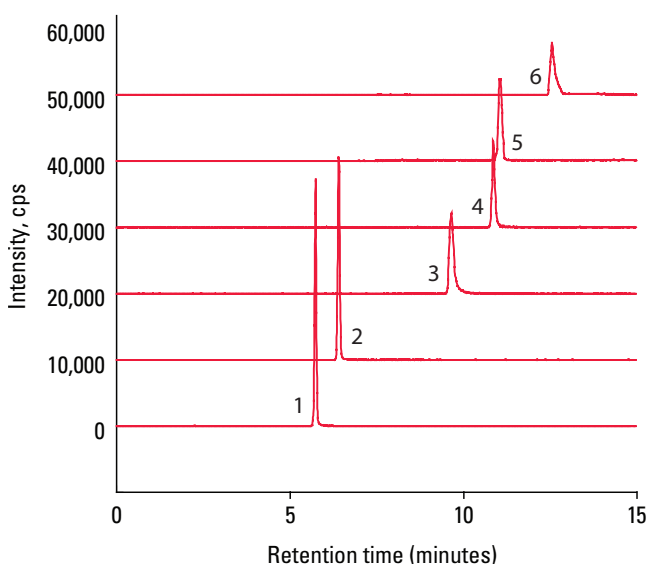


Figure 2

Column: TSKgel Amide-80 3 μm, 2mm ID x 15 cm L
 Mobile phase: A: 30 mmol/L ammonium formate, pH 4.0
 B: Acetonitrile; Flow rate: 0.2 mL/min; Temperature: 50°C;
 Injection Vol.: 2 μL; Gradient: 90% B to 40% B in 12 min;
 Sample: (1) Tryptamine (50 μg/L; 161.0/115.0 amu),
 (2) tyramine (20 μg/L; 138.0/121.0 amu); (3) histamine
 (50 μg/L; 112.0/95.0 amu); (4) cadaverine (250 μg/L;
 103.1/86.1 amu); (5) putrescine (250 μg/L; 89.1/72.1 amu);
 (6) spermidine (50 μg/L; 146.3/72.1 amu);
 Detection: ESI pos. MRM (QTRAP®, AB SCIEX)

We describe a simple, highly sensitive and direct analytical method that does not require derivatization and uses a TSKgel Amide-80 column under HILIC conditions with subsequent electrospray ionization MS/MS detection. Experimental conditions are shown in Figure 2.

RESULTS AND DISCUSSION

Figures 2 and Table 3 detail the results of the analysis of six biogenic amines. Excellent separation of the analytes and linearity of the calibration curves were obtained. When compared to a fluorescence method using a reversed phase column (data not shown), this LC-MS/MS method can be completed in half the analysis time (20 minutes versus 40 minutes). The limits of quantitation for the analytes were 0.001-0.02 mg in 100 g sample, an improvement of 5 to 1,300 times compared with the fluorescence method.

A study was conducted to compare the LC-MS/MS method to the fluorescence method in the evaluation of tuna samples under differing storage conditions. Figure 4 lists the quantitative values of biogenic amines in tuna preserved for two days at freezing and room temperatures. Good correlation was obtained between the two methods.

CONCLUSIONS

A new LC-MS/MS method for the analysis of biogenic amines was investigated by Tosoh scientists. Six biogenic amines were analyzed in 15 minutes under HILIC conditions using a TSKgel Amide-80 column without the need for a complex and time-consuming derivatization procedure. Limits of quantitation of the analytes were improved 5 - 1,300 times using this new method compared with the conventional fluorescence method.

Since good correlation between the two methods was obtained in a comparative study of quantitative values, the LC-MS/MS method is a proven alternative method that is both simple and sensitive for the analysis of biogenic amines in food products.

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COMPARISON OF LIMIT OF DETECTION AND QUANTITATION VALUES (LC-MS/MS VERSUS FLUORESCENCE METHOD)

Analytes	Calibration curve		RSD (n=5) (at 10 g/L)	LOD (g/L)	LOQ		LOQ (Fluorescence method) (mg/100 g; in fish)
	Range (g/L)	r ²			(g/L)	(mg/100 g; in fish)	
Spermidine	1.0-500	0.998	2.1	0.10	0.20	0.004	0.36
Putrescine	5.0-500	0.995	1.7	0.70	2.10	0.040	0.08
Cadaverine	1.0-500	0.992	1.5	0.30	1.00	0.020	0.10
Histamine	1.0-500	0.993	0.8	0.10	0.20	0.004	2.20
Tyramine	1.0-500	0.999	0.6	0.01	0.05	0.001	1.40
Tryptamine	1.0-500	0.996	0.9	0.03	0.10	0.002	0.18

➤ TABLE 1

COMPARISON OF QUANTITATIVE VALUES OF BIOGENIC AMINES IN TUNA SAMPLE (MG/100 G)

Sample		Spermidine (mg/kg)	Putrescine (mg/kg)	Cadaverine (mg/kg)	Histamine (mg/kg)	Tyramine (mg/kg)	Tryptamine (mg/kg)
Tuna	MS method	n.d.	n.d.	n.d.	16	1	2
	Fluorescence method	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tuna	MS method	6	36	117	774	50	2
	Fluorescence method	n.d.	26	143	684	65	n.d.

1) (2 days freezing)

2) (2 days RT)

➤ TABLE 2