Simultaneous and Direct Analysis of Biogenic Amines in Food by LC-MS/MS using Hydrophilic Chromatography

TSKgel
APPLICATION NOTE

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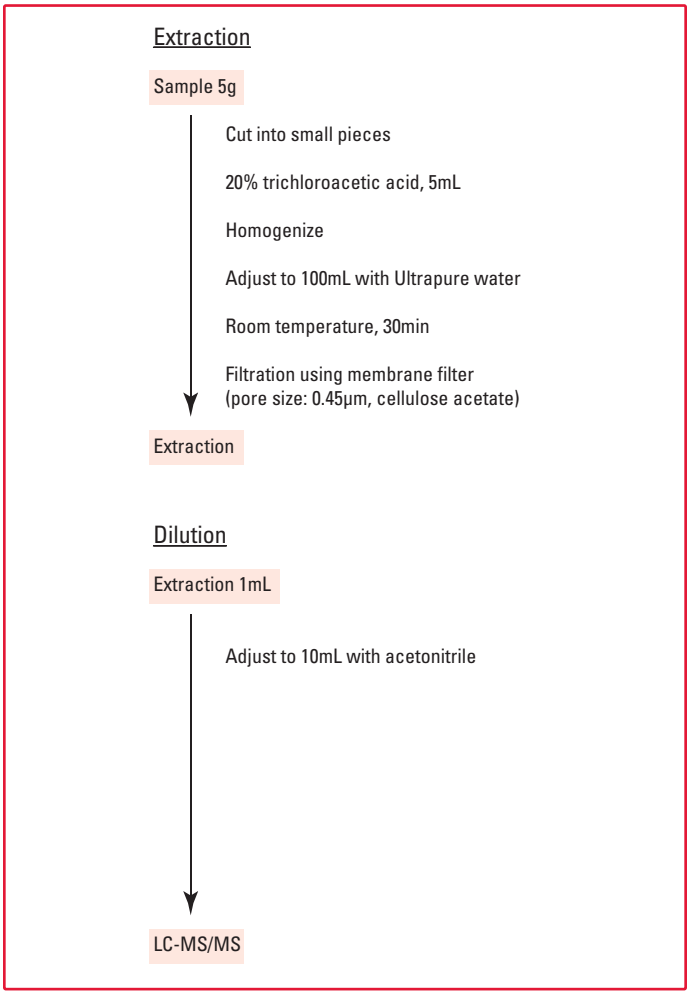
Introduction

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. Analysis methods for biogenic amines include HPLC with fluorescence detection – using derivatization with dansyl chloride or *o*-phthalaldehyde. The drawbacks to these methods are a 40 minute analysis time, sample pretreatment that requires fluorescence derivatization, and liquid phase extraction. In this study, we investigated a simple, highly sensitive and direct analytical method that does not require derivatization and uses a TSKgel Amide-80 column under HILIC conditions followed by MS/MS detection.

Experimental Conditions

Figure 1. Sample Pretreatment Procedure of LC-MS/MS Method



LC System:	Agilent 1200SL Series
Column:	TSKgel Amide-80, 3µm, 2.0mm ID x 15cm
Mobile phase:	A: 30mmol/L ammonium formate in H_2O, pH 4.0 B: ACN
Gradient:	0min (90%B), 12min (40%B), 14min (40%B), 16min (90%B)
Flow rate:	0.2mL/min
Temperature:	50°C
Injection vol.:	2µL
MS:	QTRAP® (AB SCIEX)
Ion source:	ESI
Polarity:	Positive
Mode:	MRM
Precursor ion/ Product ion:	
Spermidine (Spd):	146.3/72.1
Putrescine (Put):	89.1/72.1
Cadaverine (Cad):	103.1/86.1
Histamine (His):	112.0/95.0
Tyramine (Tyr):	138.0/121.0
Tryptamine (Trp):	161.0/115.0

Results and Discussion

Figures 2 and 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 0.001-0.02mg in 100g sample, an improvement of 5 to 1,300 times compared with the fluorescence method.

Figure 2. Chromatograms of Biogenic Amines

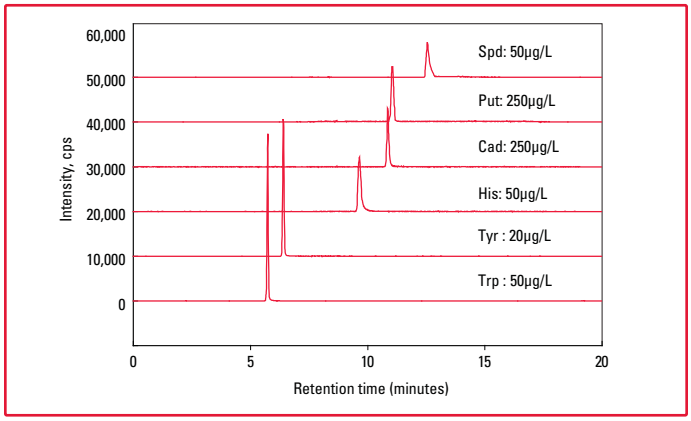


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Figure 3. Comparison of Limit of Detection and Quantitation Values (LC-MS/MS versus Fluorescence method)

Analytes	Calibration curve		RSD(n=5)	LOD	LOQ		LOQ (Fluorescence method)
	Range(µg/L)	r2	(at 10µg/L)	(µg/L)	(µg/L)	(mg/100g; in fish)	(mg/100g; in fish)
Spd	1.0-500	0.998	2.1	0.10	0.20	0.004	0.36
Put	5.0-500	0.995	1.7	0.70	2.10	0.040	0.08
Cad	1.0-500	0.992	1.5	0.30	1.00	0.020	0.10
His	1.0-500	0.993	0.8	0.10	0.20	0.004	2.20
Tyr	1.0-500	0.999	0.6	0.01	0.05	0.001	1.40
Trp	1.0-500	0.996	0.9	0.03	0.10	0.002	0.18

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.

Figure 4. Comparison of Quantitative Values of Biogenic Amines in Tuna Sample (mg/100g)

Sample		Spd	Put	Cad	His	Tyr	Trp
tuna[1)]	MS method	n.d.	n.d.	n.d.	1.6	0.1	0.2
	(RSD(%): n=5)	-	-	-	(1.3)	(1.8)	(1.4)
	Fluorescence method	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
tuna[2)]	MS method	0.6	3.6	11.7	77.4	5.0	0.20
	(RSD(%): n=5)	(2.1)	(1.2)	(1.1)	(0.8)	(0.9)	(1.1)
	Fluorescence method	N.D.	2.6	14.3	68.4	6.5	N.D.

1) preserved for 2 days under freezing
2) preserved for 2 days under room temperature

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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