Quick, Easy and Reliable Detection of Histamine in Food Using the Agilent 6490 Triple Quadrupole LC/MS with Jet Stream Technology

Application Note

Food Testing

Author

Nick Byrd

Campden BRI

Chipping Campden, Gloucestershire

United Kingdom

Introduction

Biogenic amines (BAs) are basic nitrogenous compounds that are generated in the course of microbial, vegetable, and animal metabolism. These compounds are found in varying concentrations in a wide range of foods, including fish, cheese, meat, wine, beer, vegetables, and chocolate. When present in food, biogenic amines can induce toxic effects and health problems. The most frequent foodborne biogenic amine induced health issues are caused by histamine.

Campden BRI has developed various multi compound biogenic amine methods using liquid chromatography/triple quadrupole mass spectrometry, covering compounds such as histamine, tyramine, and tryptamine. However, it is often only histamine for which a result is required, driven by the legislation described below. This application note describes that specific analysis.

Histamine contamination is referred to as scombroid fish poisoning because of the association of this illness with the consumption of scombroid fish such as tuna. In fact, in a 12 month period spanning 2011 and 2012, there were seven food poisoning alerts and 10 border rejections issued for fish products by the European Commission Rapid Alert System for Food and Feed (RASFF). The maximum legal limits in the European Union (EU) under Regulation No. 1441/2007 for histamine in the *Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae*, and *Scombresosidae* fish species range from 100 to 400 mg/kg.

Agilent Technologies

Current methods for determination of biogenic amines in food use high-pressure liquid chromatography (HPLC) and fluorescence detection after derivatization. They are time-consuming (an approximate 60-minute run time), and due to incomplete extractions and interferences in the derivatization procedure they typically produce a risk of inaccuracy and inconsistency in the results.

This application note describes a simple and rapid method using UHPLC-MS/MS and multiple reaction monitoring (MRM). This is combined with a quick acid extraction and filter procedure which has been shown in our laboratory to be suitable for subsequent LC/MS analysis not only of histamine but also of tyramine and tryptamine. Use of mass spectrometry eliminates the need for derivatization. Indeed, mass spectrometry also enables further reliability in the results to be achieved through the use of a deuterated internal standard which tracks the complete analysis.

Experimental

Reagents and Standards

Native and deuterated histamine standards were obtained from Qmx Laboratories (Thaxted, UK). The native standard was used to make up a working solution at 1,000 ppm in acetonitrile. That was then used to construct calibration curves in 0.1 M HCl.

Instruments

This method was developed on the Agilent 1290 Infinity LC System coupled to the Agilent 6490 Triple Quadrupole LC/MS System with Agilent Jet Stream Technology. The instrument conditions are listed in Table 1.

Sample Preparation

A food sample (10 g) was added to 50 mL of 0.1 M HCl and deuterated internal standard was added. The mixture was vortexed and filtered through a 0.2-µm nylon filter. Since a solvent calibration is made between 1 and 50 mg/L, this 1/5 dilution of samples allows for quantitative results when biogenic amine contamination is in the range from 5-250 mg/kg, which is a range often encountered in these analyses. For responses above this range, a greater dilution is performed on the sample to obtain a result within the calibration range.

Table 1. Agilent 1290 Infinity LC System and Agilent 6490 Triple Quadrupole LC/MS System Instrument Conditions

LC conditions		
Analytical column	Cogent Diamond Hydride column 100A 4 µm 150 × 2.1 mm 7000-15P-2	
Column temperature	30 °C	
Injection volume	0.5 µL	
Mobile phase	A = Water, 0.1 % formic acid B = Acetonitrile, 0.1 % formic acid	
Flow rate	400 µL/min	
Gradient program	Time (minutes)	% Solvent B
	0	70
	2	65
	6	10
	8	10
	9	70
	Stop time: 9 minutes	
MS conditions		
Acquisition parameters	ESI with Agilent Jet Stream, positive ionization,	
Sheath gas temperature	400 °C	
Sheath gas flow rate	12 L/min	
Drying gas flow rate	11 L/min	
Drying gas temperature	200 °C	
Nebulizer pressure	45 psig	
Nozzle voltage	1,250 V	
Vcap voltage	5,500 V	

Analysis Parameters

The Triple Quadrupole LC/MS System analysis parameters for histamine are shown in Table 2.

Table 2. Acquisition Parameters for Histamine

Target	Precursor ion	Product ion	Collision energy	Cell acceleration
Histamine (Quantifier)	112	68.1	24	7
Histamine	112	95.1	16	7
Histamine	112	54.1	48	7
Histamine D4	116	99.1	12	7
Histamine D4	116	85.1	16	7

Results and Discussion

Chromatographic Separation

Employing a C3 column results in good histamine retention, while minimizing matrix effects and ensuring separation from interferences. If looking at low concentrations, other compounds with the same transitions can be seen as shown. The internal standard leaves no doubt as to the correct assignment in such chromatography.

Hard Cheese Samples

Sample A is below the standard calibration, but, since the sensitivity of the method is clearly fit for purpose, a semi-quantitative result can be provided based on calibration fit. Since the extraction in this case involved 10 g into 50 mLs, the estimate is of about 1 mg/kg natural contamination. Sample B shows a 300 ppm spiking recovery experiment. Since the extraction in this second case involved 10 g into 100 mLs, the result of this experiment is a result of 289 mg/kg (>96 % recovery).

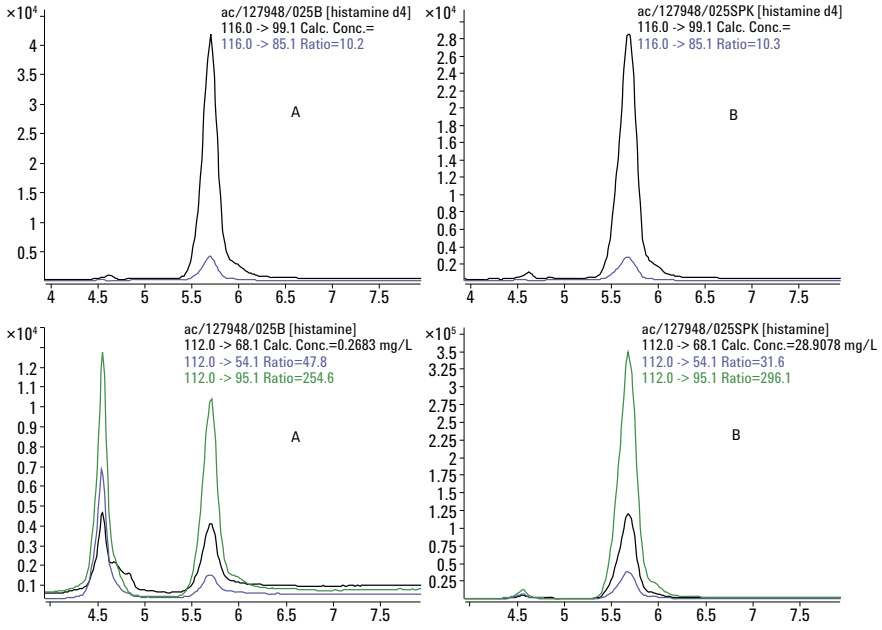

Figure 1. Quantifier and qualifier transitions for histamine in hard cheese samples.

Quantitation and Sensitivity

The calibration curve for histamine in 0.1 M HCl demonstrates excellent linearity of quantitation between 1 and 50 mg/L, with R^2 values typically >0.999 (Figure 2). This range goes down to a level which is in fact significantly below the legal limits required by EU regulations. However, it is useful to see the onset of biogenic amine formation in a food matrix, and indeed the method has the potential to quantify at even lower levels as illustrated in Figure 1.

Other Work

Recovery experiments were done in fish tissue spiked at 100 mg/kg (n=10). Recoveries for histamine were 99 ± 4.6 %. Data for two additional biogenic amines is not shown but is available from the author if required.

Conclusions

A simple, quick and easy-to-perform analytical technique has been developed to determine histamine in food. It combines a fast and inexpensive extraction procedure with a high sensitivity LC/MS/MS determination on the 6490 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology. The method provides detection limits that are well below the EU legal limits. It is therefore very plausible to apply this method on other Agilent Triple Quadrupole systems. The same transitions would be suitable, though Agilent should be approached for source condition recommendations on other models. The method is ideally suited to incorporate simultaneous determination of additional biogenic amines. Transitions and conditions for some of these have also been established to support other food work at Campden BRI.

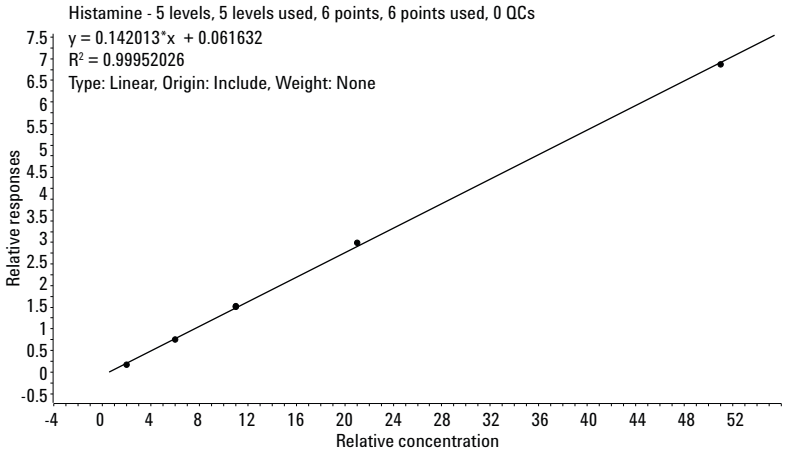

Figure 2. Calibration curve for histamine from 1– 50 mg/L in 0.1 M HCl.

www.agilent.com/chem

Printed in the USA
March 22, 2013
5991-1286EN

Agilent Technologies