Food & Beverage Application

Histamine

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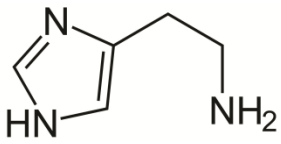


Histamine

Figure 1

INTRODUCTION

Histamine is a naturally occurring compound that is metabolized via histidine decarboxylase from the amino acid histidine (see Figure 1 for structure). Although it plays a wide variety of beneficial biochemical roles in the body, it is also the chemical released in allergic reactions involving sneezing, itching, migraines, and so on. Furthermore, ingestion of histamine-containing foods or beverages can produce these adverse effects in individuals with low amounts of the enzyme diamine oxidase, which breaks down histamine. This is referred to as *histamine intolerance*.

Due to the possibility of an adverse reaction for these individuals, manufacturers of food and beverages would prefer their products to be free of histamine. For example, some wine producers may market their wines as "histamine-free" and thereby potentially gain a competitive edge over other brands. However, often this conclusion is not based on actual quantitative analysis of histamine content, but rather on the observed absence of a reaction for histamine-intolerant individuals when they try these wines.

Part of the problem is that histamine is not readily analyzed by traditional analytical methods. Using conventional reversed phase HPLC for example, the compound is not easily retained due to its polar nature. Often a derivatization procedure is required, which complicates the analysis. Here we present a novel method for histamine analysis using the Cogent Diamond Hydride™ column. This unique stationary phase is able to operate in the Aqueous Normal Phase (ANP) mode, in which retention of analytes is based on polarity. In order to demonstrate the versatility of the column, a variety of food and beverage samples were investigated. These included wine, cheese, and tuna.

EXPERIMENTAL

Materials

Food and beverages were purchased from commercially available sources (supermarket, winery, etc.). Formic acid LC-MS ultra-grade and histamine reference standard were from Sigma–Aldrich (St. Louis, MO, USA). Deionized water (DI H_2O) was prepared on a Milli-Q™ purification system from Millipore (Bedford, MA, USA). Acetonitrile (ACN) (HPLC grade) was obtained from GFS Chemicals, Inc. (Powell, OH, USA).

Instrumentation

An Agilent (Little Falls, DE, USA) 1200SL Series LC system, including degasser, binary pump, temperature-controlled autosampler, and temperature-controlled column compartment was used. The mass spectrometer system was an Agilent (Santa Clara, CA, USA) Model 6210 MSD TOF with a dual sprayer electrospray source (ESI). The analytical column was a Diamond Hydride™ stationary phase (MicroSolv Tech. Corp. Eatontown, NJ, USA), which had dimensions 2.1 x 150 mm, a particle diameter of 4 µm, and a pore size of 100Å. Mobile phase A was DI H_2O + 0.1% formic acid and mobile phase B was ACN + 0.1% formic acid. For the cheese and tuna analyses, the A solvent was 50/50/0.1 DI water/isopropanol/formid acid. The gradient program is shown to the left.

Gradient:

Time (min)	%B
0	80
5	10
7	10
8	80

Post time: 5 min
Flow Rate: 0.4 mL/min
Injection Volume: 1.0 µL

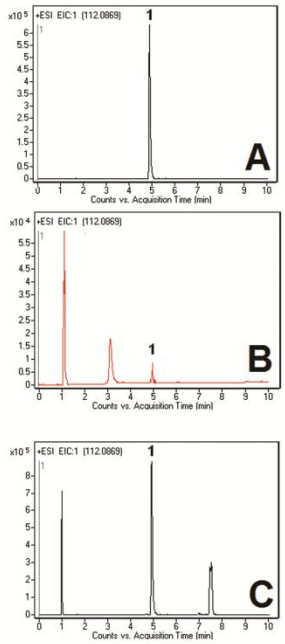


Figure 2

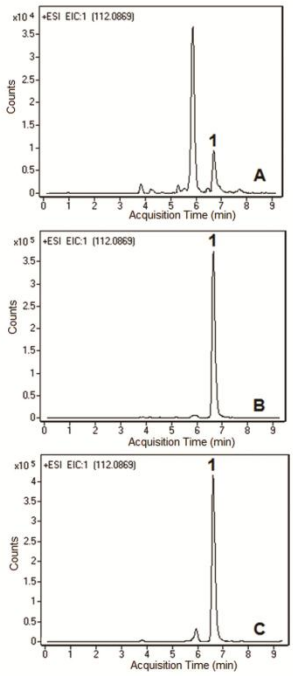


Figure 3

RESULTS AND DISCUSSION

The EIC for a histamine standard is shown in Figure 2A. Using the Diamond Hydride™ column, the analyte peak was both well-retained and symmetrical (peak 1 = histamine). At this point, the method was ready for real food and beverage samples.

The first study was verification of a winemaker's claim that their wine had virtually no histamine. This wine (Fig. 2B) was compared to a "normal" wine in which had average histamine levels (Fig. 2C). The normal wine was diluted 1:5 due to the large histamine peak (not shown) and it was still much larger than the one observed in Fig. 2B. Hence, the winemaker's claim could be analytically verified since the histamine concentration was found to be many times lower than the regular wine.

In another application, histamine was analyzed in canned tuna. The method was the same in this case except that 50% isopropanol was added to the A solvent. This was added in order to keep the column clean between runs such that strongly adsorbed compounds from the sample matrix would not accumulate. Using this method, histamine was detected in an unspiked tuna sample (Fig. 3A). Figures 3B and 3C depict EICs of spiked tuna before and after extraction, respectively. The recovery and retention times were consistent, and therefore matrix effects did not adversely affect the data. Based on calibration curve data (not shown), the amount of histamine in the unspiked tuna sample was 320 ± 4 ng/gram.

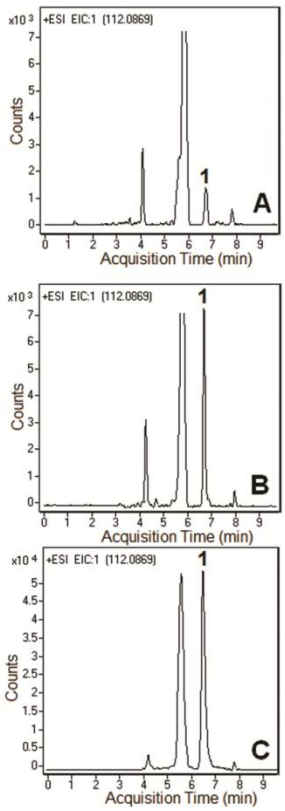
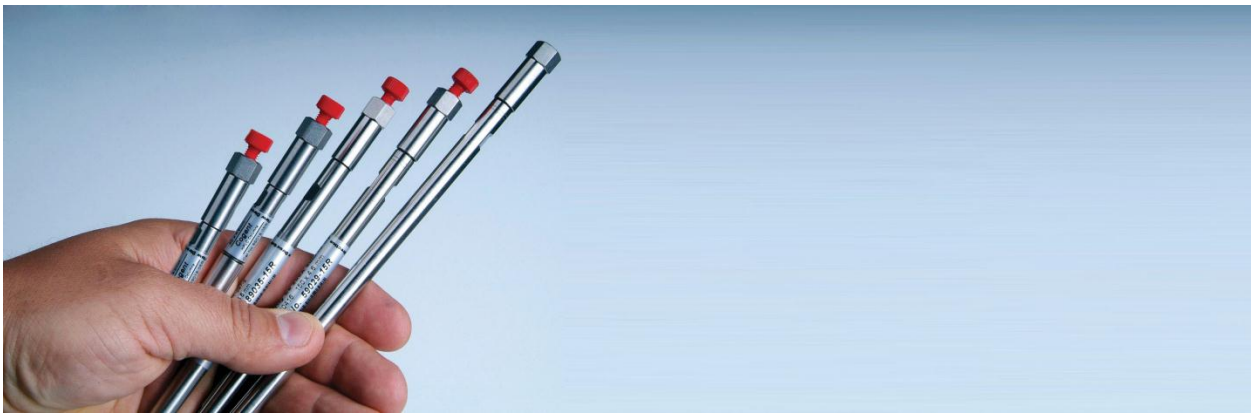


Figure 4

A similar investigation was carried out using cheese samples this time. A small but detectable amount of histamine was observed in an unspiked cheese extract (Fig. 4A). As with canned tuna, spiked samples were also prepared in which the spike was added before or after extraction. The EIC for cheese spiked before extraction is shown in Fig. 4B and the one for after extraction is shown in Fig. 4C. From these figures, it can be concluded that the identification of histamine by mass or retention time is not affected by the cheese matrix or the extracted material.

CONCLUSION

The Cogent Diamond Hydride™ column can be successfully used in the analysis of histamine. Good retention and peak shape were obtained without derivatization for a histamine standard. Subsequently, analyses involving three types of food and beverage products demonstrated the suitability of the method for real samples. Spiked samples illustrated the absence of matrix effects in affecting the data.



Catalog Number	Description
70000-02P-2	Cogent Diamond Hydride HPLC column 100A 4um 2.1mm x 20mm.
70000-03P-2	Cogent Diamond Hydride HPLC column 100A 4um 2.1mm x 30mm.
70000-05P	Cogent Diamond Hydride HPLC Column, 100A 4um 4.6mm x 50mm.
70000-05P-2	Cogent Diamond Hydride HPLC Column, 100A 4um 2.1mm x 50mm.
70000-10P	Cogent Diamond Hydride HPLC Column, 100A 4um 4.6mm x 100mm.
70000-10P-2	Cogent Diamond Hydride HPLC Column, 100A 4um 2.1mm x 100mm.
70000-15P	Cogent Diamond Hydride HPLC Column, 100A 4um 4.6mm x 150mm.
70000-15P-2	Cogent Diamond Hydride HPLC Column, 100A 4um 2.1mm x 150mm.
70000-25P	Cogent Diamond Hydride HPLC Column, 100A 4um 4.6mm x 250mm.
70000-25P-2	Cogent Diamond Hydride HPLC Column, 100A 4um 2.1mm x 250mm.
70000-7.5P	Cogent Diamond Hydride HPLC Column, 100A 4um 4.6mm x 75mm.
70000-7.5P-2	Cogent Diamond Hydride HPLC Column 100A 4um 2.1mm x 75mm.

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