Ion Chromatographic Determination of Histamine in Fish with Series Bulk Acoustic Wave Detection

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Abstract

The determination of histamine in fish using an unsuppressed ion chromatographic (IC) method with series bulk acoustic wave (SBAW) detection is developed. The advantages of the good selectivity of IC and the highly sensitive response of SBAW are combined to improve detection limit, accuracy, and reproducibility. The detection limit is 8 ng, recoveries are 98–106%, and the relative standard deviation is 1.6%. For the IC analysis, the analytical column is a Shim-pack IC C1 column, and the mobile phase is a 2.5mM HNO_3 solution. The fish samples are treated with trichloroacetic acid and purified with either *n*-pentanol or *n*-hexanol. Histamine is finally isolated with a 0.2M HCl solution. The method is applied for the determination of histamine levels in black carp.

Introduction

Histamine is produced as a result of microbial decarboxylation of histidine under appropriate conditions. The presence of histamine in fish is associated with fish deterioration, and therefore histamine contents have been proposed as an indicator of fish spoilage (1). Histamine is considered to be responsible in food poisoning. It causes allergies, headaches, vomiting, and diarrhea (2). As a result, much attention has been given to the characterization and determination of histamine in fish.

In general, histamine has been measured by fluorometry (3), biological method (3), thin-layer chromatography (4), radioenzymatic transfer assay (5), and high-performance liquid chromatography (HPLC) (6–11). In particular, various HPLC methods have been developed for the analysis of histamine. However, these methods lack specificity and/or sensitivity and require long reaction times. HPLC with fluorescence derivatization has the disadvantage of either the relative instability of the derivations, the need for carefully controlled reaction conditions, or a lack of an internal standard. Draisci et al. (12) reported a method based on ion-exchange chromatography with integrated pulsed amperometric detection and suggested that this could be applied to the analysis of cadaverine, putrescine, spermidine, and histamine in fish products. This method avoids the need for cumbersome derivatization procedures; however, it requires a complex eluent system, and the construction of the detector is more complex. The detection limit for histamine is 12 ng.

Conventional ion chromatography (IC) with conductometric detection cannot prevent such phenomena as double-layer capacitance formation or Faradaic impedance, which affect the sensitivity and accuracy of ion determination (13,14). This paper involves the determination of histamine in fish using an IC method with a novel detection system.

Bulk acoustic wave (BAW) sensing devices have been developed rapidly and utilized widely as chemical and biomedical microsensors (15,16). The frequency of the BAW detector in liquid was affected by the properties of liquids such as density, viscosity, specific conductivity, and permittivity (17–19).

A new type of BAW sensor called a series bulk acoustic wave (SBAW) has been developed, and its theory has been summarized (20–22). An SBAW detection system for IC was designed and constructed in our laboratory (13). In this work, an SBAW detector was used to develop a procedure for the determination of histamine in fish when incorporated into an unsuppressed cation-exchange chromatographic system.

Experimental

Reagents and materials

Biochemical-grade histamine hydrochloride was purchased from the Shanghai Biochemistry Research Institute of the Chinese Academy of Science. All other chemicals were of analytical-reagent grade and were used as received. Deionized water was used for preparation of the mobile phase and standard solutions. A 0.100-mg/mL histamine stock solution was prepared in water (16.91 mg histamine hydrochloride per 100 mL water), and work solutions were diluted as necessary. The mobile phase, standard, and sample solutions were filtered through a 0.45-µm membrane (Millipore, Bedford, MA).

SBAW detector

A new SBAW detection system for IC was designed and constructed in our laboratory (13,14). The SBAW detector was composed of a quartz crystal oscillator connected in series and a conductivity detection cell. The oscillator was assembled by cou-

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pling a 9-MHz AT-cut quartz crystal to a TTL-IC oscillating circuit. The detection cell was made as follows. Two 1-mm platinum wires (pretreated with 6M HNO_3 and then with water and acetone) were inserted oppositely in the detection cell as conductivity electrodes with a distance of 0.5 mm between them. The body of the detector was made of two plexiglass plates with a thickness of 5 mm within which a cylindrical trough (2-mm diameter and 8-mm length) was used as a detection cell. The detector volume was 15 µL. The cell constant 3.2 cm^{-1} was used. Two 0.5-mm stainless steel tubes were connected oppositely to the detection cell and used as the mobile phase inlet and outlet, separated by 7 mm. One of the platinum electrodes was connected to the quartz crystal, which, together with another platinum wire, served as the feedback network of the IC-TTL oscillator. The frequency signal from the detector was sent either to the digital counter to give direct frequency recording or to the F/V conversion circuit connected to the chromatography workstation to get voltage information. The base noise and hence sensitivity of the SBAW is influenced by temperature. When the SBAW detector temperature is equal to that of the column, the lowest noise and drift levels are obtained. Hence, the temperature of the SBAW detector was kept the same as that of the column: 40°C.

Table I. Effects of Mobile Phase Concentration

Mobile phase concentration (mM)	Peak area (µV × S)	Retention time (min)	Background conductivity (µS/cm)
0.5	1991	26.97	231
0.75	2640	17.89	354
1.0	2858	13.74	483
1.25	2807	10.79	742
2.0	2774	6.70	1040
2.5	2660	5.31	1800
3.0	2607	4.80	2001
3.5	1996	4.11	2115
4.0	1580	3.84	2350
5.0	1065	3.56	2568

Table II. Comparison of the Detection Limits of IC–SBAW and Other Methods

Method	Detection limit	Reference
TLC	50 ng	4
HPLCF	1 ng	4
HPLCF	13 pg	8
HPLCF	20 pg	6
HPLCF	25 ng	11
ICPA	12 ng	12
IC–SBAW	8 ng	

Table III. Recoveries of Histamine Added to Three Fish Extracts

Sample number	Amount added (µg)	Amount found (µg)	Recovery (%)
1	19.3	19.1	99
	67.6	70.3	104
	7.7	7.4	102
2	19.3	19.9	103
	67.6	68.9	99
	7.7	8.1	105
3	19.3	19.9	103
	67.6	71.6	106
	7.7	7.6	98

F/V converter

A universal frequency counter (model SC-7201, Iwatsu, Tokyo, Japan) was used to investigate the performance of the SBAW sensor with respect to frequency shift. The frequency data must be transformed into a format that can be used by the chromatographic workstation, so a frequency-to-voltage converter (made in our laboratory) was used to transform the frequency signal of the SBAW detector to a CR-4A Chromatopac data processor (Shimadzu, Kyoto, Japan), which is used to record chromatograms in real time and integrate peak areas.

Chromatographic apparatus

For chromatographic separation, a Shimadzu LP-6A liquid delivery pump, an SLC-6B system controller, an SIL-6B auto injector, and a CTO-6AS column oven were used. The analytical column was a Shim-pack IC-C1 column (15 cm × 5.0-mm i.d., stainless) filled with a resin of polystyrene–divinylbenzene (10-µm particle size) incorporating a sulfonic acid base as a functional group. A Shim-pack IC-GC1 guard column (10 mm × 4.0-mm i.d.) preceded the analytical column.

Chromatographic conditions

The mobile phase was 2.5mM nitric acid used at a flow rate of 1.5 mL/min, and the injection volume was 10 µL. To obtain the optimum response of the SBAW detector and lowest noise level, the column and detector temperatures were set at 40°C.

Sample processing

The homogenized sample (5 g) was treated with 10% trichloroacetic acid to precipitate protein. After adjusting the sample pH to 8.5 with a 10% NaOH solution, the supernatant was extracted with either *n*-pentanol or *n*-hexanol. The organic phase was back-extracted with a 0.2M HCl solution. The sample solution was diluted with water as necessary and applied to the IC column.

Results and Discussion

Effects of mobile phase concentration

The SBAW response is a linear function of the conductivity change between the sample solution and the mobile phase, provided that all other parameters are kept unchanged, which was always the case in our work (13,14). The detector sensitivity is

mostly affected by the conductivity of the mobile phase (G_0). According to the reference literature (13), the SBAW detector was sensitive over a G_0 range of 150–1200 µS. In order to achieve a sensitive response and fast separation, various dilutions of the HNO_3 solution were evaluated (Table I). From Table I, the 2.5mM HNO_3 solution was used in this work because it resulted in a reasonable retention time and background conductivity.

Method sensitivity

Under the optimum conditions, the responding sensitivity of the SBAW detector to histamine was 18.89 Hz/ppm, and the method detection limit, defined as a signal-to-noise ratio of 3, was 8 ng.

Comparison with other methods

In comparison with other methods, the proposed method had adequate selectivity and avoided the need for tedious fluorescence derivatization and/or a complex eluent system. The SBAW detector is simple to construct and has a long lifespan. Under the conditions described in the Experimental section, the limit of quantitative determination (LQ) for histamine in fish is 1.8 mg/100g, which is below the tolerance limit in fish proposed by the EEC guidelines 91/493 (10 mg/100g). Using IC-SBAW, the detection limit (DL), defined as a signal-to-noise ratio of 3, was 8 ng on the column.

With respect to the DL, a comparison of IC with SBAW detection, IC with pulsed amperometric detection (ICPA), HPLC with fluorometric detection (HPLCF), and thin-layer chromatography (TLC) was made, as can be seen from Table II. The proposed IC–SBAW was more sensitive than ICPA and TLC and less sensitive than HPLCF, except for the HPLCF described in the reference (11). However, although HPLCF was very sensitive, these methods were cumbersome because they required fluorescence derivatization of histamine.

Linearity of response, precision, and recovery

The calibration curve for the determination of histamine was linear over the tested concentration range of 7.725–772.25 µg/mL with a correlation coefficient of 0.9959 (seven replicates). The instrument reproducibility of the IC-SBAW was tested using a standard solution of 38.625 ppm histamine analyzed nine times on different days. The obtained relative standard deviation (RSD) was 1.6%. To investigate the accuracy of this method, the standard solutions were added to practical samples. The experiments indicated that the recoveries were 98–106%. (Table III).

Analysis of practical samples

The proposed IC–SBAW method was used to determine histamine levels in black carp. Figure 1 shows a typical chromatogram. Table IV shows the results of the fish samples analyses. The contents of histamine in black carp increased with stored days.

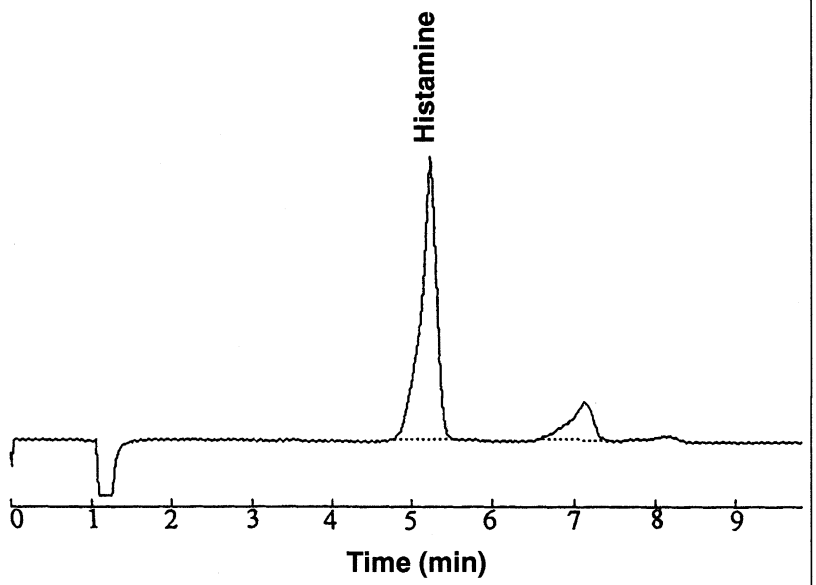

Figure 1. Chromatogram of 2.0 µg/mL histamine in extracts of black carp samples.

Table IV. Determination of Histamine in Black Carp Fish

Number of days stored at 25°C	Results by this method (mg/100g)					Average	RSD (%)
0	0.196	0.185	0.199	0.198	0.183	0.192	3.9
1	160	167	163	157	168	163	2.8
2	262	257	270	254	268	262	2.6
3	320	326	334	318	319	313	4.2
4	592	534	570	603	580	576	4.6
5	741	765	752	791	751	756	3.0

Conclusion

The proposed unsuppressed IC method using an SBAW detector has been successfully applied to the determination of histamine in fish. The method is advantageous in its rapidity, simplicity, sensitivity, and accuracy.

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