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Short communication

Use of capillary electrophoresis with UV detection as a screening method to determine histamine in fish samples

Wenta Liao[a,*], Han Paek[b], Clayton Mabuni[a], Steven Angold[b], Mary Soliman[a]

[a]*California Department of Health Services, Division of Food, Drug and Radiation Safety, Food and Drug Laboratory Branch, 1521 W. Pico Boulevard, Los Angeles, CA 90015, USA*

[b]*US Food and Drug Administration, Pacific Regional Laboratory Southwest, 1521 W. Pico Boulevard, Los Angeles, CA 90015, USA*

Abstract

Histamine levels in fish, extracted with methanol, were determined by capillary electrophoresis (CE) using phosphate buffer pH 2.5 and U.V. detection at 210 nm. Histamine was well separated from the other co-extracted components under the given CE condition without any cleanup of the methanol extract. The average recovery of spiked histamine in various types of fish samples was 96%. Using the same methanol extracts from various fish samples, we then compared histamine concentration obtained by CE and fluorometric methods. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Decomposition in fish, such as tuna and mahi-mahi, is detected by organoleptic evaluation. Decomposition is also indicated by elevated histamine levels in the muscle tissue of these fish. The presence of 50 parts per million or more of histamine is indicative of *unacceptable* decomposition in these fish whether or not decomposition is detected by organoleptic examination. Histamine forms by bacterial action on the amino acid, L-histidine. Histamine is heat stable and survives thermal processing. Odors that normally signal decomposition to the organoleptic analyst may be modified, reduced, or eliminated by thermal processing, therefore histamine is a useful indicator of decomposition in scombroid and certain other fish.

In addition to being an indicator of decomposition, ingestion of sufficiently high levels of histamine causes scombroid poisoning, in some instances leading to death.

Sensitive methods for the determination of histamine in a variety of food products have been developed [1]. Converting histamine to fluorescence derivatives followed by fluorometric measurement [2] or liquid chromatographic analysis [3] has been the most widely used technique, but the procedure requires extensive sample cleanup and is time-consuming.

Recently, capillary electrophoresis (CE) has exhibited powerful capability for the analysis of complex samples [4,5]. Mooper and Sciacchitano [6] were among the first to report CE as a rapid and sensitive method to determine histamine in fish. They also conducted an interlaboratory study on their method and have identified sources of problems associated with reproducibility and accuracy encountered by other laboratories [7].

In this study, we have validated the application of the CE method for the analysis of histamine in fish

*Corresponding author.

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samples. Included is a comparison of the analytical results between the CE method and the fluorometric method.

2. Experimental

2.1. Chemicals

Histamine dihydrochloride was obtained from Sigma (St. Louis, MO, USA). Methanol was from Burdick & Jackson (Muskegon, MI, USA). Potassium dihydrogen phosphate was from Mallinckrodt (Paris, KY, USA).

2.2. Capillary electrophoresis

CE separations were performed using a Bio-Rad BioFocus 2000 CE System (Hercules, CA, USA). An uncoated capillary (24 cm×50 μm I.D.) from Bio-Rad was used throughout. Prior to its use, the capillary was rinsed with 1.0 *M* sodium hydroxide, water, methanol and the buffer. Between analyses, the capillary was purged with run buffer for 45 s. The sample extracts were introduced by pressure injection (8 p.s.i.·s, approx. 45 nl, 1 p.s.i.=6894.76 Pa). The electrode voltage was set at 8 kV and the capillary temperature was maintained at 22°C. Detection was monitored at 210 nm. Separation buffer was 0.1 *M* phosphate (pH 2.5) in 50% methanol.

2.3. Sample preparation

A 10-g amount of fish sample was blended with 100 ml of methanol with a tissuemizer at high speed for 2 min in a graduated cylinder. The cylinder was warmed in a 60°C water bath for 15 min. The sample was then set aside and allowed to cool. After reaching ambient temperature, the supernatant was filtered through a Whatman ashless (8 μm) No. 40 filter paper. A small portion of the extract was then diluted with 0.1 *M* phosphate buffer at pH 2.5 (1:1) and utilized for CE analysis. The remaining extract was subject to histamine determination by the fluorometric method [2].

3. Results and discussion

A typical electropherogram for fish extract is shown in Fig. 1. Histamine appeared at migration

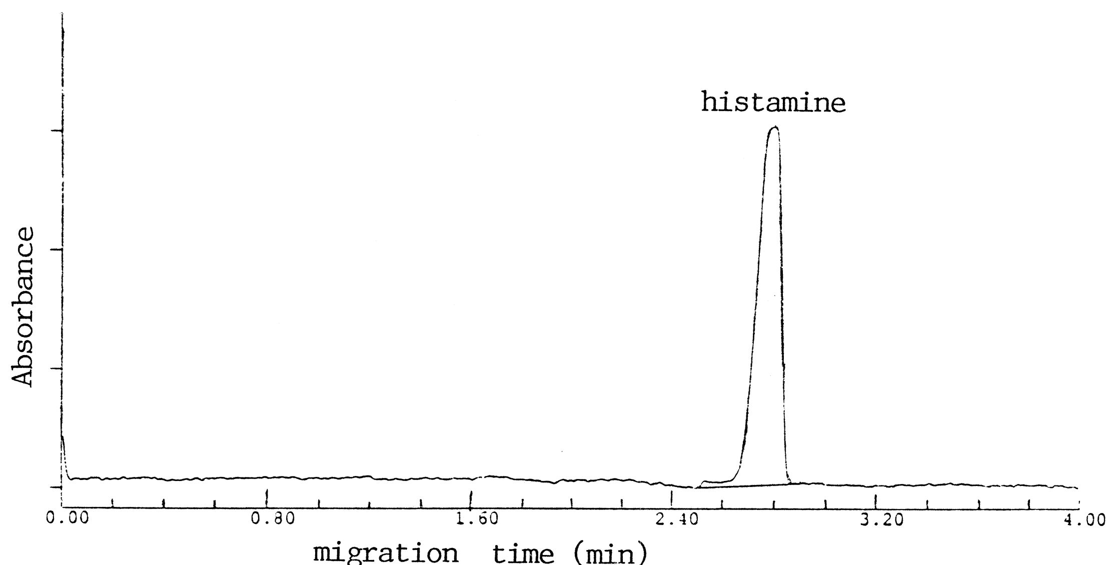

Fig. 1. Electropherogram of tuna fish extract for histamine analysis. Conditions: uncoated capillary, 24 cm×50 μm I.D.; run buffer, 0.1 *M* phosphate (pH 2.5) in 50% methanol; injection 8 p.s.i.·s; voltage, 8 kV (+ to −); detection, UV at 210 nm.

time 2.80 min without any apparent interference. Since the migration time of the histamine peak was very reproducible (±0.05 min), the peak area, instead of the ratio of the peak area to the migration time, was used for quantification by the external standard method. A linear calibration curve was obtained using histamine standards at 2.5, 5.0, 10, 25, and 50 ppm. The minimum correlation coefficient of linearity was 0.999.

Interestingly, without any cleanup of the extract, histamine was well separated from the other co-extracted components under the given CE conditions. A single CE analysis can be completed in 4 min. Most of the other components, which could be observed after the 4 min stop-run time, were purged prior to the next sample analysis. Each sample analysis, including capillary purge, injection, CE separation, and data processing, could be completed

Table 1
Comparative data of the determination of histamine (ppm) in fish samples by CE method and fluorometric method [2]

Sample	Number	Histamine (ppm)		
		CE analysis	Fluorometric analysis	% difference
Frozen tuna	1	100	90	5.3
	37	not detected	not detected	
Canned tuna	16	not detected	not detected	
Frozen mahi-mahi	1	64	58	4.9
	1	590	515	6.8
	1	not detected	5	
	1	detected[a]	15	
	1	not detected	8	
	1	91	82	5.2
	1	102	86	8.5
	1	74	85	6.9
	1	90	84	3.4
	1	80	88	3.4
	1	76	85	5.6
	28	not detected	not detected	
Frozen mackerel	1	92	84	4.5
	18	not detected	not detected	
Canned anchovies	1	25	31	10.7
	1	154	190	10.5
	1	88	100	6.4
	1	98	119	9.7
	1	25	32	12.3
	1	76	100	13.6
	1	138	142	1.4
	1	164	182	5.2
	1	164	190	7.3
	1	132	148	5.7
	1	76	94	10.6
	1	28	35	11.1
	1	68	80	8.1
	1	136	154	6.2
	1	25	27	3.8
	1	detected[a]	23	
	1	detected[a]	27	
	1	not detected	18	
	1	not detected	17	
				Average 6.9

[a] Detected but below quantitation limit.

sequentially in less than 6 min. The estimated detection limit was 25 ppm in the original sample.

The electropherograms generated from extracts obtained from various types of fish were quite reproducible, considering that the extracts were prepared without any cleanup and many of the extracts apparently contained a large amount of co-extracted materials. The CE conditions allowed the separation of histamine from other co-extracted materials which were essentially removed from the capillary by the 45 s flushing step between analyses. Except for oily samples, the relative standard deviation of the migration time and the peak area were less than 2.5% and 6.0%, respectively, within an 8-h working period. We observed no carry-over problems. The capillary did not need replacement even after 200 analyses. The peak profile of histamine, however, depends significantly on the sample matrix. As expected, when the extracts were prepared in a lower ionic strength than the run buffer (0.1 *M* phosphate, pH 2.5, in 50% methanol), the histamine peak shape was sharper and resulted in better resolution and detection. But the sample matrix from different sources could vary, which would cause varying ionic strength in the extracts. This would affect the peak area value and consequently the quantitation result. To minimize the ionic strength variation we added an equal volume of 0.1 *M* phosphate (pH 2.5) buffer to the methanol extract before CE injection. In the case of samples containing excessive amounts of co-extracted material, e.g. oily canned anchovies, the histamine peak was distorted at all concentration levels. This was probably due to overloading of the capillary. Measurement of histamine content in this oily sample type was expected to be qualitative. Cleanup of the extract may improve the peak profile.

In order to validate the capillary electrophoresis method and to demonstrate that it can be used as a routine and reliable means to measure histamine levels in fish samples, we have conducted a comparison study by analyzing over 150 fish samples using both the CE method and the fluorometric method over a two-month period. The fish samples included frozen and canned tuna, frozen mahi-mahi, mackerel and canned anchovies. The quantitative results are summarized in Table 1. Generally, there was good agreement between the CE method and the fluorometric method. For 26 samples containing measurable levels of histamine (>25 ppm), the average difference between the two methods for these samples was 6.9%. Although its detection limit is higher, the CE method has a much larger linear range (25–1000 ppm) than the fluorometric method (10–150 ppm). For 12 samples spiked at 100 ppm, the average recoveries by the CE and fluorometric methods were 95% and 87% with standard deviations of 6% and 5%, respectively (Table 2). Furthermore, there were no false negative or false positive findings reported by either method in this study.

Table 2
Comparative recovery data of the determination of histamine in fish samples (spiked at 100 ppm) by CE method and fluorometric method [2]

Sample	Number	Recovery (%)	
		CE analysis	Fluorometric analysis
Frozen tuna	3	94±4	86±4
Canned tuna	2	97±10	90±5
Frozen mahi-mahi	4	96±8	85±3
Frozen mackerel	2	94±2	91±9
Canned anchovies	1	88	85
	Average	95±6	87±5

The use of capillary electrophoresis for histamine analysis has several advantages: it is simple, rapid, cost-effective and reliable, making it a very useful tool for screening a large number of samples in a short period of time. The results we have presented show that CE can be an effective alternative to the fluorometric procedure for the determination of of histamine at 25 ppm or higher in fish samples.

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