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Research Article

OCCURRENCE OF HISTAMINE IN CANNED TUNA FISH PRODUCED OF TWO MAJOR MANUFACTORIES IN KHUZESTAN PROVINCE BY HPLC METHOD

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Abstract

Introduction: Histamine is a microbial degradation product of the decarboxylation of amino acid histidine. Histamine in seafood can cause histamine scombroid) fish poisoning (HFP). The aim of the present work was to determine of histamine concentration in canned tuna fish produced of Poolak (fadalak manufactory) and Majid (Azin shooushtar food product) at Khuzestan province using HPLC method. **Materials and Methods:** Tuna fish samples of each factory were collected 20 ml of 1 mol/L $HClO_4$ was added to an adequate amount of each homogenized fish sample, vortex and then was placed in an ultrasound bath for 15 min; centrifuged; the supernatant was then drawn off and filtered through whatman filter into a 50 ml round bottomed flask and aliquot of internal standard (1, 1-dimethlybiguanide hydrochloride) added to obtain 5mg/l concentration, was brought to volume with deionized water. Then, it was filtered through syringe filter and 20 µl of filtrate injected into HPLC equipped with column C18 (250 mm × 4.6 mm ID, 5µm) and UV detector at 214 nm. The mobile phase included of 85% (15% methanol +85% phosphate buffer solution at pH 6.9) and 15% acetonitrile at flow rate of 0.7 ml/min LOD, LOQ method were 1mg/kg and 2mg/kg respectively. **Results:** The mean concentrations of histamine in Poolak and Majid tuna fish samples were 9.43795 and 11.01230 mg/kg, respectively. The maximum level of histamine belonged to Majid tuna fish in 85.00 mg/kg. Mean concentration of histamine in Majid and Polak Tuna fish samples were significantly lower than maximum tolerable level of histamine (50 mg/kg) set by the Institute of Standards and Industrial Research of Iran (ISIRI) and FDA.

Keywords: Histamine, Tuna fish, HPLC, 1, 1- dimethlybiguanide hydrochloride

1. Introduction

Histamine (2-(1H-imidazol-4-yl) ethanamine) is a hydrophilic vasoactive amine and organic nitrogenous compound involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter. Histamine is naturally present in many vegetables in amounts without toxicological significance. (1). The presence of histamine is due to the bacterial decarboxylation of histidine, the corresponding amino acid, (Figure 1), which is present in high amounts especially in fish tissues of the Scomberesocidae and Scombridae families, e.g., tuna fish. Histamine in seafood can cause histamine fish poisoning (HFP) or scombroid poisoning syndrome, a type of food poisoning similar to those associated with seafood allergies (2). The consumption of fish containing 500 mg/kg of histamine was reported to cause the toxicological symptoms of HFP(3).The most common ichythyotoxicosis worldwide and results was from the ingestion of histamine-contaminated tuna fish in the Scombroidae family (4). Histidine decarboxylating bacteria such as *Citrobacter, Proteus, Enterobacter, Serratia, Escherichia coli, Clostridium, Vibrio, Acinetobacter, Pseudomonas, and Photobacterium* produced histamine in fish(5). Variety of symptoms including rash, diarrhea, urticaria, nausea, flushing, and tingling and itching of the skin(6) dry mouth, lightheadedness, vomiting, rarely wheezing or loss of consciousness due to hypotension and bitter, or peppery taste.(7). Several quantification methods for determine histamine levels in food, have been developed such as capillary electrophoresis(8), Enzyme-Linked Immunosorbent Assay (ELISA) (9), Enzyme-Based

Screening Assay(10), Polymerase Chain Reaction (PCR) assay (11), Gas Chromatography (GC) Thin-Layer Chromatography (12) and High Performance Liquid Chromatography (HPLC) (13). Amongst mentioned techniques, HPLC is most frequently used because of its high sensitivity and wide range of linearity (4, 14, 15).

The aim of present study was conducted to analysis and determination of the histamine content in Canned Tuna Fish Produced in two major food factories (Fadalak manufactory and Azin shooushtar food product), Poolak and Majid brand names, located in Khuzestan Province, Iran, by HPLC-UV method.

Materials and Methods

2.1. Sample preparation

Five grams of canned tuna fish samples of each brand were weighed out into a glass centrifuge test tube and 20 ml of 1 mol/L $HClO_4$ added for the HPLC method. The mixture was vortex and then was placed in an ultrasound bath for 15 min. After centrifuged at +4 ◦C for 15 min at 4160 RCF×*g*, the supernatant was then drawn off and filtered through whatman filter into a 50 ml round bottomed flask and aliquot of internal standard (1, 1-dimethlybiguanide hydrochloride) added to obtain 5mg/l concentration, was brought to volume with deionized water. Then, it was filtered through syringe filter and 20 µl of filtrate injected into HPLC equipped with column C18 (250 mm × 4.6 mm ID, 5µm) and UV detector at 214 nm. The mobile phase consisted of 85% (15% methanol +85% phosphate buffer solution at pH 6.9) and 15% acetonitrile.

2.2. Chemical reagents

The solvents used for HPLC methanol and acetonitrile were supplied by Merck (Germany). The reference standard histamine dichlorohydrate (99% pure) was supplied by Sigma–Aldrich the chromatographic reference standard 1, 1-dimethylbiguanide hydrochloride was supplied by Sigma–Aldrich. Analytical grade reagents (potassium dihydrogen phosphate, potassium hydrogen phosphate trihydrate, perchloric acid) were supplied by Merck (Germany).1-decanesulfonic acid sodium salt (98% pure) by Sigma–Aldrich.

2.3. Preparation of standard solutions

The histamine stock solution containing 2000 mg/l was prepared by dissolving 10 mg in 5 ml of a 0.1 mol/l solution of HCl. The histamine stock solution was stable for 2 months at +4 ◦C. The inner standard stock solution of 1, 1-dimethylbiguanide hydrochloride was prepared by dissolving 128.2 mg in 100 ml of a 0.1 mol/l solution of HCl. The inner standard stock solution was stable for 6 months at +4 ◦C. The histamine standard solutions at a concentration of 2–5–10–20–50–100 mg/l were obtained by suitably diluting the histamine stock solution in HCl 0.1 mol/l. The 1, 1-dimethylbiguanide hydrochlorides (chromatographic reference standard) was added to all the working solutions so as to obtain a final concentration of about 5 mg/l.

2.4. Extracting solutions

Solution of HClO4 1 mol/l was the extraction solvent used for HPLC method.

2.5. HPLC eluents

The buffer solution used was prepared by weighing out 1.70 g of KH2PO4, 2.85 g of K2HPO4·3H2O and 0.49 g of C10H21O3SNa in 1 l deionized water, obtaining a final pH of 6.9. The buffer solution was prepared at the time of use. Eluent A: 85% of buffer solution and 15% of methanol. Eluent B: acetonitrile.

2.6. HPLC equipment and conditions

The histamine was analyzed by HPLC system (Shimadzu, Japan) The separations were performed under isocratic conditions using a mobile phase composed of 85% eluent A and 15% eluent B. Using a ODS(C18)-H-OL 5-36060 column, 250mm×4.6mm ID, particle diameter 5µm, coupled with a Security Guard (Shimadzu، Japan). The flow rate was 1 ml/min, the injection volume 20µl and the UV detector was positioned at a wavelength of 214 nm (SPD-10AVP, Shimadzu, Japan) at flow rate of 0.7 ml/

3. Results

Calibration Curve of histamine standard and 1, 1-dimethylbiguanide standard while intra-day and inter-days (n=10) (Figure 2, 3), excellent linear correlation coefficient were R^2 = 0.999138 and R^2 = 0.998623 respectively, observed above the range of 2 to 100 ppm (Figure 2, 3). Retention times for histamine and 1, 1-dimethylbiguanide standard were 8.24 and 9.57 min, respectively. The average recoveries and relative standard deviation (RSD %) for intra-day and inter-days of the applied analytical methods for has been shown (Tables 1). All recoveries were more than 89%, indicating the high accuracy of the method. The limit of detection (LOD) and limit quantification (LOQ) for histamine determined were 1 and 2 mg/kg. The mean of histamine concentration in Majid and Polak tuna fish were 11.01230 and 9.43795 mg/kg respectively (Table 2).

4. Discussion

Histamine as one of the most important biogenic amines was identified by the Food and Drug Administration (FDA) as the most important chemical hazard of seafood products. It is the main causative factor of scombroid poisoning and is formed by time/temperature abuse of

Table 1.Descriptive Statistics of Data of Investigated Tuna fish Samples

Tuna fish Product	N	Minimum	Maximum	Mean	Std. Deviation	Std. Error of Mean
Majid	20	.000	85.000	11.01230	19.673463	4.399120
Polak	20	.000	56.000	9.43795	14.247878	3.185922
Total	40	.000	85.000	10.22512	16.973344	2.683721

Table 2. Recoveries for histamine in tuna fish samples

Spiked level (mg/kg)	Measurable levels (mg/kg)	Average recovery (%)
2	1.8 1.9 1.6	88.3
50	47.3 46.8 45.9	93.3
100	102 99 103	100

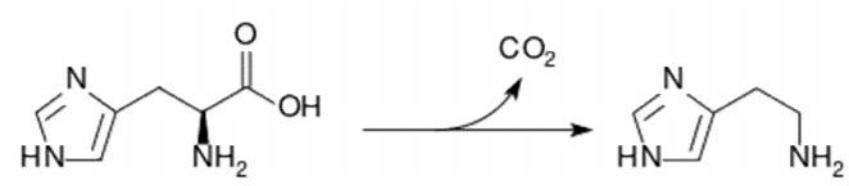

Figure 1.Conversion of histidine to histamine by histidine decarboxylase

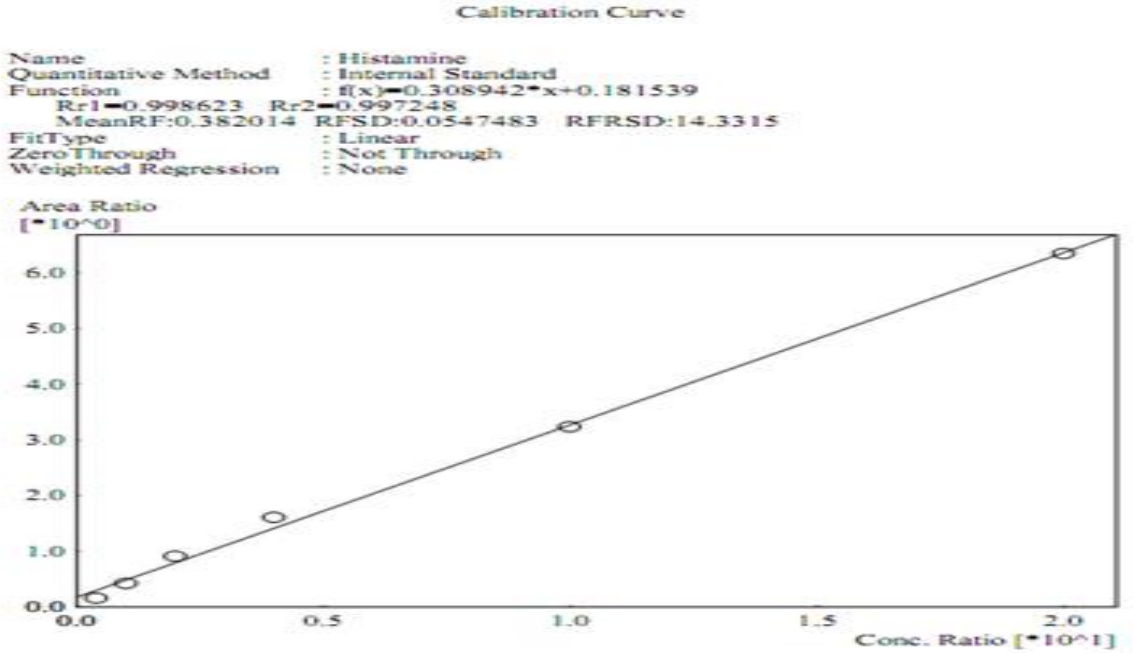

Figure 2.Calibration Curve of histamine standard and 1, 1-dimethylbiguanide standard while intra-day monitored at 214 nm; conditions: ODS (C18)-H-OL 5-36060 column, 250mm×4.6mm ID, particle diameter 5µm, eluents A–B (85:15, v/v) at a flow-rate of 0.7 ml/min, injection loop 20µl

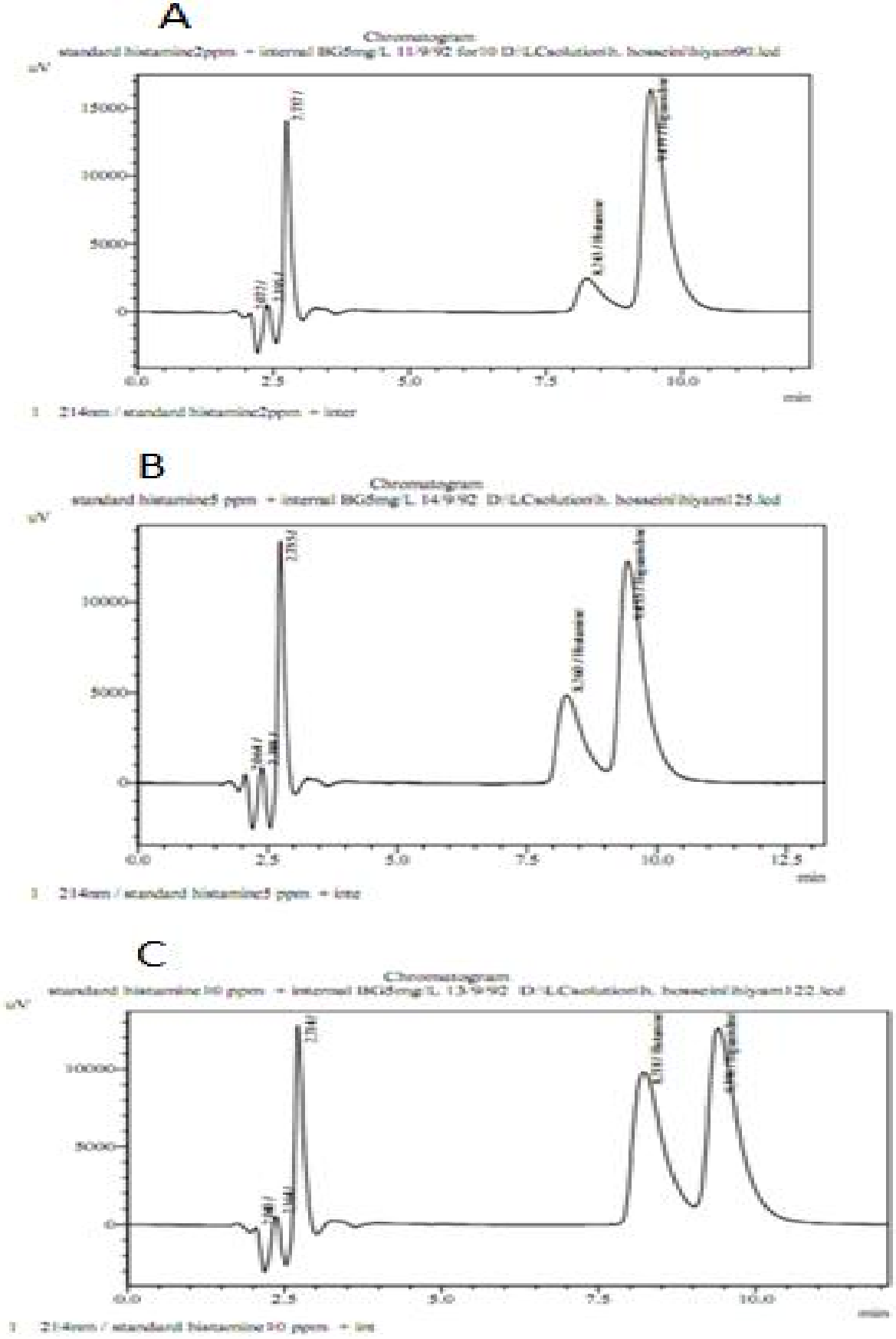

Figure 3a. HPLC chromatograms of Histamine standard A) 2ppm, B) 5ppm, C)10ppm, and 1, 1-dimethylbiguanide standard 5mg/l

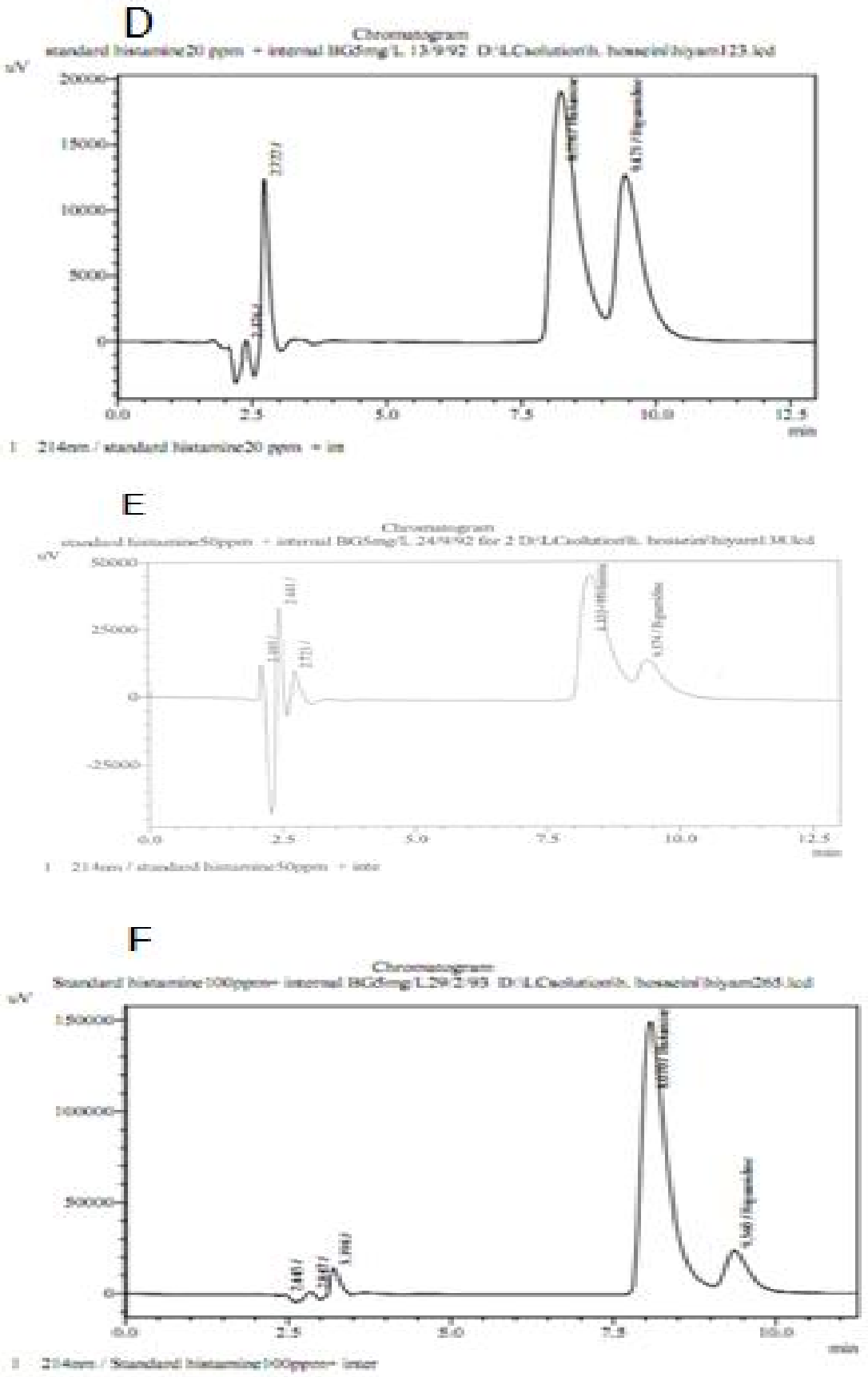

Figure 4b.HPLC chromatograms of Histamine standard D) 20ppm, E)50ppm, F)100ppm and 1, 1-dimethylbiguanide standard 5mg/l

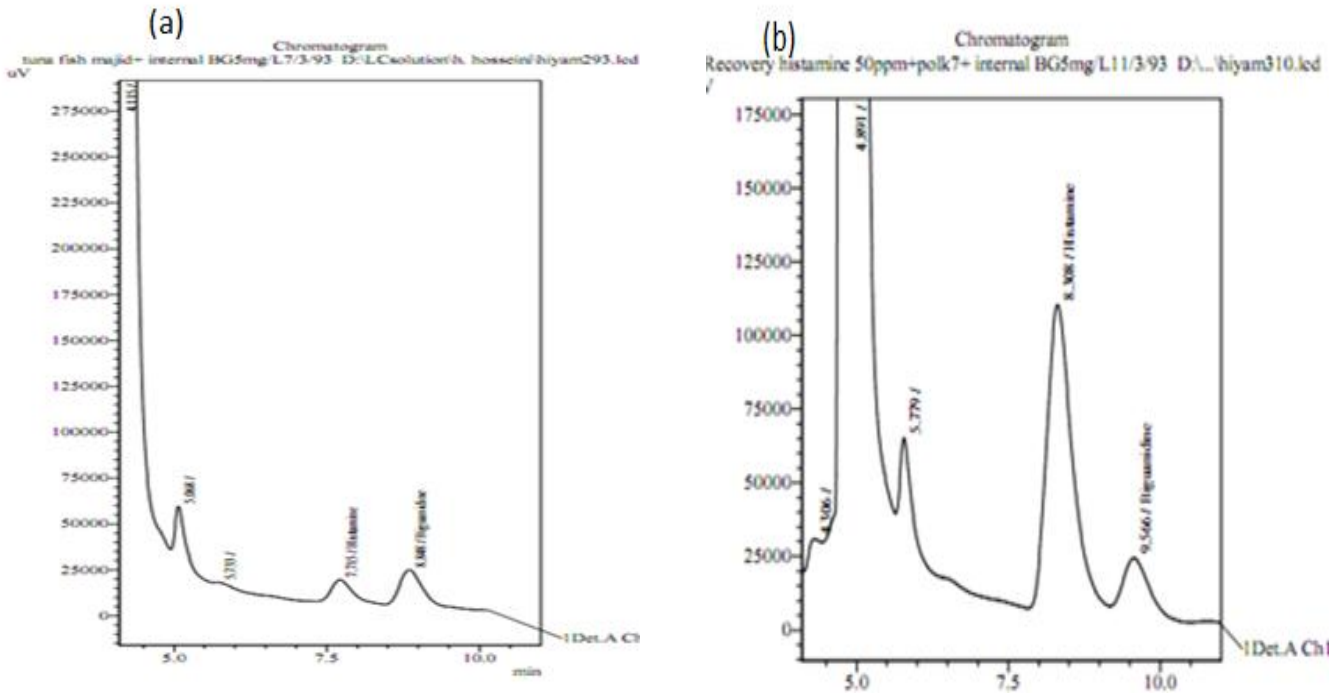

Figure 5.HPLC chromatograms of tuna tissues: (a) blank, (b) recovery

fish muscle(16). Rapid cooling of fish after catching it and maintenance properly until get the consumer to reduce the production of histamine levels. Despite the huge development in trade in the 21st century, great progress has been made in ensuring the quality and safety of fish products. This is largely the result of the introduction of international standards of food hygiene and the application of risk analysis and hazard analysis and critical control point (HACCP)(17).

As a general rule, 500 mg/kg (ppm) causes histamine fish poisoning(16). In Institute of Standards and Industrial Research of Iran (ISIRI) that level of histamine in tuna fish not exceed 100 mg/kg of fish(18). The FDA require and the European Union (EU) that histamine levels not exceed 50mg/kg.

In the current study, the histamine content was measured in canned tuna and then it compared with ISIRI and FDA standards.

Mean concentration of histamine in two brands (Majid and Polak) tuna fish samples were significantly lower than maximum tolerable level of histamine set by the ISIRI and FDA. In the present study, histamine was detected in Majid and Polak tuna fish 75% and 15% respectively. Histamine level in one sample of Majid tuna fish (5%) was above the maximum the allowable limit suggested by FDA. The maximum level of histamine belonged to Majid tuna fish in 85.00 mg/kg.

In August 2003, an outbreak of scombroid fish poisoning occurred in California, USA. Samples of fish contained markedly elevated histamine levels (from 2000 to 3800 mg/kg). This is one of the largest reported outbreaks of scombroid fish poisoning in the United States (19). A large outbreak of scombroid fish poisoning associated with eating Yellowfin tuna (*Thunnus albacares*) due to high histamine concentration 4900 mg/kg occurred in Dakar, Senegal (7).

Histamine was detected in 78.9% of canned fish and 91.6% of dried fish samples and the mean histamine levels were 2.6, 5.8, 3.1 and 104 mg/kg, respectively. A total of 3.7% and 0.79% of the total samples exceeded the FDA and EU regulatory limits for histamine(16). It was in the range of 0.12-648.20 mg/kg. 25 % of the samples contained more than 50 mg histamine/kg fish, the allowable limit suggested by US FDA(20) One sample has histamine content (52.3 mg/100 g) greater than the hazard action level of 50 mg/kg(21). In view of the importance of tuna fish several studies have been done and there are many reports and documents that has been contaminated with histamine(16) only one can of tuna and one can of anchovy had histamine (18.7 and 7.5 mg/100 g, respectively) greater than the US FDA allowable limit of 5 mg/100 g(22). The finding that high contents of histamine (>40 mg/100 g) were detected in the suspected fish samples (3) Histamine detected in Spanish canned fish products as follows: 1.35 mg/kg in herring, 30.9 mg/kg in tuna, 16.3 mg/kg in sardine, and 28.3 mg/kg in mackerel. Similar results were reported by(23) The quantitation limit was 5.00 mg/kg for histamine in canned fish by HPLC.

A comparison of the current results with the literature on method validation revealed that the recoveries in the current study were considerably higher than the range of those investigations. For example, in a study conducted, an average recovery of 92% was determined for the recovery of added histamine at 5, 10, and 20 mg/100 g levels(6), which is below the results obtained in this research.

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