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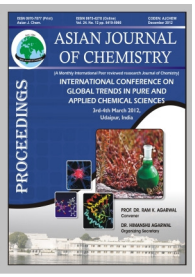

Liquid Chromatographic Method for Determination of Biogenic Amines in Imported Fish and Meat Products†

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Efficient liquid chromatographic method by employing *o*-phthaldehyde derivative for separation of biogenic amines *e.g.*, (**1**) histamine, (**2**) tyramine, (**3**) tryptamine, (**4**) putrescine, (**5**) 2-phenylethylamine, (**6**) cadaverine, (**7**) 1,7-diaminoheptane in aqueous extract from imported fish and meat products available in Iraq markets. The fish and meat samples were extracted by using 8 % trichloroacetic acid, the aqueous solution were purified by passing through disposable filter paper 0.45 µm pore diameter. The aqueous solutions were pre-concentration using rotary evaporator reaching adjusted volume of 2 mL. 20 µL was injected on HPLC ISC07/5/504Na (100 × 4.6 mm Id) column, mobile phase 0.6 N sodium citrate + 0.1 M boric acid, (pH 10) the eluted peak were detected by using pre-column *o*-phthaldehyde derivative. The concentrations of biogenic amines were determined by comparison the peak area of the sample with that of authentic standard. In this work, HPLC method was applied for measurements of most biogenic primary amine by using pre-column *o*-dialdehyde (OPA) derivative, to form high sensitive isoindole derivative of all amines. These derivatives gave low detection limit of 2 ng/mL. The results observed relatively high concentration of biogenic amine in imported fish and meats products due to manufacturer source and unsuitable storage method.

Key Words: Determination, Biogranic amines, Fish, Meat.

INTRODUCTION

The challenge in production of public food with high quality and more save, promote the search for evolution the trace compounds of biogenic amine that can affect human health, then put restrictions to not exceed the European limit (< 10 ppm). Biogenic amines (mono and di-amine) represent group of low molecular weight organic substances that possess biological activity. Biogenic amines formed and degraded as a result of normal metabolic activity in animals, plants and microorganisms[1,2].

Biogenic amines were generated by decarboxylation of free amino acids or by amination and transamination of aldehydes and ketones. Removal of the α-carboxyl group from a precursor amino acid leads to the corresponding biogenic amine. The names of many biogenic amines correspond to the names of their originating amino acids. For example, histidine is decarboxylated to produce histamine, tryptophane to tryptamine, tyrosine to tyramine, lysine to cadaverine and putrescine can be produced from glutamine, arginine and agmatine[3].

The formation of biogenic amines in variety of foods such as fish and meat products by the microbial decarboxylation of amino acids, can result allergic reactions, difficulty in breathing, itching, rash, vomiting, fever and hypertension.

Many methods have been developed for the determination of biogenic amines. Several methods are developed for specific analysis of a single amine, while high-performance liquid chromatography is the most widely used technique[4] using *o*-phthaldehyde derivatives. It comprises a condensation of *o*-phthaldehyde with an aliphatic thiol and the amine under formation of an isoindole derivative. It is highly sensitive to ultra violet-visible light. This method has found widespread application, which had established reference method in many countries for the determination of biogenic amines in protein-rich food samples[5].

Excessive oral intake of biogenic amine especially histamine and tyramine, can results in nausea, respiratory distress and several toxicological problems[6-8]. Most of the intoxications produced by biogenic amines generated by histamine, due to their effect in a dilation of blood vessels, capillaries and arteries, causing headaches, hypertension, edemas and gastrointestinal

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distress[9,10]. While tyramine increases the secretion of noradrenaline in blood as an indirect effect, acting like a vasoconstrictor, hypertension and migraine. The presence of monoamine and diamine oxidase inhibitors or the presence of diamines (putrescine or cadaverine) can potentiate their toxicity[11,12]. In addition they have potential precursors of carcinogenic nitrosamines[13,14]. For this reason it is particularly important to prevent the accumulation of these amines in food products. Biogenic amines could be used as excellent indicator for food quality and hygiene during food processing[15-17].

EXPERIMENTAL

The standard biogenic amines (histamine, tyramine, tryptamine, putrescine, 2-phenylethylamine, cadaverine, 1,7-diaminoheptane) were obtained from sigma Ltd. all used solvents were HPLC grade degassed by stream of helium before used as a mobile phase.

The sample of several types of fish and meat were homogenized in Moulinex blender, an aliquot (20 g ± 0.1 mg) was then put into 85 mL test tube, 0.5 mL of internal standard (1,7 diaminoheptane concentrations 1 mg/mL) was added and the sample was extracted for 2 min with 15 mL of 8 % Trichloroacetic acid (TCA), then added distilled water up to 100 mL. And blend for 5 min with homogenizer and used the centrifuge to obtain the supernatant after that we used filter (using Shimadzu disposable filter paper 0.45 µm pore diameter for HPLC) to obtain Leachate.

***o*-Phthaldialdehyde derivatization:** Derivatization reagents (OPA) were prepared by Mopper method[17] by dissolving 27mg *o*-phthaldehyde in 0.5 mL 99 % ethanol then, 20 µL of 98 % 2-mercapto ethanol were added and making up to 5 mL with 0.4 M borate buffer (pH 9.5, adjusted by 1 M NaoH) 0.5 µL of extract was mixed with 2.5 µL of *o*-phthaldehyde and after reaction time 2 min, 20 µL from the mixture was injected onto chromatographic column according to optimal separation conditions.

The separation of biogenic amines were occurred on high performance liquid chromatograph (HPLC) Shimadzu (Kyoto, Japan) model LC-20A equipped with binary delivery pumps model LC-20A, the eluted peak were monitored using Shimadzu UV-VIS 20A detector. Pre-column *o*-phthaldehyde derivative of biogenic amine were isocratically separated according the following optimum conditions, column: ISC07/5/504Na (100Í 4.6 mm Id), mobile phase: 0.6 N sodium citrate + 0.1 M boric acid, pH: 10, flow rate: 0.6 mL/min, column temp.: 65 ºC, detection: *o*-phthaldehyde.

RESULTS AND DISCUSSION

In this work the biogenic amines were determined by HPLC using pre-column derivatization method, due to ability of UV to absorb only hetrocyclic and aromatic amine properly, the optimum condition were applied to get complete separation for seven biogenic amine at low detection limit of less than 2 ng/mL as shown in typical chromatogram (Fig. 1). The same optimum condition were applied to separation of the sample extract from several samples of meat and fish. The peaks of the biogenic amines were satisfactorily resolved and no interfering peaks appeared, the calibration curves between the response of the injected standard (peak areas) and the

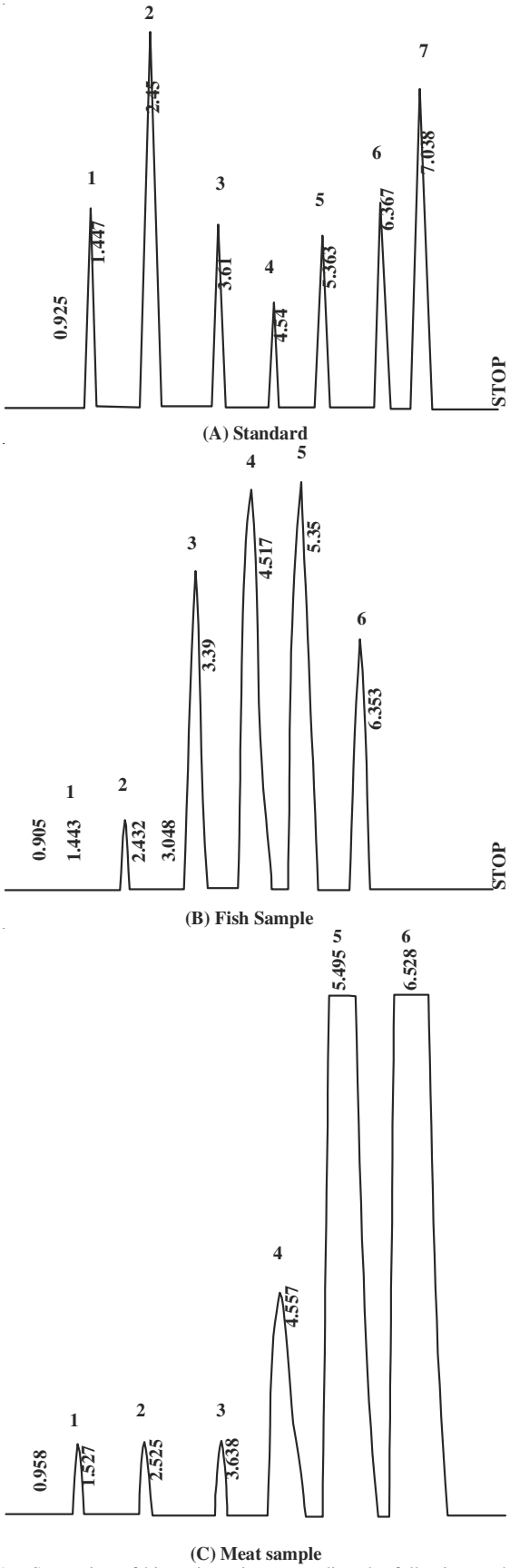

Fig. 1. Separation of biogenic amines according the following analytical separation conditions. Column: ISC07/5/504Na (100Í4.6 mm Id), mobile phase: 0.6N Sodium citrate + 0.1M Boric acid, pH: 10.0, Flow rate: 0.6 mL/min, Column Temp.: 65 ºC, Detection: *o*-phthaldehyde. 1) histamine, 2) tyramine, 3) tryptamine, 4) putrescine, 5) 2-phenylethylamine, 6) cadaverine, 7) 1,7-diaminoheptane

TABLE-1
CONCENTRATION OF BIOGENIC AMINE mg/kg (MEAN ± STANDARD ERROR OF THE MEAN; n=3) IN VARIOUS SAMPLE OF MEATS

	Meat samples						
No	HIS	TYR	TRP	PUT	PEA	CAD	1,7-Diamino heptane
M1	15.7 ± 1.20	30.20 ± 0.9	1.2 ± 0.16	61.5 ± 5.21	1.2 ± 0.5	3.4 ± 0.2	-
M2	12.4 ± 12.4	51.6 ± 0.4	1.5 ± 0.14	74.2 ± 6.25	-	3.1 ± 0.2	2.0 ± 0.17
M3	18.6 ± 2.10	102.3 ± 5.2	2.6 ± 0.21	114.2 ± 8.9	-	27.6 ± 2.3	4.6 ± 1.2
M4	17.2 ± 0.76	112.6 ± 7.92	-	145.9 ± 10.2	-	20.6 ± 1.6	3.7 ± 1.7

TABLE-2
CONCENTRATION OF BIOGENIC AMINE mg/kg (MEAN ± STANDARD ERROR OF THE MEAN; n = 3) IN VARIOUS SAMPLE OF FISH PRODUCT

	Fish Samples						
No	HIS	TYR	TRP	PUT	PEA	CAD	1,7Diaminoheptane
F1	20 ± 1	28.2±3.2	2.1±0.36	44.2±10.2	1.0±0.1	4.0±1.4	3.3±0.22
F2	25 ± 1.7	15.6±3.6	1.8±0.24	16.7±2.1	1.0±0.2	2.7±0.7	3.8±0.61
F3	18.6±3.2	22.9±4.2	1.9±0.24	18.5±2.50	1.9±0.3	55±6.2	4.2±0.27
F4	30.5±2.3	13.9±1.7	1.3±0.31	85±12.65	2.21±0.2	70±4.2	5.6±0.76
F5	54.6±4.2	127.3±60	1.4±0.24	115.7±2.1	2.60±0.6	75±6.2	8.6±0.21
F6	18.4±3.5	120.6±1.6	1.6±0.71	118.5±60	3.60±1.1	80.2±4.2	9.1±0.60

corresponding amines concentrations were linear in the range of 2-150 ng/mL in the injected volume 20 µL.

Biogenic amines can accumulate as the result of uncontrolled microbial enzymatic activity[16-19]. Foods likely to contain high levels of biogenic amines especially fish and fish products, meat and meat products. The results in Tables 1 and 2 indicate that, the most common biogenic amines in meat and fish are histamine, tyramine, putrescine and cadaverine. The synthesis and accumulation of biogenic amines in fish and meat require the availability of the substrate amino acids, bacteria with the appropriate aminoacyl decarboxylase activity and the environmental conditions facilitate the enzyme action and bacterial growth. these factors influence the varieties and quantities of biogenic amines present in fish and meat products contain high amounts of histamine in the range between (12-54 mg/kg), tyramine range between (12-127 mg/kg and putrescine between (16-145 mg/kg), fishes are most frequently associated with cases of histamine poisoning[20,21].

Several previous studies indicates that the toxicological effects for some biogenic amines-even in small amounts-following their oral administration[22,23]. The intake of these toxic compounds can induce several digestive, circulatory and respiratory symptoms, The most conspicuous symptoms of consumption of high doses of biogenic amines are vomiting, respiratory difficulties, perspiration, palpitation, hypo- or hypertension and migraine[24,25].

The oxidation way was the main route of biogenic amines detoxification following ingestion while methylation and acetylation have been also implicated in the detoxification of histamine[26].

The results given in Tables 1 and 2 show high elevated in tyramine, histidine, putrescine and cadaverine, tyramine is the most toxic, the toxicological level being 100-800 mg/kg[27]. therefore the meat sample M3 and M4 contain tyramine 102 and 112 mg/kg and putrescine 113 and 145 mg/kg respectively. While the fish sample especially F5 and F6 samples have elevated level of tyramine between 120-127 mg/kg and putrescine between 115-118 mg/kg in the present study toxic level of tyramine and putrescine were found in this products.

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