# SIMULTANEOUS ANALYSIS OF TAURINE AND HISTAMINE IN FISHES SAMPLES

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**Abstract—** This study investigated the taurine and histamine content of some fishes that sold in Thailand. Quantitative analysis of taurine and histamine were studied by high-performance liquid chromatography using fluorescence detection (HPLC-FLD) and derivatization with pre-column *o*-phthalaldehyde (OPA). The method of the analysis was validated. The correlation coefficients ($R^2$) of taurine and histamine were 0.9975 and 0.9980 respectively. Limits of detection were 0.001 mg $L^{-1}$ for taurine and 0.03 mg $L^{-1}$ for histamine. Limits of quantitation were 0.005 mg $L^{-1}$ for taurine and 0.05 mg $L^{-1}$ for histamine. The method was showed high sensitivity, selective and rapid for the determination of taurine and histamine. The method was applied to quantity of taurine and histamine in fish samples. Taurine in Thai fishes were found in the range of 490.81-2,180.88 mg $kg^{-1}$, and the highest level of taurine were found in Barramundi Giant Seaperch. Histamine in Thai fishes were found in the range of 7.41-19.07 mg $kg^{-1}$, and the highest level of histamine were found in Red Mullet. This work proved that both taurine and histamine could be separated and detected in the same time, so this modified method also used to analyse taurine and histamine in fishes samples.

**Keywords—** Taurine, Histamine, high-performance liquid chromatography, seafish, freshwater fish.

## I. INTRODUCTION

Fish is consumed worldwide, it contains several health beneficial components such as omega-3, fatty acid, protein, vitamin D and including the amino acid taurine.

Taurine (2-aminoethanesulfonic acid) is a free amino acid, sulfur-containing β-amino acid and non-protein in mammals tissue (Shifen M., 2012). It is a major constituent of bile. In humans, taurine are synthesized from metabolism of methionine and cysteine in liver (Wójcik O. P., 2010). Taurine plays an important roles in many biological systems such as conjugation of bile acids, antioxidation, osmoregulation, membrane stabilization and modulation of calcium signaling. It is essential for cardiovascular function, and development of skeletal muscle, the retina and the central nervous system (Kothandam H., 2012). Taurine occurs naturally in food, especially in seafood, fish, and chicken. Other sources of taurine including eggs, cows milk, yogurt and ice cream (Stpleton P. P., 1997). The mean daily taurine intake for adult human non-vegetarians has been estimated between 40 and 400 mg (Wójcik O. P., 2010). Although, consuming fish have benefits from taurine but also have detrimental compound such as histamine.

Histamine is organic nitrogenous compound known as biogenic amines. The formation of histamine in food related to the decarboxylation of histidine by microorganism (Valeria F., 1998).

Histamine has been implicated as the causative agent in syndrome of allergy-like food poisoning known as scombroid poisoning, which can lead to death in very sensitive subjects (Shalaby A. R., 1996; Tahmouzi S., 2011). Histamine occurs naturally in high amounts especially in fish tissues of the Scomberiscida and Scombridae families, e.g., tuna fish, mackerel, sardine, anchovy include fermented foods (beer, wine, cheese) (Valeria F., 1998). To protect public health, the US Food and Drug Administration (FAD) has established a guideline level of 50 mg $kg^{-1}$ of histamine in fish (FDA, 1995). The European Union has established an acceptable level of 100 mg $kg^{-1}$ of histamine in fish (Commission Regulation(EC), 2005). Thus, histamine use as indicator of fish quality, and is the biomarkers for quality control during the food production and transportation.

An important of taurine and histamine content in fish should be determined for benefits of consumers. The aim of this work was to simultaneous determination of taurine and histamine content in Thai fishes by high performance chromatography and to comparison quantitative of taurine and histamine between seafish and freshwater fish.

## II. DETAILS EXPERIMENTAL

### *2.1 Chemicals reagents*

Taurine and histamine standard (AR. Grade) were purchased from Sigma-Aldrich and Fluka. Acetonitrile of HPLC grade was purchased from Merck. Analytical-grade reagents consisting of potassium dihydrogen phosphate, trichloroacetic acid (TCA), sodium carbonate, 2-mercaptoethanol were purchased from Merck and *o*-phthalaldehyde (OPA) from Sigma.

### *2.2 Preparation of standard solution*

A stock standard solution of taurine and histamine (100 mg $L^{-1}$) were prepared in deionized water and stored at 4 $^{o}$C. The working standard solutions were prepared in the range of 0.1 – 10 mg $L^{-1}$ for construct the standard calibration curve by diluting from this

stock solution. All solutions were filtered through a 0.45 micron nylon syringe and collected in a 1 mL microcentrifuge tubes before analysis.

### *2.3 Fish samples preparation for taurine and histamine extraction*

Seafishes such as skipjack tuna (*Katsuwonus pelamis*), Short-bodied Mackerel (*Rastrelliger brachymosa*), Whisker Sheatfish (*Kryptopterus bleekeri*)**,** Indo-Pacific Spanish Mackerel (*Scomberomorus guttatus*) and Red Mullet (*Parupeneus cinnabarins*). Freshwater fishes such as Nile Tilapia (*Alutera monceros*), Striped Snake-Head *(Channa striata*), catfish (*Clarias spp.*), Iridescent shark (*Pangasianodon hypophthalmus*) and Barramundi Giant Seaperch (*Lates calcarifer*). All fishes were purchased from the Huai Khwang Fresh Market in Bangkok. One grams of fish sample was homogenised with 10 mL of 5% TCA and extracted in ultrasonic bath for 10 minutes, and then centrifuged at 4500 rpm/min for 20 minutes. The supernatant was filtered through a Whatman No. 1 filter paper and adjusted pH to 7 with 5% sodium carbonate solution. Then, the filtrate was filtered through a 0.45 micron nylon syringe and collected in a 1 mL microcentrifuge tubes for analysis by HPLC**.**

### *2.4 OPA derivatization for fluorescence detection*

The pre-column derivatization was performed by OPA. A 0.0500 g OPA was dissolved in 2 mL of methanol and 40 µL of 2-mercaptoethanol. The mixture was diluted to 10 mL with 0.04 M borate buffer (pH 9.5) after kept in the dark, at $4^0$C. (modified from Singthong W., 2013). The OPA derivative was added to the extract and vortexed for 10 s before injected for HPLC analysis.

### *2.5 Quantitative Analysis of taurine and histamine by HPLC*

*2.5.1 Study on the optimization condition for HPLC*

The analysis was performed using a HPLC unit (HP model 1100, Agilent) equipped with a fluorescence detector (HP model 1100, Agilent). The fluorescence detector was measured the excitation wavelength of 333 nm and emission wavelength of 451 nm. The separation of taurine and histamine compound was performed using a reversed-phase fortis analytical column (250 × 4.6 mm I.D., 5 µm). The isocratic mobile phase was 0.02 M phosphate buffer pH 4.8 (A) and acetonitrile (B) (65:35, v/v), at a flow rate of 0.8 mL $min^{-1}$.

*2.5.2 Analysis of taurine and histamine in fish samples*

The extract of fish samples were added with 20 µL of OPA derivatives and vortexed for 10 s before injected to HPLC column by using condition from optimized HPLC in part 2.5.1.

## III. RESULTS AND DISCUSSION

### *3.1 Analytical performance of Reverse phase high performance liquid chromatography*

Optimum chromatographic for quantitative analysis of taurine and histamine were obtained. The best condition of mobile phase system was used 0.02 M phosphate buffer pH 4.8: acetonitrile as 65:35 and controlled flow rate at 0.8 mL $min^{-1}$. The chromatogram of taurine and histamine standard solutions were separated within 10 mins as shown in Fig.1

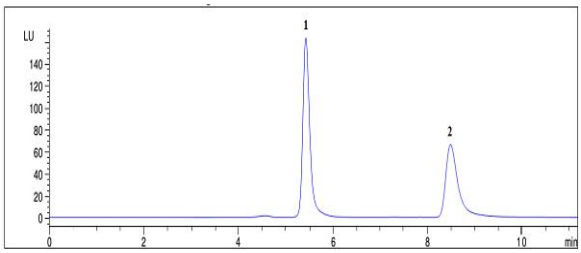


**Fig. 1. shown HPLC chromatogram taurine and histamine standard as OPA derivative.(Peak 1) Taurine (Retention time = 5.415 min), Peak 2) Histamine (Retention time = 8.483 min)**

The linearity range of taurine and histamine calibration curve were in the range of 0.1 to 10 $mgL^{-1}$ as shown in fig. 2.

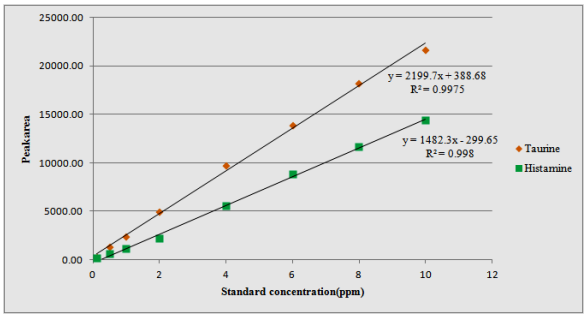


**Fig. 2 Standard calibration curve of taurine and histamine**

The modified optimized method showed high efficiency to analyse taurine and histamine with a good correlation coefficients ($R^2$) = 0.9975 and 0.998 respectively.

Under the optimized HPLC condition, the analytical performance including the limit of detection (LOD) and limit of quantitation (LOQ)were try out. The results showed that LOD for analysis of taurine and histamine were 0.001 mg $L^{-1}$ and 0.03 mg $L^{-1}$,respectively. The LOQ for analysis of taurine and histamine were 0.005 mg $L^{-1}$ and 0.05 mg $L^{-1}$, respectively

### *3.2 Analysis of taurine and histamine in fishes sample.*

In the analysis of taurine and histamine content in Thai-fish samples showed in table 1 and examples of chromatographic peak of both parameters showed in fig 3.

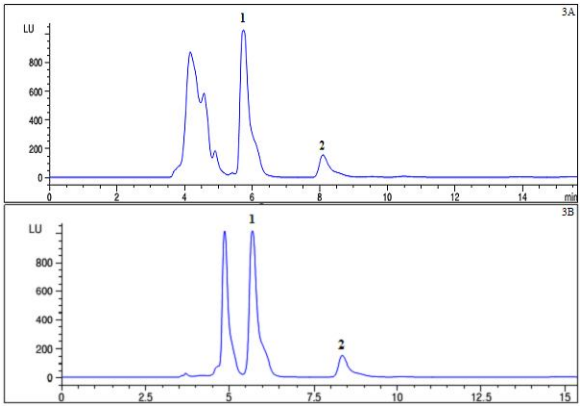


**Fig. 3. HPLC chromatogram of 3A) Longtail-tuna fish sample and 3B) Nile tilapia fish sample.**

The results from table1 showed that taurine was detected in seafishes sample in the range of 490.81-1,721.92 mg $kg^{-1}$ and skipjack tuna contained the highest level of taurine. In the freshwater fishes sample contained taurine in the range of 520.66-2,180.88 mg $kg^{-1}$ and Barramundi Giant Seaperch contained the highest level of taurine. Taurine content in freshwater fish samples trended to showed higher concentration than founded in seafish samples. For quantification of histamine in seafishes sample contained histamine in the range of 7.41-19.07 mg $kg^{-1}$ and the highest level of histamine were found in Red Mullet. In the freshwater fishes sample contained histamine in the range of 7.88-10.76 mg $kg^{-1}$ and the highest level of histamine were found in catfish. Consequently, non-scombroid fish (freshwater fish) have also been implicated in histamine, because species of fish have contained naturally high levels of histidine. Histamine was detected in Thai-fish samples is less than the maximum level established by Euopean Union( 100 mg $kg^{-1}$) and FDA ( 50 mg $kg^{-1}$ ).

**Table 1 Taurine and histamine content in fresh fishes (mg $kg^{-1}$).**

| fish samples | | Mean ± SD | |
|---|---|---|---|
| | | Taurine | Histamine |
| Seafishes | skipjack tuna | 1721.92±8.0 | 11.30±4.8 |
| | Short-bodied Mackerel | 907.92±2.5 | 7.41±5.7 |
| | Whisker Sheatfish | 593.40±11.1 | 11.37±3.1 |
| | Indo-Pacific Spanish Mackerel | 490.81±3.7 | 9.38±0.6 |
| | Red Mullet | 1628.76±6.4 | 19.07±1.4 |
| freshwater fishes | | | |
| | Barramundi Giant Seaperch | 2180.88±13.3 | 8.85±4.7 |
| | Nile Tilapia | 1380.97±14.0 | 10.14±7.6 |
| | Striped Snake-Head | 1295.79±15.4 | 9.15±5.4 |
| | catfish | 520.66±10.3 | 10.76±0.2 |
| | Iridescent shark | 1137.73±17.3 | 7.88±0.1 |

## CONCLUSIONS

This work presented the method for quantitative analysis of taurine and histamine with OPA derivative. This method was high sensitivity, selectivity and rapid analysis. The method was applied to quantify taurine and histamine content in fresh fishes. The levels of taurine and histamine in Thai fishes were found 490.81-2,180.88 and 7.41-19.07 mg kg-1, respectively. From result proved taurine and histamine was found both in freshwater fishes and seafishes, and histamine levels in freshwater fish were lower than seafish.

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