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Study of Histamine Detection using Liquid Chromatography and Gas Chromatography

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Histamine is a heterocyclic amine shaped by decarboxylation of the histidine. It is a compound that lack chromophore and involatile. However, the detection of histamine is imperative due to the characteristic of histamine has given several disadvantages in food industry. This paper describes methods for histamine detection by employing high performance liquid chromatography and gas chromatography. The derivatization techniques required for both methods in order to increase the sensitivity of chromatography analysis. Two derivatizing agents were applied in this study such as 9-flourenilmethyl chloroformate (FMOC – Cl) for HPLC analysis whereas for GC analysis a *N,O*-bis (trimethylsilyl)acetamide (BSA) was used. Method validation was in accordance to Commission Decision 657/2002/CE. The validation of specificity, linearity, precision, accuracy, detection limit and quantitation limit results indicate that the methods were acceptable. The linear range for both methods were at 0.16 – 5.00 $\mu g \cdot mL^{-1}$. The determination of histamine using GC showed the superiority of this instrument compared to HPLC. Method applicability was also checked on real sample namely mackerel in order to acquire a satisfactory recovery for both methods.

Keywords: histamine; HPLC; GC; fluorescence; FID; derivatizing reagent

I. INTRODUCTION

Histamine is a compound that containing nitrogenous and having low molecular weight. It is also considered as one of the biogenic amines which are very toxic and easily found in food protein according to the European Food Safety Authority (EFSA) (Parchami *et. al.*, 2017; Liu *et al.*, 2020). The presence of histamine in foods formed through histidine decarboxylation with the presence of a specific bacteria or shaped by amination and transamination of ketones and aldehydes (Gama & Rocha, 2020).

Histamine detection is very needed due to several reasons such as to modify the current methods so the best method acquired and to analyse histamine content of foods protein from different countries and dealing with it (Bogdanovic *et al.*, 2020). Food and Drug Administration (FDA) has reported that histamine can be safely consumed below 50 ppm, it has also related to Scombroid poisoning owing to fish consumption that related to *Scombroidae* family such as sardine, mackerel, tuna and mahi – mahi (Qiao *et al.*, 2020).

Several approaches such as chromatography and electrochemical techniques have been applied and studied to detect histamine in foods. Liquid and gas chromatography are the techniques that have been applied in decades. These methods are well developed, sensitive and very selective (Plakidi *et. al.*, 2020; Zhang *et. al.*, 2020; Bogdanovic *et. al.*, 2020; Kamankesh *et. al.*, 2019; Wojnowski *et. al.*, 2019; Jia *et al.*, 2020). However, histamine has a significant shortage when analysed using chromatography approaches such as involatile and the absence of a specific chromophore. These conditions setting off histamine need to be derivatized before analysed using liquid or gas chromatography. Furthermore, the derivatizing agents applied will also influence the application of chromatography (Munir & Badri, 2020), 9 – fluorenylmethyl chloroformate chloride (FMOC–Cl), 6–aminoquinolyl-*N*-hydroxysuccinimidy (AQC), O – phthaldialdehyde (OPA), dansyl-, dabsyl-, and benzoyl chloride are derivatizing reagents generally used to derivatize histamine before analyse using HPLC (Angulo *et. al.*, 2020; Bogdanovic *et. al.*, 2020; Lkhagva *et. al.*, 2020; Plakidi *et al.*,

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2020), whereas for GC analysis difference reagents used to derivatize histamine such as silylation, alkylation and acylation groups (Munir & Badri, 2020; Wojnowski *et. al.*, 2019; Papageorgiou *et al.*, 2018) as shown in Figure 1.

In this study, histamine was derivatized using FMOC–Cl before analyzed using HPLC that equipped with fluorescence detector whereas for GC analysis, histamine was derivatized using one of the silylation compounds namely *N,O* – bis (trimethylsilyl) acetamide (BSA) that equipped with flame ionization detection (FID) and verify the structure of histamine – derivatizing agent using mass spectrometry (MS). The recovery study of both methods were also studied by using fish mackerel.

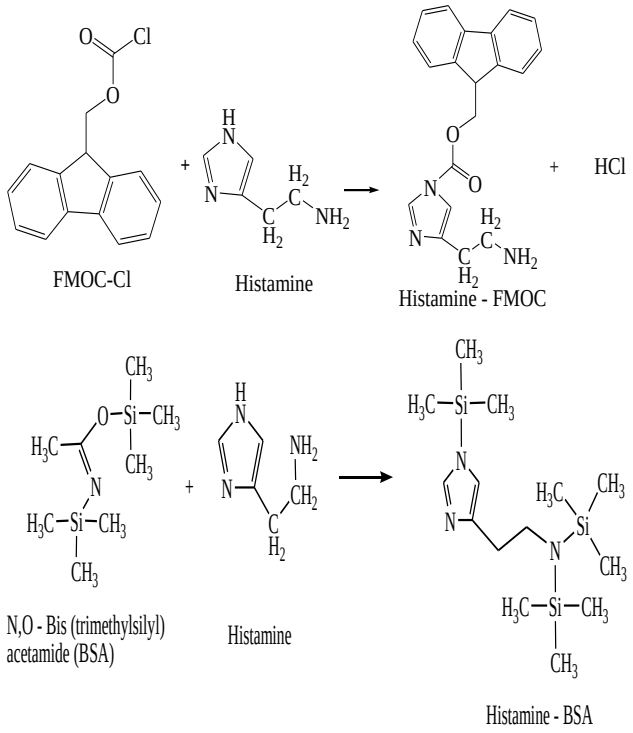

Figure 1. Reaction of FMOC – Cl and *N,O*–bis (trimethylsilyl)acetamide (BSA) as derivatizing reagents with histamine

II. MATERIALS AND METHOD

A. Standard, Chemicals and Reagents

Histamine dihydrochloride (HIS), 9-flourenilmethyl chloroformate (FMOC-Cl), N, O – bis (trimethylsilyl) acetamide (BSA), trichloroacetic acid (TCA), dichloromethane (DCM), HPLC grade acetonitrile and HPLC grade methanol, hydrochloric acid (HCl), potassium borate, glycine, sodium hydroxide, sodium hydrogen carbonate, acetone and glutamic acid were purchased from Sigma-Aldrich Sdn. Bhd. Deionized water was prepared with Premier equipment and used for all solutions. Standard (stock) solution of histamine was prepared at a concentration around 10 $mg \cdot L^{-1}$ in 0.1 M HCl and was further diluted for experiments.

B. Preparation of Histamine Standard Solution

Stock solution (10 $mg \cdot L^{-1}$) of a solution of histamine was prepared 0.1 M HCl in a volumetric flask (10 mL). The solution was stored in the fridge at 4°C. Standard solutions were prepared by diluting from this stock solution and used to obtain the calibration curves (0.16 – 5.00 $\mu g \cdot mL^{-1}$) and for validation purposes.

C. Methods for Derivatized Histamine

1. Histamine derivatized by FMOC - Cl

The derivatization of histamine using FMOC – Cl was performed by the methods of Lkhagva *et al.* (2020) with some modifications. 10 µL of histamine were mixed with a saturated solution of 2 M NaOH (10 µL), $NaHCO_3$ (100 µL) and 1 mL of FMOC – Cl. The mixture was heated on a hot plate (80°C) for 10 min. After the reaction, the excess of FMOC – Cl was removed by using 1 mL of glutamic acid (50 mg of glutamic acid in 1 mL of deionized water). After 30 minutes, the mixture was adjusted to 5 mL with acetonitrile. The solution was filtered using Whatman paper before injected onto HPLC.

2. Histamine derivatized by BSA

The derivatization method was adapted from Jia *et al.* (2020) with several modifications. Precisely 10 µL of histamine was put into the vial and evaporated with nitrogen gas. Afterward, 1 µL of BSA was added into the vial and heated at 80°C for 10 min. Then, cooled for 15 min and the derivatized solution was evaporated using nitrogen gas and the residue was dissolved with 1 mL of dichloromethane. 1 µL of the solution was injected into GC.