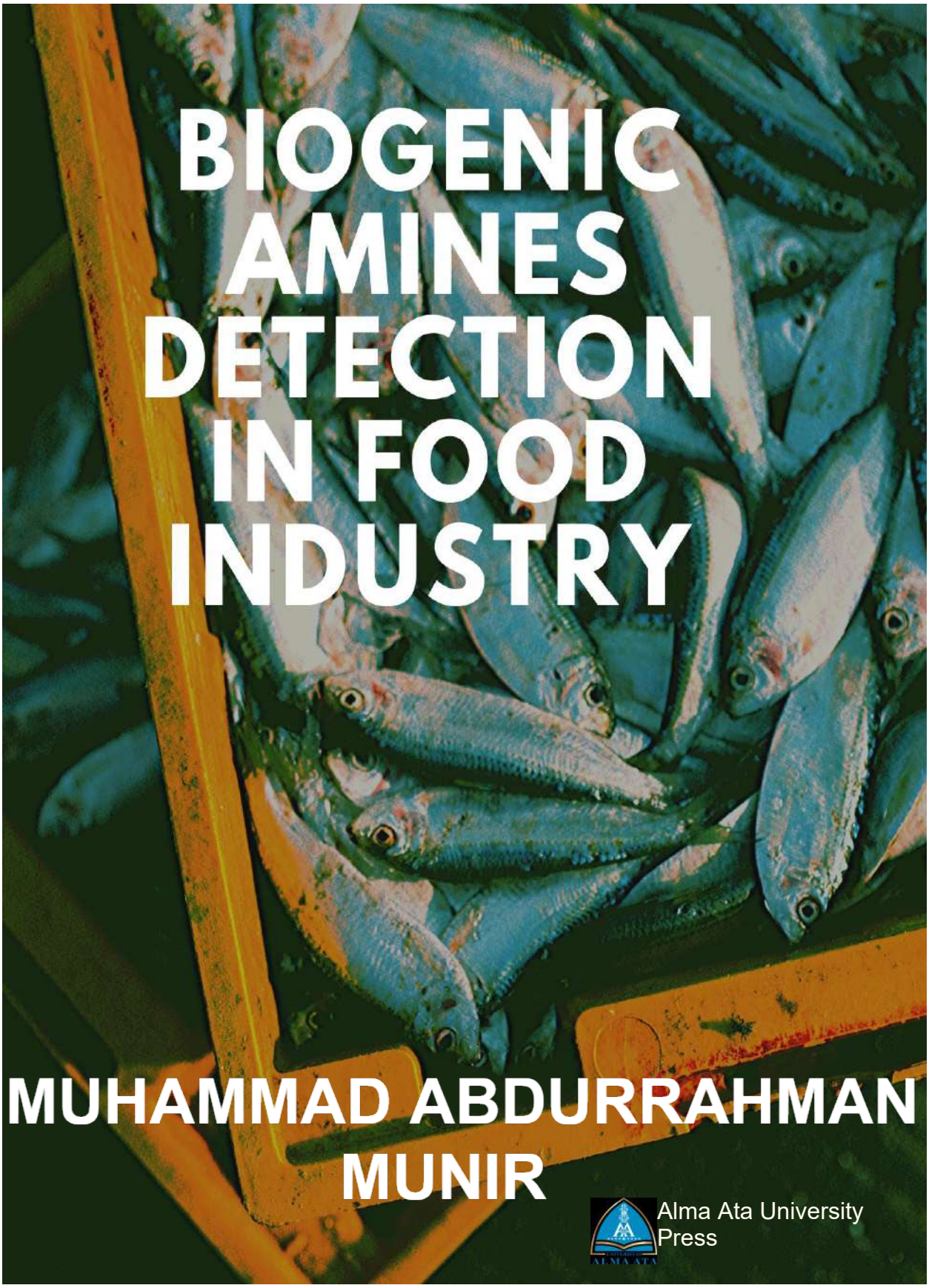
BIOGENIC
AMINES
DETECTION
IN FOOD
INDUSTRY
MUHAMMAD ABDURRAHMAN
MUNIR
Alma Ata University
Press

Biogenic amines detection in food industry

An inspirational book for analysis industry

MUHAMMAD ABDURRAHMAN MUNIR

Alma Ata University Press (AAUP)

Biogenic amines detection in food industry

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List of Abbreviations

BAN	Bromoacetonitrile
BSA	(N,O-bis(trimethylsilyl)acetamide)
BSTFA	N,O-bistrifluoro acetamide
CE	Capillary Electrophoresis
COC	Cold-On-Column
DCL	Dansyl Chloride
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
HFP	Histamine Fish Poisoning
HPLC	High Performance Liquid Chromatography
ICH	The International Council for Harmonization
ISO	International Organization for Standardization
GC-MS	Gas Chromatography- Mass Spectrometer
GC-FID	Gas Chromatography- Flame Ionization Detector
LC-MS	Liquid Chromatography-Mass Spectrometry
LoD	Limit of Detection
LoQ	Limit of Quantitation
MS	Mass Spectrometer
OPA	O-phthalaldeyhde
RSD	Relative Standard Deviation
SIM	Selected Ion Monitoring
TCA	Trichloracetic Acid
TLC	Thin Layer Chromatography
TMCS	Trimethylchlorosilane
USP	United States Pharmacopeia
WHO	World Health Organization

List of Appendices

Preface

First of all, I would like to thank God for giving me life and the opportunity to complete this book, entitled “Biogenic amines detection in food industry”.

Biogenic amines are nitrogenous compounds with low molecular weights. Biogenic amines can be found in food and beverages which contained protein. Biogenic amines, particularly histamine, with the presence of cadaverine and putrescine can cause scombroid food poisoning. Gas chromatography coupled with flame ionization detector (GC-FID) have been validated for the determination of biogenic amines (heptylamine, histamine, tyramine, cadaverine and spermidine) in fish and fish product samples, whereas gas chromatography coupled with mass spectrometer (GC-MS) has been used to identify biogenic amines. Analysis of biogenic amines using GC is difficult owing to their lack of volatility and therefore, biogenic amines need to be derivatized before analyzed using GC-FID and GC-MS. A mixture of N, O-bis (trimethylsilyl) acetamide (BSA) and trimethylchlorosilane (TMCS) were employed in this book.

This book contains 7 Chapters. Chapter 1 gives various information about the introduction on biogenic amines in food industry while Chapter 2 focuses about biogenic amines in food industry. Histamine, cadaverine, spermine, putrescine, tryptamine, tyramine and spermidine are the most amines that can be discovered in foods and important compounds for physiological and metabolic functions in human body and biologically active compounds. When they consumed with high concentration, they can lead to food-born illness. So, the substances of biogenic amines in foods are extremely important to be analyzed because due to toxicity for consumers but can also be used as an indicator of food quality. Next, the determination of biogenic amines using HPLC and GC is discussed in Chapter 3.

Derivatization methods used for biogenic amines are silylation, alkylation, acylation and esterification. This chapter also discussed about parameters for analytical methods validation and recovery efficiency of extraction method. Biogenic amines are polar and nonvolatile compound. Thus, before measured by instruments particularly GC, biogenic amines must be derivatized to increase the volatility and reduce the polarity. All the the validation method and procedure of biogenic amines is described in detailed in Chapter 4. The result of measurement of derivatized biogenic amines standard using GC-MS measurement is focused in Chapter 5 while Chapter 6 is about validated method using GC-FID measurement of derivatized biogenic amines standard. Lastly, the application methods in determination of biogenic amines in fish and fish product samples are discussed in detail in Chapter 7.

Special thanks to my family, especially my parents Badrul Munir Muhammad Nur and T. Asmah Zatun Matabin, my siblings Maryam, Hamid Al-Khair, Ahlam Inayatullah, Abdul Jawwad, Tahani, Dalaal Mahmudah, Ahmad Abdul Aziz, my wife Helwina Erfiani and my daughter Adinda Latifah Rahman for their love, support, guidance and for never giving up on me until the end. Special thanks also to all lectures, staff and students of Department of Analytical Chemistry who are involved directly or indirectly during this study.

MUHAMMAD ABDURRAHMAN MUNIR

Chapter I

Introduction

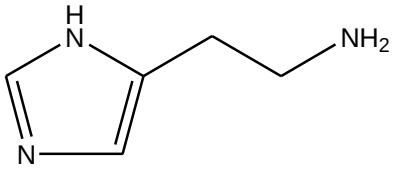
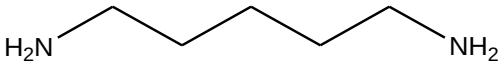
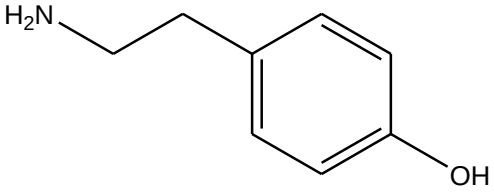
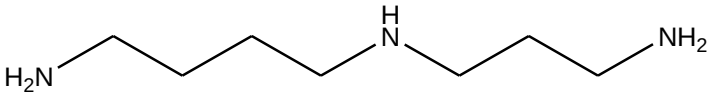
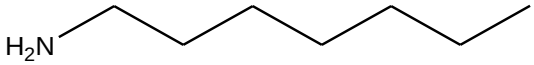
Amines are a derivative compound from ammonia with one, two or three aryl or alkyl groups bond to the nitrogen atom. They are categorized as primary, secondary or tertiary. A large dipole moment from the lone pair of electrons enhances to the dipole moments of the C– H and H – N bonds makes amines become a very polar compound. Amines are extremely bases, therefore, their solutions are basic. All amines could be dissolved by water and alcohol because they have the capacity to form hydrogen bonds with hydroxylic solvents (Wade, 2013).

Biogenic Amines

Biogenic amines are nitrogenous compounds and based on the chemical structure can either as aliphatic, aromatic or heterocyclic (Rabie & Toliba, 2013). The aliphatic structure are heptylamine, cadaverine, putrescine, spermine and spermidine, tyramine and phenythylamine are aromatic structure, while typtamine and histamine are heterocyclic (Kalac, 2009; Kim *et al.,* 2009; Mohamed *et al.,* 2009) and also they could be categorized based on the amine groups amount such as, phenylethylamine, tyramine and heptylamine as monoamines, putrescine and cadaverine for diamines and spermine and spermidine for polyamines (Table 1.1) (Spano *et al.,* 2010). Biogenic amines can be found in food that containing protein such as meat, fish, cheese and vegetables (Lorenzo *et al.,* 2007) and also low molecular weight where can be shaped by means of amino acids decarboxylation which relies on the particular bacterial or by amination and transamination of ketones or aldehydes and the presence of amino acid substrate (Zhai *et al.,* 2012; Linares *et al.,* 2011; Rivas *et*

al., 2008). Biogenic amines accumulation in food is not acceptable owing to give impacts when consumed in high concentration such as headache, heart palpitations, respiratory, hypo- or hypertension, itching, rash, fever, emesis, nausea and even death in serious cases (Li *et al.*, 2014; Zaman *et al.*, 2010).

Table 1.1 Structures of five biogenic amines

Amine	Structure
Histamine	
Cadaverine (1,5 pentanediamine)	
Tyramine	
Spermidine	
Heptylamine (1-aminoheptane)	

Factors of Biogenic Amines Formation

The factors which able to influence biogenic amines formation in food, such as raw material quality, food physico-chemical parameters (ripening temperature, NaCl and pH), distribution conditions, decarboxylase-positive microorganisms or availability of free amino acids, storage and manufacturing processes (Linares *et al.*, 2012; Pons-Sanchez- Cascado *et al.*, 2006). The low temperature can influence the biogenic amines formation via the reduction of enzyme activity and inhibition of microbial growth (Naila *et al.*, 2010).

Biogenic amines formation by mesophilic bacteria has been investigated between 20 - 37^{o}C is an optimal temperature range, while lower temperature below 5^{o}C and higher temperature above 40^{o}C will decrease their growth (EFSA, 2011). Histidine will convert to histamine at temperature above 16^{o}C where the enteric bacteria produces enzyme histidine decarboxylase.

Determination Procedure

Some procedure have been determined to control the production of biogenic amines such as, heating, low temperature storage, microbial modeling, modified atmosphere packaging, high hydrostatic pressure, irradiation and preservatives. In spite of the usage of freezer is more effective for avoiding biogenic amines accumulation (Naila *et al.,* 2010), that impossible to always control the growth of biogenic amines through the temperature, because some bacteria able to shape biogenic amines at temperature below 5^{o}C (Emborg & Dalgaard, 2006). Even for biogenic amines like histamine, is not easy to disappear by cooking (Cohen, 2015) and also reported, the toxicity of histamine will not disappear from food because they are resistant to heat (Tapingkae *et al.,* 2010; Duflos, 2009; Gonzaga *et al.,* 2009). Furthermore, these procedure have side effects such as decrement in the nutritional value of food, food functionality and organolpetic properties (Eom *et al.,* 2015).

Technique of Determination of Biogenic Amines

Several techniques were proposed to determination and investigation of biogenic amines, such as thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography (GC), (Onal, 2007; Smela *et al.,* 2003). GC is one of the most versatile techniques for analytical chemistry. The popularity of GC because of it is the fast, relatively inexpensive, simple and reproducible nature (McNair & Miller, 2009). Some of organic compounds cannot be detected by GC because they have polar functional groups and non-volatile. Amines also are not straight-forward to investigate by GC because it will

lead to significant tailing, poor reproducibility owing to interaction of analyte with the GC column and the lack of volatility. Therefore, components which have the problem with volatility or highly polar need to be derivatized. After the addition of derivatizing reagent, components with active groups such as hydroxyl, amine, carboxyl, olefin, and others can be identified and make them more volatile or less polar and consequently suitable for GC measurement (Grob, 2004).

The purpose of derivatization reactions is to transform an analyte to become detectable in GC or other equipment analytical procedure. In order to allow chromatographic separations that can be construed as a technique that modifies a functionality of compound. That is also possible if the derivative has the same structure, but not resemble as the original non-modified chemical compound (Orata, 2012).

Challenges

Measurement is of food and beverages that containing biogenic amines particularly histamine is important since they can lead toxicity and used as indicators to check the spoilage or freshness of food and beverages (Awan *et al.*, 2008). Furthermore, biogenic amines measurement using GC is difficult due to the complexity of the real matrices to be analyzed (Onal, 2007) and GC is not commonly used owing to inherent tailing problems (Karovicova & Kohajdova, 2005). Biogenic amines also deficiency of a suitable fluorophoric or chromophoric group. Thus, derivatization is needed for detection of biogenic amines (Moret *et al.*, 2005). Derivatization procedure that recommended to derivatize biogenic amines are acylation, carbamate and silylation procedure (Kataoka, 1996).

Although the derivatization increases the volatility and reduces tailing, derivatization has several disadvantages, such as (a) the agent may interfere with the measurement is and very difficult to remove, (b) the derivatization condition may lead chemical changes in a compound and (c) the derivatization step increases the measurement time (Nollet, 2006). Also, derivatization can produce by-product interferences and sometimes increase or decrease the amount of amines (Bouchereau *et al.*, 2000). Moreover, determination of

biogenic amines in food is important to develop sensitive, fast and simpler analytical techniques (Onal, 2007).

The GC measurement of derivatized biogenic amines needs to be validated. The validation includes determination of specificity, precision, accuracy, linearity, detection limit, limit of quantitation and recovery. To prove that an analytical method is satisfactory and acceptable, validation method is required for its intended purpose (Harris, 2007). It is also needed to demonstrate the reliability and method performance of analytical results.

The objectives of these book are to verify the biogenic amines standard after derivatized with a mixture of BSA+TMCS using gas chromatography – mass spectrometer, to validate a method for determination of biogenic amines standard using gas chromatography-flame ionization detector, to determine the extraction efficiency of biogenic amines from fish and fish product samples and to qualitatively and quantitatively measurement is of the biogenic amines in fish and fish product samples using validated procedures.

Conclusion

Biogenic amines particularly histamine that contained in food and beverages is important to be measure. The measurement can be used as indicator to check the spoilage or freshness of food and beverages as they can lead the toxicity.

Chapter 2

Biogenic Amines

Biogenic amines are important compounds for physiological and metabolic functions in human body (Eom *et al.*, 2015). Histamine, cadaverine, spermine, putrescine, tryptamine, tyramine and spermidine are the most general amines that can be discovered in food (Naila *et al.,* 2010). Biogenic amines are involved in connection to public health and food hygiene. They are also biologically active compounds and when were consumed with high concentrations can lead to food-borne illness (Papavergou *et al.,* 2012).

Effects to Human Health

Consuming of food which containing biogenic amines particularly histamine with concentration at above 50 mg/kg, may give effects to the human body such as flushing, nausea, hypertension, headache, cardiac palpitations, inflammation and diarrhea, even for some severe incidents the intoxication may dangerous for consumers (Naila *et al.,* 2010). Concentration of biogenic amines intoxication relies on the biogenic amines category and level. The consumption of food with low level of biogenic amines are similarly metabolized in the human being body by means of the activity of the monoamine or diamine oxidases and amine oxidizing enzymes. Thus, the poison of biogenic amines level is not straightforward to establish, because it relies on the condition of human being and individual sensitivity (Suzzi & Torriani, 2015).

The concentration of histamine in fresh fish is low which is below 0.01 mg/kg (Auerswald *et al.,* 2006). Histamine poisoning or commonly known as scombroid poisoning is a serious issue, can be happened after human consume food or beverages which containing biogenic

amines, especially histamine at the level above 500 mg/kg (Gonzaga *et al.*, 2009). The United States Food and Drug Administration has established that histamine at below 50 mg/kg in fish could be consumed (FDA, 2011) and for other countries such as Europe, South Africa and Australia are 100 mg/kg, 100 mg/kg and 200 mg/kg, respectively (Auerswald *et al.*, 2006). Although histamine is the one that cause for food intoxication (Zaman *et al., 2010*), it is not poison at a low concentration but will increase its toxicity because the presence of cadaverine and putrescine with concentrations 5 times higher than histamine (Emborg & Dalgaard, 2006). Tyramine with higher concentration able to lead an intoxication or usually known as the cheese reaction where the symptoms alike scombroid poisoning (Naila *et al.*, 2010) and also cause heart failure and brain haemorrhage (Standarova *et al.*, 2008). The reaction between cadaverine, putrescine, spermidine and spermine with nitrite can shape carcinogenic nitrosamines (Aflaki *et al.*, 2015).

Production of Biogenic Amines Controlled Method

The production of biogenic amines in food can be controlled by using two methods such as, emerging and existing method. The emerging method is a method to control or disappear biogenic amines with the temperature combination. These methods such as microbial modelling, high hydrostatic pressure, irradiation, modified atmosphere packaging and preservatives addition, whereas for existing method is a method to control biogenic amines formation with low temperature, usually using refrigerator as an instrument (Naila *et al.*, 2010). The formation of biogenic amines can be seen in Figure 2.1. This book used five biogenic amines as a standard, those can be seen in Table 2.1. It also displays the molecular weight, boiling point (^{o}C) and structures of biogenic amines standard.

Functions and Physiological Significance of Biogenic Amines

The sources of biogenic amines are precursors and nitrogen, not merely for the synthesis of proteins, nucleis acids, hormones and alkaloids but also effect the processes in the organism

such as body temperature regulation, decrease or increase of blood pressure and nutrition intake (Shukla *et al.*, 2011). Active amines are substances which shaped and broken down by the metabolic process in cells. Endogeneously synthesized biogenic amines fulfil an array of roles in cellular metabolism of human. Histamine and tyramine act as hormonal mediators in human and animals (Onal, 2007).

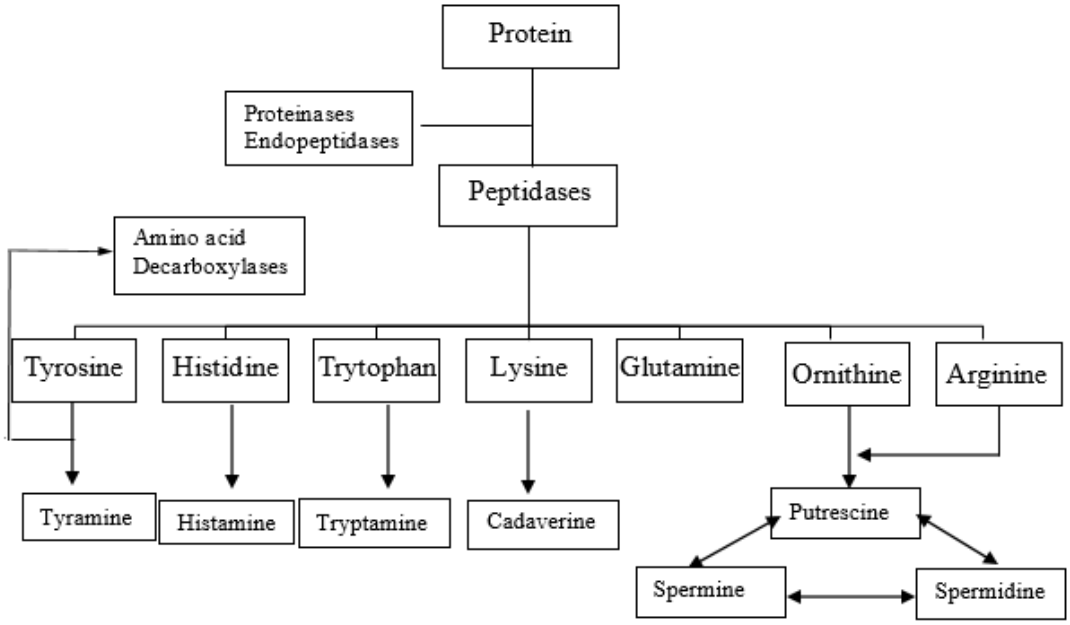


Figure 2.1 Biogenic amines formation (adopted from Ruiz-Capillas & Jimenez-Colmenero, 2004)

Histamine is released as a response to an allergic reaction (Shukla *et al*., 2011). The functions of biogenic amines are particularly alteration of DNA structure and regulation of gene expression by modulating signal transduction pathways (Linsalata & Russo, 2008). Polyamines are taken up preferentially by tissues with high demands where could be used during wound healing or for post-operation patients and for development and growth of the digestive system of neonatal. They also important for general properties of the adult digestive region and maintenance of normal growth (Kalac & Krausova 2005; Kalac *et al*., 2005).

Table 2.1 Molecular weight and boiling point of five biogenic amines

Amine	Molecular weight	Boiling point (^{0}C)
Histamine	112	167
Cadaverine	102	178-180
Tyramine	139	175-181
Spermidine	145	128-130
Heptylamine	115	154-156

Occurrence of Biogenic Amines in Food

Free amino acids or protein in food are subject to conditions that enabling microbial or biochemical activity that have biogenic amines (Karovicova & Kohajdova, 2005). The substances of biogenic amines in food are extremely important to be analyzed because not merely due to toxicity for consumers but also can be used as an indicator of food quality (Awan *et al.,* 2008).

Fish and Fish Products

Fish is one of the most necessary protein sources than other food and is an extremely perishable food which means biogenic amines can be found in food. During the spoiling process, bacterial decarboxylases catalyse will convert amino acids to biogenic amines (Chong *et al.,* 2014). Biogenic amines level depend on the species of fish, degree of microbiological contamination, temperature and storage time. The time and temperature are the main factors for the biogenic amines formation during storage and handling of fresh fish (Aflaki *et al.,* 2015). Bacteria which associated with biogenic amines are similarly present in the saltwater environment (FDA, 2011). The biogenic amines formation has been found in fish, such as anchovies, herring, tuna and sardine (Chong *et al.,* 2014).

Histamine fish poisoning (HFP) known as scombroid fish poisoning is seafood poisoning related to the improper storage of fish. The term of scombroid is derived from the family *Scombridae*, such as; tuna and mackerel. Non-scombroid that containing scombroid fish poisoning such as herring (*Clupea* spp.), bluefish (*Pomatomus* spp.), sword fish (*Xiphias gladius*) sardines (*Sardinella* spp.) and mahi-mahi (*Coryphaena* spp.) (Visciano *et al.*, 2012).

Zhai et al. (2012) detected histamine in Spanish mackerel sample at concentration between 15.74-28.70 mg/kg, where in canned anchovies, canned sardines and the other canned samples, the histamine level were 26.95, 22.38 and 10 mg/kg, respectively. Biogenic amines were investigated in food, such as the traditional food "cincalok" and "budu", canned and salt-cured fish. The biogenic amines concentrations of tyramine, histamine, tryptamine, putrescine and spermidine were found in eight "budu" samples at 174.7, 187.7, 82.7, 38.1 and 5.1 mg/kg, respectively. Histamine, putrescine and tyramine were detected in cincalok with concentrations at 126.1, 330.7 and 448.8 mg/kg, respectively. Concentrations of biogenic amines were acquired lower in canned and salt-cured fish samples (Saaid *et al.*, 2009).

Fish samples, such as canned sardines, mackerel, tuna and marinated anchovies were contained biogenic amines. The amount of biogenic amines concentration ranged between 26.58 - 406.55 mg/kg where cadaverine and histamine were the dominant. Tyramine and putrescine levels were below than 100 and 50 mg/kg, respectively (Bilgin & Genccelep, 2015). According to these scientists, there were none of scombroid poisoning cases or histamine toxicity after distributed to market or consumed by consumers.

Meat and Meat Products

Biogenic amines in meat are a freshness marker or otherwise. Biogenic amines study in meat can be considered as a function of conservation time that used to control the spoilage of meat. The increase of concentration of amines have occurred in meat owing to degrading process in food (Lazaro *et al.*, 2013). Biogenic amines can be found in fermented food like

sausages. Dry fermented sausages have potential to support the biogenic amines accumulation owing to the existence of spermine and spermidine but also because of the microbial growth (Suzzi & Gardini, 2003).

Biogenic amines have been investigated in the products of meat and detected histamine, putrescine, cadaverine and tyramine at concentration between 0-515, 0-505, 0-690 and 0-510 mg/kg, respectively (Papavergou *et al.,* 2012). Cadaverine and putrescine concentrations have been found at 87% and 93% of the samples, respectively. Spermidine, spermine and tryptamine were found in range from 0 - 10.7, 0 - 16.4 and 1.2 - 82.3 mg/kg, respectively. Phenyethylamine was detected with concentrations were lower than 25 mg/kg in 17 of the 30 samples. Histamine was discovered in 17% of the samples between 50 and 100 mg/kg (Genccelep *et al.,* 2008).

Dairy Products

Milk and milk products are very necessary as a nutrition for human-being, where cheese is one of the milk products deemed as a good resource of proteins, mineral and vitamins (Loizzo *et al.,* 2013) otherwise, cheese provides a sufficient environment for biogenic amines production and accumulation (Linares *et al.,* 2012). Cheese can be considered as an ideal environment to biogenic amines production but the concentration of biogenic amines rely on some factors, such as the cheese type, microbial growing, storage temperature, the conditions and length of the ripening process (Rohani *et al.,* 2013; Linares *et al.,* 2012).

Concentrations of biogenic amines, such as putrescine, cadaverine, histamine and spermidine, in fermented milk cow were 20.26, 29.09, 17.97 and 82.07 mg/kg, respectively and in fermented milk goat were 22.92, 29.09, 34.85 and 53.85 mg/kg, respectively (Costa *et al.,* 2015). Biogenic amines in cheese have been examined by Mascaro *et al*. (2010) in Pecorino di Fossa and found that the concentrations were 1302.86 and 579.6 mg/kg for cadaverine and putrescine, respectively. Putrescine concentration between 9.9 - 394 mg/kg and cadaverine from 26.8 - 276.1 mg/kg found in Pecorino di Farindola (Schirone *et al.,* 2011). Biogenic amines also were investigated in traditional cheeses in Iran, where five Red

Salmas samples, ten Lighvan samples and seventy Koopeh cheese samples were analysed. The lowest concentration of biogenic amines was in Koopeh cheese with 517.71 mg/kg followed by Lighvan and Red Salmas cheese with 1008.98 and 1426.91 mg/kg, respectively.

Histamine, cadaverine, putrescine and tyramine found in Red Salmas samples were 105.21, 701.05, 438.03 and 182.62 mg/kg, respectively, while in Lighvan cheese samples were 277.53, 342.74, 37.58 and 351.12 mg/kg, respectively and for Koopeh cheese samples were 156.09, 282.34, 70.80 and 8.48 mg/kg, respectively (Rohani *et al.,* 2013).

Incident of Scombroid Poisoning in Fish and Fish Products

Scombroid poisoning occurs worldwide, specifically in countries that consuming of certain types of fish. Histamine poisoning disease was emerged following consumption of fish as early as the 1800 (Taylor, 1986). Investigation of biogenic amines toxicity is not straightforward since the toxicity does not rely on the presence of biogenic amines, but also affected by the specific efficiency of detoxifying mechanisms in distinction of human-being and other compounds (Ruiz-Capillas & Jimenez-Colmenero, 2004). The incident of scombroid poisoning in the world perhaps due to toxicity caused by the consumption of fish (Visciano *et al.*, 2012).

The countries with the most reported scombroid poisoning incidents since 1970 are England, Japan and the United States and less frequent occasions have been found in several states, such as New Zealand, Canada, German, France, Netherlands, Sweden, Australia, Indonesia, South Africa, Egypt and Sri Lanka (Taylor, 1986). The cases occurred between 2005 - 2010 using Rapid Alert System for Food and Feed (RASFF) were above 100 incidents (EFSA, 2011).

Table 2.2 Scombroid fish poisoning cases reported (WHO, 2013)

Country	Year	Numbers of effected	Causative Food	Biogenic amines concentration (mg/100 g)
Australia	1990	3	Western Australian Salmon	8080
	1991	4	Salmon	254
	1991	6	Yellow-fin tuna	470-490
UK	1991	4	Smoked mackerel	250
USA	1998	11	Tuna burgers	274-325
	2003	42	Escolar fish	200-380
Taiwan		59		257
	2004		Fried billfish	
		43		157-270
	2006	7	Tuna dumpling	161
	2007	347	Fried fish cubes	40 and 52
Thailand	2007	91	Fried fermented tuna	446

An incident occurred in Taiwan owing to ingestion of fried fish cubes. The incident caused 347 victims to fall sick. They suffered some allergic symptoms such as, nausea, flushing, rash and diarrhea, but the patients recuperated within 24 hours (Chen *et al.*, 2010). In 1998-2008, 89 incidents in Japan affecting 1577 people (with no deaths) were reported, where 29 involved tuna, 16 involved mackerel and billfish, 7 involved sardine, 9 involved yellowtail, 8 involved pacific saury, 3 involved horse mackerel and dolphin (WHO, 2013). Table 2.2 below is shown the several scombroid fish poisoning that has been reported, where no cases of scombroid poisoning were found in Malaysia.

Conclusion

Histamine, cadaverine, spermine, putrescine, tryptamine, tyramine and spermidine are the most general amines that can be discovered in food and important compounds for physiological and metabolic functions in human body. Biogenic amines are involved in

connection to public health and food hygiene. They are biologically active compounds and when were consumed with high concentrations can lead to food-borne illness. So, the substances of biogenic amines in food are extremely important to be analyzed because due to the toxicity for consumers but can also be used as an indicator of food quality.

Chapter 3

Determination of Biogenic Amines in Foods

The goals for determination of biogenic amines in food are; the potential toxicity of biogenic amines and a probability to use as indicators of food quality. Several steps of biogenic amines measurement such as; monitoring fermentation process, quality control of raw materials, process control, intermediates and end products, and research and development (Onal, 2007). Analytical approaches for measurement biogenic amines have aims, such as (1) improving the current methods or developing new methods; (2) using the measures of biogenic amines in food preparation to control the effectiveness of developed methods, packaging and storage to decrease accumulation of biogenic amines and; (3) by using known methods that reporting the biogenic amines levels of product from different countries (4) understanding the relation between the contents of biogenic amines and biogenic amine-producing microorganisms (Bedia, 2013).

Extraction should be considered as an important step in the determination of biogenic amines. Inappropriate techniques may cause erroneous results, particularly during recovery studies. It is necessary to ensure the precision and accuracy during recovery processes (Onal, 2007; Moret & Conte, 1996). Fish samples extraction by methanol-HCl showed good recovery of cadaverine, putrescine and histamine (Richard *et al.*, 2008).

The scientists have found several chemicals reagent for extraction biogenic amines (Dadakova *et al.,* 2009). Reagents such as perchloric acid (Lazaro *et al.,* 2013; Rodrigues *et al.*, 2013; De Mey *et al.*, 2012; Mayer *et al.*, 2010; Bomke *et al.,* 2009), trichloroacetic acid (Sagratini *et al.,* 2012; Triki *et al.*, 2012; Saaid *et al.,* 2009), hydrochloric acid (Jia *et al.*,

2011; Mazzucci *et al.,* 2010; Ozdestan & Uren, 2010) and organic solvents (Karovicova & Kohajdova, 2005; Hwang *et al.,* 2003) are generally applied to extract biogenic amines from food and beverages. Furthermore, various analytical techniques have been established in order to determine biogenic amines, such as, thin layer chromatography (Tao *et al.,* 2011; Latorre-Moratalla *et al.,* 2009; Lapa-Guimares & Pickova, 2004), high performance liquid chromatography (Aflaki *et al.,* 2015; Bilgin & Genccelep, 2015; Costa *et al.,* 2015), gas chromatography (Awan *et al.,* 2008; Hwang *et al.,* 2003; Nakovich, 2003) and capillary electrophoresis (Bricio *et al.,* 2004; Sun *et al.,* 2003). These analytical methods are proposed for separation and identification of biogenic amines in food and beverages samples.

High-Performance Liquid Chromatography (HPLC)

HPLC is the fittest and frequently used for the detection of biogenic amines (Lazaro & Conte-Junior, 2013). The procedures of HPLC include pre- or post-column derivatization step (Karovicova & Kohajdova, 2005). The issues of HPLC are related to on-column and pre- or post-column derivatization process where it can lead to an overall long measurement time and low reproducibility due to the stability of the derivatization reagents (Favaro *et al.,* 2007). Extraction of histamine is a crucial step before separate from biogenic amines. Extraction with 5% TCA offered rapid extraction of amines, otherwise, using TCA, a good recovery was acquired (Hosseini *et al.,* 2014). The use of derivatization is to increase the sensitivity. Several derivatizations have been used, whereby o-phthalaldeyhyde (OPA) and dansyl chloride are the most generally derivatizing reagent. OPA is the best for primary amines and dansyl chloride is the best for primary and secondary, however dansyl chloride has low sensitivity and limited stability. Benzoyl chloride is low-cost, stable, easily accessible and has purity is less critical than dansyl chloride (Awan *et al.,* 2008; Karovicova & Kohajdova, 2005). Otherwise, the compounds are more stable by using OPA (Lapa-Guimares & Pickova, 2004).

Tyramine, histamine, cadaverine, putrescine and spermidine were examined using HPLC, which extraction was perchloric acid and for derivatizae reagent benzoyl chloride has been

used. Acetonitrile: water (42: 58) (% v/v) was used as the mobile phase, produced a peak at 198 nm. The standard curves with correlation coefficients of 0.9343, 0.9997, 0.9977, 0.9981 and 0.9921 for histamine, cadaverine, putrescine, tyramine and spermidine, respectively. The LOD and LOQ ranged from 0.03-1.30 µg/mL and 0.20-5.00 µg/mL, respectively and recovery for these biogenic amines were 91%-107%. (Costa *et al.,* 2015).

Five biogenic amines were found in sixty three fish samples by HPLC and 1.7-diaminoheptane was used for internal standard. Samples were extracted using perchloric acid and derivatized using dansyl chloride. The gradient elution system was 0.1 M ammonium acetate as the solvent A and acetonitrile as the solvent B. Only some biogenic amines were detected in these samples, which showed less than 20 mg/kg of each biogenic amines. Otherwise, seven tuna samples indicated the level of histamine higher than 100 mg/kg. The LOD was 0.005-0.050 µg/mL and LOQ was 0.010-0.100 µg/mL (Bilgin & Genccelep, 2015).

In another information, nine biogenic amines such as cadaverine, histamine, putrescine, tryptamine, spermine, spermidine, tyramine, phenylethylamine and agmantine were investigated in 95 fish samples of 8 species using HPLC. Biogenic amines were extracted using perchloric acid and derivatized using benzoyl chloride. C18 column was used and the mobile phase was methanol (A) and water (B). The correlation coefficient (R^2) of biogenic amines were, 0.9999 (putrescine), 0.9995 (cadaverine), 0.9997 (tryptamine), 0.9991 (phenylethylamine), 0.9998 (spermine), 0.9997 (spermidine), 0.9995 (histamine), 0.9999 (tyramine) and 0.9989 (agmantine). The LOD ranged from 0.04 mg/kg-0.09 mg/kg and LOQ ranged from 0.13 mg/kg-0.28 mg/kg. Furthermore, for recovery percentage ranged from 94.23%-101.14% (Aflaki *et al.,* 2015).

Gas Chromatography (GC)

GC is a unique and versatile technique which can be used for measurement volatile compounds. The advantages of GC include resolution, sensitivity, measurement time, automatic, convenience, cheap, high separating power, an assortment of sensitive detecting

systems and ease of recording data. This tool can also be used for the direct measurement and separation of volatile solids, liquid solutions and gaseous samples. For non-volatile samples, derivatization technique or pyrolysis GC can be applied. When analyzing an unknown peak of the compounds, the identification of peak must not be based on retention time of the compounds. Two methods to identify unknown peaks of the compounds, such as mass spectrometry (MS) and infrared spectroscopy (IRS). MS is better than IRS because it can acquire a mass number that can be compared and matched with a mass number in a library of mass spectra of known compounds (Grob, 2004). Hwang *et al.* (2003) were measured of histamine in tuna and shrimp using GC/MS without derivatized and used three megapore capillary columns with different polarity (CP- SIL 19CB, CP-SIL 8CB and CP-SIL 5CB). Internal standard used was 1,9-nonanediol. CP- SIL 19CB column was obtained the retention times of histamine and internal standard at 5.24 and 4.90 min, respectively. CP-SIL 8CB column was acquired the retention times of histamine and internal standard at 4.77 and 5.56 min, respectively, whereas CP-SIL 5CB column was obtained the retention times at 4.66 and 5.22 min for histamine and internal standard, respectively and also indicated the recovery study for histamine at 98-111% for tuna and 99-102% for shrimp. Detection limit for histamine was 5 µg/mL. The histamine content in tuna ranged from 114-267 mg/kg, but for shrimp was not detectable.

Nakovich (2003) was measured of biogenic amines using GC/FID and GC/MS, whereabouts the samples were extracted by methanol and HPLC grade-water and derivatized by propyl chloroformate. Six derivatized biogenic amines such as heptylamine, tyramine, cadaverine, histamine, putrescine and spermidine) were measured by GC/MS. To acquire the good yields, a Cold-On-Column (C.O.C.) inlet with a short column (15 meters), thick film stationary phase (ZB-5, 1.00 µm) and to cut 40 cm from the inlet end the column after 25 injections have been suggested. It used the rule of FDA where the level of histamine is below 50 mg/kg. Histamine concentration below 50 mg/kg for days 0 and 3 and showed the increment for the fifth day, levels of cadaverine, histamine, tyramine were 160, 1000 and 350 mg/kg, respectively. Six biogenic amine standards at 50 mg/kg showed good precision, 5.2, 9.9, 5.1, 5.6, 5.0 and 6.2% for heptylamine, histamine, tyramine, putrescine, cadaverine and spermidine, respectively.

Derivatization Methods for Biogenic Amines

The polar compounds cannot be detected by GC due to they have polar functional groups. Organic acids, amides, hydroxyl compounds, amino acids are instances of polar compounds that should be derivatized (Knapp, 1979). Nevertheless, derivatization has a number of disadvantages, such as; the derivatizing agent may be difficult to remove and interfering in the measurement. The use of derivatization agents also will increase the measurement cost and time. Several derivatization methods are performed. The principal of this method can be categorized into four general groups based on the reaction achieved and the reagents used such as, silylation, alkylation, acylation and esterification (Nollet, 2006).

Silylation

The silylation reaction is a leaving group with a low basicity, a capability to stabilize a negative charge, weak bonding between the silicon atom and leaving group (Knapp, 1979). The process is the substitution between the active hydrogens (in -COOH, -NH, -NH_2, –SH and –OH groups) and a trimethylsilyl group. The process of silylation reaction by means of a nucleophilic attack (SN_2). This outcomes in the production of the biomolecular transition state (Kuhnel *et al*., 2007) in the intermediate step of the reaction process. The leaving group in TMCS is the Cl atom. The reaction for the trialkylsilyl derivatives formation is shown in Figure 3.1.

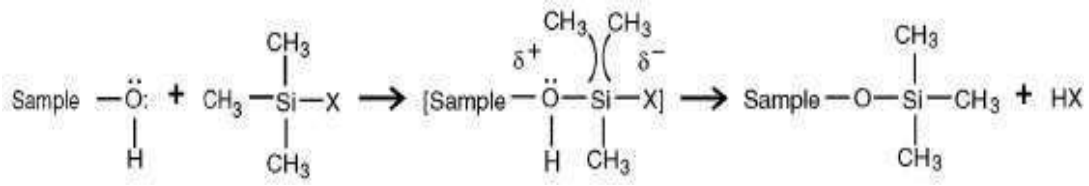


Figure 3.1 General reaction mechanism for trialkylsilyl derivatives the formation for trimethylchlorosilane, X = Cl

Szyrwinska *et al*. (2007) used BSTFA (N,O-bistrifluoroacetamide) and 1% TMCS added by bromoacetonitrile (BAN) reagents to investigate Bisphenol-A and powdered milk extracts. 200 µL of bisphenol-A solution was put into a vial 1 mL and dried with nitrogen at 60^{o}C. 100 µL of BSTFA + 1% TMCS was added to the residue and heated at 80^{o}C for 30 minutes and cooled. Afterwards, the derivatized solution was dried and re-dissolved with 100 µL of chloroform. Afterwards, 1 µL of derivatized solution was analyzed by GC–MS.

Alkylation

Alkylation decreases the compounds polarity by changing active hydrogens with an alkyl group. Alkylation usually used to modify carboxylic acids and phenols become ethers, esters, alkyl amides and alkyl amines. The disadvantages of this reagent are more limited applicability and selectivity of this method (Knapp, 1979).

The triclosan was investigated in milk and human plasma, where that was achieved by the alteration of triclosan into pentafluorobenzyl ester with enhancing 50 µL of 2 M KOH, 2 mL of H_2O, and 0.3 g NaCl (if emulsion shaped upon mixing, sodium chloride addition is recommended) and 10 µL pentafluorobenzyl chloride in 10% toluene to the extracted sample and whisking for 2 min. Afterwards, extraction of the aqueous phase by 2 mL n-hexane. 3 mL of 98% H_2SO_4 was added to the extract, whisked for 60 times and followed with another extraction using 2 mL of n-hexane. The extract solution was evaporated until 2 mL by using nitrogen gas. 0.5 mL derivatized extract solution was measured using GC (Allmyr *et al.*, 2006).

According to Chien *et al.* (1998) and Galceran *et al.* (1995) for organic acids derivatization, whereabouts the sample extracts for organic acid/phenol derivatization, firstly evaporated using nitrogen gas in the sample. Acetone was added in order to make every sample to a volume of 500 µL. 20 µL of 10% pentafluorobenzyl bromide was added to each sample. Each extract was added by 10 mg of potassium and sonicated for 3 hours. Afterwards the extract was dried using nitrogen gas and dissolved the residue by hexane prior measured by GC.

Acylation

Acylation reduces the polarity of hydroxyl, amino and thiol groups. Compare with silylation, acylation reagents good for highly polar, multifunctional compounds, such as amino acids and carbohydrates. Acylation will convert compounds to become amides, thioesters and esters. They are shaped with acyl halide, amide and anhydride. The acyl halide will form acid as a by products, whereby that must be removed prior measured by GC (Knapp, 1979).

Fluorinated anhydrides was used as a derivatize agent and triethylamine as a catalyst. 50 µg of a sample was dissolved in 0.5 mL benzene. Afterwards, 0.1 mL of triethylamine in benzene was added by 10 µL of heptafluorobutyric anhydride into vial and heated for 15 minutes at 50^{o}C. 1 mL of a 5% aqueous ammonia solution was added, then the mixture was centrifuged for 5 minutes in order to detach the benzene layer. 1 µL of solution was measured by GC (Palmer *et al.*, 2000).

Parameters for Analytical Methods Validation

Validation method is an important part to acquire consistent, accurate and reliable data. The results of method validation are employed to judge the quality and reliability of analytical methods. The validation process was applied and also to ensure that the analytical method used for a specific test is fit for its intended use. Validation method is an evidence of the specificity, suitability and repeatability of the method. Validation is needed in order to establish the reliability of analytical results and method implementation (Wieling *et al*., 1996).

Specificity and Linearity

Specificity is the analytical method that able to separate the analyte from others that might be presence in the sample (Harris, 2007). The task is not straightforward for chromatography to ensure that the sample peak is pure or impurity, where contain more than one substance.

It is impossible to find out a number of substances present in the sample. In order to solve this problem, the peak of compound must be verified for identifying the purity. Mass spectra is more specific compare to UV spectra and commonly must be applied to assessment of chromatographic selectivity (Huber, 2007).

Linearity is how satisfactory a calibration curve follows a straight line. That is also a capability to acquire yields to concentration of analyte in sample. It can be established by dilution of a standard stock solution or distinguishingly weighing synthetic moistures of the test product compounds (Harris, 2007; Huber, 2007). Five concentrations have been recommended in order to establish linearity. The response must be good to the concentrations or analyte or proportional through well-defined mathematical calculation. A linear regression equation applied to the results must have an intercept not significantly different from zero. If a significant nonzero intercept is acquired, it must be established and has no impact on the method accuracy (Huber, 2007). Measure of linearity in equation as stated 2.1 is the square of the correlation coefficient, R^2, where that initiate the good linearity for analytical method:

$$R^2 = \frac{[\sum(x_i - x)(y_i - y)]^2}{\sum(x_i - x)^2 \sum(y_i - y)^2} \qquad \text{Equation 3.1}$$

Precision and Accuracy

Precision is the reproducibility of the results. There are 4 precisions can be applied in validation method such as 1) instrument precision is the reproducibility inspected when the similar amount of the same sample is repeatedly detected into an equipment, 2) intra-assay is evaluated by measuring sample more than one time by one person on the same day using the same instrument, 3) intermediate (ruggedness) is the different inspected when a measurement is done by different analysts, days and instruments but in the same laboratory and 4) inter laboratory precision is the most common measure of reproducibility observed

where one sample is measured by different analysts in the different laboratories (Harris, 2007). Measure of precision as stated in Equation 3.2.

$$\% \text{ RSD} = \frac{\text{standard deviation} \times 100}{\text{mean}} \qquad \text{Equation 3.2}$$

Several factors may affect reproducibility such as distinction of humidity and ambient temperature, or instrument with different characteristic such as a columns with different batches or suppliers and operators with various thoroughness and experience. Repeatability needs to be measured at least six replications injected at 100% of the concentration of analyte or at least nine replications to complete the specified range (Huber, 2007). The purpose to measure samples six until nine replicates to make sure that the analytical instrument used for this study in a good condition, owing to wide range of peak area between every analyte or sample measured can be caused a defective syringe, leaks or low carrier gas flow and also low injection port or column temperature.

Accuracy is the proximity value between the true value with the value detected in any measurement. To acquire the accuracy value such as, firstly is by comparing the yields of the developed technique with established reference method results. It presumes the uncertainty of the method of reference is known, whereas, secondly the value gauged by investigating a sample with known concentrations and the measured value is compared with the true value as provided with the material (Huber, 2007). Spiking external standard is the general technique to evaluate accuracy due to unavailable of reference materials. Spiking also confirms that the matrix remains nearly constant (Harris, 2007).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD is the lowest level of analyte in a sample which able to detect but not importantly quantitated as an exact value. The sensitivity is the method that ability to distinguish the small distinctions in concentration or mass of the test analyte. LOD in chromatography is a result

of total injection in a peak with a height at least two or three times as high as the baseline noise level (Huber, 2007).

Whereas LOQ is one of an analytical method where the lowest analyte level in a sample able to determine quantitatively with appropriate accuracy and precision. LOQ is an indicator of quantitative tests for low concentrations of analytes in sample matrices and is employed especially to determination of degradation products or impurities. That is commonly decided with measuring the concentrations of sample that has been known and establishing the lowest concentration as which the analyte can be quantified with acceptable accuracy and precision (Huber, 2007).

LOQ is specified by comparing the estimated signals of samples where known analyte low concentrations with blank sample signals. It shows that the minimum concentration at which the analyte can be reliably quantified. Every outcome from LOD and LOQ measurements should be examined by testing the samples containing the analytes at values across the two areas. This is necessary to evaluate other validation method, such as accuracy, reproducibility and precision (Huber, 2007). The LOD and LOQ are measured with Equations 3.3 and 3.4 respectively.

$$\text{LOD} = 3\frac{SD}{M} \qquad \text{Equation 3.3}$$

$$\text{LOQ} = 10\frac{SD}{M} \qquad \text{Equation 3.4}$$

SD is a standard deviation blank, whereas M is a slope of the calibration curve. The value of standard deviation of blank signal is acquired by establishing calibration curve from three concentrations.

Recovery Efficiency of Extraction Method

Recovery of known amounts of compounds that added to the samples was used to validate the analytical procedure. Good recovery reflects good quantification of the inherent

compounds of interest (Richard *et al.,* 2008). Scientists determine recovery studies of biogenic amines from seafood products as a reflection of the accuracy and reproducibility of the analytical procedure (Custodio *et al.,* 2007; Moret & Conte, 1996). The addition of a known amount of standard solution was performed in a few different ways, such as, the spike is added to the extraction solvent to the sample and the mixture was homogenized (Rogers & Straruszkiewicz, 1997). The spike is added directly to the sample followed by addition of solvent and the mixture was homogenized (Marks & Anderson, 2005) or the spike was added directly to the filtrate (Moret & Conte, 1996). Equation 3.5 is shown below, it used to measure the recovery efficiency (%).

$$\text{Recovery efficiency (\%)} = \frac{\text{peak area of spiked sample} \times 100}{\text{peak area biogenic amines standard}} \qquad \text{Equation 3.5}$$

Recovery is the measured response of spiked standard that can be stated as a percentage of the pure standard response (Bressolle *et al.,* 1996). The matrix can be measured by means of contrasting the extracted samples spiked response prior extraction with the extracted blank matrix sample response, where analyte is enhanced with similar concentration prior injected (Causon, 1997). The sufficient of LOD is achieved with satisfactory accuracy and precision, the extent of recovery must not be deemed a problem in development and validation method (Dadgar *et al.*, 1995).

Conclusion

The goals for determination of biogenic amines in food are; the potential toxicity of biogenic amines and a probability to use as indicators of food quality.

Chapter 4

Validation Procedures of Biogenic Amines

The purpose of analytical procedure is to acquire accurate, reliable and consistent data. Validation plays an important role to attain this goal. Validation comes from the Latin term *validus*, means worth/strong, which suggest that something is true, reliable and useful. The definition of validation was provided by ISO 9000:2000 as the confirmation, by means of a thorough examination and acquiring firm and realistic evidences where the procedure is effectively applicable for its intended purpose (Magnusson & Omermark, 2015; Araujo, 2009; Huber, 2007). Validation procedure should demonstrate that an analytical procedure is able to measure the correct compound with high accuracy. The analyst should comprehend the behaviour of the procedure and to establish its performance limits. Therefore, the goal of validation is to establish acceptance criteria for procedure system suitability and identify the critical parameters (Khan *et al.,* 2012; Julia *et al.,* 2011; Blanchet *et al.,* 2009).

Validation procedure is a technique to prove that an analytical procedure can be accepted for its intended purpose. Validation procedure in analytical chemistry requirements for regulatory submission include studies of linearity, dynamic range, specificity, precision, accuracy, LOD, LOQ and robustness (Harris, 2007).

Biogenic amines are polar and nonvolatile compound. Thus, before measured by other instruments particularly GC, biogenic amines must be derivatized to increase the volatility and reduce the polarity. A mixture of BSA and TMCS was used as a derivatizing reagent. However, the addition of derivatizing reagent also has disadvantage as adduced Nollet (2006). During the derivatization process, the derivatizing reagent may interfere with the

analyte and will shape by-products. Yamamoto *et al.,* (1984) proposed a procedure for determination of polyamines where the sample preparation was reproducible and easy and used GC-MS in order to verify the derivatives. GC-FID technique was validated, while GC-MS was only used for verification of derivatized biogenic amines standard. The purposes of this book as described in this chapter were to identify and confirm the structure of biogenic amines standard after derivatized with a mixture of BSA + TMCS using GC-MS and to validate a procedure for determination of biogenic amines standard using GC-FID. Validation procedure were employed for derivatized biogenic amines standards such as specificity, linearity, accuracy, precision, LOD and LOQ. Recovery study was also applied to determine whether the procedure is feasible or otherwise.

Five biogenic amines standard, such as heptylamine, histamine, tyramine, Cadaverine and spermidine and also a mixture of BSA + TMCS as a derivatize agent were purchased from Sigma-Aldrich. HPLC grade water, methanol, dichloromethane, Whatman filter paper, Erlenmeyer (Pyrex) 250 mL, Beaker glass (Pyrex) 100 mL, graduated cylinder (Pyrex) 10 mL, funnel, vial 1 mL, syringe (Hamilton) 1 µL were obtained from UNIMAS laboratory.

Derivatization Procedure

The derivatization of biogenic amines was performed at room temperature according to procedure outlined by Szyrwinska *et al.* (2007). Precisely 200 µL mixture of biogenic amines standard solution in a vial was evaporated until dry using nitrogen gas. Approximately a 100 µL mixture of BSA+TMCS solution was added into the vial and heated for 30 minutes at 80^{o}C then was cooled. Afterwards, the derivatized solution was then dried and the residue was re-dissolved in 100 µL of dichloromethane.

GC Measurement for Biogenic Amines Standard Preparation of Stock Biogenic Amines Standard Solutions

Stock solutions for each biogenic amines standard were prepared by dissolving approximately 500 mg of each biogenic amines standard in 50 mL HPLC grade water. A stock solution of 10000 µg/mL of each biogenic amines standard was diluted to 500 µg/mL. A mixture of 500 µg/mL biogenic amines standard solutions was stored in the freezer until further measurement.

Preparation of Biogenic Amines Calibration Standard Solutions

A mixture of 500 µg/mL biogenic amines standard solution was derivatized based on the derivatization procedure, namely silylation procedure. Five different concentrations were prepared from a 500 µg/mL derivatized biogenic amines standard in the range concentrations: 150, 100, 75, 50 and 25 µg/mL.

Optimization of Gas Chromatographic Approach

Derivatized mixture of biogenic amines was analyzed using two units of gas chromatography coupled into different detector, flame ionization detector (FID) and mass spectrometer (MS). The GC-FID and GC-MS measurement were carried out using separation on similar temperature program. GC-FID was carried out on HP-5 capillary column while GC- MS was performed on BPX-5 capillary column. These columns have stationary phases with different polarity.

GC-FID Measurement

Measurement of derivatized biogenic amines standard was performed on a Hewlett Packard model 6890 gas chromatography and equipped with a FID and splitless injector. A mixture of derivatised biogenic amines were separated on HP-5 (30 m x 0.25 mm x 0.25 µm stationary phase thickness) capillary column and hydrogen was used as the carrier gas. The initial oven temperature was programmed at 110°C maintained for 2 min, increased to 190^{o}C at the rate of 5^{o}C/min and held at 190^{o}C for 2 min. Exactly 1 µL of sample was injected onto the column using micro-syringe with splitless injection mode. Prior to measurement, GC-FID capillary column was conditioned at 300^{o}C overnight to ensure that no retained compounds at high temperature range of column. Measurement on blank sample was performed before any sample injected in order to confirm that the instrument system was uncontaminated and stable.

GC-MS Measurement

GC-MS measurement of derivatized biogenic amines standard was conducted on a Shimadzu GC-MS model QP5000 Plus equipped with a quadrupole mass spectrometer. The separation was carried out on the BPX-5 column (30 m x 0.25 mm x 0.25 µm stationary phase) with helium as the carrier gas. Sample was injected onto the column in splitless inject mode with the injector maintained the temperature at 280^{o}C. The initial temperature was set at 100°C held for 2 min, increased to 190°C at the rate of 5^{o}C/min and held for 2 min at the 190°C. Exactly 1 µL of 100 µg/mL mixture of derivatized biogenic amines solution was injected into GC-MS.

Validation of GC-FID Procedure for Detection of Derivatized Biogenic Amines Standard

Validation of GC-FID techniques for determination of biogenic amines was assessed on several parameters such as specificity, linearity, accuracy, precision, LOD and LOQ.

Specificity and Linearity

Specificity was checked by measuring derivatized biogenic amines standard using GC- FID. Five biogenic amines standard were measured, thus five peaks in GC-FID should be significantly detected. Specificity is to distinguish the peaks in GC-FID chromatogram which corresponded to derivatized biogenic amines standard from other peaks that might also be detected in the sample.

Linearity was assessed by measuring five different concentrations of derivatized biogenic amines standard (150, 100, 75, 50 and 25 µg/mL) on GC-FID. Measurement for each concentration was measured in six replicates. Linearity was evaluated by construction of plot of peak area versus concentration.

Precision and Accuracy

Precision was verified based on six replicates of measurement of derivatized biogenic amines standard solutions and the relative standard deviation of every derivatized biogenic amines standard solutions were then calculated. The precision of this procedure evaluated by measuring a derivatized biogenic amines standard in six replicates for each concentration and standard deviation (SD) were reported. The accuracy was assessed using relative standard deviation (% RSD) of the peak area. The precision and accuracy value were calculated using equation 3.1 and 3.2, respectively.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD is the lowest concentration of an analyte in a sample which can be detected but not importantly quantitated as an exact value. In order to determine the value of LOD and LOQ, the concentrations of derivatized biogenic amines standard in the lower part of the linear range of the calibration curve was used, such as 25, 50 and 75 µg/mL. The values of LOD and LOQ were calculated using equation 3.3 and 3.4, respectively.

Validation of Analytical Procedure for Extraction of Biogenic Amines from Fish Sample

Validation of analytical procedure was performed to determine whether the procedure of extraction was eligible or otherwise. Using recovery study in order to validate the analytical procedure, spiking a particular amount of biogenic amines to fish muscle and then recovered its by extracting with a particular solvent. The extract was then derivatised prior analyzed using GC-FID. Muscle of fish raw mackerel (*Scomberomorus guttatus*) was used as a matrix due to raw mackerel had good structure of muscle. The extraction procedure was performed according to Nakovich (2003) with slight modification. Approximately 5 grams of fish muscle was chopped and placed into an Erlenmeyer flask. Afterwards, fish muscle was spiked with 1 mL of 100 µg/mL biogenic amines standard solution and homogenized for 1 min. The sample was then dissolved with 10 mL mixture of methanol 99.8% and 10 mL HPLC grade water. The solution was then sonicated by sonicator for 20 min with temperature at 45^{o}C. The extract was cooled to 30^{o}C and filtered with Whatman filter paper (15) into vial. Exactly 200 µL of the supernatant was derivatized using derivatizing agent as described in Section 3.2.3. The procedure for derivatizing of the fish sample has similar steps as done for derivatizing derivatized biogenic amines standard. 1 µL from derivatized extract fish was injected into the GC-FID using microsyringe. The equation 3.5 was used to calculate recovery efficiency.

Biogenic amines formed in food via decarboxylation of specific free amino acids by

exogenous decarboxylase released by microbial species associated with food and by transamination of aldehyde and ketone (Karovicova & Kohajdova, 2005). High biogenic amines contents in food can cause psychoactive and vasoactive health problems, such as hypo- or hypertension, respiratory distress, headache, nausea, sweating, bright red rash and heart palpitation (Moon *et al.,* 2010). The presence of biogenic amines in food owing to improper salt maturation or fermentating stage, poor handling of the raw materials and the condition of storage (Kose, 2010). Histamine poisoning is the major problem hygiene linked with the high level of biogenic amines in fish. Scombroid fish species such as mackerel, bonito and tuna, furthermore for non-scombroid fish species such as salmon, sardine, mahi-mahi and herring have histidine in their muscle (Richard *et al.,* 2008). Several countries have established regulations and maximum permission level of biogenic amines, particularly histamine. The European Union (EU) has suggested the level of histamine in scombridae fish should be below than 100 mg/kg (Bilgin & Genccelep, 2015). FDA guidelines for testing fish samples has set the maximum permissible for histamine concentration in fish is 50 mg/kg (FDA, 2011). Histamine is the one that responsible for scombroid poisoning, but it should be considered owing to the presence of cadaverine and putrescine can increase the toxicity of histamine (Zaman *et al.,* 2010; Emborg & Dalgaard, 2006). Several procedure have been developing to control biogenic amines production such as, irradiation, microbial growth reduction by freezing, control atmosphere packaging, hydrostatic pressures and preservatives addition. (Latorre-Moratalla *et al.,* 2012), yet these procedure also have disadvantage effects such as decrement in the food functionality, nutritional value of food and organoleptic properties (Eom *et al.,* 2015).

The extraction of amines from real matrices is the most critical in terms of acquiring recoveries for all biogenic amines. However, determination of biogenic amines is difficult owing to the complexity of the real matrices to be analyzed. In analytical process, derivatization process is time consuming, so it is important to develop a sensitive, faster and simpler analytical procedure to determination of biogenic amines in food (Onal, 2007). The objectives for this book were to extract biogenic amines from fish and fish product samples where was following the recovery study and to qualitatively and quantitatively measurement the biogenic amines in fish and fish product samples using GC-FID.

Fish and Fish Product Samples

Three types of samples were investigated; raw and salted fish samples were purchased from Riyal Market at Kota Samarahan and canned fish products purchased from Everrise supermarket at Kota Samarahan.

Raw Fish Samples

Five raw fish samples that have been analyzed were mackerel (*Scomberomorus guttatus*), sardine (*Sardinella gibbosa*), toli shad (*Tenualosa toli*), whiptail stingray (*Himantura walga*) and gourami (*Trichogaster pectoralis*). Figure 4.1 shows the photos of five raw fish samples studied.

(a)

(b)

(c)

(d)

(e)

Figure 4.1 Raw fish samples (a) sardine (*S. gibbosa*), (b) mackerel (*S. guttatus*), (c) gourami (*T. pectoralis*), (d) toli shad (*T. toli*) and (e) whiptail stingray (*H. walga*) were analyzed for determination of biogenic amines using GC-FID and GC-MS

Salted Fish Products

Figure 4.2 shows the photos of five salted fish products such as mackerel (*S. guttatus*), sardine (*S. gibbosa*), toli shad (*T. toli*), whiptail stingray (*H. walga*) and gourami (*T. pectoralis*) were analyzed.

(a)

(b)

(c)

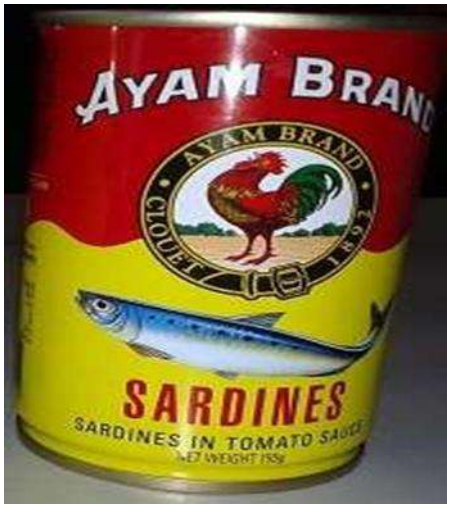
(d)

(e)

Figure 4.2 Salted fish products (a) sardine (*S. gibbosa*), (b) mackerel (*S. guttatus*), (c) gourami (*T. pectoralis*), (d) toli shad (*T. toli*) and (e) whiptail stingray (*H. walga*) were investigated to determine the level of biogenic amines using GC-FID and GC-MS

Canned Fish Products

Four pictures of canned fish products were measured to determine the biogenic amines concentration were canned mackerel (*Scomberomorus* sp.) (ayam brand – the expiry date: March 2017), sardine (*Sardinella* sp.) (ayam brand– the expiry date: September 2017), salted- sardine (*Sardinella* sp.) (seafresh brand– the expiry date: January 2017) and dace (*Leuciscus leucisus*) (tan lung brand– the expiry date: June 2017) as shown in Figure 4.3.



(a)

(b)

(c) **(d)**

Figure 4.3 Canned fish products (a) sardine (*Sardinella* sp.), (b) salted-sardine (*Sardinella* sp.), (c) makcerel (*Scomberomorus* sp.) and (d) dace (*L. leucisus*) used to determine the level of biogenic amines using GC-FID and GC-MS

Application Validated Procedure for Determination of Biogenic Amines in Fish and Fish Product Samples

The optimized extraction procedure have been described in Chapter 3 where all fish and fish product samples were extracted using HPLC grade water and methanol. The extract was then derivatized using silylation procedure followed the procedure that has been described in Chapter 3. Derivatizing reagent used was a 100 µL mixture of BSA + TMCS. Each derivatized extract of fish and fish product samples were measured in six replicates using GC-FID to obtain quantitative data and were measured one time using GC-MS to acquire qualitative data.

Measurement derivatized of extract fish and fish product sample were performed using GC-FID Hewlett Packard model 6890 equipped with HP-5 capillary column (30m x 0.25 mm x 0.25 µm). The initial temperature of GC-FID was 110^{o}C held for 2 minutes and elevated to 190^{o}C, rate of 5^{o}C/min and held for 2 minutes at 190^{o}C and the carrier gas was hydrogen. Fourteen fish samples were used and measured in this study consist of five raw and five salted fish such as mackerel (*S. guttatus*), sardine (*S. gibbosa*), gourami (*T. pectoralis*), whiptail stingray (*H. walga*) and toli shad (*T.toli*) and four canned fish products were

mackerel (*Scomberomorus* sp.), sardine (*Sardinella* sp.), salted sardine (*Sardinella* sp.) and dace (*L. leuciscus*).

Measurement on blank sample was performed before every derivatized extract fish and fish product samples being measured in order to ensure that the GC-FID instrument was stable and uncontaminated. Each derivatized extract fish and fish product samples also measured using GC-MS to identify the analytes in samples. Optimization for GC-MS measurement of derivatized extract fish and fish product samples were followed the procedure that has been described in Chapter 3.

Validation procedures of biogenic amines on fish and fish products samples were determined using the calibration equations of derivatized biogenic amines standard and the occurrence of histamine in fish and fish product samples was also assessed.

Conclusion

The purpose of analytical procedure is to acquire accurate, reliable and consistent data. Validation plays an important role to attain this goal. Therefore, the goal of validation is to establish acceptance criteria for procedure system suitability and identify the critical parameters. The purposes of this book is to identify and confirm the structure of biogenic amines standard after derivatized with a mixture of BSA + TMCS using GC-MS and to validate a procedure for determination of biogenic amines standard using GC-FID. Validation procedure were employed for derivatized biogenic amines standards such as specificity, linearity, accuracy, precision, LOD and LOQ. Recovery study was also applied to determine whether the procedure is feasible or otherwise.

Chapter 5

Results of Validation Procedures I

Biogenic amines was derivatized before measured using Gas Chromatography-Mass Spectrometry (GC-MS) and GC-FID. This step is necessary owing to the polarity and volatility the biogenic amines, thus the purpose of derivatize biogenic amines standard was to enable the analyte can be detected by GC.

Figure 5.1 shows a GC-MS chromatogram of 100 µg/mL derivatized biogenic amines standard where 10 significant peaks were appeared in the GC-MS chromatogram. However, only 5 peaks are corresponded to the biogenic amines standard. The first peak was heptylamine at retention time 5.091 min and then for the second, third and fourth were histamine, tyramine and cadaverine at retention time of 10.718, 11.770 and 12.274 min, respectively. Spermidine was appeared at the tenth peak with retention time 19.241 min. Several peaks were considered as by-products also appeared at peaks fifth, sixth seventh, eighth and ninth for retention times 13.025, 14.720, 15.530, 16.295 and 17.030 min, respectively and based on the library spectra those can be benzene ethanamine, n – butylamine, ethyl ester, acetic acid and 1,4 butane diamine, respectively.

Table 5.1 shows the retention times of five derivatized biogenic amines standard after measured using GC-MS, while Figure 5.2 shows a GC-MS chromatogram for derivatized blank samples where several peaks were appeared in the chromatogram but none of them was identified as derivatized bioganic amine.

Table 5.1 Retention times for derivatized biogenic amines measured by GC – MS

Biogenic amine	Retention time (minute)
Heptylamine	5.091
Histamine	10.718
Tyramine	11.770
Cadaverine	12.274
Spermidine	19.241

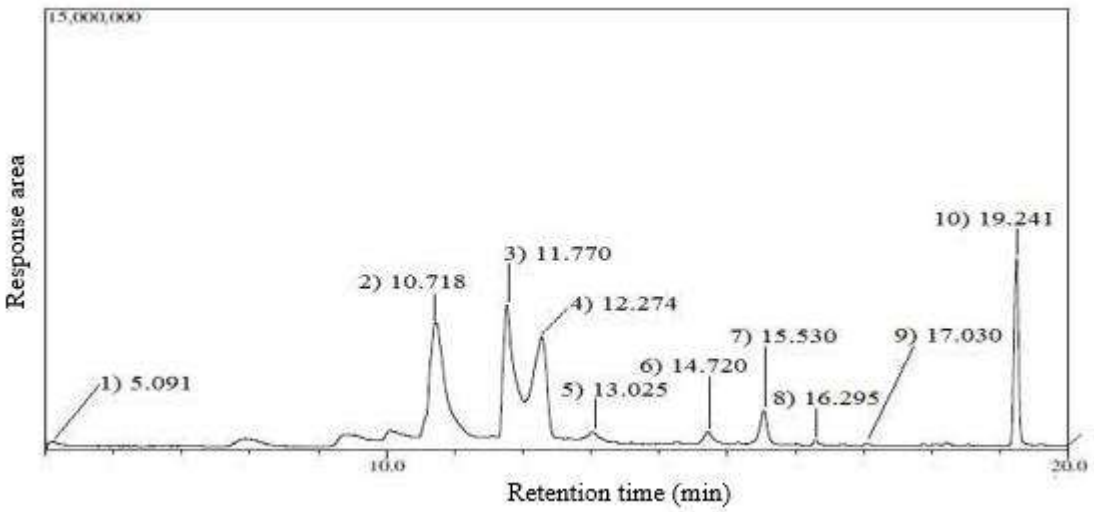


Figure 5.1 Gas chromatrogram traced by GC-MS for concentration of derivatized 100 µg/mL biogenic amines standard

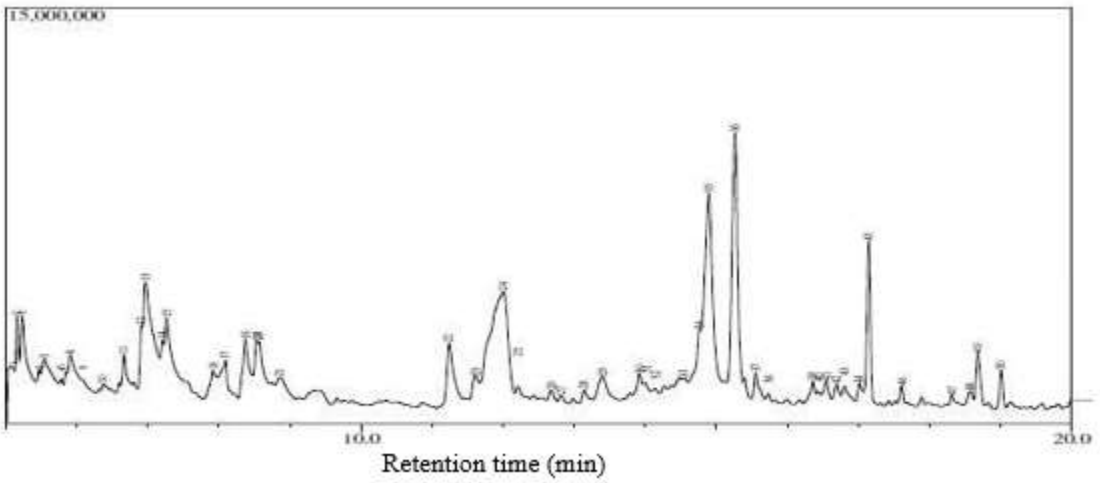


Figure 5.2 Gas chromatrogram traced by GC-MS for derivatized blank sample

FID was a detection technique that lacks of the capability to confirm peak identity where it relies on the comparison between the retention times of both samples and derivatized biogenic amines standard. Furthermore, in this study GC-MS was used to identify and confirm the structure of derivatized biogenic amines standard, because the use of derivatize agent produced by-product together with the derivatized biogenic amines standard itself. Mass spectrometer is an excellent qualitative technique that providing confirmatory measurement guidelines to investigate the presence or absence of analyte(s) in a sample. Measurement of derivatized biogenic amines standard by GC-MS is very important to ensure that the derivatization of biogenic amines are actually exist or otherwise.

Mass Spectra of Derivatized Biogenic Amines Standard

Figures 5.3 to 5.7 show the mass spectra for five derivatized biogenic amines standard such as, hepthylamine, histamine, tyramine, cadaverine and spermidine, respectively. The molecular and fragment ions of derivatised biogenic amines are shown in Tables 5.2 – 5.6, respectively. Figure 5.3 shows electron impact of mass spectrum for derivatized heptylamine while Table 5.2 shows the structures for molecular and fragment ions in mass spectrum of derivatized heptylamine.

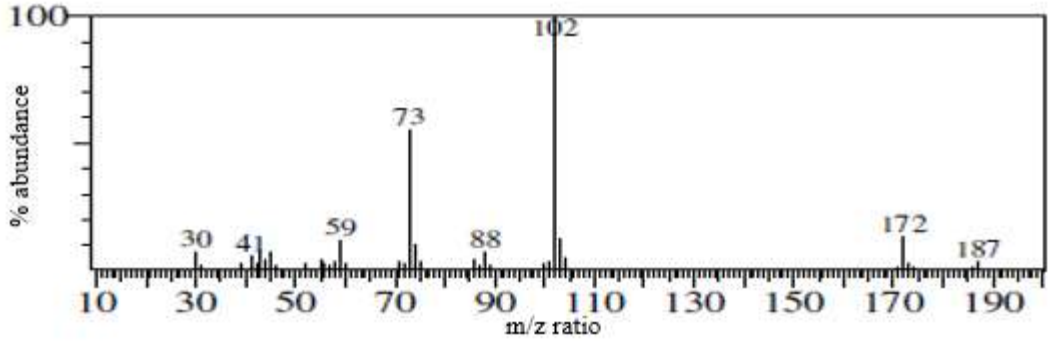
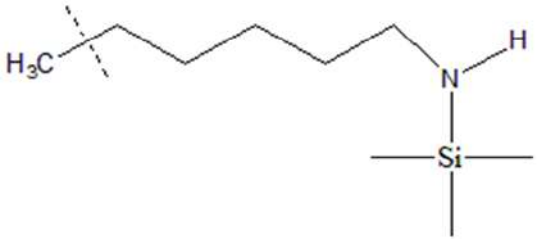
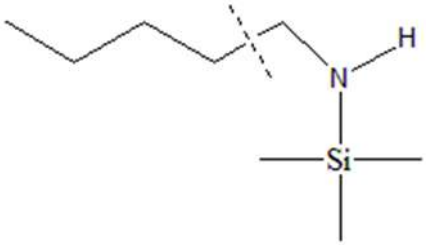
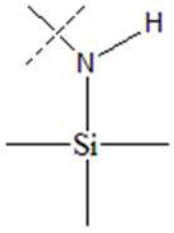
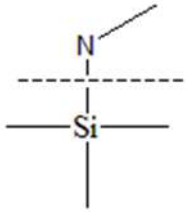
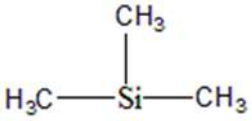


Figure 5.3 Mass spectrum of derivatized heptylamine

Table 5.2 Molecular and fragment ions in mass spectrum of derivatzied heptylamine

Ion	m/z	Suggested structure
Molecular ion	187	
Fragment ion	172	
Fragment ion	102	
Fragment ion	88	
Derivatizing reagent ion	73	

The mass spectrum shown in Figure 5.3 indicate the molecular ion (m/z 187) with four significant fragment ions observed at 172, 102, 88 and 73. The loss of CH_3 (methyl) from a molecular ion m/z = 187 resulted a fragment ion with m/z = 172 and the loss of C_4H_9 (butyl)

resulted a fragment ion with m/z = 102. The base peak was also observed m/z = 102, which assigned as N,1,1,1 – tetramethylsilanamine.

Figure 5.4 shows the electron impact (EI) mass spectrum for derivatised histamine and the structure of molecular ion and several fragment ions are listed in Table 5.3.

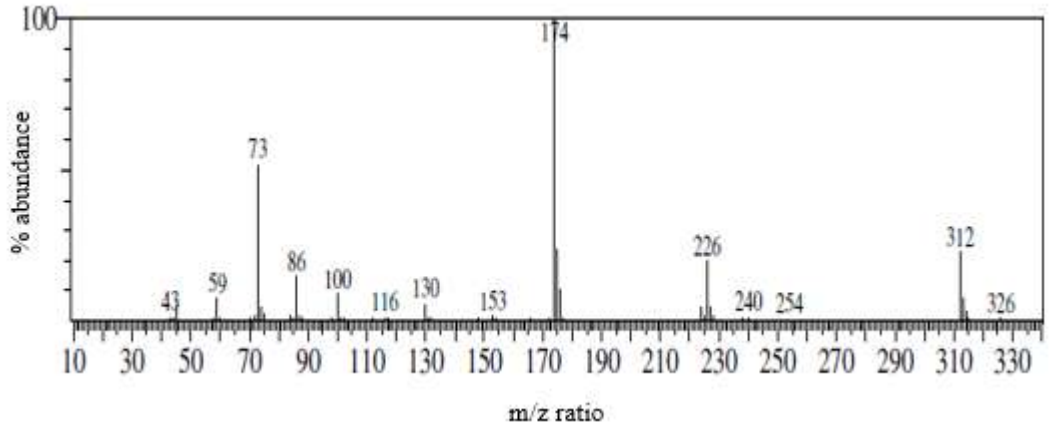
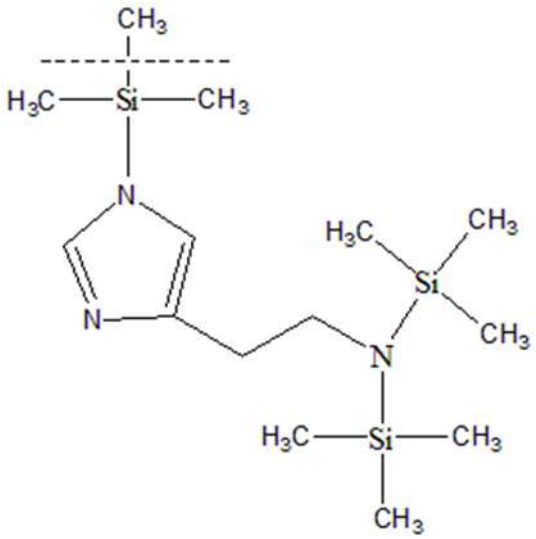
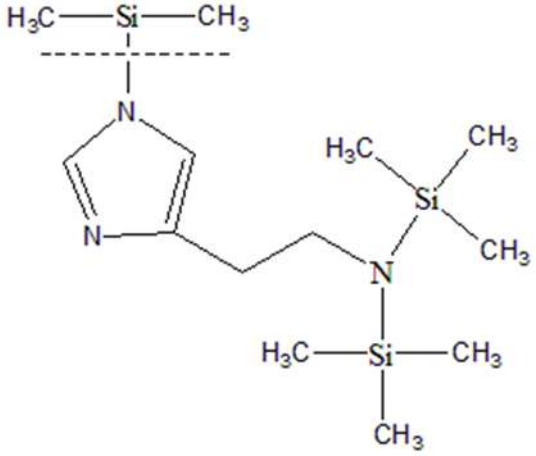
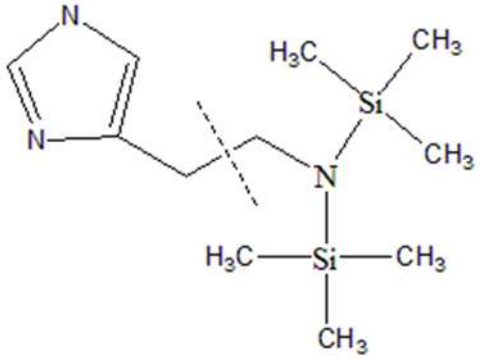


Figure 5.4 Mass spectrum of derivatized histamine

Figure 5.4 shows the electron impact (EI) mass spectrum for derivatised histamine and the structure of molecular ion and several fragment ions are listed in Table 5.3.

Mass spectrum of histamine derivatized by BSA+TMSC has a molecular ion with m/z = 326 which can be seen in mass spectrum. The loss of CH_3 (methyl) from a molecular ion (m/z=326) resulted a fragment ion with m/z = 312. The loss of C_2H_6Si (dimethylsilylane) from a fragment ion with m/z = 312 resulted a base peak with m/z = 174. Osorio *et al.* (2011) stated the molecular ion is not be the most abundant ion or even not appear in the mass spectrum. Other fragment ions with structural information are shown in Table 5.3. The base peak was observed at m/z = 174 which correspond to $C_7H_{21}NSi_2$ or N,1,1,1 – tetramethyl-N (trimethylsilyl) silanamine.

Table 5.3 Molecular and fragment ions in mass spectrum of derivatized histamine

Ion	m/z	Suggested structure
Molecular ion	326	
Fragment ion	312	
Fragment ion	254	

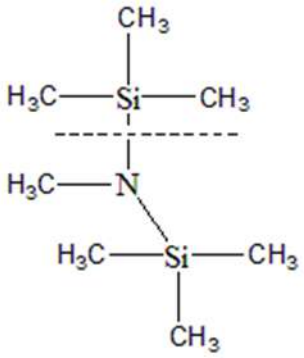
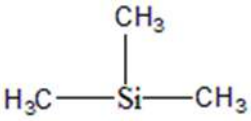
Fragment ion	174	
Derivatizing reagent ion	73	

Figure 5.5 shows mass spectrum of derivatized tyramine, while Table 3.4 listed molecular and fragment ions and their structure.

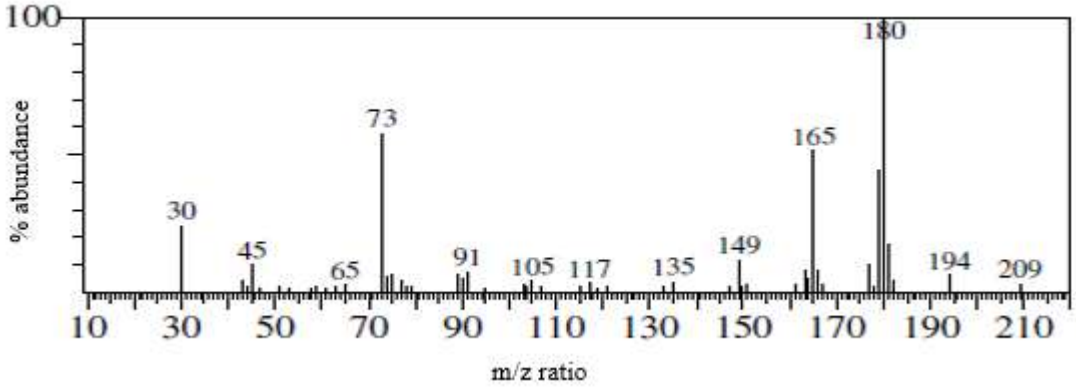
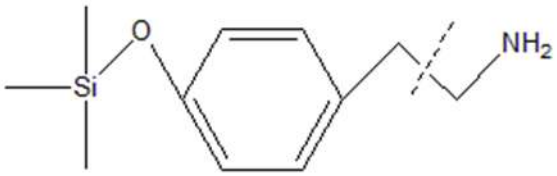
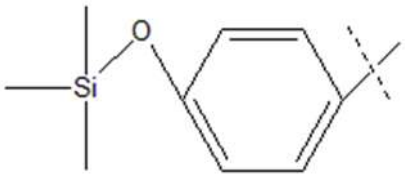
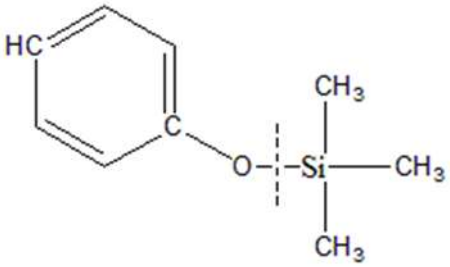
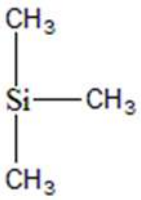


Figure 5.5 Mass spectrum of derivatized tyramine

Table 5.4 Molecular and fragment ions in mass spectrum of derivatized tyramine

Ion	m/z	Suggested structure
Molecular ion	209	
Fragment ion	180	
Fragment ion	165	
Derivatizing reagent ion	73	

The base peak for derivatized tyramine was observed at m/z = 180 in mass spectrum which is corresponded to $C_{10}H_{18}OSi$ or trimethyl (4 – methylphenoxy)silane. Not all ions in mass spectrum for derivatized tyramine were fragmented, which have m/z at 209, 180 (base peak), 165 and 73. The loss of CH_4N (aminomethyl) from a molecular ion with m/z = 209 resulted a base peak with m/z = 180. Further loss of CH_3 (methyl) from base peak ion with m/z = 180 resulted a fragment ion with m/z = 165.

Figure 5.6 has shown the molecular and several ions which can be considered. The

molecular and fragments ions for cadaverine derivative are listed in Table 5.5.

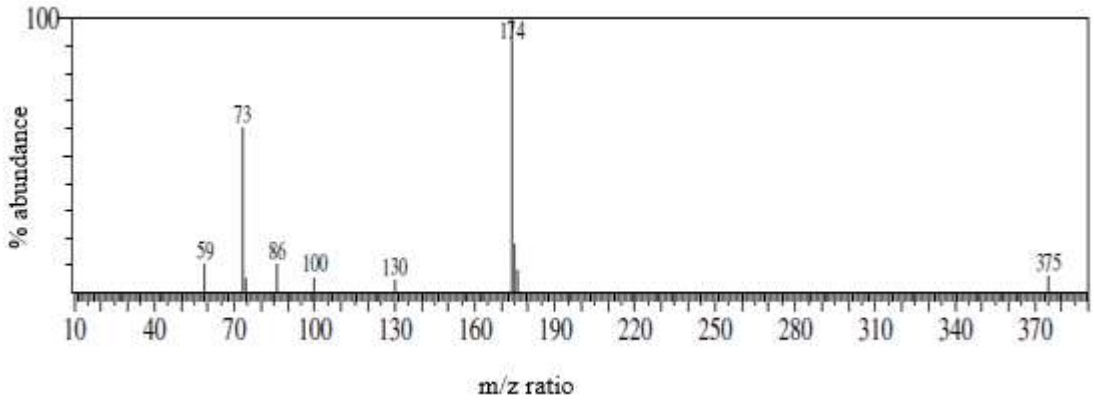
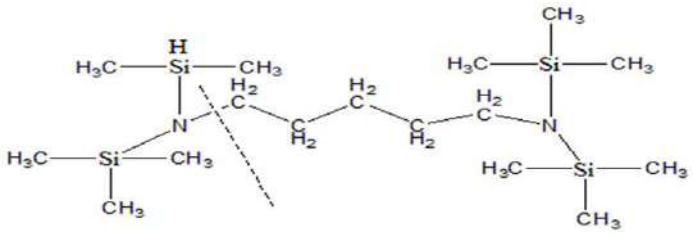


Figure 5.6 Mass spectrum of derivatized cadaverine

The base peak of mass spectrum for cadaverine is at m/z = 174 similar to histamine which corresponds to $C_7H_{21}NSi_2$ or N,1,1,1 – tetramethyl- N (trimethylsilyl) silanamine.The molecular ion at m/z = 375 and for fragmentat ions at m/z 174 (base peak) and 73, where that can be seen in Table 5.5 where all ions has fragmented very well.

Table 5.5 Molecular and fragment ions in mass spectrum of derivatized cadaverine

Ion	m/z	Suggested structure
Molecular ion	375	

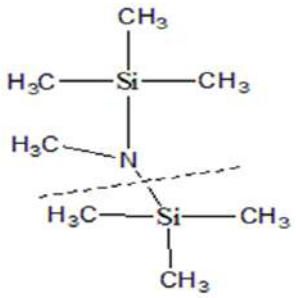
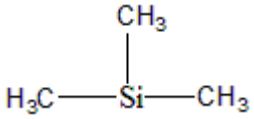
Fragment ion	174	
Derivatizing reagent ion	73	

Figure 5.7 has shown the several ions which can be considered. The ions for derivatized spermidine are listed in Table 3.6.

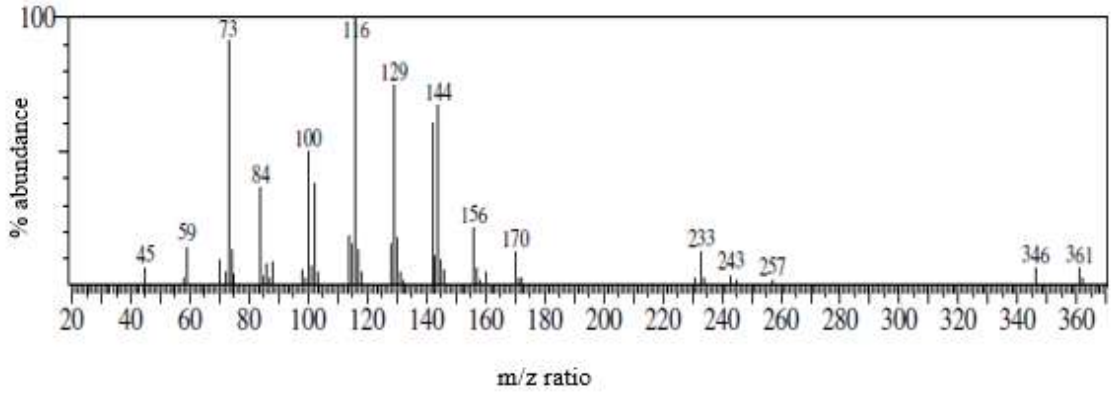
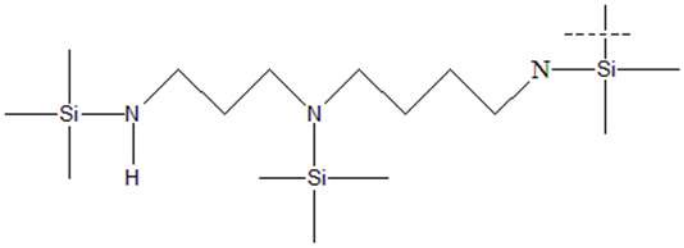
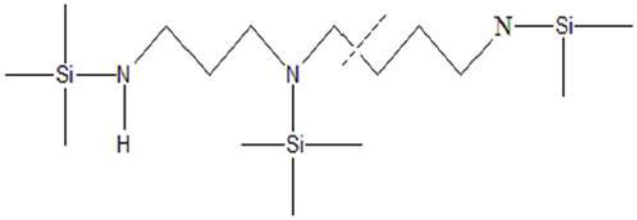
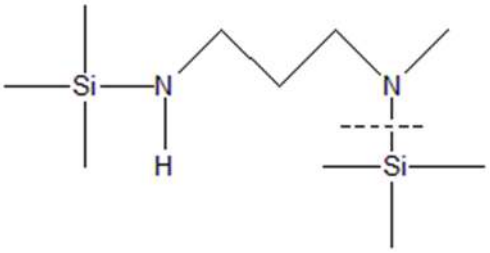
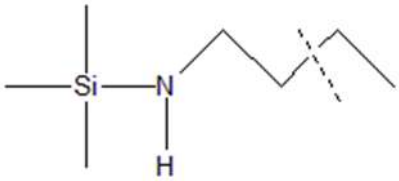
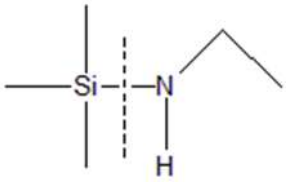
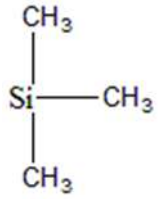


Figure 5.7 Mass spectrum of derivatized spermidine

Table 5.6 Molecular and fragment ions in mass spectrum of derivatized spermidine

Ion	m/z	Suggested structure
Molecular ion	361	
Fragment ion	346	
Fragment ion	233	
Fragment ion	144	
Fragment ion	116	
Derivatizing reagent ion	73	

The base peak ion was observed at m/z = 116 which considered as $C_5H_{15}Nsi$ (trimethylsilyl – N,N-dimethylamide). The molecular ion was observed at m/z = 361 in mass spectrum of spermidine and fragment ions at m/z 233, 144, 116 (base peak) and 73 that can be seen in Table 3.6. The loss of CH_3 (methyl) from molecular ion with m/z = 361 resulted a fragment ion with m/z = 346. Further loss of $C_6H_{16}NSi$ (1, 1, 1 – trimethyl – N – propylsilanamine) in fragment ion (m/z = 346) resulted a fragment ion with m/z = 233. While, loss of C_2H_5 (ethyl) from fragment ion with m/z = 144 resulted a fragment ion (base peak) with fragment ion at m/z = 116.

Gas Chromatography- Flame Ionization Detector (GC-FID) Measurement of Derivatized Biogenic Amines Standard

A total of 5 different concentrations of derivatized biogenic amines were prepared at 150, 100, 75, 50 and 25 µg/mL together with a derivatized blank sample. All derivatized biogenic amines standard were measured using GC-FID. The blank sample was prepared by preparing a solvent of HPLC-grade water without addition of biogenic amines standard solution and then was derivatized with a mixture of BSA + TMCS.

Figure 5.8 shows the GC-FID chromatogram of 100 µg/mL of derivatized biogenic amines. The retention times for individual derivatized biogenic amines are listed in Table 5.7. Several peaks were appeared in gas chromatogram of derivatized blank sample which can be seen in Figure 3.9 at retention time of 7.308, 10.835, 11.33, 15.091 and 17.001 min. However, these peaks did not match to the retention times of derivatized biogenic amines peaks.

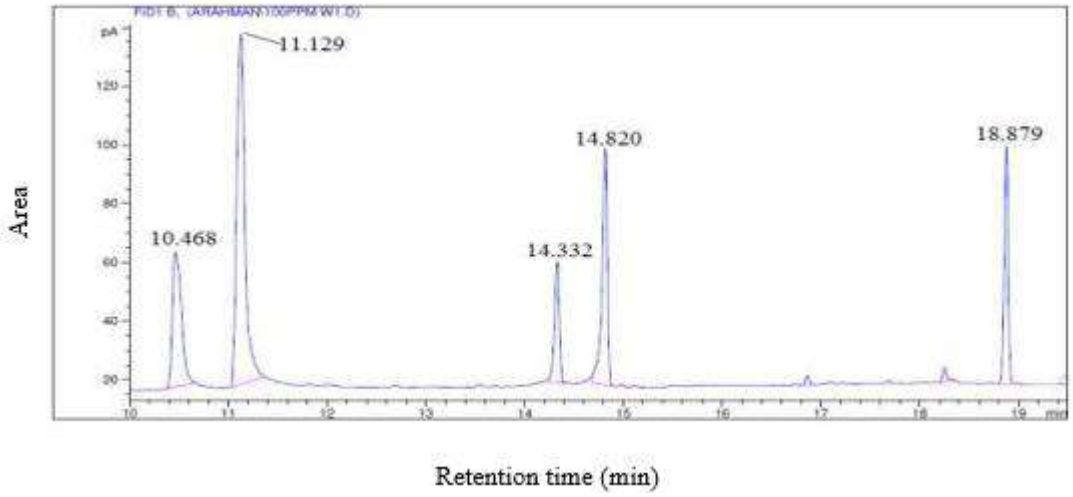


Figure 5.8 GC-FID chromatogram of 100 µg/mL derivatized biogenic amines standard

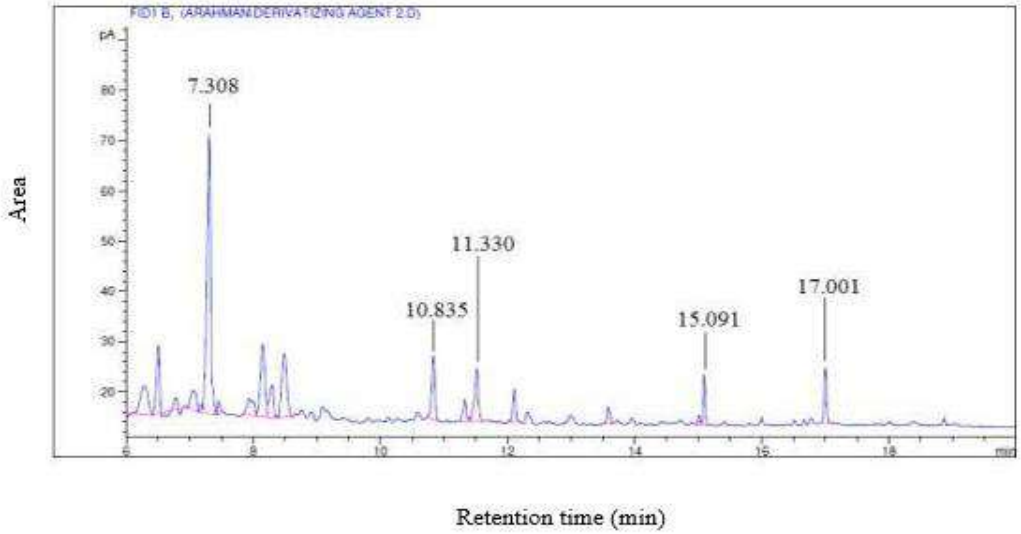


Figure 5.9 GC-FID chromatogram of derivatized blank sample

Table 5.7 The retention times of derivatized biogenic amines analyzed by GC – FID

No.	Biogenic amines	Retention time (n = 6)
1	Heptylamine	10.46 ± 0.12
2	Histamine	11.13 ± 0.08
3	Tyramine	14.33 ± 0.11
4	Cadaverine	14.81 ± 0.15
5	Spermidine	18.88 ± 0.11

Table 3.7 shows the precision of retention time of derivatized biogenic amines standard analyzed by GC-FID. This was to verify whether the range of retention time can be accepted for the determination of biogenic amines in fish samples using GC-FID. The retention time of heptylamine was obtained at 10.46±0.12 min which means the retention time between 10.34 – 10.58 min is deemed as heptylamine. Histamine retention time was acquired at 11.13±0.08 min that means the retention time between 11.05 – 11.21 min was can be agreed and considered as histamine. Tyramine retention time was acquired 14.33±0.11 min which means the range of retention agreed is 14.22 – 14.44 min. The retention time of cadaverine was obtained at 14.81±0.15 min that means the retention time between 14.66 – 14.96 min was considered as cadaverine. Lastly for spermidine where the retention time was acquired at 18.88±0.11 min which means the range of retention time between 18.77 – 18.99 min can be considered as spermidine.

Conclusion

Determination of biogenic amines using GC-FID was successfully validated. Good specifity was considered in this study, where five derivatized biogenic amines standard appeared in GC-FID chromatogram without any impurity peaks.

Chapter 6

Results of Validation Procedures II

Specificity and Linearity

Good specificity was obtained as can be seen in gas chromatogram (Figure 5.8) where five significant related peaks of biogenic amines appeared without impurities peaks. Five peaks emerged at retention time of 10.47, 11.13, 14.33, 14.82 and 18.88 min were derivatized biogenic amines which corresponded to heptylamine, histamine, tyramine, cadaverine and spermidine, respectively.

All calibration curves of derivatized biogenic amines standard were evaluated for concentration ranged between 25-150 µg/mL. Measurement was performed in six replicates and calibration curves were constructed using a Microsoft Excel. The correlation coefficient for calibration curve should be 0.95 or greater (FDA, 2000). The calibration curves heptylamine, histamine, tyramine, cadaverine and spermidine are depicted in Figures 3.10 – 3.12, respectively.

Good linearity with good correlation coefficients were obtained for derivatized biogenic amines standard. R^2 values were 0.9995, 0.9999, 0.9999, 0.9998 and 0.9998 for heptylamine, histamine, tyramine, cadaverine and spermidine, respectively. The linearity data can be assessed by constructing the y-intercept of the linear regression line and correlation coefficient for the plot of peak area versus concentration. Green (1996) adduced that a correlation coefficient of >0.9999 is commonly deemed as proven that data is satisfactory to the regression line.

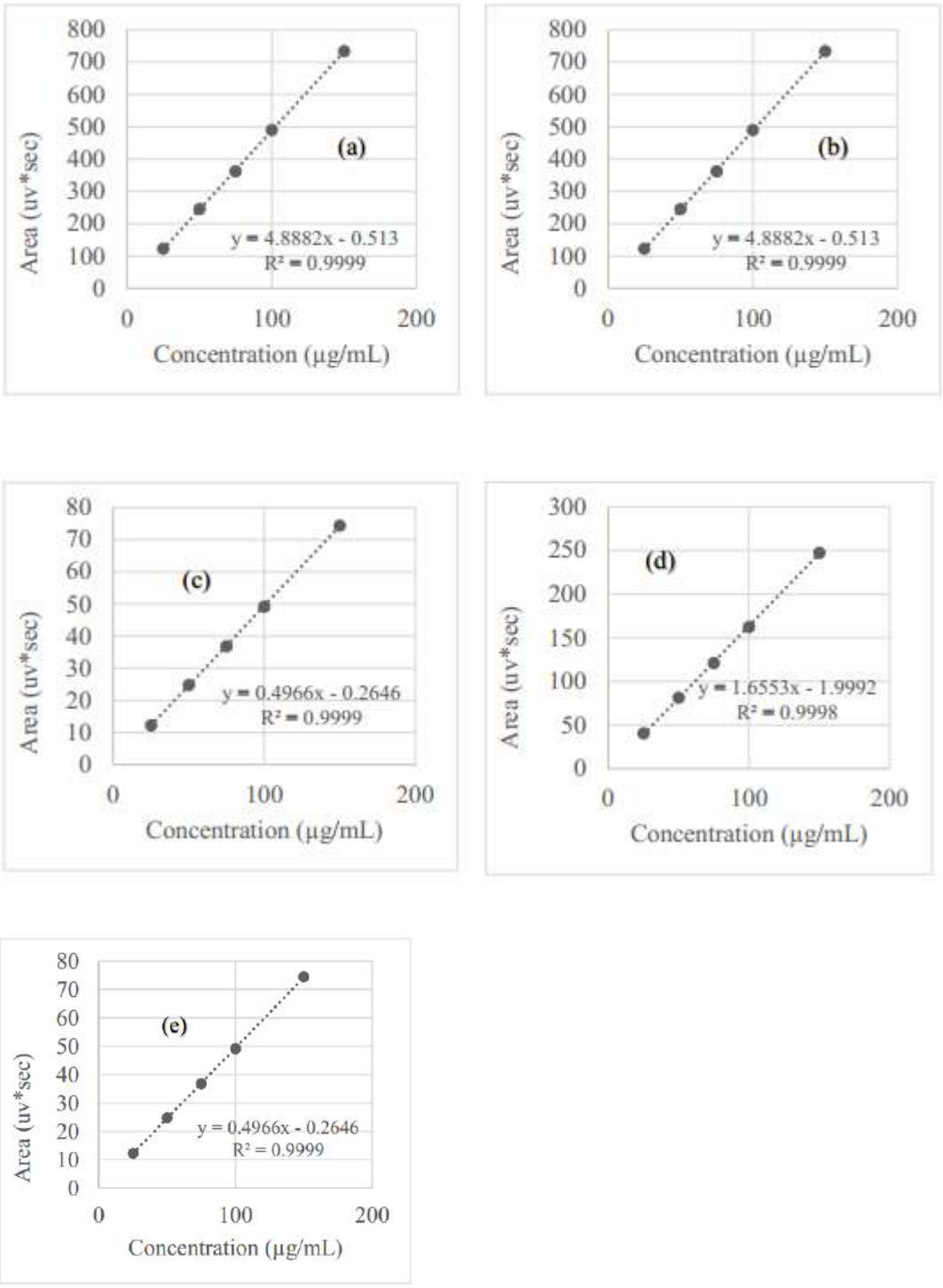


Figure 6.1 Calibration curve for GC-FID analysis of (a) derivatized heptylamine, (b) derivatized histamine, (c) derivatized tyramine, (d) derivatized cadaverine and (e) derivatized spermidine

Assessment on the linearity of calibration curve is required to testify the acceptability of analytical procedure (Green *et al.*, 1996). Linearity can be assessed based on correlation coefficient (R^2) which is used as linearity measure of the calibration curve (Mohammed *et al.*, 2014). The International Council for Harmonization (ICH) has recommended the linearity curve of the y-intercept, correlation coefficient, slope of the regression line and residual sum of squares are required for accuracy. A plot data must be included in the report. The analytical response must be described by a suitable function of the analyte concentration in a sample. Minimum five concentrations are needed in order to establish the linearity (Huber, 2007).

Precision and Accuracy

The values of precision and accuracy listed in Table 6.1 demonstrates the precision and accuracy values based on their peak areas for six replicates analysis of derivatized biogenic amines standard using GC-FID were considered accurate and precise. The estimation of the precision for the GC-FID method is very necessary to accomplish quantitative data.

The lack of a precision in retention time can lead to an erroneous identification. The precision of retention time in GC is controlled by both carrier flow and oven temperature. Area precision determines the degree of accuracy of a quantitative measurement. Even though there is no connection between retention and area precision, but they can be affected by several of the same variables (Agilent Technologies, 2005).

Good precision and accuracy was obtained for peak area, where all %RSD were below 7.87%. Table 3.8 shows the C.V. values of derivatized biogenic amines standard that show significant degradation from the lowest concentration to highest concentration. The C.V. values of derivatized biogenic amines standard such as, heptylamine, histamine, tyramine, cadaverine and spermidine at concentration 25 µg/mL were 3.75, 3.66, 7.87, 5.98 and 5.11% respectively. While at concentration 150 µg/mL, the C.V. values were 0.92, 0.78, 2.01, 1.36 and 0.79%, respectively. This can be deduced, for the high concentration, the error can be minimalized during measured using GC-FID.

Table 6.1 The precision and accuracy value of derivatized biogenic amines standard at different concentrations based on peak area (n = 6)

Biogenic amines	**Concentrations (µg/mL)**				
	25	**50**	**75**	**100**	**150**
Heptylamine	44.77 ± 1.68	90.27 ± 2.83	134.57 ± 3.37	183.26 ± 4.06	279.43 ± 2.56
Histamine	123.02 ± 4.50	247.13 ± 8.32	365.9 ± 11.92	489.94 ± 2.52	735.44 ± 5.75
Tyramine	12.70 ± 1.00	26.34 ± 1.44	38.96 ± 1.16	48.07 ± 1.10	76.49 ± 1.54
Cadaverine	41.63 ± 2.49	81.51 ± 2.93	120.33 ± 3.31	165.27 ± 4.33	247.63 ± 3.36
Spermidine	33.84 ± 1.73	68.25 ± 1.85	100.45 ± 1.89	136.06 ± 1.36	203.36 ± 1.62

Two aspects need to be considered when using GC, were mode of injection and sensitivity of detector. The choice of detector is an important factor that must be considered when developing an analytical method based on GC techniques. For instance, injection of 1 µL of sample using 10 µL capacity of microsyringe can contribute 2-5% error (Grob, 2004). Several situations can lead to a poor response such as column conditioning at very high temperature, partial adsorption of the sample by the packing, tubing or end plugs and use of a column that has simply worn out. The small sample size, a defective syringe, poor injection technique, leaks or low carrier gas flow, deposits or residues in the inlet, low injection port or column temperature and an incorrect detector sensitivity setting also will cause poor response (Grob, 1985). The precision of data usually relies on the ability of the instrument to control the temperature of the column and the flow rate of the carrier gas. A change in the temperature approximately 30^{o}C will affect the retention time, it will increase or decrease. In order to maintain a 1% repeatability in retention time measurements, the column temperature must be adjusted and hold within ±0.3^{o}C. Alteration in the carrier-gas flow rate at 1% effects the retention time by approximately 1% (Grob, 2004).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ values were calculated by plotting a calibration curve using three different concentrations of derivatized biogenic amines standard. The LOD were established from the lowest concentration of the amine required to give a signal to noise ratio of three, whereas the LOQ was established with a signal to noise ratio of ten (Gosetti *et al.,* 2007; Shakila *et al*., 2001). Three lowest concentration of derivatized biogenic amines standard in this study were 25, 50 and 75 µg/mL. These statistical data consist of the standard deviation (SD) and the slope of the regression line. Analytical procedure was validated by establishing the linear range, detection and quantitation limit.

The sensitivity of the method reflected by the LOD and LOQ values are comparable to those reported in the literature (Gosetti *et al.,* 2007; Shakila *et al*., 2001). LOD values ranged between 1.20 – 2.90 µg/mL, whereas for LOQ ranged between 3.98 – 9.65 µg/mL. The values of LOD and LOQ indicate that the sensitivity of the purposed method is satisfactory. These values are listed in Table 6.2.

Table 6.2 Sensitivity and standard deviation of blank, LOD and LOQ acquired from calibration curve

Biogenic amines	**Parameters**			
	Sensitivity (M) (n = 3)	**S.D. for blank**	**LOD (µg/mL)**	**LOQ (µg/mL)**
Heptylamine	1.78 ± 0.05	1.45	2.44	8.13
Histamine	4.86 ± 0.13	2.36	1.46	4.85
Tyramine	0.52 ± 0.02	0.50	2.90	9.65
Cadaverine	1.61 ± 0.02	1.09	2.03	6.77
Spermidine	1.34 ± 0.01	0.53	1.20	3.98

Recovery Efficiency of Extraction Method

Recovery of known amount of added compound to another steps sample is used to validate the procedure. Good recovery reflects good quantification of the compounds (Richard *et al.*, 2008). The recovery efficiency was determined by injecting 100 µg/mL of biogenic amines standard solution into fish muscle sample. The recovery percentage (%) of derivatized biogenic amines standard at that concentration was found to be satisfactory. The recovery percentage (%) of biogenic amines standard are shown in Table 6.3.

Table 6.3 The added and found amount of biogenic amines standard in sample and recovery efficiency for biogenic amines standard (n=3)

No.	Biogenic amines	Added amount (area)	Found amount (area)	Recovery (%)
1	Heptylamine	183.26	188.26	102.73 ± 1.73
2	Histamine	489.94	517.92	105.71 ± 6.65
3	Tyramine	48.07	47.31	98.41 ± 2.76
4	Cadaverine	165.27	180.29	109.09 ± 0.71
5	Spermidine	136.06	158.36	116.39 ± 0.92

A Proposed Analytical Procedures of GC-FID and GC-MS for Determination of Biogenic Amines in Fish and Fish Product Samples

Analysis of biogenic amines in fish and fish product samples using GC-FID and GC- MS were applied the analytical method that has been validated. All procedures such as extraction, derivatizing reagent, and instrument for analysis are listed in Table 6.4.

Table 6.4 The analytical procedure for analysis of biogenic amines using GC-FID and GC-MS in fish and fish product samples

Solvent used to extract	A mixture of HPLC grade water and methanol
Derivatization agent	A mixture of BSA + TMCS
GC-FID and GC-MS instruments for detection of biogenic amines in fish and fish products	
Column	1. Capillary HP-5 column (30 meter, 0.25 mm and 0.25 µm stationary phase thickness) for GC-FID 2. Capillary BPX-5 column (30 meter, 0.25 mm and 0.25 µm stationary phase) for GC-MS
Temperature program for GC-FID and GC-MS	
Initial temperature	110^{o}C maintained for 2 minutes
Ramp rate	5^{o}C for every minute
Final temperature	190^{o}C maintained for 2 minutes
Injector temperature	280^{o}C
Detector temperature	320^{o}C
Injection technique into GC-FID and GC-MS	
Mode of injection	Split-less
Volume injected	1 µL
Solvent	Dichloromethane

Conclusions

Determination of biogenic amines using GC-FID was successfully validated. Good specifity was considered in this study, where five derivatized biogenic amines standard appeared in GC-FID chromatogram without any impurity peaks. The value of linearity (R^2) were obtained with range of 0.9995 - 0.9999 with respect to the peak area in the concentration range of 25 – 150 µg/mL. Good accuracy and precision were obtained where all % C.V. of derivatized biogenic amines standard below 7.87%. While, LOD of derivatized biogenic amines standard was acquired ranged between 1.20 – 2.90 mg/kg, whereas the value of LOQ ranged between 3.98 – 9.65 mg/kg. Recovery (%) of five derivatized biogenic amines

standard were also obtained with ranged between 98.41 – 116.39 %. Since validation method produced good results, the analytical procedures have been applied as a reference for detection of biogenic amines in fish and fish product samples. A mixture of HPLC grade water and methanol has been used as the solvent of extraction the biogenic amines from fish and fish product samples. Fish muscle was used as a matrix during extraction process. Prior analysis using GC, extract of fish samples were derivatized using a mixture of BSA and TMCS. This derivatizing reagent has been added into extract fish and fish product samples, heating using hot plate at 80^{o}C has been done. The instruments for analysing and identifying biogenic amines from fish and fish product samples such as GC- FID with a capillary HP-5 column and GC-MS with capillary BPX-5 column can be employed. The following temperature program can be performed for GC-FID and GC-MS analysis of biogenic amines in fish and fish product samples where the initial oven temperature was programmed at 110^{o}C maintained for 2 min, increased to 190^{o}C with the rate at 5^{o}C/min and maintained at 190^{o}C for 2 min. Prior to analysis of extract fish and fish product samples, GC column was conditioned at 300^{o}C for overnight in order to ensure that no retained compounds at high temperature range of column.

Chapter 7

Application of Validated Procedure in Determination of Biogenic Amines in Fish and Fish Product Samples

Identification of biogenic amines by using GC-FID in fish and fish product samples were performed by comparing the retention times of peaks detected in derivatized extract sample to the peaks of derivatized biogenic amines standard. Furthermore, to calculate the level of biogenic amines in fish and fish product samples the regression equations of derivatized biogenic amines standard were applied. The GC-FID chromatograms and biogenic amines concentration of derivatized extract sample are presented as follows.

Raw Fish Samples

The GC-FID chromatograms of derivatized extracts from muscle of *S. guttatus*, *S. gibbosa*, *H. walga*, *T. pectoralis* and *T. toli* are presented in Figure 7.1. Three biogenic amines were detected in mackerel extract such as histamine, tyramine and spermidine. Five biogenic amines were detected in sardine extract such as, heptylamine, histamine, tyramine, cadaverine and spermidine. Only cadaverine and spermidine were detected in whiptail stingray extract. Heptylamine, tyramine and spermidine were detected in gourami extract. Tyramine and spermidine have been detected in toli shad extract.

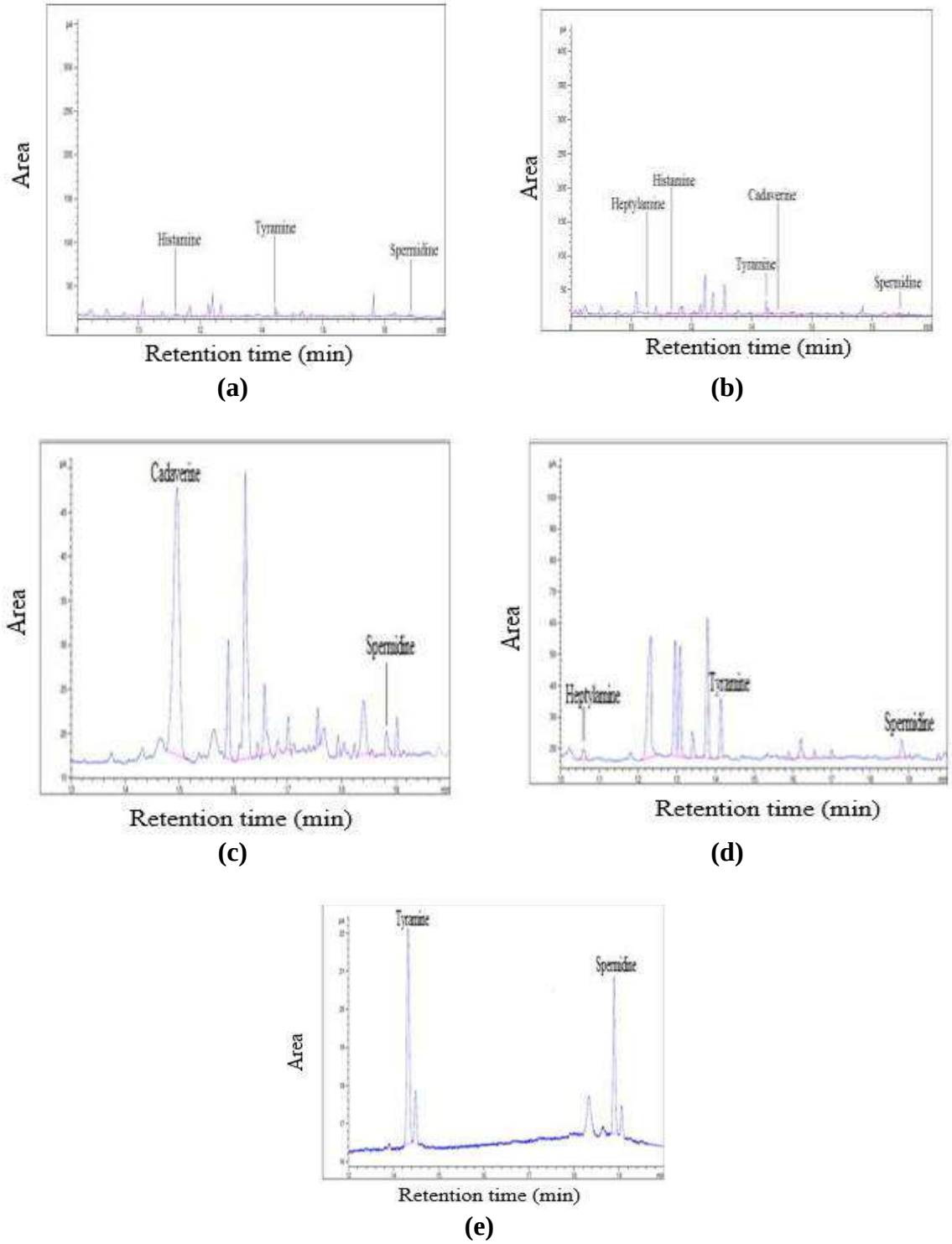


Figure 7.1 GC-FID chromatograms of derivatized extract from raw fish samples such as (a) makerel (*S. guttatus*), (b) sardine (*S. gibbosa*), (c) whiptail stingray (*H. walga*), (d) gourami (*T. pectoralis*) and (e) toli shad (*T. toli*)

There were several significant peaks appeared in gas chromatograms shown in Figure 7.1. However, their retention times did not correspond to retention times of derivatized biogenic amines standard. Derivatized extracts fish was measured using GC-MS in order to verify the presence of biogenic amines in the raw fish samples. The GC-MS chromatograms of raw fish samples are shown in Appendix 3 - 7. Biogenic amines concentration in raw fish samples studied are presented in Table 7.1. All derivatized extract raw fish samples were injected 6 replicates and all of them showed similar peaks. This can be deduced that good precisions of derivatized extract raw fish samples were obtained during measurement process using GC-FID.

Biogenic amines concentration in derivatized extract mackerel was 5.96±0.72, 103.29±4.86 and 7.38±0.75 mg/kg for histamine, tyramine and spermidine, respectively. Five biogenic amines were detected in derivatized extract sardine with concentration ranged between 2.69-106.95mg/kg. While, the concentration of cadaverine and spermidine were found at level 146.39±3.03 and 6.18±0.24 mg/kg, respectively. Derivatized extract gourami has been detected three biogenic amines, such as heptylamine, tyramine and spermidine with concentration at 15.11±0.38, 135.24±6.85 and 19.97±1.70 mg/kg, respectively. Only tyramine and spermidine were detected in derivatized extract toli shad.

Fish with high histidine content in sardines exceeded 50 mg/kg for less than 24 hour stored at 22^{o}C was reported by Prester *et al.* (2009). A study in Iran has reported the level of histamine in sardine (*Sardinella* spp.) ranged from 5–47 mg/kg (Kamkar *et al.,* 2003). While, Bilgin & Genccelep (2015) reported biogenic amines in sardine (*Sardinella* spp.) from Turkey were concentration at 51.66, 29.09, 61.09, 2.78 and 18.08 mg/kg for histamine, putrescine, cadaverine, tyramine and tryptamine, respectively. High concentrations of histamine in commercial fish from Asian regions have been highlighted in various FAO reports (Rahimi *et al.,* 2012). Mahmoudi & Norian (2014) have reported the very high concentration of histamine in some dried, fermented and salted products from Asia.

Table 7.1 Concentration (mg/kg) of biogenic amines in raw fish samples (n = 6)

Samples	Concentration of biogenic amines (mg/kg)				
	Heptylamine	**Histamine**	**Tyramine**	**Cadaverine**	**Spermidine**
Mackerel	n.d.	5.96 ± 0.72	103.29 ± 4.86	n.d.	7.38 ± 0.75
Sardine	6.08 ± 0.22	2.69 ± 0.75	106.95 ± 1.99	4.96 ± 0.26	4.04 ± 0.28
Whiptail stingray	n.d.	n.d.	n.d.	146.39 ± 3.03	6.18 ± 0.24
Gourami	15.11 ± 0.38	n.d.	135.24 ± 6.85	n.d.	19.97 ± 1.70
Toli shad	n.d.	n.d.	46.53 ± 3.94	n.d.	11.30 ± 0.76

n.d.: non - detected

Histamine was reported in Indian mackerel (*Scomber* spp.) after 16 hours stored at room temperature with concentration at 363.5 mg/kg (Chong *et al.,* 2014). Spermine and spermidine were the dominant biogenic amines detected and no biogenic amines were detected in mackerel (Katikou *et al.,* 2006; Krizek *et al.,* 2004). Histidine content in Atlantic mackerel (*Scomber* spp.) was detected to increase 50 mg/kg for less than 24 hours kept at 22^{o}C (Prester *et al.,* 2009). Histamine was also reported in mackerel with the concentration at 38 mg/kg (Gonzaga *et al.,* 2009).

The differences in histamine concentration in fish could be related to the their species, situations such as conditions during storage and handling, storage in the boat, during fish catching, hygienic condition of the environment and refrigerated for fish processing and handling (Rahimi *et al.,* 2012). Biogenic amines concentrations in raw fish samples from other studies are shown in Table 7.2. Although some studies have reported for containing histamine concentration beyond the FDA regulation, no cases of scombroid poisoning have been reported.

Table 7.2 Concentration of biogenic amines (mg/kg) in raw fish samples from other studies

Samples	Biogenic amines	Concentrations (mg/kg)	Ref.
Tuna (*Thunnus thynnus*)	Histamine	267 ± 11	Hwang *et al*. (2003)
Pacific saury (*Coloabis saira*)		168 ± 8	
Mackerel (*Scomber australasicus*)		149 ± 8	
Japanese anchovy (*Engraulis japonicus*)		115 ± 9	
White pomfret (*Pampus argentus*)		10 ± 1	
Indo-pacific mackerel (*S. Guttatus*)	Cadaverine	17.9 ± 3.5	This book.
	Spermidine	6.3 ± 1.4	
	Histamine	12.6 ± 2.7	
	Tyramine	11.7 ± 2.9	
Sardine (*Dussumieria acuta*)	Cadaverine	19.8 ± 1.4	Aflaki *et al*. (2015)
	Spermidine	5.6 ± 1.5	
	Histamine	15.8 ± 1.6	
	Tyramine	4.8 ± 1.3	
Narrow barred Spanish mackerel (*S. commerson*)	Cadaverine	5.8 ± 0.9	
	Spermidine	0.4 ± 0.1	
	Histamine	9.8 ± 1.9	
	Tyramine	4.5 ± 1.9	
Salmon (*Oncorhynchus* spp.)	Cadaverine	312.8 ± 12	Nakovich (2003)
	Histamine	311.9 ± 9.3	
	Tyramine	124 ± 3.6	
Mackerel (*S. japonicas peruanus*)	Histamine	86.0	Gonzaga *et al*. (2009)
Bonito (*Sarda sarda*)		6.4	
Mackerel (*S.* spp.)	Histamine	175 ± 0.15	Tao *et al.* (2011)
Mackerel (*S. guttatus*)	Histamine	5.96 ± 0.72	This book.
	Tyramine	103.29 ± 4.86	
	Spermidine	7.38 ± 0.75	
Whiptail stingray (*H. walga*)	Cadaverine	146.39 ± 3.03	
	Spermidine	6.18 ± 0.24	
Toli shad (*T. toli*)	Tyramine	46.53 ± 3.94	
	Spermidine	11.30 ± 0.76	
Gourami (*T. pectoralis*)	Heptylamine	15.11 ± 0.38	
	Tyramine	135.24 ± 6.85	
	Spermidine	19.97 ± 1.70	
Sardine (*S. gibbosa*)	Histamine	2.69 ± 0.75	
	Tyramine	106.95 ± 1.99	
	Cadaverine	4.96 ± 0.26	

Salted Fish Products

The GC-FID chromatograms of derivatized extract from toli shad, whiptail stingray, mackerel, sardine and gourami are presented in Figure 4.5.

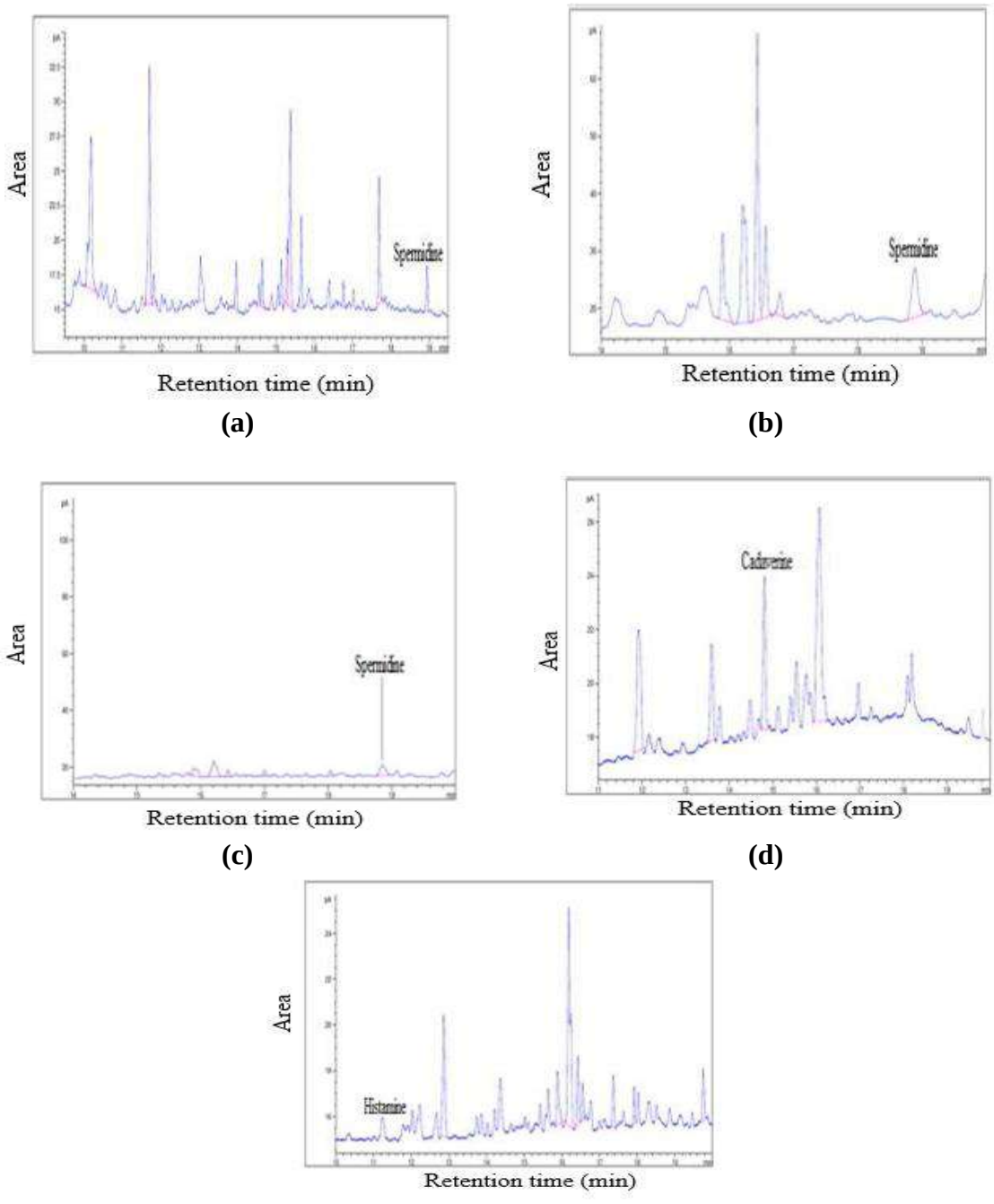


Figure 7.2 GC-FID chromatograms of derivatized extract from salted fish samples such as (a) toli shad (*T. toli*), (b) whiptail stingray (*H. walga*), (c) mackerel (*S. guttatus*), (d) gourami (*T. pectoralis*) and (e) sardine (*S. gibbosa*)

Spermidine was the only biogenic amines detected in salted fish products such as toli shad, mackerel and whiptail stingray. Several peaks in GC-FID chromatograms did not correspond to biogenic amines standard. While histamine has been detected in salted sardine, whereas cadaverine has been detected in salted gourami. GC-MS measurement also was perfomed in order to specify the identity of biogenic amines in salted fish samples after derivatized using a mixture of BSA + TMCS. The GC-MS chromatograms are shown in Appendix 8 – 12. All derivatized extract salted fish samples were injected 6 replicates and all of them have showed resemble peaks and area value. This can be deduced that good precisions of derivatized extract salted fish sample were acquired during measurement process using GC-FID.

The concentration of biogenic amines in salted fish products for mackerel, sardine, whiptail stingray, gourami and toli shad are listed in Table 7.2. Spermidine was only detected in salted toli shad, mackerel and salted whiptail stingray with concentration 8.75±0.34, 19.08±0.28 and 45.29±1.11 mg/kg, repectively. While, histamine was the only biogenic amines detected in salted sardine with concentration 8.95±0.38 mg/kg. While, cadaverine was the only biogenic amines detected in salted gourami with concentration 18.80±0.97 mg/kg.

Table 7.3 Concentration (mg/kg) of biogenic amines in salted fish samples (n = 6)

Samples	**Concentration of biogenic amines (mg/kg)**				
	Heptylamine	**Histamine**	**Tyramine**	**Cadaverine**	**Spermidine**
Mackerel	n.d.	n.d.	n.d.	n.d.	8.75 ± 0.34
Sardine	n.d.	8.95 ± 0.38	n.d.	n.d.	n.d.
Whiptail stingray	n.d.	n.d.	n.d.	n.d.	19.08 ± 0.28
Gourami	n.d.	n.d.	n.d.	18.80 ± 0.97	n.d.
Toli shad	n.d.	n.d.	n.d.	n.d.	45.29 ± 1.11

n.d.: non - detected

The absence of biogenic amines in several salted fish products may due to the concentration of biogenic amines were below detection limit. The addition of salt into fish also responsible for the absence of biogenic amines in salted fish. Salt as a preservative is a factor that can control biogenic amines accumulation. Salt usually used to control the growth of pathogens with aims of avoiding the spoilage and food poisoning (Linares *et al.*, 2012). Salt or sodium chloride can be considered as a preservative because it is non-toxic and inexpensive and is often applied as a preservative since ancient times to maintain the food against mold, spoiling and bacteria. Biogenic amines concentrations in salted fish samples from other studies are shown in Table 7.4.

Table 7.4 The concentration of biogenic amines (mg/kg) in salted fish samples from other studies

Samples	Biogenic amines	Concentrations (mg/kg)	Ref.
Anchovy (*Engraulis* spp.)	Histamine	3.2	Saaid *et al*. (2009)
	Tyramine	30.5	
	Spermidine	61.8	
Mackerel (*S.* spp.)	Histamine	111.8	
	Spermidine	3.6	
Gourami (*T.* spp.)	Histamine	3.5	
	Tyramine	5.9	
	Spermidine	3.7	
Anchovy (*E.* spp.)	Histamine	106.1 ± 3.7	Kose *et al*. (2012)
	Cadaverine	2.8 ± 0.2	
	Tyramine	3.9 ± 0.2	
	Spermidine	79.1 ± 0.5	
Salmon (*O.* spp.)	Histamine	9.1 ± 0.1	
	Cadaverine	11.5 ± 0.1	
	Tyramine	17.7 ± 0.1	
	Spermidine	19.9 ± 0.2	
Tuna (*T.* spp.)	Histamine	7.9 ± 0.2	
	Cadaverine	4.0 ± 1.2	
	Tyramine	6.5 ± 0.6	
	Spermidine	22.2 ± 0.6	
Herring (*Clupea* spp.)	Histamine	1.3 ± 0.1	
Toli shad (*T. toli*) Whiptail stingray (*H. walga*) Mackerel (*S. guttatus*) Sardine (*S. gibbosa*) Gourami (*T. pectoralis*)	Spermidine Histamine Cadaverine		This book.

Canned Fish Products

The GC-FID chromatograms of derivatized extract from canned products are presented in Figure 7.3. Cadaverine and spermidine were detected in derivatized extract from canned mackerel. Three biogenic amines detected in canned sardine were heptylamine, cadaverine and spermidine. Three biogenic amines also detected in canned salted sardine were heptylamine, tyramine and spermidine. GC-FID chromatogram of canned dace showed four biogenic amines, such as, heptylamine, histamine, tyramine and spermidine.

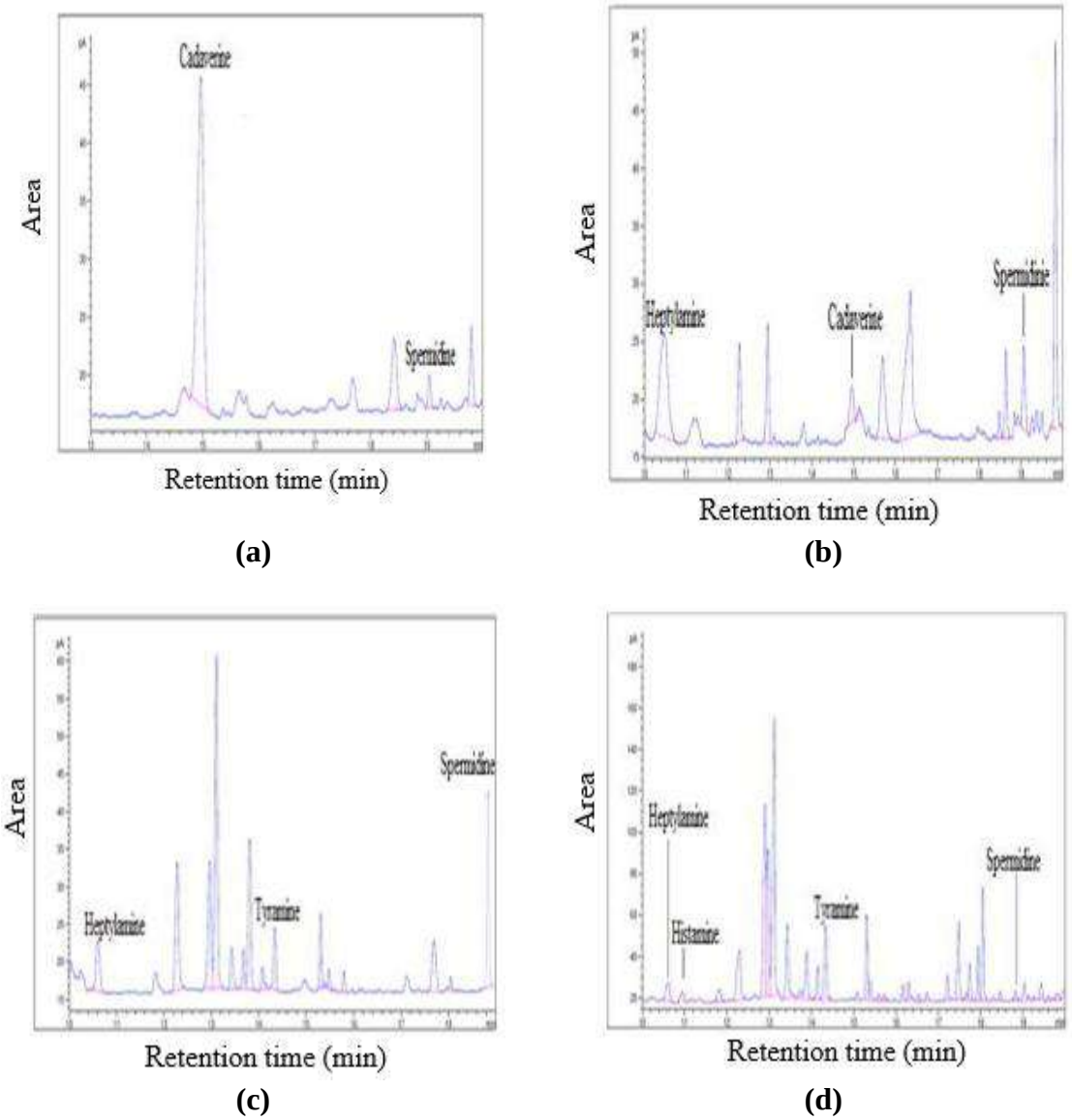


Figure 7.3 GC-FID chromatograms of derivatized extract from canned fish samples such as (a) mackerel (*Scomberomorus* sp.), (b) sardine (*Sardinella* sp.), (c) salted sardine (*Sardinella* sp.) and (d) dace (*L. leuciscus*)

All derivatized extract canned fish samples were injected 6 replicates and all of them have showed resemble peaks and area value. This can be deduced that good precisions of derivatized extract canned fish samples were acquired during measurement process using GC-FID. The concentration of biogenic amines for canned fish products in mackerel, sardine, salted sardine and canned dace are listed in Table 4.5. GC-MS chromatograms of canned products can be seen in Appendix 13 – 16. Canned mackerel has been detected for cadaverine and spermidine. While, in canned sardine was detected three biogenic amines such as, heptylamine, cadaverine and spermidine. Three biogenic amines also have been detected in canned salted sardine, such as heptylamine, tyramine and spermidine. While, four biogenic amines such as, heptylamine, histamine, tyramine and spermidine were detected in canned dace. Only canned dace contained histamine but the level did not contravene the FDA regulation. High level for cadaverine as an indicator that increasing the toxicity of histmine also was found in canned mackerel at concentration of 150.33 mg/kg but without histamine, the scombroid poisoning incident will not occur. Biogenic amines concentrations in canned fish samples from other studies are shown in Table 7.6. Some studies have reported for containing histamine concentration beyond the limit of FDA at below 50 mg/kg, yet no cases of scombroid poisoning have been reported in these studies.

Table 7.5 Concentration (mg/kg) of biogenic amines in canned fish products (n = 6)

Samples	**Concentration of biogenic amines (mg/kg)**				
	Heptylamine	**Histamine**	**Tyramine**	**Cadaverine**	**Spermidine**
Mackerel	n.d.	n.d.	n.d.	150.33 ± 2.33	6.65 ± 0.57
Sardine	97.79 ± 3.05	n.d.	n.d.	24.65 ± 1.59	23.75 ± 1.45
Salted sardine	29.30 ± 2.47	n.d.	78.89 ± 3.14	n.d.	79.93 ± 2.50
Dace	31.94 ± 0.91	6.18 ± 0.61	600.81 ± 14.64	n.d.	39.70 ± 1.97

n.d.: non - detected

Table 7.6 The concentration of biogenic amines (mg/kg) in canned fish samples from other studies

Samples	Biogenic amines	Concentrations (mg/kg)	Ref.
Sardine (*S.* spp.)	Histamine	51.66	Bilgin & Genccelep (2015)
	Cadaverine	61.09	
	Tyramine	2.78	
Mackerel (*S.* spp.)	Histamine	26.02	
	Cadaverine	4.37	
	Tyramine	14.20	
Anchovy (*E.* spp.)	Histamine	23.37	
	Cadaverine	45.70	
	Tyramine	2.03	
Tuna (*T.* spp.)	Histamine	8.59	Mahmoudi & Norian (2014)
Mackerel (*S.* spp.)	Tyramine	54.4	Saaid *et al*. (2009)
Salmon (*O.* spp.)	Spermidine	3.6	
	Tyramine	1.2	
Tuna (*T.* spp.)	Histamine	18.0	
	Tyramine	1.8	
	Spermidine	1.4	
Anchovy (*E.* spp.)	Histamine	2.0	
	Tyramine	2.1	
	Spermidine	1.6	
Sardine (*S.* spp.)	Spermidine	2.4	This book.
Dace (*L. leuciscus*)	Heptylamine	31.94	
	Histamine	6.18	
	Tyramine	600.81	
Sardine (*Sardinella.* sp.)	Spermidine	39.70	
	Cadaverine	24.65	
	Spermidine	23.75	
Mackerel (*Scomberomorus* sp.)	Spermidine	6.65	
	Cadaverine	150.33	

Conclusions

The validated procedures using GC-FID for determination of biogenic amines in fish and fish product samples have been successfully applied to measure of raw fish, salted fish products and canned fish products. The significant reduction of biogenic amines found between raw fish and salted fish products. This occurs owing to the addition of salt can decrease the biogenic amines concentration in fish. Although some salted fish samples have contain biogenic amines, content and concentration of biogenic amines have disparity with the raw fish samples. The level of biogenic amines in raw fish samples were found where almost all biogenic amines such as, heptylamine, histamine, tyramine, cadaverine and spermidine were detected. The level of histamine in raw fish was below 50 mg/kg, which means it safe to be consumed by human. Salted fish samples also contained biogenic amines, but the concentration of biogenic amines in salted was extremely different compare to raw fish samples, whereby almost all biogenic amines did not appeared in salted fish samples, eventhough some biogenic amines concentration appeared, the concentrations have been decreased. It occurred owing to the added of salt as preservative, where salt is one of a preservative that usually used to control biogenic amines in food. In this book, for determination biogenic amines in fish and fish product samples, only three samples contain histamine such as mackerel (*S. guttatus*), sardine (*S. gibbosa*), salted sardine (*S. gibbosa*) and canned dace (*L. leuciscus*), but the concentration of histamine at below 50 mg/kg. The disparity of histamine concentration was also found in raw sardine and salted sardine where histamine in salted sardine (8.95±0.38 mg/kg) was higher than raw sardine (2.69±0.75) but with concentration below 50 mg/kg and the absence of others biogenic amines particularly cadaverine which can enhance the risk of scombroid poisoning, thus raw and salted fish can be consumed by human. Consuming the canned dace (*L. leuciscus*) routinely is not recommended, because the concentration of tyramine in canned dace (*L. leuciscus*) was too high which was 600.81 mg/kg. In spite of some biogenic amines concentration were extremely high in several fish and fish product samples, but poisoning incidents have been reported. Nevertheless, routinely consume of these fish in this book that containing biogenic amines, particularly histamine with concentration above 50 mg/kg are not recommended.

References

1. Aflaki, F., Ghoulipour, V., Saemian, N., Sheibani, S. and Salahinejad, M. (2015). Determination of biogenic amines in Persian gulf fish: application of stirrer bead milling extraction method. *Food Measure,* 9: 86-94.
2. Agilent Technologies. (2005). The Importance of Area and Retention Time Precision in Gas Chromatography. USA. Agilent Technologies, Inc., pp 1-6.
3. Allmyr, M., McLachlan, M. S., Sandborgh, E. G. and Adolfsson, E. M. (2006). Determination of triclosan as its pentafluorobenzoyl ester in human plasma and milk using electron capture negative ionization mass spectrometry. *Analytical Chemistry,* 78: 6542-6546.
4. Araujo, P. (2009). Key aspects of analytical method validation and linearity evaluation. *Journal of Chromatography,* 877: 2224-2234.
5. Auerswald, L., Morren, C. and Lopata, A. L. (2006). Histamine levels in seventeen species of fresh and processed South African seafood. *Food Chemistry,* 98: 231-239.
6. Awan, M. A., Fleet, I. and Thomas, C. L. P. (2008). Determination of biogenic diamines with vaporisation derivatization approach using solid-phase microextraction gas chromatography- mass spectrometry. *Food Chemistry,* 111 (2): 462-468.
7. Bedia, E. F. (2013). Recent analytical approaches to analysis of biogenic amines in food samples. *Trends in Analytical Chemistry,* 52: 239-247.
8. Bilgin, B. and Genccelep, H. (2015). Determination of biogenic amines in fish products. *Food Science and Biotechnology,* 24 (5): 1907-1913.
9. Blanchet, B., Sabourea, C., Benichou, A. S., Billemont, B., Taieb S. R. and Alain, D. (2009). Development and validation of an HPLC-UV-visible method for sunitinib quantification in human plasma. *Clinica Chimica Acta,* 404: 134-139.
10. Bomke, S., Seiwert, B., Dudek, L., Effkemann, S. and Karst, U. (2009). Determination of biogenic amines in food samples using Derivatization followed by liquid chromatography/mass spectrometry. *Analytical and Bioanalytical Chemistry,* 393 (1): 247-256.
11. Bressolle, F., Bromet-pitit, M. and Audran, M. (1996). Validation of liquid chromatography and gas chromatography method application to pharmacokinetics. *Journal of Chromatography Biomedical Application,* 686: 3-10.
12. Bricio, B. M. S., Rios, A. and Valcarcel, M. (2004). Direct automatic determination of biogenic amines in wine by flow injection-capillary electrophoresis-mass spectrometry. *Electrophoresis,* 25: 3427-3433.
13. Causon, R. (1997). Validation of chromatographic methods in biomedical analysis viewpont and discussion. *Journal of Chromatography and Biomedical Applications,* 689: 175-180.
14. Chen, H. C., Huang, Y. R., Hsu, H. H., Lin, C. S., Chen, W. C., Lin, C. M. and Tsai, Y. H. (2010).

15. Determination of histamine and biogenic amines in fish cubes (*Tetrapturus angustirostris*) implicated in a food-borne poisoning. *Food Control,* 21: 13-18.
16. Chien, C. J., Charles, M. J., Sexton, K. G. and Jeffries, H. E. (1998). Analysis of airborne carboxylic acids and phenols as their pentafluorobenzyl derivatives: gas chromatography/ion trap mass spectrometry with a novel chemical ionization reagent, PFBOH. *Environmental Science and Technology,* 32: 299-309.
17. Chong, C. W., Abu Baker, F., Rahman, R. A., Bakar, J. and Zaman, M. Z. (2014). Biogenic amines, amino acids and microflora changes in Indian mackerel (*Rastrellinger kanagurta*) stored at ambient (25-29^{O}C) and ice temperature (0^{O}C). *Journal Food and Science Technology,* 51: 1118-1125.
18. Cohen, G., Rudnik, D. D., Laloush, M., Yakir, D. and Karpas, Z. (2015). A novel method for determination of histamine in tuna fish by ion mobility spectrometry. *Food Analytical Methods,* 8: 2376-2382.
19. Costa, M. P., Balthazar, C. F., Rodrigues, B. L., Lazaro, C. A., Silva, A. C. O. Cruz, A. G. and Junior, C. A. C. (2015). Determination of biogenic amines by high-performance liquid chromatography (HPLC-DAD) in probiotic cow's and goat's fermented milks and acceptance. *Food Science and Nutrition,* 3 (3): 172-178.
20. Custodio, F. B., Tavares, E. and Gloria, M. B. A. (2007). Extraction of bioactive amines from grated Parmesan cheese using acid, alkaline and organic solvents. *Journal of Food Composition and Analysis,* 20 (4): 280-288.
21. Dadakova, A., Krizek, M. and Pelikanova, T. (2009). Determination of biogenic amines in foods using ultra-performance liquid chromatography (UPLC). *Food Chemistry,* 116: 365-370.
22. Dadgar, D., Burnett, P. E., Choc, M.G., Gallicano, K. and Hooper, J.W. (1995). Application issues in bioanalytical validation, sample analysis and data reporting, *Journal of Pharmaceutical and Biomedical Analysis.* Volume 13, 2: 89-97.
23. De Mey, E., Drabik, M. G., De Maere, H., Peeters, M. C., Derdelinckx, G., Paelinck, H. and Kowalska, T. (2012). Dansyl derivatization as an alternative for dansylation in the detection of biogenic amines in fermented meat products by reversed phase high performance liquid chromatography. *Food Chemistry,* 130 (4): 1017-1023.
24. Duflos, G. (2009). Histamine risk in fishery products. *Bulletin Academia Veterinaire de France,* 162 (3): 241-246.
25. European Food Safety Authority (EFSA). (2011). Panel on Biological Hazards (BIOHAZ). Scientific opinion on risk based control of biogenic amine formation in fermented foods. *EFSA Journal,* 9 (10): 2393-2486.
26. Eom, J. S., Seo, B. Y. and Choi, H. S. (2015). Biogenic amine degradation by *Bacillus* species isolated from traditional fermented soybean food and detection of decarboxylation-related genes. *Journal of Microbiology and Biotechnology,* 25 (9): 1519-1527.
27. Emborg, J. and Dalgaard, P. (2006). Formation of histamine and biogenic amines in cold-smoked tuna:

an investigation of psychrotolerant bacteria from samples implicated in cases of histamine fish poisoning. *Journal of Food Protein,* 69: 897-906.

28. Favaro, G., Pastore, P., Saccani, G. and Cavalli, S. (2007). Determination of biogenic amines in fresh and processed meat by ion chromatography and integrated pulsed amperometric detection on Au electrode. *Food Chemistry,* 105 (4): 1652-1658.
29. Food and Drug Administration (FDA). (2011). *Fish and Fishery Products Hazards and Control Guidance,* 4th edition, Washington DC: Department of Health and Human Services, FDA, Center for Food Safety and Applied Nutrition., 476pp.
30. Food and Drug Administration (FDA). (2000). *Analytical Procedures and Method Validation, Chemistry, Manufacturing and Controls Documentation,* 2nd edition, Washington DC. Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research.
31. Galceran, M. T., Moyano, E. and Poza, J. M. (1995). Pentafluorobenzyl derivatives for the gas chromatographic determination of hydroxyl-polycylic aromatic hydrocarbons in urban aerosols. *Journal of Chromatography A,* 710: 139-147.
32. Genccelep, H., Kaban, G., Aksu, M. I., Oz, F. and Kaya, M. (2008). Determination of biogenic amines in sucuk. *Food Control,* 19 (9): 868-872.
33. Gonzaga, V. E., Lescano, A. G., Huaman, A. A., Salmn-Mulanovich, G. and Blazes, D. L. (2009). Histamine levels in fish from markets in Lima, Peru. *Journal of Food Protein,* 72: 1112-1115.
34. Gosetti, F., Mazzucco, E., Gianotti, V., Polati, S. and Gennaro, M. C. (2007). High performance liquid chromatography/tandem mass spectrometry determination of biogenic amines in typical Piedmont cheeses. *Journal of Chromatography A,* 1149: 151-157.
35. Green, M. J. (1996). A practical guide to analytical method validation. *Analytical Chemistry,* 68: 305-309.
36. Grob, R.L. (2004). *Modern Practice of Gas Chromatography*, 4th edition. New York. A Wiley - Interscience Publication, John Wiley & Sons., 1054pp.
37. Grob, R.L. (1985). *Modern Practice of Gas Chromatography*, 2nd edition. New York. A Wiley - Interscience Publication, John Wiley & Sons., 897pp.
38. Harris, D. C. (2007). *Quantitative Chemical Analysis*, 7th edition. New York. W.H. Freeman and Company., 828 pp.
39. Hosseini, A. R., Rahimi, R. and Mirghaed, A. T. (2014). Determination of histamine content in muscle tissue of rainbow trout (*Oncorhynchus mykiss*) during ice storage. *Comparative Clinical Pathology,* 23: 775-778.
40. Huber, L. (2007). *Validation and Qualification in Analytical Laboratories*, 2nd edition. New York, USA. Informa Healthcare., 76pp.
41. Hwang, B. S., Wang, J. T. and Choong, Y. M. (2003). A rapid gas chromatographic method for the

determination histamine in fish and fish products. *Food Chemistry*, 82: 329-334.

42. Jia, S., Kang, Y. P., Park, J. H., Lee, J. and Kwon, S. W. (2011). Simultaneous determination of 23 amino acids and 7 biogenic amines in fermented food samples by liquid chromatography/quadrupole time of flight mass spectrometry. *Journal of Chromatography A,* 1218 (51): 9174-9182.
43. Julia, T., Mena, A. J., Aucoin, M. G. and Kamen, A. A. Development and validation of a HPLC method for the quantification of baculovirus particles. *Journal of Chromatography,* 879: 61- 68.
44. Kalac, P. (2009). Recent advanced in the research on biological roles of dietary polyamines in man. *Journal of Application Biomedical,* 7 (2): 65-74.
45. Kalac, P. and Krausova, P. (2005). *A review of dietary polyamines*: Formation, Implication For Growth and Health and Occurrence in Foods. *Food Chemistry*, 90: 219-230.
46. Kalac, P., Krizek, M., Pelikanova, T., Langova, M. and Veskrna, O. (2005). Contents of polyamines in selected foods. *Food Chemistry*, 90: 561-564.
47. Kamkar, A. A. F., Hosseini, S. A. A. F., Alavi, A. and Bahonar, H. (2003). The study of nitrite residue in meat products consumed in Tehran. *Animal Fish and Science.* 63 (2): 60-65.
48. Karovicova, J. and Kohajdova, Z. (2005). Biogenic amines in food. *Chemical Papers*, 59 (1): 70-79.
49. Kataoka, H. (1996). Derivatization reactions for the determination of amines by gas chromatography and their applications in environmental analysis. *Journal of Chromatography (*2010). Strategies to reduce biogenic amine accumulation in traditional sausage manufacturing. *LWT- Food Science and Technology*, 43: 20-25.
50. Katikou, P., Georgantelis, D., Paleologos, E. K., Ambrosiadis, I. and Kontominas, M. G. (2006). Relation of biogenic amines formation with microbiological and sensory attributes in Lactobacillus-inoculated vacuum-packed rainbow trout (*Oncorhynchus mykiss*) fillets. *Journal of Agricultural and Food Chemistry,* 54: 4277-4283.
51. Khan, M. C., Redd, N. K., Ravindra, G., Reddy, K. V. S. R. K. and Dubey, P. K. (2012). Development and validation of a stability indicating HPLC method for simultaneous determination of four novel fluoroquinolone dimers as potential antibacterial agents. *Journal of Pharmaceutical and Biomedical Analytical,* 59: 162-166.
52. Kim, M. K., Mah, J. H. and Hwang, H. J. (2009). Biogenic amine formation and bacterial contribution in fish, squid and shellfish. *Food Chemistry,* 116 (1): 87-95.
53. Knapp, D. R. (1979). *Handbook of Analytical Derivatization Reaction*. New York. Jhon Wiley & Sons., 741pp.
54. Kose, S. (2010). Evaluation of seafood safety health hazards for traditional fish products: preventive measures and monitoring issues. *Journal Fish Aquatic and Science,* 10: 139-160.
55. Kose, S., Koral, S., Tufan, B., Pompe, M., Scavnicar, A. and Kocar, D. (2012). Biogenic amine contents of commercially processed traditional fish products originating from European countries and Turkey. *European Food Research and Technology,* 235: 669-683.
56. Krizek, M., Vacha, F., Vorlova, L., Lukasova, J. and Cupakova, S. (2004). Biogenic amines in vacuum-

packed and non-vacuum-packed flesh of carp (*Cyprinus carpio*) stored at different temperatures. *Food Chemistry,* 88: 185-191.

57. Kuhnel, E., Laftan, D. D. P., Llyod-Jones, G. C., Martinez, D. C. T., Shepperson, I. R. and Slaughter, J. L. (2007). Mechanism of methyl esterification of carboxylic acids by trimethylsilydiazomethane. *Angewandte Chemie International Edition,* 46 (37): 7075-7078.
58. Lapa-Guimaraes, J. and Pickova, J. (2004). New solvent systems for thin-layer chromatographic determination of nine biogenic amines in fish and squid. *Journal of Chromatography,* 1045 (2): 223-232.
59. Latorre-Moratalla, M. L., Bover-Cid, S., Veciana-Nogues, M. T. and Vidal-Carou, M. C. (2012). Control of biogenic amines in fermented sausages: role of starter cultures. *Frontiers in Microbiology,* 3: 169 – 178.
60. Latorre-Moratalla, M. L., Bosch-Fuste, J., Lavizzari, T., Bover-Cid, S., Veciana-Nogues, M. T. and Vidal-Carou, M. C. (2009). Validation of an ultra high pressure liquid chromatographic method for the determination of biologically active amines in food. *Journal of Chromatography,* 1216: 7715-7720.
61. Lazaro, C. A. and Conte-Junior, C. A. (2013). Chromatographic methods for biogenic amines determination in foods of animal origin. *Brazil Journal of Veterinary Research Animal Science,* 50 (6): 430-446.
62. Lazaro, C. A., Conte-Junior, C. A., Cunha, F. L., Marsico, E. T., Mano, S. B. and Franco, R. M. (2013). Validation of an HPLC methodology for the identification and quantification of biogenic amines in chicken meat. *Food Analytical Methods,* 6: 1024-1032.
63. Li, G., Dong, L., Wang, A., Wang, W., Hu, N. and You, J. (2014). Simultaneous determination of biogenic amines and estrogens in foodstuff by an improved HPLC method combining with fluorescence labelling. *LWT-Food Science and Technology,* 55: 355-361.
64. Linares, D. M., Rio, D. B., Ladero, V., Martinez, N., Fernandez, M. and Martin, M. C. (2012). Factors influencing biogenic amines accumulation in dairy products. *Frontiers in Microbiology,* 3: 180-190.
65. Linares, D. M., Martin, M., Ladero, V., Alvarez, M. A. and Fernandez, M. (2011). Biogenic amines in dairy products. *Critical reviews in Food Science and Nutrition,* 51 (7): 691-703.
66. Linsalata, M. and Russo, F. (2008). Nutritional factors and polyamine metabolism in colorectal cancer. *Nutrition,* 24 (4): 382-389.
67. Loizzo, M. R., Menichini, F., Picci, N., Puoci, F., Spizzirri, U. G. and Restuccia, D. (2013). Technological aspects and analytical determination of biogenic amines in cheese. *Trends in Food Science and Technology,* 30 (1): 38-55.
68. Lorenzo, J. M., Martinez, S., Franco, I. and Carballo, J. (2007). Biogenic amine content during the manufacture of dry-cured lacon, a Spanish traditional meat product: Effect of some additives. *Meat Science,* 77 (2): 287-293.
69. Mahmoudi, R. and Norian, R. (2014). Occurrence of histamine in canned tuna fish from Iran. *Journal of Counsumer Protection and Food Safety,* 9: 133-136.
70. Magnusson, B. and Omemark, U. (2014). *The Fitness for Purpose of Analytical Methods: A Laboratory*

Guide to Method Validation and Related Topics, 2nd edition. Europe: Eurochem Guide., 70pp.

71. Marks, R. H. S. and Anderson, C. R. (2005). Determination of putrescine and cadaverine in seafood (finfish and shellfish) by liquid chromatography using pyrene excimer fluoroscene. *Journal of Chromatography,* 1094 (1): 60-69.
72. Mascaro, N., Stocchi, R., Ricciutelli, M., Cammertoni, N., Renzi, F., Cecchini, S., Loschi, A. R. and Rea, S. (2010). Biogenic amine content and chemical and physical features of Italian formaggio di Fossa. *Associazione Italiana Veterinari Igienisti,* 8: 49-53.
73. Matsuhisa, M., Yamasaki, Y., Shiba, Y., Nakahara, I., Kuroda, A. and Tomita, T. (2000). Important role of hepatitis vagus nerve in glucose uptake and production by the liver metabolism. *Clinical and Experimental,* 1: 11-16.
74. Mayer, H. K., Fiechter, G. and Fischer, E. A. A new ultra-pressure liquid chromatography method for the determination of biogenic amines in cheese. *Journal of Chromatography,* 1217 (19): 3251- 3257.
75. Mazzucci, E., Gosetti, F., Bobba, M., Marengo, E., Robotti, E. and Gennaro, M. C. (2010). High-performance liquid chromatography-ultraviolet detection method for the simultaneous determination of typical biogenic amines and precursor amino acids. Applications in food chemistry. *Journal of Agricultural and Food Chemistry,* 58 (1): 127-134.
76. McNair, H.M and Miller, J.M. (2009). *Basic Gas Chromatography*, 2nd edition. John Wiley & Son., 256pp.
77. Mohammed N. S., Flowers, T. H. and Duncan, H. J. (2014). HPLC-UV method development and validation of potato sprout inhibitor 1,4-dimethylnaphthalene using different systems. *Chromatography Research International,* 2014: 1-7.
78. Mohamed, R., Livia, S. S., Hassan, S., Soher, E. S. and Ahmed-Adel, E. B. (2009). Changes in free amino acids and biogenic amines of Egyptian salted-fermented fish (Feseekh) during ripening and storage. *Food Chemistry,* 115: 635-638.
79. Moon, J. S., Kim, Y., Jang, K. I., Cho, K., Yang, S., Yoon, G., Kim, S. and Han, N. S. (2010). Analysis of Biogenic Amines in Fermented Fish Products Consumed in Korea. *Food Science and Biotechnology,* 16 (6): 1689-1692.
80. Moret, S., Smela, D., Populin, T. and Conte, L. S. (2005). A survey on free biogenic amine content of fresh and preserved vegetables. *Food Chemistry,* 89: 355-361.
81. Moret, S. and Conte, L. S. (1996). High-performance liquid chromatography evaluation of biogenic amines in foods and analysis of different methods of sample preparation in relation to food characteristics. *Journal of Chromatography A,* 729: 363-369.
82. Naila, A., Flint, S., Fletcher, G., Bremer, P. and Meerdink, G. (2010). Control of biogenic amines in food – existing and emerging approaches. *Journal of Food Science,* 75: 139-150.
83. Nakovich, L. (2003). *Analysis of Biogenic Amines by GC/FID and GC/MS.* MSc Thesis of Virginia Polytechnic Institute, Blacksburg., 81 pp.

84. Nollet, L. M. L. (2006). *Chromatographic Analysis of the Environment*, 3rd edition. London: Taylor and Francis Group., pp 82-85, pp 382-392.
85. Onal, A. (2007). A review: Current analytical methods for the determination of biogenic amines in foods. *Food Chemistry*, 103 (4): 1475-1486.
86. Orata, F. (2012). *Derivatization reactions and reagents for gas chromatography analysis*, *Advanced Gas Chromatography*. Shanghai: InTech., 460pp.
87. Osorio, M. T., Moloney, A. P., Schmidt, O. and Monahan, F. J. Beef authentication and retrospective dietary verification using stable isotope ratio analysis of bovine muscle and tail hair. *Journal of Agricultural and Food Chemistry,* 59 (7): 3295-3305.
88. Ozdestan, O. and Uren, A. (2010). Biogenic amine content of kefir: a fermented dairy product. *European Food Research and Technology,* 231 (1): 101-107.
89. Palmer, R. B., Kim, N. H. and Dasgupta, A. (2000). Simultaneous determination of fenfluramine and phentermine in urine using gas chromatography-mass spectrometry with pentafluoropropionic anhydride derivatization. *Therapeutic Drug Monitoring*, 22: 418-422.
90. Papavergou, E. J., Savvaidis, I. N. and Ambrosiadis, I. A. (2012). Levels of biogenic amines in retail market fermented meat products. *Food Chemistry*, 135: 2750-2755.
91. Pons-Sanchez-Cascado, S., Vesciana-Nogues, M., Bover-Cid, S., Marine-Font, A. and Vidal-Carau, M. (2006). Use of volatile and non-volatile amines to evaluate the freshness of anchovies stored in ice. *Journal of Science and Food Agriculture,* 86 (5): 699-705.
92. Prester, L., Macan, J., Varnai, V. M., Orct, T., Vukusic, J. and Kipcic, D. (2009). Endotoxin and biogenic amine levels in Atlantic mackerel (*Scomber scombrus*), sardine (*Sardine pilchardus*) and Mediterranean hake (*Merluccius merluccius*) stored at 22^{O}C. *Food Analytical Chemistry,* 26: 355-362.
93. Rabie, M. and Toliba, A. (2013). Effect of irradiation and storage on biogenic amine content in ripened Egyptian smoked cooked sausage. *Journal of Food and Science Technology,* 50 (6): 1165-1171.
94. Rahimi, E., Nayebpour, F. and Alian, F. (2012). Determination of histamine in canned tuna fish using ELISA method. *The Journal of Toxicological Science,* 4 (2): 64-66.
95. Richard, N., Pivarnik, L., Ellis, P. C. and Lee, C. (2008). Effect of matrix on recovery of biogenic amines in fish. *Journal of AOAC International,* 91 (4): 768-776.
96. Rivas, B., Goncalez, R., Landete, J. M. and Munoz, R. (2008). Characterization of a second ornithine deacarboxylase isolated from *Morganell morganii. Journal of Food Protein,* 71 (3): 657-661.
97. Rodrigues, B. L., Alvares, T. S., Costa, M. P., Sampaio, G. S. L., Lazaro, T. C. A., Marsico, E. T. and Conte-Junior, C. A. (2013). Concentration of biogenic amines in rainbow trout (*Oncorhynchus mykiss*) preserved in ice and its relationship with physicochemical parameters of quality. *Journal of Aquaculture Research & Development,* 4 (3): 174-177.
98. Rogers, P. L. and Straruszkiewicz, W. F. (1997). Gas chromatographic method for putrescine and Cadaverine in canned tuna and mahimahi and fluorometric method for histamine: collaborative study.

Journal of AOAC International, 80 (3): 591-602.

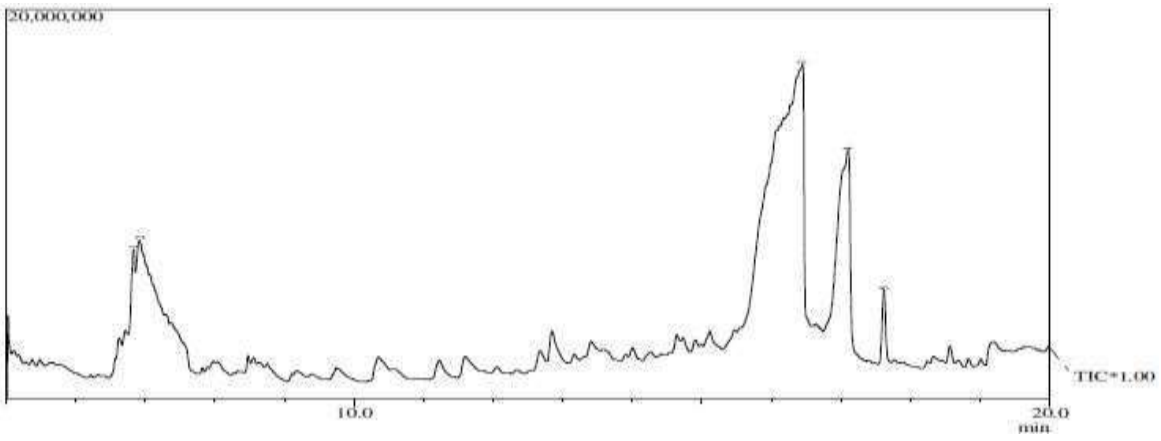
99. Rohani, S. M. R., Aliakbarlu, J., Ehsani, A. and Hassanzadazar, H. (2013). Biogenic amines determination in some traditional cheeses in West Azerbaijan province of Iran. *Veterinary Research Forum,* 4 (2): 115-118.
100. Ruiz-Capillas, C. and Jimenez-Colmenero, F. (2004). Biogenic amines in meat and meat products. *Critical Reviews in Food Science and Nutrition,* 44 (8): 489-499.
101. Saaid, M., Saad, B., Hashim, N. H., Ali, A. S. M. and Saleh, M. I. (2009). Determination of biogenic amines in selected Malaysian food. *Food Chemistry,* 113 (4): 1356-1362.
102. Sagratini, G., Fernandez-Frazon, M., Berardinis, F., Font, G., Vittori, S. and Manes, J. (2012). Simultaneous determination of eight underivatised biogenic amines in fih by solid phase extraction and liquid chromatography-tandem mass spectrometry. *Food Chemistry,* 132 (1): 537-543.
103. Schirone, M., Tofalo, R., Mazzone, G., Corsetti, A. and Suzzi, G. (2011). Biogenic amine content and microbiological profile of Pecorino di Farindola cheese. *Food Microbiology,* 28: 128-136.
104. Shakila, R. J., Vasundhara, T. S. and Kumudavally, K. V. (2001). A comparison of the TLC-densitometry and HPLC method for the determination of biogenic amines in fish and fishery products. *Food Chemistry,* 75: 255-259.
105. Shukla, S., Park, H. K., Kim, J. K. and Kim, M. (2011). Determination of biogenic amines in Japanese miso products. *Food Science and Biotechnology,* 20 (3): 851-854.
106. Smela, D., Pechova, P., Komprda, T., Klejdus, B. and Kuban, V. (2003). Liquid chromatography determination of biogenic amines in a meat product during fermentation and long-term storage. *Czech Journal of Food Science,* 21 (5): 167-175.
107. Spano, G., Russo, P., Lonvaud-Funel, A., Lucas, P., Alexandre, H., Grandvalet, C., Coton, E., Coton, M., Barnavon, L., Bach, B., Rattray, F., Bunte, A., Magni, C., Ladero, V., Alvarez, M., Fernandez, M., Lopez, P., Corbi, A., Trip, H. and Lolkema, J. S. (2010). Biogenic amines in fermented foods. *European Journal of Clinical Nutrition,* 64 (3): 95-100.
108. Standarova, E., Borkovcova I. and Vorlova, L. (2008). The occurrence of biogenic amines in dairy products on the Czech market. *Acta Scientiarum Polonorum Medicina Veterinaria,* 7 (4): 35- 42.
109. Sun, X., Yang, X. and Wang, E. (2003). Determination of biogenic amines by capillary electrophoresis with pulsed amperometric detection. *Journal of Chromatography A,* 1005 (2): 189-195.
110. Suzzi, G. and Gardini, F. (2003). Biogenic amines in dry fermented sausages: a review: *International Journal of Food Microbiology,* 88 (1): 41-54.
111. Suzzi, G. and Torriani, S. (2015). Biogenic amines in foods. *Frontiers in Microbiology,* 6: 472-473.
112. Szyrwinska, K., Kolodziejczak, A., Rykowska, I., Waisak, W. and Lulek, J. (2007). Derivatization and gas chromatography-low resolution mass spectrometry of bisphenol. *Acta Chromatographica,* 18: 49-58.
113. Tao, Z., Sato, M., Han. Y., Tan. Z., Yamaguchi, T. and Nakano, T. (2011). A simple and rapid method for histamine analysis in fish and fishery products by TLC determination. *Food Control,* 22: 1154-1157.

114. Tapingkae, W., Tanasupawat, S., Parkin, K. L., Benjakul, S. and Visessanguan, W. (2010). Degradation of histamine by extremely halophilic archaea isolated from high salt-fermented fishery products. *Enzyme Microbiology Technology,* 46 (2): 92-99.
115. Taylor, S. L. (1986). Histamine food poisoning: toxicology and clinical aspects. *Criticals Review Tocixology,* 17: 91-128.
116. Triki, M., Jimenez-Colmenero, F., Herrero, A. M. and Ruiz-Capillas, C. (2012). Optimization of a chromatographic procedure for determining biogenic amine concentrations in meat and meat products employing a cation-exchange column with a post-column system. *Food Chemistry,* 130 (4): 1066-1073.
117. Visciano, P., Schirone, M., Tolafo, R. and Suzzi, G. (2012). Biogenic amines in raw and processed seafood. *Frontiers in Microbiology,* 3: 188-190.
118. Wade, L. G. (2013). *Organic Chemistry,* 8th edition. Boston: Pearson Education Inc., 1326pp.
119. World Health Organization (WHO) Publication (2013). Meeting on public health risks of histamine and other biogenic amines from fish and fishery products. Joint Food and Agriculture Organization (FAO)/ Expert Report. http://www.who.int/foodsafety/publications/ histamine_risk/en/.
120. Wieling, J., Hendriks, G., Tamminga, W. J., Hempenius, J., Mensink, C. K., Oosterhuis, B. and Jonkman, J. H. G. (1996). Rational experiment design for bioanalytical methods validation illustration using an assay method for total captopril in plasma. *Journal of Chromatography,* 730: 381-394.
121. Yamamoto, S., Iwado, A., Hashimoto, Y., Aoyama, Y. and Makita, M. (1984). Gas chromatography-mass spectrometry of polyamines as their N-ethyloxycarbonyl derivatives and identification of *sym*-homospermidine and *sym*-norspermine in mosses and ferns. *Journal of Chromatography,* 303: 99-108.
122. Zaman M. Z., Abu Bakar, F., Selamat, J. and Bakar, J. (2010). Occurrence of biogenic amines and amines degrading bacteria in fish sauce. *Czech Journal of Food Science,* 28: 440-449.
123. Zhai, H., Yang, X., Li, L., Xia, G. Cen, J., Huang, H. and Hao, S. (2012). Biogenic amines in commercial fish and fish prodcuts sold in southern China. *Food Control*, 25: 303-308.

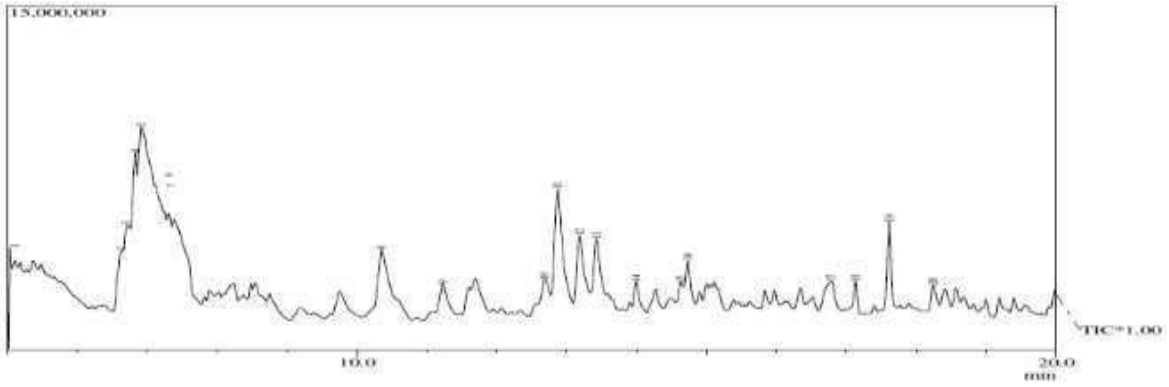
Appendix 1. The Instrument of Gas Chromatography-Flame Ionization Detector.

Appendix 2. The Instrument of Gas Chromatography – Mass Spectrometry.

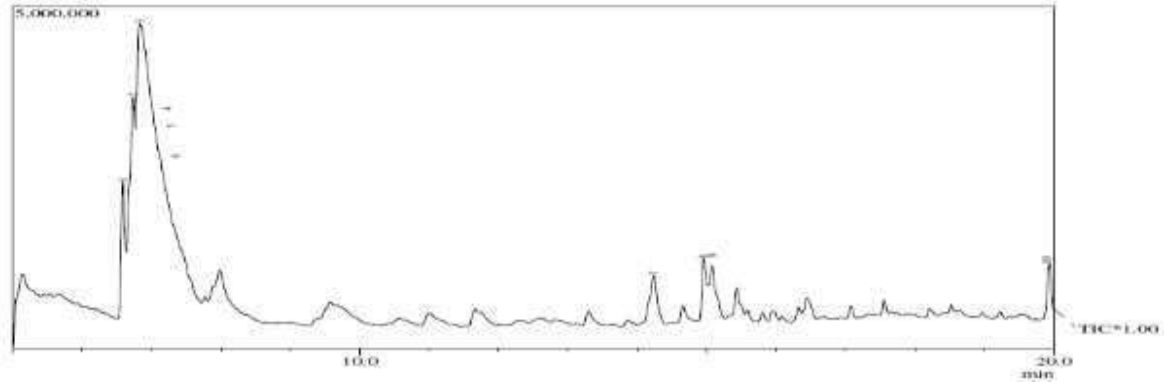
Appendix 3. The GC-MS chromatogram of derivatized extract from muscle of mackerel (*S. guttatus*).



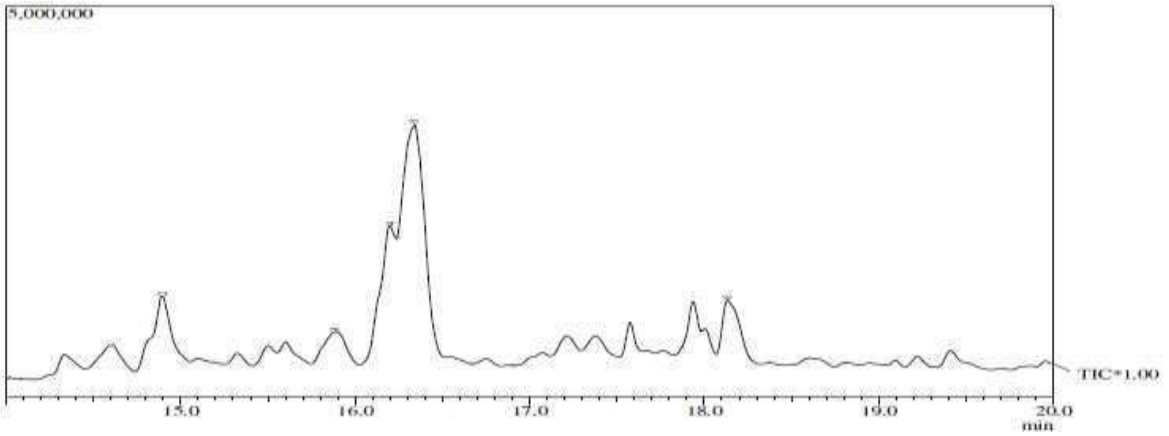
Appendix 4. The GC-MS chromatogram of derivatized extract from muscle of sardine (*S. gibbosa*).



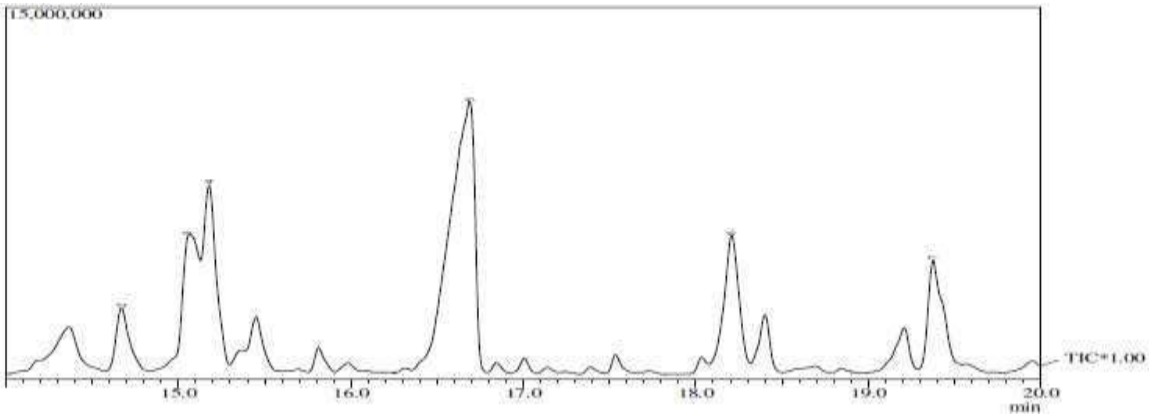
Appendix 5. The GC-MS chromatogram of derivatized extract from muscle of toli shad (*T. toli*).



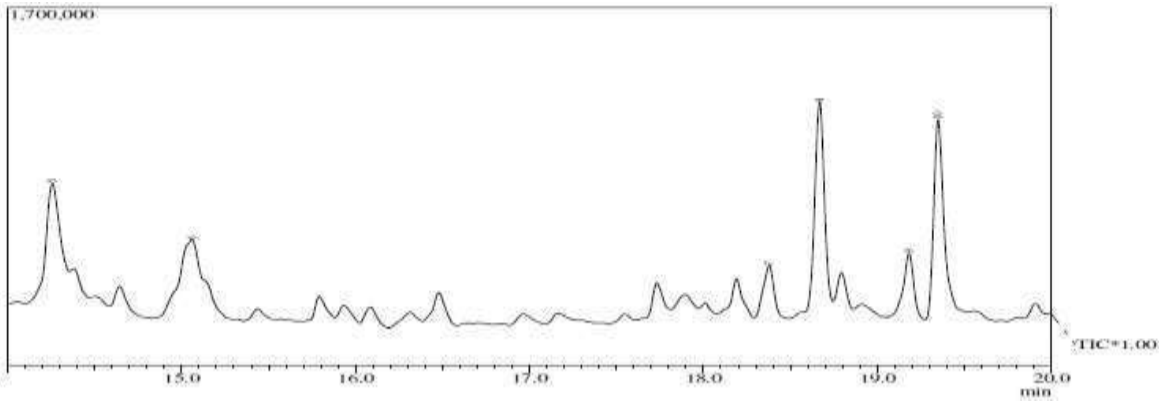
Appendix 6. The GC-MS chromatogram of derivatized extract from muscle of gourami (*T. pectoralis*).



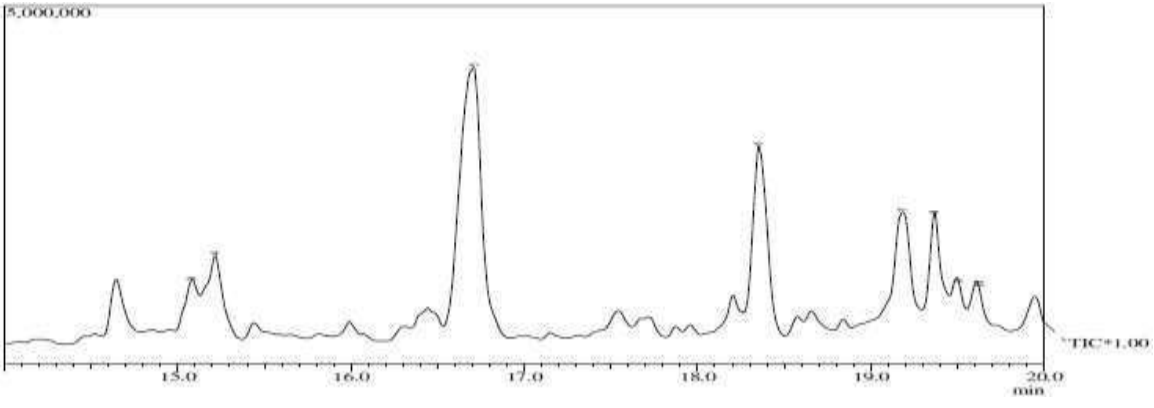
Appendix 7. The GC-MS chromatogram of derivatized extract from muscle of whiptail stingray (*H. walga*).



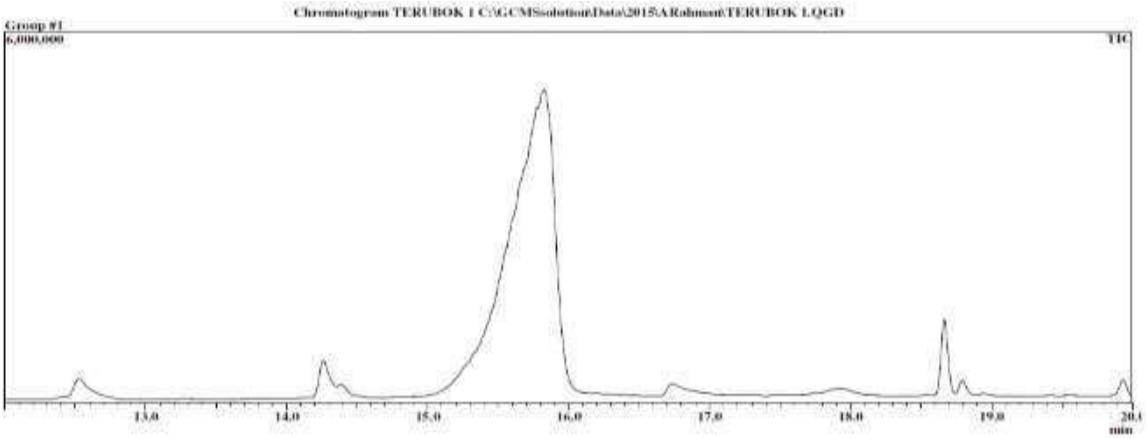
Appendix 8. The GC-MS chromatogram of derivatized extract from muscle of salted mackerel (*S. guttatus*).



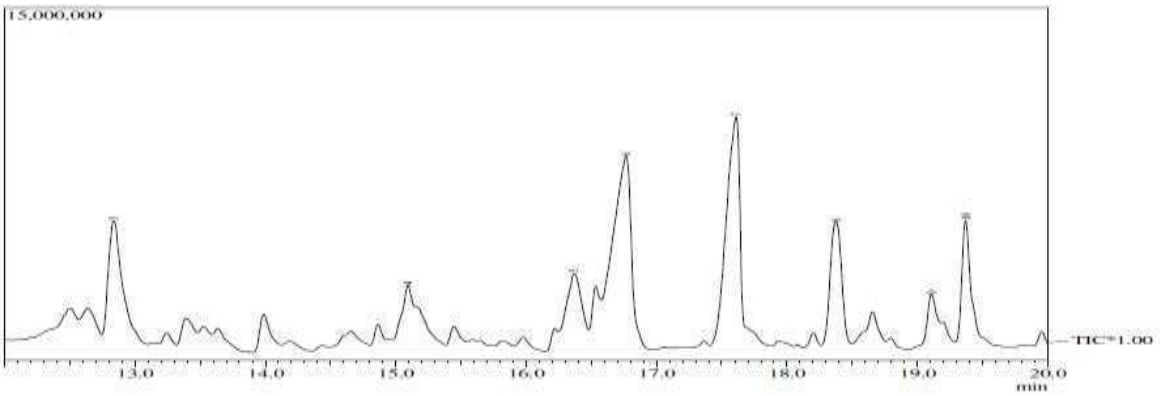
Appendix 9. The GC-MS chromatogram of derivatized extract from muscle of salted sardine (*S. gibbosa*).



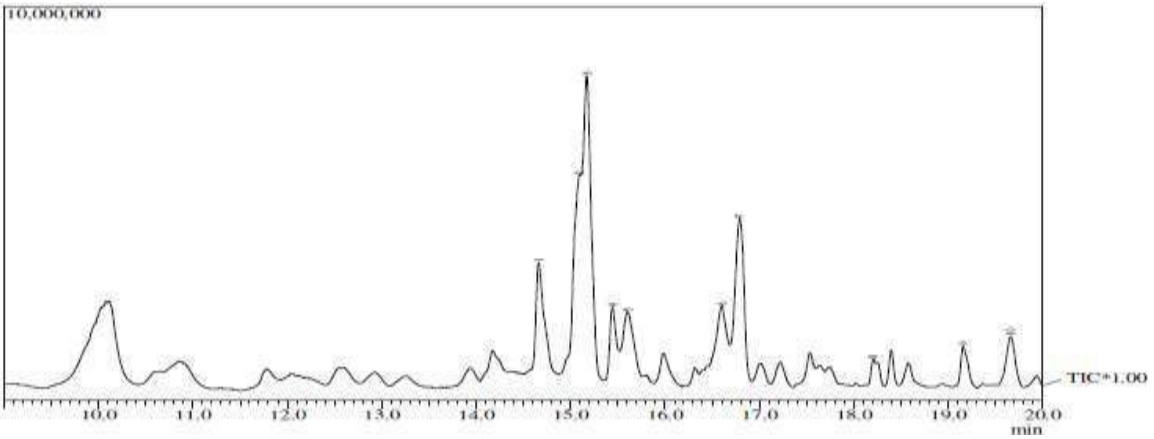
Appendix 10. The GC-MS chromatogram of derivatized extract from muscle of salted toli shad (*T. toli*).



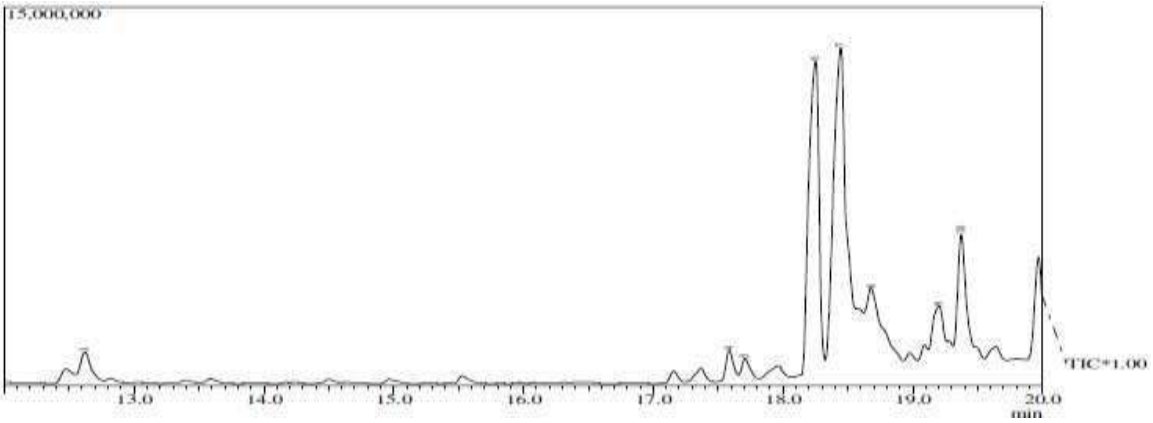
Appendix 11. The GC-MS chromatogram of derivatized extract from muscle of salted gourami (*T. pectoralis*).



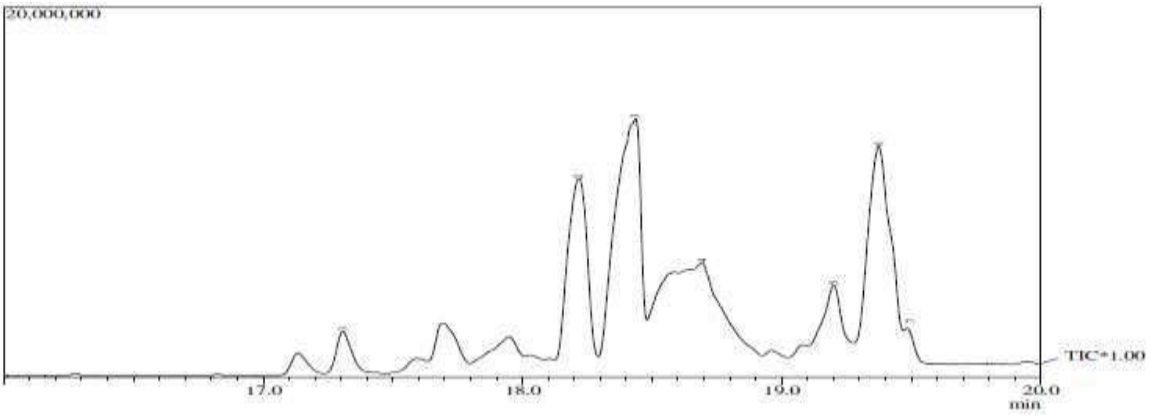
Appendix 12. The GC-MS chromatogram of derivatized extract from muscle of salted whiptail stingray (*H. walga*).



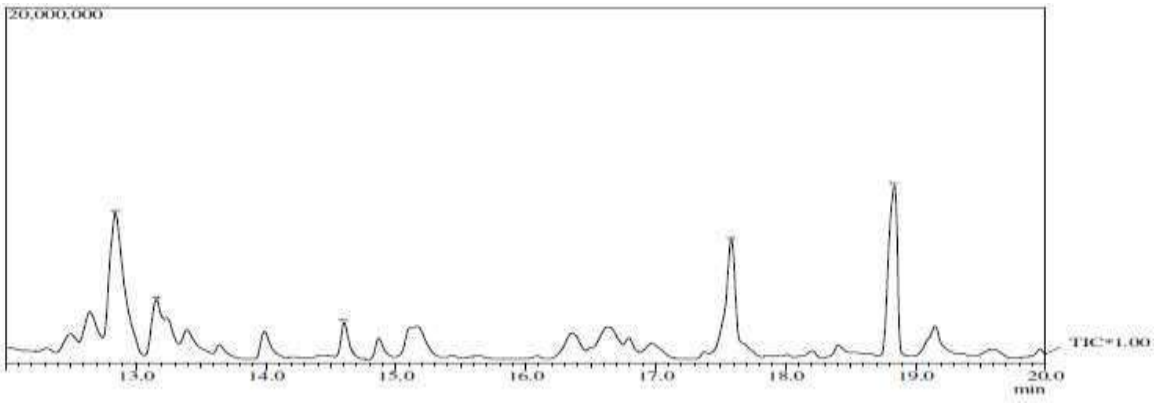
Appendix 13. The GC-MS chromatogram of derivatized extract from muscle of canned mackerel (*Scomberomorus* sp.).



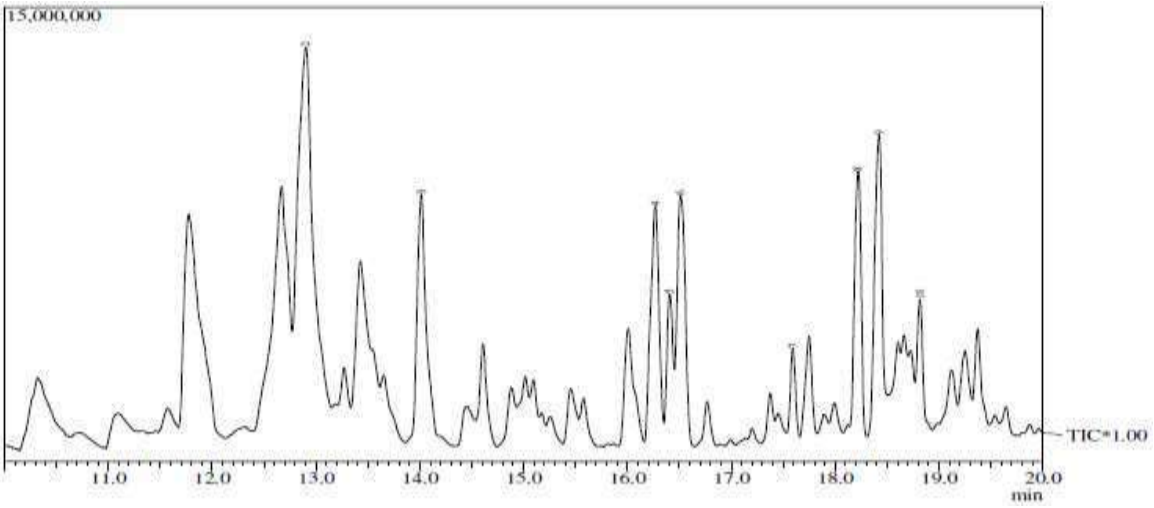
Appendix 14. The GC-MS chromatogram of derivatized extract from muscle of canned sardine (*Sardinella* sp.).



Appendix 15. The GC-MS chromatogram of derivatized extract from muscle of canned salted sardine (*Sardinella* sp.).



Appendix 16. The GC-MS chromatogram of derivatized extract from muscle of canned dace (*L. leuciscus*).



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