METHOD DEVELOPMENT FOR FORENSIC ANALYSIS OF BODY ODOUR USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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METHOD DEVELOPMENT FOR FORENSIC ANALYSIS OF BODY ODOUR USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

by

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DECLARATION

I declared that the material presented in this thesis is all my own work. The thesis has not been previously submitted for any other degree.

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemist
API-MS	Atmospheric Pressure Source-Mass Spectrometer
DNA	Deoxyribonucleic Acid
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometer
HIV	Human Immunodeficiency Virus
IS	Internal Standard
IUPAC	International Union of Pure and Applied Chemistry
LLE	Liquid-liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
MHC	Major Histocompactibility Complex
PCA	Principal Component Analysis
PTFE	Polytetrafluoroethylene
PVF	Polyvinyl Fluoride
SD	Standard Deviation

SESI	Secondary Electrospray Ionization
SPME	Solid Phase Microextraction
STU	Scent Transfer Unit
SVOC	Semi Volatile Organic Compounds
VOC	Volatile Organic Compounds
VVOC	Very Volatile Organic Compounds

PEMBANGUNAN KAEDAH UNTUK ANALISA FORENSIK BAU BADAN MENGGUNAKAN KROMATOGRAFI GAS-SPEKTROMETRI JISIM

ABSTRAK

Bau badan seseorang individu mempunyai kandungan yang unik disebabkan oleh peranan yang dimainkan oleh faktor genetik, cara pemakanan, persekitaraan dan juga bahan-bahan kosmetik yang digunakan oleh individu tersebut dalam menentukan bau badannya. Sifat bau badan manusia ini boleh digunakan sebagai cap jari kimia seseorang individu dalam penyiasatan forensik. Fokus kajian ini adalah untuk membangun satu kaedah yang mudah untuk mengumpul, menyediakan dan juga menganalisis sampel peluh seseorang individu. Sampel peluh daripada subjek dikumpul dengan menggunakan kapas yang telah disterilkan dengan menggunakan alkohol. Subjek melakukan senaman selama 30 minit sebelum persampelan. Setelah selesai melakukan senaman, subjek diminta mengelap lengan kanan dengan kapas yang diberikan sebelum kapas tersebut ditutup ketat dalam tiub eppendorf bersaiz 5 mL. Sebelum ditutup *dihexyl ketone* (standard dalaman), natrium sulfat kering dan juga 1 mL metanol ditambahkan ke dalam tiub tersebut dan disimpan pada suhu bilik selama satu malam. Instrumen kromatografi gas-spektrometer jisim (GC-MS) telah digunakan untuk mengesan dan mengenal pasti analit-analit kimia yang terdapat dalam peluh seseorang individu. Dua jenis sistem suntikan sampel telah dikaji kesesuaiannya dalam menjalankan analisa peluh ini. Sistem suntikan “headspace” tanpa pemekatan analit dan suntikan sampel cecair telah

dibandingkan untuk mengkaji keberkesanannya dalam analisis ini. Pelbagai teknik penyediaan sampel untuk suntikan cecair seperti penyulingan diikuti oleh pengekstrakan cecair-cecair dan juga proses pengestrakan secara langsung menggunakan pelbagai jenis pelarut telah dibandingkan bagi melihat keberkesanan dalam pengestrakan analit daripada kapas. Kajian ini menunjukkan bahawa kaedah pengestrakan analit secara langsung menggunakan metanol adalah kaedah lebih berkesan untuk analisis peluh. Pengesahan kaedah kualitatif telah dilakukan dengan menguji pengkhususan, pemilihan, kepekaan, kelinearan dan kestabilan kaedah ini. Parameter yang dinilai untuk mengesahkan kaedah ini adalah sesuai untuk analisis peluh. Antara analit-analit yang ditelah dikesan dalam peluh manusia adalah asid-asid organik yang mempunyai kandungan karbon lebih daripada tiga belas, sebatian alkana, sebatian alkena dan alkohol. Analisis sebatian-sebatian yang terdapat dalam peluh manusia menggunakan kaedah yang dibangunkan telah menunjukkan bahawa komponen peluh yang utama iaitu komponen primer boleh digunakan untuk pengenalpastian individu. Sebatian-sebatian kimia yang terdapat dalam peluh juga boleh digunakan untuk mengenal pasti tabiat individu tersebut seperti merokok, ubat-ubatan yang diambil dan juga penyalahgunaan dadah. Secara kesimpulannya, cap jari kimia berdasarkan bau badan seseorang individu adalah sejenis kaedah yang boleh digunakan untuk membantu penyiasatan forensik dan mengecilkan senarai saspek.

METHOD DEVELOPMENT FOR FORENSIC ANALYSIS OF BODY ODOUR USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

ABSTRACT

Body odour of an individual has unique composition due to the influence of specific combination of genetic, diet and environment on the production of the compounds responsible for giving this odour. The characteristic of human odour could be used as a human chemical fingerprint for forensic identification purposes. This research focused on developing an easy, fast and reliable method to collect, prepare and analyse the components of human body odour. The odour was collected from male subjects using a pre-treated gauze material. The subject was requested to exercise for 30 minutes prior to sampling by using the gauze to wipe over the right arm before sealing the gauze in a 5 mL eppendorf tube. Before sealing dihexyl ketone (internal standard), anhydrous sodium sulphate and also 1 mL methanol were added into the tube and left overnight at room temperature. The gas chromatography-mass spectrometer was used for the detection and identification of the components of human body odour. Two different injection systems; direct static headspace injection without pre-concentration and also the liquid injection system were compared for their suitability for this analysis. Sample preparation techniques for liquid injection by steam distillation followed by liquid-liquid extraction as well as direct extraction method using several different solvents were compared for the effectiveness to extract analyte from the collection material. This study demonstrated that direct extraction method using methanol as extraction solvent and analysis through

liquid injection system via GC-MS were the optimum analysis method for body odour. Qualitative method validation was performed to evaluate the selectivity, specificity, sensitivity, linearity and stability of this method. The evaluated parameters validated the method fitness for its purpose. The analysis of the body odour revealed that it contains majority of organic acids with thirteen carbons and above, alkanes, alkenes, alcohol and others. Analysis of the compounds in the odour of the subjects by applying the developed method reveals that primary odour components can be used to identify an individual. In addition, the compounds present in the sweat of an individual are also useful to identify specific habit of a person, for example; smoking, medications consumed, and drug abuse. In conclusion, chemical fingerprint of body odour may be used to aid forensic investigation to identify and also narrow down suspect pool.

CHAPTER 1:

INTRODUCTION

1.1 Research Background

Human skin secretions are composed of unique combination of organic compounds which can be used as a biometric identification method. In recent years numerous researches have been conducted to gain better understanding of the human skin secretion production, composition and its unique chemical fingerprint for each individual(Curran, 2005; Penn *et al.*, 2007). Advancement in the scientific instrument with very low level sensitivity such as gas chromatography coupled with mass spectrometer or tandem mass spectrometer has enormous impact on the studies of the composition of human body odour (Curran *et al.*, 2005). The odour emanated from the human skin secretion is commonly termed as the body odour.

Theories have been presented to identify the origin of the odour compounds and its composition. Among the odour source that have been previously discussed is the natural skin secretion and its interaction with microorganism on the skin (Zhang *et al.*, 2005) and also the genetic influence of the major histocompactibility complex (MHC) proteins on the production of MHC-associated odours (Curran *et al.*, 2010b).

Body odour is composed of long chain fatty acids and many other compounds from other functional groups (Ostrovskaya *et al.*, 2002). Body odour also differs in composition according to different parts of the human body such as back and the forearm (Gallagher *et al.*, 2008). However, the primary odour compounds present in any part of the body are consistent and does not differ from day to day regardless of the influence from diet and environment (Curran *et al.*, 2005). This primary odour

compounds are the components responsible for individual identification (Curran *et al.*, 2005).

1.2 Body Odour and Forensic Investigation

The human scent in trailing and identification of suspects with the help of the canine species is a widely applied technique in the crime scene investigation globally (Schoon and Bruin, 1994). The olfactory acuity of dogs and their responses to odour have been extensively studied by several researchers (Schoon and Bruin, 1994; Hirano *et al.*, 2000; Lasseter *et al.*, 2003; Oesterhelweg *et al.*, 2008; Curran *et al.*, 2010a; Pinc *et al.*, 2011). However, there is definite lack of understanding of the odour identification mechanism by the canine species (Curran *et al.*, 2010a).

The advantages of using canine for the detection of body odour include their superior olfactory ability and also the easy and uncomplicated process by which the detection is done. In spite of this, using canine in crime investigation can cause other problems such as biological and physical contamination of the crime scene. Training the canine unit also requires extensive funds and training. The detection accuracy depends on the subjective evaluation of the odour by canine with differing olfactory ability and training it has undergone (Lasseter *et al.*, 2003). External influence on the dog's performance such as weather, temperature, humidity, handler's belief and cues given by the handler also can be the downside of using canine based scent identification (Lasseter *et al.*, 2003; Lit *et al.*, 2011).

The analysis of body odour using Gas Chromatography Mass Spectrometer (GC-MS) has not been previously used in Malaysia for crime investigation purpose because proving the uniqueness of the body odour and characterising it using a scientific instrument is still in its early research stages. Research into the uniqueness and

stability of the body odour is imperative to establish body odour fingerprinting as a tool that may aid in forensic investigations.

1.3 Problem statement

The primary odour components of each individual have been identified using scientific instruments in several findings (Curran, 2005; Curran *et al.*, 2005; Penn *et al.*, 2007; Gallagher *et al.*, 2008), but this method of the odour analysis is yet to be incorporated into crime investigation. The lack of standard operating guide and exposure of the law authorities to the science of this evidence is the main reason for the very limited application of electronically defined human odour in forensic field (Prada and Furton, 2008). The methods that have been previously published require specialized injection autosampler such as Solid Phase Microextraction (SPME), Purge and Trap or automated thermal desorption chamber coupled with GC-MS. These samplers are not commonly available in all laboratory units.

There are limited understanding on the origin of body odour (Curran *et al.*, 2005). The problem in understanding the origin of body odour is due to the complexity of the human skin secretion (Curran *et al.*, 2010a), and various external and internal factors influencing the odour production (Oesterhelweg *et al.*, 2008; Hudson *et al.*, 2009; Curran *et al.*, 2010a; Pinc *et al.*, 2011). Different sample collection method and sample extraction method produces different type of compounds detected in the same individual. Since there is no definitive prove of the origin of body odour and its primary odour components, analysis of the body odour using different methods of analysis is required to gain better understanding of the body odour.

Previous researches have been conducted using concentrated headspace analysis of adsorbent material containing odour which focuses more on identifying the more

volatile analyte and analyte with high vapour pressure in the body odour (Curran, 2005; Curran *et al.,* 2005). Other research which employed solvent extraction method, used acetone as the extraction solvent and the number of compounds detected was not enough for identification purposes (Natale *et al.*, 2005).

A method for the analysis of different components of body odour needed for identifying other characteristics of body odour. Primary odour component responsible for the uniqueness of body odour have to be identified. Hence, the method can be incorporated in the workflow for forensic investigation and identification of individual using body odour.

1.4 Phases of the research

The first part of this research investigates the suitable sample injection method for the instrument GC-MS to analyse the compounds in body odour. Two sampling systems; the static headspace injection system and also the liquid injection system were tested for this purpose. Comparison of the two systems can provide better understanding of the nature of the body odour components.

The second part of the study examines the appropriate sample collection and preparation method that fits the purpose of the research. Although compounds were detected in some of the experimental parameters attempted, the number of compounds was not sufficient for identification purpose using headspace injection analysis. Hence, the collection, preparation and analysis using liquid injection system were optimised to give the highest sensitivity level, consistency and reproducibility of the results.

The third part of the study focuses on the analysis of the compounds in body odour of the male human subjects by applying the developed method. The compounds detected were studied to determine intra and inter individual variations. The distribution of the body odour profile for the subjects was evaluated using Principal Component Analysis (PCA) to assess whether human body odour can be used as a biometric marker in forensic investigation.

1.5 Objectives

The main objective of this research was to develop a user friendly method which can be adapted by forensic laboratories for the analysis of body odour. This research was carried out to develop new method to collect, preserve and analyse the components of the human body odour using scientific instrument.

It was also aimed to evaluate the intra-individual and inter- individual variations of the human body odour components in order to provide preliminary scientific information for the intra individual consistency of the odour and the uniqueness of odour to each individual. In this research, the GC-MS was used to analyse the odour specimen obtained from representative male subjects from the three major ethnics in Malaysia; Malay, Chinese and Indian.

Specific objectives of this research were:

i) To evaluate different sample collection and preparation methods for the analysis of body odour.

ii) To evaluate different GC-MS sampling systems such as static headspace GC-MS and liquid injection GC-MS for best suited method for analysis of body odour.

iii) To evaluate the effect and also the difference in compounds extracted using different solvents used for analyte extraction from the collection material.

iv) To conduct qualitative validation on the developed method.

v) To study intra and inter individual variations based on the compounds detected in the analysis of the body odour using appropriate statistical tools.

vi) To determine primary odour components in an individual's body odour and evaluate its use in establishing body odour as a biometric marker.

At the end of this research, it was expected to discover more properties of the body odour and its potential application in the field of forensic as a biometric marker for identification of human.

1.6 Thesis Outline

This study is organised in five main chapters. The first chapter, the introduction chapter, illustrates the overview of the body odour analysis and the difficulties in identifying the components using scientific instruments and establishing it as a useful forensic evidence. The objectives and also the phases of research are covered in this chapter.

The second chapter is an overview of the literature study into related studies conducted previously. The definition of body odour and its characteristics, previous researches conducted to analyse body odour and its properties, the instruments and the sample collections as well as the preparation methods are addressed accordingly in this chapter. Overview into the principles of the GC-MS operation system and method development and validation guidelines is elaborated as well.

The third chapter is the detailed description of the analyses conducted and different stages of the method development, validation and also analysis of the results obtained. Chapter 4 outlines in detail the results obtained through this research. The results are tabulated and presented in diagrams for better understanding. Chapter 5 covers the discussion of the results obtained, the justification of the methods used and attempted.

The last chapter, Chapter 6 concludes the research findings and further explains the limitation of this research as well the further work warranted in this area of study.

CHAPTER 2:

LITERATURE REVIEW

2.1 Definition of the Term Identification and Racial Variations

Identification refers to discrimination or perception of the individual within a group or species (Cole, 2009). Forensic identification or "individualisation" sciences include dentition, fingerprints, writings, firearms, footwear and deoxyribonucleic acid profiling (DNA). Forensic identification begins with the recognition of the fine features and based on the belief that no two objects could leave indistinguishably similar markings (Saks, 2010). Many of the methods adopted for forensic identification are based on probability and statistics such as DNA analysis.

Each identification method has advantages and disadvantages. However, the combination of this evidence can lead to positive identification of the suspect. Development of another identification method can vastly improve the probability of positive identification of the suspect as the perpetrator.

Racial variations in human genetics are the direct result of global dispersal of anatomically modern human. The differences in racial, ethnic, and ancestral origins are results of differing allele frequencies and a little influence from socially mediated mechanisms (Barnshad *et al.*, 2005). Although some researchers believe that race is socially constructed and not related to genetic differences (Braun, 2002; Hunt and Megyesi, 2008), narrowing down the possible racial classification of a suspect may aid in the identification of the individual.

2.2 Body Odour

Odour is a combination of volatile and semi volatile organic compounds (VOC and VVOCs) produced by the human skin. These odour produced by the human body is secreted onto the skin surface via the sweat glands. The use of canine in the crime investigation mainly relies on the principle that each individual has unique odour fingerprint. The uniqueness of the odour is because of the different combination of VOCs and its abundance in every person's odour (Curran, 2005; Curran *et al.*, 2005).

Hence, the concept of odour fingerprinting as a biometric marker can be used for human identification using scientific instrument as the odour analyser instead of employing dogs. Prior to delving into the chemical analysis of odour, it is imperative to understand about the odour production mechanism and characteristics of odour.

2.2.1 Human Integumentary System and the Distribution of Sweat Glands in the Body

Human skin can be divided into two main parts: the superficial part epidermis and the deep part, the dermis. The epidermis is made up of stratified epithelium which becomes flattened as they rise to the surface. The thickness of the epidermis varies at different parts of the body. The dermis consists of dense connective tissue rich with blood vessels, connective vessels and nerves. The dermis is then connected to the deep fascia or bones by the subcutaneous tissue (Snell, 1992).

The skin has a few appendages like nails, hair follicles, sebaceous glands and sweat glands. The nails are keratinised plates on the dorsal surfaces of the tips of the fingers and toes. Hair follicles are the invaginations of the epidermis into the dermis. The follicles lie obliquely to the skin surface and the deeper end is expanded to form

hair bulbs. A band of smooth muscle, the arrector pili, connects the undersurface of the follicle to the superficial part of the dermis. The sympathetic nerve fibre innervates the muscle and causes contraction which moves the hair into a more vertical position. This action also causes the compression of the sebaceous gland and thus the secretion of its content (Chung and Chung, 2012).

Sebaceous glands are simple or compound alveolar glands that produce sebum, an oily white substance which is rich with lipids. These glands are located in the dermis of the skin. The sebaceous glands are categorised as holocrine glands because the sebum is released by the lysis and death of secretory cells. Most sebaceous glands are connected to the upper part of hair follicles by a duct, from which the sebum oils the hair and the skin surface (Eckman *et al.*, 2007). A few sebaceous glands located in the lips, the eyelids and the genitalia are not associated with hairs but open directly onto the skin surface. The sebaceous gland covers the entire body except for some part of the feet, the palms of the hands, the palm sides of the fingers and between the fingers.

Sebum can be found all over the body, including where there are no sebaceous glands because sebum flows over the skin very rapidly (Tebrich, 1993). Sebum is liquid at body temperature, and solid at room temperature. The chemical combination of the sebum is very complex mixture of free and combined fatty acids, wax alcohols, sterols, terpenoids and hydrocarbons, with compounds of relatively high molecular weight predominating. The replacement of sebum on the skin occurs very rapidly and it flows over wet skin in about 1.3 inches per second (Wingerd, 2014).

The subcutaneous layer which lies beneath the dermis consists of loose connective tissues and adipose tissues. It functions to attach dermis to the underlying tissues.

This layer also contains two types of glands that are sweat (sudoriferous) glands and ceruminous glands (in the external ear). The secretions of these glands are passed to the skin surface via ducts that lie longitudinally along the dermis and epidermis (Eckman *et al.*, 2007).

Sweat or perspiration, is a mixture of water, sodium chloride, potassium, bicarbonate and products of metabolism (Murota *et al.*, 2014). Examples of products of metabolism are urea, uric acid, amino acids, ammonia and lactic acid. The type of sweat produced and the structure of the gland can be used for the subdivision of the sweat glands. There are two types of glands, apocrine and the eccrine sweat glands. Apocrine glands are simple tubular structures found in the skin of the armpit (axillae), pubic areas and the areolar areas of the breast. The viscosity of the apocrine gland's secretion is due to the presence of metabolic substances such as fatty acids. These secretions provide an ideal growth environment for the bacteria and other odour causing organism (Seeley *et al.*, 2011).

Apocrine sweat also contains pheromones, particularly those that are sexual attractants (Clancy and McVicar, 2002). Apocrine sweat glands become active at the time of puberty because of the sex hormones. An organic substance such as 3-methyl-2-hexanoic acid, an odourless chemical, is usually present in the apocrine secretions. The quick metabolisation of this substances by bacteria is said to cause the body odour (Applegate, 2011).

The most common type of sweat gland that is present in the skin is the eccrine or merocrine sweat gland. They are simple, coiled, tubular glands that open directly onto the surface of the skin through sweat pores. Eccrine sweat glands can be divided into two parts. The deep coiled portion is located mostly in the dermis and the duct,

which passes to the skin surface. The palm of the hands and the soles of feet contains most numerous amount of eccrine sweat glands, which is absent from the margin of the lips, the labia minora and the tips of the penis clitoris (Seeley *et al.*, 2011).

The coiled part of the gland produces an isotonic fluid that is mostly water but also contains some salts (mainly sodium chloride) and small amounts of ammonia, urea, uric acid and lactic acid. The sodium chloride present in the sweat fluid is transported back into the body by active transport as the fluid moves through the duct. The resulting hyposmotic fluid that leaves the duct is called the sweat. The sweat is released to reduce the body temperature by cooling effect and also as a result of emotional stress. Emotional sweating is used in lie detector (polygraph) test because the sweat gland activity is increased when a person is lying (Wingerd, 2014). A polygraph test is executed by recording the changes in the heart rate, blood pressure breathing and the galvanic skin response. Galvanic skin response are measured by using electrodes that are connected to the hands which records skin conductance, or sweating (Coon and Mitterer, 2010).

Other glands that are present on the skin are ceruminous glands and the mammary glands. The ceruminous glands are modified eccrine sweat glands located in the ear canal. Ceruman or ear wax is composed of both ceruminous and sebaceous secretions. The mammary glands, on the hand are modified apocrine glands located in the breast that functions to produce milk (Eckman *et al.*, 2007).

2.2.2 Postulations on the Odour Production Mechanism

The exact mechanism of odour production is not fully understood yet (Curran *et al.*, 2005), however several postulations have been made over the years regarding the origin of odour. The first postulation is by Syrotuck in the year 1972. According to this postulation, the human integumentary system constantly sheds the outer epithelial layer of cells into the environment. The shed skin cells are approximately 14 microns in size and weighs approximately 0.07 micrograms. These cells are referred to as rafts. The "raft" is composed of three major components, where the combination of these three components contributes to the uniqueness of the raft. The components are one or more dead cells, approximately four microbial bacteria and body secretions (Stockham *et al.*, 2004a; Curran, 2005; Curran *et al.*, 2010a; Curran *et al.*, 2010b). The bacterial action catalysed by the secretory products on the dead skin cells is said to produce a minute vapour cloud and these combination is unique to each individual (Curran, 2005).

According to Doyle (1970), a current of warm air that surrounds the body carries the rafts from the body into the surrounding area allowing for the deposit of scent into the environment (Curran, 2005). The air coating of the human body is approximately one third to half inch thick and it contains four or five times more germ in comparison to air in the rest of the sampling room (Curran, 2005).

The second postulation for the production of human odour is by using the elimination mechanism based on characteristics of human odour that were discovered. Assuming the characteristic odour recognised is the property of some specific combination of material, it is also reasonable to deduce that these compounds are produced by a specific or unique source. It has been proven that the skin or hair transfers the odour

to other objects. Thus, this hypothesis proposed that the logical source of the human odour might therefore be one of the various secretions normal to the human skin (Tebrich, 1993; Penn *et al.*, 2007).

The three secretions that are normal to the human skin come from the eccrine, apocrine and the sebaceous glands. The eccrine sweat secretions contains one percent of fat soluble components and the rest is made up of water (Tebrich, 1993; Yamazaki *et al.*, 2010). Human urine also contains very similar composition to the eccrine secretions but experiments proved that canines cannot identify the person through their urine (Tebrich, 1993). This eliminates the eccrine sweat as the possible source of the human door (Penn *et al.*, 2007).

Considering apocrine sweat glands as the candidate for the human odour production also seems unlikely although apocrine glands are mostly corroborated with the body odour smelled by humans. This is because canines can readily take odours from a person's hand, crook of elbow, pant pockets, hair on head or armpit. The presence of apocrine sweat secretions can only be justified in case of odour detected from armpit, groin or the areolar area, thus excluding this gland as the contributor to the characteristic odour of the human (Tebrich, 1993).

Decomposing skin cells are also a poor candidate since the process takes a long time and cannot be immediately replaced. A person can be identified by canine even after a series of bath. This leaves sebum as the last remaining option for the source of the individualising odour. Unlike decomposing skin cells, sebum production is very rapid and it covers the whole body even the areas which does not contain sebaceous glands. The components of the sebum are heavy alcohols and hydrocarbons which has properties corresponding to those of human odour. Among the properties are the

limited volatility, the persistence and the solubility. However it cannot be concluded that this secretion is the only one that is recognised by the canine and contributes to the uniqueness of the human odour (Tebrich, 1993).

The other postulation for the origin of human odour is the interaction between the secretion by the skin's sebaceous gland and sweat glands with the waste products plaques from the stratum corneum. Metabolism of these products by bacterial action or by atmospheric oxidation is assumed to produce the volatile odour perceived. According to this author body odour is primarily composed of low molecular weight fatty acids, mainly aldehydes, ketones, nitrogen containing compounds and sulphur containing compounds (Yamazaki *et al.*, 2010).

The fourth hypothesis is that the human odour is distinctively linked to highly polymorphic gene within the major histocompactibility complex (MHC). The extreme nucleotide diversity in that particular region is believed to lead to the distinctive odour characteristic of each individual. There are two possible pathways for the influence of MHC in producing the human odour. The first pathway is that the MHC molecules binds to specific allele subsets of peptides and their volatile metabolites such as carboxylic acids and provide the odorants. The second pathway is that the MHC gene may influence the microbial flora populations (Curran *et al.*, 2010b). These two factors will ultimately influence the production of odour in an individual.

2.2.3 Three Categories of Odour

Body odour of an individual can be determined by several factors that are either stable over long period of time or factors which are influenced by environmental, diet and cosmetic applications. Based on these factors the body odours can be categorised into three components.

The first group is the primary odour (Curran, 2005). The primary odour is resultant of the genetic makeup of the person. Research has been conducted to study the connection between MHC genes and an individual's odour signature (Jacob *et al.*, 2002). It is well established that the MHC gene somehow affects the production of body odour in several vertebrates including humans (Santos *et al.*, 2005). A study also suggests that there could be a difference in odour intensity between MHC heterozygotes and MHC homozygotes. The homozygotes is theorised to smell more intensely especially to MHC dissimilar smellers (Wedekind *et al.*, 2006).

The second group is the secondary odour which is produced as the result of dietary and environmental influence on an individual (Curran, 2005). A study conducted by Mebazaa et al, 2011 proved that several odorant compound were found in sweat as the result of fenugreek ingestion (Mebazaa *et al.*, 2011b). It has also been said that the garlic ingestion could produce distinct body odour (Lenochova *et al.*, 2009; Mebazaa *et al.*, 2011b).

The third group of body odour is the tertiary odour, which is the result of cosmetic usage such as perfumes, body shampoo, fragranced soap, facial wash, shampoo, conditioner and make-up (Curran, 2005). Most of these products contain lipid based volatiles that is similar to odoristic compound found in natural body odour. This component of the odour is called the tertiary body odour (Curran *et al.*, 2005).

2.2.4 The Characteristics of Human Odour

An article published by the Central Intelligence Agency regarding human odour and its detection listed a few characteristics of human odour based on experiment conducted by Lohner, of the Physiological Institute of the University of Graz in 1926. Lohner conducted a series of experiments using trained canines to define the characteristic of human odour (Löhner, 1924; Tebrich, 1993).

First, Lohner postulated that the odorous substance must be a VOC. The researcher deduced this postulation based on the observation that the human odour can be removed by exposure to hot air. Odour of a human present at a distance also can be sensed by the canine. This means that the odorous substance is usually airborne for a considerable length of time, depending on the environmental condition. Thus the odour material is made up of moderately VOCs (Tebrich, 1993).

The second characteristic that he deduced is that the odorant substance must be persistent. This means that the substance is chemically stable and also relatively dense in comparison to air. The third postulation is that the odour is not readily soluble in water since detection of the odour is still possible after submersion into warm water (Tebrich, 1993).

Following Lohner's experiments, the Central Intelligence Agency conducted a few experiment of their own and came up with few other characteristics of human odour (Tebrich, 1993). They found out that canines appear to find some common individual odour produced by different parts of the human body except for urine (Tebrich, 1993). Experiment done by using the Dutch police canines also concluded that odour collected from elbow, hand and pocket of pants can be associated as similar by the canines (Schoon and Bruin, 1994). Besides, Central Intelligence Agency also found

that the human odour is susceptible to fat solvents and can be extracted from hair by the use of these. Their final finding is that the human odour does not change from day to day (Tebrich, 1993).

The human odour is also genetically controlled to some extent. This can be proven by the experiments done using identical twins (Pinc *et al.*, 2011). In the case where the identical twins are from the same diet and environment the canines could not differentiate the odour article given (Pinc *et al.*, 2011). However, the canines are able to differentiate identical twins coming from different diet and environment (Stockham *et al.*, 2004b). This indicates that although primary odour is controlled by genetic make-up of a person, diet and environment a person lives in can also influence their odour (Curran *et al.*, 2005).

2.3 Volatile Organic Compounds

According to previous researches, the components of the human skin secretion are VOCs (Gallagher *et al.*, 2008; DeGreeff *et al.*, 2011). Volatile organic compounds as defined by Minnesota Department of Health are *"Volatile Organic Compounds (VOCs) are a large group of carbon-based chemicals that easily evaporate at room temperature"* (Sethi *et al.*, 2013). There are three categories of VOCs that is, very volatile organic compound (VVOC), volatile organic compounds (VOC) and semi-volatile organic compounds (SVOC). The boiling point of the VVOC is from below 0ºC to 100ºC, while the VOC has boiling in the range 100ºC to 260ºC. The boiling point of SVOC is above 260ºC to 400ºC (Hodgson *et al.*, 1994).

2.4 Gas Chromatography Mass Spectrometer

2.4.1 Gas Chromatography

The standard definition of chromatography by the International Union of Pure and Applied Chemistry (IUPAC) is “Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, stationary and mobile phase”. The chromatographic process are named according to the physical state of the mobile phase, which is gas in gas chromatography (McNair and Miller, 1998).

Since the analyte of interest are in gaseous phase, gas chromatography was chosen for the separation of the analyte and subsequent detection by mass spectrometry which is very fast and easy way for identification of the compounds separated by the gas chromatography.

The separation process using gas chromatography starts with the gas tight syringe which transfers a portion of the sample onto the column. The constant flow of the carrier gas, the mobile phase, moves the sample along the column. The different compounds in the sample interact differently with the stationary phase in the column, separating each compound along the length of the column. The continuously streaming carrier gas will eventually move the compounds to the detector but at different retention time. The compounds with least interaction with the stationary phase will elute first (McNair and Miller, 1998; Scott, 1998; Slack *et al.*, 2003; Skoog *et al.*, 2007).

The length of a column directly affects the separation efficiency and analysis time. The separation efficiency of a column is proportional to the square root of the column length. However, the use of a very lengthy column will also increase the

analysis time. The internal diameter of a column affects the analyte separation and the amount of sample that can be loaded onto a column. Smaller internal diameter gives better separation but reduces the maximum amount of sample that can be loaded onto the column (Grob and Barry, 2004). The column chosen for every analysis must be able to balance the advantages and the disadvantages and yield good peak resolution and shape in the resulting chromatogram.

Mass spectrometer is the detector which functions by first ionising the separated analyte using either electron ionisation or chemical ionisation. The compounds bombarded with high energy electrons in case of electron ionisation will break into several groups. The vulnerable bonds in the structure of the compound such as functional group will break. The charged particles are repelled or attracted by charged lenses into mass analyser. The magnetic or electrical plates present in this chamber will deflect the ionic species according to the mass to charge ratio. Finally the detector is used to count the ions and generate a mass spectrum for each compound separated by the gas chromatograph (McNair and Miller, 1998). The identification of the compounds can be done by comparing the mass spectrum of each peak to the existing library.

2.4.2 Sample Injection Systems in Gas Chromatography

The samples for gas chromatography analysis can be in either gas or liquid state. The chemical separation method for samples based on water matrix which contains volatile analyte of interest can be divided into two, the purge and trap method and the static headspace method (McNair and Miller, 1998). The purge and trap method, also known as the dynamic headspace method involves purging the analyte of interest from the aqueous phase to gas phase using inert gas and then separating the analyte

from the stream of gas by adsorbent filters. The filters are then heated to release the compounds into the GC carrier gas.

The static headspace method is operated by collecting analyte of interest from the headspace of the vial containing the sample using a gas tight syringe and then analysed using gas chromatography (Jacq *et al.*, 2008a). Although this method is less sensitive compared to the purge and trap method, the operation is relatively simpler and easily automated which saves time. Static headspace method also offers higher robustness, less carryover, cross contamination, foam formation and water management problems (Jacq *et al.*, 2008a).

2.4.3 Method Development and Validation for Gas Chromatography-Mass Spectrometry Analysis

2.4.3.1 Static Headspace Analysis

The sample preparation optimisation for static headspace analysis includes obtaining the optimum incubation time and temperature of the sample prior to analysis, vial pressure and studying the effect of salt addition (Sugaya *et al.*, 2001; Jacq *et al.*, 2008a). The static headspace technique is an equilibrium technique where the optimum condition for sampling is when equilibrium is reached between concentration of the solute in the sample and in the headspace gas phase (Jacq *et al.*, 2008a). The equilibrium can be affected by two factors that are the incubation time and temperature of the sample vial prior to injection into the GC-MS.

An optimum incubation time and temperature can be determined by gradually increasing the time and temperature until the overall highest peak for the analyte compounds in the chromatogram is achieved for the same concentration of sample (Sugaya *et al.*, 2001; Sakata *et al.*, 2004; Jacq *et al.*, 2008a). Too much increase in

the incubation temperature will result in higher vial pressure. The resulting internal pressure in the vial is transferred into the syringe during sample extraction and finally lost of analyte occurs to the atmosphere. The incubation time influences the method sensitivity and repeatability (Sakata *et al.*, 2004)

The vial pressure is another factor to be considered during sample preparation optimisation. When equilibrium is reached, the vial is pressurised with the carrier gas and then pressurised headspace is vented to a gas sampling valve with sample loop. Subsequently, the analyte is injected into the GC-MS for analysis. The pressure functions to drive the sample to the loop. Fewer samples will enter the loop for analysis if there is lack of pressure, meanwhile excessive dilution of sample will occur if too much pressure is used (Jacq *et al.*, 2008a).

The sensitivity of the static headspace method can be increased by increasing the amount of analyte in the headspace during equilibrium (Sugaya *et al.*, 2001; Jacq *et al.*, 2008a). The concentration of VOC in the headspace depends upon a few factors such as initial concentration in the sample, the phase ratio between liquid phase and gas phase and the water/air distribution constant. The water/air distribution constant in turn depends upon solute characteristics (vapour pressure, water solubility), temperature, pressure, pH and salt concentration. By increasing the salt concentration in the sample, the VOC in the sample is driven out into the headspace to maintain the equilibrium (Jacq *et al.*, 2008a).

The extraction efficiency of VOCs from biological samples can be improved by adding inorganic salt. Salting out can be used to lower the detection limit of an analysis and to buffer random salt concentration. A study was conducted to test the salting out ability of five inorganic salts, sodium chloride, potassium chloride,

magnesium sulphate, potassium carbonate and sodium carbonate. Out of all the five salt, sodium chloride and potassium chloride showed significant improvement, with sodium chloride being the best due to hydration energies (Kusano *et al.*, 2011).

2.4.3.2 Steam Distillation

Steam distillation is a process that is often used to extract essential oil from plant material (Nixon and McCaw, 2001). The same concept can be used to extract human odour for the gauze used to collect the swab sample. Since only a trace amount of analyte is present in the sweat absorption material, a sample clean up method and concentration method is needed for a successful analysis. The human odour can also be viewed as the perfume of an individual or a concentrated essence of the person.

There are three methods of steam distillation. The first method is to place the sample in water, bring the water to a boil and recover the oil and vapour by condensing the vapours. The essential oil which has lower density compared to water will form a layer on top which can be siphoned using a pipette or extracted using solvents. The second method is executed by boiling the water in separate vessel and passing steam over the sample placed in a different flask. The third method is called dry distillation, where the sample is also heated during the process so that the steam would not condense on the sample material (Nixon and McCaw, 2001).

The VOCs (with wide range of boiling point) in the human odour can be extracted from the gauze into a liquid medium without compromising the chemical structures of heat sensitive compounds through distillation. Steam distillation produces localised high temperature for a short period of time and effectively extracts materials like essential oils. Dalton’s Law states that if two substances are immiscible, the total vapour pressure in a system of gases is the same as the sum of

the vapour pressures of its components. If a mixture of two liquids is heated, the boiling will occur when combined vapour pressures of the liquid equals the atmospheric temperature. Condensation of the vapours gives a two phase mixture (condensate or distillate) of the organic species and water (Nixon and McCaw, 2001).

The ability of this method to separate thermally labile, high-boiling substances from relatively non-volatile material makes it very useful and easy sample preparation method for the analysis of the human odour (Nixon and McCaw, 2001). The water insoluble human odour can then be extracted from the distillate with water immiscible solvents.

2.4.3.3 Liquid-Liquid Extraction

Liquid-liquid extraction (LLE) is a process of extraction of analyte based on their relative solubility in two different immiscible liquids. Liquid-liquid extraction is also known as solvent extraction partitioning. The common liquid used in this process is water and organic solvent. The analyte of interest is extracted from water into the solvent which can then be separated physically (Müller *et al.*, 2002).

2.4.3.4 Analytical Parameter Optimisation for Gas Chromatography-Mass Spectrometry

Following sample preparation optimisation, the analytical parameters for the GC-MS analysis should be optimised as such that the chromatogram consists of peaks with good resolution and less dispersion (Sakata *et al.*, 2004). The analytical parameters to be optimised are the injector mode and temperature, carrier gas type and flow rate and the column temperature programming.