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Comparison of Medical and Forensic Profiling Potential of Volatile Biomarkers from Different Biological Specimens from Individuals and Across Populations

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COMPARISON OF MEDICAL AND FORENSIC PROFILING POTENTIAL OF VOLATILE BIOMARKERS FROM DIFFERENT BIOLOGICAL SPECIMENS FROM INDIVIDUALS AND ACROSS POPULATIONS

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Maiko Kusano

2010

To: Dean Kenneth Furton
College of Arts and Sciences

This dissertation, written by Maiko Kusano, and entitled Comparison of Medical and Forensic Profiling Potential of Volatile Biomarkers from Different Biological Specimens from Individuals and Across Populations, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: October 28, 2010

The dissertation of Maiko Kusano is approved.

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DEDICATION

This dissertation is dedicated to my parents, Yoichi and Keiko Kusano.

Without their unconditional love and silent yet continuous support from the other side of the world, the completion of this work would not have been possible.

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ABSTRACT OF THE DISSERTATION

COMPARISON OF MEDICAL AND FORENSIC PROFILING POTENTIAL OF VOLATILE BIOMARKERS FROM DIFFERENT BIOLOGICAL SPECIMENS FROM INDIVIDUALS AND ACROSS POPULATIONS

by

Maiko Kusano

Florida International University, 2010

Miami, Florida

Professor Kenneth Furton, Major Professor

There is limited scientific knowledge on the composition of human odor from different biological specimens and the effect that physiological and psychological health conditions could have on them. There is currently no direct comparison of the volatile organic compounds (VOCs) emanating from different biological specimens collected from healthy individuals as well as individuals with certain diagnosed medical conditions. Therefore the question of matching VOCs present in human odor across various biological samples and across health statuses remains unanswered.

The main purpose of this study was to use analytical instrumental methods to compare the VOCs from different biological specimens from the same individual and to compare the populations evaluated in this project. The goals of this study were to utilize headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME-GC/MS) to evaluate its potential for profiling VOCs from specimens collected using standard forensic and medical methods over three different populations: healthy group with no

diagnosed medical or psychological condition, one group with diagnosed type 2 diabetes, and one group with diagnosed major depressive disorder.

The pre-treatment methods of collection materials developed for the study allowed for the removal of targeted VOCs from the sampling kits prior to sampling, extraction and analysis. Optimized SPME-GC/MS conditions has been demonstrated to be capable of sampling, identifying and differentiating the VOCs present in the five biological specimens collected from different subjects and yielded excellent detection limits for the VOCs from buccal swab, breath, blood, and urine with average limits of detection of 8.3 ng.

Visual, Spearman rank correlation, and PCA comparisons of the most abundant and frequent VOCs from each specimen demonstrated that each specimen has characteristic VOCs that allow them to be differentiated for both healthy and diseased individuals. Preliminary comparisons of VOC profiles of healthy individuals, patients with type 2 diabetes, and patients with major depressive disorder revealed compounds that could be used as potential biomarkers to differentiate between healthy and diseased individuals. Finally, a human biological specimen compound database has been created compiling the volatile compounds present in the emanations of human hand odor, oral fluids, breath, blood, and urine.

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1. RESEARCH INTRODUCTION

1.1. Basis for research

Cases have been reported where pet dogs have detected cancers on their owners[1,2]. In each case, the patient's dog paid excessive attention to a particular part of the owner's body and, upon seeking medical advice, the owners were diagnosed with cancer. Cases like these have triggered interest in the scientific community to investigate the odors emitted by the human body. In the forensic science field, this is especially true in terms of understanding how canines differentiate people by their scents.

Volatile organic compounds (VOCs) are naturally occurring chemical compounds that are characterized by having low boiling points. The human body produces odors made up of a variety of VOCs. The individual odor hypothesis theorizes that each individual possesses a unique scent, referred to as "human scent", which allows for scent identification using canines in law enforcement and forensic fields. However, if persons with certain diseases are known to emit disease-specific odors, it is possible that problems may arise in scent identification line-ups as a result of diseases or disorders which could result in false matching of persons of interest. Therefore, the analysis of the scent biomarkers among different populations can provide valuable information as to what impact it could have on law enforcement in terms of matching people. Identification of such biomarkers is also useful for the medical field for diagnostic purposes. To date, no studies have been published that compare the volatile organic compounds obtained from different biological specimens collected from healthy volunteers, those with type 2 diabetes, and clinically depressed patients to establish a profile that can be used to

differentiate between healthy and diseased persons. Results obtained from this research will provide valuable information not only for the medical perspectives but also for the law enforcement/person detection perspectives helping to determine the uniqueness of odor profiles from different individuals for diagnostic and/or identification purposes.

1.2. Research Objectives

This research was conducted to test two main hypotheses:

Hypothesis I: Individuals can be differentiated using any biological fluids.

Hypothesis II: Subjects can be grouped based on being healthy or having a medical or psychiatric condition.

The main objectives of this research were to employ analytical instrumental methods to compare the VOCs from different samples taken from the same individual and to compare among the populations evaluated in this project. The content of this report focuses on data obtained via instrumental evaluation of human scent samples from different biological specimens, as well as samples taken from different populations. The different tasks that were addressed are listed below.

a. Optimization of collection and analysis methods for human scent samples from different biological specimens using SPME to extract the volatile compounds and GC/MS as separation and identification technique
b. Evaluation of odor profiles of different specimens of individuals over time
c. Evaluation of the similarities/differences in sampling from different biological specimens using SPME-GC/MS

d. Population analyses of the VOCs present above collected odor samples using SPME-GC/MS
    i. Healthy individuals
    ii. Patients with type 2 diabetes
    iii. Patients with Major Depressive Disorder (MDD)
e. Creation of human biological specimen compound database

1.3. Forensic Science and Human Scent

1.3.1. Admissibility of Scientific Evidence

For scientific evidence to be admissible in a United States court of law, it must satisfy the requirements of either the *Frye standard*, the *Daubert standard*, or the Federal Rules of Evidence. The appropriate standard is dependent on the case and in which jurisdiction the case is being handled. The Federal Rules of Evidence govern the admission of evidence in civil and criminal proceedings in federal courts. These rules are applied through the cases of *Frye v. United States* (1923) and *Daubert v. Merrell Dow Pharmaceuticals* (1993)[3].

The *Frye standard*, or the general acceptance test, is applied to determine the admissibility of scientific evidence. Under the *Frye standard*, scientific evidence is admissible if the technology is "generally accepted" in that field. *Frye* defines general acceptance as follows:

> Just when a scientific principle or discovery crosses the line between the experimental and demonstrable stages is difficult to define. Somewhere in this twilight zone the evidential force of the principle must be recognized, and while courts will go a long way in admitting expert testimony deduced from a well recognized scientific principle or discovery, the thing from which the deduction is

> made must be sufficiently established to have gained general acceptance in the particular field in which it belongs[3].

The issue with the *Frye* standard is that it does not specify in which field the technology is accepted. It also does not distinguish between science and pseudo-science. In 1975, the *Frye* test was superseded by the Federal Rules of Evidence (Rule 702) governing expert testimony:

> If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise, if (1) the testimony is based upon sufficient facts or data, (2) the testimony is the product of reliable principles and methods, and (3) the witness has applied the principles and methods reliably to the facts of the case[4].

While many states (including Florida, New York, Alaska, California, and Washington) still follow the *Frye* standard, the U.S. federal court and half of the states now use the *Daubert* standard in their proceedings governing the admissibility of expert testimony. With the *Daubert* standard, scientific expert testimony's admissibility is regulated by several guidelines. The judge is given the task of "gatekeeping" in which s/he must ensure the relevance and reliability of the expert's testimony to the case. Beyond "general acceptance" as is the case with *Frye*, *Daubert* requires that the scientific knowledge is a product of a scientific methodology which has been tested for its validity and reliability, subjected to peer review and publication, tested for its error rate, have standards and controls of operation, and has gained widespread acceptance in the relevant scientific community[3].

1.3.2. Human Scent as Scientific Evidence

Human scent as scientific evidence is still very controversial and often challenged in court[5]. While research on human scent has been a growing interest in the scientific and law enforcement communities, there are still many unanswered questions concerning the use of human scent and there is not a common method of evidence collection, storage, and analysis that is implemented across different law enforcement agencies and research groups. For this reason, introduction of human scent evidence in a United States court is not an easy task; however, there are instances wherein human scent evidence has been accepted as scientific evidence in court cases.

The most recent U.S. court ruling pertaining to human scent evidence was a court case in 2009 of *U.S. v. Wade* where the defendant requested a *Daubert* hearing regarding the reliability of human scent trailing canines[6]. The prosecution introduced evidence that the scent of the victim was trailed to the defendant's residence by a trained trailing dog. Scent trailing was performed using scent collected with the scent transfer unit from the victim's clothing. The introduction of this human scent (dog trailing) evidence was challenged by the defendant on the durability of human scent, the reliability of the scent transfer unit, and the dog trailing methods in general. The court ruled that there was no prejudice from the admission of the scent evidence because of other sufficient corroborating pieces of evidence that justified the issuance of the search warrant. In this case, the dog trail evidence met the threshold for reliability under Evidence Rule 702 and *Daubert* and was ruled admissible. Table 1 lists several court rulings pertaining to the use of human scent evidence.

Table 1. U.S. Court Cases with Human Scent Evidence

Court Case	Year	Standard	
People v. Gonzalez 218 Cal.App.3d 403	1990	*Frye*	Corroborating evidence is required when using canine human scent evidence.
People of the State of California v. Jeffrey Dewyane Mitchell[7]	2003	*Frye*	Evidence was scent collected from scent transfer unit. Issues on the novelty of the device as well as the degradation of human scent after collection were challenged.
People v. Ryan Willis[8]	2004	*Frye*	Dog scent identification was challenged on the persistence and uniqueness of human scent, as well as the adequacy of the certification procedures for scent identification.
People of the State of California v. Benigo Salcido[9]	2005	*Frye*	Scent identification line-up by canines was challenged on the basis of the uniqueness and survivability of human scent. Court ruled that human scent evidence can be admissible if "the person performing the technique used the correct scientific procedures, the training and experience of the dog and dog handler prove them to be proficient, and the methods used by the dog handler in the case are reliable.[9]"
U.S. v. Wade	2008	*Daubert*	Dog trailing evidence was accepted as meeting the standard for admissibility under Rule 702 and *Daubert* because dog trail evidence is "based on scientifically valid principles[6]".

1.3.3. Canines and Human Scent

Specialized use of human scent in criminal investigations is well-established by detector canines. Scent-discriminating canines have been used in criminal investigations for over 100 years. Many European countries have been using trained dogs for scent identification line-ups, where the dogs match the scent sample from a crime scene to scent collected from the hands of a suspect. Human scent identification line-ups have gained widespread acceptance in Europe, and have been admitted as evidence in the court. However, it is still very controversial and debated upon in the United States. Studies have indicated that with sufficient training, detector canines are capable of matching scents emanating from different areas of the body[10,11]. In addition to criminal investigations, canines are being trained on human scent for search and rescue to search for missing persons, disaster site survivors, and decomposing human remains[12].

1.4. Alternative Biological Specimens as Scent Sources

There is currently no direct comparison made between different biological specimens as scent sources. In the forensic field, skin odor and sweat (on clothing articles) are the main sources used for human scent identification. Forensic research on human scent have utilized scent collected on gauze, stainless steel bars, and glass beads from skin contact[10,12-14]. In the medical field, blood and urine are the specimens of choice for testing, diagnostics, and metabolic profiling. The advantages and disadvantages of the collection and testing for the five biological specimens of interest to this study are outlined in Table 2.

Table 2. Advantages and disadvantages of hand odor, buccal swabs, breath, blood, and urine testing

	Hand Odor	Buccal Swab	Breath	Blood	Urine
Sample Collection	Noninvasive	Noninvasive	Noninvasive	Invasive	Privacy concerns
Sample Availability	Microliters of insensible sweat	1 – 5 mL	> 50 mL	> 5 mL	> 50 mL
Speed of Collection	20 minutes with hand-washing procedure	< 1 minute	< 1 minute	Minutes	Minutes
Currently Routine Method?	No	For DNA	For breath-alcohol only	Yes	Yes

Published research on VOC detection and identification on different specimens generally focuses on only one specimen. The VOC research on the biological specimens of interest to this study will be discussed in detail in subsequent sections. Research on VOC detection across multiple specimens is usually only limited to two, at most three specimens[15-17]. Statheropoulos et al. reported the use of VOCs from human expired air, blood, and urine for locating entrapped people in earthquakes to determine whether a relatively small target group of VOCs common to all three matrices can be determined[18]. The VOCs from blood and urine were analyzed by headspace SPME-GC/MS and breath samples were analyzed by thermal desorption GC/MS. Acetone was found in all three matrices, and isoprene was found in both breath and blood samples.

Identification of target odor compounds can provide valuable information to both the medical and forensic communities. From the medical perspective, analysis of VOCs in biological fluids can reveal interesting diagnostic properties of different biomarkers. In addition to the disease diagnostic potential, analysis of VOCs in biological samples may

be useful in differentiating populations (i.e. healthy vs. illness). The differences found among the different populations can lead to potential for early diagnosis of certain medical diseases.

From the forensic perspective, biological evidence collected may be useful for human identification in terms of matching individuals to odor from a crime scene. Canines have the ability to discriminate human scent because people vary in their odors. However, if persons with certain diseases are known to emit disease-specific VOCs, it is possible that problems may arise in scent identification line-ups which could result in false matching between two people with the same disease. Therefore, the analysis of the volatile biomarkers among different populations can provide valuable information as to the impact of matching people for law enforcement purposes.

1.5. Theory of Instrumental Techniques

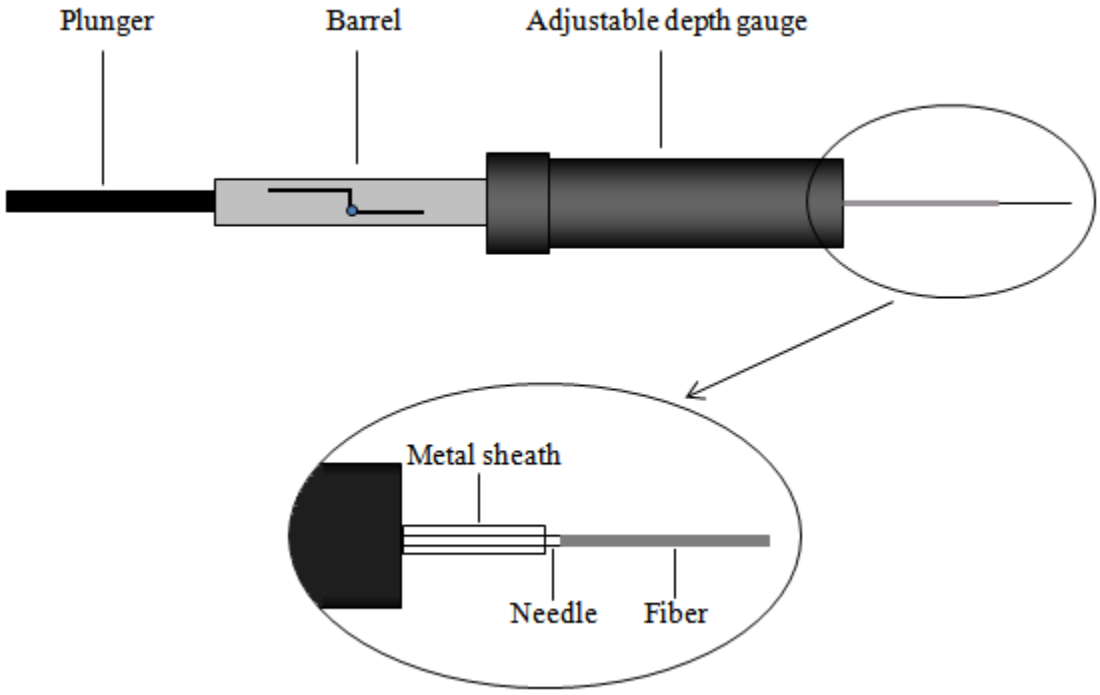
1.5.1. Solid Phase Microextraction (SPME)

Solid phase microextraction was invented in 1989 by Pawliszyn as a new preconcentration technology[19]. Since then, SPME methods have been developed and widely adopted for various applications. Solid phase microextraction gained its popularity over the recent years for numerous advantageous characteristics. The extraction process does not require a solvent, making SPME economically and environmentally friendly. Minimal sample volume is required. SPME is known for its high sensitivity when coupled to analytical instruments. It is also field portable, providing a simple and convenient mean for sampling on-site. SPME can be used for the extraction

of volatile and semi-volatile components in a sample matrix from both liquid and gaseous phases.

A SPME device has a very simple yet sophisticated configuration similar to a syringe (Figure 1). The SPME fiber, a fused silica fiber coated with an absorbent polymer about 1.0cm in length, is attached to a stainless steel needle and encased by a metal sheath for the purpose of fiber protection in between extractions. The body of the SPME holder consists of a spring loaded plunger, barrel, and an adjustable depth gauge. By pushing the plunger down the fiber is exposed out of the needle and can perform extraction of the sample. Extraction can be done by immersing the SPME fiber directly into a sample, by exposing the fiber to the headspace of a sample, or by membrane protection where the fiber is separated from the sample with a selective membrane[20]. In this study, extraction mode of interest was headspace sampling.

Figure 1. Schematic of a SPME fiber



Fiber coating varies in terms of polarity and thickness. Choosing the appropriate fiber type depends on the properties of the analytes of interest. Commercially available fibers come in various polymeric phases, some of which are shown in Table 3. Polar compounds have a higher affinity to polar coatings than non-polar coatings, where non-polar compounds will be retained more effectively with non-polar coatings. Therefore, fiber optimization and choosing the correct fiber type is essential in analysis using the SPME sampling method.

Table 3. SPME Fiber Characteristics

Fiber Coating	Polarity	Extraction	Applications
Polydimethylsiloxane (PDMS)	Non-polar	Absorbent	Volatiles, mid- to non-polar semi-volatiles
Polydimethylsiloxane/divinylbenzene (PDMS/DVB)	Bi-polar	Adsorbent	Polar volatiles
Polyacrylate (PA)	Polar	Absorbent	Polar semi-volatiles
Carbowax/Polyethylene Glycol (PEG)	Polar	Adsorbent	Polar analytes
Polydimethylsiloxane/carboxen (PDMS/CAR)	Bi-polar	Adsorbent	Gases and volatiles
Divinylbenzene/carboxen/polydimethyl siloxane (DVB/CAR/PDMS)	Bi-polar	Adsorbent	Odors and flavors

SPME is a multiphase equilibration process involving two main steps. The first step involves the partitioning of the analytes between the fiber coating and the sample matrix via adsorption or absorption. Extraction is considered complete when equilibrium is reached between the coating and the matrix. The second step is desorption of the extracted analytes from the fiber into an analytical instrument. In headspace sampling, the phases in the SPME equilibrium system are the solid or liquid sample, the headspace above the sample, and the fiber coating. The equilibrium condition can be described with the following equation:

$$n_f = \frac{K_{fs}V_fV_sC_0}{K_{fs}V_f+K_{hs}V_h+V_s} \quad \textbf{Equation 1}$$

where n_f is the amount of analyte extracted by the fiber coating, K_{fs} is the fiber coating-sample matrix distribution constant, V_f is the fiber-coating volume, $K_{hs}V_h$ is the analyte capacity of the headspace, V_s is the sample volume, and C_0 is the initial concentration of a

given analyte in the sample matrix[21]. When equilibrium is reached, the sample concentration is directly proportional to the amount of analyte extracted.

1.5.2. Gas-Chromatography/Mass Spectrometry (GC/MS)

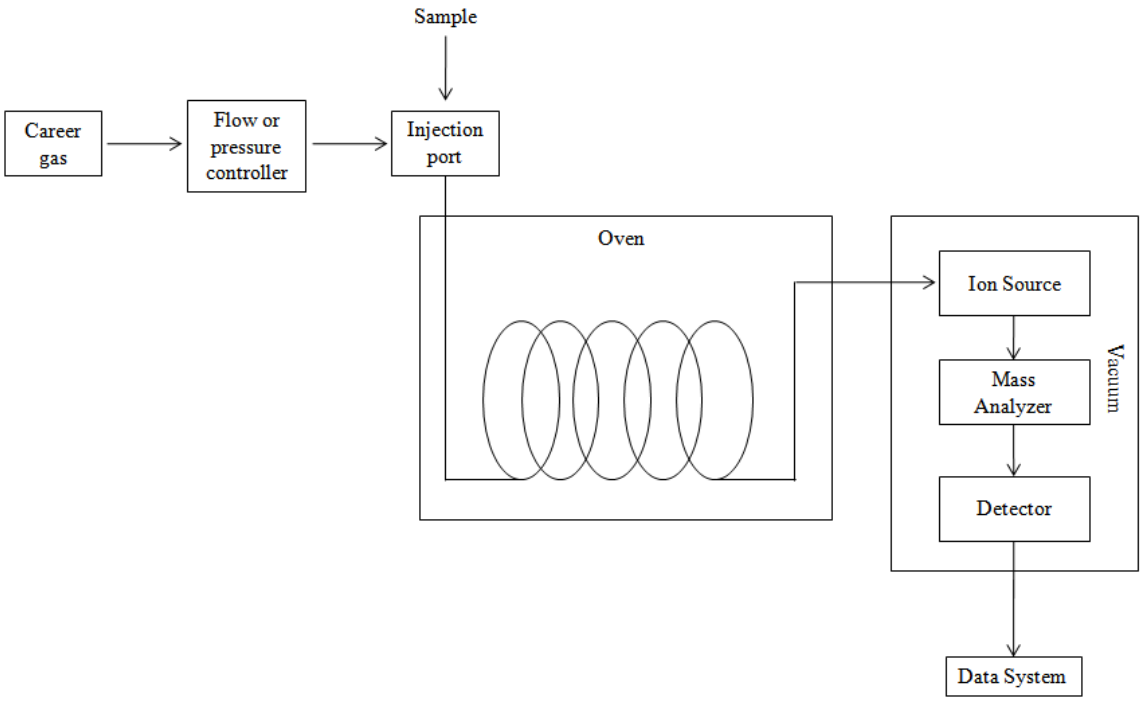
Gas chromatography/mass spectrometry is the synergistic combination of two well-established and powerful analytical instruments. Separation of analytes in complex mixture samples is performed by the gas chromatograph; structural identification of these analytes is then provided by the mass spectrometer through the measurement of mass and abundance of ions. The complimentary characteristics of GC and MS allow for the separation, quantification, and identification of substances at low concentrations and have led to the general acceptance of GC/MS as the "gold standard" in forensic and clinical laboratories.

Gas chromatography was first introduced in 1952 by James and Martin[22]. The basic operation sequence of the gas chromatograph is as follows: the volatilization of the sample introduced at the heated injection port, the separation of the analytes in the sample through selective distribution between the mobile phase (inert carrier gas, typically helium or hydrogen) and the stationary phase (coating of the column), and the detection of the separated analytes by the detector. The amount of time the analyte resides in the column, the retention time, is based on the interaction of the analyte with the stationary phase. Because the samples require volatilization at the time of sample introduction, separation by GC requires that the sample be volatile and thermally stable. Optimization of the column parameters such as polarity, phase thickness, internal diameter, and column length determine the selectivity and efficiency of the separation of

the analytes of interest[22-24]. Separated analytes are then introduced into the mass spectrometer downstream for further identification.

The first mass spectrometer was constructed by J.J. Thomson in 1912[25]. In the mass spectrometer, neutral sample molecules are ionized to generate a charged molecule or fragments of a molecule and the mass-to-charge (*m/z*) ratio of gaseous ions is measured. The sample is introduced into the ion source where the vaporized analyte is converted into ions via electron, chemical, or field ionization. The ions are then accelerated into and sorted by the mass analyzer according to their *m/z* ratio in either time or space. The sorted ions are detected by the detector in proportion to their abundance. The resulting mass spectrum of a molecule is thus a plot of *m/z* ratio against ion abundance.

Figure 2. Block diagram of a gas chromatograph coupled to a mass spectrometer



2. BIOLOGICAL SPECIMENS

2.1. Human Scent

2.1.1. Production of Human Scent

Human odor production is a complex process that is yet to be fully understood. The most common interpretation of the human odor production rests on the idea that glandular secretions, volatile organic compounds synthesized by metabolism, and bacterial action on dead skin cells play key roles in the generation of human scent. There is a current of warm air that surrounds the human body carrying germs that come from the bacteria that are shed with dead skin cells (called "rafts")[26]. This warm current of air, or the human thermal plume, allows for the deposition of human scent to the environment.

2.1.2. Distinctiveness of Human Scent

The concept that individuals have their own distinctive scent that is retained by a variety of factors such as diet, exercise, circadian and seasonal changes, menstrual cycle, emotional and physical health status has been around for over a century[27]. This individual odor hypothesis has triggered many researchers to investigate the fingerprint characteristics in and discriminatory power of compounds found in human scent. A large-scale study involving 197 subjects whose scent samples were collected five times each over a ten-week period revealed strong evidence of individual fingerprints, particularly from axillary sweat samples[28]. Statistical evaluations of data demonstrated repeat samples of individuals clustering closely. In another study, subjects were sampled during different seasons and the results showed that although emission behavior of the

scents were different between seasons, the ratios of the significant fingerprint peaks did not fluctuate as much[29].

A very recently published study by a research group at Florida International University also demonstrated that distinguishable human scent profiles can be produced by utilizing the relative ratio patterns of a combination of compounds in hand scent profiles which vary in degree of frequency of detection[13]. This research group has categorized human scent components into three distinguishing categories: primary, secondary, and tertiary[5]. The primary odor of a person contains constituents that are stable over time regardless of diet or environmental factors. Secondary odor constituents are present as a result of diet and environmental factors. Tertiary odor constituents are those which are present because of outside source influences (i.e., lotions, shampoos, and perfumes). Individual scent is likely to be a combination of various primary odor compounds differing in ratio from person to person as well as other compounds that vary among individuals. To date, no specific marker has been found that is unique or exclusive to one gender. There is still much work to be done to classify what specific compounds are exclusively "human scent", or to determine how and from where these human scent compounds originate. While fingerprint characteristics of human scent are being researched extensively, current knowledge on human scent indicates that human odor may be analogous to facial features in that there is no single measurement on the face that characterizes an individual, and characterization requires the combination of features and inclusion of other traits. The same can be said for human odor. Hence, human odor can be considered an "extended

phenotype" where the variations may be genetically or environmentally induced, or by combination of both[28].

2.1.3. Persistence and Stability of Human Scent

The persistence of human scent has also been investigated by examining the effects of aging on scent samples. In a scent-weight dissipation study, results revealed that measurable amounts of human scent compounds were still present in a controlled environment three months after the scent compounds were deposited onto sterile gauze and sealed in a glass vial[30]. However, aging of samples seems to affect the scent profiles to some extent, especially depending on the storage conditions under which they were kept. In an aging study on crime scene objects performed by the Netherlands National Police, trials were conducted where canine performance was tested to determine how well dogs could match fresh and stored (aged) scent samples[31]. Dogs were able to faultlessly match fresh scent samples to the originating subject, but when tested with stored scent samples, their performances decreased. A storage and scent stability study conducted in a laboratory setting demonstrated that scent profiles changed over time, with the greatest profile variations seen between week 0 and week 3[12]. Excessive exposure of samples to UVA/UVB also altered the human scent profile, indicating that choosing the appropriate storage conditions is vital in human scent sample storage[12].

2.1.4. Hand odor sampling

Scent collected from hand odor sampling using the method previously established served as a base human scent sample to which odor profiles of the remaining biological specimens were compared.

2.1.4.1. Materials & Methods

DUKAL brand, 100% cotton, sterile, 2 X 2, 8 ply, gauze pads used to collect hand odor were purchased from DUKAL Corporation (Syosset, NY, USA). The soap used for hand washing was Natural, Clear Olive Oil Soap from Life of the Party (North Brunswick, NJ, USA). Ten ml glass, clear, screw top headspace vials with PTFE/Silicone septa were used to hold the samples (SUPELCO, Bellefonte, PA, USA). The methanol used for the pre-treatment of gauze pads was HPLC grade (Fisher Scientific, Pittsburgh, PA, USA). Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) (50/30 μm film thickness) SPME fibers and SPME fiber holders were obtained from SUPELCO (Bellefonte, PA, USA).

Prior to sample collection, DUKAL gauze pads were pretreated by spiking 1000 μL of HPLC-grade methanol and baking in a 105°C oven for 45 minutes in an Isotemp Oven, Model 655G (Fisher Scientific, Pittsburgh, PA, USA). Pretreated gauze pads were analyzed to ensure analytical cleanliness by SPME-GC/MS.

The hand odor sampling protocol is as follows: 30 seconds washing of the hands and forearms with olive oil based soap, two minutes rinsing of the washed areas with water, two minutes air drying, and five minutes of rubbing the palms of the hands over the forearms. Subjects were given a pre-treated sterile gauze pad to hold between the palms

of their hands for ten minutes. Samples were collected in triplicates with ten minute-breaks in between each sampling. The gauze pad was re-sealed back into the 10 ml glass headspace vial. All samples were stored at room temperature and were allowed to sit for approximately 24 hours prior to extraction. No attempt was made to control microbial interactions with the samples as that may make contributions to the overall odor profile.

The GC/MS analysis was carried out using an Agilent Technologies 6890N gas chromatograph coupled to an Agilent Technologies 5973N mass selective detector (Palo Alto, CA, USA). The column used to separate the analytes was a HP5-MS, 30 m, 0.25 um, 0.25 mm with helium as the carrier gas with a flow rate of 1.0mL/min. The extracted VOCs were desorbed in the injection port of the GC with a temperature of 250°C for five minutes in splitless mode. The GC oven temperature program was as follows: an initial oven temperature of 40°C for five minutes, 10°C/min ramp to a final temperature of 250°C, followed by a final hold for two minutes for a total run time of 28 minutes. The mass spectrometer used was an HP 5973 MSD with a quadrupole analyzer operated in electron ionization mode. The mass spectrometer transfer line was maintained at 280°C and the source temperature was 230°C. The analytes were acquired in full-scan mode in 41-550 *m/z* range.

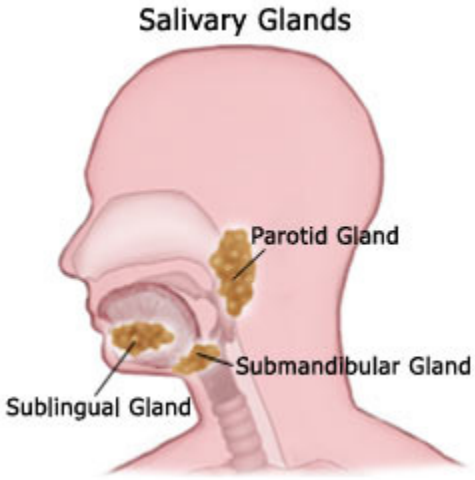
2.2. Oral Fluids (Saliva)

Saliva is an exocrine secretion composed of water, salts, mucus and the digestive enzyme α-amylase[32-35]. Human saliva has several functions pertaining to oral health and homeostasis. Protective functions of saliva include lubrication, antimicrobial, mucosal integrity, cleansing, buffering, and remineralization of the teeth. Digestive functions

include food preparation, digestion, and tasting. When food is introduced into the mouth, as chewing starts the process of digestion, saliva moistens the food and provides lubrication so that the food can be swallowed. Preliminary digestion of food starts by the polysaccharide-digesting enzyme α-amylase.

Saliva is secreted by three main salivary glands, the parotid, sublingual, and submandibular glands which are located in the mouth (Figure 3[36]). The parotid gland is found wrapped around the ramus of the mandible, and the sublingual and submandibular glands are found below the tongue. There are also numerous minor salivary glands including Von Ebner glands and Blandin-Nühm mucous glands. Submandibular glands contribute the greatest to the unstimulated saliva secretion at 65%-70%, followed by 20% by the parotid glands, 7%-8% by the sublingual glands, and less than 10% by the minor salivary glands[32,33,37]. The contributions of these salivary glands change with stimulated saliva secretion, where the parotid glands contribute the greatest at over 50% of the total saliva secretion. Saliva production from a healthy adult ranges from 500 mL to 1500 mL per day. Salivary pH is close to neutral (about six or seven), and changes with the salivary flow where the pH becomes more acidic (pH 5.3) at low salivary flow and more basic (pH 7.8) at high salivary flow[33]. Quantitative and qualitative saliva production can be affected by different conditions, pathological and physiological, including chewing, health status, stimulation by smell or taste, age, and oral hygiene[32].

Figure 3. Schematic of human salivary glands (source: Causes of Dry Mouth by Morefocus Group, Inc.)



In addition to the fluids secreted from the salivary glands, components of whole saliva also include serum and blood derivatives, oral bacterial and viral products, fluids from bronchial and nasal secretions, epithelial lining cells, and exogenous substances like food debris. There are both inorganic and organic components in saliva. Inorganic components of saliva are ions and electrolytes (Na^+, K^+, Cl^-, Ca^{2+}, H^+, HCO_3^-) that help with the buffering capacity (bicarbonate and phosphate ions), remineralization of teeth enamels (calcium and phosphate ions), and maintenance of mucosal integrity. Organic components of saliva include a wide range of proteins and glycoproteins that contribute to the protective and food- and speech-related functions of saliva. Proline-rich proteins (PRPs) are the proteins that contain high levels of proline that account for about 70% of the parotid saliva protein content[38]. Acidic PRPs contribute to the protection of oral tissues and surfaces by forming pellicle, and by maintaining saliva calcium in equilibrium with the enamel. Other acidic and basic PRPs also play roles in food processing as well

as microbial management in the mouth. Statherin, histatins, and mucins are other proteins that are major contributors to the protective, food processing, and microbial management functions of saliva. Some of the other proteins that are present in lower concentrations but also play anti-microbial roles include lysozyme, lactoferrins, cystatins, peroxidases, and secretory immunoglobulin A[33]. Most of the aforementioned proteins are multifunctional.

There are some constituents of saliva that are not normally a part of salivary secretion. Drugs and hormones are examples of such non-ordinary saliva constituents. These constituents are serum constituents that are transported from the blood to saliva via different transport mechanisms. The transport mechanisms can either be intracellular or extracellular[34]. Intracellular transport mechanisms include passive diffusion across a concentration gradient and active transport through protein channels, of which passive diffusion is the more common of the two. The most common route of extracellular transport is ultrafiltration through tight gap junctions between the cells. Constituents transported to saliva must have some water solubility to be retained in saliva, since saliva is 99% water. The saliva/plasma concentrations of the non-salivary constituents depend on the pH of the saliva[37].

Analysis of odiferous volatile saliva compounds have generally been in relation to disease of the mouth, such as periodontitis[39-41]. Volatile sulfur compounds and aromatic nitrogen containing compounds have been found at elevated levels in the mouth air and saliva samples of patients with moderate to severe preriodontitis[40,42]. More recently, saliva analysis has gained interest for its diagnostic tool potential for cancer, infections

from virus and bacteria, hormonal abnormality, and drug testing[32,37,43]. Saliva sampling is noninvasive and simple. In the clinical and forensic fields, buccal swab sampling is a routine sampling method for the collection of DNA. Analysis of VOCs from such swab samples has not yet been investigated.

2.2.1. Materials & Methods

Sterile cotton-tipped applicators to collect cheek cells and saliva were purchased from Solon Manufacturing Co (Skowhegan, ME, USA). Reagent quality (200 proof) ethyl alcohol used for the pre-treatment of cotton swabs was purchased from Florida Distillers Co (Lake Alfred, FL, USA). Ten ml glass, clear, screw top headspace vials with PTFE/Silicone septa were used to hold the samples (SUPELCO, Bellefonte, PA, USA). Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) (50/30 μm film thickness) SPME fibers and SPME fiber holders were obtained from SUPELCO (Bellefonte, PA, USA).

The GC/MS analysis was carried out using an Agilent Technologies 6890N gas chromatograph coupled to an Agilent Technologies 5973N mass selective detector (Palo Alto, CA, USA). The column used to separate the analytes was a HP5-MS, 30 m, 0.25 um, 0.25 mm with helium as the carrier gas with a flow rate of 1.0mL/min. The extracted VOCs were desorbed in the injection port of the GC with a temperature of 250°C for five minutes in splitless mode. The GC method begins with an initial oven temperature of 40°C for five minutes, then ramped at 10°C/min to a final temperature of 270°C, and held at 270°C for two minutes for a total run time of 30 minutes. The mass spectrometer used was an HP 5973 MSD with a quadrupole analyzer operated in electron ionization mode.

The mass spectrometer transfer line was maintained at 280°C and the source temperature was 230°C. The analytes were acquired in full-scan mode in 41-550 *m/z* range.

2.2.2. Pre-treatment of buccal swab collection material

2.2.2.1. Methods

Sterile cotton-tipped swabs were pre-treated prior to sample collection to eliminate any background compounds. Swabs were spiked with 200 µL of ethanol, then baked in a 105°C oven for one hour, and the procedure was repeated again. Pre-treated swabs were analyzed using SPME-GC/MS with a 21-hour extraction time to ensure they were free of undesired compounds and analytically clean.

2.2.2.2. Results

Pretreated cotton swabs were analyzed using SPME-GC/MS to ensure they were free of undesired human scent compounds. Example of a chromatogram of pre-and post-cleaning treatment of the sterile cotton swabs prior to sample collection are shown in Figure 4. As seen in Figure 5, the pre-treatment procedure effectively removes the numerous compounds that were originally present in the scent collection materials.

Figure 4. Comparison of untreated and pre-treated cotton swab

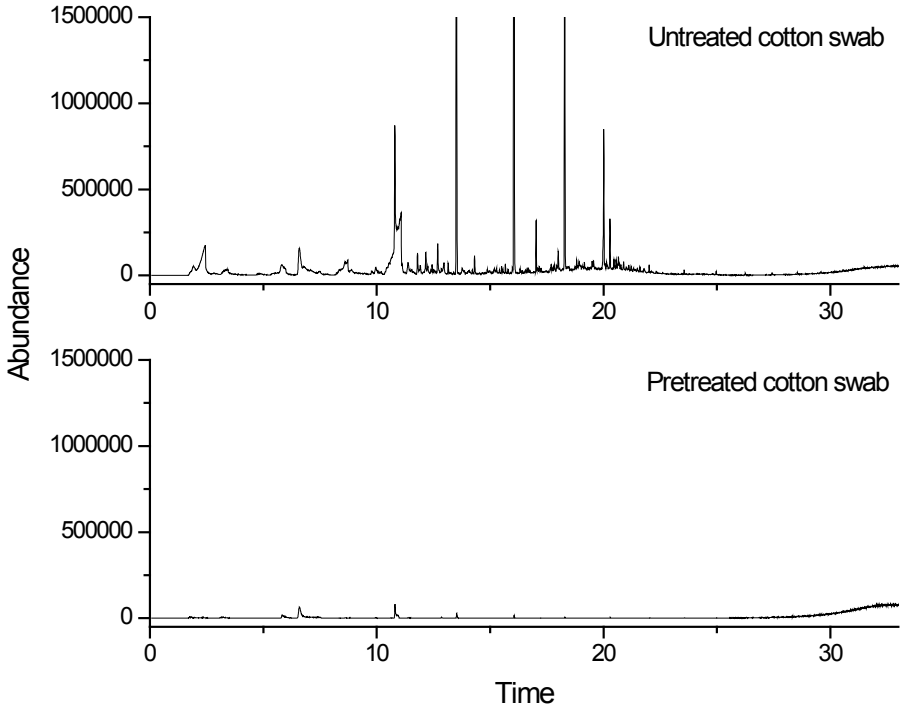
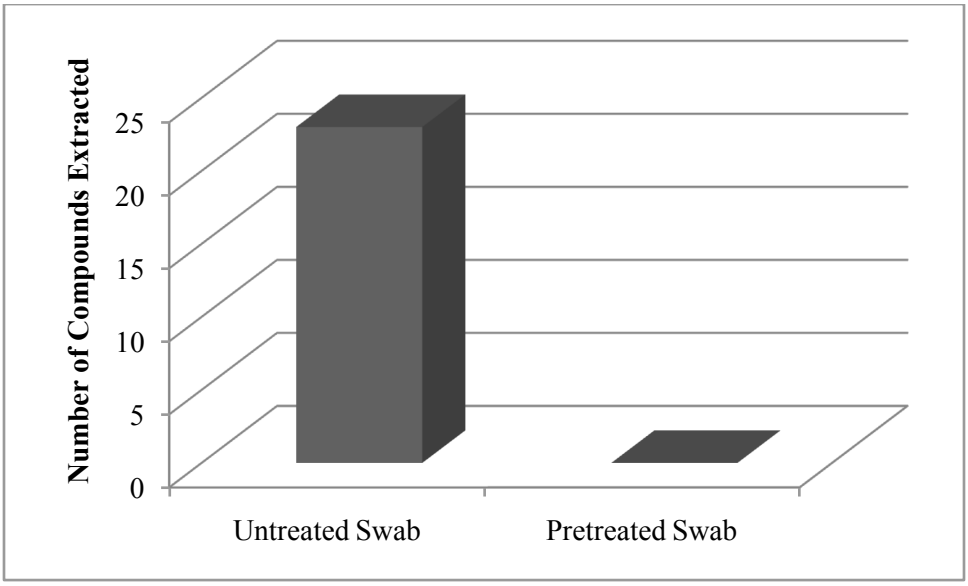


Figure 5 Comparison of cotton swab pretreatment for the removal of undesired VOCs



2.2.3. Buccal swab sampling procedure

Each subject was asked not to eat or drink anything other than water at least two hours prior to buccal swab and breath samplings. Subjects sampled themselves with a pre-treated cotton-tipped sterile swab. Subjects followed a set sampling procedure of rinsing their mouths with water for 30 seconds followed by rubbing the swabs on the inside of both cheek sides (rubbing 20 times up and down per side of cheek). Samples were collected in triplicates with ten minute breaks in between each sampling. Swabs were re-sealed back into the ten ml glass headspace vial. All samples were stored at room temperature and were allowed to sit for approximately 24 hours prior to extraction. No attempt was made to control microbial interactions with the samples as that may make contributions to the overall odor profile.

2.2.4. Determination of optimal extraction conditions for buccal swab

2.2.4.1. Methods

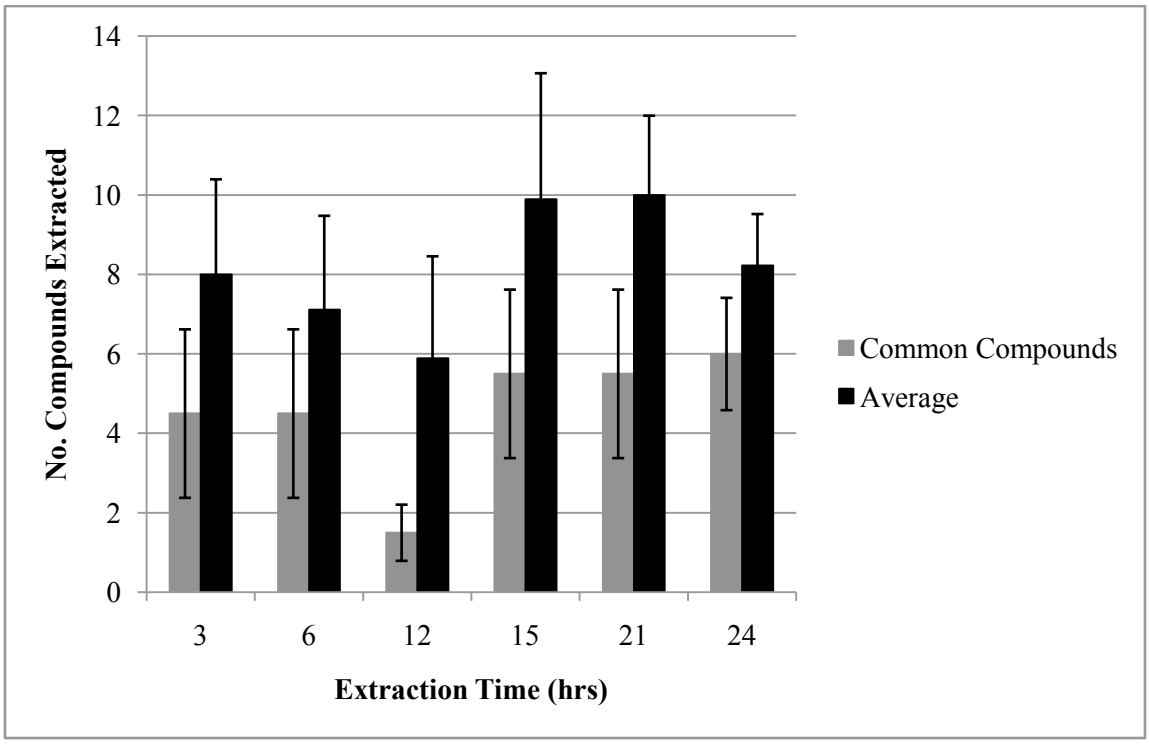
Buccal swab samples were collected from subjects Male 1, Male 5, and Female 4 following a set sampling procedure as described in section 2.2.3. Each subject sampled themselves using a pre-treated sterile cotton-tipped swab. Samples were taken in triplicates with 10 minutes break in between each sampling. Swabs were re-sealed back into the 10 ml glass vial. All samples were stored in the ten ml glass vials at room temperature, and were allowed to equilibrate for approximately 24 hours prior to extraction. During optimization, the odor exposures were done at room temperature on multiple samples for 3, 6, 12, 15, 21, and 24 hours. The 50/30 μm DVB/CAR/PDMS SPME fibers (SUPELCO, Bellefonte, PA, USA) were used to extract the VOCs from the

headspace of the buccal swab samples in the vials. All samples were run using the GC/MS method for buccal swab samples previously mentioned in section 2.2.1.

2.2.4.2. Results

Figure 6 shows the number of compounds that have been previously reported as "human scent compounds" extracted from buccal swab samples against extraction times. "Common compounds" denote the number of VOCs present in all triplicate samples. The optimal extraction time for buccal swab samples was evaluated on a combination of the number of human scent compounds extracted as well as the abundances of six selected common buccal swab compounds (1-octen-3-ol, acetophenone, benzaldehyde, nonanal, decanal, and nonanoic acid methyl ester) present in the collected samples. Twenty-one (21) hours was determined to be the optimal extraction time for collected buccal swab odor compounds.

Figure 6. Number of human scent compounds extracted from buccal swab samples vs. extraction time



2.3. Breath

Human breath is "a bulk matrix consisting of nitrogen, oxygen, carbon dioxide, water vapor, inert gases and some trace components; volatile organic compounds" that is produced in the concentrations of nmol/l to pmol/l range[44,45]. For humans, breathing is the process that exchanges oxygen and carbon dioxide between the body and the external environment and is governed by the respiratory system. Some of the major functions of the respiratory system are to provide oxygen, eliminate carbon dioxide, regulate the blood pH, and to defend the body against microbes[46]. The anatomical features of the human respiratory system include the lungs, the airways, and the respiratory muscles.

The trachea branches into two bronchi, one entering the right lung and the other entering the left lung. The bronchi branch further into smaller, narrower bronchioles. At the end of the bronchioles are the round air sacs called alveoli where the gas exchange occurs between the capillaries and alveoli. The mechanics of breathing follows Boyle's law, which states that at in a closed system at constant temperature, pressure and volume of a gas are inversely proportional to each other[46]. The closed system in the respiratory system is the thoracic cavity, and the mechanics of breathing depends on the changes in volume and pressure of the thoracic cavity. Breathing occurs in two phases, inspiration (inhaling) and expiration (exhaling). The diaphragm contracts during inspiration, increasing the thoracic cavity volume and thereby decreasing the pressure within. The lungs expand; the air pressure inside the alveoli becomes subatmospheric resulting in the air rushing in to fill the lungs until equilibrium is reached. Conversely, during expiration, the diaphragm relaxes, decreasing the thoracic volume and increasing the pressure within (alveolar pressure becomes larger than atmospheric pressure), and, as a result, the lungs contract and air is expelled out into the atmosphere. The VOCs are also transported in the bloodstream and expired through the lungs along with the other gases that compose the "bulk matrix" of the human breath.

The VOCs in breath may be of endogenous or exogenous origins. Volatile substances of exogenous origins are contaminants from the environment that are breathed in and absorbed by the body. Inorganic gases such as nitric oxide and carbon monoxide are endogenous compounds that are generated in the airways. Other endogenous VOCs that are exhaled can be produced virtually anywhere in the body as a result of metabolic and

biochemical processes. These VOCs are transported through the bloodstream and eventually exhaled through the lungs. The VOCs can diffuse across the pulmonary alveolar membrane which separates the alveolar air from the blood in the capillaries, thereby crossing from blood to air and air to blood[44]. The exhalation rates and route of transport of individual blood-borne VOCs depends on whether the VOC has a high or low Henry's constant, as having a low Henry's constant means that the VOC is not readily soluble in water[44]. In other words, the exhalation rates of the VOCs in human breath depend on their molecular weight and hydrophobicity.

Modern breath analysis started in 1971 when Pauling et al. determined more than 200 components in human breath using gas chromatography[15]. Since then, variations in the VOCs as well as the effect of age and gender on the profile of volatile components in normal human breath have been studied widely resulting in the detection of over 3000 compounds[47,48]. Some known endogenous VOCs present in human breath include ethane, pentane, isoprene, and acetone[48-53]. Acetone and isoprene are two of the most common VOCs in human breath, and the biochemical pathways of these compounds are well known. Acetone production is a result of decarboxylation of excess acetyl-CoA which generates ketone bodies[45,49]. Isoprene generation is a result of mevalonate metabolism of cholesterol synthesis[49]. However, the source and the biochemical pathways of most VOCs that have been detected in human expired breath are still not known.

There has been an increasing interest in human breath analysis for its investigation potential for biomarkers for diseases such as certain types of cancer (lung and breast), oxidative stress, pulmonary tuberculosis, diabetes mellitus, and kidney impairment[52,54-57].

The interest in human exhaled breath is based on the non-invasiveness and safeness in the sampling process as compared to blood testing. Breath VOC measurements can be used to estimate the body burden and different processes in the body because there exists a dynamic equilibrium between the blood VOCs and the expired breath VOC concentrations. However, a standard breath sampling and analysis protocol for clinical settings has yet to be settled. Studies on the analysis of endogenous compounds found in humane exhaled breath are still challenged because of the low concentrations of the VOCs. Currently, breath-alcohol testing is the only typical routine breath analysis application implemented in the medical and forensic fields.

2.3.1. Materials & Methods

Breath samples were collected using a Teflon Bio-VOC® breath sampler (Markes International Ltd., Rhondda Cynon Taff, UK). Disposable cardboard pediatric mouthpieces attached to the Bio-VOC® apparatus were purchased from Alliance Tech Medical, Inc.™ (Grandbury, TX, USA). To seal the apparatus, 11mm crimp seals with PTFE/Silicone septa (Sun Sri, Rockwood, TN, USA) were used. The acetone used for the pre-treatment of Bio-VOC® breath sampler apparatus was ACS grade acetone purchased from Fisher Scientific, Pittsburgh, PA, USA).

The GC/MS analysis was carried out using an Agilent Technologies 6890N gas chromatograph coupled to an Agilent Technologies 5973N mass selective detector (Palo Alto, CA, USA). The column used to separate the analytes was a HP5-MS, 30 m, 0.25 um, 0.25 mm with helium as the carrier gas with a flow rate of 1.0mL/min. The extracted VOCs were desorbed in the injection port of the GC for five minutes in splitless mode.

The injection port temperature was set at 280°C for breath samples. The GC method begins with an initial oven temperature of 40°C for five minutes, then ramped at 10°C/min to a final temperature of 270°C, and held at 270°C for two minutes for a total run time of 30 minutes. The mass spectrometer used was an HP 5973 MSD with a quadrupole analyzer operated in electron ionization mode. The mass spectrometer transfer line was maintained at 280°C and the source temperature was 230°C. The analytes were acquired in full-scan mode in 41-550 *m/z* range.

2.3.2. Pre-treatment of Bio-VOC® Breath Sampler

2.3.2.1. Methods

Sample collection materials were pre-treated prior to sample collection to eliminate any background compounds. The Bio-VOC® apparatus was rinsed with acetone and placed in an oven of 40°C for at least one hour followed by 30 minutes in a 105°C oven, then pure nitrogen gas was passed through the bulb for two minutes. The breath sampler was crimp sealed until time of breath sampling.

2.3.2.2. Results

Pretreated Bio-VOC® breath samplers were analyzed using SPME-GC/MS to ensure they were free of undesired human scent compounds. Example of a chromatogram of pre- and post-cleaning treatment of the breath samplers prior to sample collection are shown in Figure 7. As seen in Figure 8, the pre-treatment procedure effectively removes the numerous compounds that were originally present in the scent collection materials.

Figure 7. Comparison of untreated and pre-treated Bio-VOC® breath sampler

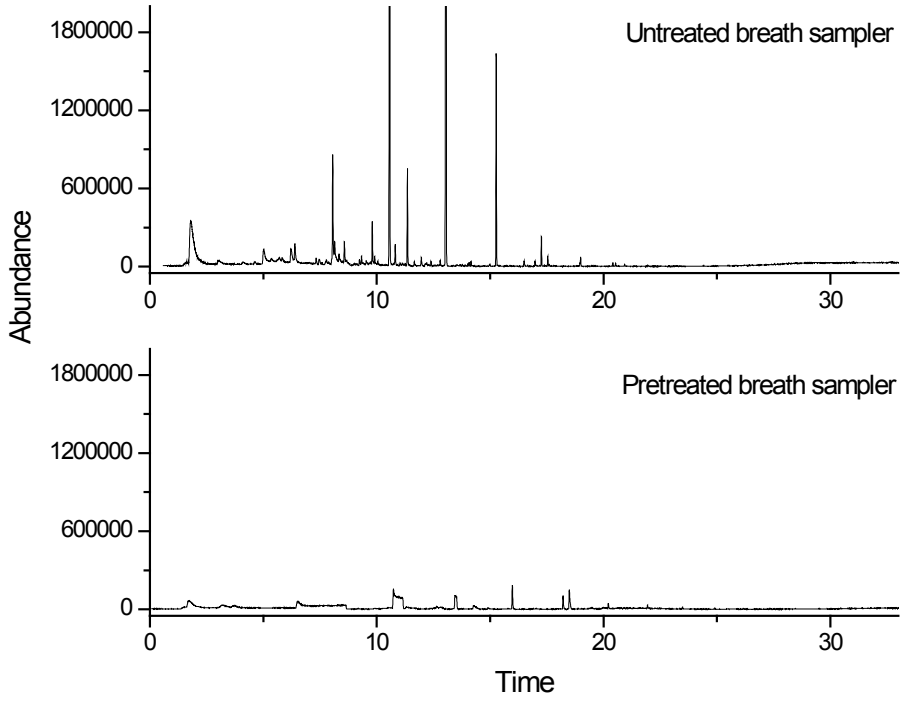
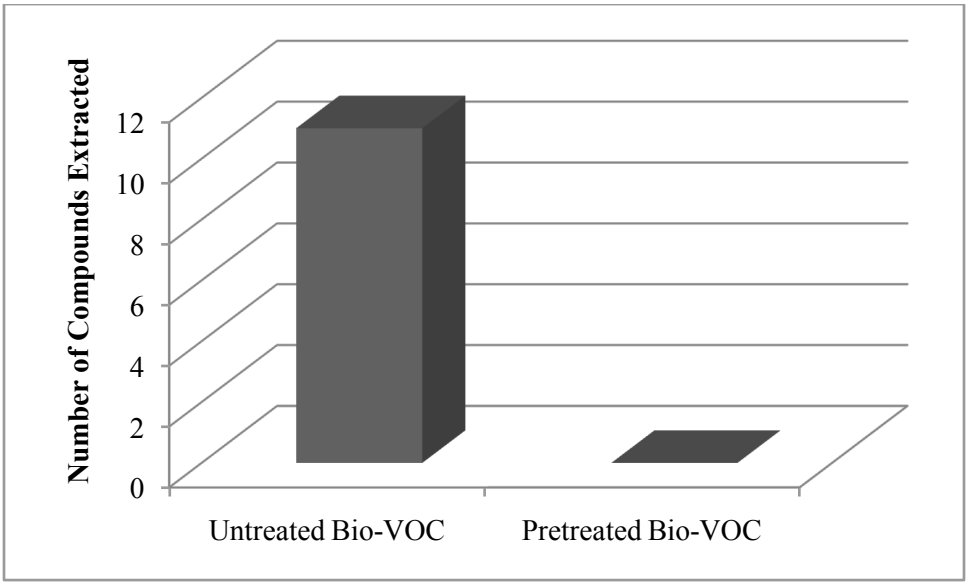


Figure 8. Comparison of breath sampler pretreatment for the removal of undesired VOCs



2.3.3. Breath sampling procedure

Breath samples were collected following a set sampling procedure. Subjects were asked to perform a single slow vital capacity breath into a disposable cardboard mouthpiece connected to a 150 ml Teflon®-bulb, which traps the end-expired air. The Teflon®-bulb was immediately sealed after sampling.

2.3.4. Determination of optimal extraction conditions for breath

2.3.4.1. Methods

Breath samples were collected from subjects Male 1 and Female 4 following a set sampling procedure as described in section 2.3.3. Each subject sampled themselves by performing a single slow vital capacity breath into a disposable cardboard mouthpiece connected to a 150 ml Teflon® Bio-VOC® breath sampler (Markes International Ltd., Rhondda Cynon Taff, UK), which traps the end-expired air. The Teflon®-bulb was immediately sealed using an 11 mm crimp seal with PTFE/Silicone septa (Sun Sri, Rockwood, TN, USA) after sampling. The exhaled breath VOCs were extracted by 50/30 μm DVB/CAR/PDMS SPME fibers (SUPELCO, Bellefonte, PA, USA) directly inserted into the Teflon®-bulb. During extraction optimization, the breath exposures were done at room temperature on multiple samples for 10 min, 15 min, 30 min, 45 min, 1 hr, 3 hrs, 6 hrs, 12 hrs, 15 hrs, 18 hrs, 21 hrs, and 24 hrs. Optimization of sample equilibration time was also performed at 15 min, 1 hr, and 24 hrs. All samples were run using the GC/MS method for breath samples previously mentioned in section 2.3.1.

2.3.4.2. Results

2.3.4.2.1. Optimal Extraction Time

The exhaled breath VOCs from four subjects (Male 1, Male 5, Female 2, and Female 4) were extracted by SPME fibers inserted directly into the Teflon®-bulb portion of the BioVOC® Breath Sampler. During optimization, the breath exposures were done at room temperature on duplicate samples taken from subjects Male 1 and Female 4 for 10 min, 15 min, 30 min, 45 min, 1 hr, 3 hrs, 6 hrs, 12 hrs, 15 hrs, 18 hrs, 21 hrs, and 24 hrs (Figure 9). Extraction time optimization was only investigated for the 15, 18, 21, and 24 hours exposures for subjects Male 5 and Female 2, since fewer compounds were extracted at extraction times below 12 hours (Figure 10). The optimal extraction time for breath samples was evaluated on a combination of the number of human scent compounds extracted as well as the abundances of selected common breath VOCs present in the subject breath samples: phenol, styrene, nonanal, decanal, and acetophenone. Figure 11 shows an example of the abundances of common breath VOCs extracted for the 15, 18, 21, and 24-hour extraction times for subject M5. Similar results were seen for the other three subjects. Twenty-one (21) hours was determined to be the optimal extraction time for collected breath odor compounds using the Bio-VOC® breath sampler through the evaluation parameters stated.

Figure 9. Number of common human scent compounds extracted vs. extraction time for breath samples for subjects M1 and F4

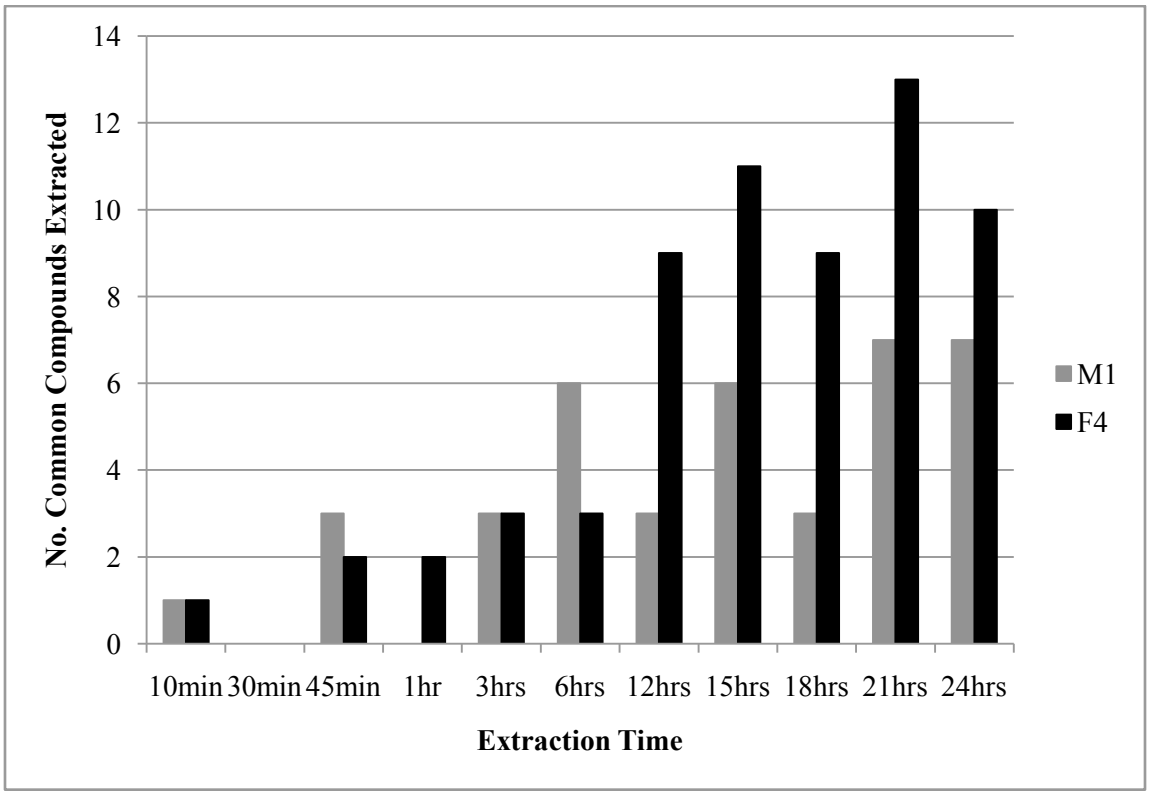


Figure 10. Number of common human scent compounds extracted vs. extraction time for breath samples for subjects M5 and F2

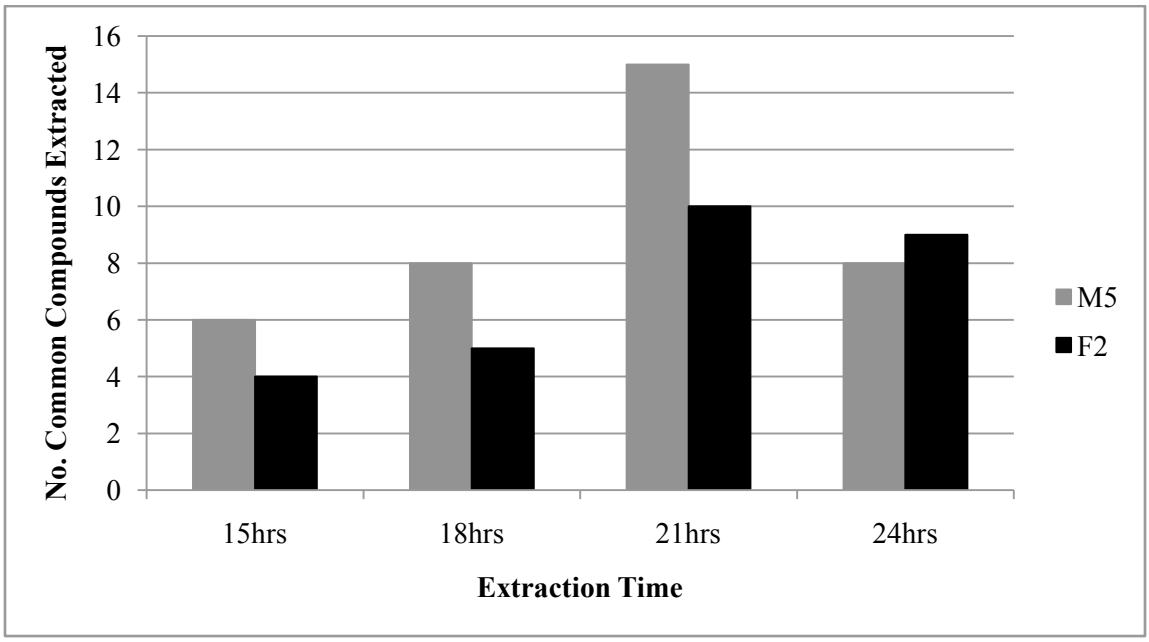
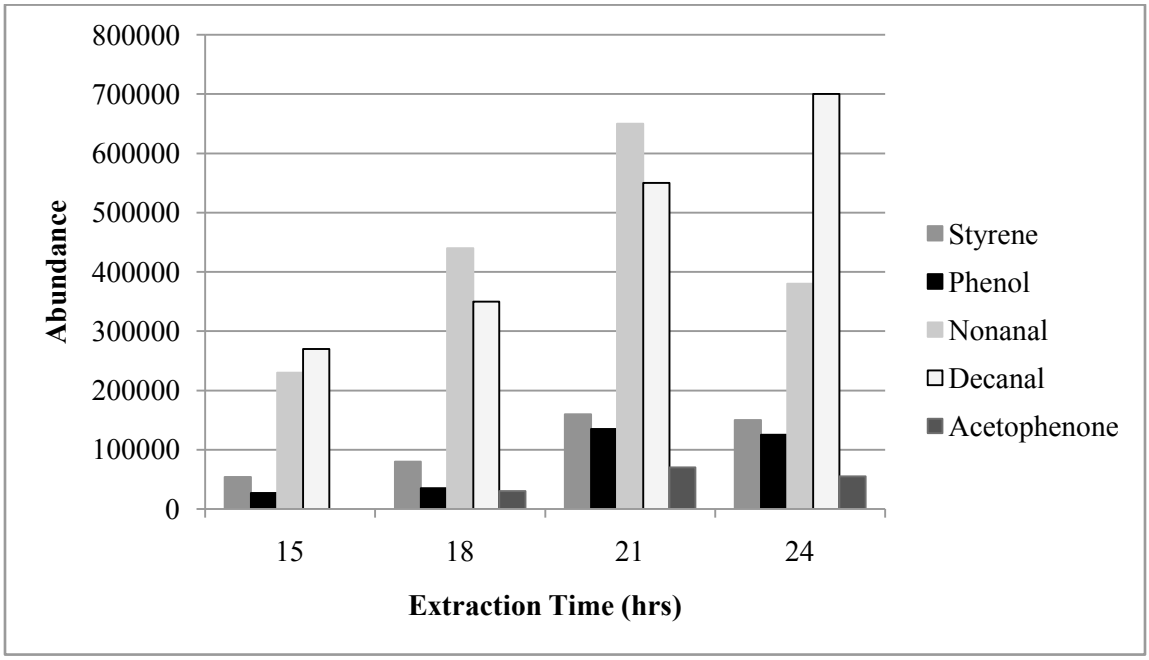


Figure 11 Abundances of selected common breath VOCs extracted vs. extraction time for Male 5



2.3.4.2.2. Optimal Equilibration Time

Equilibration time is the time which the collected sample is allowed to sit for in order for the VOCs in the sample to reach a steady state between the headspace and the sample collection matrix/material. The optimal equilibration time for breath samples collected in Bio-VOC® breath sampler was evaluated with the previously-determined 21-hr extraction time. Optimal equilibration time was evaluated on a combination of the number of human scent compounds extracted and the abundances of selected common human scent compounds, phenol, styrene, nonanal, decanal, and acetophenone, present in the breath samples. Fifteen (15) minutes was determined to be the most optimal equilibration time as shown in Figure 12 and Figure 13. At longer equilibration times, fewer compounds were extracted. There is a potential loss of compounds at longer equilibration times; with longer equilibration times it is possible that some of the compounds dissipate through the breath sampler and, therefore, the compounds are no longer present in the breath sampler at the time of extraction by SPME.

Figure 12. Number of compounds extracted vs. equilibration time for breath

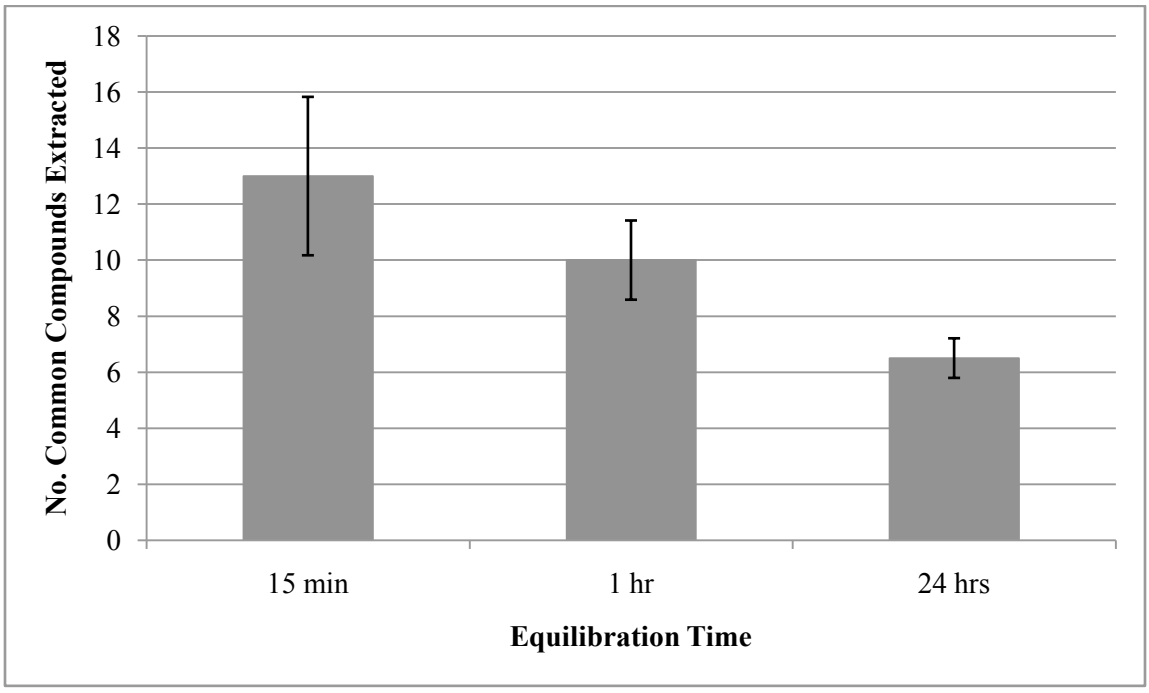
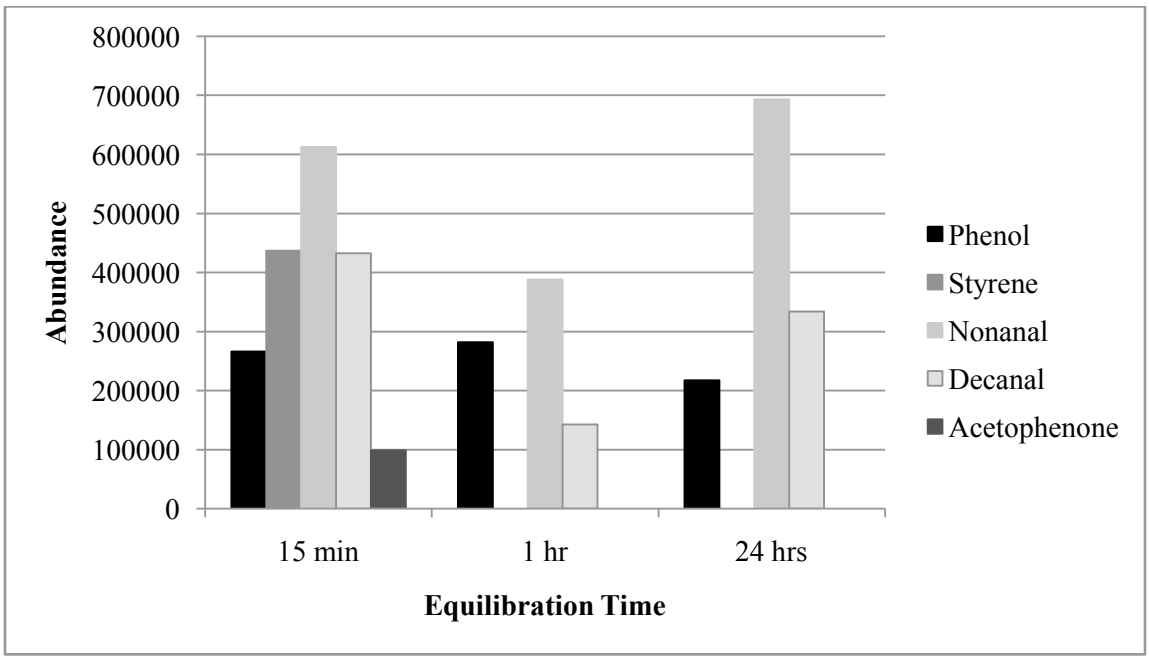


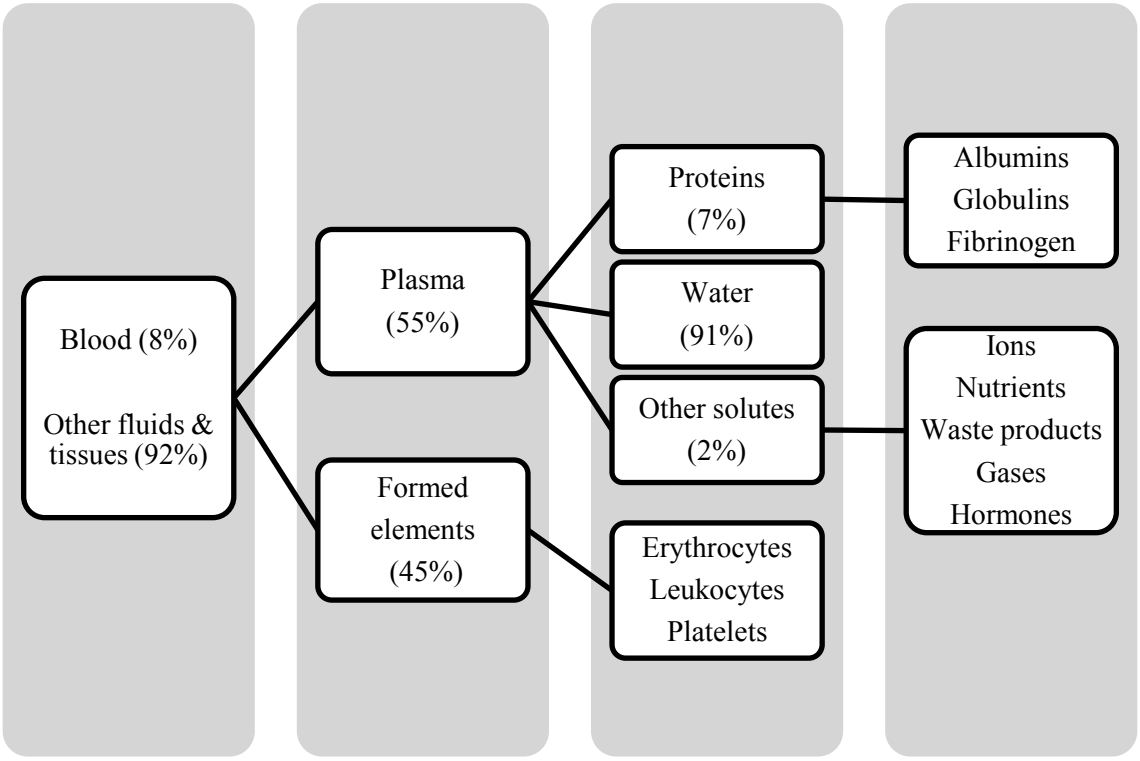
Figure 13. Abundances of selected common breath VOCs extracted vs. equilibration time



2.4. Blood

Blood is the essential body fluid which transports oxygen and nutrients to the body cells and carries carbon dioxide and waste materials away from the cells. Blood accounts for about 7% to 9% of a person's total body weight, and the volume is about 4-6 L of blood for an average adult[58]. Blood is composed of cells and plasma; plasma constitutes about 55% of blood volume and is the liquid portion of the blood in which the cells are suspended. The blood cells, called formed elements, are erythrocytes (red blood cells), leukocytes (white blood cells), and platelets (fragment cells). Erythrocytes carry oxygen and carbon dioxide and account for majority of the blood cells (>99%). Erythrocytes contain the protein hemoglobin which binds the oxygen and carbon dioxide molecules, resulting in the characteristic red color of blood. The functions of leucocytes and platelets are infection/cancer protection and blood clotting, respectively. Plasma is mostly water (91% of plasma by weight), and a variety of inorganic electrolytes, proteins, gases, nutrients, hormones, and waste products[46] (Figure 14). The other functions of blood include immunological functions, self-repair mechanism (blood clotting and coagulation), messenger functions, homeostatic functions such as pH and body temperature regulations. Blood pH is maintained via homeostasis between 7.35 and 7.45[59].

Figure 14. Components of blood



Blood, nutrients, gases, ions, and hormones are transported among the cells in the body via the circulatory system. The circulatory system is made up of the cardiovascular system and the lymphatic system; blood flow is through the former. The cardiovascular system is further composed of two pumping systems: pulmonary circulation and systemic circulation[58,60]. The pulmonary circuit involves the right side of the heart where oxygen-depleted blood is pumped to the lungs. Carbon dioxide and waste products are eliminated from the blood via the pulmonary circuit. Deoxygenated blood is carried from the right heart chamber to the lungs via the pulmonary arteries; oxygenated blood is transported from the lungs back to the heart via the pulmonary veins. Returned oxygenated blood is then pumped from the left side of the heart and is circulated to the rest of the body by the

systemic circuit. The blood moves from the heart into aorta which feeds into the arteries, to arterioles, and finally to capillaries where the two-way exchange of gases, nutrients, and waste materials between blood and cells occurs. The blood-cell exchange of water (via osmosis) and dissolved solutes mainly occurs by process of diffusion, and to a lesser extent, by the blood pressure exerted against the capillary walls as blood flows through the capillaries.

Blood VOCs have been studied for the health effects of occupational and environmental exposures[61-63]. When increased levels of volatile aromatic compounds such as benzene, toluene, and xylene isomers are present in most human blood, they are indicative of occupational or environmental VOC exposures and/or smoking[16,61-64]. Benzene and toluene concentrations in the blood of smokers (median concentration of 493 ng/l and 2001 ng/l for benzene and toluene, respectively) have been reported to be significantly different ($p<0.0001$ for benzene, $p<0.05$ for toluene)from those of non-smokers (median concentrations of 190 ng/l and 1141 ng/l for benzene and toluene, respectively)[61]. Blood sampling is the most invasive technique compared to collection of other biological specimens, as it requires needles to withdraw blood or breaking of the skin with a lancet, causing physical pain and psychological discomfort to the subject. Despite the sampling method being more invasive compared to breath or urine, blood VOC analysis is more representative of the internal environment of human biological activities. For this reason, analyses of volatile biomarkers for diseases have been gaining interest in the recent years[65-68]. Hexanal and heptanal were found in higher levels (greater than 1.8μM) in lung cancer blood compared to normal control blood (lower than 0.20 μM)[66,67]. In a study

comparing blood VOCs of healthy participants and liver cancer patients, hexanal, 1-octen-3-ol, and octane were found with positive rates greater than 84% in lung cancer patient blood ($p \leq 0.0001$)[68].

Blood testing is still the most definitive testing method for DNA testing and disease diagnostics and therefore is widely performed in medical, toxicological, and forensic fields. Blood testing normally involves venipuncture to obtain venous blood, and requires several milliliters of blood in quantity. FTA cards have been used as a blood DNA storage method, and only require several drops of blood. To date, blood VOC detection using FTA cards as the collection medium has not been performed.

2.4.1. Materials & Methods

Blood samples were collected on Whatman FTA® MiniCard (Whatman International Ltd, Maidstone, UK). Unistik2 Super (21G, 0.81mm) single-use capillary sampling devices used for obtaining blood were purchased from Fisher HealthCare (Houston, TX, USA). Human whole blood containing anticoagulant sodium EDTA was obtained from Bioreclamation Inc. (Hicksville, NY, USA). Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) (50/30 μm film thickness) SPME fibers and SPME fiber holders were obtained from SUPELCO (Bellefonte, PA, USA).

The GC/MS analysis was carried out using an Agilent Technologies 6890N gas chromatograph coupled to an Agilent Technologies 5973N mass selective detector (Palo Alto, CA, USA). The column used to separate the analytes was a HP5-MS, 30 m, 0.25 um, 0.25 mm with helium as the carrier gas with a flow rate of 1.0mL/min. The extracted

VOCs were desorbed in the injection port of the GC with a temperature of 250°C for five minutes in splitless mode. For blood, the GC method begins with an initial oven temperature of 30°C ramped at 10°C/min to a final temperature of 200°C where it is held for two minutes, for a total run time of 19 minutes. The mass spectrometer used was an HP 5973 MSD with a quadrupole analyzer operated in electron ionization mode. The mass spectrometer transfer line was maintained at 280°C and the source temperature was 230°C. The analytes were acquired in full-scan mode in 41-550 *m/z* range.

2.4.2. Pre-treatment of FTA card

2.4.2.1. Methods

Sample collection materials were pre-treated prior to sample collection to eliminate any background compounds. One half of the Whatman FTA® MiniCard (one circle) was inserted in a sterile ten ml glass headspace vial and baked in a 105°C oven for 45 minutes. Pre-treated materials were analyzed using SPME-GC/MS to make sure they were free of undesired compounds and analytically clean.

2.4.2.2. Results

Pretreated FTA cards were analyzed using SPME-GC/MS to ensure they were free of undesired human scent compounds. An example of a chromatogram of pre-and post-cleaning treatment of the FTA cards prior to sample collection is shown in Figure 15. As seen in Figure 16, the pre-treatment procedure effectively removes the numerous compounds that were originally present in the scent collection materials.

Figure 15. Comparison of untreated and pre-treated FTA card

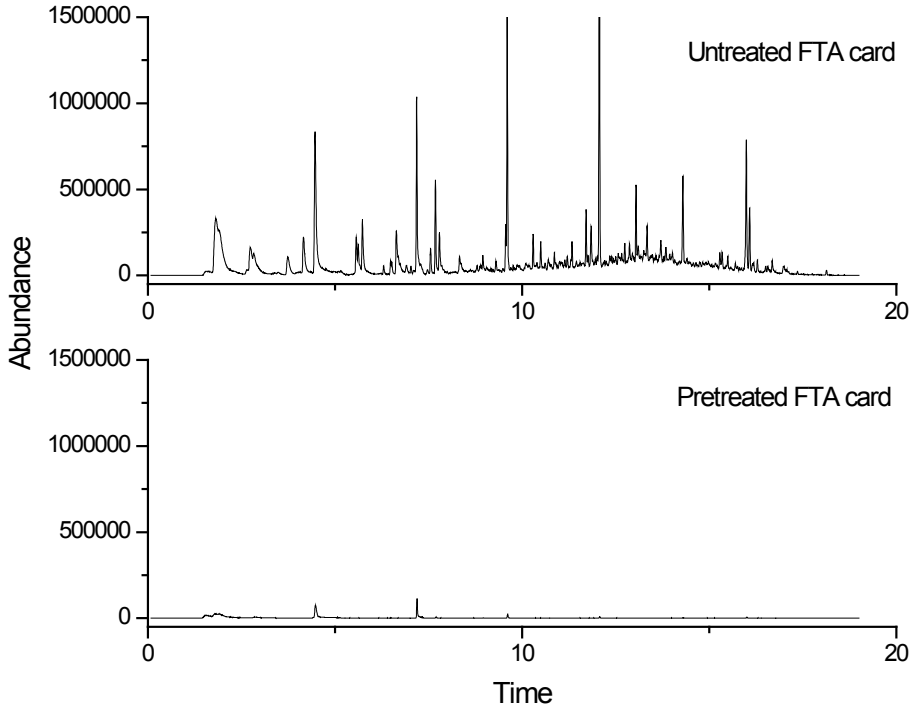
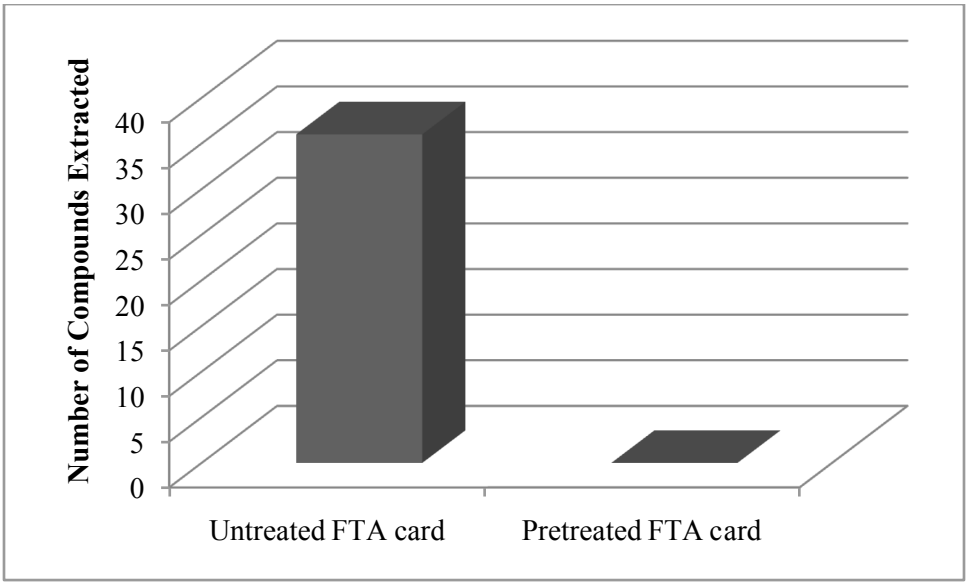


Figure 16. Comparison of FTA card pretreatment for the removal of undesired VOCs



2.4.3. Blood sampling procedure

Blood samples were collected from two fingers (right and left ring fingers) using a fingerstick apparatus. Subjects sampled themselves by filling the circle of the FTA card with their blood. All samples were stored in the ten ml vials at room temperature.

2.4.4. Determination of optimal extraction conditions for blood

2.4.4.1. Methods

Human whole blood containing the anticoagulant sodium EDTA (Bioreclamation Inc., Hicksville, NY, USA) was used for the optimization study for blood. Whole blood samples were collected on pre-treated Whatman FTA® MiniCard (Whatman International Ltd, Maidstone, UK). To each individual FTA card, 200 µL of whole blood was added, where blood was evenly distributed within the FTA circle. FTA cards were then resealed in their respective vials. During optimization, the blood exposures were done in a 37°C sand bath in triplicate samples for 1 min, 5 min, 10 min, 30 min, 1 hr, 3 hrs, 12 hrs, 18 hrs, 21 hrs, and 24 hrs. Optimization studies were also performed for sample equilibration time at 5 min, 1 hr, 8 hrs, 18 hrs, and 24 hrs as well as the effect of sample heating. The 50/30 µm DVB/CAR/PDMS SPME fibers (SUPELCO, Bellefonte, PA, USA) were used to extract the VOCs from the headspace of the blood samples in the vials. All samples were run using the GC/MS method for blood samples previously mentioned in section 2.4.1.

2.4.4.2. Results

2.4.4.2.1. Optimal Extraction Time

During optimization, the blood sample exposures were done in a 37°C sand bath in triplicate for one min, 5 min, 10 min, 30 min, 1 hr, 3 hrs, 12 hrs, 18 hrs, 21 hrs, and 24 hrs. The optimal extraction time for blood samples was evaluated on a combination of the number of human scent compounds extracted as well as the abundance of selected human scent compounds of various functional groups (decane, toluene, heptanal, and 1-octen-3-ol). Eighteen (18) hours was determined to be the optimal extraction time for collected blood VOCs as shown in Figure 17 and Figure 18. No compounds were detected below the one hour extraction time. Beyond 18 hours, the abundance of majority of the extracted compounds decreased. The 24-hour extraction resulted in half the abundance of what was extracted under the 18-hour extraction time.

Figure 17. Number of compounds extracted vs. extraction time for whole blood

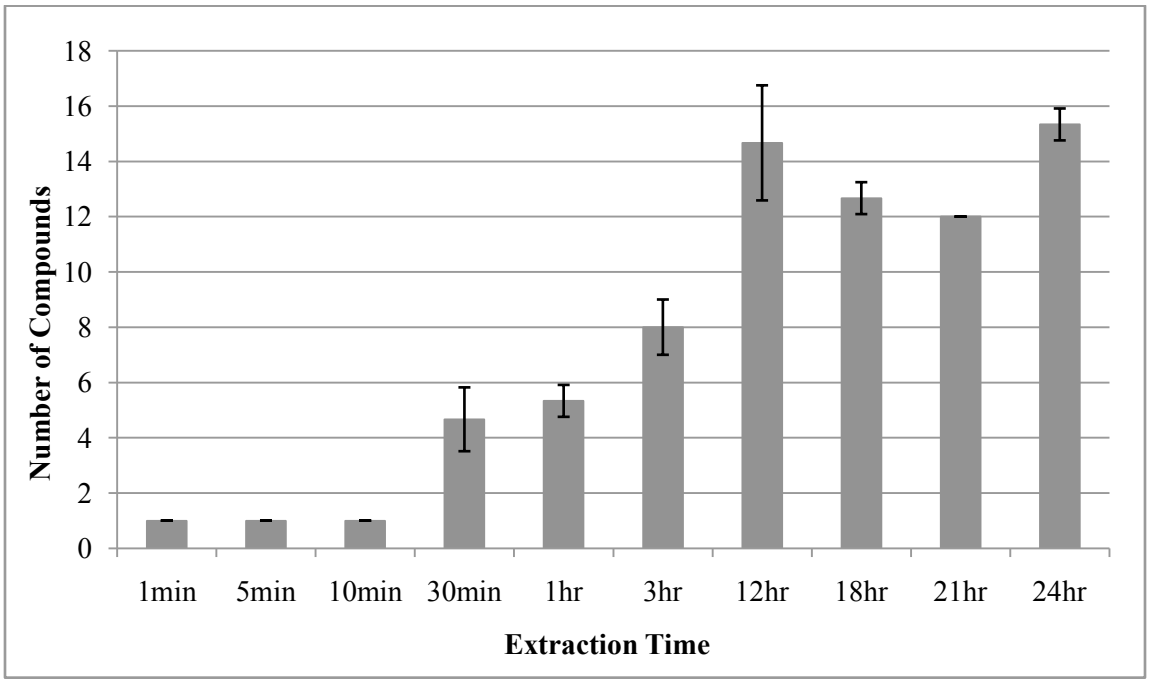
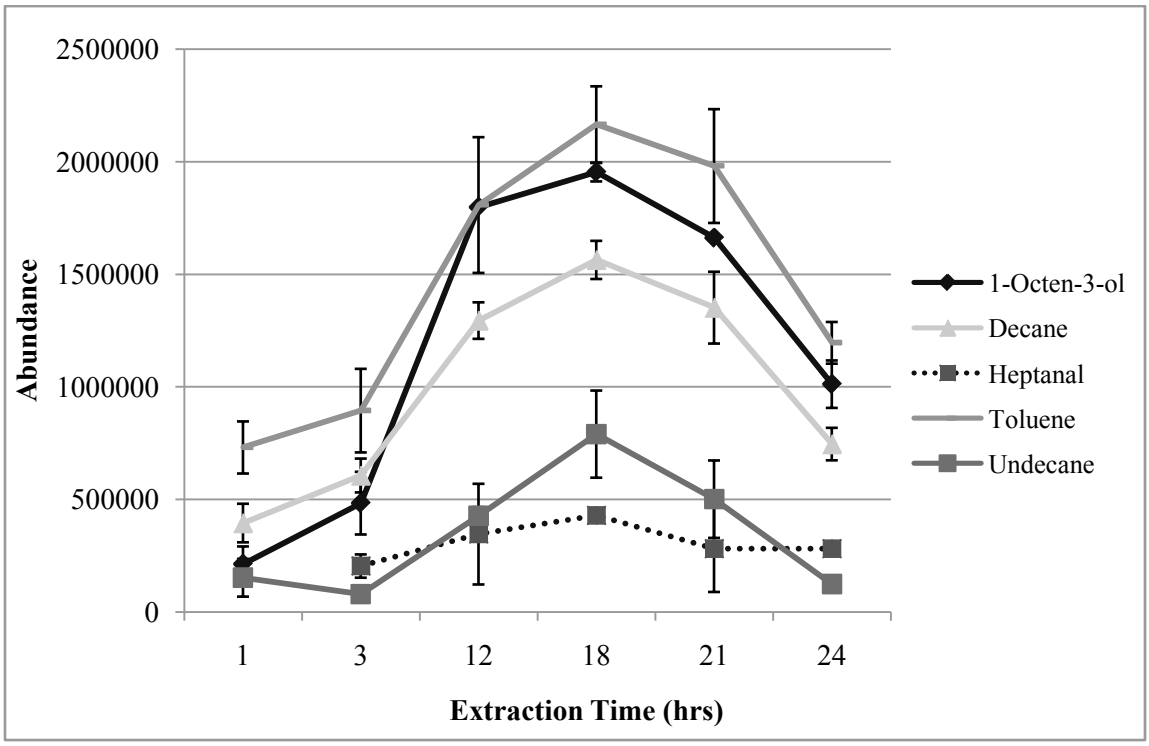


Figure 18. Abundance of selected common compounds extracted vs. extraction time for whole blood



2.4.4.2.2. Optimal Equilibration Time

Once the optimal extraction time was determined, the optimal equilibration time was investigated. Various equilibration times (5 min, 1 hr, 8 hrs, 18 hrs, and 24 hrs) were investigated with the optimized 18 hour extraction time in a 37°C sand bath. One hour equilibration resulted in the greatest number of compounds extracted with SPME (Figure 19). The optimal equilibration time for blood samples were evaluated on a combination of the number of human scent compounds extracted as well as the abundance of selected human scent compounds of various functional groups (decane, undecane, toluene, heptanal, and 1-octen-3-ol). The abundance of the compounds detected was not greatly affected by the different equilibration times (Figure 20). No single obvious trend was

seen in the abundances of compounds with varying equilibration times; however, with the exception of 1-octen-3-ol, a general decrease in the abundance was seen with increasing equilibration times. While abundances of extracted compounds were slightly higher for the 5-minute equilibration time than for the 1-hour equilibration time, the number of compounds extracted was greater for the latter. Therefore, the combination of a 1-hour equilibration time and 18-hour extraction time was chosen for the blood samples.

Figure 19. Number of compounds extracted vs. equilibration time for whole blood

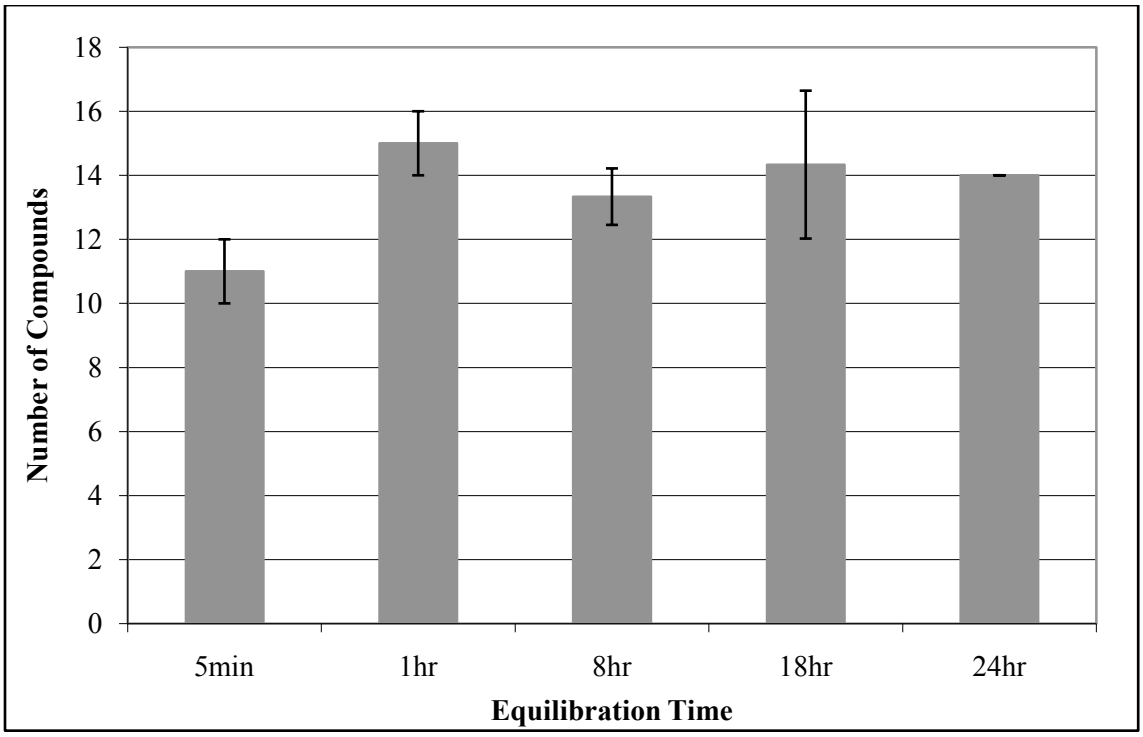
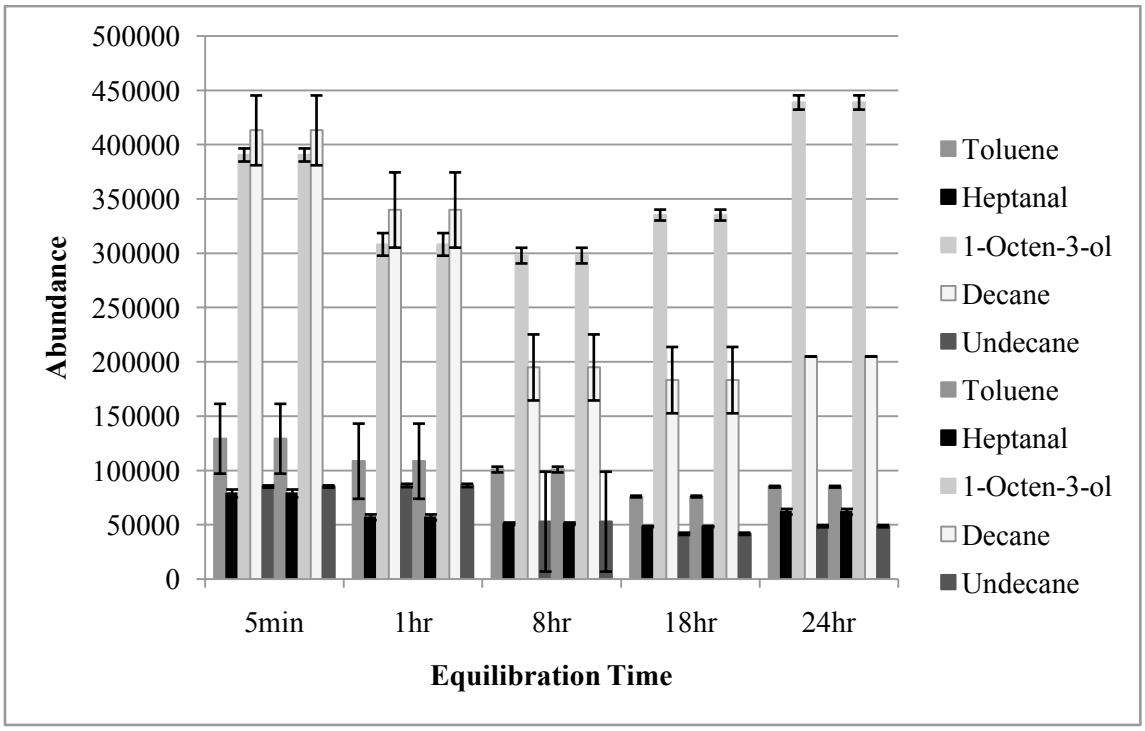


Figure 20. Abundance of selected common blood VOCs extracted vs. equilibration time



2.4.4.2.3. Effect of Sample Heating

The extraction efficiency of heating the blood samples was investigated. High temperatures degrade the blood samples so a comparison was made between unheated samples (room temperature equilibration and extraction) and samples heated to 37°C (human body temperature). More VOCs were extracted from the samples heated in the 37°C sand bath regardless of the extraction time, although the difference decreased as extraction time increased (Figure 21). Abundance of compounds significantly increased for the heated samples also. An example is shown for a common VOC extracted from blood samples, undecane, in Figure 22.

Figure 21. Number of compounds extracted at room temperature and 37°C for whole blood

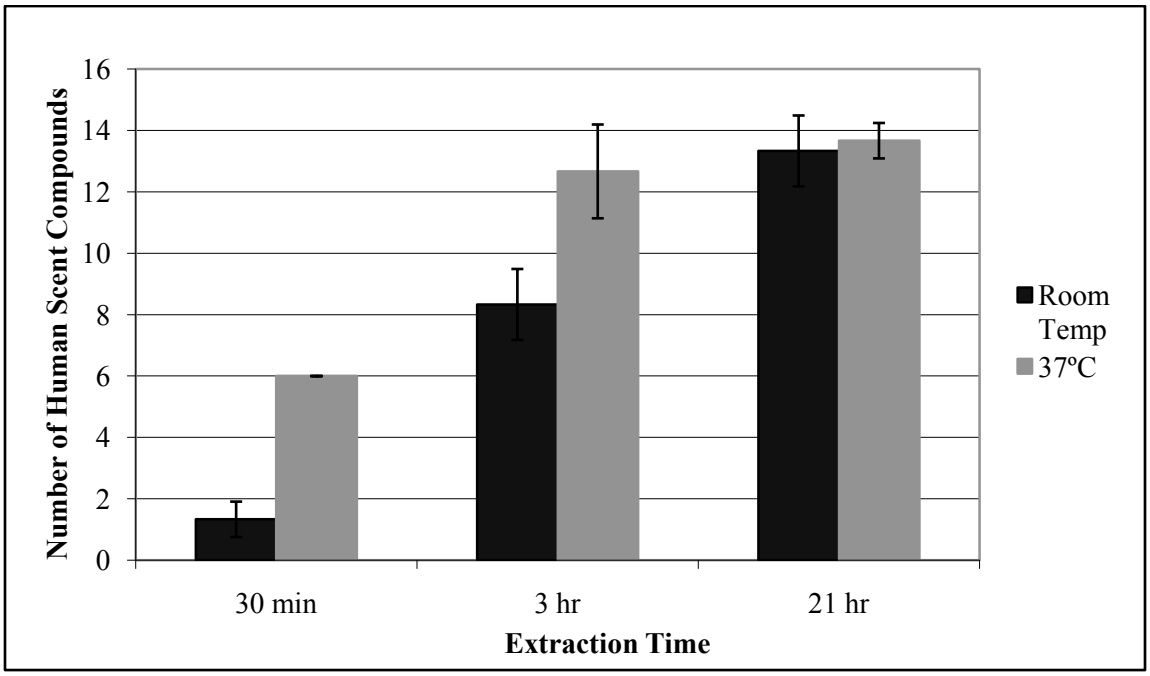
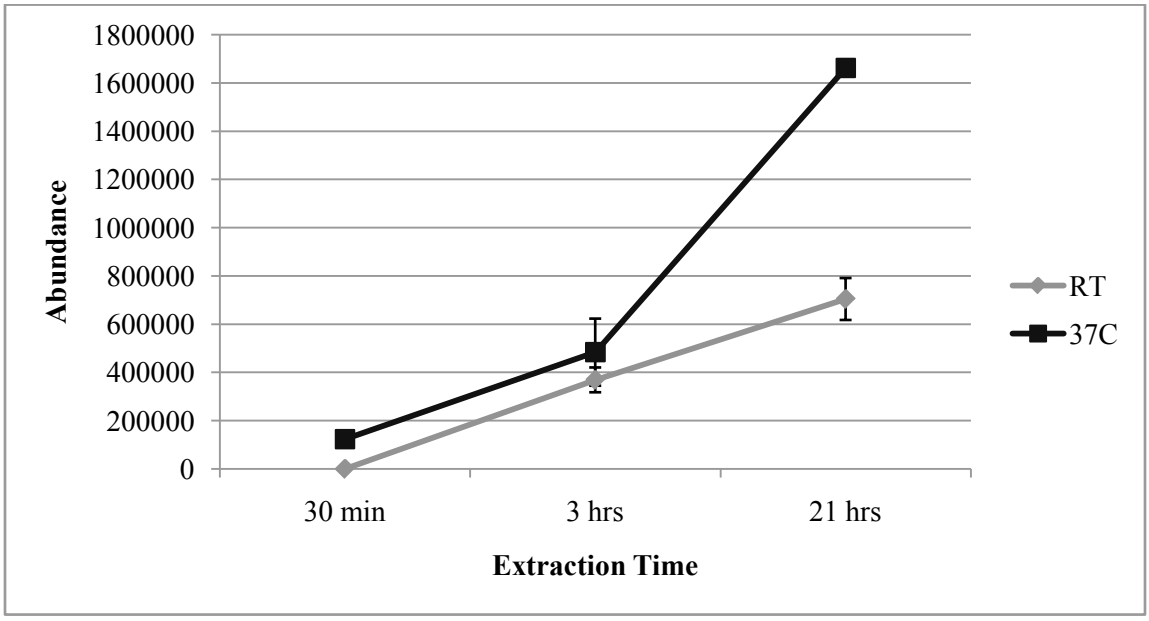


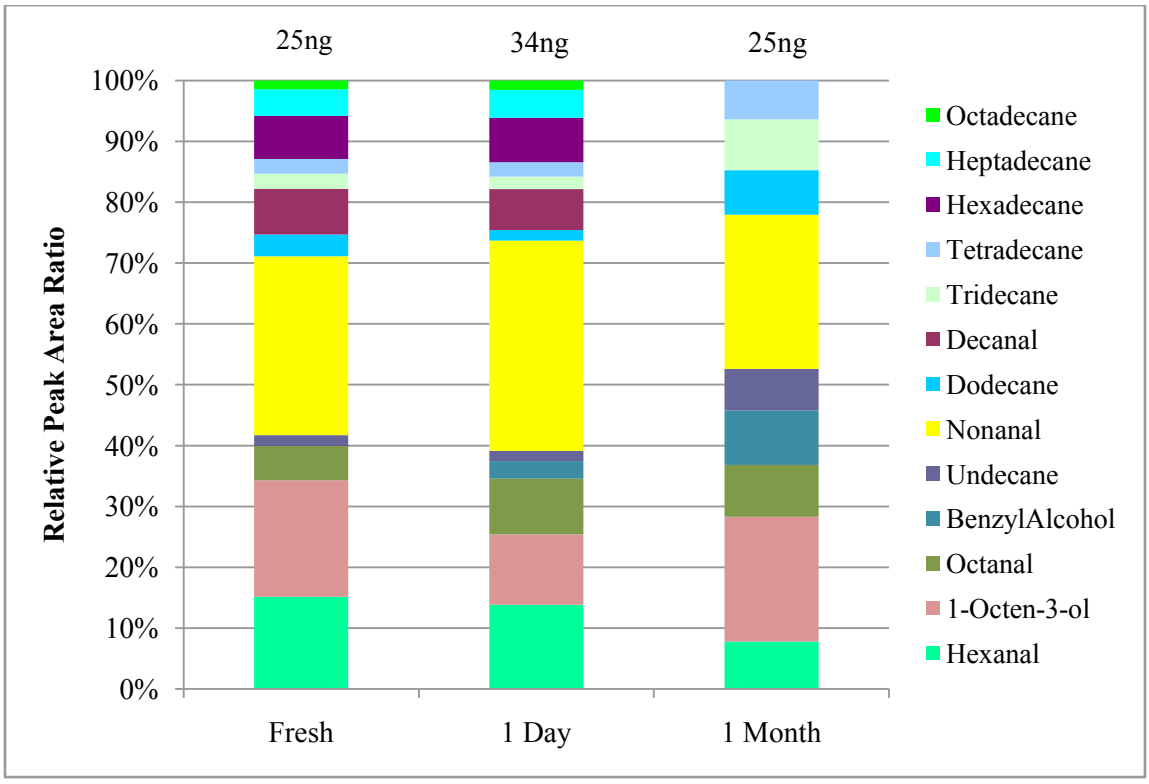
Figure 22. Abundance vs. extraction time for undecane at room temperature and 37°C



2.4.4.2.4. Blood Sample Stability

The stability of blood stored over time below 3°C was investigated. Blood collection tubes used to collect and store the blood only contained the anticoagulant K_2EDTA, and no preservatives. The VOC profiles of subject Male 5 were shown to be relatively stable even after one month of storage below 3°C as shown in Figure 23. Decanal, hexadecane, heptadecane, and octadecane were not present after one month of storage.

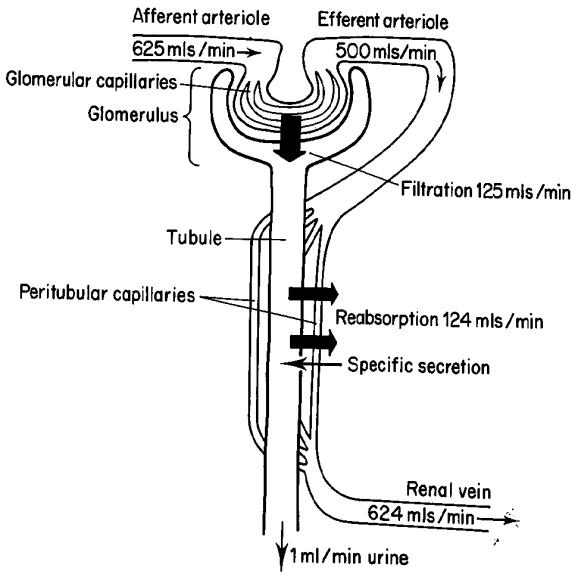
Figure 23. VOC profiles of M5 blood fresh, 1 day, and 1 month after storage



2.5. Urine

Urine is a transparent, aqueous liquid of mostly metabolic waste products that is secreted by the kidneys. Urine is composed of mostly water (95%), and the rest are dissolved organic waste products, inorganic salts, and gases[60]. Organic waste materials include urea, creatinine, ammonia, and uric acid[46,69]. Inorganic constituents of urine include sodium, potassium, calcium, magnesium, phosphates, and sulfates. It is usually pale-yellow in color; however, the color may range from yellow to amber, depending on the solute concentration in the urine and ingestion of certain foods. Urine secreted by the kidneys flows to the bladder through the ureters and is stored there until it is eliminated by the urethra during micturition.

Figure 24. Schematic of urine production



The urinary excretion mechanism begins by filtration by the nephrons of the kidneys (Figure 24). Initially, urine is an ultrafiltrate of the plasma from the glomerular capillaries into the glomerular capsule[58,59]. The glomerular filtration process is a bulk flow

movement of the solvent (water) and the solutes within. Molecular weight determines what molecules are allowed to pass through the filter. The ultrafiltrate that results from the process is essentially all of the plasma constituents in almost exactly the same concentration, with the exception of proteins. The ultrafiltrate does not contain any cells. Following filtration, kidney tubular transport mechanisms of reabsorption and secretion occur. Reabsorption of tubular fluid, either by active transport or passive diffusion, moves water and solutes and transports back into the bloodstream[59]. There are two potential routes of reabsorption, the paracellular pathway where solutes are reabsorbed between tight junctions, and the transcellular pathway where solutes are reabsorbed through epithelial cells. Passive reabsorption via diffusion depends on the polarity and therefore the lipid solubility of the solute. Secretion is the opposite of reabsorption, where substances are moved from the blood into the tubular fluid to be excreted. The few mLs of urine containing water and solutes that were not reabsorbed are excreted. Table 4 shows the solute concentrations in plasma, ultrafiltrate in the glomerular capsule, and final urine. On average, humans produce approximately one to two liters per day. The average pH of freshly collected urine is 6.0, but it can range between 4.5 and 8.0[60].

Table 4. Plasma, initial ultrafiltrate and final urine concentrations of major solutes[54]

	Plasma (mmol/L)	Bowman's capsule (mmol/L)	Urine (mmol/L)
Sodium	142	142	50-150
Potassium	4.0	4.0	20-100
Chloride	103	113	50-150
Bicarbonate	24-27	27-30	0-25
Glucose	5.5	5.9	0
Protein	6g/100mL	0.020g/100mL	< 0.010g/100mL

Urine as collected in the bladder is sterile until it reaches the urethra[59]. However, bacterial colonies reside in the epithelial cells of the urethra, and when urine passes through the urethra and comes in contact with the bacteria, the interaction between the urine and the bacterial community can result in strong odor. Urinary odor depends on the volume and concentration of the solutes excreted by the kidneys. Dilute urine is nearly odorless; concentrated urine may result in strong odor of ammonia, resulting from the breakdown of urea. Diet and physiological conditions may also affect the odor of urine. Unusual urinary odor may be related to pathological conditions, infections, or renal failure.

Volatile organic compounds in urine result from food, drinks, air pollutants, drugs, bacterial interaction within the body, and metabolic processes[70,71]. Studies on the profiles of volatile metabolites in urine have demonstrated the presence of compounds of wide range of functional groups including alcohols, aldehydes, ketones, oxygen- and nitrogen-containing compounds, sulfur-containing compounds, and other heterocyclic compounds[70-73]. The VOCs in the headspace of urine have been studied in a variety of fields including toxicology (drug detection and recovery)[74-76], occupational and

environmental exposures[77-80], and metabolic investigations[70,71,81]. Early studies on the urinary VOC profiles showed that there was a significant variance between different individuals, but intra-person urinary VOC profiles remained constant over time even with variation factors such as diet, exercise, circadian and seasonal changes[73]. Disturbances of volatile compounds found in urine have been associated with physiological disorders including diabetes mellitus, liver and kidney disease, and trimethylaminuria[71,82,83].

2.5.1. Materials & Methods

Sterile, disposable specimen collection cups were purchased from Dynarex Corporation (Orangeburg, NY, USA). Laboratory grade artificial urine was obtained from WARD'S Natural Science (Rochester, NY, USA). NaCl, KCl, $MgSO_4$, K_2CO_3, and Na_2CO_3 used for the urine optimization studies were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ten ml glass, clear, screw top headspace vials with PTFE/Silicone septa were used to hold the samples (SUPELCO, Bellefonte, PA, USA). Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) (50/30 μm film thickness) SPME fibers and SPME fiber holders were obtained from SUPELCO (Bellefonte, PA, USA).

The GC/MS analysis was carried out using an Agilent Technologies 6890N gas chromatograph coupled to an Agilent Technologies 5973N mass selective detector (Palo Alto, CA, USA). The column used to separate the analytes was a HP5-MS, 30 m, 0.25 um, 0.25 mm with helium as the carrier gas with a flow rate of 1.0mL/min. The extracted VOCs were desorbed in the injection port of the GC with a temperature of 250°C for five minutes in splitless mode. The GC oven temperature programming was as follows: the

initial oven temperature of 40°C held for five minutes, and then ramped at 15°C/min to a final temperature of 280°C where it was held for two minutes, for a total run time of 23 minutes. The mass spectrometer used was an HP 5973 MSD with a quadrupole analyzer operated in electron ionization mode. The mass spectrometer transfer line was maintained at 280°C and the source temperature was 230°C. The analytes were acquired in full-scan mode in 41-550 m/z range.

2.5.2. Urine sampling procedure

Subjects sampled themselves by collecting urine in a disposable sterile specimen collection cup. Urine samples were stored in a 4°C refrigerator until ready for use.

2.5.3. Determination of optimal extraction conditions for urine

2.5.3.1. Methods

Laboratory grade artificial urine (WARD'S Natural Science, Rochester, NY, USA) was spiked with acetone, 2-pentanone, dimethyl disulfide, 1H-pyrrole, toluene, hexanal, 4-heptanone, 3-heptanone, 2-heptanone, benzaldehyde, phenol, 1-octanol, octanoic acid, and nonanoic acid for the optimization study. Urine samples were prepared in sterile 10 ml glass headspace vials with 2 ml urine, 0.5 ml deionized water, 1.5 g NaCl (except during salting-out study), and spiked with 25 µL of 200 ppm standard mix comprised of the 14 volatile compounds mentioned above. Samples were made in triplicates and vortexed at 2800 rpm using lab dancer vortex (IKA® Works, Inc., Wilmington, NC, USA) for 30 seconds. Optimization studies were performed for SPME fiber exposure time (1, 5, 15, 30 min, and 1hr), sample equilibration time (5, 15, and 30 min), extraction temperature (room temperature, 40°C, 50°C, 60°C, and 70°C), and salting-out effects of

5 different inorganic salts (NaCl, KCl, $MgSO_4$, K_2CO_3, and Na_2CO_3). The 50/30 μm DVB/CAR/PDMS SPME fibers (SUPELCO, Bellefonte, PA, USA) were used to extract the VOCs from the headspace of the urine samples in the vials. All samples were run using the GC/MS method for urine samples previously mentioned in section 2.5.1.

2.5.3.2. Results

2.5.3.2.1. Optimal Extraction Time

Laboratory grade artificial urine was spiked with 25 μl of 200 ppm standard mix comprised of 14 volatile compounds listed in section 2.5.3.1. Urine samples were exposed for 1 min, 5 min, 15 min, 30 min, and 1 hour to determine the optimal extraction time in a 50°C water bath with a 30 minute equilibration time. Signal increased with increasing extraction time until 30 minutes. The signal remained the same for the 30 minute- and one hour-extraction times. As shown in Table 5, 13 out of the 14 spiked compounds were recovered for both 30 minute- and one hour-extraction times. No acetone was detected in any of the samples, as it was masked by the acetonitrile solvent peak. From the results, 30 minutes was determined to be the optimal extraction time.

Table 5. Compounds detected under different extraction times for urine SPME optimization

RT	Compound	Extraction Time				
		1 min	5 min	15 min	30 min	1 hr
1.89	Acetone					
3.28	2-Pentanone	x	x	x	x	x
4.44	Dimethyl Disulfide	x	x	x	x	x
4.76	Pyrrole		x	x	x	x
5.07	Toluene	x	x	x	x	x
6.02	Hexanal	x	x	x	x	x
7.73	4-Heptanone	x	x	x	x	x
8.05	3-Heptanone	x	x	x	x	x
8.13	2-Heptanone	x	x	x	x	x
9.25	Benzaldehyde	x	x	x	x	x
9.58	Phenol	x	x	x	x	x
10.86	1-Octanol		x	x	x	x
12.15	Octanoic acid				x	x
12.94	Nonanoic acid			x	x	x

2.5.3.2.2. Optimal Equilibration Time

Equilibration times were varied (5, 15, and 30 minutes) with a 30 minute extraction time at 50°C. Results demonstrated that peak areas for the three equilibration times were comparable with one another (Figure 25 through Figure 29). Longer equilibration time did not affect the signal strength or peak area; therefore, the shortest equilibration time of five minutes was chosen.

Figure 25. Abundance vs. equilibration times of aldehydes for urine optimization

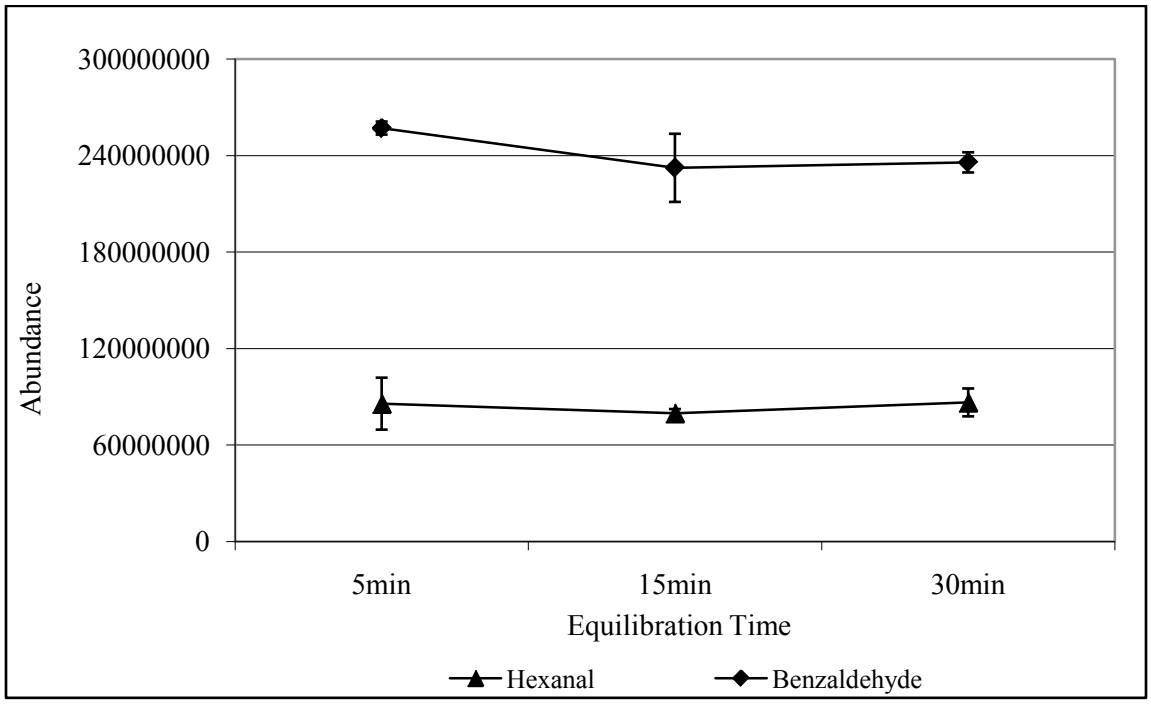


Figure 26. Abundance vs. equilibration times for acids for urine optimization

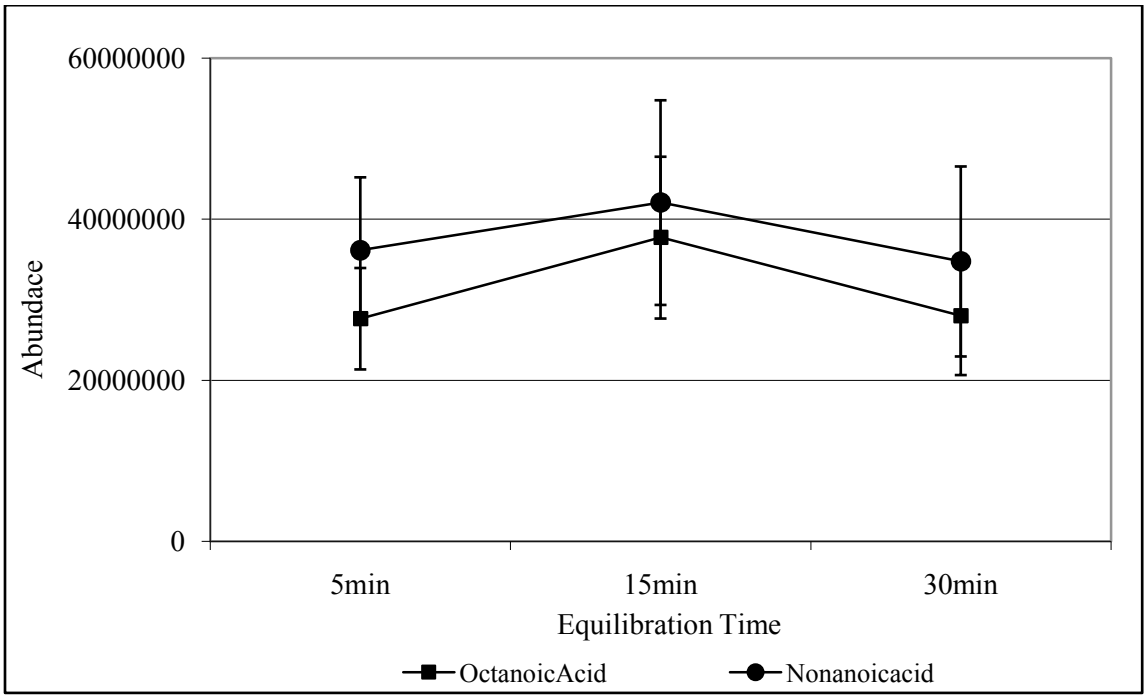


Figure 27. Abundance vs. equilibration times for alcohols for urine optimization

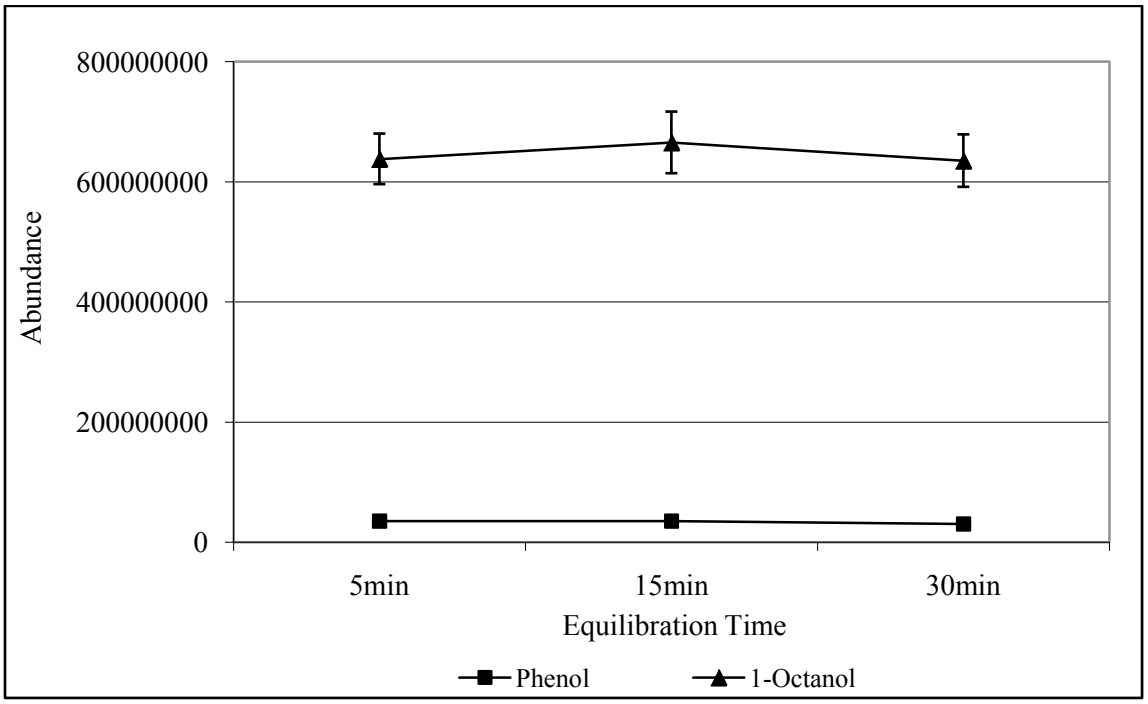


Figure 28. Abundance vs. equilibration times for ketones for urine optimization

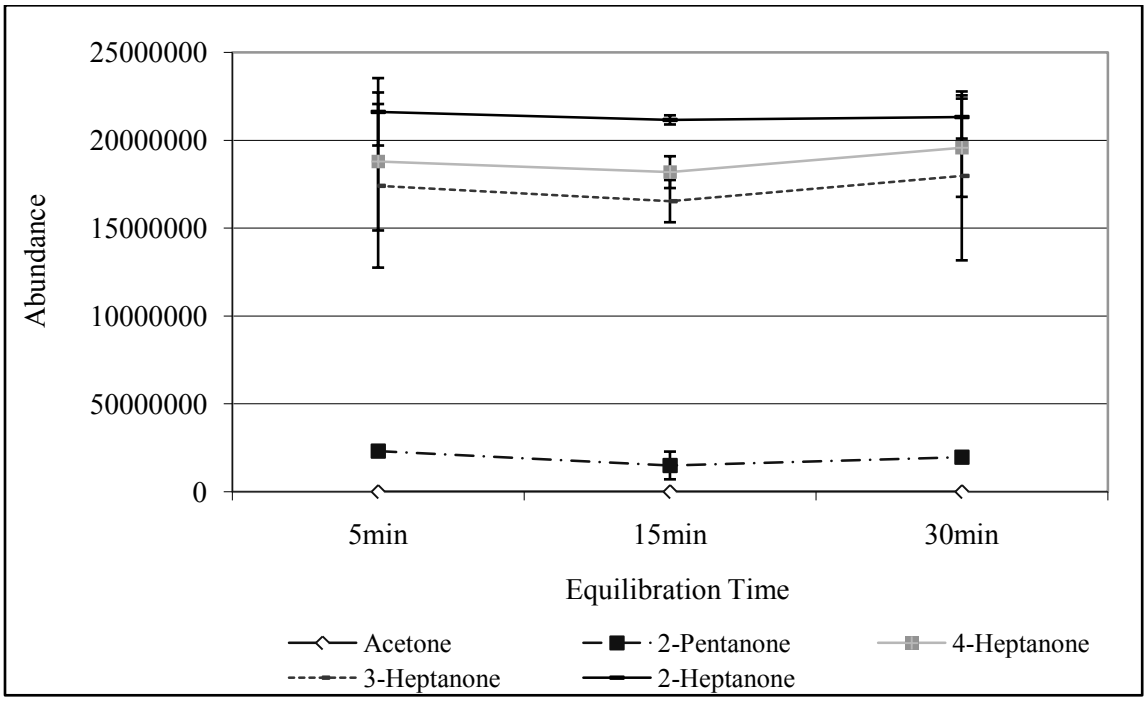
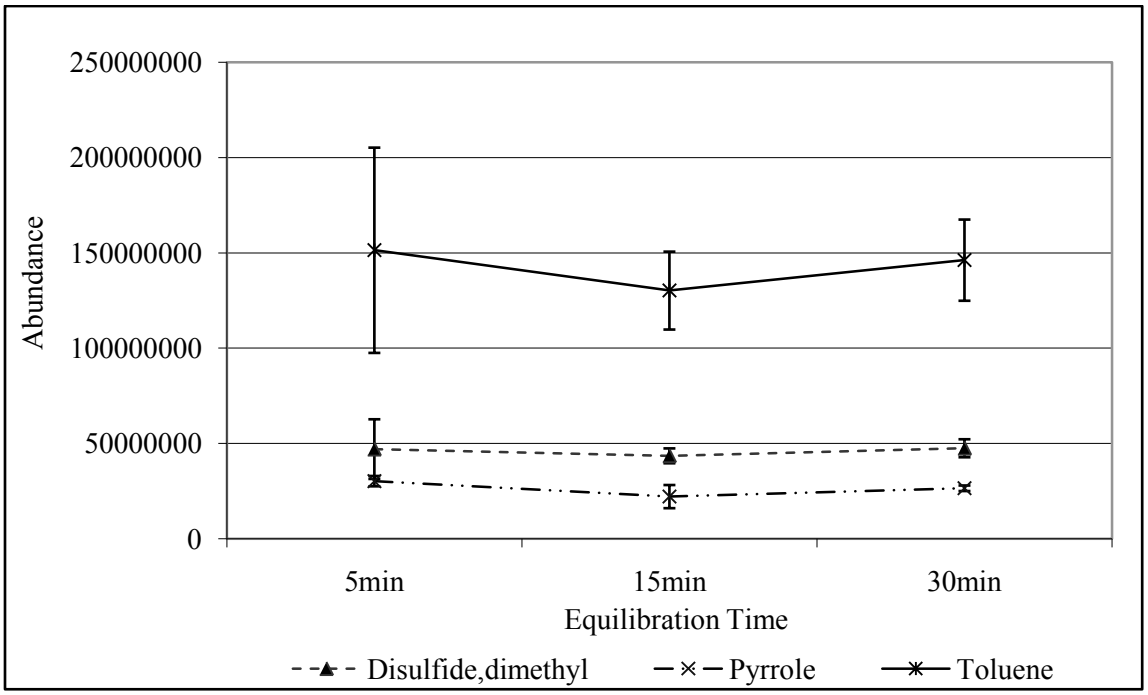


Figure 29. Abundance vs. equilibration time for other functional group compounds for urine optimization



2.5.3.2.3. Optimal Extraction Temperature

Temperature of the hot water bath was varied (room temperature (~25°C, 40°C, 50°C, 60°C, and 70°C) using the five minute equilibration and 30 minute extraction times. Results demonstrated that 60°C gave the best results in terms of the abundance of the compounds extracted for the majority of the compounds. As shown in Figure 31, for the carboxylic acids nonanoic acid and octanoic acid, the abundance of extracted compounds was highest at 70°C. However, for aldehydes, alcohols, ketones, and pyrrole, the abundance of the extracted compounds decreased at 70°C. For dimethyl disulfide and toluene, there was a slight increase in the abundance of the extracted compounds. Raising the temperature beyond 60°C introduces the additional concern of fiber coating swelling and powdering which could risk damage to the fiber through fragmentation

when it is withdrawn back into the needle. Therefore, 60°C was chosen as the optimal extraction temperature.

Figure 30. Abundance vs. extraction temperatures of aldehydes for urine optimization

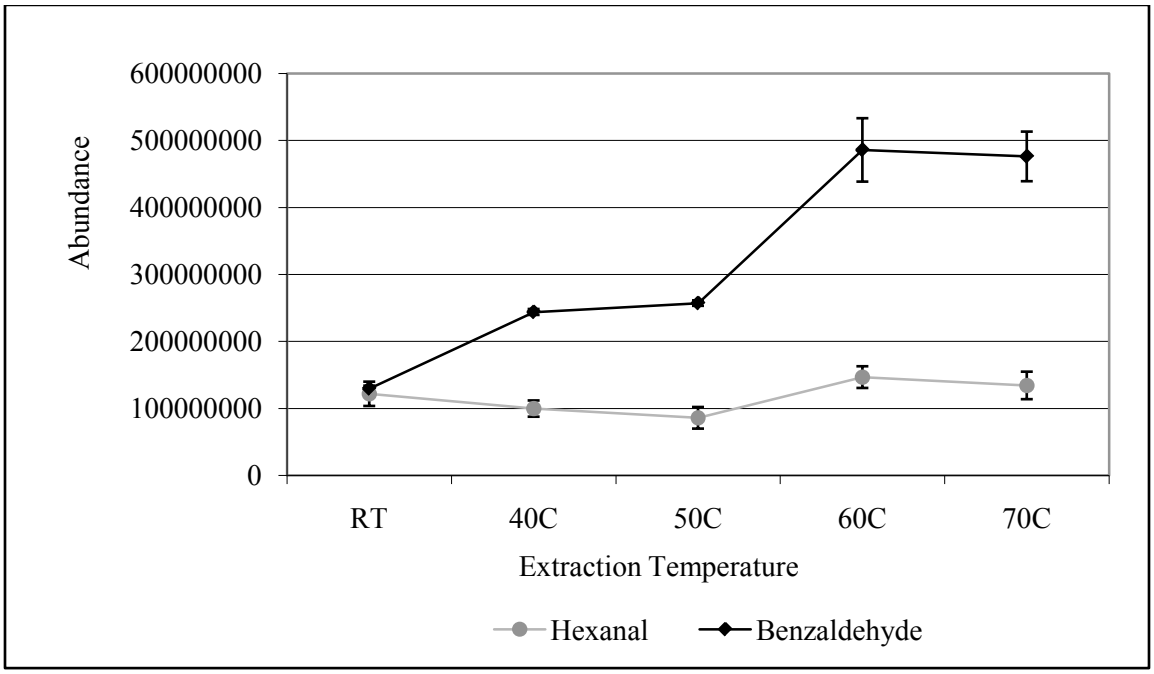


Figure 31. Abundance vs. extraction temperatures of acids for urine optimization

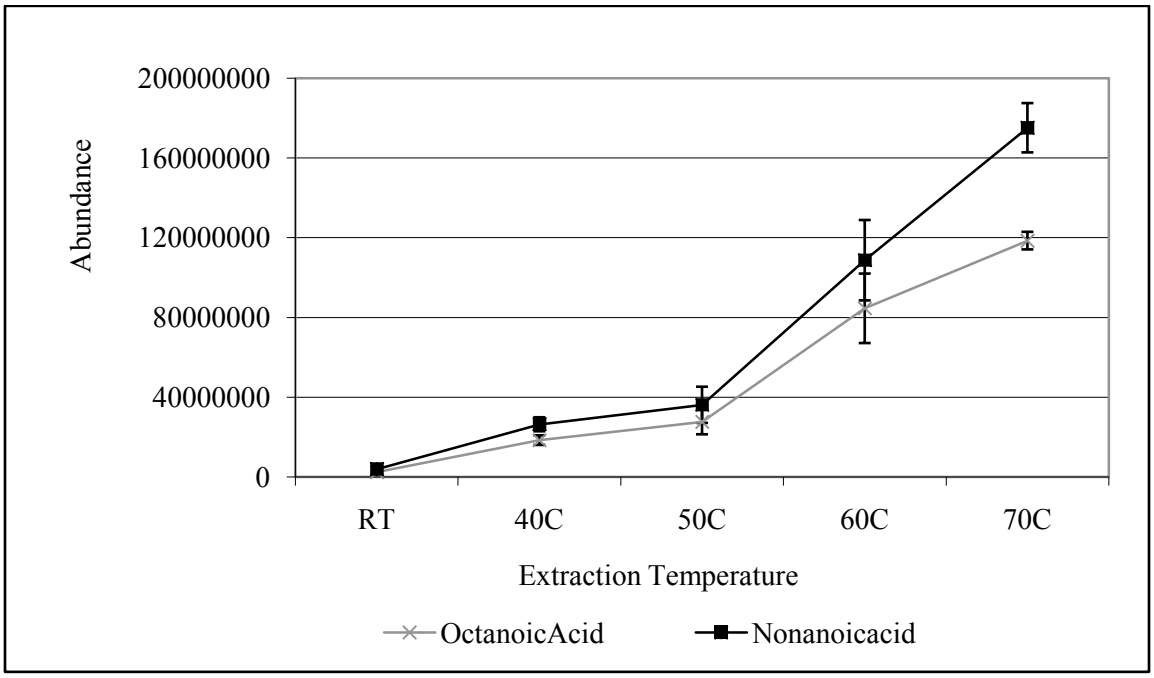


Figure 32. Abundance vs. extraction temperatures of alcohols for urine optimization

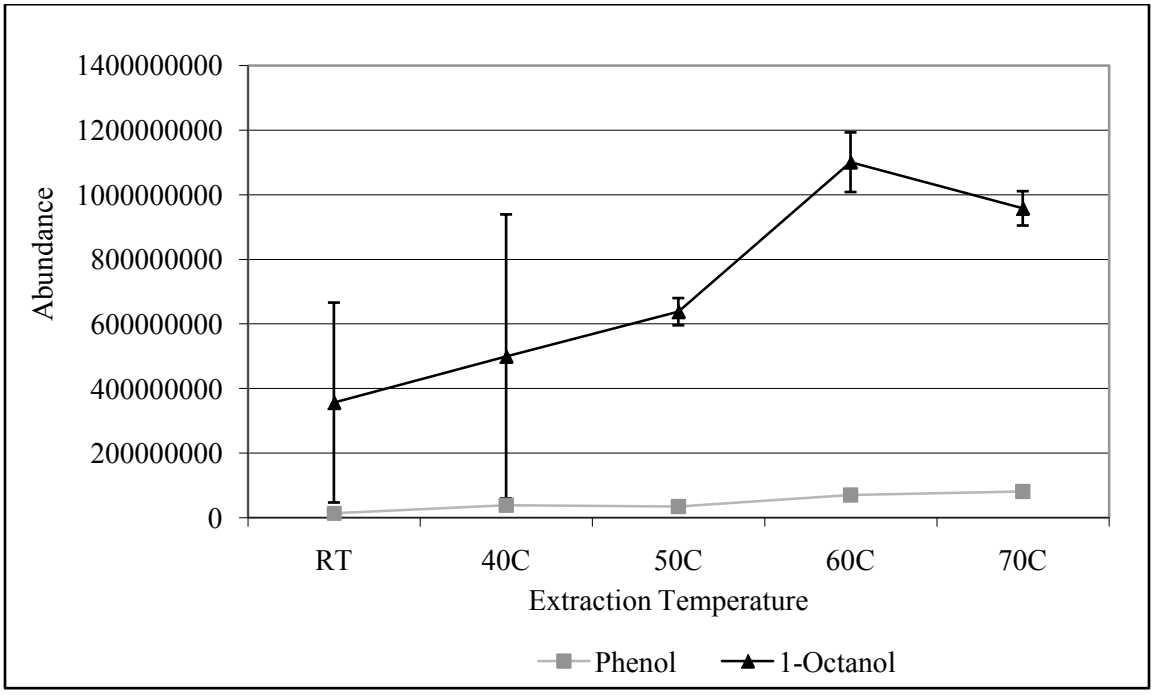


Figure 33. Abundance vs. extraction temperatures of ketones for urine optimization

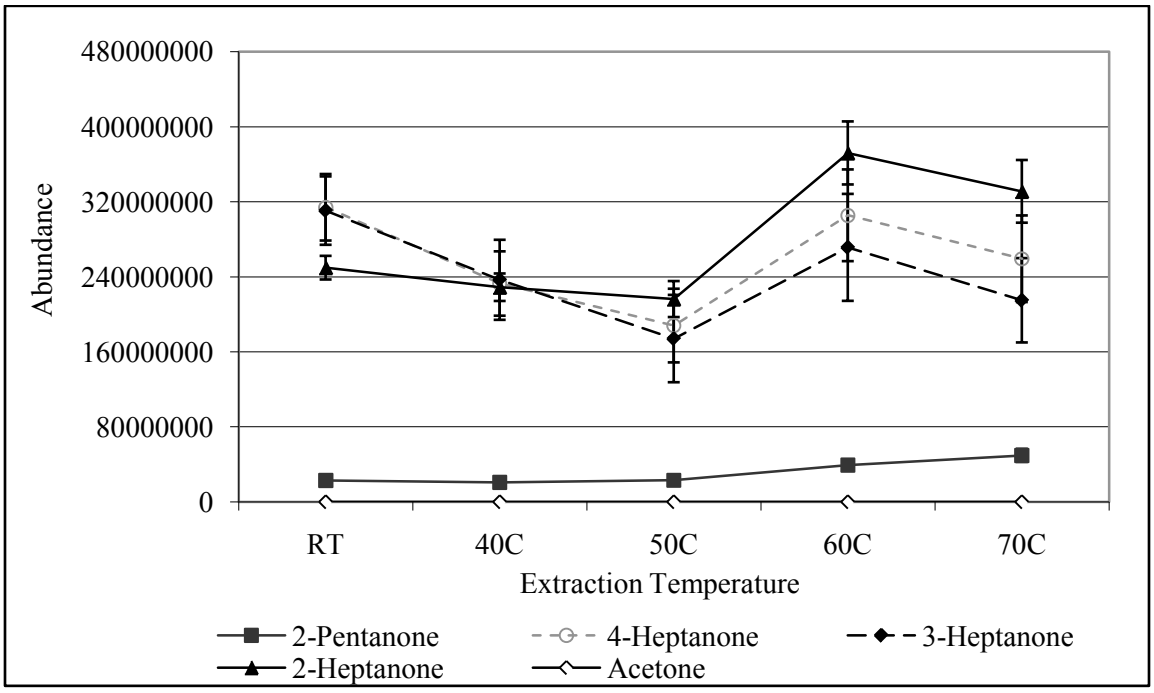
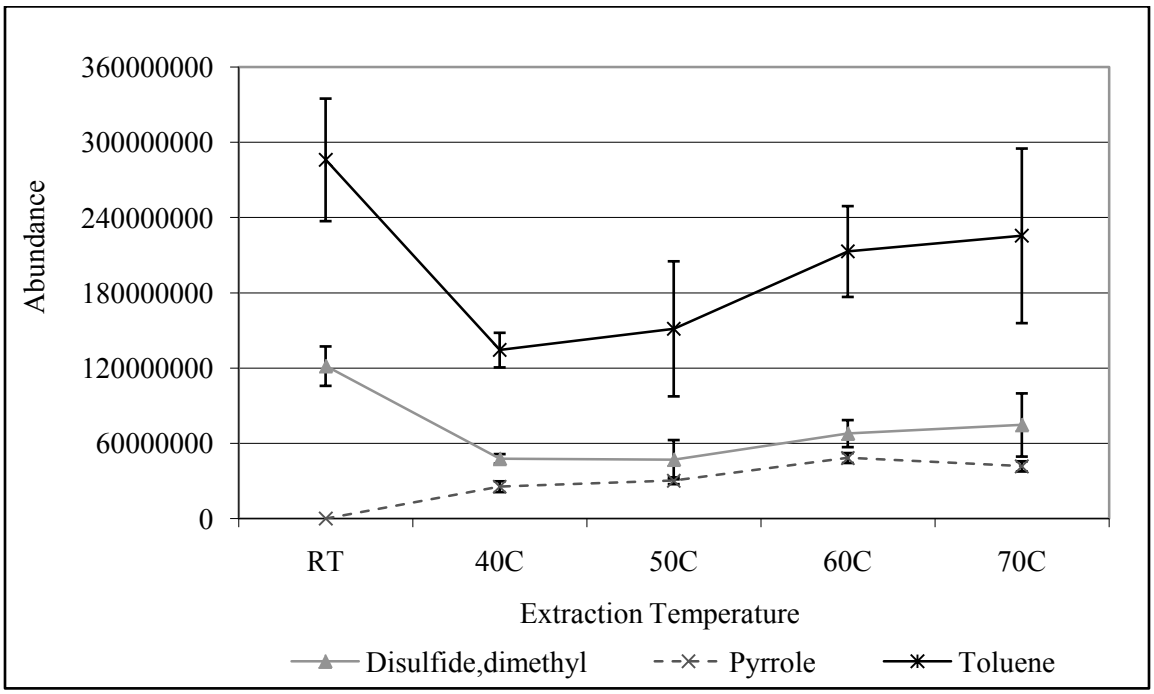


Figure 34. Abundance vs. extraction temperatures of other functional group compounds for urine optimization



2.5.3.2.4. Effect of Salting Out

Five inorganic salts (NaCl, KCl, $MgSO_4$, K_2CO_3, and Na_2CO_3) were tested for their salting-out abilities. Adding an inorganic salt to a sample solution improves extraction efficiency for volatile compounds in biological fluids. Salting out can be used not only to lower the detection limits, but also to buffer random salt concentration in body fluids. Salt (1.5g) was added to completely saturate the sample solution. Samples were equilibrated for five minutes and SPME fibers were exposed for 30 minutes in a 60°C hot water bath. As shown in Figure 35 through Figure 39, NaCl improved the extraction efficiency of the VOCs best out of the five inorganic salts investigated. No acids were detected with the addition of K_2CO_3 or Na_2CO_3 (Figure 36).

Figure 35. Effect of salt addition for urine optimization for aldehydes

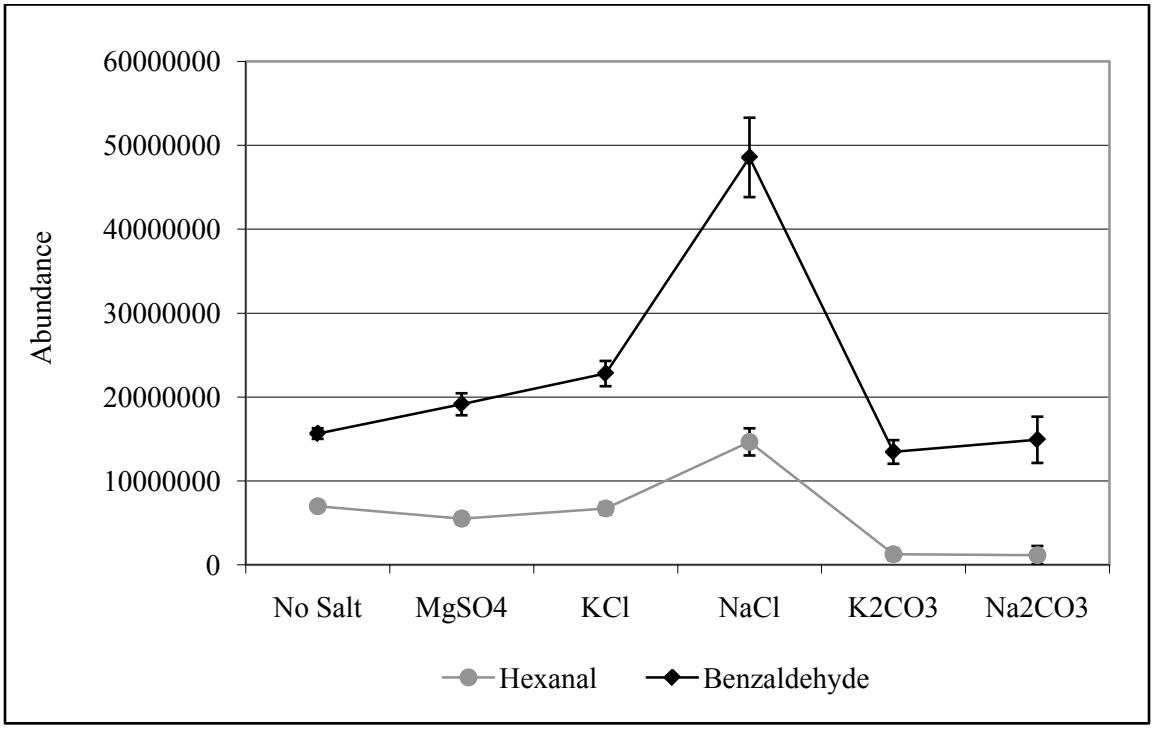


Figure 36. Effect of salt addition for urine optimization for acids

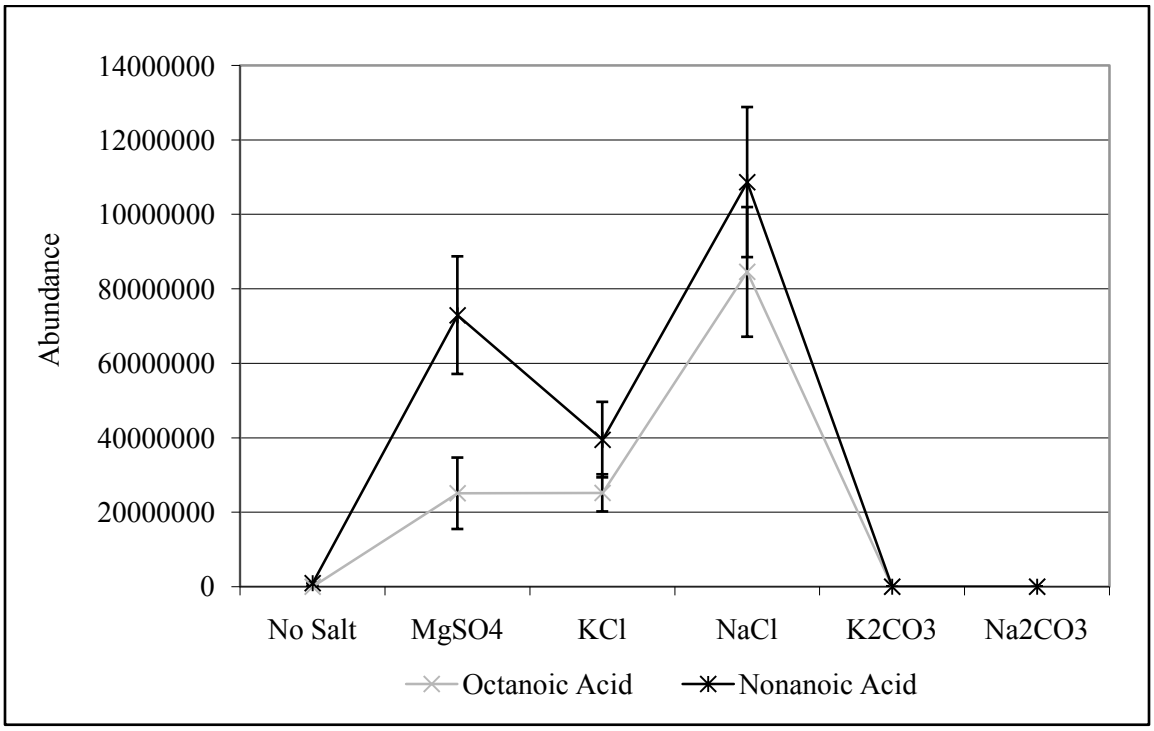


Figure 37. Effect of salt addition for urine optimization for alcohols

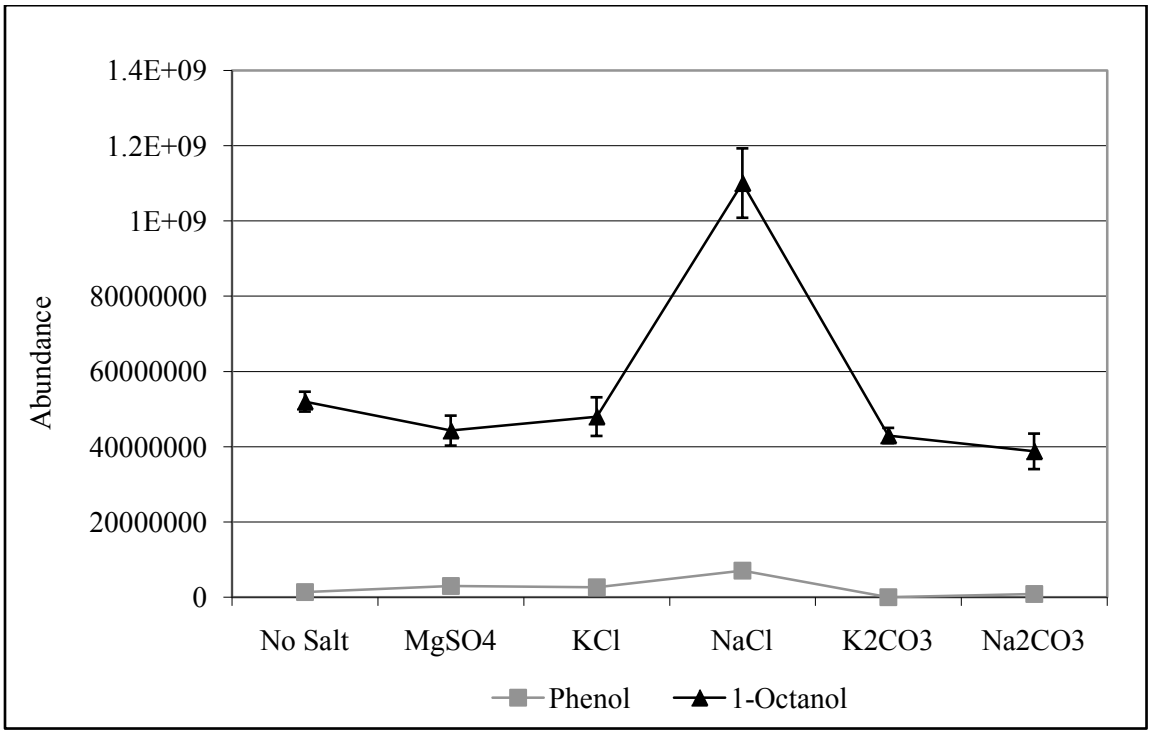


Figure 38. Effect of salt addition for urine optimization for ketones

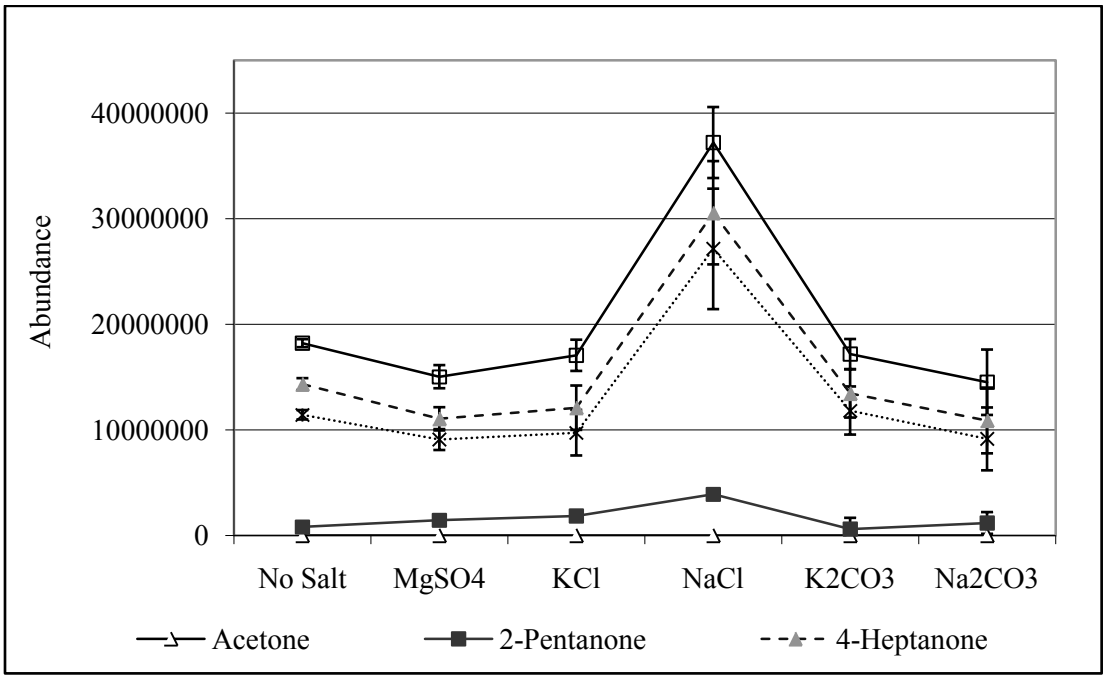
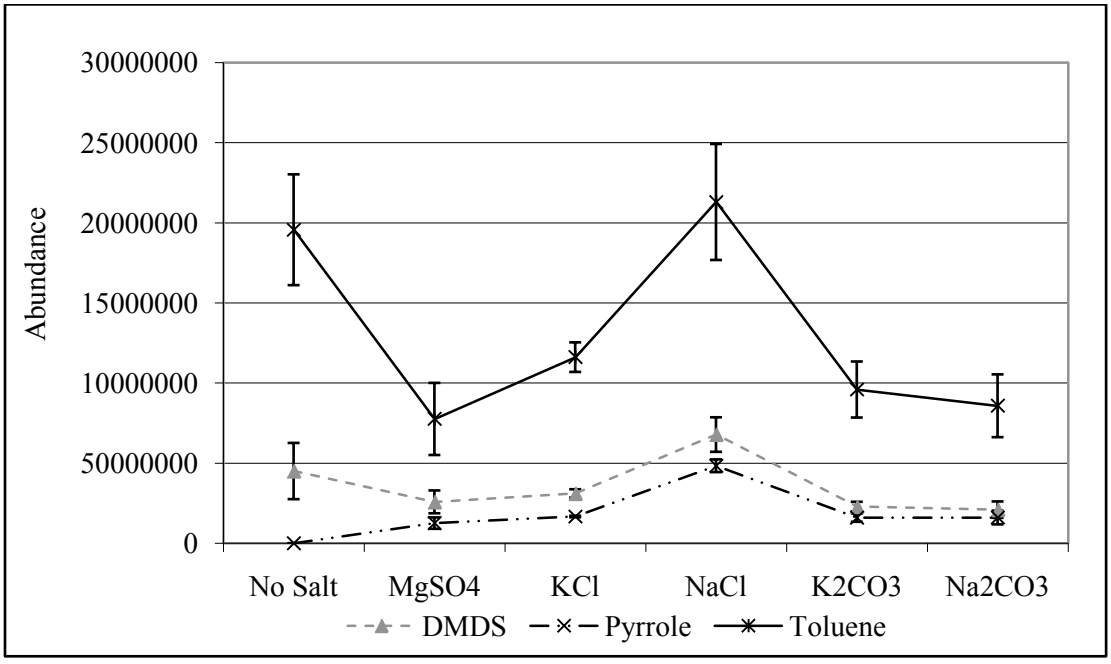


Figure 39. Effect of salt addition for urine optimization for other functional group compounds



2.6. Comparison of Five Specimens from Individuals

2.6.1. Methods

Five biological specimen samples were collected from four subjects: two females (F2 and F4) and two males (M1 and M5) ranging in age from 27 to 31 years old. Each specimen sample was collected in duplicate (hand odor and breath) or triplicate (buccal swab, blood, and urine) and analyzed by the SPME-GC/MS methods mentioned in the preceding sections of this chapter and subjected to chromatographic and statistical analyses as described below to determine the similarities and differences in the VOCs extracted from the five samples. Hand odor, buccal swab, breath, and urine samples were collected using the specimen sampling procedures as described previously. Blood was obtained using venipuncture method from subjects F4, M1, and M5 in a BD Vacutainer ® blood collection tube containing K_2EDTA (BD, Franklin Lakes, NJ, USA) for this portion of study. Subject F2 was not able to give the blood sample, therefore human whole blood containing anticoagulant sodium EDTA (Bioreclamation Inc., Hicksville, NY, USA) was used in place of F2 blood sample for the VOC profile comparison of blood. Hand odor, buccal swab, and breath samples were stored at room temperature. Blood and urine samples were stored at 4°C until ready for use. The optimized conditions are summarized in Table 6. Extraction conditions for hand odor were determined previously by Curran[84]. All subsequent studies involving human subject samplings utilized the optimized SPME conditions established here. Methods for GC/MS analyses of samples from each specimen are described in the previous sections also.

Table 6. Summary of optimized SPME conditions for biological specimens

	Hand Odor	**Buccal Swab**	**Breath**	**Blood**	**Urine**
Equilibration Time	24 hours	24 hours	15 minutes	1 hour	5 minutes
Extraction Time	21 hours	21 hours	21 hours	18 hours	30 minutes
Extraction Temperature	RT	RT	RT	37°C	60°C
Other	--	--	--	--	NaCl

2.6.2. Results

Using the optimized sample collection and SMPE conditions, preliminary human subject odor profiles were compared for the five biological specimens prior to larger population sampling. Examples of a typical chromatogram for each specimen are shown in Figure 40. Specimen samples were compared among four subjects (two female subjects F2 and F4, two male subjects M1 and M5). Figure 41 through Figure 44 show the chromatogram expanded and siloxane peaks removed to compare the odor profiles produced from the four subjects (F4, F2, M1, and M5) for the four specimens (buccal swab, breath, blood, and urine). As seen from these figures, qualitatively there are commonalities in the compounds present in the headspace of the collected samples from different individuals. Figure 45 shows the comparison of the relative peak area ratios for the common VOCs present in triplicate samples of buccal swabs collected from the four subjects. Figure 45 also demonstrates the reproducibility between samples collected from the same individual. For buccal swab samples, hexanoic acid and decanal were common to all subjects; however, the ratios of all compounds demonstrate unique overall VOC profiles per individual.

Figure 40. Typical odor profile chromatogram for a) hand odor, b) buccal swab, c) breath, d) blood, and e) urine

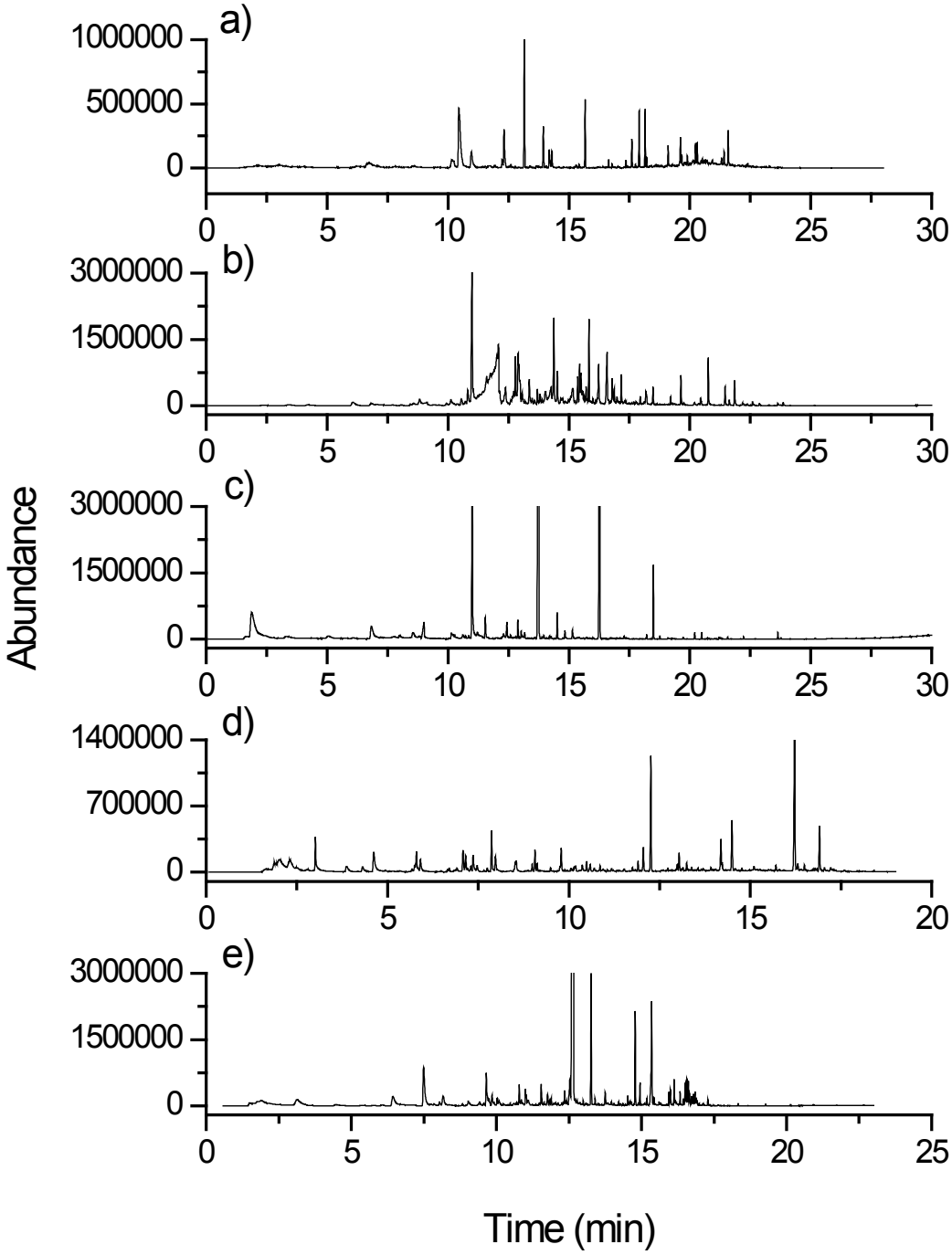


Figure 41. Comparison of buccal swab odor profiles from subjects F4, F2, M1, and M5

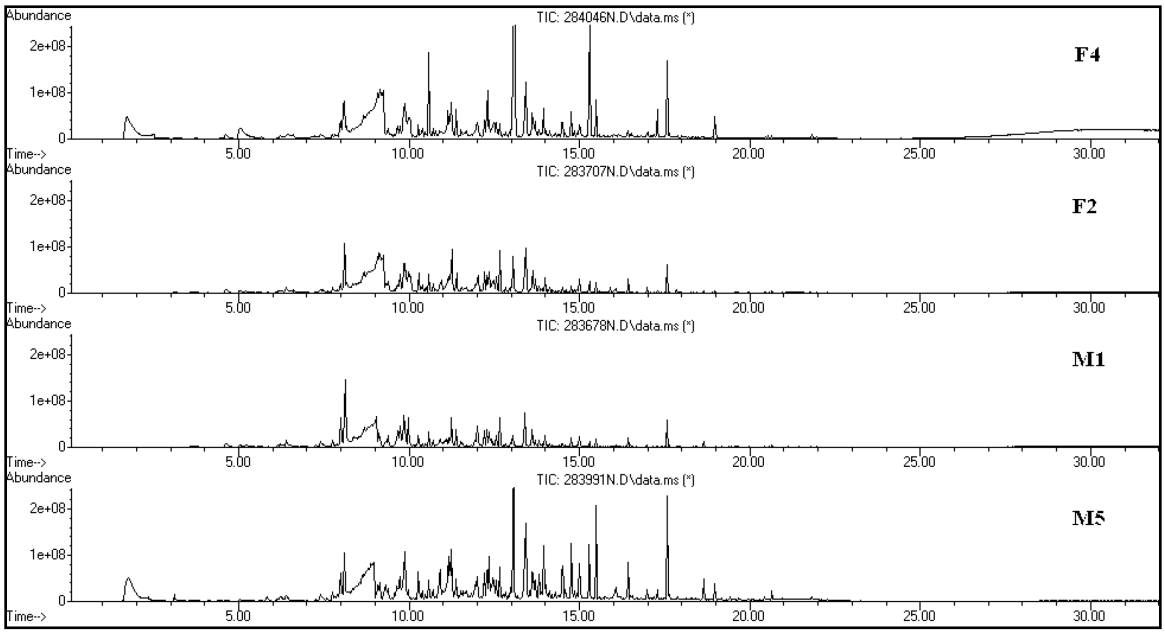


Figure 42. Comparison of breath odor profiles from subjects F4, F2, M1, and M5

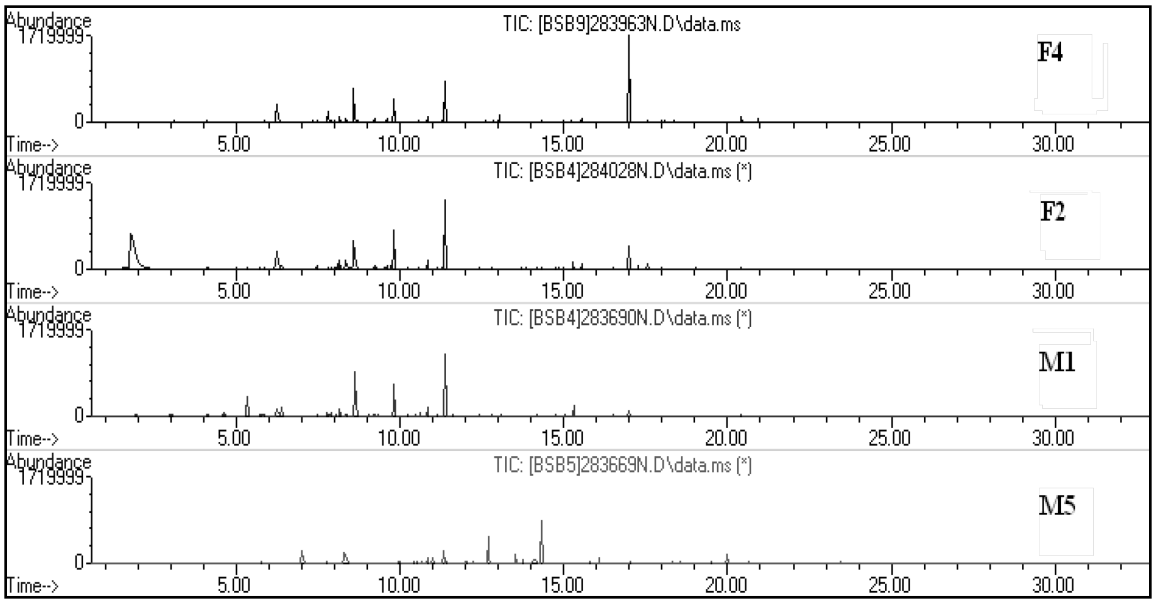


Figure 43. Comparison of blood odor profiles from subjects F4, F2, M1, and M5

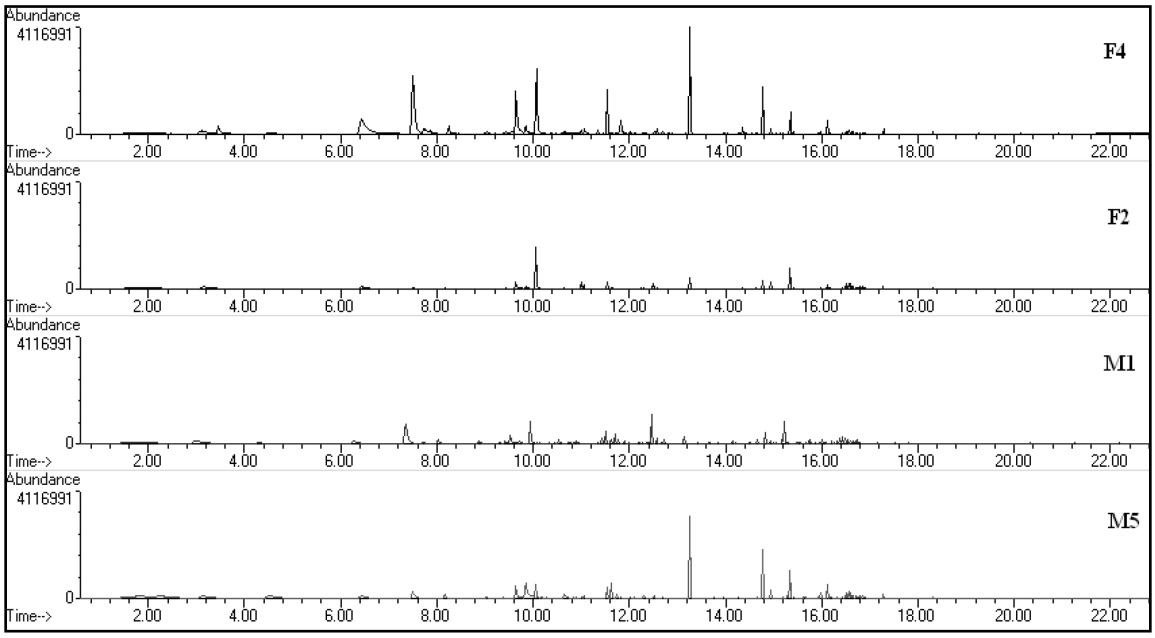


Figure 44. Comparison of urine odor profiles from subjects F4, F2, M1, and M5

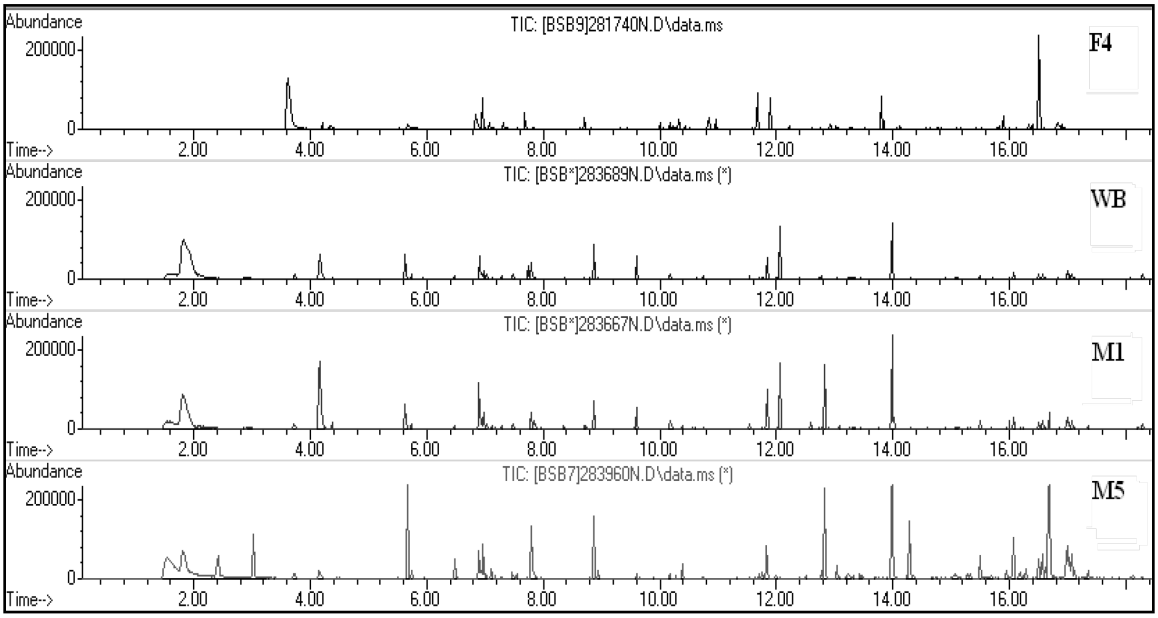


Figure 45. Buccal swab VOC profile comparison between 4 subjects

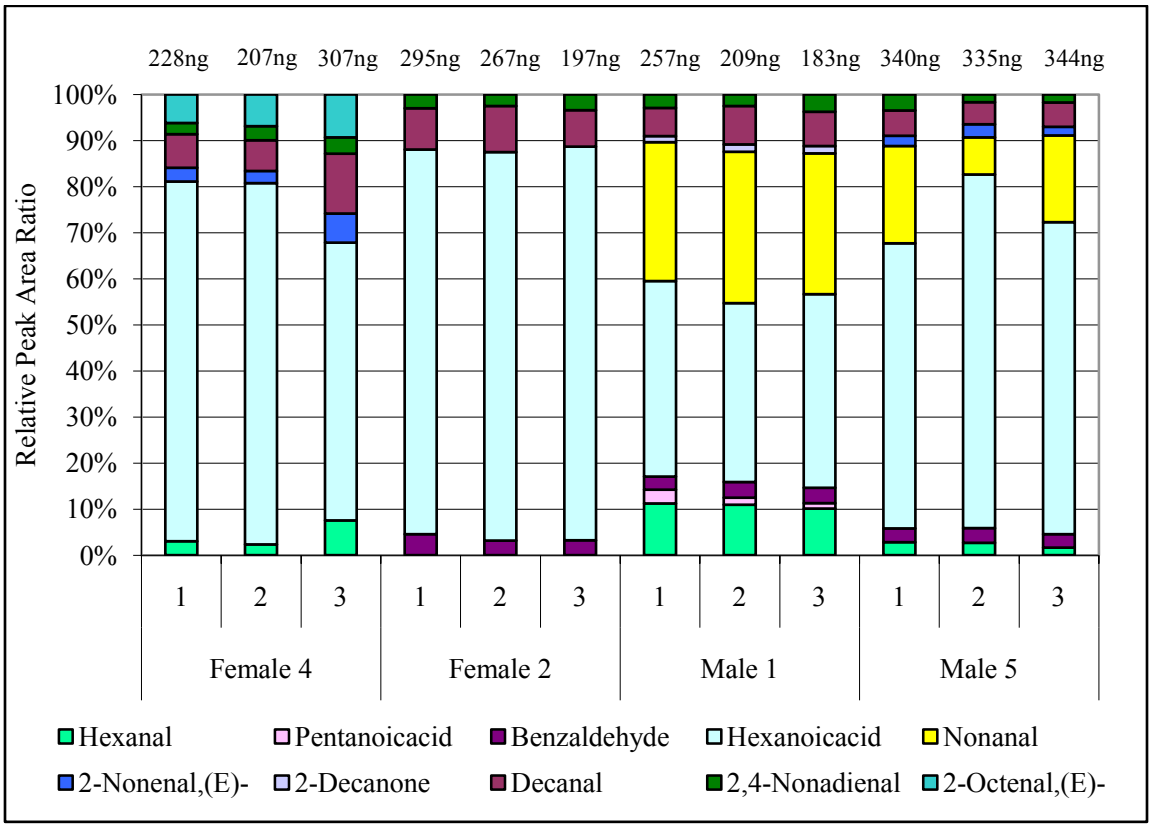


Figure 46 through Figure 48 show the comparisons of the VOC profiles for breath, blood, and urine for the four subjects compared side by side. For breath, acetone, nonanal, decanal, and 2-ethyl-1-hexanol were common to all subjects, but in varying ratios (Figure 46). Blood was collected from subjects Female 4, Male 1, and Male 5 in a BD Vacutainer® blood collection tube containing K_2EDTA. The blood VOC profiles were compared along with the whole blood (WB) used during the optimization studies. Compounds common to all blood samples were undecane, nonanal, dodecane, tridecane, and tetradecane (Figure 47). For urine, only 4-heptanone was present in all subject samples, and was also the predominant VOC in urine (Figure 48). For each specimen, reproducibility of VOC profiles was demonstrated between the multiple samples

collected from the same individual. Compounds that were present in all four subjects varied in the ratios of the compounds present in the profile, demonstrating the individuality of the overall VOC profiles per individual.

Figure 46. Subject comparison of breath VOC profiles from subjects F4, F2, M1, and M5

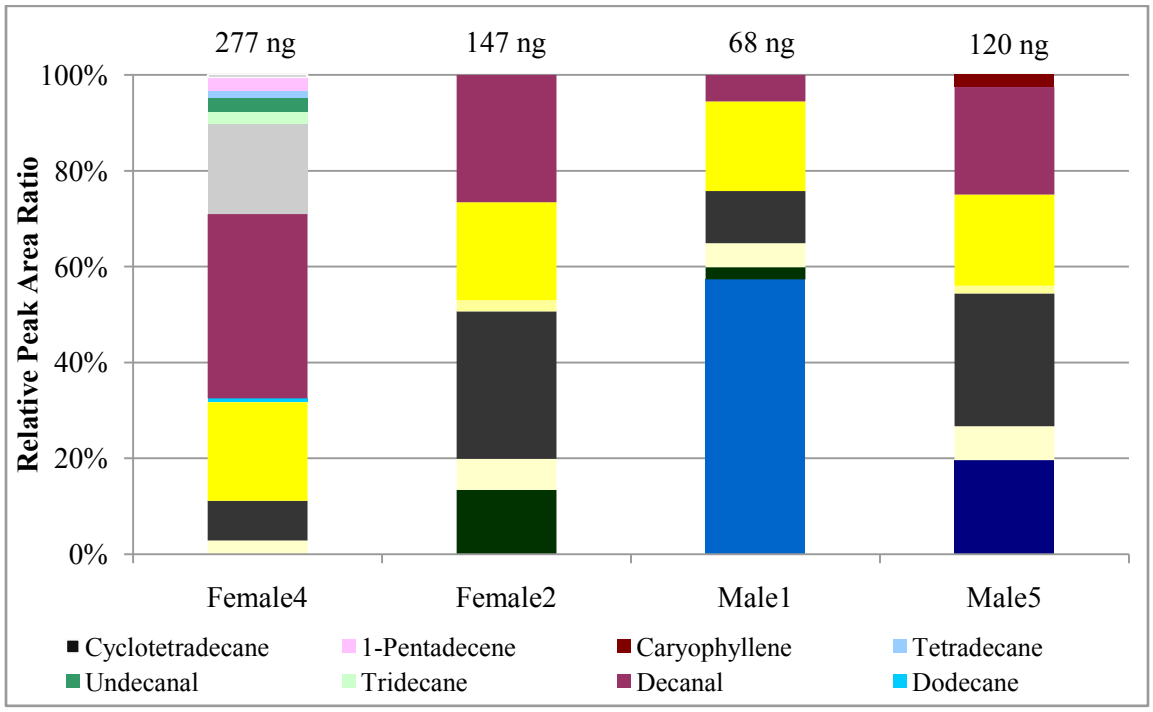


Figure 47. Subject comparison of blood VOC profiles from subjects F4, WB, M1, and M5

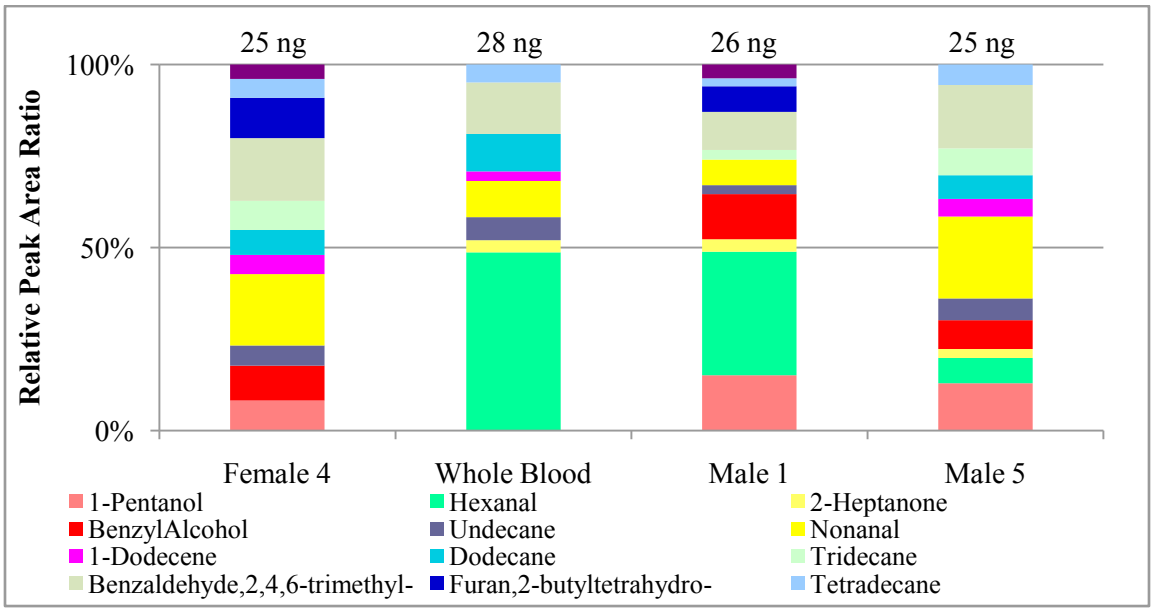
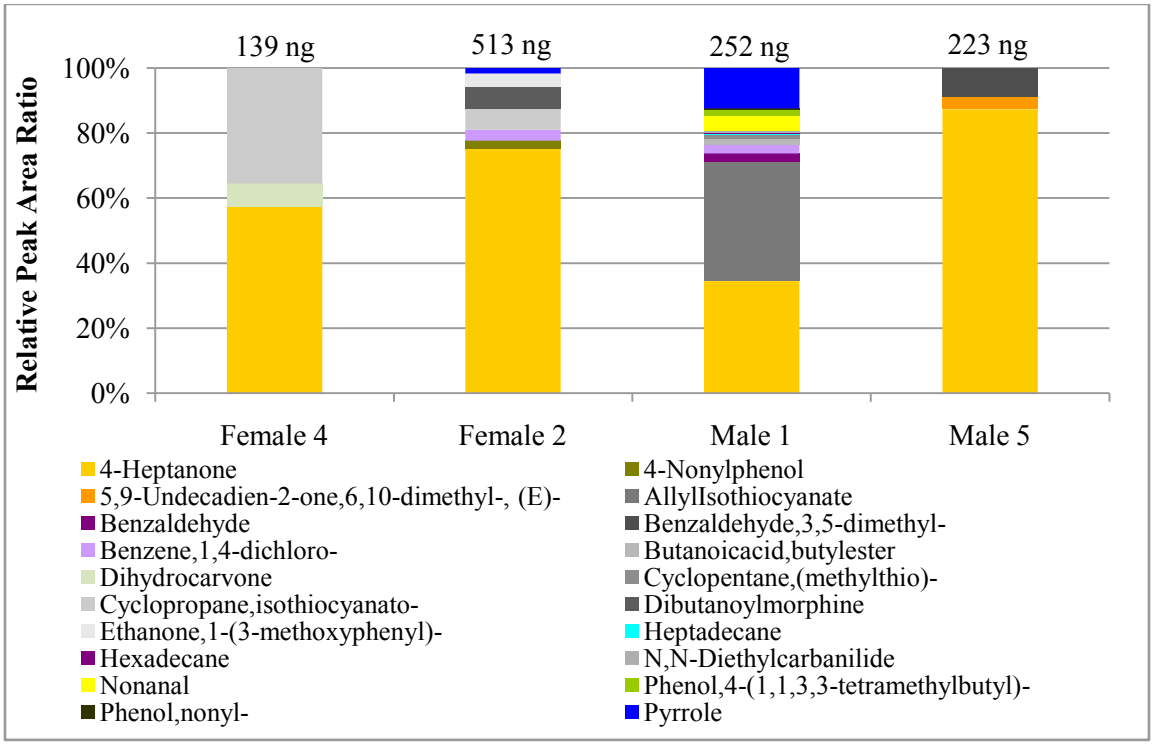


Figure 48. Subject comparison of urine VOC profiles from subjects F4, F2, M1, and M5



2.6.3. Spearman Correlation Ranking

Spearman's rank correlation is a statistical technique that is used to test the statistical dependence, in terms of direction and strength of the relationships, between two variables[85]. The Spearman rank correlation coefficient method is a nonparametric, or distribution-free technique, that allows for ranked data to be compared to determine the correlation between the variables. The null hypothesis is that there is no relationship between the variables being compared. The Spearman correlation coefficient ranges from -1 to +1. The Spearman rank correlation coefficient is defined by the following equation:

$$r_s = 1 - \frac{6\sum d^2}{n(n^2-1)} \qquad \textbf{Equation 2}$$

The correlation coefficient, r_s, is determined in terms of the difference between the ranked compounds, d, and the number of compounds being compared, n. The Spearman rank correlation determined the possible correlation between the chemical odor profile (set of VOCs) of a single individual or a single specimen with a population or other specimens[13].

Spearman correlation coefficient comparisons were conducted utilizing the VOCs detected in the headspace of the four specimens investigated in this study. Using a Spearman correlation macro developed in-house[13], analysis of specimen profiles from subjects demonstrated that intra-subject samples have a high correlation (correlation coefficient > 0.9) while inter-subject samples comparison shows a low correlation (correlation coefficient < 0.6), reiterating the individuality of odor profiles characteristic for each subject (Figure 49). Similarly, Spearman rank correlation comparisons for breath

(Figure 50), blood (Figure 51), and urine (Figure 52) were performed and showed similar results as that for buccal swab samples shown above. High correlation (correlation coefficient > 0.9) was seen between the samples from the same subject, while low correlation (correlation coefficient ≤ 0.6) was seen between different subjects, reiterating the individuality of odor profiles for each subject.

Figure 49. Spearman rank correlation with respect to Female 4, sample 1 for buccal swab

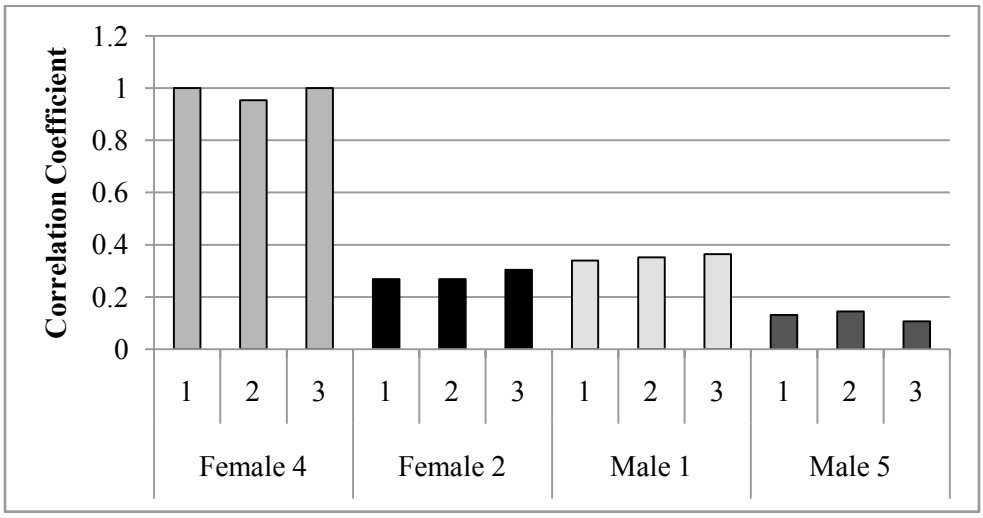


Figure 50. Spearman rank correlation with respect to Female 2, sample 1 for breath

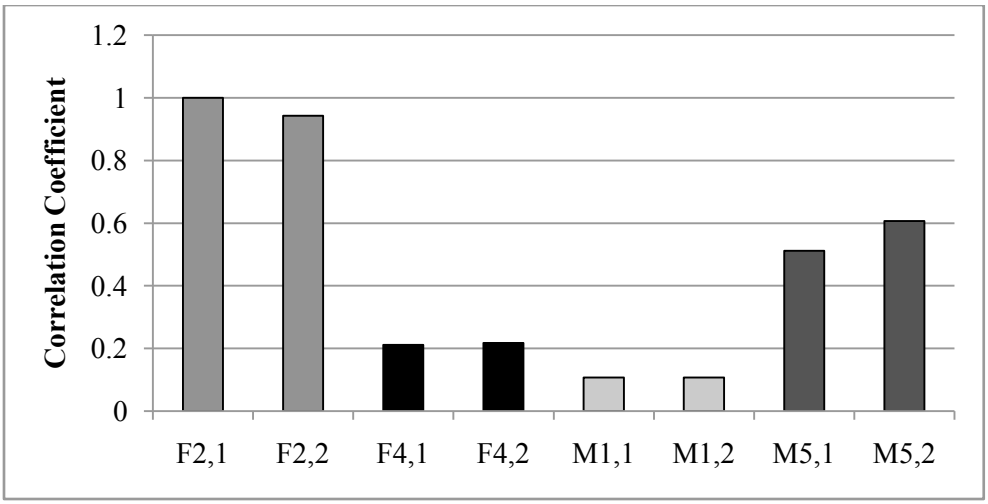


Figure 51. Spearman rank correlation with respect to Male 1, sample 1 for blood

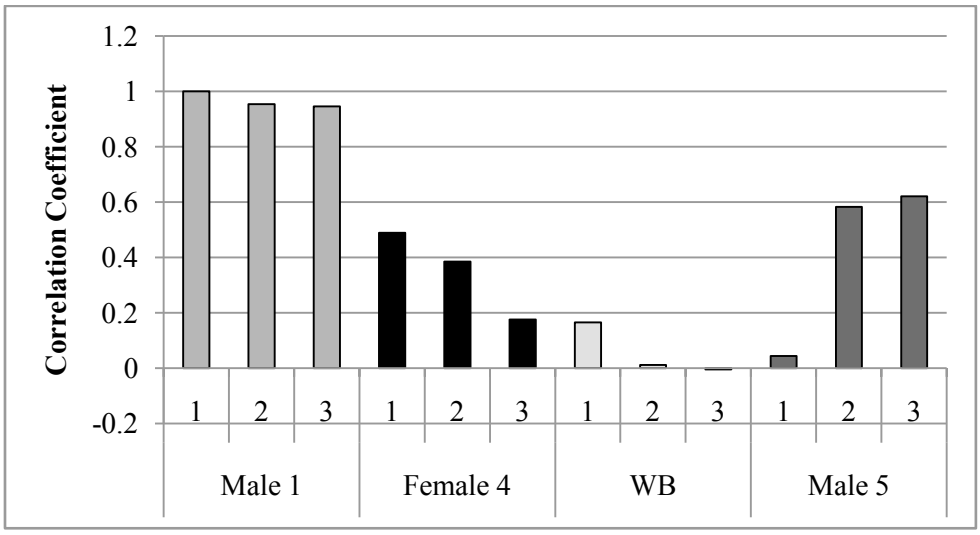
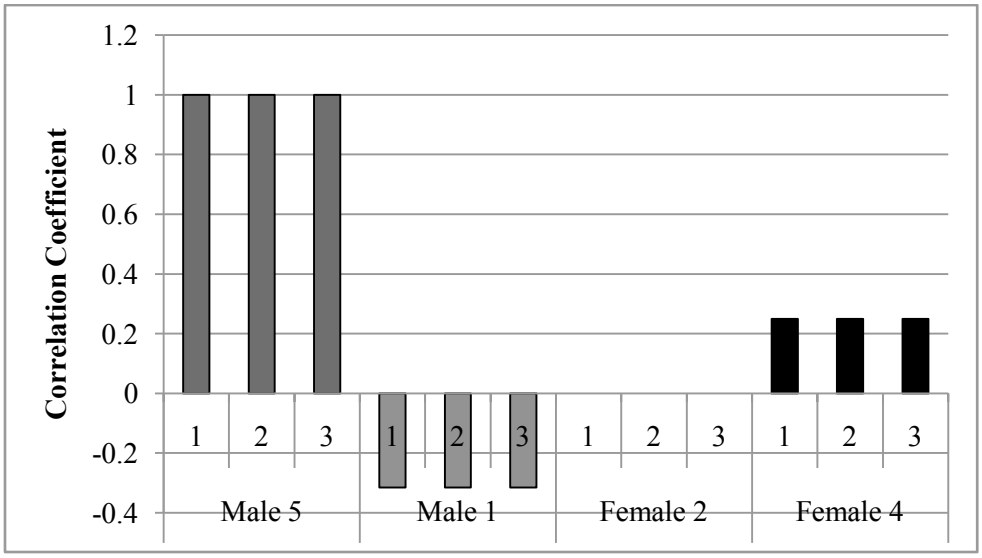


Figure 52. Spearman rank correlation with respect to Male 5, sample 1 for urine



2.7. Quantitation of VOCs Collected from Specimens

It is important to determine the level of the signal of the analyte where it could be distinguished from the background noise of the instrument. With human scent where the amounts of volatile organic compounds emitted from the specimens are very low. The limit of detection (LOD) and limit of quantitation (LOQ) must be determined for each analyte using the instrument of analyses. In analytical chemistry, the limit of detection is defined as the concentration of the analyte which gives an instrument a signal three times greater than the background noise. The limit of quantitation is defined as the lower limit of the analyte concentration for precise quantitative measurements. LOD and LOQ can be determined by Equation 3 and Equation 4, respectively:

$$y_{lod} = a + 3s_{x/y} \qquad \textbf{Equation 3}$$

$$y_{loq} = a + 10s_{x/y} \qquad \textbf{Equation 4}$$

where y_{lod} is the y-coordinate of the limit of detection for the calibration curve, a is the y-intercept, and $3s_{x/y}$ is the standard error for the predicted y value for all x values in the regression.

Limits of detection and quantitation were investigated for selected compounds most commonly found in the five biological specimens. To determine the LODs and LOQs of the analytes found in each specimen, a calibration graph was prepared for each analyte using 5, 10, 20, 40, 60, and 80 ng/uL concentrations of standard solutions prepared in dichloromethane and analyzed by GC/MS. The LODs and LOQs were calculated using the slopes from the calibration curves of each analyte in conjunction with a regression

equation as shown in Equation 3. The LODs and LOQs for all analytes tested are presented in Table 7 through Table 11. The average LOD values of the VOCs commonly found in hand odor, buccal swab, breath, blood, and urine were 3.65ng, 7.98ng, 6.88ng, 5.07ng, and 8.46ng, respectively. The average LOQ values were 12.15ng (hand odor), 29.60ng (buccal swab), 22.92ng (breath), 16.90ng (blood), and 28.20ng (urine). Sample calibration curves of selected compounds of various functional groups for each specimen can be found in the Appendices section.

Table 7. Limits of detection and quantitation of VOCs found in the headspace of hand odor

RT (min)	Compound Name	LOD (ng)	LOQ (ng)
6.25	Furfural	4.86	16.19
6.93	2-Furanmethanol	4.08	13.60
8.10	Heptanal	4.57	15.23
8.85	Propanedioicacid,dimethylester	3.78	12.59
9.44	Benzaldehyde	2.24	7.48
10.01	Phenol	1.58	5.27
10.06	5-Hepten-2-one,6-methyl-	2.44	8.13
10.25	Hexanoicacid	ND	ND
10.36	Octanal	6.88	22.92
10.97	BenzylAlcohol	2.32	7.74
11.56	Acetophenone	1.82	6.06
12.13	Undecane	3.83	12.78
12.16	1,6-Octadien-3-ol,3,7-dimethyl-	5.77	19.22
12.22	Nonanal	3.97	13.24
12.56	Octanoicacid,methylester	1.68	5.60
13.14	2-Nonenal,(E)-	2.99	9.95
13.31	Nonanol	3.05	10.17
13.65	2-Decanone	1.59	5.30
13.75	Dodecane	2.20	7.32
13.85	Decanal	1.85	6.16
15.21	Tridecane	2.15	7.18
15.33	Undecanal	2.25	7.51
16.57	Tetradecane	2.40	8.01
16.70	Dodecanal	2.22	7.41
17.04	5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	2.18	7.27
17.74	1-Pentadecene	2.61	8.69
17.83	Pentadecane	3.11	10.35
18.65	Dodecanoic acid	4.33	14.42
19.02	Hexadecane	3.11	10.36
20.16	Heptadecane	5.38	17.92
23.23	Eicosane	3.22	10.73
24.17	Heneicosane	4.11	13.71
25.06	Docosane	18.09	60.30
	Average	**3.65**	**12.15**

Table 8. Limits of detection and quantitation of VOCs found in the headspace of buccal swab

RT (min)	Compound Name	LOD (ng)	LOQ (ng)
5.24	Hexanal	10.20	34.00
6.25	Furfural	6.60	22.01
7.28	1-Hexanol	7.54	25.14
8.16	Pentanoicacid	12.42	41.41
9.44	Benzaldehyde	5.78	19.27
9.95	Phenol	9.13	30.44
10.12	Furan,2-pentyl-	9.23	30.77
10.13	1-Decene	ND	ND
10.40	Hexanoicacid	5.19	17.29
11.41	2-Octenal,(E)-	5.61	18.69
11.56	Acetophenone	9.96	33.21
11.64	1-Octanol	6.78	22.60
11.83	Heptanoicacid	4.88	16.27
12.12	Heptanoicacid,ethylester	8.47	28.23
13.14	2-Nonenal,(E)-	5.06	16.87
13.37	Menthol	6.51	21.69
13.48	OctanoicAcid	4.79	15.98
13.63	1-Dodecene	6.89	22.96
13.71	Octanoicacid,ethylester	16.98	56.60
13.85	Decanal	5.84	19.47
13.99	2,4-Nonadienal,(E,E)-	5.43	18.10
15.16	Nonanoicacid,ethylester	12.11	40.36
15.21	Tridecane	5.73	19.10
15.46	Naphthalene,1-methyl-	7.19	23.98
16.46	1-Tetradecene	7.55	25.17
16.51	Decanoicacid,ethylester	10.54	35.14
16.57	Tetradecane	6.49	21.62
16.70	Dodecanal	5.82	19.41
16.97	Caryophyllene	7.56	25.20
17.03	5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	9.92	33.05
17.74	1-Pentadecene	6.82	22.72
18.13	Dodecanoicacid,methylester	9.36	31.19
18.59	Dodecanoicacid	9.47	31.58
19.02	Hexadecane	7.05	23.51
19.48	Benzophenone	10.09	33.65
20.16	Heptadecane	5.87	19.56
23.23	Eicosane	6.99	23.29
24.17	Heneicosane	7.20	24.02
25.06	Docosane	14.16	47.19
	Average	**7.98**	**26.60**

Table 9. Limits of detection and quantitation of VOCs found in the headspace of breath

RT (min)	Compound Name	LOD (ng)	LOQ (ng)
4.16	Octane	6.93	23.09
4.28	Hexanal	ND	ND
5.28	Ethylbenzene	5.73	19.11
5.42	Xylenes	11.00	36.67
5.75	3-Heptanone	5.72	19.06
5.79	Styrene	6.14	20.46
6.52	1R-.alpha.-Pinene	6.18	20.60
6.89	Benzene,propyl-	5.27	17.55
7.03	Benzaldehyde	6.03	20.09
7.37	Phenol	4.38	14.61
7.47	5-Hepten-2-one,6-methyl-	4.89	16.29
8.16	Limonene	6.12	20.40
8.26	Benzene,1,2-dichloro-	5.26	17.54
8.80	Acetophenone	4.96	16.53
9.29	Undecane	6.14	20.46
9.37	Nonanal	4.47	14.90
9.58	Benzene,1,2,4,5-tetramethyl-	5.10	16.99
10.46	Menthol	6.54	21.79
10.65	Naphthalene	22.41	74.70
10.69	1-Dodecene	3.73	12.42
10.81	Methyl Salicylate	5.16	17.19
10.81	Dodecane	4.69	15.62
10.91	Decanal	5.30	17.67
12.23	Tridecane	5.65	18.83
12.35	Undecanal	4.90	16.34
12.49	Naphthalene,1-methyl-	5.79	19.29
13.12	n-Decanoicacid	9.04	30.13
13.57	Tetradecane	6.00	20.00
13.71	Dodecanal	5.11	17.02
13.98	Caryophyllene	6.22	20.74
14.04	5,9-Undecadien-2-one,6,10-dimethyl-	5.54	18.45
14.28	5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	11.39	37.98
15.07	ButylatedHydroxytoluene	5.63	18.76
15.57	Dodecanoicacid	11.07	36.89
16.02	Hexadecane	6.59	21.95
16.48	Benzophenone	6.16	20.55
17.16	Heptadecane	6.71	22.37
17.57	2,6-Diisopropylnaphthalene	6.17	20.57
19.70	n-Hexadecanoicacid	17.13	57.11
20.02	Eicosane	6.98	23.26
	Average	**6.88**	**22.92**

Table 10. Limits of detection and quantitation of VOCs found in the headspace of blood

RT (min)	Compound Name	LOD (ng)	LOQ (ng)
4.80	1-Hexanol	2.73	9.11
4.88	4-Heptanone	3.69	12.29
5.08	3-Heptanone	2.81	9.38
5.14	2-Heptanone	4.19	13.95
5.20	Cyclohexanone	4.34	14.47
5.23	Nonane	5.88	19.61
5.30	Heptanal	3.21	10.68
6.23	Benzaldehyde	3.74	12.45
6.33	1-Heptanol	3.31	11.02
6.49	1-Octen-3-ol	18.66	62.18
6.53	Phenol	17.14	57.14
6.62	5-Hepten-2-one,6-methyl-	5.04	16.81
6.67	1-Decene	10.84	36.14
6.68	Furan,2-pentyl-	16.89	56.31
7.28	Limonene	2.81	9.38
7.38	Benzene,1,2-dichloro-	1.58	5.28
7.39	Benzyl Alcohol	4.63	15.42
7.89	Acetophenone	4.66	15.52
7.92	1-Octanol	3.34	11.15
8.35	Undecane	2.68	8.95
8.43	Nonanal	2.57	8.58
8.64	Benzene,1,2,4,5-tetramethyl-	4.39	14.64
9.49	Menthol	4.68	15.61
9.69	Naphthalene	10.50	35.01
9.71	1-Dodecene	5.71	19.03
9.74	2-Decanone	5.39	17.97
9.84	Dodecane	2.76	9.21
9.94	Decanal	3.22	10.73
11.25	Tridecane	2.47	8.22
11.51	Naphthalene,1-methyl-	3.71	12.37
12.48	1-Tetradecene	2.36	7.85
12.58	Tetradecane	2.41	8.04
12.71	Dodecanal	2.49	8.31
13.05	5,9-Undecadien-2-one,6,10-dimethyl-,(E)-	5.01	16.69
13.52	1-Dodecanol	4.78	15.92
15.02	Hexadecane	2.45	8.16
16.16	Heptadecane	1.63	5.42
16.57	2,6-Diisopropylnaphthalene	3.92	13.08
	Average	**5.07**	**16.90**

Table 11. Limits of detection and quantitation of VOCs found in the headspace of urine

RT (min)	Compound Name	LOD (ng)	LOQ (ng)
	Toluene	ND	ND
	2-Pentanone	ND	ND
	Pyrrole	ND	ND
	4-Nonylphenol	ND	ND
7.08	4-Heptanone	7.91	26.36
7.38	3-Heptanone	9.52	31.73
7.46	2-Heptanone	7.54	25.15
8.69	Benzaldehyde	10.07	33.56
8.80	Dimethyltrisulfide	7.15	23.83
9.15	1-Decene	7.59	25.30
9.77	Benzene,1,2-dichloro-	8.23	27.42
10.24	1-Octanol	7.89	26.28
10.30	Phenol,4-methyl-	9.39	31.30
10.60	1,6-Octadien-3-ol,3,7-dimethyl-	7.98	26.59
10.65	Nonanal	5.21	17.36
11.29	2-Nonenal,(E)-	8.25	27.51
11.46	Menthol	7.41	24.71
11.71	MethylSalicylate	8.65	28.85
11.77	Decanal	6.50	21.66
12.67	1,3-Benzodioxole,5-(2-propenyl)-	8.06	26.85
14.10	5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	7.44	24.79
14.64	ButylatedHydroxytoluene	7.00	23.34
14.95	Dodecanoicacid	16.52	55.08
15.26	Hexadecane	8.09	26.98
15.38	Phenol,4-(1,1,3,3-tetramethylbutyl)-	7.92	26.40
15.55	Diphenylamine	9.78	32.58
15.63	Benzophenone	10.34	34.47
16.02	Heptadecane	8.59	28.62
	Average	**8.46**	**28.20**

2.8. Summary of Biological Specimen Optimization

Thus far, SPME-GC/MS conditions of VOCs above the headspace of collected buccal swab, breath, blood, and urine specimens have been optimized. Data obtained using these optimized conditions yielded promising results as VOC profiles of different subjects are distinct from one another and reproducible within themselves. The specimen sampling methods described through the present chapter were utilized for the simultaneous collection of hand odor, buccal swab, breath, blood, and urine for the subsequent population studies on healthy, diabetic, and depressed individuals. The optimized SPME-GC/MS conditions were used for the extraction, separation, and analysis of the VOCs extracted above the headspace of the collected specimen samples.

3. POPULATION STUDIES

3.1. Scent Biomarkers for Diseases

"By the sense of smell we can recognize the peculiar perspiration of many diseases, which has an important bearing on their identification.[86]" – Susruta Samhita

The above quote was taken from an ancient ayurvedic medicine transcript over 2000 years ago. Thus it is clear that people have long used the smell of individuals to diagnose diseases, and the above statement holds true today. The difference is that today, instead of physicians simply using the sense of smell or taste, there are analytical methods and instruments available to detect and identify such potential biomarkers. The VOC analysis in biological specimens is of research interest because of its potential to identify diagnostic biomarkers for various diseases. The appearance of specific biomarkers can indicate physiological malfunction. Odor can be a diagnostic marker that can lead to early diagnosis of diseases. Identification of target VOCs for a particular disease would be a useful screening tool. Over the last couple decades, research describing the detection and identification of VOCs that are specific to certain diseases has been growing in published literature (Table 12).

Since the initial demonstration of a peculiar odor emanating from schizophrenic patients, there have been several studies attempting to identify the odoriferous substance which could perhaps provide an approach to an etiologic diagnosis of schizophrenia[87,88]. It has been reported that the mean alveolar gradients of carbon disulfide and pentane were significantly higher in schizophrenic patients compared to patients with other psychiatric disorders and subjects with no psychiatric disorder.

There are numerous studies on the investigation of volatile biomarkers for cancer. Deng et al. have developed a procedure for headspace SPME-GC/MS with on-fiber derivatization to determine the levels of hexanal and heptanal in the blood of cancer patients, as aldehydes with low molecular weight have been proposed to be cancer biomarkers[66]. It was found that high levels of hexanal and heptanal were found only in blood taken from patients with lung cancer. Their results corresponded with other studies that also found that aldehyde compounds were only present in breath and urine of cancer patients[67]. These findings suggest that early screening of lung cancer may be carried out by analysis of these aldehydes in biological fluids. Other studies on volatile biomarkers in breath samples of lung cancer patients have also identified potential VOCs that could discriminate between individuals with and without lung cancer, although there is no single compound that marks the disease[54,57,89,90].

There are many published studies on the profiling potential of VOCs in biological specimens in attempt to associate disturbances of volatile compounds with many other metabolic illnesses (Table 12). While many of the reported VOCs are also present in healthy individuals, studies have reported an elevation in the levels of these compounds in patients with asthma[91-93], diabetes[56,94], liver and kidney disease/impairment[70,71,83], gastrointestinal diseases[95], and oxidative stress[56,96].

Table 12. Published studies on VOCs in biological specimens identified as potential diagnostic markers of various diseases

Diseases	VOCs Detected	Specimen	References
Lung or breast cancer	Alkanes, monomethylated alkanes, benzene and benzene derivatives	Breath, blood	[54,57,66-68,89,90,97]
Oxidative stress	Ethane, pentane, other alkanes and methylated alkanes	Breath	[56,87,96]
Asthma	NO, pentane, ethane, 8-isoprostane	Breath	[91-93]
Diabetes mellitus	Acetone, ethanol, methyl nitrate, 4-heptanone	Breath, urine	[55,94]
Schizophrenia	Carbon disulfide, pentane, ethane	Breath	[87,88]
Cystic fibrosis	Carbonyl sulfide, alkanes	Breath	[98]
Kidney impairment	Nitrogen-containing compounds (ammonia, dimethylamine, trimethylamine)	Urine	[71]
Liver impairment/failure	Benzaldehyde and phenolics (phenylketonuria), dimethyl disulfide and sulfur-containing compounds (hepatitis),	Urine	[70,71,83]
Gastrointestinal disease	Sulfur-containing compounds, p-menth-1-en-8-ol	Feces	[95]

3.1.1. Medicine and Human Scent

3.1.1.1. Disease-Specific Scent

Certain medical conditions are known to cause odor symptoms. Patients of acidosis have acetone smelling breath[86]. Maple syrup urine disease is an inherited disease of amino acid metabolism, which causes urine to have a characteristic sweet smell like maple syrup[99]. This disease is an autosomal recessively inherited disorder in which branched-chain amino acids and their α-keto and α-hydroxy acids accumulate in the body fluids, leading to mental retardation and neurological damage. The odor of this disease is a result of the presence of a ketone called sotolone, which is a component of maple syrup. Sotolone is only present in the urine of patients with maple syrup urine disease, and not in urine of healthy individuals. Trimethylaminuria, or fish odor syndrome (or fish malodor syndrome), is a genetically inherited enzyme deficiency where the patient fails to metabolize trimethylaminuria, causing the body to emit a persistent fish odor[100]. Unusual amounts of unoxidized trimethylamine are excreted in the sweat, urine, breath, and other bodily secretions. Patients with diabetes mellitus may have a breath odor resembling the smell of fruity acetone. The smell of fresh baked brown bread is associated with typhoid fever[86]. Other diseases known to exhibit characteristic (mostly unpleasant, putrid) odors include foetor hepaticus of the liver, smallpox, scurvy, scrofula (tuberculosis of the neck), yellow fever, gout, and diseases of the respiratory tract such as bronchiectasis, lubabscessea and ozaena[86]. Examples of metabolic disorders characterized by unusual odors are shown in Table 13.

Table 13. Metabolic disorders with characteristic odors

Disease	Characteristic Odor
Phenylketonuria	Musty or mousey odor
Maple syrup urine disease	Maple syrup
Isovaleric academia	Sweaty feet
Trimethylaminuria	Fish odor
Diabetes Mellitus	Sweet fruity acetone
Acidosis	Acetone smelling breath

3.1.1.2. Canines and Cancer

In 1989, Williams and Pembroke described a case of malignant melanoma detected by the patient's pet dog[2]. The dog constantly sniffed and even tried to bite a specific mole on her leg. Upon seeking medical advice, the patient was diagnosed with basal cell carcinoma. More instances of this kind have been reported in the recent years. In 2001, a case was reported by Church where a pet Labrador constantly pushing and sniffing at his owner's lesion on his left thigh led to the owner to have the lesion examined[101]. The lesion was found to be a basal cell carcinoma. After the lesion was surgically removed, the dog showed no more interest in the area. In 2005, Welsh et al. reported yet another similar case where a patient's dog sniffed and poked at her left axilla which was later found to be infiltrating ductal carcinoma: breast cancer[1]. Following these reports, researchers at Cambridge Universality Veterinary School in England in collaboration with the Cambridge Institute of Dog Behavior & Training are testing the viability of dogs to be trained to detect cancer – what is now referred to as "dognoseis".

3.1.1.3. Rats and Schizophrenia

Patients with schizophrenia have been reported to emit a characteristic unpleasant odor that is unrelated to hygiene. In 1959, Smith and Sines' study of the peculiar odor in the sweat of schizophrenic patients demonstrated the differences to that of non-schizophrenic individuals' sweat through the use of trained rats[102]. A human panel testing the odor was also able to discriminate between the sweat of the patients and controls.

3.2. Diabetes Mellitus

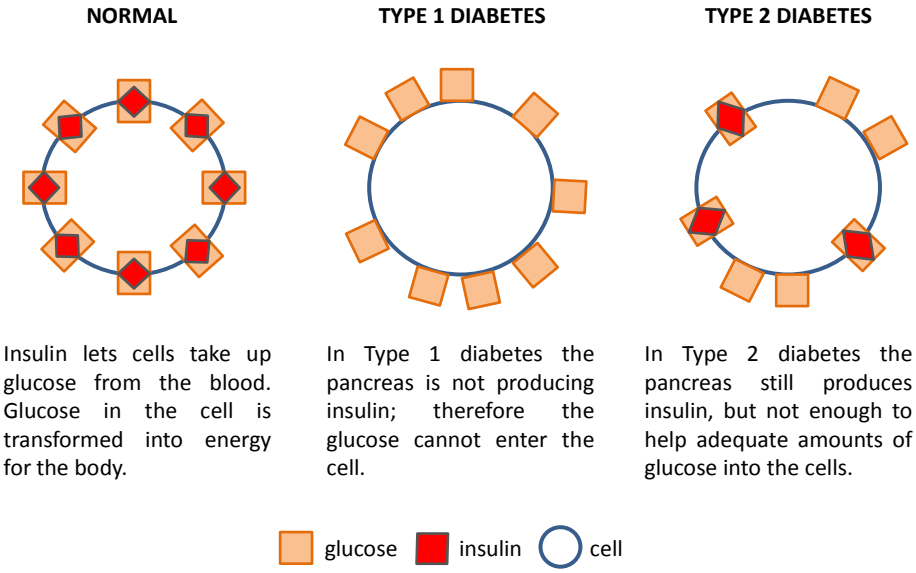
Diabetes mellitus (commonly just called diabetes) is a metabolic disorder characterized by an insulin deficiency or to a hyporesponsiveness to insulin[46,103]. These conditions result in high blood glucose levels to the point where glucose spills over into the urine – hence the name *diabetes* ("syphon" or "running through") and *mellitus* ("sweet") in Greek. Despite the high blood glucose levels, the body cells do not absorb the glucose and therefore "starve" while the glucose accumulates in the blood, a condition known as hyperglycemia. High blood glucose results in the acceleration of other metabolic pathways like triacylglycerol hydrolysis, gluconeogenesis, fatty acid oxidation, and ketone body formation. When plasma ketone body level becomes abnormally high, blood pH-buffering capacity and renal functions are impaired. Ketosis leads to increased plasma H^+ concentration, where excess H^+ together with other important ions (Na^+, K^+) is excreted into the urine. Severe dehydration may occur from loss of these ions and water excretion. In addition, H^+ accumulation causes brain dysfunction. Complications of diabetes include heart disease, high blood pressure, blindness, kidney disease, nervous system disease, circulatory problems, dental disease, and complication of pregnancy[104].

Uncontrolled diabetes often results in acute life-threatening emergencies such as diabetic ketoacidosis, hypotention, coma, and death[105]. Diabetes is the third leading cause of death in the U.S., following heart disease and cancer. The 2007 National Diabetes Fact Sheet estimated 23.6 million children and adults in the U.S. (7.8% of the population) having diabetes, of which 17.9 million are diagnosed diabetes patients and the remaining 5.7 million are undiagnosed[106].

3.2.1. Type 1 Diabetes

Type 1 diabetes or insulin-dependent diabetes mellitus (IDDM) is caused by a deficiency of pancreatic β cells resulting in insulin deficiency[46]. Type 1 diabetes was previously also known as juvenile-onset diabetes mellitus, for its onset is most often during childhood. Pancreatic β cells are selectively destroyed as an autoimmune response, and individuals with type 1 diabetes require insulin therapy with daily injections and a specific diet. The cause of this autoimmune destruction is not yet fully understood. Type 1 diabetes is the less common type of diabetes, affecting about 5-10 percent of the diagnosed diabetic patients[107]. Diabetic ketoacidosis occurs predominantly in type 1 diabetes patients, because it results from a total inability of insulin secretion. The body responds to this insulin shortage by converting to burning fatty acids and producing acidic ketone bodies. As noted before, management of type 1 diabetes involves daily insulin replacement therapy. Pancreas transplantation and a lesser invasive islet cell transplantation are treatments of extreme cases, although these still do not fully cure the disease. The lifespan of type 1 diabetic are generally reduced by up to one-third as a result of degenerative complications mentioned before.

Figure 53. Diagram of cell glucose regulation for normal, type 1 and type 2 diabetes



3.2.2. Type 2 Diabetes

Type 2 diabetes or non-insulin-dependent diabetes mellitus accounts for over 90% of the diagnosed diabetes cases[106]. Unlike type 1 diabetes, type 2 diabetics have normal or even elevated insulin levels. The issue with type 2 diabetes lies in that there is a lack of insulin receptors on normally insulin-responsive cells (Figure 53). The cells do not respond appropriately to insulin, hence these cells are said to be insulin-resistant. Consequently, the blood glucose level becomes elevated, particularly after a meal. Table 14 shows the criteria for diagnosing diabetes.

Type 2 diabetes is also commonly known as adult-onset diabetes and usually occurs in obese individuals with a predisposition for this condition. High-cholesterol, hypertension, high-fat diet, and low exercise lifestyle are all factors that increase the risk of type 2 diabetes. In addition to insulin resistance, type 2 diabetes is characterized by impaired regulation of hepatic glucose production and declining β-cell function. In extreme cases,

the conditions could lead up to β-cell failure where the patient will require insulin therapy for survival. Major complications from improperly managed type 2 diabetes include retinopathy leading to blindness, nephropathy, neuropathy, and cardiovascular diseases such as coronary artery disease[46]. Treatment (and prevention for onset) for type 2 diabetes mainly involves diet therapy for weight reduction, and exercise. There are also oral therapeutic agents that are used in the management of type 2 diabetes (Table 15)[105,108]. Since type 2 diabetes accounts for the majority of diabetic patients in the U.S., the type 2 will be the diabetic population of interest in this study.

Table 14. Diabetes diagnosis criteria

Stage	Test		
	Fasting Plasma Glucose	Random Plasma Glucose	Oral Glucose Tolerance Test (75-g)
Normal	< 100 mg/dL		2hPG[a] < 140 mg/dL
Diabetes	≥ 126 mg/dL	≥ 200 mg/dL plus typical diabetes symptoms	2hPG ≥ 200 mg/dL

[a] 2-hour plasma glucose

Table 15. Current oral therapeutic agents for type 2 diabetes

Drug Class	Mechanism of Action	Indication(s)
Sulphonylureas and repaglinide	Increase insulin secretion	Deficient pancreatic secretion of insulin resulting in hyperglycemia
Biguanides (Metformin)	Decrease peripheral insulin resistance Decrease hepatic gluconeogenesis	Obesity Insulin resistance
Thiazolidenediones	Decrease peripheral insulin resistance Reduce fatty acids	Insulin resistance
α-glucosidase inhibitors	Slow absorption of carbohydrates	Hyperglycemia after meal

3.3. Mood Disorders

Mood disorders are mental disorders in which the predominant feature is having a disturbance in mood. The National Institute of Mental Health estimates 26.2 percent of Americans ages 18 and older suffer from some form of diagnosable mental disorder in a given year[109]. Of these mental disorders, the prevalence of mood disorders (major depressive disorder (MDD), dysthymic disorder, and bipolar disorder) is approximately 9.5 percent of the U.S. population age 18 and older, or about 20.9 million American adults. In particular, in a given year, approximately 14.8 million American adults or about 6.7 percent of the U.S. population (age 18 or older) suffer from MDD. Worldwide, MDD affects about 121 million people and is among the leading causes of disability[110].

3.3.1. Major Depressive Disorder

Depression is common. It is an episodic medical disorder in which patients are overwhelmed by feeling sad, hopeless, helpless, and worthless[111-113]. Depression commonly affects sleep, appetite, and general interest in life. The latest edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) defines MDD to be characterized by one or more Major Depressive Episodes where the depressed mood must be present for most of the day, almost every day, for at least a 2-week period[111]. Major Depressive Disorder can be a single episode where there is only a single presence of a Major Depressive Episode, or recurrent if two or more Major Depressive Episodes are present. To be classified as having MDD, there could not have been a Manic Episode, a Mixed Episode, or a Hypomanic Episode, as the diagnosis would be changed to Bipolar Disorder if this were the case. It is also different from a depressive mood disorder as a

result of a medical condition, side effect of medications or drugs of abuse. Lastly, Dysthymic Disorder is very similar to MDD in terms of symptoms and, therefore, is often difficult to distinguish from MDD. The difference between the two mood disorders lies in severity, chronicity, and persistence. Usually Dysthymic Disorder consists of chronic (depressed mood present for most days over at least two years), less severe depressive symptoms compared to MDD.

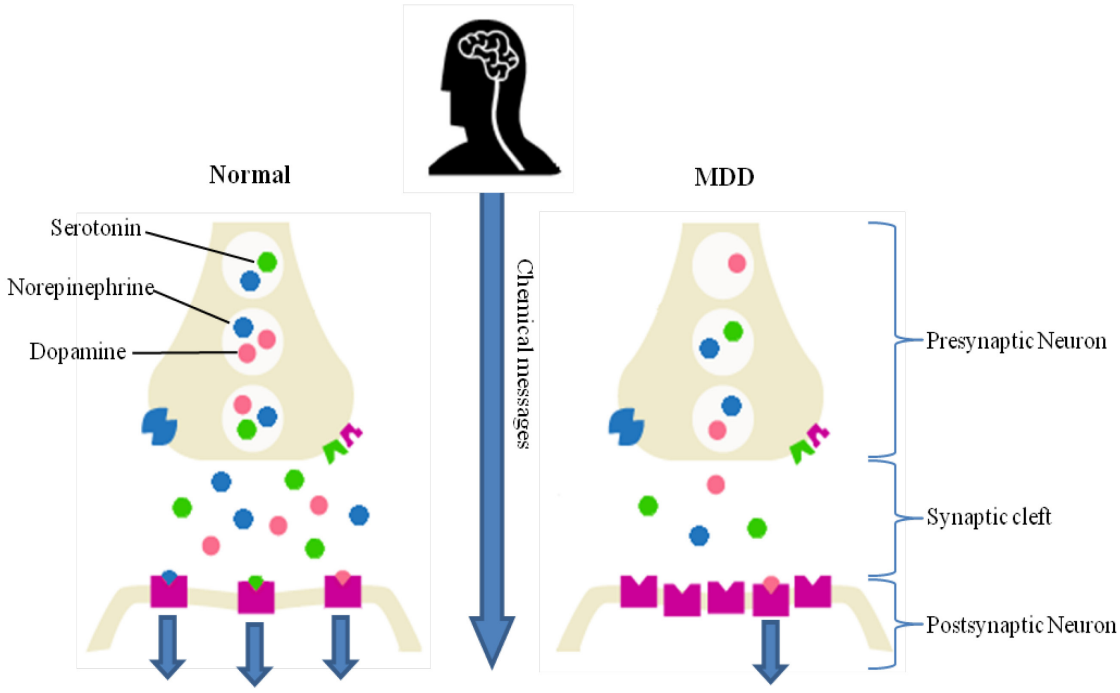
Other mental disorders not under the Mood Disorders category may have depressive symptoms also; however, they are different from MDD. Such disorders include Schizophrenia, Delusional Disorder, and Psychotic Disorder Not Otherwise Specified. Schizoaffective Disorder is also similar to MDD with Psychotic Features, but the difference is that to be diagnosed with Schizoaffective Disorder, "there must be at least 2 weeks of delusions or hallucinations occurring in the absence of prominent mood symptoms.[111]"

The average onset age of MDD is in the early 30s and the peak age of risk is between 25 and 44, although it can develop at any age, even prepubescent[113]. Major Depressive Disorder is approximately twice as common in women as men, with prevalence of lifetime risk for MDD varying around 10-25% for women and 5-12% for men (adolescent and adult)[111] . There is a high suicide risk and high mortality rate associated with MDD, as completed suicide occurs in up to 15% of individuals with severe MDD[114]. Death rates increase even more for elder individuals suffering MDD[115].

While depression is so common and treatment of the disorder has been very successful, the exact cause of depression is yet to be understood. Many hypotheses have been

presented to suggest the cause of depression to be the result of genetic, biological, psychological, and environmental factors; however, depression most likely results from a combination of these factors and not from one single cause[112,113]. Family, twin, and adoption studies support the idea that there are genetic influences that affect development of depression. Prevalence of depression is greatly increased among blood relatives of individuals with diagnosed MDD when compared to the general population[112]. Biochemically, unusual levels of monoamine neurotransmitters, especially norepinephrine, serotonin, and dopamine, have been accounted as causal factors of depression. In the brain, chemical messages and information are communicated between neurons. Information from one neuron is transmitted to another neuron at the synaptic cleft by neurotransmitters that are released by the presynaptic neuron. Altered neurotransmitter activities such as depletion or complete absence of one or all of these monoamine transmitters, or increases in them are thought to result in depression (Figure 54).

Figure 54. Difference in the chemical message transduction in the brain cells of normal individual and individual with MDD (adapted from Pfizer Japan Inc.)



Treatment of MDD usually involves psychotherapy and/or pharmacotherapy (antidepressant medications). Psychological treatments include cognitive-behavioral therapy, interpersonal psychotherapy, and behavioral activation which all focus on the relationship between thoughts, perceptions, and behaviors and depressed mood. Other forms of treatment include electroconvulsive therapy (ECT) and light therapy. Antidepressant drugs have proven to be very effective in the treatment of depression. There are over 20 antidepressant drugs available today, and most of them work by normalizing the chemical imbalance in the brain. Major classes of antidepressant drugs are:

- Tricyclic antidepressants (TCAs)
- Monoamine oxidase inhibitors (MAOIs)
- Selective serotonin reuptake inhibitors (SSRIs)
- Serotonin-norepinephrine reuptake inhibitors (SNRIs)
- Others
    - Lithium
    - Dopamine-norepinephrine reuptake inhibitors
    - Serotonin modulators
    - Norepinephrine-serotonin modulator

Tricyclic antidepressants and MAOIs are first generation antidepressants. Tricyclic antidepressants work by slowing down the reabsorption of norepinephrine and serotonin by brain cells. Monoamine oxidase inhibitors are one of the oldest classes of antidepressant drugs and are recommended to be restricted to patients who have not responded well to other antidepressant medications due to their potential adverse side effects and requirement for strict dietary restrictions[116]. Monoamine oxidase inhibitors work by inhibiting the enzyme monoamine oxidase from breaking down the monoamine neurotransmitters, thereby increasing the level of active neurotransmitters in the brain. Selective serotonin reuptake inhibitors, as the name suggests, selectively inhibits the reabsorption of serotonin by the nerve cells, thereby providing higher levels of serotonin at the brain receptor site.

3.4. Comorbidity between Diabetes and Depression

Since 1684, it has been recognized that there is an association between diabetes and depression[117]. Since then, the persistence and prevalence of MDD in diabetic adults have been studied through a number of epidemiological surveys, longitudinal, follow-up, and prospective studies[118-122]. The exact factors that link the two illnesses still remain uncertain; however, there are hypotheses on the interrelationship between MDD and diabetes. One hypothesis suggests that MDD in diabetics results from biochemical factors related to the illness and the treatments. Another hypothesis suggests that the psychosocial or psychological burden from having the chronic illness (diabetes) accountable for MDD in diabetes. Intensive review of the current literature published on the covariance of MDD and diabetes found that the latter hypothesis is not supported[122]. Studies have reported that MDD doubles the risk for onset of type 2 diabetes in otherwise healthy individuals and over the general population[117,122].

3.5. Methods

For all subjects sampled in the population studies, hand odor, buccal swabs, breath, blood, and urine were collected using the specimen sampling methods as described in the previous chapter. All collected specimens except for urine were stored at room temperature under normal laboratory conditions until ready for SPME analysis. Urine samples were stored at 4°C until ready for SPME analysis. The volatile organic compounds from the headspace of collected specimen samples were extracted using 50/30 μm DVB/CAR/PDMS SPME fibers. Single headspace extractions were performed

for each sample. VOC extraction conditions using SPME are described in Table 6 under section 2.6.1.

3.5.1. Evaluation of Odor Profiles of Individuals over Time

Unrelated subjects Male 5 and Female 4 were sampled over six months. No attempt was made to control the diet of the subjects during the course of sampling. Samples were collected on a monthly interval (month 0 to month 6). The average temperature and the average humidity of the sampling environment during the samplings in the laboratory were 22.5°C and 75%, respectively.

3.5.2. Evaluation of the Effect of Fasting Prior to Sampling

Four subjects were evaluated: two females (F4 and F15) and two males (M2 and M5) ranging in age from 24 to 29. Each subject was sampled twice during the course of a day. The first sample was collected at least two hours after the subject had consumed any food or beverage (except water) and the second sample was collected immediately after consuming a meal. No attempt was made to control the meal consumed by the subjects. The average temperature and the average humidity of the sampling environment during the samplings in the laboratory were 23.6°C and 56%, respectively.

3.5.3. Population Analysis of the Volatile Organic Compounds Present Above Collected Odor Samples

3.5.3.1. Healthy Individuals

All sampling protocols were approved by the Florida International University Institutional Review Board (IRB) for research involving human subjects. Subjects were asked to read and sign an IRB-approved informed consent form prior to starting the

sampling protocol. Thirty-one healthy subjects (15 males, 16 females), ages 19 to 36, were sampled. Subjects were also asked to answer a confidential medical questionnaire form prior to sampling to ensure that they were not diagnosed with any major physiological or psychological illnesses (particularly diabetes and depression) and to be aware of any over-the-counter or prescription medication they may be taking at the time of sampling that could account for any unusual metabolites in the extracted VOCs. No attempt was made to control the diet of the subjects being sampled.

3.5.3.2. Patients with Type 2 Diabetes

All sampling protocols were approved by the Florida International University Institutional Review Board (IRB) for research involving human subjects as well by the Jackson Health System Clinical Trials Office. Subjects were asked to read and sign an IRB-approved informed consent form prior to starting the sampling protocol. Nineteen diabetic subjects (11 males, 8 females), ages 25 to 60 were sampled. Nine of these subjects were inpatients at Jackson North Medical Center (North Miami Beach, FL) and were sampled at the hospital. Subjects were also asked to answer a confidential medical questionnaire form. No attempt was made to control the diet of the subjects being sampled. Blood glucose level was tested and noted at the time of sampling for all subjects.

3.5.3.3. Patients with Major Depressive Disorder

All sampling protocols were approved by the Florida International University Institutional Review Board for research involving human subjects as well by the Jackson Health System Clinical Trials Office. Subjects were asked to read and sign an IRB-

approved informed consent form prior to starting the sampling protocol. Twenty healthy subjects (10 males, 10 females), ages 18 to 49 were sampled. Nine of these subjects were inpatients at Jackson North Medical Center (North Miami Beach, FL) and were sampled at the hospital. Subjects were also asked to answer a confidential medical questionnaire form. No attempt was made to control the diet of the subjects being sampled.

3.6. Results

3.6.1. Evaluation of Odor Profiles of Individuals over Time

The VOC profiles of subjects Female 4 and Male 5 were followed over a six-month period (month 0 to month 6). Fifty-five (55) compounds were extracted across the five biological specimens over six monthly samplings. Compounds that did not appear at least five times over the monthly samplings were disregarded. Table 16 and Table 17 show the common compounds that were extracted over the six-month period for Female 4 and Male 5, respectively. The VOCs from the five biological specimens included a variety of compounds including aldehydes, alcohols, alkanes, acids, esters, ketones, and aromatics. Specimen-specific compounds were detected, such as 4-heptanone and 4-nonylphenol for urine, which did not appear in the other four specimens. No single compound was found to be present across all five specimens in all six months. The variability of compounds present between subjects Female 4 and Male 5 for each specimen can be seen in Table 18 through Table 22, which lists the common compounds that were extracted among the monthly samplings of these subjects. Ten human compounds were extracted from hand odor samples, of which seven were common to both subjects Female 4 and Male 5. These common compounds were: nonanal, decanal, undecanal, tetradecane, hexadecane,

heptadecane, and octadecane. Twenty-five compounds were extracted from buccal swab samples, of which fourteen were common to both subjects Female 4 and Male 5. These common compounds were: 2-pentylfuran, hexanoicacid, (E)-2-nonenal, (E,E)-2,4-nonadienal, decanal, nonanoic acid, nonanoic acid ethyl ester, 6-dodecanone, 1-tetradecene, (E)-6,10-dimethyl-5,9-undecadien-2-one, dodecanoic acid ethyl ester, 2,2'-diethyl-1,1'-biphenyl, tetradecanoic acid ethyl ester, and hexadecanoic acid ethyl ester. Ten compounds were extracted from breath samples, of which eight were common to both subjects: styrene, phenol, 1,2-dichlorobenzene, 2-ethyl-1-hexanol, nonanal, decanal, butylated hydroxytoluene, and benzophenone. Sixteen compounds were extracted from blood samples, of which ten were common to both subjects. The ten common compounds were: hexanal, 2-pentylfuran, 1,2-dichlorobenzene, undecane, nonanal, decanal, tridecane, 2,4,6-trimethylbenzaldehyde, hexadecane, and 2,6-diisopropylnaphthalene. Finally, nine compounds were extracted from urine samples, of which two were common to both subjects: 4-heptanone and 4-nonylphenol. Figure 55 through Figure 59 show the variability of the relative peak area ratio patterns for the common compounds present in the monthly samplings of subjects Female 4 and Male 5.

Curran determined that multiple sampling of one individual's odor profile over time does not contain as much variation as that seen amongst members of a population using hand odor[84]. Similar results have been obtained with different biological specimens, showing that peak area arrays for the common human compounds extracted among six monthly samples for an individual have greater correlation than when compared between subjects. Table 23 through Table 27 show the correlation coefficients calculated between six

monthly samplings for subjects Female 4 and Male 5. Correlation coefficients are much higher when comparing samples of the same subject over the six-month period, as compared to the low coefficients when comparing samples between the subjects.

Table 16. VOCs extracted over 6 months for Female 4

Compound Name	HD							CH							BR							BL							UR						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6
1,1'-Biphenyl,2,2'-diethyl-								x	x		x	x	x																						
1-Hexanol,2-ethyl-															x	x	x	x	x																
1-Pentanol																						x	x	x	x		x								
1-Tetradecene								x	x		x	x	x																						
2,4-Nonadienal,(E,E)-								x	x	x	x	x	x																						
2,6-Diisopropylnaphthalene																						x	x	x	x	x	x	x							
2-Nonenal,(E)-								x	x	x	x	x	x																						
4-Heptanone																													x	x	x	x	x	x	x
4-Nonylphenol																														x	x	x	x	x	x
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-								x	x	x	x	x	x	x		x	x	x	x	x															
6-Dodecanone								x	x	x		x	x	x																					
Benzaldehyde,2,4,6-trimethyl-																						x	x	x	x	x	x	x							
Benzene,1,2-dichloro-															x	x	x	x	x	x	x	x	x	x		x	x								
Benzene,1,4-dichloro-																													x	x	x	x	x	x	
Benzophenone															x	x	x	x	x	x															
BenzylAlcohol	x	x	x	x	x	x	x															x	x	x	x		x								
ButylatedHydroxytoluene															x	x	x	x	x		x														
Decanal	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x							
Decanoicacid,ethylester								x	x		x	x	x	x																					
Dodecanoicacid,ethylester								x	x	x	x	x	x	x																					
Furan,2-pentyl-								x	x	x		x	x	x								x	x	x	x		x								
Heptadecane	x	x	x		x	x	x																												
Heptanoicacid,ethylester									x	x	x	x	x	x																					
Hexadecane	x	x	x	x	x	x	x															x	x	x	x	x	x	x							
Hexadecanoicacid,ethylester								x	x	x	x	x	x	x																					
Hexanal								x		x	x	x	x									x	x	x	x		x	x							
Hexanoicacid								x	x	x	x	x	x	x																					
Hexanoicacid,ethylester								x	x	x	x	x	x	x																					
Nonanal	x	x	x	x	x	x	x								x	x	x	x	x	x	x	x	x	x	x	x	x	x							
Nonanoicacid								x	x	x		x	x	x																					
Nonanoicacid,ethylester								x	x	x	x	x	x	x																					
Octadecane		x	x	x	x	x	x																												
Octanoicacid,ethylester								x	x	x	x	x	x	x																					
Phenol															x	x	x	x	x	x	x														
Styrene															x	x	x	x	x		x														
Tetradecane	x	x	x	x	x	x	x															x	x	x	x	x	x	x							
Tetradecanoicacid,ethylester								x	x	x	x	x	x	x																					
Tridecane																						x		x	x	x	x								
Undecanal	x	x			x	x	x																												
Undecane																						x		x	x	x	x								
Xylenes															x	x	x	x	x		x														

Table 17. VOCs extracted over 6 months for Male 5

Compound Name	HD							CH							BR							BL							UR						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6
1,1'-Biphenyl,2,2'-diethyl-									x	x	x	x	x	x																					
1-Dodecene								x	x	x		x	x	x																					
1-Hexanol,2-ethyl-															x	x		x	x	x	x														
1-Tetradecene								x	x	x	x	x	x	x																					
2,4-Nonadienal,(E,E)-								x	x	x	x	x	x	x																					
2,6-Diisopropylnaphthalene																						x	x		x	x	x								
2-Heptanone																						x	x	x		x	x								
2-Nonenal,(E)-								x	x	x		x	x	x																					
2-Undecanone																						x			x	x	x								
4-Heptanone																													x	x	x	x	x	x	x
4-Nonylphenol																													x	x	x	x	x	x	x
4-Terpineol																														x	x	x		x	x
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	x		x	x	x	x	x	x	x	x	x	x	x	x																					
6-Dodecanone								x			x	x	x	x																					
6-Methyl-3,5-heptadiene-2-one								x	x	x		x	x																						
Benzaldehyde,2,4,6-trimethyl-																						x	x		x	x	x	x							
Benzene,1,2-dichloro-															x	x	x			x	x	x	x		x	x	x	x							
Benzophenone															x			x	x	x	x														
ButylatedHydroxytoluene															x	x	x	x	x	x	x														
Carvone																													x	x	x	x	x	x	x
Caryophyllene										x	x	x	x	x																					
Decanal	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x	x	x	x	x							
Dodecane																						x			x	x	x	x							
Dodecanoicacid																													x	x	x	x		x	
Dodecanoicacid,ethylester								x	x	x	x	x	x	x																					
Furan,2-pentyl-								x	x	x	x	x	x	x								x	x	x	x	x	x	x							
Heptadecane	x	x	x	x	x	x	x																												
Hexadecane	x	x	x	x	x	x	x															x	x	x	x	x	x	x							
Hexadecanoicacid,ethylester								x	x	x	x	x	x																						
Hexanal																						x	x	x	x	x	x								
Hexanoicacid								x	x	x	x	x	x	x																					
Linalool Oxide								x	x	x	x		x	x																					
Menthol																													x	x	x	x	x	x	
Naphthalene,2-methyl-								x	x	x	x	x	x	x																					
Nonanal	x	x	x	x	x	x	x								x	x	x	x	x	x	x	x	x	x	x	x	x	x							
Nonanoicacid								x	x	x	x	x	x	x																					
Nonanoicacid,ethylester								x	x	x	x	x	x	x																					
Octadecane	x	x	x	x	x	x	x	x		x	x	x	x																						
Phenol															x		x	x	x	x	x														
Phenol,4-(1,1,3,3-tetramethylbutyl)-																													x	x		x	x	x	
Pyrrole																													x	x		x	x	x	x
Styrene															x	x	x	x	x	x	x														
Tetradecane	x	x	x	x	x	x	x																												
Tetradecanoicacid,ethylester								x	x	x	x		x																						
Tridecane	x			x	x	x	x															x			x	x	x								
Undecanal	x	x	x	x	x	x	x																												
Undecane																						x			x	x	x								

Table 18. VOCs extracted from hand odor from F4 and M5 over 6 months

	RT	Compound Name	F4							M5						
	(min)		0	1	2	3	4	5	6	0	1	2	3	4	5	6
	11.39	BenzylAlcohol	x	x	x	x	x	x	x							
	12.64	Nonanal	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	14.27	Decanal	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	15.62	Tridecane								x			x	x	x	x
	15.74	Undecanal	x	x			x	x	x	x	x	x	x	x	x	x
	16.49	Hexadecane	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	17.63	Heptadecane	x	x	x		x	x	x	x	x	x	x	x	x	x
	17.69	5,9-Undecadien-2-one,6,10-dimethyl-, (E)-								x		x	x	x	x	x
	20.17	Tetradecane	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	21.65	Octadecane		x	x	x	x	x	x	x	x	x	x	x	x	x

Figure 55. Common compounds extracted among monthly samplings of hand odor from subjects F4 and M5

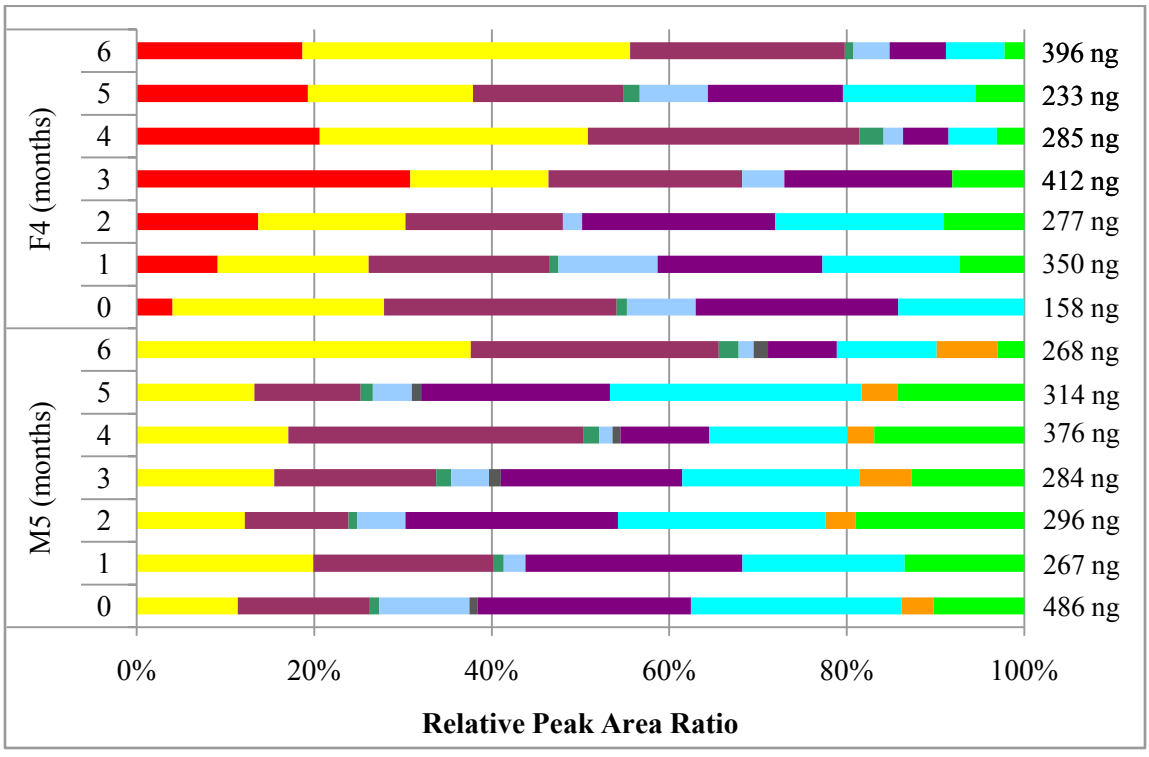


Table 19. VOCs extracted from buccal swabs from F4 and M5 over 6 months

	RT	Compound Name	F4							M5						
	(min)		0	1	2	3	4	5	6	0	1	2	3	4	5	6
	4.63	Hexanal	x		x	x	x	x								
	7.98	Furan,2-pentyl-	x	x	x		x	x	x	x	x	x	x	x	x	x
	8.12	Hexanoicacid,ethylester	x	x	x	x	x	x	x							
	9.25	Hexanoicacid	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	9.37	Linalool Oxide								x	x	x	x		x	x
	9.73	Heptanoicacid,ethylester		x	x	x	x	x	x							
	9.87	6-Methyl-3,5-heptadiene-2-one								x	x	x		x	x	
	10.70	2-Nonenal,(E)-		x	x	x	x	x	x	x	x	x		x	x	x
	11.25	Octanoicacid,ethylester	x	x	x	x	x	x	x							
	11.36	2,4-Nonadienal,(E,E)-		x	x	x	x	x	x	x	x	x	x	x	x	x
	11.39	Decanal	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	12.48	Nonanoicacid	x	x	x		x	x	x	x	x	x	x	x	x	x
	12.67	Nonanoicacid,ethylester	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	12.76	Naphthalene,2-methyl-								x	x	x	x	x	x	x
	13.71	6-Dodecanone	x	x	x		x	x	x	x			x	x	x	x
	13.91	Decanoicacid,ethylester	x	x		x	x	x	x							
	13.95	1-Tetradecene		x	x		x	x	x	x	x	x	x	x	x	x
	14.49	Caryophyllene										x	x	x	x	x
	14.76	5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	15.00	1-Dodecene								x	x	x		x	x	x
	16.44	Dodecanoicacid,ethylester	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	17.70	1,1'-Biphenyl,2,2'-diethyl-		x	x		x	x	x		x	x	x	x	x	x
	18.57	Tetradecanoicacid,ethylester	x	x	x	x	x	x	x	x	x	x	x		x	
	18.70	Octadecane								x		x	x	x	x	
	20.66	Hexadecanoicacid,ethylester	x	x	x	x	x	x	x	x	x	x	x	x	x	

Figure 56. Common compounds extracted among monthly samplings of buccal swabs from subjects F4 and M5

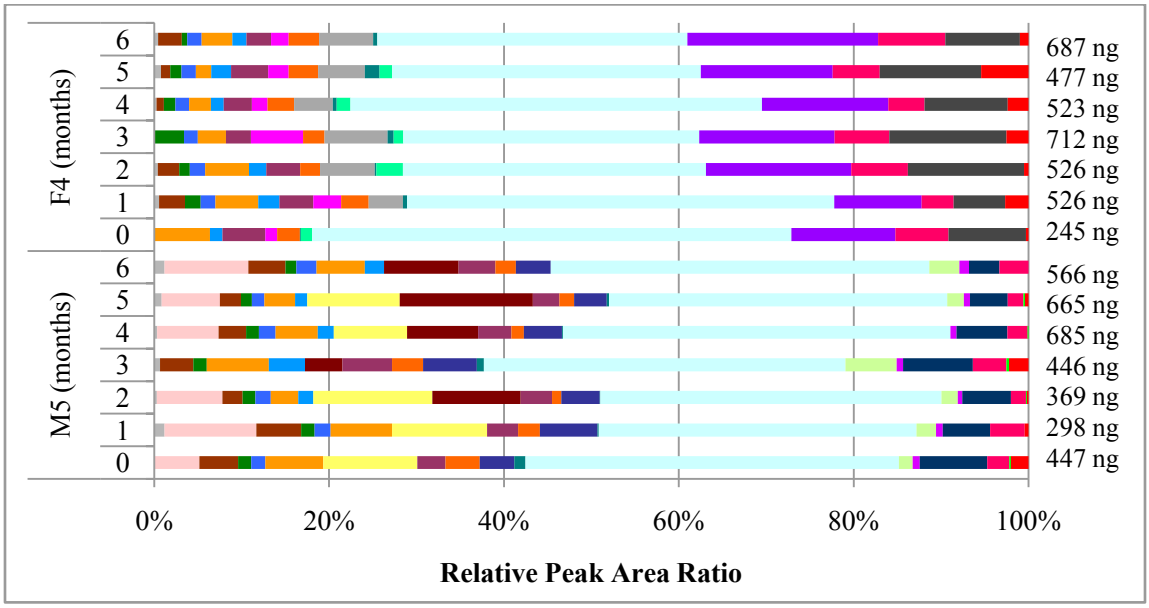


Table 20. VOCs extracted from breath from F4 and M5 over 6 months

	RT	Compound Name	F4							M5						
	(min)		0	1	2	3	4	5	6	0	1	2	3	4	5	6
	5.85	Xylenes	x	x	x	x	x		x							
	6.23	Styrene	x	x	x	x	x		x	x	x	x	x	x	x	x
	7.79	Phenol	x	x	x	x	x	x	x	x		x	x	x	x	x
	8.35	Benzene,1,2-dichloro-	x	x	x	x	x	x	x	x	x	x			x	x
	8.60	1-Hexanol,2-ethyl-		x	x	x	x	x		x	x		x	x	x	x
	9.83	Nonanal	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	11.38	Decanal	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	14.75	5,9-Undecadien-2-one,6,10-dimethyl-, (E)-		x	x	x	x	x								
	15.56	ButylatedHydroxytoluene	x	x	x	x	x		x	x	x	x	x	x	x	x
	16.99	Benzophenone	x	x	x	x	x	x		x			x	x	x	x

Figure 57. Common compounds extracted among monthly samplings of breath from subjects F4 and M5

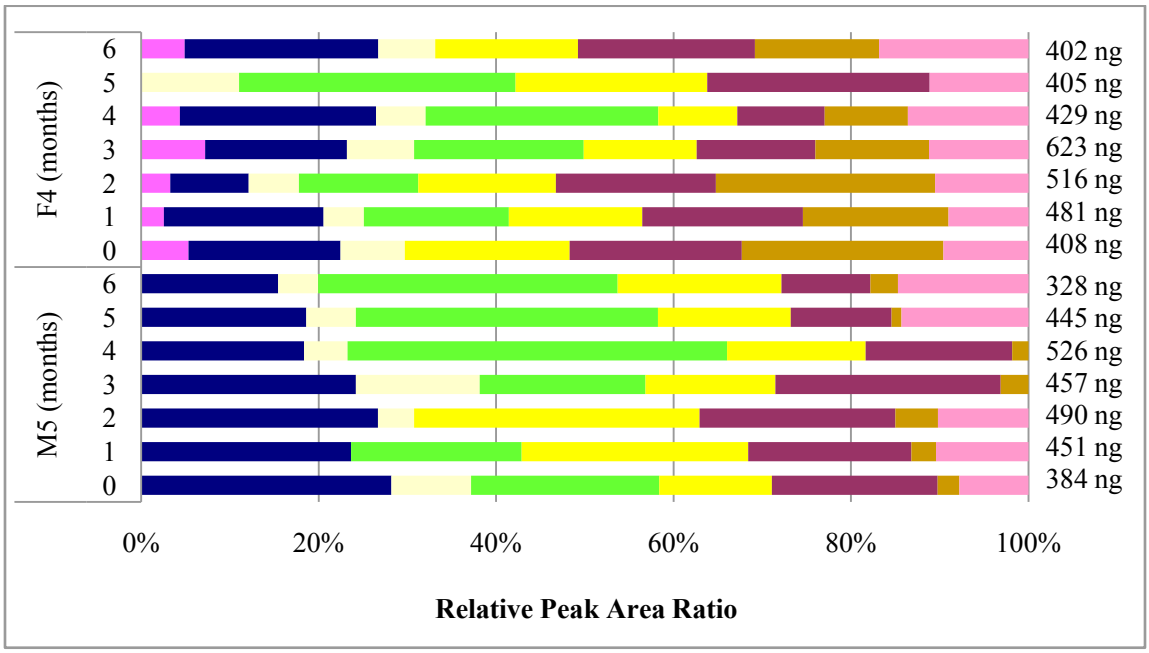


Table 21. VOCs extracted from blood from F4 and M5 over 6 months

	RT	Compound Name	F4							M5						
	(min)		0	1	2	3	4	5	6	0	1	2	3	4	5	6
	3.72	1-Pentanol	x	x	x	x		x								
	4.14	Hexanal	x	x	x	x		x	x	x	x	x	x	x	x	
	5.50	2-Heptanone								x	x	x		x	x	
	7.1	Furan,2-pentyl-	x	x	x	x		x		x	x	x	x	x	x	x
	7.47	Benzene,1,2-dichloro-	x	x	x		x	x		x	x		x	x	x	x
	7.79	BenzylAlcohol	x	x	x	x		x								
	8.77	Undecane	x		x	x	x	x		x			x	x	x	x
	8.8	Nonanal	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	10.29	Dodecane								x			x	x	x	x
	10.39	Decanal	x	x	x	x		x	x			x	x	x	x	x
	11.63	2-Undecanone								x			x	x	x	x
	11.63	Tridecane	x		x	x	x	x		x			x	x	x	x
	11.84	Benzaldehyde,2,4,6-trimethyl-	x	x	x	x	x	x	x	x	x		x	x	x	x
	13.04	Tetradecane	x	x	x	x	x	x	x							
	15.49	Hexadecane	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	16.99	2,6-Diisopropylnaphthalene	x	x	x	x	x	x	x	x	x		x	x	x	

Figure 58. Common compounds extracted among monthly samplings of blood from subjects F4 and M5

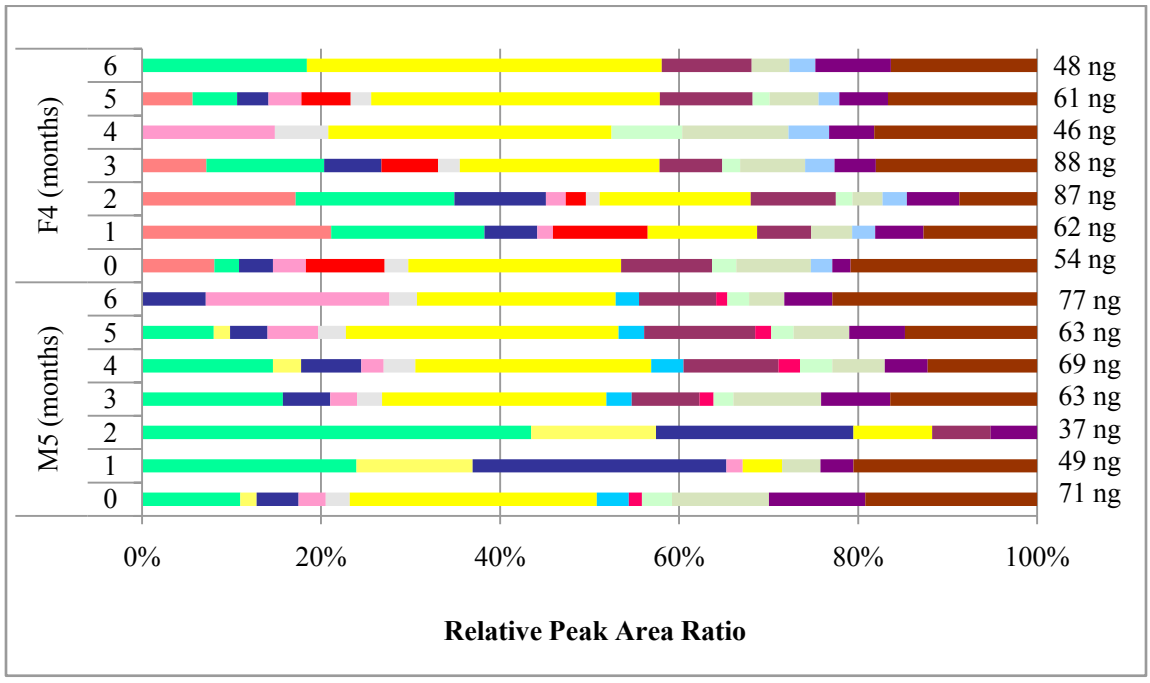


Table 22. VOCs extracted from urine from F4 and M5 over 6 months

	RT	Compound Name	F4							M5						
	(min)		0	1	2	3	4	5	6	0	1	2	3	4	5	6
	4.50	Pyrrole								x	x		x	x	x	x
	7.51	4-Heptanone	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	9.85	Benzene,1,4-dichloro-	x	x	x	x	x	x								
	11.82	Menthol								x	x	x	x	x	x	
	11.88	4-Terpineol									x	x	x		x	x
	12.58	Carvone								x	x	x	x	x	x	x
	15.30	Dodecanoicacid								x	x	x	x		x	
	16.51	Phenol,4-(1,1,3,3-tetramethylbutyl)-								x	x		x	x	x	
	16.62	4-Nonylphenol		x	x	x	x	x	x	x	x	x	x	x	x	x

Figure 59. Common compounds extracted among monthly samplings of urine from subjects F4 and M5

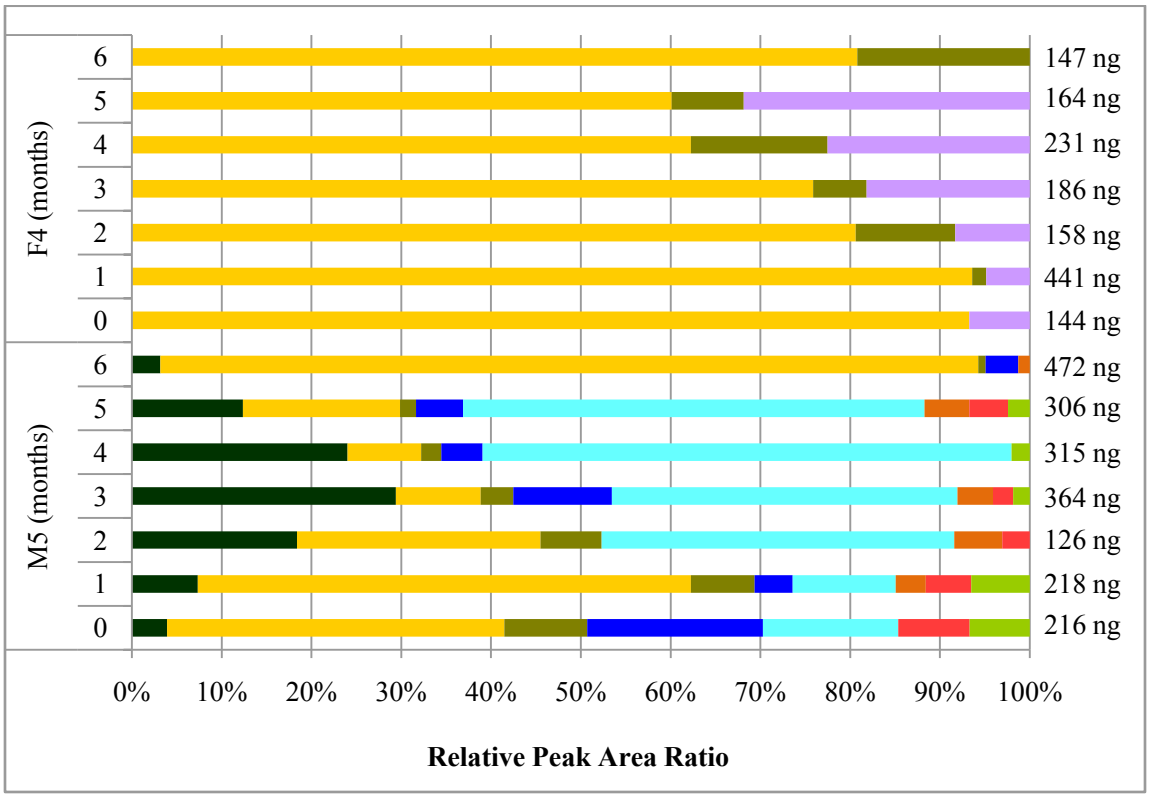


Table 23. Spearman rank correlation coefficients for the peak areas of hand odor VOCs across 6 monthly samplings of F4 and M5

	Spearman Rank Correlation Coeffient Matrix (Hand Odor)													
	F4 (m0)	F4 (m1)	F4 (m2)	F4 (m3)	F4 (m4)	F4 (m5)	F4 (m6)	M5 (m0)	M5 (m1)	M5 (m2)	M5 (m3)	M5 (m4)	M5 (m5)	M5 (m6)
F4 (m0)	1	0.740	0.721	0.000	0.572	-0.139	0.572	0.214	0.401	0.000	0.313	0.324	-0.077	0.686
F4 (m1)	0.740	1	0.910	0.271	0.265	-0.364	0.265	0.407	0.557	0.233	0.459	0.175	0.100	0.592
F4 (m2)	0.721	0.910	1	0.323	0.560	0.000	0.560	0.280	0.436	0.126	0.341	0.132	0.000	0.560
F4 (m3)	0.000	0.271	0.323	1	0.407	0.447	0.407	0.103	0.188	-0.077	0.113	-0.211	-0.201	0.172
F4 (m4)	0.572	0.265	0.560	0.407	1	0.728	1	0.000	0.173	-0.166	0.099	0.175	-0.233	0.518
F4 (m5)	-0.139	-0.364	0.000	0.447	0.728	1	0.728	-0.232	-0.129	-0.342	-0.181	-0.141	-0.402	0.165
F4 (m6)	0.572	0.265	0.560	0.407	1	0.728	1	0.000	0.173	-0.166	0.099	0.175	-0.233	0.518
M5 (m0)	0.214	0.407	0.280	0.103	0.000	-0.232	0.000	1	0.925	0.815	0.963	0.000	0.815	0.410
M5 (m1)	0.401	0.557	0.436	0.188	0.173	-0.129	0.173	0.925	1	0.815	0.983	0.278	0.772	0.552
M5 (m2)	0.000	0.233	0.126	-0.077	-0.166	-0.342	-0.166	0.815	0.815	1	0.830	0.154	0.964	0.078
M5 (m3)	0.313	0.459	0.341	0.113	0.099	-0.181	0.099	0.963	0.983	0.830	1	0.256	0.830	0.534
M5 (m4)	0.324	0.175	0.132	-0.211	0.175	-0.141	0.175	0.000	0.278	0.154	0.256	1	0.231	0.503
M5 (m5)	-0.077	0.100	0.000	-0.201	-0.233	-0.402	-0.233	0.815	0.772	0.964	0.830	0.231	1	0.116
M5 (m6)	0.686	0.592	0.560	0.172	0.518	0.165	0.518	0.410	0.552	0.078	0.534	0.503	0.116	1

Table 24. Spearman rank correlation coefficients for the peak areas of buccal swab VOCs across 6 monthly samplings of F4 and M5

	Spearman Rank Correlation Coeffient Matrix (Buccal Swab)													
	F4 (m0)	F4 (m1)	F4 (m2)	F4 (m3)	F4 (m4)	F4 (m5)	F4 (m6)	M5 (m0)	M5 (m1)	M5 (m2)	M5 (m3)	M5 (m4)	M5 (m5)	M5 (m6)
F4 (m0)	1	0.933	0.917	0.783	0.967	0.967	0.933	-0.184	-0.128	-0.145	-0.145	-0.145	-0.139	-0.100
F4 (m1)	0.933	1	0.983	0.867	0.933	0.933	1.000	-0.161	-0.100	-0.156	-0.117	-0.156	-0.128	-0.067
F4 (m2)	0.917	0.983	1	0.833	0.950	0.950	0.983	-0.172	-0.083	-0.133	-0.106	-0.133	-0.111	-0.044
F4 (m3)	0.783	0.867	0.833	1	0.700	0.700	0.867	-0.083	-0.011	-0.106	-0.033	-0.106	-0.061	0.028
F4 (m4)	0.967	0.933	0.950	0.700	1	1	0.933	-0.206	-0.133	-0.145	-0.150	-0.145	-0.139	-0.100
F4 (m5)	0.967	0.933	0.950	0.700	1	1	0.933	-0.206	-0.133	-0.145	-0.150	-0.145	-0.139	-0.100
F4 (m6)	0.933	1.000	0.983	0.867	0.933	0.933	1	-0.161	-0.100	-0.156	-0.117	-0.156	-0.128	-0.067
M5 (m0)	-0.184	-0.161	-0.172	-0.083	-0.206	-0.206	-0.161	1	0.855	0.764	0.891	0.800	0.882	0.736
M5 (m1)	-0.128	-0.100	-0.083	-0.011	-0.133	-0.133	-0.100	0.855	1	0.936	0.945	0.945	0.955	0.827
M5 (m2)	-0.145	-0.156	-0.133	-0.106	-0.145	-0.145	-0.156	0.764	0.936	1	0.882	0.991	0.909	0.727
M5 (m3)	-0.145	-0.156	-0.133	-0.106	-0.145	-0.145	-0.156	0.800	0.945	0.991	1	0.909	0.973	0.882
M5 (m4)	-0.145	-0.156	-0.133	-0.106	-0.145	-0.145	-0.156	0.800	0.945	0.991	0.909	1	0.945	0.755
M5 (m5)	-0.139	-0.128	-0.111	-0.061	-0.139	-0.139	-0.128	0.882	0.955	0.909	0.973	0.945	1	0.855
M5 (m6)	-0.100	-0.067	-0.044	0.028	-0.100	-0.100	-0.067	0.736	0.827	0.727	0.882	0.755	0.855	1

Table 25. Spearman rank correlation coefficients for the peak areas of breath VOCs across 6 monthly samplings of F4 and M5

	Spearman Rank Correlation Coefficient Matrix (Breath)													
	F4 (m0)	F4 (m1)	F4 (m2)	F4 (m3)	F4 (m4)	F4 (m5)	F4 (m6)	M5 (m0)	M5 (m1)	M5 (m2)	M5 (m3)	M5 (m4)	M5 (m5)	M5 (m6)
F4 (m0)	1	1	1	0.800	0.400	1	0.400	-0.143	0.143	-0.191	0.238	-0.143	-0.191	-0.048
F4 (m1)	1	1	1	0.800	0.400	1	0.400	-0.143	0.143	-0.191	0.238	-0.143	-0.191	-0.048
F4 (m2)	1	1	1	0.800	0.400	1	0.400	-0.143	0.143	-0.191	0.238	-0.143	-0.191	-0.048
F4 (m3)	0.800	0.800	0.800	1	0.200	0.800	0.800	-0.191	0.191	-0.143	0.095	-0.191	-0.143	0.048
F4 (m4)	0.400	0.400	0.400	0.200	1	0.400	0.400	-0.477	-0.286	-0.524	-0.191	-0.477	-0.524	-0.429
F4 (m5)	1	1	1	0.800	0.400	1	0.400	-0.143	0.143	-0.191	0.238	-0.143	-0.191	-0.048
F4 (m6)	0.400	0.400	0.400	0.800	0.400	0.400	1	-0.524	-0.238	-0.477	-0.334	-0.524	-0.477	-0.334
M5 (m0)	-0.143	-0.143	-0.143	-0.191	-0.477	-0.143	-0.524	1	0.771	0.943	0.829	0.943	0.886	0.714
M5 (m1)	0.143	0.143	0.143	0.191	-0.286	0.143	-0.238	0.771	1	0.829	0.714	0.600	0.657	0.600
M5 (m2)	-0.191	-0.191	-0.191	-0.143	-0.524	-0.191	-0.477	0.943	0.829	1	0.657	0.886	0.943	0.829
M5 (m3)	0.238	0.238	0.238	0.095	-0.191	0.238	-0.334	0.829	0.714	0.657	1	0.771	0.600	0.486
M5 (m4)	-0.143	-0.143	-0.143	-0.191	-0.477	-0.143	-0.524	0.943	0.600	0.886	0.771	1	0.943	0.829
M5 (m5)	-0.191	-0.191	-0.191	-0.143	-0.524	-0.191	-0.477	0.886	0.657	0.943	0.600	0.943	1	0.943
M5 (m6)	-0.048	-0.048	-0.048	0.048	-0.429	-0.048	-0.334	0.714	0.600	0.829	0.486	0.829	0.943	1

Table 26. Spearman rank correlation coefficients for the peak areas of blood VOCs across 6 monthly samplings of F4 and M5

	Spearman Rank Correlation Coefficient Matrix (Blood)													
	F4 (m0)	F4 (m1)	F4 (m2)	F4 (m3)	F4 (m4)	F4 (m5)	F4 (m6)	M5 (m0)	M5 (m1)	M5 (m2)	M5 (m3)	M5 (m4)	M5 (m5)	M5 (m6)
F4 (m0)	1	0.771	0.829	0.943	0.943	0.943	0.829	0.524	0.238	0.238	0.524	0.477	0.524	0.477
F4 (m1)	0.771	1	0.943	0.886	0.886	0.886	0.943	0.572	0.238	0.238	0.572	0.429	0.572	0.429
F4 (m2)	0.829	0.943	1	0.943	0.943	0.943	1.000	0.715	0.334	0.334	0.715	0.572	0.715	0.572
F4 (m3)	0.943	0.886	0.943	1	1	1	0.943	0.620	0.286	0.286	0.620	0.524	0.620	0.524
F4 (m4)	0.943	0.886	0.943	1	1	1	0.943	0.620	0.286	0.286	0.620	0.524	0.620	0.524
F4 (m5)	0.943	0.886	0.943	1	1	1	0.943	0.620	0.286	0.286	0.620	0.524	0.620	0.524
F4 (m6)	0.829	0.943	1.000	0.943	0.943	0.943	1	0.715	0.334	0.334	0.715	0.572	0.715	0.572
M5 (m0)	0.524	0.572	0.715	0.620	0.620	0.620	0.715	1	0.400	0.400	1.000	0.800	1.000	0.800
M5 (m1)	0.238	0.238	0.334	0.286	0.286	0.286	0.334	0.400	1	1	0.400	0.800	0.400	0.800
M5 (m2)	0.238	0.238	0.334	0.286	0.286	0.286	0.334	0.400	1	1	0.400	0.800	0.400	0.800
M5 (m3)	0.524	0.572	0.715	0.620	0.620	0.620	0.715	1.000	0.400	0.400	1	0.800	1	0.800
M5 (m4)	0.477	0.429	0.572	0.524	0.524	0.524	0.572	0.800	0.800	0.800	0.800	1	0.800	1
M5 (m5)	0.524	0.572	0.715	0.620	0.620	0.620	0.715	1.000	0.400	0.400	1.000	0.800	1	0.800
M5 (m6)	0.477	0.429	0.572	0.524	0.524	0.524	0.572	0.800	0.800	0.800	0.800	1	0.800	1

Table 27. Spearman rank correlation coefficients for the peak areas of urine VOCs across 6 monthly samplings of F4 and M5

	Spearman Rank Correlation Coefficient Matrix (Urine)													
	F4 (m0)	F4 (m1)	F4 (m2)	F4 (m3)	F4 (m4)	F4 (m5)	F4 (m6)	M5 (m0)	M5 (m1)	M5 (m2)	M5 (m3)	M5 (m4)	M5 (m5)	M5 (m6)
F4 (m0)	1	0.980	0.750	0.540	0.960	0.750	0.829	0.350	0.110	-0.010	-0.180	-0.150	-0.080	0.260
F4 (m1)	0.980	1	0.400	0.320	0.790	0.676	0.310	0.190	0.140	-0.090	-0.290	-0.220	-0.130	-0.010
F4 (m2)	0.750	0.400	1	0.490	0.908	0.680	0.940	0.470	0.160	0.110	0.020	-0.060	0.100	0.440
F4 (m3)	0.540	0.320	0.490	1	0.460	0.930	0.260	0.390	0.120	-0.120	-0.130	-0.220	0.040	0.120
F4 (m4)	0.960	0.790	0.908	0.460	1	0.640	0.938	0.430	0.140	0.050	-0.130	-0.130	-0.040	0.310
F4 (m5)	0.750	0.680	0.680	0.930	0.640	1	0.510	0.290	0.080	-0.140	-0.280	-0.290	0.120	0.120
F4 (m6)	0.860	0.310	0.940	0.260	0.938	0.510	1	0.420	0.180	0.210	0.080	-0.010	0.120	0.480
M5 (m0)	0.350	0.190	0.470	0.390	0.430	0.290	0.420	1	0.270	0.000	0.260	0.060	0.200	0.780
M5 (m1)	0.110	0.140	0.160	0.120	0.140	0.080	0.180	0.270	1	0.510	0.420	0.685	0.520	0.300
M5 (m2)	-0.010	-0.090	0.110	-0.120	0.050	-0.140	0.210	0.000	0.510	1	0.886	0.755	0.920	0.337
M5 (m3)	-0.180	-0.290	0.020	-0.130	-0.130	-0.280	0.080	0.260	0.420	0.886	1	0.810	0.808	0.320
M5 (m4)	-0.150	-0.220	-0.060	-0.220	-0.130	-0.290	-0.010	0.060	0.685	0.755	0.810	1	0.730	0.090
M5 (m5)	-0.080	-0.130	0.100	0.040	-0.040	0.120	0.120	0.200	0.410	0.920	0.808	0.730	1	0.350
M5 (m6)	0.260	-0.010	0.440	0.120	0.310	0.120	0.480	0.780	0.300	0.337	0.320	0.090	0.350	1

3.6.2. Principal Component Analysis

Principal component analysis (PCA) was conducted on the six month time study sampling dataset. Figure 60 and Figure 61 shows a three-dimensional plot of the first three principal components of subjects Female 4 and Male 5, respectively. Groupings can be seen among the different biological specimen samples. As shown in purple in the figure, buccal swab samples group together, as do hand odor (blue), breath (green), blood (red), and urine (orange) samples. Additionally, PCA was individually conducted on the multiple-samplings dataset between Female 4 and Male 5 for each specimen. When the first three principal components of these datasets are plotted, groupings can be noted among the multiple samples collected from the same individual. Female 4's samplings from the time study group together and Male 5's samplings group together, but there is no overlap between subjects. Figure 62 to Figure 66 display PCA data for the hand odor, buccal swab, breath, blood, and urine samples from Female 4 and Male 5 over the six month time period. With the exception of a few outliers, each specimen's samples from Female 4 group together as do the samples from Male 5. These three-dimensional plots provide a convenient visual aid for identifying the groupings according to specimen or individual.

Figure 60. Three-dimensional PCA scatter plot of PCs 1, 2, and 3 of Female 4

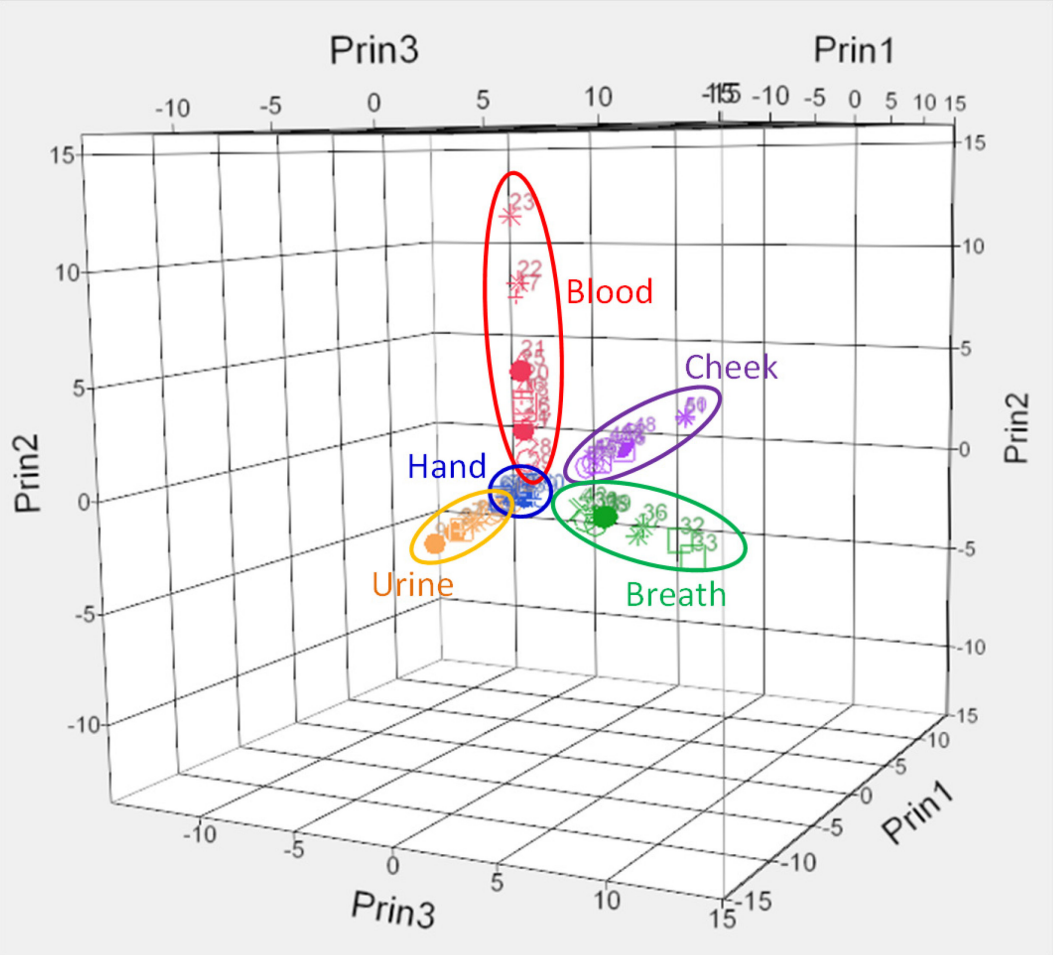


Figure 61. Three-dimensional PCA scatter plot of PCs 1, 2, and 3 of Male 5

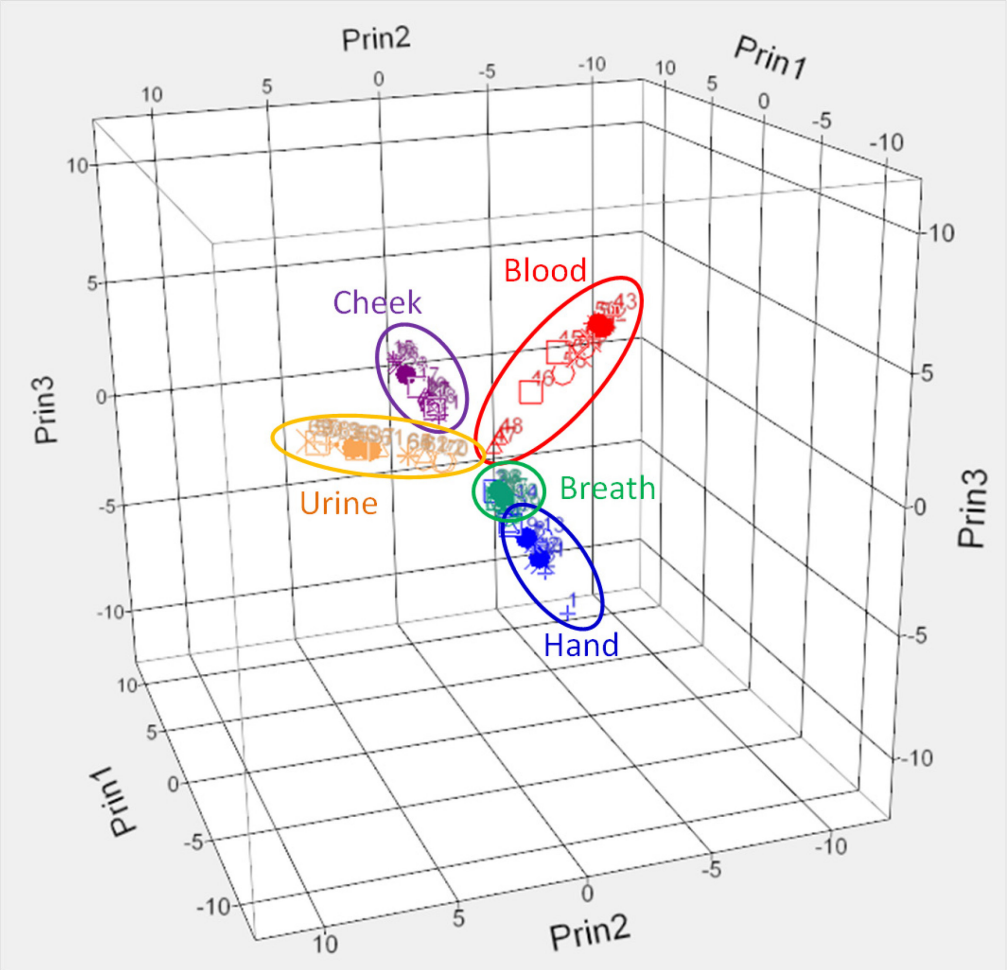


Table 28. PCA results summary for hand odor

Number	1	2	3	4	5
Eigenvalue	3.643	1.864	1.458	0.549	0.275
Percent	45.531	23.306	18.230	6.862	3.438
Cum Percent	45.531	68.838	87.068	93.929	97.367

Figure 62. Three-dimensional PCA scatter plot of PCs 1, 2, and 3 of hand odor from F4 and M5 over 6 months

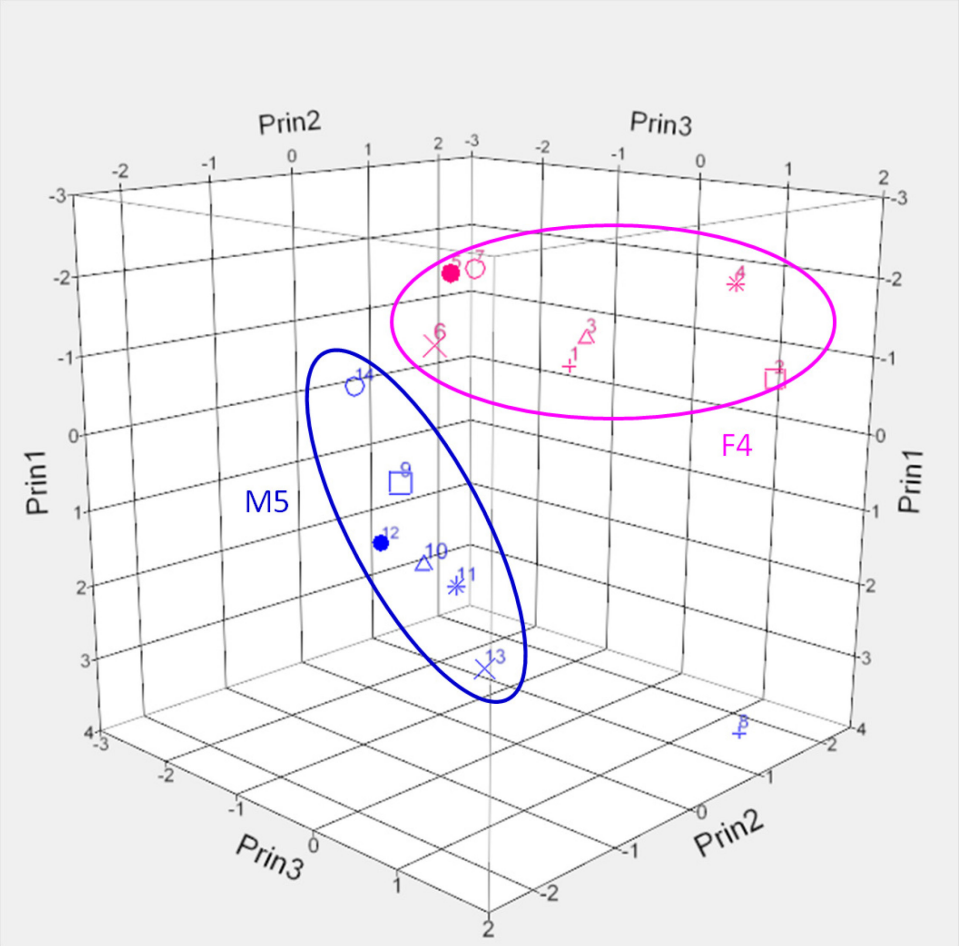


Table 29. PCA results summary for buccal swab

Number	1	2	3	4	5
Eigenvalue	9.739	3.239	0.706	0.468	0.386
Percent	64.926	21.599	4.705	3.118	2.576
Cum Percent	64.926	86.526	91.231	94.349	96.925

Figure 63. Three-dimensional PCA scatter plot of PCs 1, 2, and 3 of buccal swabs from F4 and M5 over 6 months

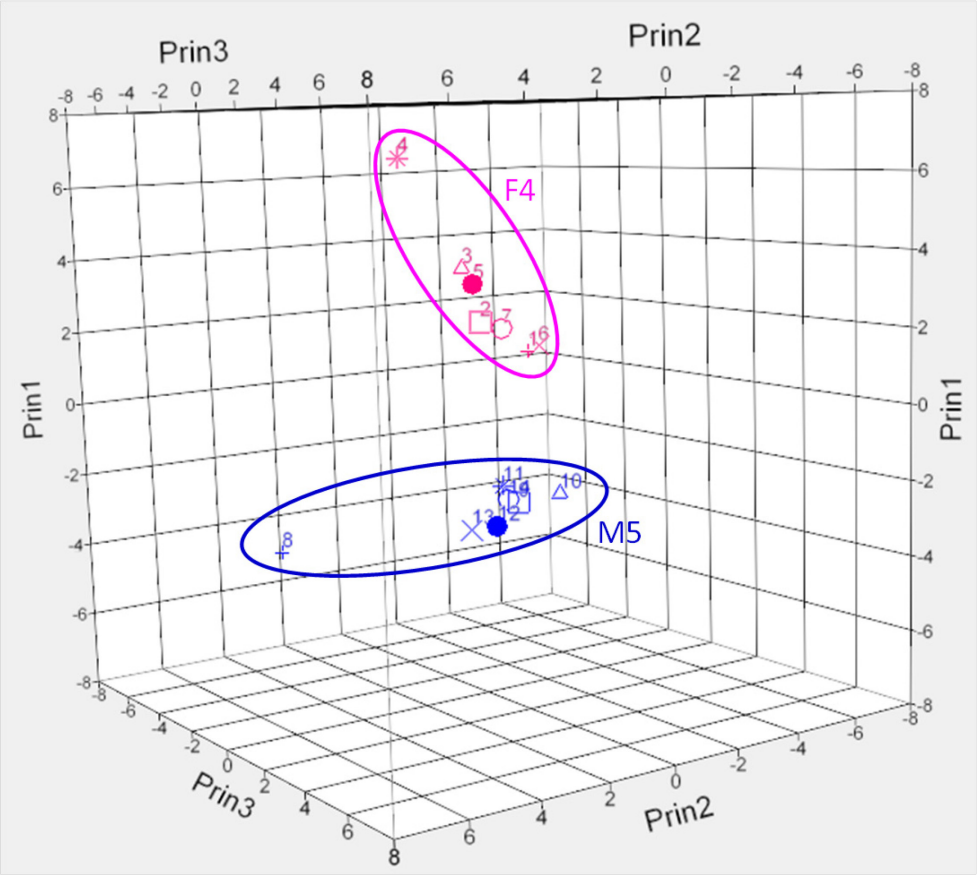


Table 30. PCA results summary for breath

Number	1	2	3	4	5
Eigenvalue	3.533	2.026	0.886	0.241	0.182
Percent	50.469	28.940	12.658	3.446	2.606
Cum Percent	50.469	79.409	92.068	95.514	98.120

Figure 64. Three-dimensional PCA scatter plot of PCs 1, 2, and 3 of breath from F4 and M5 over 6 months

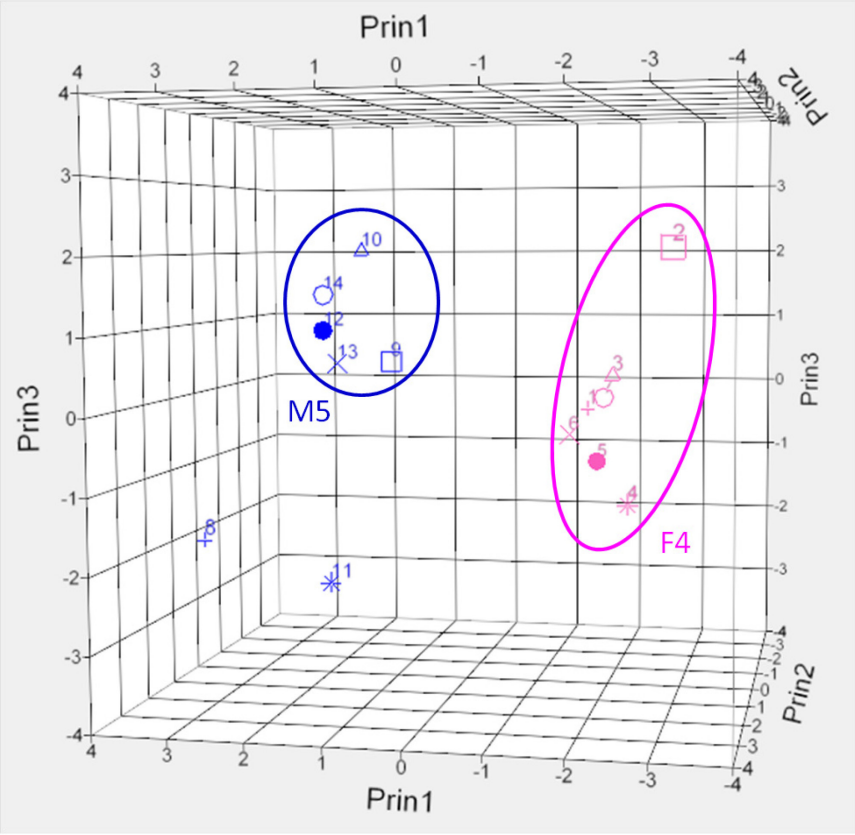


Table 31. PCA results summary for blood

Number	1	2	3	4	5
Eigenvalue	3.636	2.176	0.742	0.252	0.153
Percent	51.939	31.090	10.602	3.594	2.188
Cum Percent	51.939	83.029	93.631	97.225	99.413

Figure 65. Three-dimensional PCA scatter plot of PCs 1, 2, and 3 of blood from F4 and M5 over 6 months

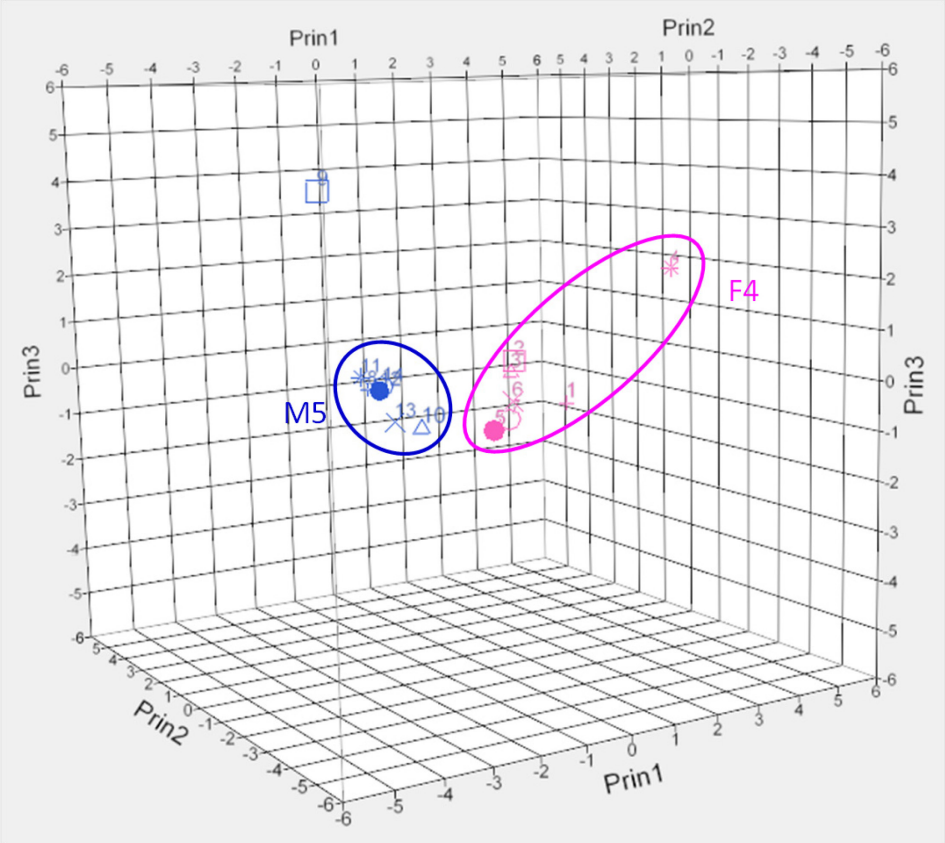
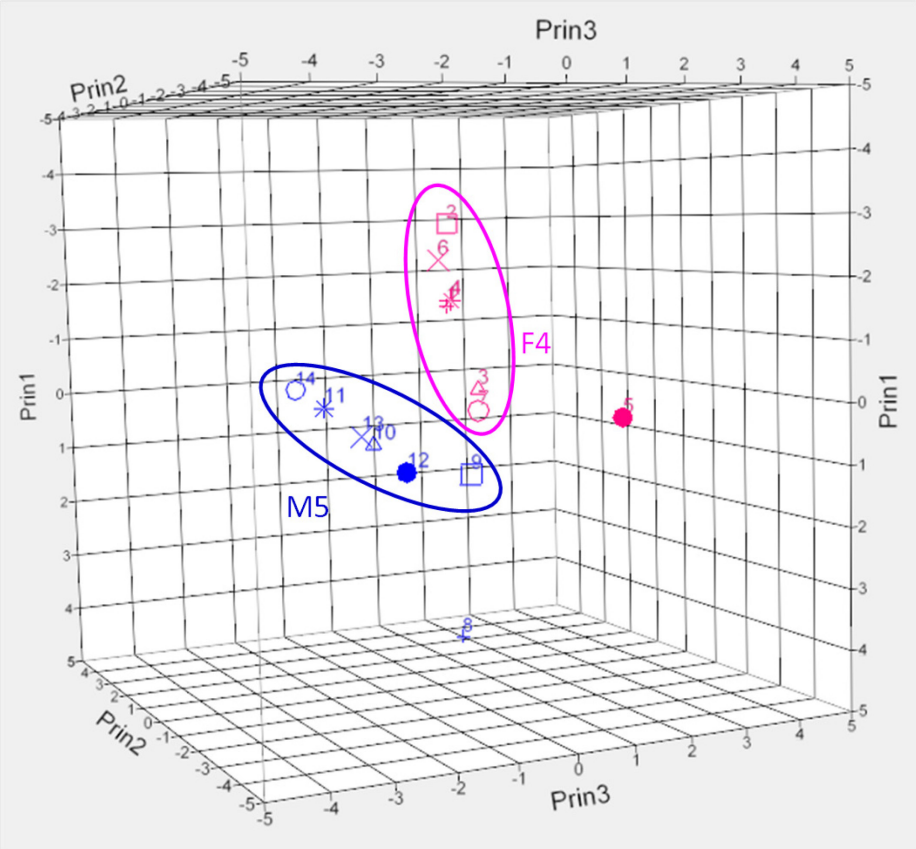


Table 32. PCA results summary for urine

Number	1	2	3	4	5
Eigenvalue	2.389	1.023	0.899	0.487	0.202
Percent	47.781	20.454	17.988	9.737	4.040
Cum Percent	47.781	68.235	86.223	95.959	100

Figure 66. Three-dimensional PCA scatter plot of PCs 1, 2, and 3 of urine from F4 and M5 over 6 months

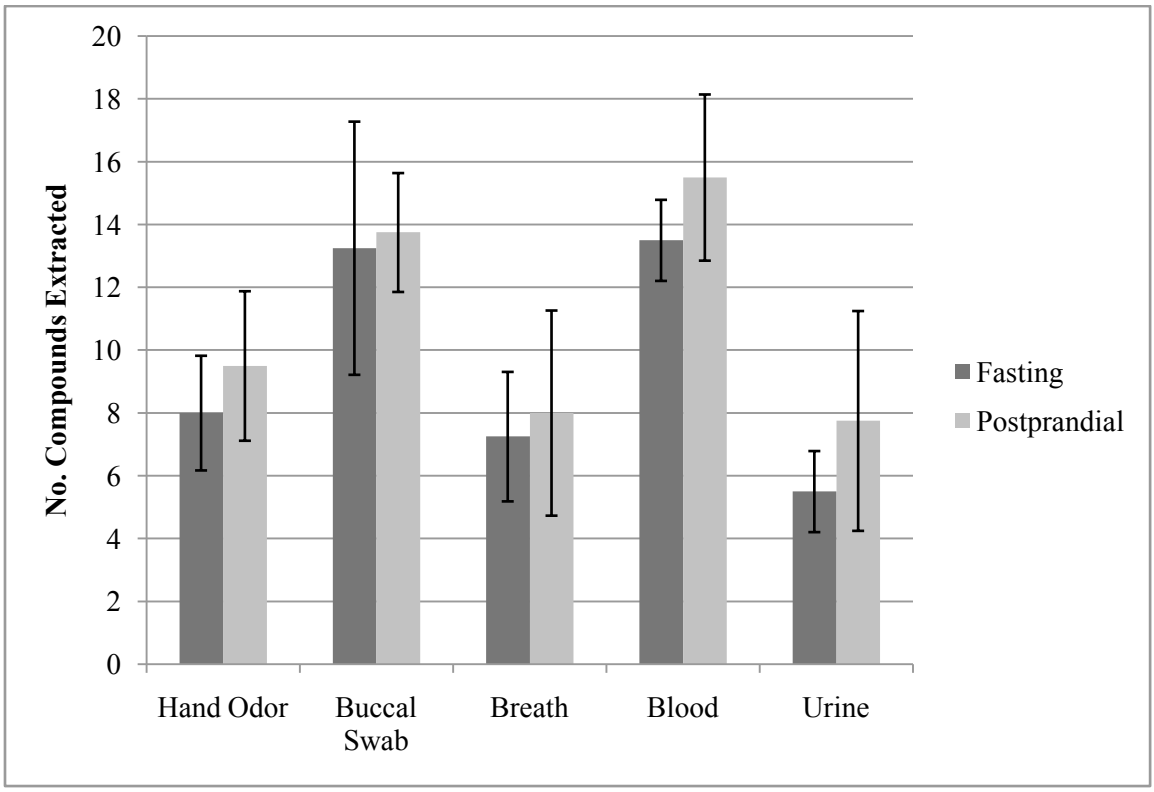


Results from the six-month study of the VOC profiles of two subjects were in agreement with the previously reported findings of the similar study done with hand odor. Relative peak area ratio comparisons, Spearman rank correlation coefficient comparisons, and PCA results all demonstrate that VOC profiles of one individual taken over a period of time do not contain as much variation as that seen between different individuals. The VOCs from the same specimen over time group together, and VOCs from the same individual for different specimens group together. These findings further support the hypothesis that human scent, not only hand odor but also extending to other biological specimens, is stable over time and distinguishable between individuals.

3.6.3. Evaluation of the Effect of Fasting Prior to Sampling

Four unrelated subjects (Female 4, Female 15, Male 2, and Male 15) were sampled intra-day to evaluate the effect of fasting prior to specimen collections. The "fasting" sampling was done in the morning where the subjects had not consumed any food or drinks, with the exception of water, since the previous night. The "postprandial" sampling was done in the afternoon, immediately after the subject had consumed a meal (within thirty minutes after eating). Figure 67 demonstrates the comparison of the average number of compounds extracted from each specimen. A small increase in the total number of common compounds extracted was observed for each of the five specimens. Buccal swab and breath samples were anticipated to have the largest differences between the fasting and postprandial samples; however, the differences in the number of compounds extracted were not drastic.

Figure 67. Average number of compounds extracted from specimens collected after fasting and postprandial



Comparisons of compounds that were extracted at fasting and postprandial sampling times from the five biological specimens for subject Female 4 are summarized in Table 33. For each specimen, the compounds that were extracted from both fasting and postprandial samplings were subjected to paired *t*-test of significance to evaluate whether the variation in the amounts of the compound extracted was statistically significant or not. For hand odor, eight out of the twelve compounds extracted were common across fasting and postprandial samplings: benzyl alcohol, nonanal, decanal, tridecane, tetradecane, (E)-6,10-dimethyl-5,9-undecadien-2-one, hexadecane, and heptadecane. The extracted amounts were statistically significant only for tridecane. For buccal swabs, nine

out of the fifteen compounds extracted were common across fasting and postprandial samplings: 2-pentylfuran, hexanoic acid, octanoic acid ethyl ester, decanal, nonanoic acid, nonanoic acid ethyl ester, (E)- 6,10-dimethyl-5,9-undecadien-2-one, 1-decene, and dodecanoic acid ethyl ester. When subjected to paired *t*-test of significance, the difference in the amounts of these nine common extracted compounds was not found to be statistically significant. For breath, four out of the six compounds extracted were common across fasting and postprandial samplings: toluene, 2-ethyl-1-hexanol, nonanal, and decanal. The differences in the amounts of these four compounds extracted between samplings were not statistically significant. For blood, nine out of the eighteen compounds extracted were common between samplings: undecane, nonanal, tridecane, 2,4,6-trimethylbenzaldehyde, 2-butyltetrahydrofuran, tetradecane, hexadecane, diisopropylnaphthalene, and 2,6-diisopropylnaphthalene. None of the common compounds were have to have a statistically significant difference in the amount extracted betweens samplings. Finally for urine, two out of the five compounds extracted were common between samplings: 4-heptanone and carvone. Carvone was found to be statistically significantly different in the amount extracted between fasting and postprandial samplings.

Table 33. Summary of volatile compounds extracted fasting (AM) vs. postprandial (PM) for Female 4

		Hand		Buccal		Breath		Blood		Urine	
		AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
	1-Decene			x	x			x			
	1-Hexanol,2-ethyl-					x	x				
	1-Octanol										x
	2,6-Diisopropylnaphthalene							x	x		
	2-Dodecene,(Z)-								x		
	4-Heptanone									x	x
	5,9-Undecadien-2-one,6,10-dimethyl-,(E)-	x	x	x	x				x		
	6-Dodecanone				x						
	Acetone					x					
	Benzaldehyde	x									
	Benzaldehyde,2,4,6-trimethyl-							x	x		
	BenzylAlcohol	x	x					x			
	Carvone									x	x
	Caryophyllene				x						
	Cyclododecane								x		
	Cyclopropane,1-ethyl-2-heptyl-										x
	Decanal	x	x	x	x	x	x		x		
	Diisopropylnaphthalene							x	x		
	Dodecane							x	x		
	Dodecanoicacid				x						
	Dodecanoicacid,ethylester			x	x						
	Furan,2-butyltetrahydro-							x	x		
	Furan,2-pentyl-			x	x				x		
	Heptadecane	x	x								
	Heptanoicacid,ethylester				x						
	Hexadecane	x	x					x	x		
	Hexanal							x			
	Hexanoicacid			x	x						
	Hexanoicacid,pentylester				x						
	Menthol									x	
	Nonanal	x	x			x	x	x	x		
	Nonanoicacid			x	x						
	Nonanoicacid,ethylester			x	x						
	Octadecane	x									
	Octanoicacid,ethylester			x	x						
	Octanoicacid,methylester		x								
	Phenol					x					
	Tetradecane	x	x					x	x		
	Toluene					x	x				
	Tridecane	x	x					x	x		
	Undecane		x					x	x		

Comparisons of compounds that were extracted at fasting and postprandial sampling times from the five biological specimens for subject Female 15 are summarized in Table 34. For each specimen, the compounds that were extracted from both fasting and postprandial samplings were subjected to paired *t*-test of significance to evaluate whether the variation in the amounts of the compound extracted was statistically significant or not. For hand odor, seven out of the eleven compounds extracted were common across fasting and postprandial samplings: benzyl alcohol, undecane, nonanal, decanal, tetradecane, (E)-6,10-dimethyl-5,9-undecadien-2-one, and octadecane. The extracted amounts were not statistically significant for any of the common compounds. For buccal swabs, nine out of the twenty-four compounds extracted were common across fasting and postprandial samplings: benzaldehyde, 2-pentylfuran, hexanoic acid, decanal, nonanoic acid, nonanoic acid ethyl ester, (E)-6,10-dimethyl-5,9-undecadien-2-one, diethyl phthalate, and isopropyl myristate. When subjected to paired *t*-test of significance, the difference in the amounts of these nine common extracted compounds was not found to be statistically significant. For breath, eight out of the nine compounds extracted were common across fasting and postprandial samplings: toluene, p-xylene, 1,2-dichlorobenzene, 2-ethyl-1-hexanol, nonanal, decanal, butylated hydroxytoluene, and benzophenone. The differences in the amounts of these eight compounds extracted between samplings were not statistically significant. For blood, eleven out of the sixteen compounds extracted were common between samplings: 2-pentylfuran, nonanal menthol, 1-dodecene, decanal, (Z)-2-dodecene, tridecane, 2-butyltetrahydrofuran, tetradecane, hexadecane, and 2,6-diisopropylnaphthalene. None of the common compounds were have to have a statistically significant difference in the amount extracted betweens samplings.

Finally for urine, four out of the eight compounds extracted were common between samplings: 4-heptanone, menthol, carvone, and N-N-diethylcarbanilide. Menthol and carvone were found to be statistically significantly different in the amount extracted between fasting and postprandial samplings.

Table 34. Summary of compounds extracted fasting (AM) vs. postprandial (PM) for Female 15

		Hand		Buccal		Breath		Blood		Urine	
		AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
	1,1'-Biphenyl,3-methyl-				x						
	1-Decene				x						
	1-Dodecanol								x		
	1-Dodecene				x			x	x		
	1-Hexanol,2-ethyl-					x	x				
	1-Tetradecene			x							
	2,4-Nonadienal				x						
	2,6-Diisopropylnaphthalene							x	x		
	2-Dodecene,(Z)-							x	x		
	3-Cyclohexen-1-one2-isopropyl-5-methyl-									x	
	4-Heptanone									x	x
	5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	x	x	x	x						
	6-Dodecanone			x							
	6-Methyl-3,5-heptadiene-2-one			x							
	β-Cadinene			x							
	Acetophenone		x								
	Benzaldehyde			x	x						
	Benzaldehyde,2,4,6-trimethyl-								x		
	Benzene,1,2-dichloro-					x	x				
	Benzene,1,3-dimethyl-					x	x				
	Benzeneacetaldehyde,.alpha.-methyl-										x
	Benzophenone					x	x				
	BenzylAlcohol	x	x								
	ButylatedHydroxytoluene					x	x				
	Carvone									x	x
	Caryophyllene			x							
	Cedrol										x
	Decanal	x	x	x	x	x	x	x	x		
	DiethylPhthalate			x	x						
	Diisopropylnaphthalene							x			
	Furan,2-butyltetrahydro-							x	x		
	Furan,2-pentyl-			x	x			x	x		
	Heptadecane		x								
	Heptanoicacid,ethylester				x						
	Hexadecane		x					x	x		
	Hexanal							x			
	Hexanoicacid			x	x						
	Hexanoicacid,pentylester			x							
	IsopropylMyristate			x	x						
	Menthol					x		x	x	x	x
	N,N-Diethylcarbanilide									x	x
	Nonanal	x	x			x	x	x	x		
	Nonanoicacid			x	x						
	Nonanoicacid,ethylester			x	x						
	Octadecane	x	x		x						
	Octanoicacid,ethylester			x							
	Pentanoicacid			x							
	Pyrrole									x	
	Tetradecane	x	x	x				x	x		
	Toluene					x	x				
	Tridecane							x	x		
	Undecanal		x								
	Undecane	x	x					x			

Comparisons of compounds that were extracted at fasting and postprandial sampling times from the five biological specimens for subject Male 2 are summarized in Table 35. For each specimen, the compounds that were extracted from both fasting and postprandial samplings were subjected to paired *t*-test of significance to evaluate whether the variation in the amounts of the compound extracted was statistically significant or not. For hand odor, five out of the seven compounds extracted were common across fasting and postprandial samplings: nonanal, decanal, tetradecane, hexadecane, and heptadecane. The extracted amounts were not statistically significant for any of the common compounds. For buccal swabs, eight out of the fourteen compounds extracted were common across fasting and postprandial samplings: 2-pentylfuran, hexanoic acid, octanoic acid ethyl ester, decanal, nonanoic acid ethyl ester, 6-dodecanone, (E)-6,10-dimethyl-5,9-undecadien-2-one, and dodecanoic acid ethyl ester. When subjected to paired *t*-test of significance, the difference in the amounts of dodecanoic acid ethyl ester extracted between fasting and postprandial samplings was found to be statistically significant. For breath, five out of the eight compounds extracted were common across fasting and postprandial samplings: phenol, 2-ethyl-1-hexanol, nonanal, decanal, and butylated hydroxytoluene. The differences in the amounts of these five compounds extracted between samplings were not statistically significant. For blood, ten out of the seventeen compounds extracted were common between samplings: hexanal, nonanal, dodecane, tridecane, 2,4,6-trimethylbenzaldehyde, tetradecane, 1-decene, hexadecane, 2,6-diisopropylnaphthalene, and octadecane. None of the common compounds were have to have a statistically significant difference in the amount extracted betweens samplings. Finally for urine, three out of the eight compounds extracted were common between

samplings: menthol, 2-isopropylbenzaldehyde, and carvone. 2-Isopropylbenzaldehyde was found to be statistically significantly different in the amount extracted between fasting and postprandial samplings.

Table 35. Summary of compounds extracted fasting (AM) vs. postprandial (PM) for Male 2

		Hand		Buccal		Breath		Blood		Urine	
		AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
	1-Decene							x	x		
	1-Hexanol,2-ethyl-					x	x				
	1-Tetradecene				x						
	2,6-Diisopropylnaphthalene							x	x		
	2-Cyclohexen-1-one,3-methyl-6-(1-methylethyl)-										x
	2-Isopropylbenzaldehyde									x	x
	4-Heptanone									x	
	5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	x	x	x	x				x		
	6-Dodecanone			x	x						
	AllylIsothiocyanate										x
	Benzaldehyde,2,4,6-trimethyl-							x	x		
	Benzene,1,3-dichloro-										x
	Benzeneacetaldehyde,.alpha.-methyl-									x	
	Benzophenone						x				
	ButylatedHydroxytoluene					x	x				
	Carvone						x			x	x
	Cyclopropane,nonyl-			x							
	Decanal	x	x	x	x	x	x	x			
	Diisopropylnaphthalene							x			
	Dodecane							x	x		
	Dodecanoicacid,ethylester			x	x						
	Furan,2-pentyl-			x	x				x		
	Heptadecane	x	x					x			
	Hexadecane	x	x					x	x		
	Hexadecanoicacid,ethylester			x							
	Hexanal							x	x		
	Hexanoicacid			x	x						
	Hexanoicacid,pentylester				x						
	Menthol						x			x	x
	Naphthalene							x			
	Nonanal	x	x			x	x	x	x		
	Nonanoicacid				x						
	Nonanoicacid,ethylester			x	x						
	Octadecane							x	x		
	Octanoicacid,ethylester			x	x						
	Phenol					x	x				
	Tetradecane	x	x					x	x		
	Tetradecanoicacid,ethylester			x							
	Tridecane							x	x		
	Undecane	x							x		

Comparisons of compounds that were extracted at fasting and postprandial sampling times from the five biological specimens for subject Male 5 are summarized in Table 36. For each specimen, the compounds that were extracted from both fasting and postprandial samplings were subjected to paired *t*-test of significance to evaluate whether the variation in the amounts of the compound extracted was statistically significant or not. For hand odor, nine out of the twelve compounds extracted were common across fasting and postprandial samplings: undecane, nonanal, decanal, tridecane, tetradecane, hexadecane, heptadecane, and octadecane. The extracted amounts were not statistically significant for any of the common compounds. For buccal swabs, ten out of the twenty compounds extracted were common across fasting and postprandial samplings: 2-pentylfuran, hexanoic acid, 6-methyl-3,5-heptadiene-2-one, 1-dodecene, decanal, nonanoic acid, 6-dodecanone, 1-tetradecene, (E)-6,10-dimethyl-5,9-undecadien-2-one, and 1-decene. When subjected to paired *t*-test of significance, the differences in the amounts of these common compounds extracted were not statistically significant. For breath, eight out of the thirteen compounds extracted were common across fasting and postprandial samplings: toluene, p-xylene, phenol, 1,2-dichlorobenzene, nonanal, decanal, tetradecane, and butylated hydroxytoluene. The amount extracted for phenol was statistically significantly different between the two samplings. For blood, ten out of the twenty-one compounds extracted were common between samplings: hexanal, dimethylsulfone, undecane, nonanal, dodecane, tridecane, tetradecane, 1-decene, hexadecane, diisopropylnaphthalene, and 2,6-diisopropylnaphthalene. None of the common compounds were found to have a statistically significant difference in the amount extracted betweens samplings. Finally for urine, five out of the fourteen

compounds extracted were common between samplings: pyrrole, 4-heptanone, dimethylsulfone, nonanal, and menthol. Menthol was found to be statistically significantly different in the amount extracted between fasting and postprandial samplings.

Table 36. Summary of compounds extracted fasting (AM) vs. postprandial (PM) for Male 5

		Hand		Buccal		Breath		Blood		Urine	
		AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
	.beta.-Phellandrene										x
	1,3-Cyclohexadiene,1-methyl-4-(1-methylethyl)-										x
	1,4-Cyclohexadiene,1-methyl-4-(1-methylethyl)-										x
	1-Decene			x	x			x	x		
	1-Dodecene			x	x				x		
	1-Heptanol								x		
	1-Hexadecene			x							
	1-Hexanol			x							
	1-Hexanol,2-ethyl-					x					
	1-Pentanol								x		
	1-Tetradecene			x	x						
	2,4-Nonadienal				x						
	2,5-Octanedione								x		
	2,6-Diisopropylnaphthalene							x	x		
	4-Heptanone									x	x
	5,9-Undecadien-2-one,6,10-dimethyl-, (E)-		x	x	x						
	6-Dodecanone			x	x						
	6-Methyl-3,5-heptadiene-2-one			x	x						
	Benzaldehyde,2,4,6-trimethyl-							x			
	Benzene,1,2-dichloro-					x	x				
	Benzene,1,3-dimethyl-					x	x				
	Benzene,1-methyl-4-(1-methylethenyl)-										x
	Benzene,1-methyl-4-(1-methylethyl)-										x
	Benzenemethanol,.alpha.,.alpha.,4-trimethyl-										x
	Benzophenone						x				
	BenzylAlcohol							x			
	ButylatedHydroxytoluene				x	x	x				
	Carvone									x	
	Caryophyllene				x						
	Cedrol										x
	Cyclopropane,nonyl-			x							
	Decanal	x	x	x	x	x	x		x		
	DiethylPhthalate				x						
	Diisopropylnaphthalene							x	x		
	Dimethylsulfone							x	x	x	x
	Dimethyltrisulfide										x
	D-Limonene						x				
	Dodecane							x	x		
	Dodecanoicacid		x								
	Furan,2-pentyl-			x	x				x		
	Heptadecane	x	x								
	Hexadecane	x	x					x	x		
	Hexanal						x	x	x		
	Hexanoicacid			x	x						
	Menthol						x		x	x	x
	Naphthalene,2-methyl-			x							
	Nonanal	x	x			x	x	x	x	x	x
	Nonanoicacid			x	x						
	Nonanoicacid,ethylester				x						
	Octadecane	x	x						x		
	Pentadecane	x									
	Phenol					x	x				
	Pyrrole									x	x
	Tetradecane	x	x			x	x	x	x		
	Toluene					x	x				
	Tridecane	x	x					x	x		
	Undecanal		x								
	Undecane	x	x					x	x		
	Δ-Cadinene			x							

3.6.3.1. Cluster Analysis

Cluster analysis is a statistical method which classifies a set of observations into subsets, or clusters, based on similarities or dissimilarities (distances). The Bray-Curtis Similarity Index (BSCI) is a normalization method which measures the resemblance, or similarity, between samples. Bray-Curtis, originally a dissimilarity measure that falls between zero and one, is multiplied by 100 to give percentage similarity. The equation for BCSI is as follows:

$$BSCI = 100\left(1 - \frac{\sum_{i=1}^{n}|X_{ij} - X_{ik}|}{\sum_{i=1}^{n}(X_{ij} + X_{ik})}\right) \quad \textbf{Equation 5}$$

X*ij* is the peak area for the *i*th compound from sample *j*. Data classified using BSCI can be displayed in the form of a two-dimensional diagram known as a dendogram. Hierarchical clusters represented by dendograms were constructed using the Minitab 15 Statistical Software (Minitab Inc., State College, PA, USA).

The following figures (Figure 68 through Figure 72) demonstrate, in semi-quantitative fashion, examples of the relative ratios of the peak areas of the compounds extracted in the headspace above the collected specimen samples along with the corresponding dendograms which show the similarity between the VOC profiles in the intra-day (fasting vs. postprandial) specimen samples from a male subject and a female subject. The greatest variation between the intra-day samples were observed in urine. Urinary volatiles should be most susceptible to variation because urinary components are filtered waste products being secreted from the body. The intra-day variation of urine is affected by the food and beverages consumed by the subject. However, sampling immediately after

consuming a meal as it was done in this study should not have influenced the VOC profiles, because the metabolites and waste products from the meal consumed prior to sampling will not have been secreted yet. Therefore, it should be noted that the intra-day variation of urine samples in this study are not results from fasting or postprandial prior to sampling.

Figure 68. Color chart and dendogram for hand odor for M2 and F15

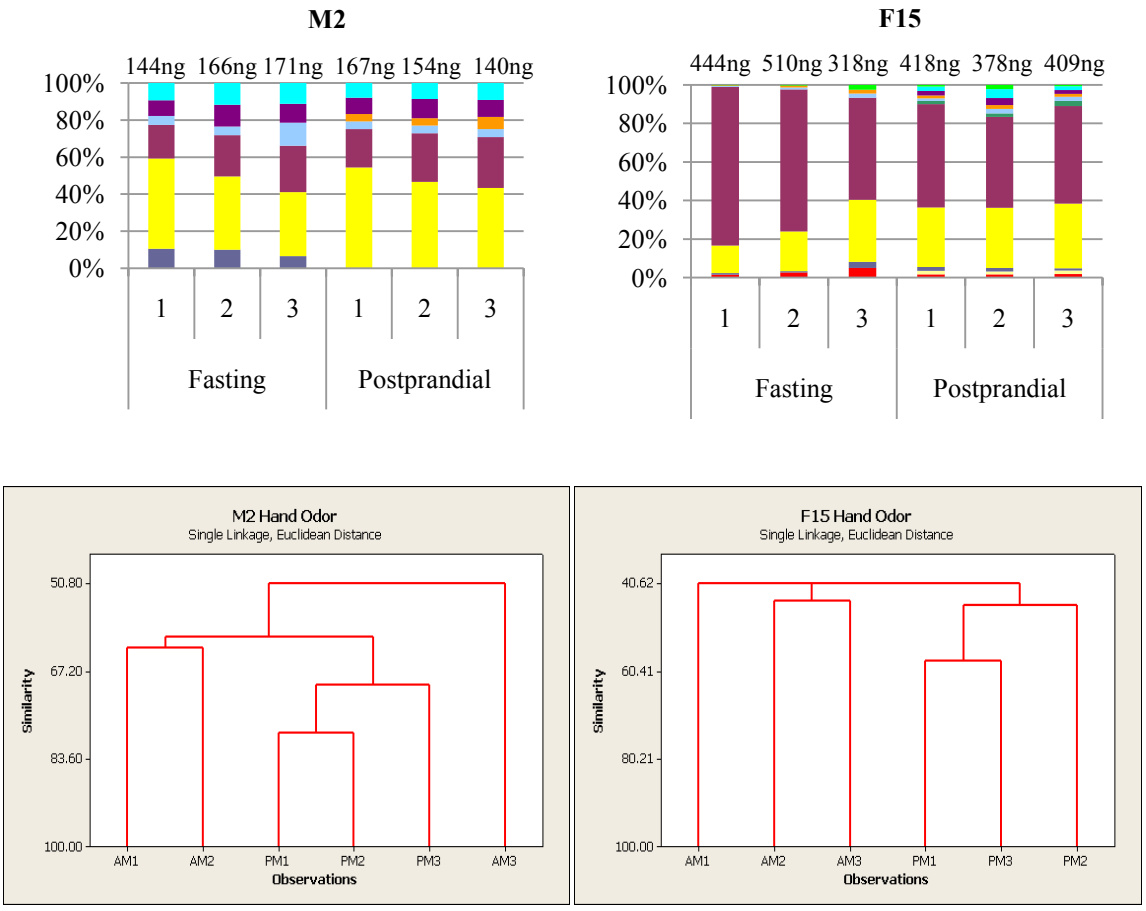
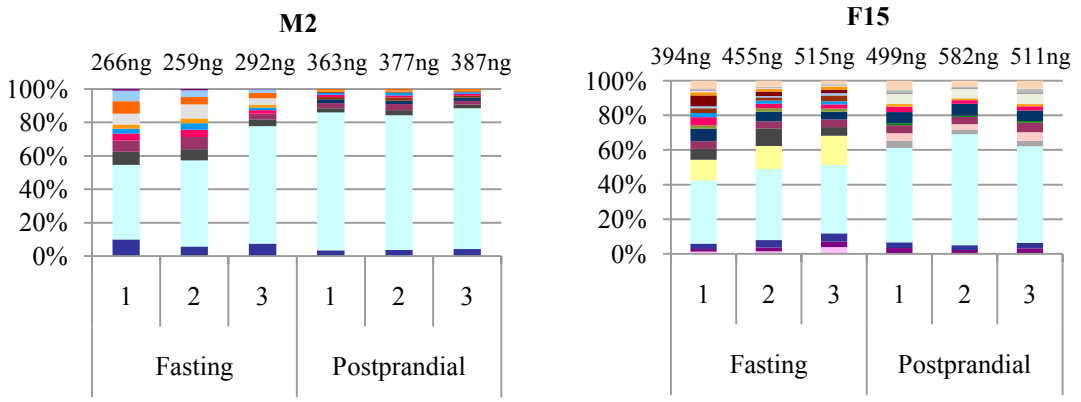
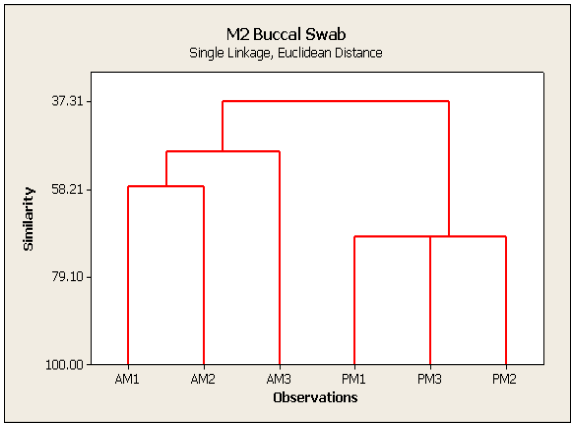


Figure 69. Color chart and dendogram for buccal swabs for M2 and F15





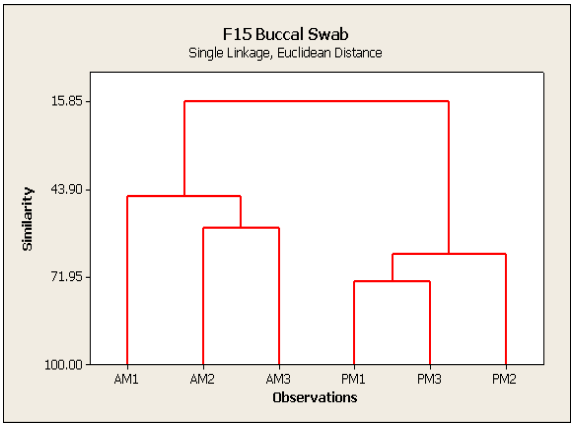


Figure 70. Color chart and dendogram for breath for M2 and F15

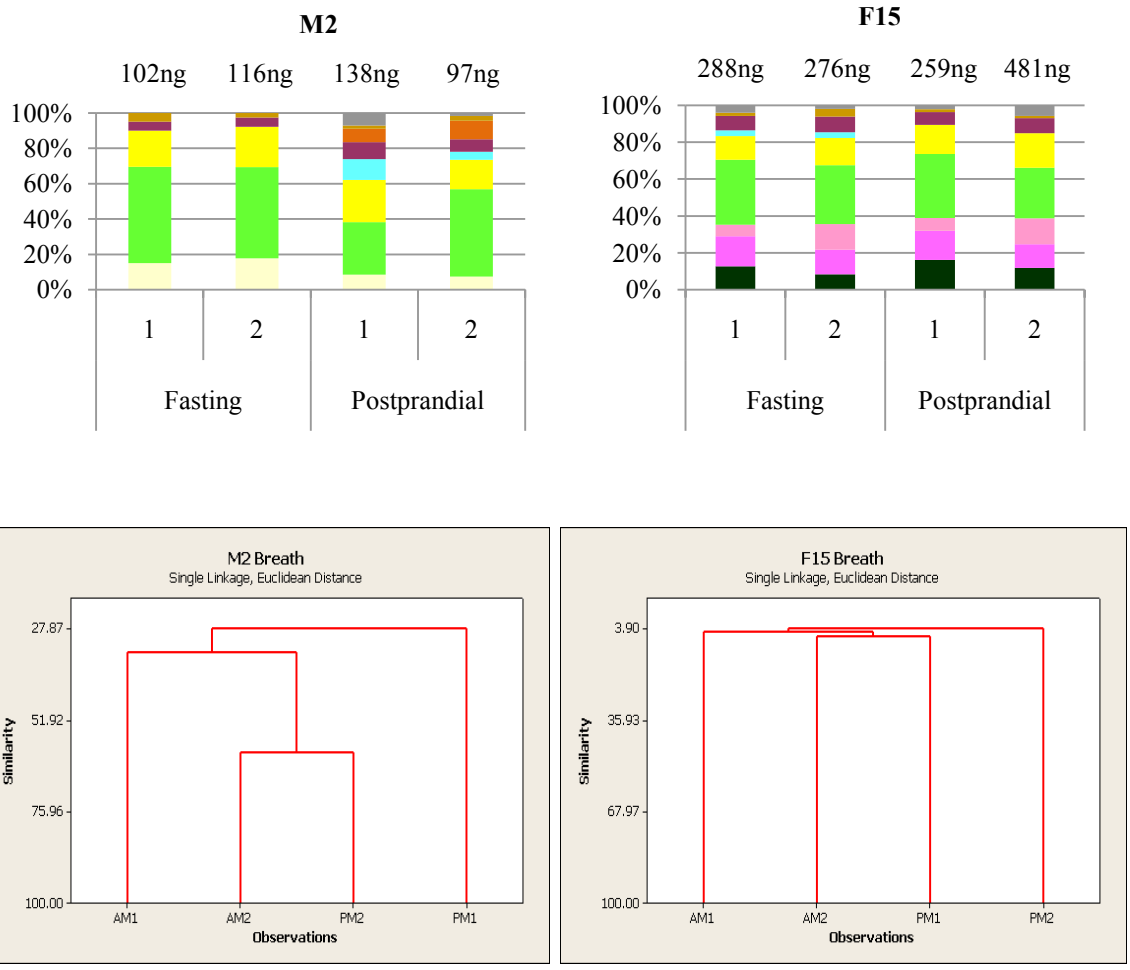


Figure 71. Color chart and dendogram for blood for M2 and F15

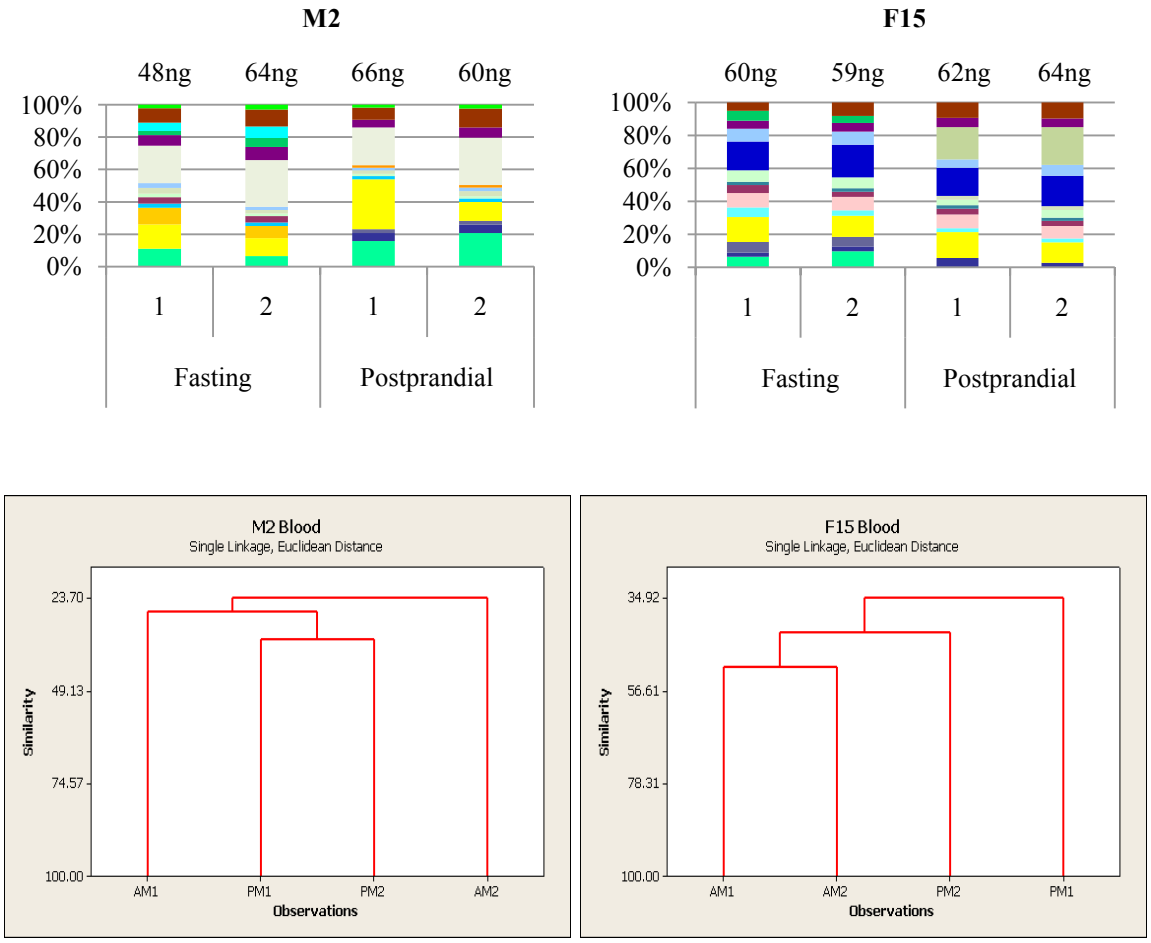
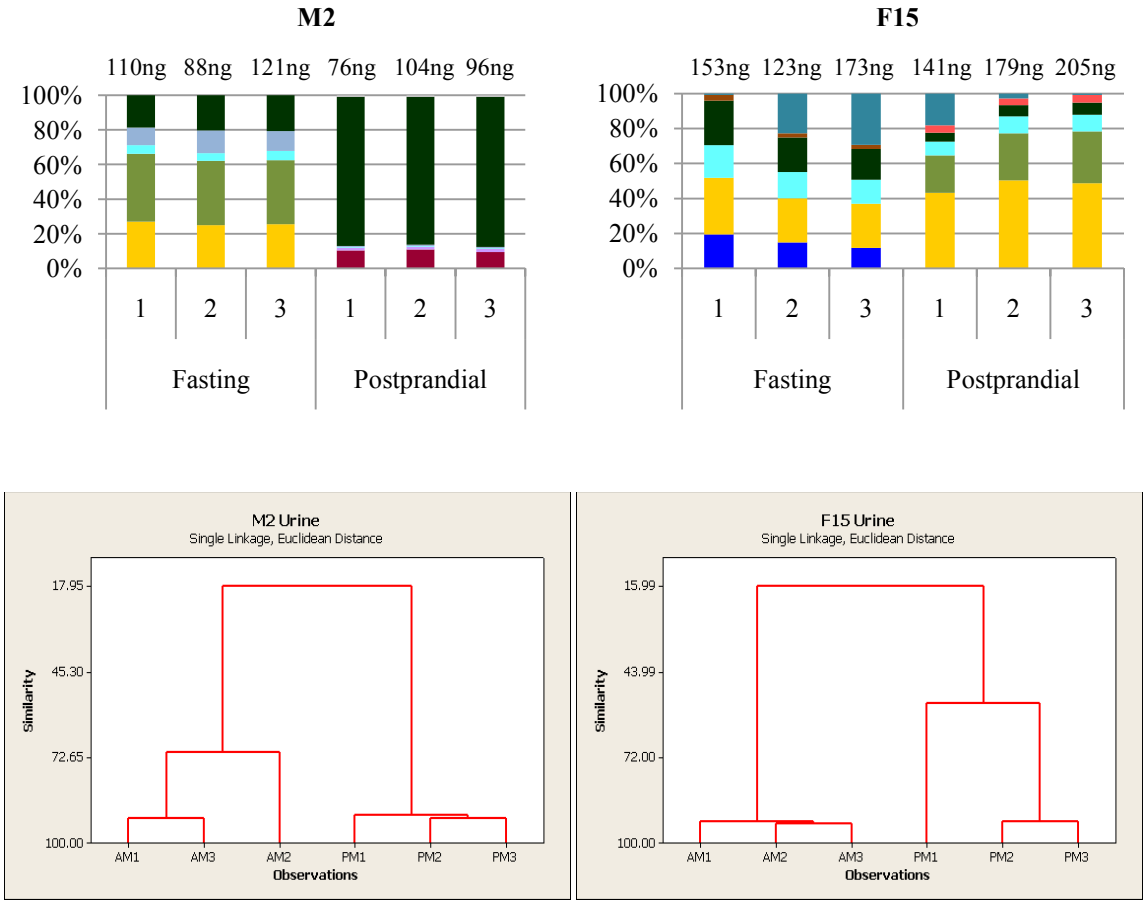


Figure 72. Color chart and dendogram for urine for M2 and F15



The effect of fasting prior to sampling was evaluated through the number of compounds extracted, paired *t*-test of significance of the amount of compounds extracted both at fasting and postprandial samplings, relative peak area ratios of the compounds extracted, and cluster analysis using BCSI. While the fasting samples and postprandial samples are more similar within the same sampling condition, the difference in the VOC profiles of samples collected at fasting and postprandial were not drastic enough to conclude that fasting is crucial prior to sampling. Fasting prior to sampling is more important for

medical purposes, particularly for blood testing (i.e., blood glucose or blood cholesterol). This is particularly true for patients with diabetes. Food intake disturbs blood glucose homeostasis and changes metabolic response of the body to ingested materials. Fasting can also affect the presence of ketone bodies in urine, resulting in higher production of ketone bodies (ketonuria). Additionally, for medical purposes, first morning urine is usually a preferred screening sample because it is most concentrated and prevents false-negative tests.

Forensically, fasting prior to sampling is not as important as it would be for medical profiling purposes. In real life scenarios, samples collected from suspects or subjects will have been sampled at random time of the day and will most likely not be fasting. With only a small increase in the number of compounds extracted and the amount of common compounds extracted between fasting and postprandial sampling being mostly not statistically significant, whether or not the suspect or subject of interest was fasting or had just consumed food should not greatly influence the specimen VOC profile in terms of forensic applications.

3.6.4. Population Analysis of the Volatile Organic Compounds Present Above Collected Odor Samples

3.6.4.1. Evaluation of Odor Profiles of Healthy Individuals

Odor profiles of thirty-one healthy individuals were evaluated using SPME-GC/MS. Compounds were identified by spectral library (NIST) and/or by standard reference comparison. Thirty-six VOCs were extracted across the hand odor samples collected from each of the thirty-one subjects. The compounds ranged in functionality, including:

acids (2.8%), alcohols (11.1%), aldehydes (27.8%), aliphatics (38.9%), esters (11.1%), and ketones (8.3%) as shown in Figure 73. The frequency of the occurrence of the VOCs extracted in healthy subjects' hand odor are listed in Table 37 and displayed in the histogram in Figure 74. Of the short to mid-chain aldehydes (C_5-C_{14}) extracted from hand odor, nonanal, decanal, and undecanal were present in more than 90% of the subjects' odor profiles. Hand odor (skin of palms of hands and forearms) is comprised of secretions from the sebaceous and the eccrine glands. Aldehydes have been reported to be oxidative degradation products of sebaceous secretion components[5,14]. Likewise, alcohols and free fatty acids derive from the interaction of sebaceous secretion components and bacteria residing on skin[14]. Hydrolysis of triglycerides from cutaneous bacterial activity results in free fatty acids (mostly shorter than C_{20}, as was the case in the present study). Acids however are not as prominent in skin odor as aldehydes or aliphatics, as many of the shorter chain free fatty acids (C_6-C_{10}) are reduced to alcohols on skin[123]. Mid- to long-chain alkanes (C_{11}-C_{20}) were observed in the present study, of which tetradecane, hexadecane, and heptadecane were present in over 90% of the subjects. Three ketones were extracted, of which (E)-6,10-dimethyl-5,9-undecadien-2-one (geranyl acetone) was present in over two thirds of the subjects.

Figure 73. Frequency distribution of functional groups of extracted volatile organic compounds for hand odor

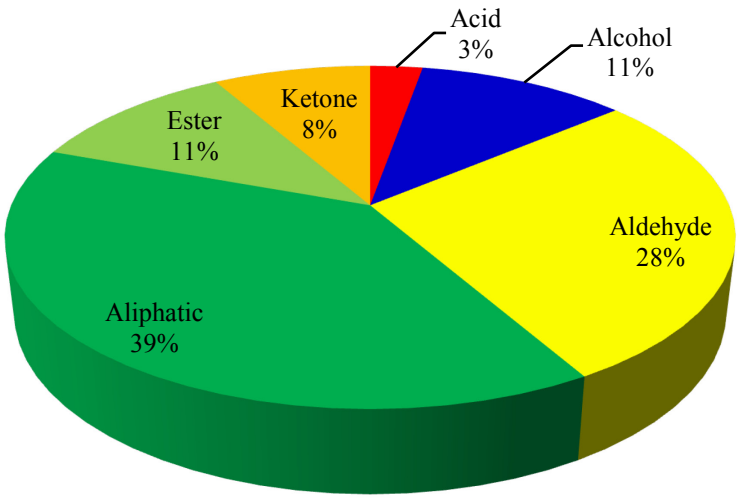
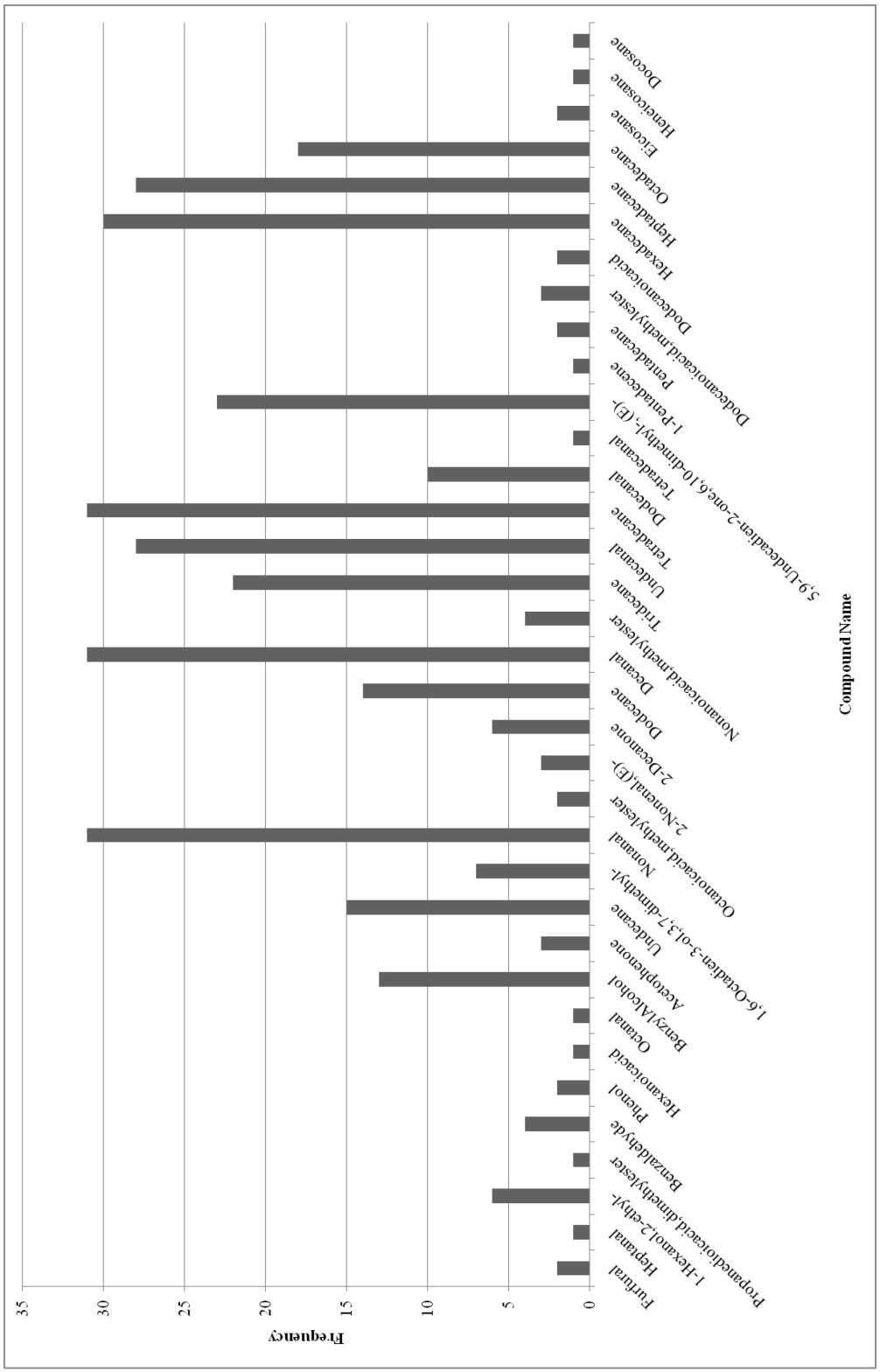


Table 37. Identified VOCs in hand odor of thirty-one healthy individuals ranked by frequency of occurrence (Note: * denotes compound was verified by reference standard)

Compound Name	Frequency			Occurrence (%)
	Female	Male	Total	
Decanal*	16	15	31	100
Nonanal*	16	15	31	100
Tetradecane*	16	15	31	100
Hexadecane*	15	15	30	96.8
Heptadecane*	14	14	28	90.3
Undecanal*	14	14	28	90.3
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-*	9	14	23	74.2
Tridecane*	9	13	22	71.0
Octadecane*	8	10	18	58.1
Undecane	8	7	15	48.4
Dodecane*	9	5	14	45.2
Benzyl Alcohol*	5	8	13	41.9
Dodecanal*	5	5	10	32.3
1,6-Octadien-3-ol,3,7-dimethyl-*	4	3	7	22.6
1-Hexanol,2-ethyl-*	4	2	6	19.4
2-Decanone*	3	3	6	19.4
Benzaldehyde*	3	1	4	12.9
Nonanoicacid,methylester	2	2	4	12.9
2-Nonenal,(E)-*	2	1	3	9.7
Dodecanoicacid,methylester	2	1	3	9.7
Dodecanoicacid*	0	2	2	6.5
Eicosane*	0	2	2	6.5
Furfural*	1	1	2	6.5
Octanoicacid,methylester*	0	2	2	6.5
Phenol*	1	1	2	6.5
1-Pentadecene*	1	0	1	3.2
Docosane*	0	1	1	3.2
Heneicosane*	0	1	1	3.2
Heptanal*	0	1	1	3.2
Hexanoicacid	0	1	1	3.2
Octanal*	1	0	1	3.2
Propanedioicacid,dimethylester*	1	0	1	3.2
Tetradecanal	1	0	1	3.2
Acetophenone*	2	1	3	0.1
Pentadecane*	0	2	2	0.1

Figure 74. Histogram of VOCs in hand odor across thirty-one healthy individuals



For buccal swab samples, 109 VOCs were extracted across the samples collected from the thirty-one healthy individuals. The functionality distribution of the extracted volatile compounds was as follows: acids (10.1%), alcohols (5.5%), aldehydes (11.0%), aliphatics (26.6%), aromatics (12.8%), esters (21.1%), ketones (11.9%), and others (0.9%). Figure 75 displays a pie chart of the functionality distribution. The frequency of the occurrence of the buccal swab VOCs from the thirty-one healthy individuals are listed in Table 38 and displayed in the histogram in Figure 76. The compounds with the highest frequency of occurrences (>80.0%) were (E)-6,10-dimethyl-5,9-undecadiene-2-one, decanal, hexanoic acid, 2-pentylfuran, (E,E)-2,4-nonadienal, nonanoic acid ethyl ester, and (E)-2-nonenal.

While aliphatic compounds have the highest frequency distribution of functional groups in buccal swab odor, top two-thirds of the compounds detected in the headspace of buccal swab samples were fatty acid ester compounds, and specifically ethyl esters of long chain fatty acids (C_8-C_{16}). Carboxylic acids were also widely present in buccal swab samples. Hexanoic acid was the most predominant compound extracted from buccal swabs (and present in 100% of the subjects); its chromatographic peaks were usually very large and with a wide peak width also. Hexanoic acid peaks in the gas chromatograms of buccal swabs were generally not a chromatographically ideal peak, making quantitation for hexanoic acid difficult.

It is difficult to specify the exact origin of the VOCs found in buccal swab, as there are many possible routes of VOC entry into the salivary flow. Volatile constituents of oral fluids can be attributed to environmental and occupational exposure through inhalation of

air and/or water vapor through the lungs, ingestion through the mouth, and transdermal absorption through the skin. Putrefactive activities of microorganisms in the mouth also affect the production of volatiles in buccal swab samples. Blood-saliva partition must be studied from the size and solubility (in water or lipids) of the volatile compounds.

Figure 75. Frequency distribution of functional groups of extracted volatile organic compounds for buccal swabs

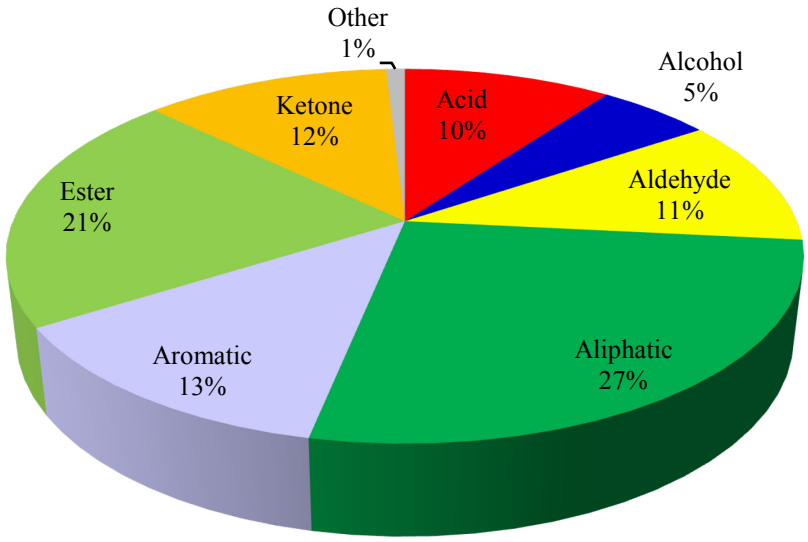


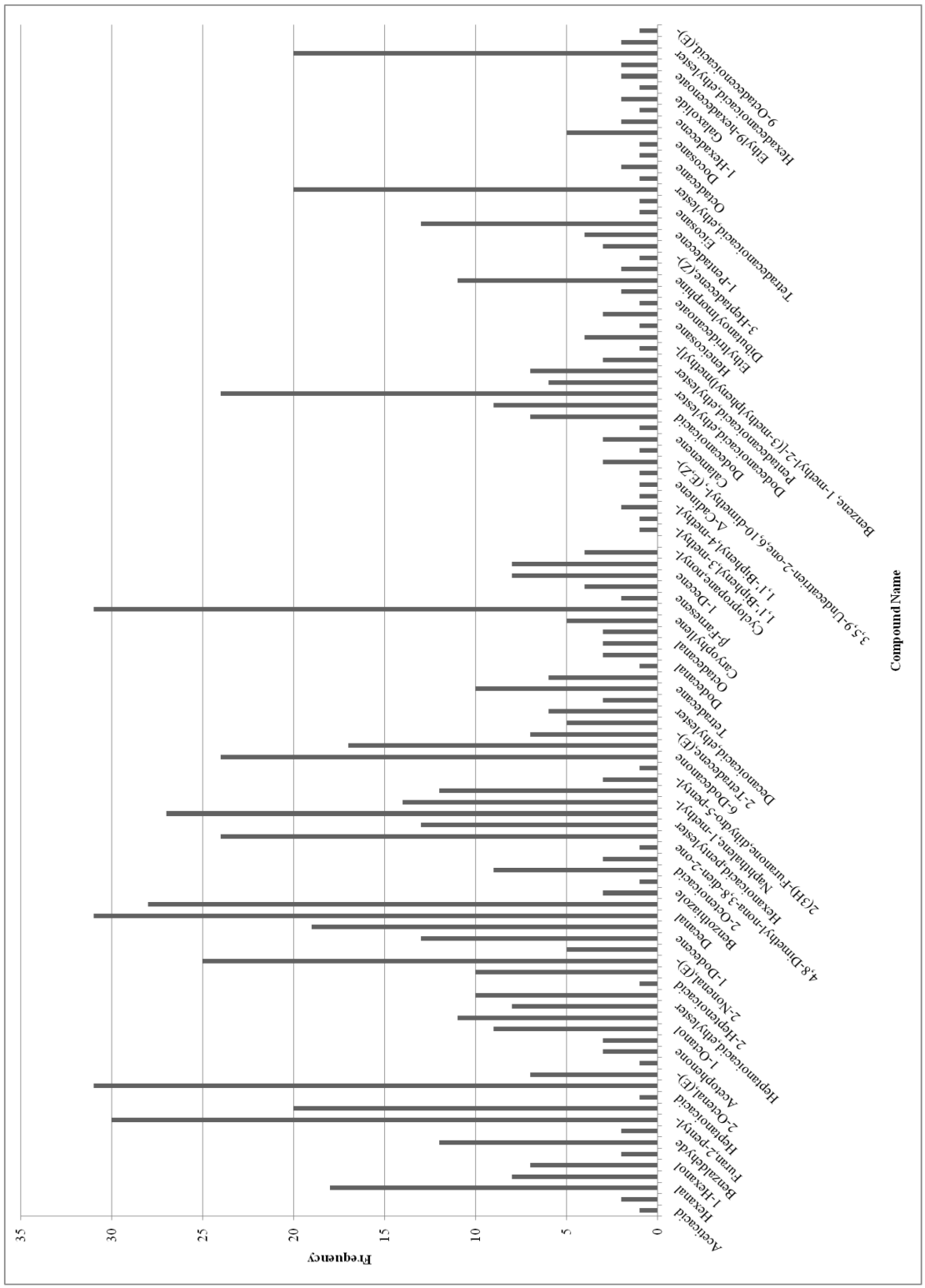
Table 38. Identified VOCs in buccal swab of thirty-one healthy individuals ranked by frequency of occurrence (*Note*: *denotes compound was verified by reference standard)

Compound	Frequency			Occurrence (%)
	Female	Male	Total	
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-*	16	15	31	100
Decanal*	16	15	31	100
Hexanoicacid*	16	15	31	100
Furan,2-pentyl-*	15	15	30	96.8
2,4-Nonadienal,(E,E)-*	14	14	28	90.3
Nonanoicacid,ethylester*	13	14	27	87.1
2-Nonenal,(E)-*	12	13	25	80.7
6-Dodecanone*	14	10	24	77.4
Dodecanoicacid,ethylester*	11	13	24	77.4
Nonanoicacid	13	11	24	77.4
Hexadecanoicacid,ethylester	8	12	20	64.5
Hexanoicacid,ethylester	7	13	20	64.5
Tetradecanoicacid,ethylester	8	12	20	64.5
Octanoicacid,ethylester*	9	10	19	61.3
Hexanal*	10	8	18	58.1
1-Tetradecene*	6	11	17	54.8
Naphthalene,1-methyl-*	8	6	14	45.2
1,1'-Biphenyl,2,2'-diethyl-	6	7	13	41.9
1-Dodecene*	4	9	13	41.9
Hexanoicacid,pentylester	6	7	13	41.9
Benzaldehyde*	9	3	12	38.7
Naphthalene,2-methyl-	6	6	12	38.7
Dibutanoylmorphine	3	8	11	35.5
Linalool oxide	5	6	11	35.5
6-Methyl-3,5-heptadiene-2-one	3	7	10	32.3
Tetradecane*	5	5	10	32.3
3-Nonen-2-one	4	6	10	32.2
1-Octanol*	4	5	9	29.0
2-Octenoicacid	5	4	9	29.0
DiethylPhthalate	3	6	9	29.0
1-Decene*	3	5	8	25.8
Cyclododecane	6	2	8	25.8
Furfural*	1	7	8	25.8
Heptanoicacid,ethylester*	4	4	8	25.8
1-Hexanol*	2	5	7	22.6
2-Octenal,(E)-*	1	6	7	22.6
2-Tetradecene,(E)-	5	2	7	22.6
Dodecanoicacid*	1	6	7	22.6
Pentadecanoicacid,ethylester	0	7	7	22.6
Decanoicacid,ethylester*	2	4	6	19.4

Hexadecane*	2	4	6	19.4
Naphthalene,2,7-dimethyl-	2	4	6	19.4
Benzoicacid,ethylester	3	2	5	16.1
Caryophyllene*	2	3	5	16.1
IsopropylMyristate	3	2	5	16.1
Vanillin	0	5	5	16.1
1-Pentadecene*	2	2	4	12.9
p-Benzoquinone	3	1	4	12.9
Benzophenone*	1	3	4	12.9
Cyclopropane,nonyl-	1	3	4	12.9
2(3H)-Furanone,5-ethyldihydro-	3	0	3	9.7
2(3H)-Furanone,dihydro-5-pentyl-	0	3	3	9.7
3,5,9-Undecatrien-2-one,6,10-dimethyl-, (E,Z)-	0	3	3	9.7
3,7-Dimethyl-octa-1,6-diene	1	2	3	9.7
Acetophenone*	2	1	3	9.7
Benzene,1,1'-methylenebis[4-methyl-	2	1	3	9.7
Benzene,1-methyl-2-[(4-methylphenyl)methyl]-	0	3	3	9.7
Benzothiazole	2	1	3	9.7
Calamenene	1	2	3	9.7
Heptadecane*	1	2	3	9.7
Hexanoicacid,anhydride	1	2	3	9.7
Octadecanal	1	2	3	9.7
OctanoicAcid*	2	1	3	9.7
β-Bourbonene	2	1	3	9.7
1,1'-Biphenyl,4-methyl-	0	2	2	6.5
1-Heptadecene	0	2	2	6.5
1-Hexadecene	0	2	2	6.5
1-Pentanol	1	1	2	6.5
Cyclodecane	0	0	0	6.5
Cyclotetradecane	1	1	2	6.5
E-11-Hexadecenoicacid,ethylester	1	1	2	6.5
Ethyl9-hexadecenoate	0	2	2	6.5
Galaxolide	1	1	2	6.5
IsopropylPalmitate	1	1	2	6.5
Octadecane	1	1	2	6.5
Pentanoicacid*	1	1	2	6.5
Phenol*	2	0	2	6.5
β-Farnesene	1	1	2	6.5
1,1'-Biphenyl,2-ethyl-	1	0	1	3.2
1,1'-Biphenyl,3-methyl-	0	1	1	3.2
2(3H)-Furanone,dihydro-5-propyl-	0	1	1	3.2
2-Dodecenal,(E)-	0	1	1	3.2
2-Heptenoicacid	0	1	1	3.2
3-Eicosene,(E)-	0	1	1	3.2
3-Heptadecene,(Z)-	1	0	1	3.2
4,8-Dimethyl-nona-3,8-dien-2-one	1	0	1	3.2
7-Hexadecene,(Z)-	1	0	1	3.2

9-Octadecenoicacid,(E)-	0	1	1	3.2
Aceticacid	0	1	1	3.2
Benzaldehyde,4-(1-methylethyl)-	1	0	1	3.2
Benzene, 1-methyl-2-[(3-methylphenyl)methyl]-	1	0	1	3.2
ButylatedHydroxytoluene	1	0	1	3.2
Docosane*	1	0	1	3.2
Dodecanal*	1	0	1	3.2
Dodecanoicacid,methylester*	0	1	1	3.2
Eicosane*	1	0	1	3.2
Ethyltridecanoate	0	1	1	3.2
Heneicosane*	1	0	1	3.2
Heptanoicacid*	0	1	1	3.2
Hexadecanoicacid,methylester	1	0	1	3.2
Nonadecanoicacid,ethylester	0	1	1	3.2
Pentadecane,7-methyl-	1	0	1	3.2
Tetradecanoicacid,2-methyl-,methyl ester	0	1	1	3.2
γ-Cadinene	1	0	1	3.2
Δ-Cadinene	0	1	1	3.2

Figure 76. Histogram of VOCs in buccal swab odor from healthy individuals



Across the breath samples collected from the healthy subjects, 83 volatile compounds were extracted. The functionality distribution of the extracted volatile compounds was as follows: acids (2.4%), alcohols (8.4%), aldehydes (10.8%), aliphatics (30.1%), aromatics (34.9%), esters (7.2%), and ketones (6.0%). Figure 77 displays a pie chart of the distribution of the seven functional groups. The frequency of the occurrence of the breath VOCs from the thirty-one healthy individuals are listed in Table 39 and displayed in the histogram in Figure 78. The highest frequency occurring VOCs for breath samples were decanal, nonanal, styrene, benzophenone, 2-ethyl-1-hexanol, and 1,2-dichlorobezene.

Exhaled breath VOCs origin can be attributed to the lungs, nasal cavity, or some systemic pathway from which the VOCs are exhaled following blood-to-alveolar diffusion. Diffusion property depends on the compound's polarity, fat solubility, volatility, and Henry's constant as described in section 0. Blood-breath partition ratio of a compound depends on the compound's lipid or water solubility. Water soluble compounds such as alcohols and ketones can be seen in breath by diffusion through the blood circulation of the respiratory tree to the water-bearing membranes. Therefore, higher concentrations of water-soluble compounds are expected to be observed in exhaled breath. However, water-insoluble (lipophilic) compounds such as straight-chain hydrocarbons can also be observed in exhaled breath because of their ability to diffuse through the lungs. High lipid soluble compounds are eliminated more slowly than lipophobic compounds because they can be deposited and stored in fat before being eliminated[62].

Straight-chain hydrocarbons (C_{10}-C_{20}) were present in the breath odor VOCs extracted from the healthy individuals. These compounds are products of lipid peroxidation of the

polyunsaturated fatty acids[49]. Oxidation by enzymes of the polyunsaturated fatty acids also produce fatty acid hydroperoxides, which are eventually converted to aldehydes, as observed across most subjects (nonanal and decanal are present in 95% of the subjects). Branched alkanes (i.e., 2,6-dimethylundecane) and highly substituted benzenes (i.e., trimethyl-benzenes) were commonly present across all subjects. These compounds are most likely of environmental contaminant origin as they possess negative polarity alveolar gradient which are consistent with VOCs that are of exogenous origin[48]. In other words, VOCs with negative alveolar gradient are eliminated faster than the rate they are synthesized in the body. Naphthalene and methylnaphthalenes have been suggested to be steroid degradation products but the exact origin is still unknown[48].

One compound that is a known volatile constituent of human breath that was not detected in the present study is isoprene. Isoprene is present in all human breath resulting from the mevalonic pathway of cholesterol synthesis. It is important to note here that under the current instrumental conditions isoprene was not detected, most likely due to its high vapor pressure (560 mmHg at 20°C) therefore making isoprene a highly volatile compound that is also extremely unstable and thermally labile to cause degradation at the GC injection port with the current settings.

Figure 77. Frequency distribution of functional groups of extracted volatile organic compounds for breath

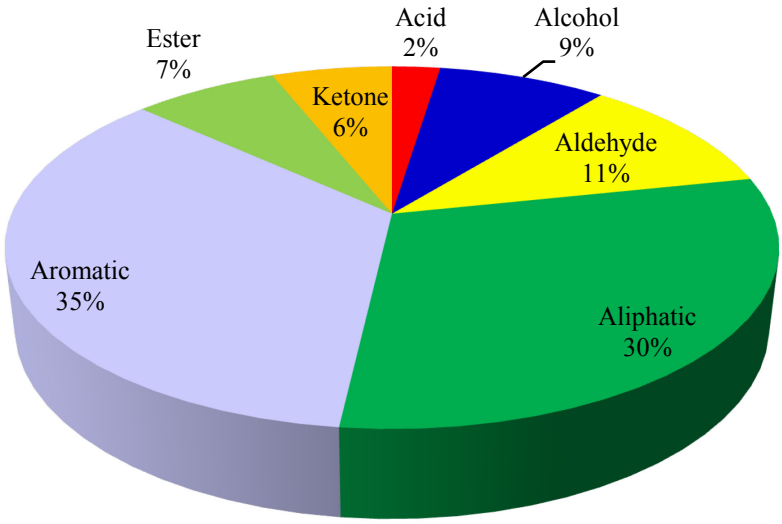
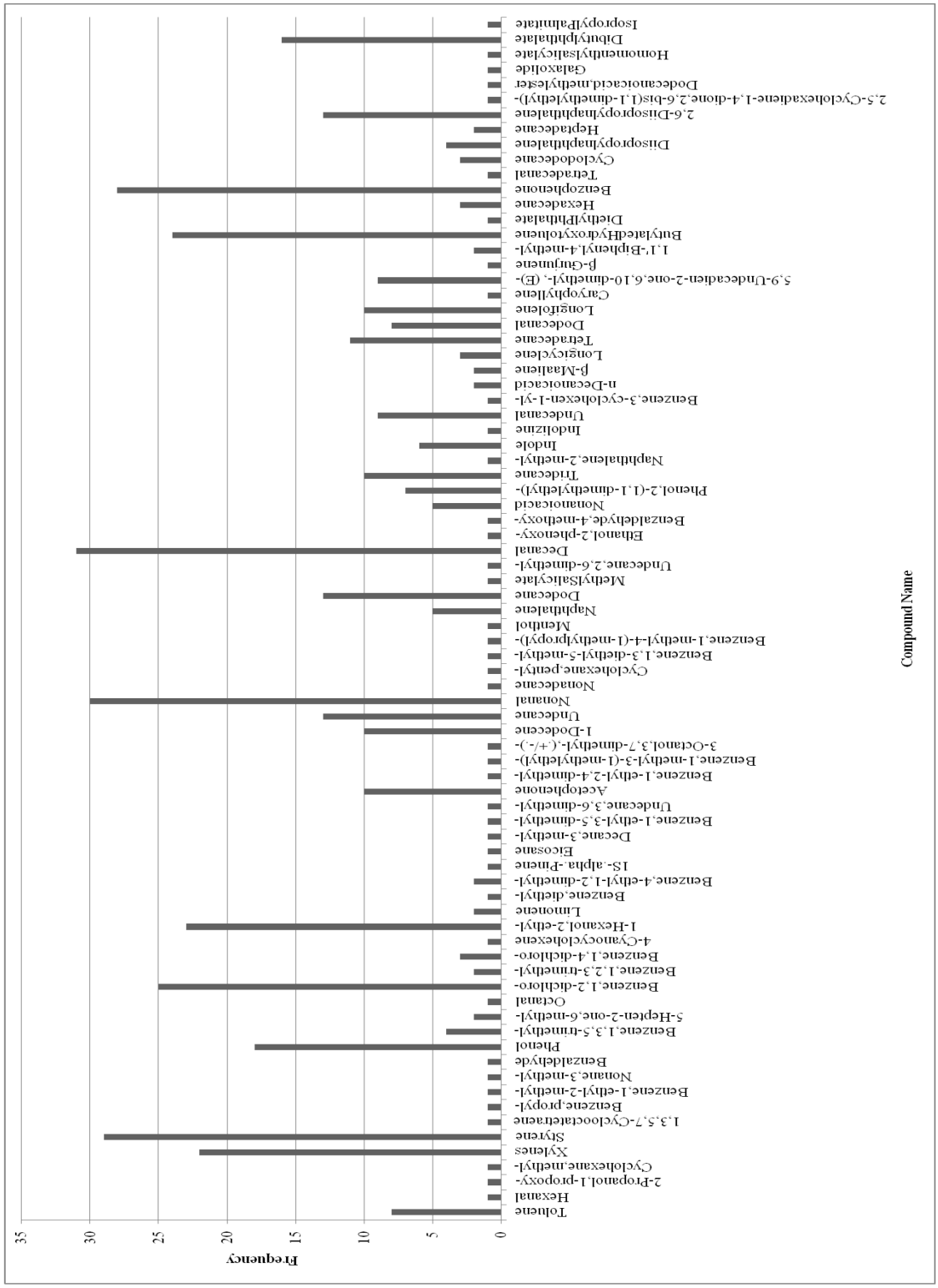


Table 39. Identified VOCs in breath odor of thirty-one healthy individuals ranked by frequency of occurrence (*Note*: *denotes compound was verified by reference standard)

Compound	Frequency			Occurrence (%)
	Female	Male	Total	
Decanal*	16	15	31	100
Nonanal*	15	15	30	96.8
Styrene*	15	14	29	93.6
Benzophenone*	13	15	28	90.3
Benzene,1,2-dichloro-*	13	12	25	80.7
1-Hexanol,2-ethyl-*	11	12	23	80.7
ButylatedHydroxytoluene*	14	10	24	77.4
Xylenes*	11	11	22	71.0
Phenol*	9	9	18	58.1
Dibutylphthalate	10	6	16	51.6
Undecane*	9	4	13	41.9
Dodecane*	9	4	13	41.9
2,6-Diisopropylnaphthalene*	7	6	13	41.9
Tetradecane*	6	5	11	35.5
Acetophenone*	4	6	10	32.3
1-Dodecene*	5	5	10	32.3
Tridecane*	6	4	10	32.3
Longifolene	6	4	10	32.3
Undecanal*	5	4	9	29.0
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-*	6	3	9	29.0
Dodecanal*	4	4	8	25.8
Toluene*	5	3	8	25.8
Phenol,2-(1,1-dimethylethyl)-	5	2	7	22.6
Indole	2	4	6	19.4
Naphthalene*	2	3	5	16.1
Nonanoicacid	3	2	5	16.1
Benzene,1,3,5-trimethyl-	2	2	4	12.9
Diisopropylnaphthalene	3	1	4	12.9
Benzene,1,4-dichloro-	1	2	3	9.7
Longicyclene	1	2	3	9.7
Hexadecane*	1	2	3	9.7
Cyclododecane	2	1	3	9.7
Limonene*	1	1	2	6.5
5-Hepten-2-one,6-methyl-*	1	1	2	6.1
Benzene,1,2,3-trimethyl-	1	1	2	6.1
Benzene,4-ethyl-1,2-dimethyl-	1	1	2	6.1
n-Decanoicacid*	2	0	2	6.1
β-Maaliene	1	1	2	6.1
1,1'-Biphenyl,4-methyl-	0	2	2	6.1
Heptadecane*	1	1	2	6.1

Hexanal*	0	1	1	3.0
2-Propanol,1-propoxy-	0	1	1	3.0
Cyclohexane,methyl-	1	0	1	3.0
1,3,5,7-Cyclooctatetraene	1	0	1	3.0
Benzene,propyl-	1	0	1	3.0
Benzene,1-ethyl-2-methyl-	1	0	1	3.0
Nonane,3-methyl-	1	0	1	3.0
Benzaldehyde*	0	1	1	3.0
Octanal	1	0	1	3.0
4-Cyanocyclohexene	0	1	1	3.0
Benzene,diethyl-	1	0	1	3.0
1S-.alpha.-Pinene	0	1	1	3.0
Eicosane*	1	0	1	3.0
Decane,3-methyl-	1	0	1	3.0
Benzene,1-ethyl-3,5-dimethyl-	1	0	1	3.0
Undecane,3,6-dimethyl-	1	0	1	3.0
Benzene,1-ethyl-2,4-dimethyl-	1	0	1	3.0
Benzene,1-methyl-3-(1-methylethyl)-	1	0	1	3.0
3-Octanol,3,7-dimethyl-,(.+/-.)-	0	1	1	3.0
Nonadecane	1	0	1	3.0
Cyclohexane,pentyl-	1	0	1	3.0
Benzene,1,3-diethyl-5-methyl-	1	0	1	3.0
Benzene,1-methyl-4-(1-methylpropyl)-	1	0	1	3.0
Menthol*	0	1	1	3.0
MethylSalicylate*	0	1	1	3.0
Undecane,2,6-dimethyl-	1	0	1	3.0
Ethanol,2-phenoxy-	0	1	1	3.0
Benzaldehyde,4-methoxy-	1	0	1	3.0
Naphthalene,2-methyl-	1	0	1	3.0
Indolizine	1	0	1	3.0
Benzene,3-cyclohexen-1-yl-	0	1	1	3.0
Caryophyllene*	0	1	1	3.0
β-Gurjunene	1	0	1	3.0
DiethylPhthalate	1	0	1	3.0
Tetradecanal	1	0	1	3.0
p-Benzoquinone	1	0	1	3.0
Dodecanoicacid,methylester	0	1	1	3.0
Galaxolide	0	1	1	3.0
Homomenthylsalicylate	0	1	1	3.0
IsopropylPalmitate	0	1	1	3.0

Figure 78. Histogram of VOCs in breath odor across thirty-one healthy individuals



For blood samples, 77 VOCs were extracted across the samples collected from each of the thirty-one individuals. The compounds ranged in functionality, including: alcohols (18.2%), aldehydes (18.2%), aliphatics (27.3%), aromatics (22.1%), esters (1.3%), ketones (14.3%), and others (3.9%) as shown in Figure 79. Volatile compounds included in the "others" category included ethers and sulfur compound (dimethylsulfone). The frequency of occurrence and histogram of the VOCs found in blood odor from thirty-one healthy individuals are shown in Table 40 and Figure 80.

Among the alcohols that were present in blood odor samples, benzyl alcohol was the only compound found at high frequency of 80.6%. Benzyl alcohol is commonly found in nature in essential oils and is very widely used in the flavor and fragrance industries and thus the exposure of normal humans to benzyl alcohol is very high. 1-Octen-3-ol is a secondary alcohol that is produced by mushrooms and also accounted for the odor of cows[68]. The alcohol 1-octen-3-ol has been reported to be a constituent of human sweat and breath and has been suggested to be an oxidation product from milk fat. The C_7-C_{10} aldehydes were commonly observed in the blood odor of healthy individuals. Hexanal and heptanal were both observed across the subjects. These two compounds have been suggested to be lung cancer biomarkers at elevated levels, but are also known to be present in healthy individuals as a breakdown product of lipid peroxidation in the body[67,68]. Aliphatic compounds included C_7-C_{25} cycloalkanes, C_{11}-C_{25} higher alkanes, and C_{10}-C_{15} higher alkenes, of which C_{11}-C_{16} straight-chain higher alkanes were present in over 90% of subjects.

Major sources of aromatic VOCs including substituted and chlorinated benzenes and xylenes that are of exogenous origin include tobacco products, automobile exhaust, and chlorinated water which today are all extremely difficult to avoid exposure from even for nonoccupationally and nonenvironmentally exposed workers. With increasing air pollutant levels especially in developed countries such as the U.S. where the present study was conducted, it is inevitable to see an above-detection level of exogenous VOCs in human biological specimens even for nonoccupational exposure workers. Alkylnaphthalenes (diisopropylnaphthalene, 2,6-diisopropylnaphthalane) have seen a significant increase in the uses as substitutes for polychlorinated biphenyls (PCBs) over the last several decades, resulting in the increase in their concentrations in the environment. Concentration of 2,6-diisopropylnaphthalene in blood has been shown to be directly proportional to the exposure dosage of the compound, as it is readily absorbed and redistributed in blood and tissues of the body and rapidly bioaccumulated[124].

Figure 79. Frequency distribution of functional groups of extracted volatile organic compounds for blood

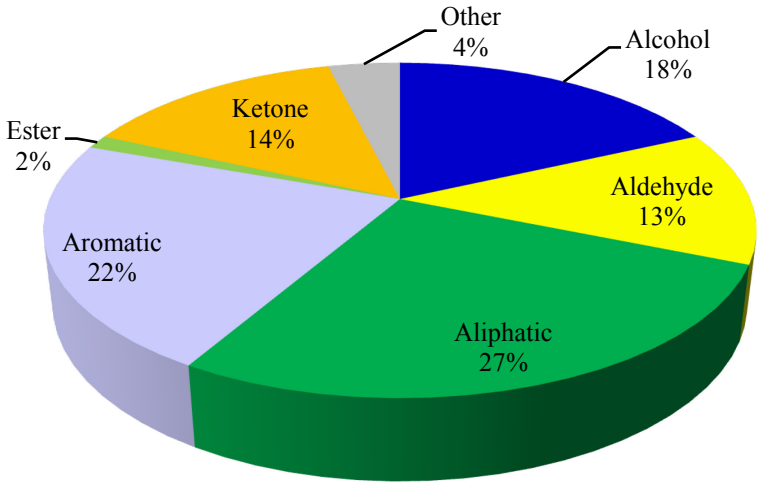
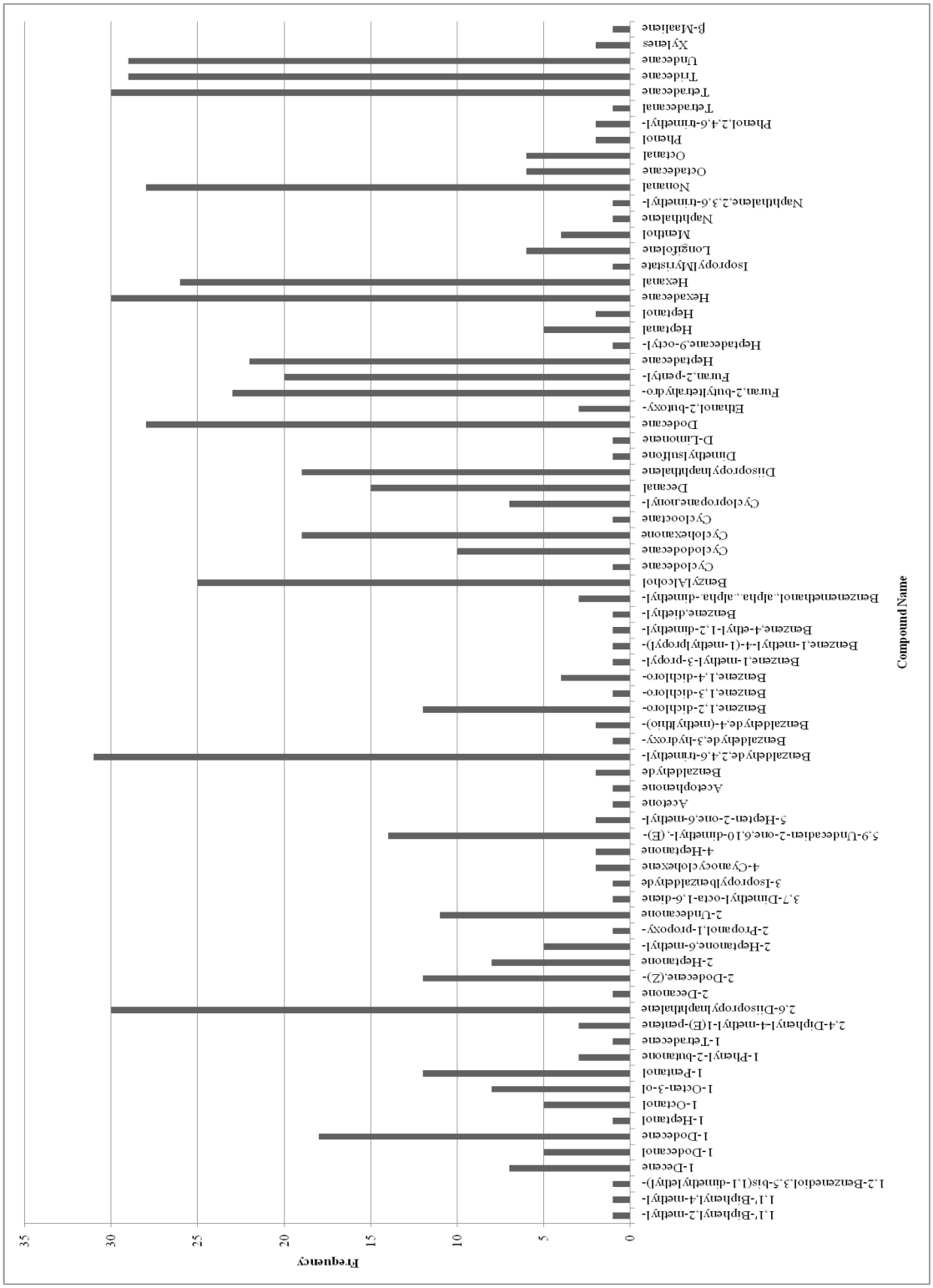


Table 40. Identified VOCs in blood odor of thirty-one healthy individuals ranked by frequency of occurrence (*Note:* *denotes compound was verified by reference standard)

Compound	Frequency			Occurrence (%)
	Female	Male	Total	
Benzaldehyde,2,4,6-trimethyl-	16	15	31	100
2,6-Diisopropylnaphthalene*	15	15	30	96.8
Hexadecane*	15	15	30	96.8
Tetradecane*	15	15	30	96.8
Tridecane*	14	15	29	93.6
Undecane*	14	15	29	93.6
Dodecane*	13	15	28	90.3
Nonanal*	14	14	28	90.3
Hexanal	15	11	26	83.9
BenzylAlcohol*	13	12	25	80.7
Furan,2-butyltetrahydro-	12	11	23	74.2
Heptadecane*	13	9	22	71.0
Furan,2-pentyl-*	9	11	20	64.5
Cyclohexanone*	10	9	19	61.3
Diisopropylnaphthalene	10	9	19	61.3
1-Dodecene*	6	12	18	58.1
Decanal*	6	9	15	48.4
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-*	6	8	14	45.2
1-Pentanol	6	6	12	38.7
2-Dodecene,(Z)-	6	6	12	38.7
Benzene,1,2-dichloro-*	6	6	12	38.7
2-Undecanone	4	7	11	35.5
Cyclododecane	5	5	10	32.3
2-Heptanone*	5	3	8	25.8
1-Octen-3-ol*	4	4	8	25.5
1-Decene*	1	6	7	22.6
Cyclopropane,nonyl-	4	3	7	22.6
Longifolene	4	2	6	19.4
Octadecane	2	4	6	19.4
Octanal	2	4	6	19.4
1-Dodecanol	3	2	5	16.1
1-Octanol*	1	4	5	16.1
2-Heptanone,6-methyl-	4	1	5	16.1
Heptanal*	3	2	5	16.1
Benzene,1,4-dichloro-	2	2	4	12.9
Menthol*	2	2	4	12.9
1-Phenyl-2-butanone	1	2	3	9.7

2,4-Diphenyl-4-methyl-1(E)-pentene	2	1	3	9.7
Benzenemethanol,.alpha.,.alpha.-dimethyl-	1	2	3	9.7
Ethanol,2-butoxy-	1	2	3	9.7
4-Cyanocyclohexene	0	2	2	6.5
4-Heptanone*	2	0	2	6.5
5-Hepten-2-one,6-methyl-*	1	1	2	6.5
Benzaldehyde*	1	1	2	6.5
Benzaldehyde,4-(methylthio)-	0	2	2	6.5
Heptanol*	1	1	2	6.5
Phenol*	2	0	2	6.5
Phenol,2,4,6-trimethyl-	2	0	2	6.5
Xylenes*	2	0	2	6.5
1,1'-Biphenyl,2-methyl-	1	0	1	3.2
1,1'-Biphenyl,4-methyl-	0	1	1	3.2
1,2-Benzenediol,3,5-bis(1,1-dimethylethyl)-	0	1	1	3.2
1-Tetradecene*	0	1	1	3.2
2-Decanone*	1	0	1	3.2
2-Propanol,1-propoxy-	1	0	1	3.2
3,7-Dimethyl-octa-1,6-diene	1	0	1	3.2
3-Isopropylbenzaldehyde	1	0	1	3.2
Acetone	1	0	1	3.2
Acetophenone*	0	1	1	3.2
Benzaldehyde,3-hydroxy-	0	1	1	3.2
Benzene,1,3-dichloro-	1	0	1	3.2
Benzene,1-methyl-3-propyl-	1	0	1	3.2
Benzene,1-methyl-4-(1-methylpropyl)-	1	0	1	3.2
Benzene,4-ethyl-1,2-dimethyl-	1	0	1	3.2
Benzene,diethyl-	1	0	1	3.2
Cyclodecane	1	0	1	3.2
Cyclooctane	1	0	1	3.2
Dimethylsulfone	0	1	1	3.2
D-Limonene*	1	0	1	3.2
Heptadecane,9-octyl-	0	1	1	3.2
IsopropylMyristate	0	1	1	3.2
Naphthalene*	0	1	1	3.2
Naphthalene,2,3,6-trimethyl-	0	1	1	3.2
Tetradecanal	1	0	1	3.2
β-Maaliene	0	1	1	3.2

Figure 80. Histogram of VOCs in blood odor across thirty-one healthy individuals



For urine, 87 volatile compounds were extracted across the samples collected from thirty-one individuals. Urinary VOCs were comprised of the widest range in functional groups compared to the other specimens. The functionality distribution of the extracted volatile compounds was as follows: acids (2.3%), alcohols (24.1%), aldehydes (11.5%), aliphatics (13.8%), alkylphenols (AP, 3.4%), aromatics (10.3%), esters (3.4%), ketones (18.4%), nitrogen compounds (4.6%), *O*-heterocyclic compounds (3.4%), and sulfur compounds (5.7%) as seen in Figure 81. Where aromatics, aliphatics, and aldehydes comprised about 75% of the entire functional group distribution other specimens, those major hydrocarbon functional groups together only contributed to approximately one-third of the total range of functional groups found in urine. Over 20% of the urinary VOCs detected were alcohols, which was much higher than the percentage of alcohols present in other specimens. Additionally, sulfur compounds, nitrogen compounds, and O-heterocyclic compounds were present in urine. These functional groups are not completely absent in other specimens, however, they are present at extremely low frequencies or they may be present only at below-detection limit levels. The frequency of occurrence and histogram of the VOCs found in urine odor from thirty-one healthy individuals are shown in Table 41 and Figure 82. 4-Heptanone was the only compound with a frequency of occurrence higher than 80.0% (96.8%).

The volatile compounds ranging in the eleven functional groups are found regularly in human urine and their presences are mostly accounted for by nutrients, intermediates, and environmental contaminants. Many compounds including furans, terpines, pyrroles, carvones, and menthols originate from food and food additives. Some compounds such as

2-ethyl-1-hexanol, p-benzoquinone, and 2,6-(1,1-dimethylethyl)-4-ethyl-phenol were omitted from the urine specimen compound database because they were also present in all water-blank samples that were stored in the same specimen collection container that the actual urine samples were collected and stored in.

Ketones are known to be prominent components of urinary volatiles and they are most of time decarboxylation products of their corresponding oxo-acids that are produced in urine. 4-Heptanone and 2-heptanone have been suggested to be exogenous compounds and their specific origins are still unknown. These ketones are widely observed in normal human urine and have been attributed to the *in vivo* metabolism of plasticizers in humans[70]. 2-Heptanone along with 2-pentanone are also known to be a naturally occurring VOCs in milk[125].

Dichlorobenzenes which were observed in most healthy subjects' urine samples are unmetabolized VOCs that result from exposure. These compounds have been reported to be biomarkers of low level exposure to these chlorinated aromatic VOCs in indoor environments[80]. Lastly, alkylphenols (AP), 4-nonylphenol (or simply nonylphenol, NP) and 4-(1,1,3,3-tetramethylbutyl)-phenol (or 4-*tert*-octylphenol, OP), are prominent constituents of urinary volatiles. These alkylphenols are used as surfactants commonly used in pesticides as "inert" ingredients and have been shown to be environmental contaminants in sewage wastewater, river water, sea water, and as a results in fish tissue[77,78,126]. Alkylphenols are detected in normal human urine due to exposure from daily activities, air, and meals.

Figure 81. Frequency distribution of functional groups of extracted volatile organic compounds for urine (*Note:* AP = alkylphenols)

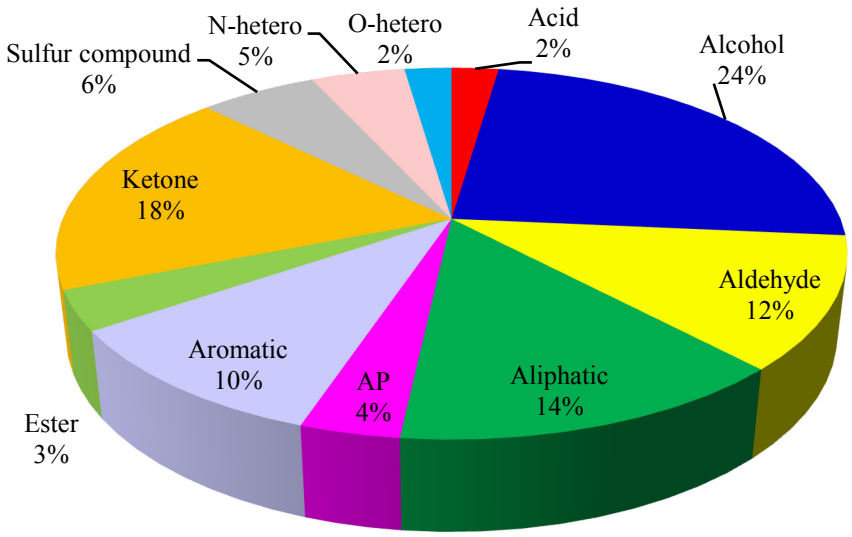


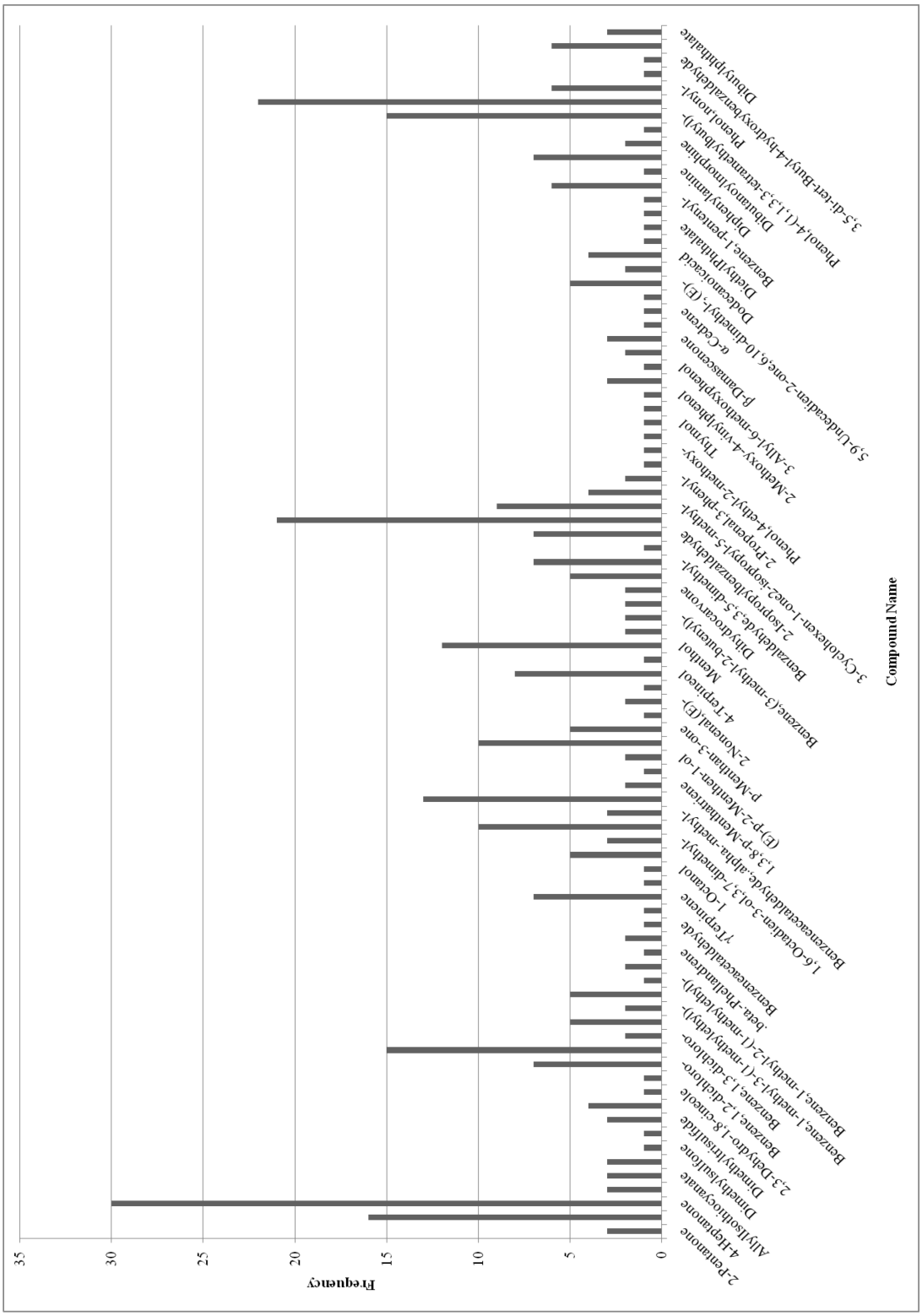
Table 41. Identified VOCs in urine odor of thirty-one healthy individuals ranked by frequency of occurrence (Note: * denotes compound was verified by reference standard)

Compound Name	Frequency			Occurrence (%)
	Female	Male	Total	
4-Heptanone*	16	14	30	96.8
4-Nonylphenol	11	11	22	71.0
Carvone	11	10	21	67.7
Pyrrole	9	7	16	51.6
Benzene,1,4-dichloro-	5	10	15	48.4
Phenol,4-(1,1,3,3-tetramethylbutyl)-*	7	8	15	48.4
Nonanal*	5	8	13	41.9
Menthol*	5	7	12	38.7
Benzene,1-methyl-4-(1-methylethenyl)-	5	5	10	32.3
.+/-.-4-Acetyl-1-methylcyclohexene	6	4	10	32.3
3-Cyclohexen-1-one2-isopropyl-5-methyl-	7	2	9	29.0
4-Terpineol	5	3	8	25.5
Benzene,1,2-dichloro-*	3	4	7	22.6
γ-Terpinene	3	4	7	22.6
Benzaldehyde,3,5-dimethyl-	4	3	7	22.6

2-Isopropylbenzaldehyde	6	1	7	22.6
Benzophenone*	3	4	7	22.6
Cedrol	3	3	6	19.4
Phenol,nonyl-	2	4	6	19.4
N,N-Diethylcarbanilide	2	4	6	19.4
α-Terpinene	1	4	5	16.1
Benzene,1-methyl-4-(1-methylethyl)-	4	1	5	16.1
Phenol,4-methyl- *(p-Cresol)**	3	2	5	16.1
p-Menthan-3-one	3	2	5	16.1
Decanal*	1	4	5	16.1
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-*	4	1	5	16.1
Benzaldehyde*	2	2	4	12.9
p-Menth-1-en-3-one	1	3	4	12.9
Dodecanoicacid*	1	3	4	12.9
2-Pentanone	2	1	3	9.7
Cyclopropane,isothiocyanato-	3	0	3	9.7
AllylIsothiocyanate	2	1	3	9.7
2-Heptanone*	3	0	3	9.7
Dimethyltrisulfide*	3	0	3	9.7
1,6-Octadien-3-ol,3,7-dimethyl-*	1	2	3	9.7
Benzeneacetaldehyde,α-methyl-	2	1	3	9.7
Ethanone,1-(3-methoxyphenyl)-	2	1	3	9.7
β-Damascenone	2	1	3	9.7
Dibutylphthalate	1	2	3	9.7
Benzene,1,3-dichloro-	2	0	2	6.5
Benzene,1-methyl-3-(1-methylethyl)-	1	1	2	6.5
β-Thujene	1	1	2	6.5
Eucalyptol	0	2	2	6.5
1,3,8-p-Menthatriene	2	0	2	6.5
(E)-p-2-Menthen-1-ol	1	1	2	6.5
2-Nonenal,(E)-*	1	1	2	6.5
Benzenemethanol,.alpha.,.alpha.,4-trimethyl-	1	1	2	6.5
Benzene,(3-methyl-2-butenyl)-	1	1	2	6.5
Ethanone,1-(4-methylphenyl)-	1	1	2	6.5
Dihydrocarvone	1	1	2	6.5
2-Propenal,3-phenyl-	1	1	2	6.5
Eugenol	1	1	2	6.5
1-Dodecanol	2	0	2	6.5
Dibutanoylmorphine	2	0	2	6.5
Dimethylsulfone	0	1	1	3.2
3-Heptanone,6-methyl-	0	1	1	3.2
2,3-Dehydro-1,8-cineole	0	1	1	3.2
2,3-Octanedione	1	0	1	3.2
Benzene,1-methyl-2-(1-methylethyl)-	0	1	1	3.2

β-Phellandrene	0	1	1	3.2
Benzeneacetaldehyde	1	0	1	3.2
Cyclopentane,(methylthio)-	0	1	1	3.2
β-Terpineol	1	0	1	3.2
1-Octanol*	1	0	1	3.2
trans-p-Mentha-2,8-dienol	1	0	1	3.2
BenzoicAcid	0	1	1	3.2
Phenol,2,5-dichloro-	0	1	1	3.2
(3E,5Z)-1,3,5-Undecatriene	0	1	1	3.2
Pulegone	1	0	1	3.2
Cyclodecane	1	0	1	3.2
Phenol,4-ethyl-2-methoxy-	1	0	1	3.2
1-Undecanol	0	1	1	3.2
Thymol	0	1	1	3.2
p-Mentha-1,8-dien-7-ol	1	0	1	3.2
2-Methoxy-4-vinylphenol	1	0	1	3.2
3-Allyl-6-methoxyphenol	1	0	1	3.2
Butanoicacid,butylester	0	1	1	3.2
α-Cedrene	0	1	1	3.2
4'-(2-Methylpropyl)acetophenone	1	0	1	3.2
α-Cedreneoxide	0	1	1	3.2
DiethylPhthalate	1	0	1	3.2
Hexadecane*	0	1	1	3.2
Benzene,1-pentenyl-	0	1	1	3.2
Diphenylamine*	0	1	1	3.2
Heptadecane*	0	1	1	3.2
Phenol,2-methyl-5-(1-methylethyl)	0	1	1	3.2
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	0	1	1	3.2

Figure 82. Histogram of VOCs in urine odor across thirty-one healthy individuals



3.6.4.1.1. Inter-Specimen Matching

The similarities and differences of the VOC profiles of each collected specimens were investigated to determine whether inter-specimen samples could be used to match an individual (i.e. could subject A's blood samples be used to match with subject A's buccal swabs?). Figure 83 is a percent stacked column color chart of the relative peak area ratios of the VOCs extracted from the five specimens from subject Male 16. The color chart shows a visual representation of the similarities among the multiple samples from the same specimen (i.e., hand odor samples 1, 2, and 3) as well as the differences among the samples from varying specimens (i.e., buccal swab samples and blood samples). A semi-quantitative method of analysis to represent these data is through Spearman rank correlation comparison. Table 42 demonstrates the correlation coefficients for the peak areas of the VOCs extracted from the five biological specimens of subject Male 16. The correlation coefficient values clearly indicate a high correlation among samples from the same specimen (> 0.9); samples from different specimens show low correlations, with the Spearman rank correlation coefficient values ranging from 0.142 to -0.495. Similar results were obtained from the other twenty-nine subjects sampled. Groupings according to specimens were seen through PCA analysis, similar to that demonstrated previously in Figure 60.

Figure 83. Relative peak area ratios of VOCs extracted from M16 specimens

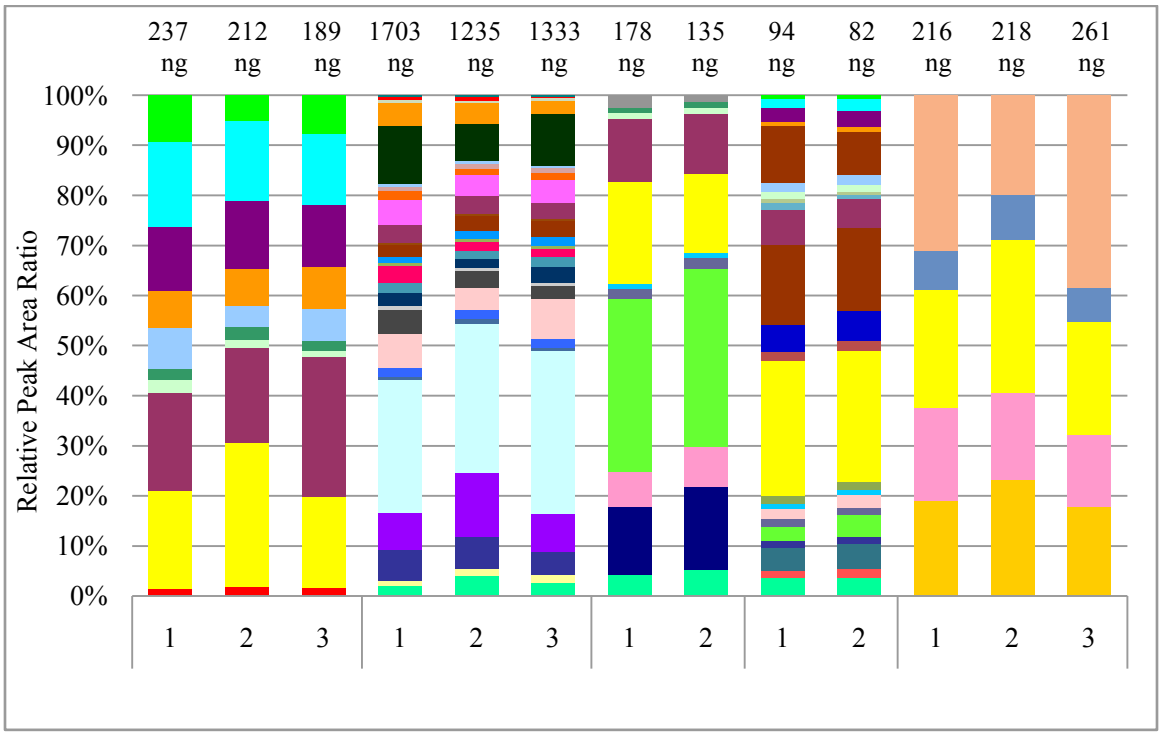


Table 42. Spearman rank correlation coefficients for the peak areas of the VOCs extracted from biological specimens from Male 16

	HD1	HD2	HD3	CH1	CH2	CH3	BR1	BR2	BL1	BL2	UR1	UR2	UR3
HD1	1.000	0.915	0.927	-0.353	-0.348	-0.358	-0.140	-0.170	0.116	0.142	-0.495	-0.439	-0.495
HD2	0.915	1.000	0.988	-0.334	-0.327	-0.352	-0.147	-0.167	0.092	0.117	-0.470	-0.409	-0.470
HD3	0.927	0.988	1.000	-0.313	-0.306	-0.329	-0.155	-0.170	0.087	0.111	-0.495	-0.439	-0.495
CH1	-0.353	-0.334	-0.313	1.000	0.960	0.947	-0.426	-0.426	-0.380	-0.392	-0.412	-0.412	-0.412
CH2	-0.348	-0.327	-0.306	0.960	1.000	0.945	-0.394	-0.394	-0.352	-0.366	-0.412	-0.412	-0.412
CH3	-0.358	-0.352	-0.329	0.947	0.945	1.000	-0.403	-0.403	-0.356	-0.373	-0.372	-0.372	-0.372
BR1	-0.140	-0.147	-0.155	-0.426	-0.394	-0.403	1.000	0.958	0.093	0.089	-0.047	0.009	-0.047
BR2	-0.170	-0.167	-0.170	-0.426	-0.394	-0.403	0.958	1.000	0.015	0.005	-0.068	-0.016	-0.068
BL1	0.116	0.092	0.087	-0.380	-0.352	-0.356	0.093	0.015	1.000	0.976	-0.248	-0.207	-0.248
BL2	0.142	0.117	0.111	-0.392	-0.366	-0.373	0.089	0.005	0.976	1.000	-0.234	-0.194	-0.234
UR1	-0.495	-0.470	-0.495	-0.412	-0.412	-0.372	-0.047	-0.068	-0.248	-0.234	1.000	0.929	1.000
UR2	-0.439	-0.409	-0.439	-0.412	-0.412	-0.372	0.009	-0.016	-0.207	-0.194	0.929	1.000	0.929
UR3	-0.495	-0.470	-0.495	-0.412	-0.412	-0.372	-0.047	-0.068	-0.248	-0.234	1.000	0.929	1.000

Results obtained through Spearman rank analysis of the relative peak areas of the common VOCs extracted across the five biological specimens from the same individual indicate that different specimens from the same individual are too different to be used for matching purposes. For instance, the blood sample and the buccal swab sample of subject A cannot be compared to one another in attempt to match the samples to be originating from subject A. Variations in the volatile organic compounds emanating from different biological specimens is most likely the result of distinct glandular secretions and their interactions with resident bacteria relative to each specimen. Even when comparing VOCs emanating from the skin, it has been previously reported that the site of emanation (skin from the back vs. skin from the forearm) influences the abundance of the same VOCs collected[14]. A number of exocrine glands are distributed throughout the body that contributes to the VOCs being excreted. Merocrine glands are found throughout the skin and include the eccrine (sweat) glands as well as salivary glands. Apocrine glands are present in the axillae and the pubic areas and are thought to function as scent glands. Holcrine glands include the sebaceous glands and are found mostly on the upper part of the body (such as the cheek mucosa, as described in the literature review section on oral fluids). Secretions from these various glands and their interactions with the different bacterial flora residing in surfaces of the body (skin vs. mouth/cheek mucosa), are the likely explanation for the differences in the VOC profiles of the different biological specimens.

3.6.4.1.2. Intra-Specimen Matching

It has been demonstrated that volatile organic compound signatures of individuals can be differentiated through SPME-GC/MS. Previous research has successfully demonstrated the differentiation ability of human scent samples from hand and armpit of individuals using SPME-GC/MS[13]. The goal of the intra-specimen matching study was to investigate whether the same holds true for other biological specimens, namely buccal swabs, breath, blood, and urine.

Figure 84 through Figure 88 shows the three-dimensional scatter-plot of principal component analysis on correlations from ten randomly selected subjects (5 females and 5 males) from the healthy population sampled. The PCA plot demonstrates that samples from the same subject group together closely. Some samples from different individuals, however, clustered closely with one another making it difficult to differentiate between these individuals using PCA analysis. An example of samples from different individuals clustering together can be seen in Figure 85 for buccal swab samples between subjects Male 11 (represented by blue X) and Male 16 (represented by blue open square). These samples will most likely be misidentified.

Figure 84. Three-dimensional PCA scatter plot of PCs 1, 2, and 3 of hand odor from 10 randomly selected healthy subjects

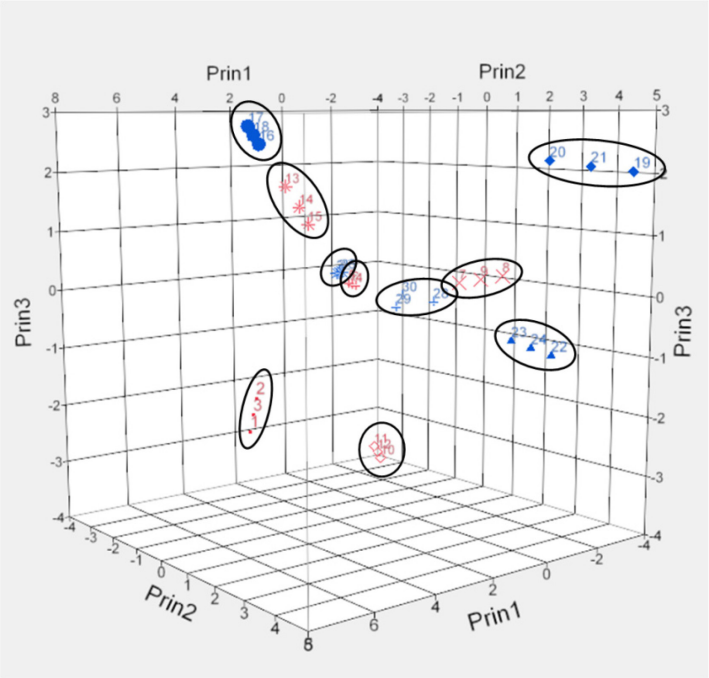


Figure 85. Three-dimensional PCA scatter plot of PCs 1, 2, and 3 of buccal swabs from 10 randomly selected healthy subjects

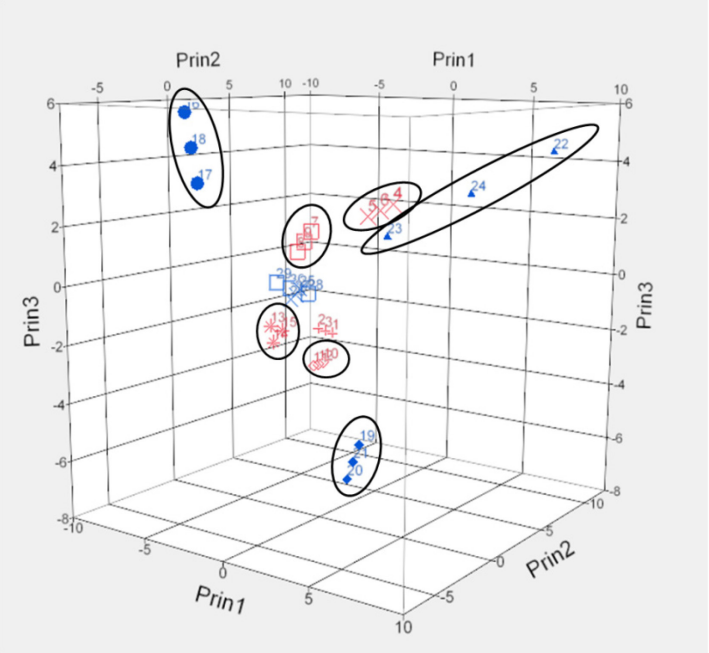


Figure 86. Three-dimensional PCA scatter plot of PCs 1, 2, and 3 of breath from 10 randomly selected healthy subjects

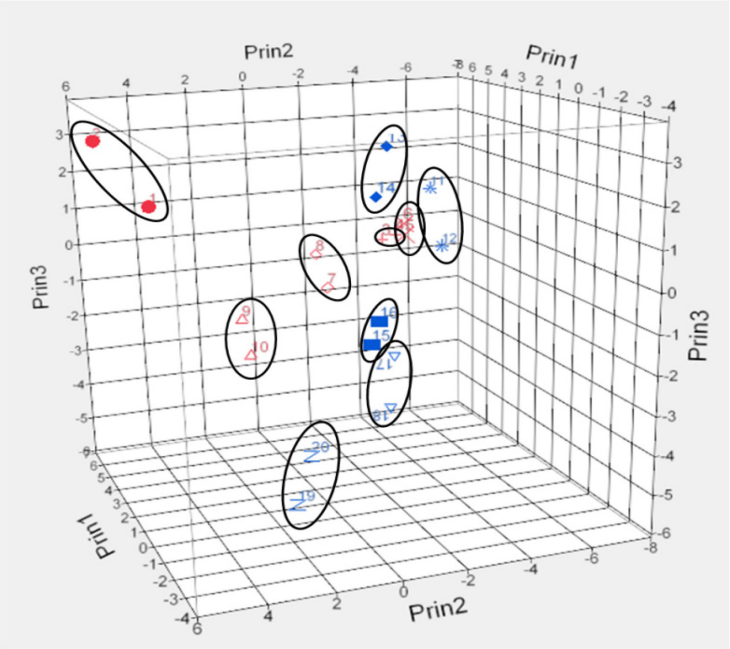


Figure 87. Three-dimensional PCA scatter plot of PCs 1, 2, and 3 of blood from 10 randomly selected healthy subjects

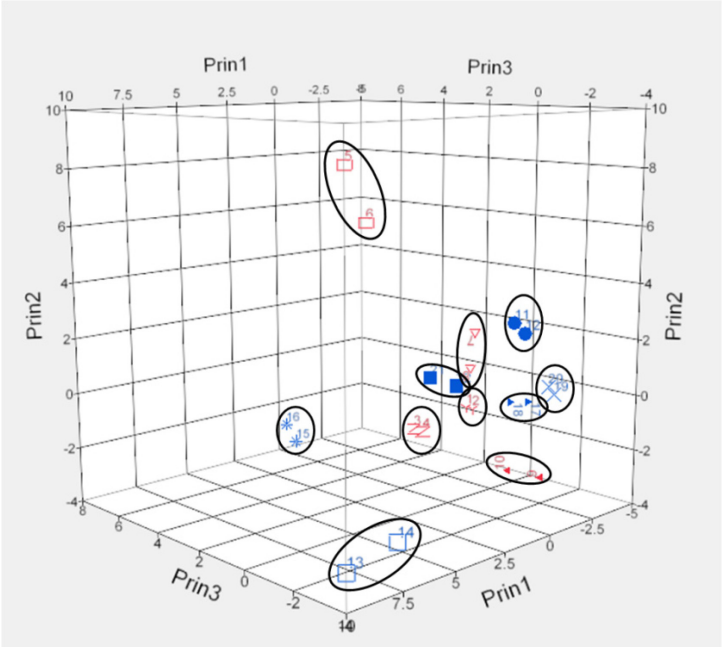
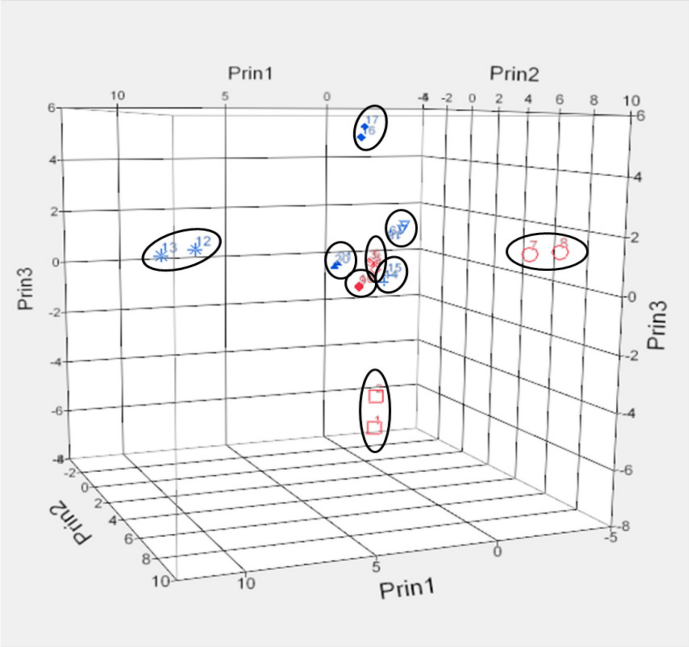


Figure 88. Three-dimensional PCA scatter plot of PCs 1, 2, and 3 of urine from 10 randomly selected healthy subjects



Spearman rank correlation coefficient comparisons were conducted with the samples obtained from the five biological specimens of the thirty-one healthy subjects. Occurrence of Type I and Type II errors were investigated under different correlation thresholds. Type I error is the process of incorrectly rejecting the null hypothesis in favor of the alternative, and is often referred to as a "false positive". In the present study, a Type I error is when two samples are implied to be from the same individual, when in reality they are from different individuals. This would result in a mismatch of samples (i.e. blood sample from subject A be positively identified as blood sample from subject B). Type II error is the opposite of Type I error and is the false acceptance of the null hypothesis. Often referred to as a "false negative", a Type II error implies that the two

samples from the same individual are not a match to one another. Blood samples 1 and 2 from subject A would be identified as a non-match in this case.

3.6.4.1.2.1. Type I Error

The comparison of triplicate samples (for hand odor, buccal swab, and urine) collected from thirty-one subjects generates 4005 possible pairs. The comparison of duplicate samples (for breath and blood) collected from thirty-one subjects generates 1770 possible pairings. Examples where Type I errors were observed are shown in Table 43. At a correlation threshold of 0.8, breath samples of Female 9 and Male 5 could not be distinguished. If the correlation threshold were lowered to 0.7, breath samples of Female 9, Male 5, Female 4, Female 12, and Male 7 were indistinguishable and may result in mismatched samples.

Table 44 is a summary of the number of Type I error occurrences for the comparison of the biological specimens scent samples from the thirty-one subjects. When considering a correlation threshold of 0.9 the individuals were distinguished in 99.98% of the cases for hand odor (or a mismatch percentage of 0.02%), and 100% of the cases for buccal swabs, breath, blood, and urine. At a correlation threshold of 0.8, these percentages were 99.93% (0.07% mismatch) of the hand odor cases, 99.99% of the buccal swab and breath cases (0.01% mismatch), and 100% of the blood and urine cases (no mismatch). These values are promising in supporting the hypothesis the emanations of VOCs are different among individuals and can be used as distinguishing profiles among individuals. With the current sample size these percentages of distinguish-ability of individuals are extremely high. With increasing number of subjects and sample size, the likelihood of the sample

pool to include individuals having similar odor profiles will increase resulting in the distinguish-ability percentage to decrease with larger sample size. In order to avoid Type I errors (false positives) as much as possible, it is recommended for the match/no-match cut-off threshold to be kept at least at 0.8, and wherever possible, a cut-off threshold of 0.9 would be best.

Table 43. Example of Type I error occurrences

F9 Breath			
F9,1	1.000	F9,2	1.000
F9,2	0.959	F9,1	0.959
M5,2	0.870	M5,2	0.839
M5,1	0.864	M5,1	0.839
F4,2	0.764	F4,2	0.785
F12,1	0.732	F4,1	0.744
F4,1	0.722	F12,1	0.743
M7,2	0.714	M7,2	0.730
F12,2	0.708	F12,2	0.725
M11,2	0.687	M7,1	0.709
M7,1	0.681	M11,2	0.688
M11,1	0.671	M11,1	0.684
M4,1	0.663	F10,1	0.665
F10,1	0.654	F10,2	0.660
F10,2	0.650	M4,1	0.654
M10,1	0.648	M10,1	0.648
F8,1	0.646	M6,2	0.646
M6,1	0.646	M6,1	0.644
M6,2	0.644	F8,1	0.625
M9,2	0.637	M12,2	0.617
M12,2	0.619	M9,2	0.614
M12,1	0.602	M12,1	0.599
M10,2	0.598	M10,2	0.598

M9 Blood			
M9,1	1.000	M9,2	1.000
M9,2	0.947	M9,1	0.947
M6,1	0.732	M6,1	0.718
F4,2	0.662	F4,2	0.682
F8,2	0.612	F4,1	0.641
F4,1	0.609	F11,1	0.547
F11,1	0.578	F9,2	0.541
F9,1	0.574	F8,2	0.538
F11,2	0.572	F11,2	0.531
F9,2	0.563	F9,1	0.530
M6,2	0.550	M6,2	0.518
F8,1	0.520	M8,2	0.469
M16,2	0.504	M16,2	0.466
M16,1	0.503	M16,1	0.464
M5,1	0.496	M5,1	0.456
F7,1	0.494	M5,2	0.442
M8,2	0.481	F8,1	0.439
M5,2	0.480	F7,1	0.438
F6,1	0.478	F6,1	0.426
M1,1	0.464	M7,1	0.421
M1,2	0.459	F15,1	0.416
M8,1	0.451	M1,1	0.413
M7,1	0.445	M8,1	0.413

Table 44. Type I error occurrences for three correlation thresholds across biological specimens

	Threshold		
	0.9	**0.8**	**0.7**
Hand Odor	5	32	73
Buccal Swab	0	1	8
Breath	0	1	25
Blood	0	0	3
Urine	0	0	0

3.6.4.1.2.2. Type II Error

Table 45 shows the Spearman rank correlation coefficients of buccal swab samples 1 and 2 from Female 5, breath samples 1 and 2 from Male 16, and blood samples 1 and 2 from Male 7. In each of these cases the duplicate samples from the same individual were identified as a non-match at all three thresholds of 0.7, 0.8, and 0.9. Table 46 summarizes the Type II error occurrences among the thirty-one subjects compared in each of the biological specimens. As is expected the number of Type II errors increases as the cut-off threshold is raised. If the match/no-match threshold is set too high (i.e. at 0.9), the samples from the same individuals that have high correlation coefficients (as they should have) would be identified as a non-match, resulting in a false-negative. Breath and urine had the lowest number of Type II errors. Blood had the highest number of Type II errors; however, this could be due to sampling protocol. For example, there was an obvious volume difference between the duplicate blood samples collected onto the two FTA cards obtained from Male 7.

Table 45. Example of Type II error occurrences

F5 Buccal Swab			
F5,1	1.000	F5,2	1.000
F5,2	0.692	F5,1	0.692
F6,2	0.673	F6,2	0.565
F6,1	0.607	F6,1	0.428
M2,2	0.425	F17,3	0.353
M2,1	0.396	F17,2	0.352
F17,3	0.311	F17,1	0.342
F17,2	0.310	M2,1	0.330
M7,1	0.309	F14,2	0.326
F17,1	0.299	M7,1	0.325
F14,2	0.291	F14,1	0.291
F7,1	0.284	M7,2	0.291
M7,2	0.280	M2,2	0.264
F14,1	0.255	F7,1	0.231
F7,2	0.237	F16,2	0.212
F16,2	0.222	F16,3	0.206
F16,3	0.213	F16,1	0.185
F16,1	0.176	F7,2	0.159
F10,2	0.125	F8,2	0.129
F10,1	0.097	F10,2	0.081
F8,2	0.092	F8,1	0.064
M6,1	0.029	F10,1	0.056
M6,2	0.026	M1,2	0.052
F8,1	0.023	M1,1	0.051

M15 Breath			
M15,1	1.000	M15,2	1.000
M15,2	0.600	M15,1	0.600
F5,2	0.442	M9,2	0.596
F5,1	0.442	F2,2	0.534
M9,2	0.440	F2,1	0.527
F2,1	0.395	F5,2	0.487
F2,2	0.388	F5,1	0.487
M9,1	0.387	M9,1	0.460
M16,1	0.290	M16,1	0.396
M16,2	0.286	F10,2	0.382
M4,1	0.260	M4,1	0.380
F14,1	0.234	F10,1	0.365
F14,2	0.207	M16,2	0.364
F17,1	0.205	F14,2	0.328
F4,2	0.197	F14,1	0.322
F15,2	0.189	F17,1	0.298
M4,2	0.184	M5,2	0.298
M14,2	0.179	F4,2	0.292
F10,1	0.179	M5,1	0.286
F15,1	0.174	F4,1	0.276
F4,1	0.166	M12,2	0.270
F10,2	0.159	F15,1	0.257
M12,2	0.157	F15,2	0.249
M14,1	0.153	F17,2	0.245

M7			
M7,1	1.000	M7,2	1.000
M7,2	0.620	M7,1	0.620
M2,1	0.518	F8,2	0.526
F7,1	0.516	M5,2	0.472
F11,1	0.502	M5,1	0.468
F11,2	0.487	F11,1	0.462
F8,2	0.486	F11,2	0.444
F7,2	0.477	M2,1	0.427
M5,1	0.459	F8,1	0.426
M10,1	0.454	F7,1	0.387
M10,2	0.449	M1,1	0.380
M9,1	0.445	F13,1	0.380
M2,2	0.437	M10,2	0.377
M5,2	0.436	M10,1	0.376
M6,1	0.422	F6,1	0.370
M9,2	0.421	M1,2	0.369
M13,1	0.408	M2,2	0.341
F9,1	0.407	F7,2	0.340
M16,1	0.402	F13,2	0.326
M16,2	0.400	M6,1	0.324
F8,1	0.395	M16,2	0.307
M12,1	0.385	M9,1	0.304
M13,2	0.383	M12,1	0.304
F9,2	0.377	M16,1	0.304

Table 46. Type II error occurrences for three correlation thresholds across biological specimens

	Threshold		
	0.9	**0.8**	**0.7**
Hand Odor	15	9	7
Buccal Swab	9	4	3
Breath	11	4	1
Blood	12	6	4
Urine	8	3	1

Table 47. Type I and Type II errors for five biological specimens amongst thirty-one healthy individuals

	Hand Odor			Buccal Swab			Breath			Blood			Urine		
Threshold	0.9	0.8	0.7	0.9	0.8	0.7	0.9	0.8	0.7	0.9	0.8	0.7	0.9	0.8	0.7
Type I	3	13	34	0	1	3	0	1	19	0	0	3	0	0	0
Type II	15	9	7	9	4	3	11	4	1	12	6	4	8	3	1
Total Error	18	22	41	9	5	6	11	5	20	12	6	7	8	3	1
% Mismatch	0.89	1.09	2.03	0.45	0.25	0.30	0.58	0.26	1.06	0.63	0.32	0.37	0.36	0.14	0.05

Prior to the current study, it was not known if one could differentiate individuals by odor components other than odor emanating from skin (mainly hand, arm, and armpits) on gauze. For the population study of thirty-one healthy individuals, the headspace of scent samples collected on various biological specimens (hand odor, buccal swabs, breath, blood, and urine) can be distinguished chromatographically based on a combination of the relative peak area ratios of the common compounds present in these samples. Through the combined methods of chromatogram comparison, Spearman rank correlation comparison, and principal component analysis, it is possible to distinguish the VOC profiles of individuals for each of the specimens with high confidence. However, VOC profiles of different biological specimens from the same individual are too different to be used for matching purposes.

3.6.4.2. Evaluation of Odor Profiles of Individuals with Type 2 Diabetes

Odor profiles of nineteen individuals with diagnosed type 2 diabetes were evaluated using SPME-GC/MS. Compounds were identified by spectral library (NIST) and/or by standard reference comparison. Figure 89 demonstrates the frequency distribution of the different functional groups of VOCs that were extracted from hand odor, buccal swab,

breath, blood, and urine across the 19 subjects. For hand odor, aldehyde and aliphatic VOCs (28% each) comprised slightly over half of the total volatile compounds extracted, followed by ketones (16%), carboxylic acids (16%), and esters (12%). No carboxylic acid VOCs were extracted from hand odor. Decanal and nonanal were extracted from all 19 diabetic subjects (Table 48). Other high-frequency occurring (>70%) compounds for hand odor for type 2 diabetics were heptadecane, hexadecane, and tetradecane, each having an 89.5% occurrence among the 19 subjects sampled.

For buccal swab odor, ester VOCs had the highest frequency of occurrence (24%), followed by aromatic (18%), aliphatic (16%), ketones (16%), carboxylic acids (9%), alcohols (8%), and ether (1%) compounds. There were eight high-frequency compounds across 19 diabetic individuals as seen in Table 49, of which hexanoic acid and decanal were present in all 19 diabetic individuals.

Aromatic compounds comprised about half of the VOCs extracted from breath odor at 45%. The remaining frequency distribution of functional groups for breath odor was as follows: aliphatics (17%), alcohols (10%), ketones (10%), aldehydes (8%), esters (8%), and other functional group compounds (2%). Only two compounds (nonanal and decanal) were high-frequency compounds for breath, although xylenes were present in 78.9% of the 19 diabetic individuals (Table 50). Typical VOCs in exhaled breath are under 1ppb concentration range. It has been reported that disease states do not typically result in higher VOC concentrations, although there are certain disease states such as untreated diabetes or severe renal impairment that do result in significant differences in the VOC concentrations in breath[49]. For the present study, acetone was only detected in the breath

samples of three of the diabetic patients sampled, and the amount of acetone extracted was not particularly higher than that of healthy individuals. The minimal difference in the acetone presence and the amount extracted can be likely explained by the fact that the diabetic subjects sampled in the present study were all fairly well-controlled (treated with diet and/or medication). Additionally, detection and measurement of acetone under the current instrumental condition are not always consistent due to acetone's nature of volatility and activity. Pre-concentration and/or on-fiber derivatization of acetone may need to be considered for future studies in order to effectively extract and accurately measure acetone in breath samples.

For blood, the frequency distribution of functional groups of the VOCs extracted was as follows: aromatics (40%), aliphatics (26%), alcohols (13%), ketones (10%), other functional groups (4%), and esters (2%). Other functional groups included ethers and nitrogen-containing compounds. There were seven high-frequency compounds, of which nonanal and hexadecane were present in all 19 diabetic individuals (Table 51).

Finally for urine, the frequency distribution of functional groups of the extracted VOCs was as follows: alcohols (22%), aromatics (20%), ketones (17%), aldehydes (11%), nitrogen-containing compounds (11%), sulfur-containing compounds (6%), aliphatics (5%), carboxylic acids (4%), and esters (4%). No compound was present in high frequency; 4-heptanone was present at highest frequency among the 19 diabetic individuals sampled at 73.7% (Table 52).

Figure 89. Frequency distribution of functional groups of extracted VOCs in hand odor, buccal swab, breath, blood, and urine across twenty individuals diagnosed with type 2 diabetes

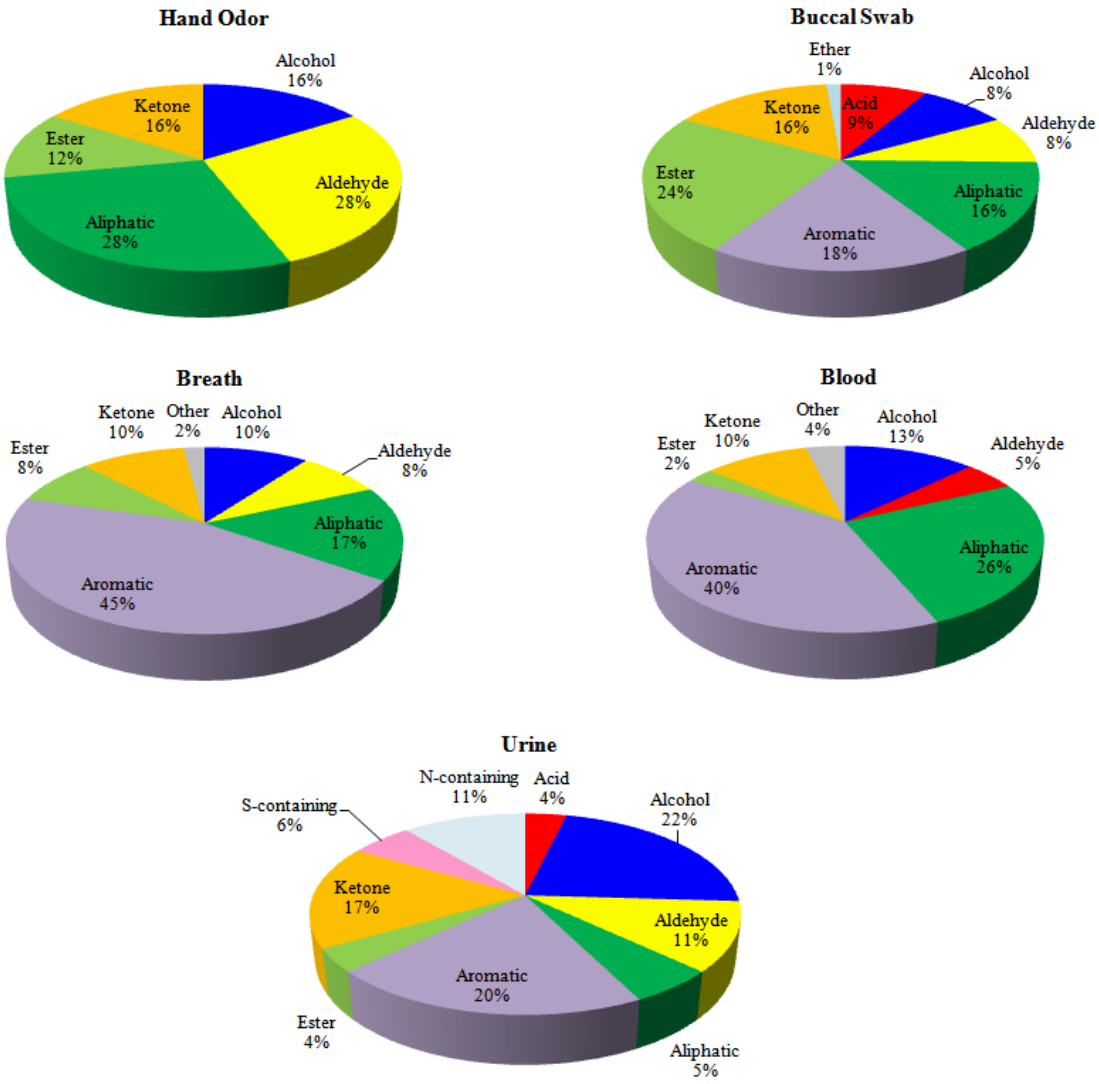


Table 48. Identified VOCs in hand odor of nineteen individuals with diagnosed type 2 diabetes ranked by frequency of occurrence (*Note*: *denotes compound was verified by reference standard)

Compound Name	Frequency			Occurrence (%)
	Female	Male	Total	
Decanal*	8	11	19	100%
Nonanal*	8	11	19	100%
Heptadecane*	7	10	17	89.5%
Hexadecane*	7	10	17	89.5%
Tetradecane*	7	10	17	89.5%
Octadecane	4	7	11	57.9%
Undecane*	5	6	11	57.9%
Tridecane*	3	6	9	47.4%
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-*	3	6	9	47.4%
Undecanal*	2	5	7	36.8%
Dodecane*	2	3	5	26.3%
2-Nonenal,(E)-*	2	2	4	21.1%
Dodecanoicacid,methylester	0	4	4	21.1%
Acetophenone*	2	1	3	15.8%
1-Hexanol,2-ethyl-*	1	1	2	10.5%
Benzyl Alcohol*	1	1	2	10.5%
Dodecanal*	1	1	2	10.5%
1,6-Octadien-3-ol,3,7-dimethyl-*	0	1	1	5.3%
Cedrol	0	1	1	5.3%
Benzaldehyde*	1	0	1	5.3%
Lilial	1	0	1	5.3%
Aceticacid,butylester	1	0	1	5.3%
Octanoicacid,methylester*	0	1	1	5.3%
2-Pentanone	1	0	1	5.3%
5-Hepten-2-one,6-methyl-*	0	1	1	5.3%

Table 49. Identified VOCs in buccal swabs of nineteen individuals with diagnosed type 2 diabetes ranked by frequency of occurrence (*Note*: *denotes compound was verified by reference standard)

Compound Name	Frequency			Occurrence (%)
	Female	Male	Total	
Hexanoicacid*	8	11	19	100%
Decanal*	8	11	19	100%
Nonanoicacid	6	11	17	89.5%
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-*	7	10	17	89.5%
Nonanoicacid,ethylester*	6	10	16	84.2%
Furan,2-pentyl-*	6	9	15	78.9%
Dodecanoicacid,ethylester	6	8	14	73.7%
6-Dodecanone	5	9	14	73.7%
1-Tetradecene*	4	9	13	68.4%
Tetradecanoicacid,ethylester	3	8	11	57.9%
1-Decene*	6	5	11	57.9%
1,1'-Biphenyl,2,2'-diethyl-	4	7	11	57.9%
2,4-Nonadienal,(E,E)-*	3	7	10	52.6%
Hexadecanoicacid,ethylester	3	7	10	52.6%
Tetradecane*	4	6	10	52.6%
6-Methyl-3,5-heptadiene-2-one	3	7	10	52.6%
Heptanoicacid*	2	6	8	42.1%
Benzaldehyde*	3	5	8	42.1%
Octanoicacid,ethylester*	3	4	7	36.8%
2-Nonenal,(E)-*	2	4	6	31.6%
1-Dodecene*	2	4	6	31.6%
1,1'-Biphenyl,4-methyl-	2	4	6	31.6%
1-Hexanol*	2	4	6	31.6%
Cyclododecane	3	2	5	26.3%
2(3H)-Furanone,dihydro-5-pentyl-	2	3	5	26.3%
Dodecanoicacid*	3	2	5	26.3%
2(3H)-Furanone,5-ethyldihydro-	3	2	5	26.3%
2-Tetradecene,(E)-	3	1	4	21.1%
2-Octenoicacid	1	3	4	21.1%
Naphthalene,2-methyl-	1	3	4	21.1%
Benzothiazole	0	4	4	21.1%
Benzene,1-methyl-2-[(4-methylphenyl)methyl]-	1	3	4	21.1%
Phenol*	1	3	4	21.1%
Hexanoicacid,ethylester	1	2	3	15.8%
Heptanoicacid,ethylester*	1	2	3	15.8%
Caryophyllene*	2	1	3	15.8%
DiethylPhthalate	1	2	3	15.8%

Pentanoicacid*	0	3	3	15.8%
Naphthalene,2,7-dimethyl-	1	2	3	15.8%
Decanoicacid,ethylester*	1	2	3	15.8%
Acetophenone*	1	2	3	15.8%
Pentadecanoicacid,ethylester	1	1	2	10.5%
IsopropylMyristate	1	0	1	10.5%
Hexanoicacid,pentylester	0	2	2	10.5%
Hexadecanoicacid,methylester	1	1	2	10.5%
Dibutanoylmorphine	1	1	2	10.5%
Tridecane	1	1	2	10.5%
Naphthalene,1-methyl-*	0	2	2	10.5%
ButylatedHydroxytoluene	1	1	2	10.5%
2(3H)-Furanone,dihydro-5-propyl-	1	1	2	10.5%
IsopropylPalmitate	1	0	1	5.3%
Hexanoicacid,propylester	0	1	1	5.3%
E-11-Hexadecenoicacid,ethylester	1	0	1	5.3%
Naphthalene,2,6-dimethyl-	0	1	1	5.3%
Benzene,1-methyl-4-nitro-	1	0	1	5.3%
Octadecane	1	0	1	5.3%
Benzene,1,2,4,5-tetramethyl-	0	1	1	5.3%
p-Menthan-3-one	0	1	1	5.3%
Hexadecane*	0	1	1	5.3%
Benzene,1,2,3,5-tetramethyl-	1	0	1	5.3%
3,5,9-Undecatrien-2-one,6,10-dimethyl-, (E,Z)-	0	1	1	5.3%
Cyclohexadecane	1	0	1	5.3%
1,1'-Biphenyl,3-methyl-	0	1	1	5.3%
Carvone	0	1	1	5.3%
p-Benzoquinone	0	1	1	5.3%
1-Octanol*	1	0	1	5.3%
Hexanal*	0	1	1	5.3%
Menthol*	1	0	1	5.3%
Furfural*	0	1	1	5.3%
Linalool Oxide	0	1	1	5.3%

Table 50. Identified VOCs in breath odor of nineteen individuals with diagnosed type 2 diabetes ranked by frequency of occurrence (*Note*: *denotes compound was verified by reference standard)

Compound Name	Frequency			Occurrence (%)
	Female	Male	Total	
Nonanal*	8	11	19	100%
Decanal*	7	11	18	94.7%
Xylenes*	5	10	15	78.9%
Tridecane*	5	8	13	68.4%
Tetradecane*	5	8	13	68.4%
Dodecane*	5	8	13	68.4%
1-Hexanol,2-ethyl-*	5	6	11	57.9%
Undecane*	3	7	10	52.6%
Benzene,1,2-dichloro-*	4	6	10	52.6%
Benzophenone*	5	5	10	52.6%
Toluene	4	2	6	31.6%
Styrene*	2	4	6	31.6%
ButylatedHydroxytoluene	3	3	6	31.6%
Benzene,1,3,5-trimethyl-	1	5	6	31.6%
p-Benzoquinone	2	4	6	31.6%
Benzene,4-ethyl-1,2-dimethyl-	2	3	5	26.3%
Benzene,2-ethyl-1,4-dimethyl-	2	3	5	26.3%
Benzene,1-methyl-3-propyl-	0	5	5	26.3%
Undecanal*	1	3	4	21.1%
Benzene,1-ethyl-2,3-dimethyl-	1	3	4	21.1%
4-Cyanocyclohexene	2	2	4	21.1%
Phenol*	1	2	3	15.8%
1,3,5,7-Cyclooctatetraene	2	1	3	15.8%
Menthol*	2	1	3	15.8%
Naphthalene*	1	2	3	15.8%
Limonene*	0	2	2	10.5%
2-Dodecene,(Z)-	1	1	2	10.5%
Diisopropylnaphthalene	0	2	2	10.5%
Benzene,2-ethyl-1,3-dimethyl-	1	1	2	10.5%
Benzene,1-ethyl-2-methyl-	1	1	2	10.5%
Benzene,1-ethyl-2,4-dimethyl-	1	1	2	10.5%
Benzene,1,2,3-trimethyl-	1	1	2	10.5%
2,6-Diisopropylnaphthalene*	0	2	2	10.5%
Hexanedioicacid,bis(1-methylethyl) ester	0	2	2	10.5%
Acetone	1	1	2	10.5%
2-Propanol,1-propoxy-	1	0	1	5.3%
Dodecanal*	0	1	1	5.3%

5-Dodecene,(E)-	0	1	1	5.3%
Naphthalene,2-methyl-	0	1	1	5.3%
Benzene,1-methyl-4-(1-methylpropyl)-	0	1	1	5.3%
Benzene,1,4-dichloro-	0	1	1	5.3%
Benzene,1,3-dichloro-	0	1	1	5.3%
Benzene,1,2,3,4-tetramethyl-	0	1	1	5.3%
Isobornylacetate	1	0	1	5.3%
DiethylPhthalate	0	1	1	5.3%
Dibutylphthalate	0	1	1	5.3%
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-*	0	1	1	5.3%
3-Heptanone*	0	1	1	5.3%

Table 51. Identified VOCs in blood odor of nineteen individuals with diagnosed type 2 diabetes ranked by frequency of occurrence (*Note*: *denotes compound was verified by reference standard)

Compound Name	Frequency			Occurrence (%)
	Female	Male	Total	
Nonanal*	8	11	19	100%
Hexadecane*	8	11	19	100%
Tetradecane*	7	9	16	84.2%
Undecane*	6	10	16	84.2%
2,6-Diisopropylnaphthalene*	6	9	15	78.9%
Tridecane*	6	8	14	73.7%
Benzophenone*	4	10	14	73.7%
Dodecane*	6	7	13	68.4%
Heptadecane*	4	8	12	63.2%
Benzaldehyde,2,4,6-trimethyl-	4	8	12	63.2%
Hexanal	4	7	11	57.9%
Decanal*	7	2	9	47.4%
Furan,2-butyltetrahydro-	4	5	9	47.4%
1-Octen-3-ol*	4	5	9	47.4%
1-Decene*	4	4	8	42.1%
Longifolene	2	6	8	42.1%
Benzene,1,3,5-trimethyl-	2	5	7	36.8%
Diisopropylnaphthalene	3	4	7	36.8%
Naphthalene*	3	3	6	35.8%
Benzyl Alcohol*	3	3	6	31.6%
1-Dodecene*	3	3	6	31.6%
2-Dodecene,(Z)-	4	2	6	31.6%
Octadecane	3	3	6	31.6%
Furan,2-pentyl-*	4	2	6	31.6%
Benzene,1,2-dichloro-*	2	3	5	26.3%
Benzene,1-ethyl-2,4-dimethyl-	1	4	5	26.3%
Naphthalene,2-methyl-	2	3	5	26.3%
Menthol*	3	1	4	21.1%
1-Dodecanol*	1	3	4	21.1%
Heptanal*	2	2	4	21.1%
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-*	1	3	4	21.1%
2-Propanol,1-propoxy-	0	3	3	15.8%
Benzene,1,2,3,5-tetramethyl-	1	2	3	15.8%
2-Undecanone	2	1	3	15.8%
3-Heptanone*	1	2	3	15.8%
Cyclohexanone*	1	2	3	15.8%
4-Cyanocyclohexene	2	1	3	15.8%

1-Octanol*	2	0	2	10.5%
1-Pentanol	0	2	2	10.5%
3-Dodecene,(Z)-	0	2	2	10.5%
Cyclododecane	2	0	2	10.5%
Benzene,1,2,3-trimethyl-	1	1	2	10.5%
Benzene,1,2,4,5-tetramethyl-*	0	2	2	10.5%
Benzene,1-ethyl-3,5-dimethyl-	2	0	2	10.5%
Benzene,2-ethyl-1,4-dimethyl-	0	2	2	10.5%
Naphthalene,1-methyl-*	1	1	2	10.5%
Naphthalene,2,7-dimethyl-	1	1	2	10.5%
p-Xylene*	1	1	2	10.5%
2,5-Octanedione	2	0	2	10.5%
2-Heptanone*	1	1	2	10.5%
1-Heptanol*	0	1	1	5.3%
2-Propanol,1-butoxy-	1	0	1	5.3%
Ethanol,2-butoxy-	1	0	1	5.3%
1-Hexadecene	1	0	1	5.3%
3,7-Dimethyl-octa-1,6-diene	1	0	1	5.3%
Cyclopropane,nonyl-	0	1	1	5.3%
Cyclotetradecane	1	0	1	5.3%
Decane	0	1	1	5.3%
Dodecane,2,6,10-trimethyl-	1	0	1	5.3%
Z-8-Hexadecene	0	1	1	5.3%
1,1'-Biphenyl,2-methyl-	0	1	1	5.3%
1,1'-Biphenyl,3-methyl-	0	1	1	5.3%
Benzene,1,2,3,4-tetramethyl-	0	1	1	5.3%
Benzene,1,3-dimethyl-5-(1-methylethyl)-	0	1	1	5.3%
Benzene,1,4-dichloro-	0	1	1	5.3%
Benzene,1-ethyl-2,3-dimethyl-	1	0	1	5.3%
Benzene,1-ethyl-2-methyl-	0	1	1	5.3%
Benzene,1-ethyl-3-methyl-	0	1	1	5.3%
Benzene,1-methyl-4-(1-methylethyl)-	0	1	1	5.3%
Benzene,1-methyl-4-(1-methylpropyl)-	0	1	1	5.3%
Benzene,2,4-dimethyl-1-(1-methylethyl)-	1	0	1	5.3%
Benzene,4-ethyl-1,2-dimethyl-	0	1	1	5.3%
Benzene,ethyl-1,2,4-trimethyl-	0	1	1	5.3%
Naphthalene,1,7-dimethyl-	1	0	1	5.3%
Naphthalene,2,3,6-trimethyl-	1	0	1	5.3%
4-tert-Butylcyclohexylacetate	0	1	1	5.3%
Hexanedioicacid,bis(1-methylethyl) ester	0	1	1	5.3%
2-Heptanone,6-methyl-	1	0	1	5.3%

Table 52. Identified VOCs in urine odor of nineteen individuals with diagnosed type 2 diabetes ranked by frequency of occurrence (*Note*: *denotes compound was verified by reference standard)

Compound Name	Frequency			Occurrence (%)
	Female	Male	Total	
4-Heptanone*	5	9	14	73.7%
Cedrol	4	7	11	57.9%
Nonanal*	2	9	11	57.9%
Menthol*	4	4	8	42.1%
Carvones	3	4	7	36.8%
ButylatedHydroxytoluene*	2	3	5	26.3%
Butanoicacid,butylester	3	2	5	26.3%
4-Terpineol	3	2	5	26.3%
Benzaldehyde*	3	1	4	21.1%
Decanal*	1	3	4	21.1%
Benzene,1-methyl-4-(1-methylethenyl)-	0	4	4	21.1%
2-Pentanone	2	2	4	21.1%
2-Isopropylbenzaldehyde	1	2	3	15.8%
1,4-Cyclohexadiene,1-methyl-4-(1-methylethyl)-	2	1	3	15.8%
Benzene,1-methyl-4-(1-methylethyl)-	1	2	3	15.8%
3-Heptanone*	1	2	3	15.8%
ValproicAcid	1	1	2	10.5%
2-Methoxy-4-vinylphenol	2	0	2	10.5%
Phenol,4-methyl- *(p-Cresol)**	2	0	2	10.5%
Benzaldehyde,3,5-dimethyl-	0	2	2	10.5%
1,3-Cyclohexadiene,1-methyl-4-(1-methylethyl)-	2	0	2	10.5%
p-Menth-1-en-3-one	1	1	2	10.5%
Cyclopropane,isothiocyanato-	0	2	2	10.5%
N,N-Diethylcarbanilide	2	0	2	10.5%
Pyrrole	2	0	2	10.5%
Nonanoicacid	1	0	1	5.3%
α-Terpineol	0	1	1	5.3%
BenzylAlcohol*	0	1	1	5.3%
E-2-Tetradecen-1-ol	1	0	1	5.3%
Eucalyptol	0	1	1	5.3%
Eugenol	1	0	1	5.3%
Phenol,2-methoxy-	0	1	1	5.3%
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	0	1	1	5.3%
α-Cubebene	0	1	1	5.3%
Benzene,-(1-formylethyl)-	1	0	1	5.3%
Benzene,(2-isothiocyanatoethyl)-	1	0	1	5.3%
Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-	1	0	1	5.3%

Benzene,1,2-dichloro-*	1	0	1	5.3%
Benzene,1,3-dichloro-	0	1	1	5.3%
Benzene,1-methyl-3-(1-methylethyl)-	1	0	1	5.3%
Naphthalene,2,3-dimethyl-	1	0	1	5.3%
Naphthalene,2-methyl-	1	0	1	5.3%
Isobornylacetate	0	1	1	5.3%
3-Nonen-2-one	1	0	1	5.3%
4-Hepten-3-one,4-methyl-	1	0	1	5.3%
Acetone	0	1	1	5.3%
Benzophenone*	1	0	1	5.3%
1-Butene,4-isothiocyanato-	0	1	1	5.3%
Dibutanoylmorphine	0	1	1	5.3%

3.6.4.3. Evaluation of Odor Profiles of Individuals with Major Depressive Disorder

Odor profiles of twenty individuals with diagnosed Major Depressive Disorder (MDD) were evaluated using SPME-GC/MS. Compounds were identified by spectral library (NIST) and/or by standard reference comparisons. Figure 90 demonstrates the frequency distribution of the different functional groups of VOCs that were extracted from hand odor, buccal swab, breath, blood, and urine across the 20 subjects. Aliphatics (alkanes, alkenes, cycloalkanes) and aldehydes constructed over half (64%) of the hand odor VOCs. Nonanal and decanal were present in all subjects, followed by tetradecane, hexadecane, heptadecane, and octadecane being present in more than 80% of the subjects sampled (Table 53).

For buccal swab, esters constructed slightly less than a third of the extracted VOCs (27%), followed by aromatics (17%) and aliphatics (14%). Hexanoic acid and decanal were present in 100% of sampled subjects. Other VOCs present in high frequency

(<70%) were 2-pentylfuran, (E)-6,10-dimethyl-5-9-undecadien-2-one, nonanoic acid, nonanoic acid ethyl ester, (E,E)-2,4-nonadienal, dodecanoicacid ethyl ester, and 2,2'-diethyl-1,1'-biphenyl (Table 54).

Aromatic VOCs comprised approximately half of the breath odor of the MDD subject group at 49%. The remaining was comprised of aliphatics (17%), ketones (9%), carboxylic acids (8%), esters (7%), aldehydes (6%), and alcohols (4%). The two compounds that were present in all subjects were both aldehydes, namely nonanal and decanal (Table 55). Other breath VOCs with high frequency of occurrence were xylenes (p-xylene and m-xylene), 1,2-dichlorobezene, and 2-ethyl-1-hexanol, and benzophenone.

For blood, two-thirds of the VOCs extracted from the MDD subjects' blood samples were aromatics (35%) and aromatics (26%). The remainder of the functional groups present in the headspace of blood odor VOCs were aldehydes (17%), alcohols (10%), ketones (9%), and ethers (3%). Hexadecane and 2,6-diisopropylnaphthalene were present in all MDD subjects. Other compounds with high frequency of occurrence were undecane, nonanal, dodecane, 2,4,6-trimethylbenzaldehyde, tridecane, tetradecane, hexane, diisopropylnaphthalene, and heptadecane (Table 56).

Lastly for urine of MDD subjects, ketones had the highest frequency (23%) in the extracted VOCs of urine odor, followed by aldehydes (17%), aromatics (16%) and alcohols (16%). Nitrogen-containing VOCs were also present in urine odor (9%), which were not found in the other specimens from the MDD group. 4-Heptanone and cedrol were the only two compounds that were present in over 70% of the depressed subjects sampled (Table 57).

Figure 90. Frequency distribution of functional groups of extracted VOCs in hand odor, buccal swab, breath, blood, and urine across twenty individuals diagnosed with Major Depressive Disorder

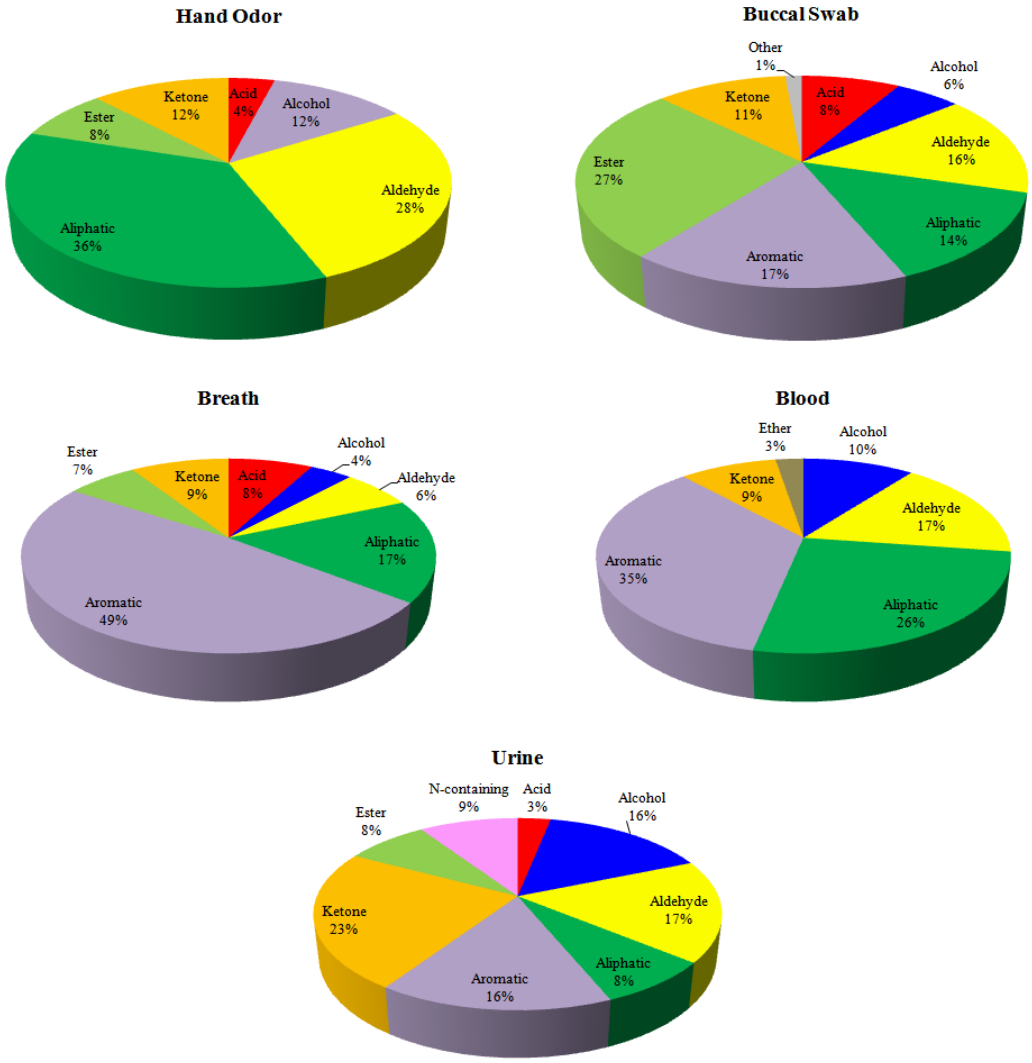


Table 53. Identified VOCs in hand odor of twenty individuals with diagnosed Major Depressive Disorder ranked by frequency of occurrence (*Note*: * denotes compound was verified by reference standard)

Compound Name	Frequency			Occurrence (%)
	Female	Male	Total	
Nonanal*	10	10	20	100
Decanal*	10	10	20	100
Tetradecane*	10	9	19	95.0
Hexadecane*	9	9	18	90.0
Heptadecane*	9	9	18	90.0
Octadecane	8	9	17	85.0
Undecane*	6	6	12	60.0
Tridecane*	6	6	12	60.0
Undecanal*	6	6	12	60.0
5,9-Undecadien-2-one-6,10-dimethyl-(E)-*	6	6	12	60.0
Dodecane*	2	5	7	35.0
Benzyl Alcohol*	3	2	5	25.0
Dodecanal*	2	2	4	20.0
1-Hexanol,2-ethyl-	3	0	3	15.0
1,6-Octadien-3-ol,3,7-dimethyl-*	2	1	3	15.0
Dodecanoicacid,methylester	0	3	3	15.0
Benzaldehyde*	2	0	2	10.0
Acetophenone*	1	1	2	10.0
Heptanal	1	0	1	5.0
5-Hepten-2-one,6-methyl-*	0	1	1	5.0
Hexanoicacid*	1	0	1	5.0
Octanoicacid,methylester*	1	0	1	5.0
2-Nonenal, (E)-*	0	1	1	5.0
1-Pentadecene*	0	1	1	5.0
Pentadecane*	1	0	1	5.0

Table 54. Identified VOCs in buccal swab of twenty individuals with diagnosed Major Depressive Disorder ranked by frequency of occurrence (*Note*: *denotes compound was verified by reference standard)

Compound Name	Frequency			Occurrence (%)
	Female	Male	Total	
Hexanoicacid*	10	10	20	100
Decanal*	10	10	20	100
Furan,2-pentyl-*	9	10	19	95.0
5,9-Undecadien-2-one,6,10-dimethyl,(E)-*	10	9	19	95.0
Nonanoicacid	8	10	18	90.0
Nonanoicacid,ethylester*	9	9	18	90.0
2,4-Nonadienal, (E,E)-v	6	10	16	80.0
Dodecanoicacid,ethylester	6	10	16	80.0
1,1'-Biphenyl,2,2'-diethyl-	5	10	15	75.0
2-Nonenal, (E)-*	5	8	13	65.0
Hexadecanoicacid,ethylester	5	8	13	65.0
1-Tetradecene*	7	5	12	60.0
Tetradecanoicacid,ethylester	4	8	12	60.0
6-Dodecanone	7	4	11	55.0
6-Methyl-3,5-heptadiene-2-one	3	7	10	50.0
1-Dodecene*	3	6	9	45.0
Octanoicacid,ethylester*	6	3	9	45.0
Heptanoicacid*	2	6	8	40.0
1-Decene*	4	4	8	40.0
1,1'-Biphenyl, 4-methyl-	2	6	8	40.0
Pentanoicacid*	1	6	7	35.0
Tetradecane*	1	6	7	35.0
Hexanoicacid,ethylester	2	4	6	30.0
Heptanoicacid,ethylester*	3	3	6	30.0
Hexanoicacid,pentylester	4	2	6	30.0
Naphthalene,1-methyl-*	3	3	6	30.0
2-Tetradecene, (E)-	1	5	6	30.0
Hexanal*	3	2	5	25.0
Benzaldehyde*	2	3	5	25.0
Decanoicacid,ethylester*	2	3	5	25.0
Dodecanoicacid*	0	5	5	25.0
Pentadecanoicacid,ethylester	2	3	5	25.0
E-11-Hexadecenoicacid,ethylester	1	4	5	25.0
Phenol*	1	3	4	20.0

Acetophenone*	2	2	4	20.0
Naphthalene,2-methyl-	2	2	4	20.0
Naphthalene,2,7-dimethyl-	2	2	4	20.0
Hexadecane*	3	1	4	20.0
Furfural*	0	3	3	15.0
1-Hexanol*	0	3	3	15.0
2-Octenal, (E)-*	3	0	3	15.0
3-Nonen-2-one	2	1	3	15.0
2-Octenoicacid	1	2	3	15.0
Benzene,1,2,4,5-tetramethyl-	1	2	3	15.0
p-Benzoquinone	2	1	3	15.0
Galaxolide	3	0	3	15.0
Homomenthylsalicylate	3	0	3	15.0
Benzothiazole	1	1	2	10.0
2(3H)-Furanone, 5-ethyldihydro-	2	0	2	10.0
2(3H)-Furanone, dihydro-5-pentyl-	1	1	2	10.0
Vanillin	2	0	2	10.0
DiethylPhthalate	2	0	2	10.0
Benzene,1-methyl-2-[(4-methylphenyl)methyl]-	1	1	2	10.0
Dibutanoylmorphine	0	2	2	10.0
IsopropylPalmitate	2	0	2	10.0
Linalool Oxide	1	0	1	5.0
1-Octanol*	1	0	1	5.0
Benzene,1-ethyl-2,3-dimethyl-	0	1	1	5.0
Benzoicacid,ethylester	0	1	1	5.0
2-Decenal, (E)-	1	0	1	5.0
Naphthalene,2,6-dimethyl-	1	0	1	5.0
Dodecanal*	1	0	1	5.0
Octadecanal	1	0	1	5.0
3,7-Dimethyl-octa-1,6-diene	1	0	1	5.0
Caryophyllene*	1	0	1	5.0
β-Cadinene	1	0	1	5.0
ButylatedHydroxytoluene	1	0	1	5.0
Octadecane	1	0	1	5.0
1-Hexadecene	1	0	1	5.0
Hexadecanoicacid,methylester	1	0	1	5.0
11-Octadecenoicacid,methylester	1	0	1	5.0

Table 55. Identified VOCs in breath of twenty individuals with diagnosed Major Depressive Disorder ranked by frequency of occurrence (*Note*: *denotes compound was verified by reference standard)

Compound Name	Frequency			Occurrence (%)
	Female	Male	Total	
Nonanal*	10	10	20	100
Decanal*	10	10	20	100
Xylenes*	7	9	16	80
Benzene,1,2-dichloro-*	6	10	16	80
1-Hexanol,2-ethyl-*	7	8	15	75
Benzophenone*	6	8	14	70
Dodecane*	5	8	13	65
Tetradecane*	5	8	13	65
Styrene*	5	7	12	60
Phenol*	5	7	12	60
Undecane*	4	8	12	60
Tridecane*	4	8	12	60
ButylatedHydroxytoluene*	6	6	12	60
Toluene	3	4	7	35
Benzene,1,3,5-trimethyl-	2	5	7	35
Benzene,1-methyl-3-propyl-	1	6	7	35
Benzene,1,2,3-trimethyl-	1	4	5	25
Benzene,4-ethyl-1,2-dimethyl-	2	3	5	25
Benzene,1-ethyl-2,4-dimethyl-	1	4	5	25
Undecanal*	1	4	5	25
p-Benzoquinone	0	5	5	25
Benzene,1-ethyl-2-methyl-	1	3	4	20
Nonanoicacid	2	2	4	20
Benzene,1,2,4,5-tetramethyl-*	2	2	4	20
Naphthalene,2-methyl-	1	3	4	20
5,9-Undecadien-2-one, 6,10-dimethyl-(E)-*	1	3	4	20
Naphthalene*	1	3	4	20
Limonene*	1	2	3	15
Benzene,1,2,3,4-tetramethyl-	0	3	3	15
Dodecanal*	0	3	3	15
1,1'-Biphenyl,4-methyl-	2	1	3	15
5-Hepten-2-one,6-methyl-*	1	1	2	10
Benzene,diethyl-*	0	2	2	10

Acetophenone*	1	1	2	10
Benzene,1-methyl-2-(1-methylethyl)-	0	2	2	10
Benzene,1-ethyl-2,3-dimethyl-	1	1	2	10
Benzene,1,2,3,5-tetramethyl-	0	2	2	10
Triacetin	0	2	2	10
DiethylPhthalate	1	1	2	10
Acetone	1	0	1	5
Aceticacid	0	1	1	5
Hexanal*	0	1	1	5
Tetrachloroethylene	1	0	1	5
2-Propanol,1-propoxy-	0	1	1	5
3-Heptanone*	0	1	1	5
Benzene,(1-methylethyl)-	0	1	1	5
Benzene,propyl-*	0	1	1	5
Benzene,1-ethyl-3-methyl-	0	1	1	5
Benzene,1,2,4-trimethyl-	0	1	1	5
Benzene,1,4-dichloro-	1	0	1	5
Benzene,2-ethyl-1,4-dimethyl-	0	1	1	5
Decane,2-methyl-	1	0	1	5
Benzene,1-methyl-4-propyl-	0	1	1	5
Benzene,2-ethyl-1,3-dimethyl-	0	1	1	5
Benzene,1-methyl-4-(1-methylethyl)-	0	1	1	5
1-Dodecene*	0	1	1	5
Benzene,1-methyl-4-(1-methylpropyl)-	0	1	1	5
Undecane,2,6-dimethyl-	1	0	1	5
2-Dodecene, (Z)-	0	1	1	5
3-Dodecene, (Z)-	0	1	1	5
Benzene,pentamethyl-	0	1	1	5
Indole	1	0	1	5
n-Decanoicacid*	0	1	1	5
Hexanedioicacid,bis(1-methylethyl) ester	0	1	1	5
Dodecanoicacid*	0	1	1	5
Hexadecane*	0	1	1	5
Methyldihydrojasmonate	1	0	1	5
Diisopropylnaphthalene	0	1	1	5
2,6-Diisopropylnaphthalene*	0	1	1	5
Tetradecanoicacid	0	1	1	5
Galaxolide	1	0	1	5
Homomenthylsalicylate	1	0	1	5
n-Hexadecanoicacid*	0	1	1	5

Table 56. Identified VOCs in blood odor of twenty individuals with diagnosed Major Depressive Disorder ranked by frequency of occurrence (*Note*: *denotes compound was verified by reference standard)

Compound Name	Frequency			Occurrence (%)
	Female	Male	Total	
Hexadecane*	10	10	20	100
2,6-Diisopropylnaphthalene*	10	10	20	100
Undecane*	9	10	19	95.0
Nonanal*	9	10	19	95.0
Dodecane*	9	10	19	95.0
Benzenaldehyde,2,4,6-trimethyl-	9	10	19	95.0
Tridecane*	9	10	19	95.0
Tetradecane*	9	9	18	90.0
Hexanal	9	7	16	80.0
Diisopropylnaphthalene	7	7	14	70.0
Heptadecane*	5	9	14	70.0
Benzyl Alcohol*	6	5	11	55.0
Longifolene	2	7	9	45.0
Benzene,4-ethyl-1,2-dimethyl-	1	7	8	40.0
Benzene,1,2,4,5-tetramethyl-	2	6	8	40.0
5,9-Undecadien-2-one,6,10-dimethyl,(E)-	2	6	8	40.0
Octadecane	2	6	8	40.0
Cyclohexanone*	4	3	7	35.0
Furan,2-pentyl-*	4	3	7	35.0
Benzene,1,3,5-trimethyl-	2	5	7	35.0
Benzene,1,2,3,5-tetramethyl-	3	4	7	35.0
1-Decene*	3	4	7	35.0
2-Dodecene,(Z)-	2	5	7	35.0
Decanal*	3	4	7	35.0
2-Undecanone	3	4	7	35.0
Furan,2-butyltetrahydro-	3	4	7	35.0
Naphthalene,2-methyl-	2	4	6	30.0
2-Heptanone*	1	4	5	25.0
Heptanal*	3	2	5	25.0
1-Octen-3-ol*	1	4	5	25.0
1-Dodecene*	3	2	5	25.0
1-Pentanol	1	3	4	20.0
p-Xylene*	1	3	4	20.0
Benzene,1-ethyl-2,4-dimethyl-	0	4	4	20.0
Naphthalene,1-methyl-*	2	2	4	20.0

Benzene,1-ethyl-2-methyl-	1	2	3	15
Benzene,1,2,3-trimethyl-	1	2	3	15
Benzene,1,2-dichloro-*	2	1	3	15
4-Cyanocyclohexene	1	2	3	15
Benzene,2-ethyl-1,4-dimethyl-	3	0	3	15
Benzene,1,2,3,4-tetramethyl-	2	1	3	15
Decane,1-chloro-	1	2	3	15
1-Dodecanol*	0	3	3	15
Naphthalene*	0	3	3	15
2-Propanol,1-propoxy-	0	2	2	10
Octanal	0	2	2	10
Benzenemethanol,α,α-dimethyl-	2	0	2	10
Benzaldehyde,4-(methylthio)-	2	0	2	10
2,4-Diphenyl-4-methyl-1(E)-pentene	1	1	2	10
Tetrachloroethylene	1	0	1	5
3-Heptanone*	0	1	1	5
Nonane*	1	0	1	5
Ethanol,2-butoxy-	1	0	1	5
Benzaldehyde*	1	0	1	5
Benzene,1-ethyl-3-methyl-	0	1	1	5
2,5-Octanedione	1	0	1	5
Phenol*	1	0	1	5
Decane	0	1	1	5
Benzene,1,4-dichloro-	1	0	1	5
Benzaldehyde,2-hydroxy-	1	0	1	5
Benzene,1-methyl-3-propyl-	0	1	1	5
3-Isopropylbenzaldehyde	1	0	1	5
3-Dodecene,(Z)-	0	1	1	5
1-Cyclohexene-1-carboxaldehyde,2,6,6-trimethyl-	1	0	1	5
Benzaldehyde,ethyl-	1	0	1	5
1-Phenyl-2-butanone	1	0	1	5
Naphthalene,1,6-dimethyl-	1	0	1	5
Naphthalene,2,7-dimethyl-	0	1	1	5
Naphthalene,2,6-dimethyl-	1	0	1	5
Naphthalene,2,3-dimethyl-	1	0	1	5
Cyclododecane	1	0	1	5
1,1'-Biphenyl,4-methyl-	0	1	1	5
1,1'-Biphenyl,2-methyl-	0	1	1	5
Z-8-Hexadecene	0	1	1	5

Table 57. Identified VOCs in urine odor of twenty individuals with diagnosed Major Depressive Disorder ranked by frequency of occurrence (*Note*: *denotes compound was verified by reference standard)

Compound Name	Frequency			Occurrence (%)
	Female	Male	Total	
4-Heptanone*	10	9	19	95.0
Cedrol	6	8	14	70.0
Nonanal*	5	7	12	60.0
Carvones	6	5	11	55.0
2-Pentanone	2	3	5	25.0
Phenol,4-methyl- *(p-Cresol)**	3	2	5	25.0
Benzene,1-methyl-4-(1-methylethenyl)-	3	2	5	25.0
2-Isopropylbenzaldehyde	1	4	5	25.0
Pyrrole	2	2	4	20.0
Menthol*	5	5	10	20.0
Decanal*	2	2	4	20.0
Benzaldehyde,3,5-dimethyl-	2	2	4	20.0
4-Nonylphenol	3	1	4	20.0
Benzene,1,4-dichloro-	2	1	3	15.0
.+/-.-4-Acetyl-1-methylcyclohexene	1	2	3	15.0
p-Menthan-3-one	3	0	3	15.0
4-Terpineol	1	2	3	15.0
ButylatedHydroxytoluene*	0	3	3	15.0
3-Heptanone*	0	2	2	10.0
Benzaldehyde*	0	2	2	10.0
Benzene,1,2-dichloro-*	2	0	2	10.0
Benzene,1-methyl-3-(1-methylethyl)-	1	1	2	10.0
Benzeneacetaldehyde,.alpha.-methyl-	2	0	2	10.0
ValproicAcid	0	2	2	10.0
α-Terpineol	2	0	2	10.0
p-Chloroaniline	0	2	2	10.0
3-Cyclohexen-1-one,2-isopropyl-5-methyl-	1	1	2	10.0
p-Menth-1-en-3-one	1	1	2	10.0
Dodecanoicacid*	1	1	2	10.0
Benzophenone*	2	0	2	10.0
Phenol,4-(1,1,3,3-tetramethylbutyl)-*	1	1	2	10.0
Toluene	0	1	1	5.0
α-Terpinene	1	0	1	5.0
Benzene,1,3-dichloro-	1	0	1	5.0
Benzene,4-ethyl-1,2-dimethyl-	0	1	1	5.0

Benzene,1-methyl-4-(1-methylethyl)-	0	1	1	5.0
Benzeneacetaldehyde	1	0	1	5.0
3-Octen-2-one	0	1	1	5.0
γ-Terpinene	1	0	1	5.0
Benzene,-(1-formylethyl)-	0	1	1	5.0
1,6-Octadien-3-ol,3,7-dimethyl-*	1	0	1	5.0
Benzaldehyde,3-chloro-	1	0	1	5.0
Benzaldehyde,4-chloro-	0	1	1	5.0
Ethanone,1-(2-hydroxyphenyl)-	1	0	1	5.0
Benzenamine,4-chloro-2-(trifluoromethyl)-	1	0	1	5.0
Phenol,2,5-dichloro-	1	0	1	5.0
Benzenamine,2,6-dimethyl-	0	1	1	5.0
Limonene	0	1	1	5.0
Dihydrocarvone	1	0	1	5.0
2,4-Cycloheptadien-1-one,2,6,6-trimethyl-	0	1	1	5.0
Isobornylacetate	0	1	1	5.0
2-Propenal,3-phenyl-	1	0	1	5.0
2-Methoxy-4-vinylphenol	0	1	1	5.0
Ethanone,1-(4-chlorophenyl)-	1	0	1	5.0
Benzoicacid,2-amino-,methylester	1	0	1	5.0
Damascenone	0	1	1	5.0
Butanoicacid,butylester	1	0	1	5.0
5,9-Undecadien-2-one,6,10-dimethyl,(E)-*	1	0	1	5.0
Dibutanoylmorphine	0	1	1	5.0
Cyclotetradecane	0	1	1	5.0
IsopropylPalmitate	1	0	1	5.0

3.6.4.4. Comparison of the Volatile Organic Compounds Present Above Collected Odor Samples from Five Biological Specimens across Populations

As investigated in sections 3.6.4.1.2.1 and 3.6.4.1.2.2, Type I and Type II errors were calculated for each of the five biological specimens for the type 2 diabetic and MDD subject groups. Summary of the total number of errors and the percentage of mismatching of individuals are shown in Table 58 through

Table 60 for healthy, type 2 diabetic and MDD subject groups, respectively. For all three subject groups, the number of total errors was highest for hand odor. However, this can be attributed to the fact that for hand odor, the volatile compounds categorized as "human scent compounds" have previously been determined and therefore the human compound database for hand odor holds a much smaller number of VOCs compared to the other specimens that were newly investigated in the present study. All extracted volatile compounds were included in the data analysis for the remaining four biological specimens investigated in the present study, resulting in a greater array of compounds and higher variations in the relative peak area ratios of compounds present in the headspace of each individual's specimen odor profiles, thereby lowering the Type I and Type II error occurrences.

The low percentages in the mismatch occurrences further support the hypothesis that VOCs emanations are different among individuals for the five biological specimens and can be used as distinguishing profiles among individuals. As previously mentioned in section 3.6.4.1.2.1 the match/no-match cut-off threshold is recommended to be kept at least at 0.8 correlation, and wherever possible, a cut-off threshold of 0.9 should be

utilized. Preliminary comparison of the three subject groups at 0.9 correlation threshold as shown in Table 61 demonstrates that having a medical condition did not greatly affect in the mismatch occurrences for any of the biological specimens. Percentages of mismatch occurrences were almost identical across the three study groups for hand odor and buccal swabs. For breath and blood, misidentification occurrences were slightly higher in the diabetic and depressed study groups than in the healthy control group. For urine, diabetics had the lowest percentage of misidentification occurrence, while the depressed study group had the highest percentage of misidentification occurrence, although all are still under 1%. There were no particular patterns in the increase or decrease in the percentage of misidentification of individuals relative to their physiological or psychological health statuses.

Thus far the present study has demonstrated that odor profiles from human biological specimens other than hand odor can differentiate individuals with high certainty. Specimen odor profiles from individuals with the same diagnosed medical condition were also found to achieve good differentiation which is important for forensic consideration. Because certain diseases are known to be associated with certain odors, it is expected that the odor profiles of individuals who are diagnosed with the same disease or disorder to have similar VOC profiles with one another than individuals who do not have the same disease or disorder. From the medical perspective this could be important as it could lead to identifying key volatile biomarkers for certain medical conditions. From the legal/forensic perspective, the ability to identify individuals with a given medical condition is potentially significant because the possibility of mismatching two individuals

with the same medical condition becomes unlikely. This leads to less chance of false identification of suspects, for example by canine detection, on the basis of the two individuals having the same medical condition. With the sample size of the present study, having a diagnosed medical/psychological condition did not affect the misidentification rate of individuals. If an argument was made where suspect mismatching occurred because of the individual's health statuses, results from this preliminary study can be used as a counterargument on the basis that a high distinguish-ability of specimen VOC profiles was still achieved regardless of the subjects' health statuses.

Further studies with larger sample sizes for the three subject groups are needed to obtain a more definitive indication on whether physiological or psychological health statuses have an effect on the misidentification occurrences of individuals. Future studies should include individuals with extreme conditions from the diabetic and depressed subject groups. For the diabetic population, it would be desirable to collect samples from uncontrolled (by medication or diet) diabetic patients and perhaps even diabetic patients recently suffered from diabetic ketoacidosis. Similarly for the depressed population, sample collection from patients who are in severe depressed state at the time of sampling is desirable. The present study was not able to include such subjects as access to diabetic inpatients admitted as a result of uncontrolled diabetes was prohibited, and the severely depressed patients were unresponsive and unable to give consent for sample collection during the course of the study.

Table 58. Type I and Type II errors for five biological specimens amongst healthy individuals

	Hand Odor		Buccal Swab		Breath		Blood		Urine	
Threshold	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8
Type I	3	13	0	1	0	1	0	0	0	0
Type II	15	9	9	4	11	4	12	6	8	3
Total Error	18	22	9	5	11	5	12	6	8	3
% Mismatch	0.89	1.09	0.45	0.25	0.58	0.26	0.63	0.32	0.36	0.14

Table 59. Type I and Type II errors for five biological specimens amongst type 2 diabetic patients

	Hand Odor		Buccal Swab		Breath		Blood		Urine	
Threshold	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8
Type I	7	39	0	0	0	0	0	0	0	0
Type II	7	4	7	2	8	3	7	4	2	0
Total Error	14	43	7	2	8	3	7	4	2	0
% Mismatch	0.88	2.69	0.44	0.13	1.14	0.43	1.00	0.57	0.13	0

Table 60. Type I and Type II errors for five biological specimens amongst individuals diagnosed with Major Depressive Disorder

	Hand Odor		Buccal Swab		Breath		Blood		Urine	
Threshold	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8
Type I	4	28	0	0	0	1	0	0	0	0
Type II	10	9	5	3	7	4	15	8	11	8
Total Error	14	37	5	3	7	5	15	8	11	8
% Mismatch	0.79	2.09	0.28	0.17	0.90	0.64	1.92	1.03	0.62	0.45

Table 61. Comparison of mismatch occurrences for the five biological specimens across populations at 0.9 correlation cutoff threshold

	Hand Odor	Buccal Swab	Breath	Blood	Urine
Healthy	0.89%	0.45%	0.58%	0.63%	0.36%
Type 2 Diabetes	0.88%	0.44%	1.14%	1.00%	0.13%
MDD	0.79%	0.28%	0.90%	1.92%	0.62%

3.6.4.4.1. Chi-Square Significance Test

Table 62 to Table 66 give the frequencies of the VOCs extracted from individuals within their population subgroups (healthy control, type 2 diabetics (T2DB), and clinically depressed (MDD)). From the entire list of compounds detected from each specimen, those compounds that appeared at least once in each subgroup were considered for further data analysis.

Table 62. Frequency of occurrence of hand odor VOCs in total population and within subgroups (*Notes*: [a]n=70, [b]n=31, [c]n=19, [d]n=20)

Compound Name	Occurrence in Total Population[a]	Occurrence within Group		
		Healthy[b]	T2DB[c]	MDD[d]
Decanal	100%	31	19	20
Nonanal	100%	31	19	20
Tetradecane	96%	31	17	19
Hexadecane	93%	30	17	18
Heptadecane	90%	28	17	18
Undecanal	67%	30	7	10
Octadecane	66%	18	11	17
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	63%	23	9	12
Tridecane	61%	22	9	12
Undecane	54%	15	11	12
Dodecane	37%	14	5	7
BenzylAlcohol	29%	13	2	5
Dodecanal	23%	10	2	4
1,6-Octadien-3-ol,3,7-dimethyl-	16%	7	1	3
1-Hexanol,2-ethyl-	16%	6	2	3
Dodecanoicacid,methylester	14%	3	4	3
2-Nonenal,(E)-	11%	3	4	1
Acetophenone	11%	3	3	2
Benzaldehyde	10%	4	1	2
2-Decanone	9%	6	0	0
Nonanoicacid,methylester	6%	4	0	0
Octanoicacid,methylester	6%	2	1	1
Pentadecane	4%	2	0	1
1-Pentadecene	3%	1	0	1
5-Hepten-2-one,6-methyl-	3%	0	1	1
Dodecanoicacid	3%	2	0	0
Eicosane	3%	2	0	0
Furfural	3%	2	0	0
Heptanal	3%	1	0	1
Hexanoicacid	3%	1	0	1
Phenol	3%	2	0	0
2-Pentanone	1%	0	1	0
Aceticacid,butylester	1%	0	1	0
Cedrol	1%	0	1	0
Docosane	1%	1	0	0
Heneicosane	1%	1	0	0
Lilial	1%	0	1	0
Octanal	1%	1	0	0
Propanedioicacid,dimethylester	1%	1	0	0
Tetradecanal	1%	1	0	0

Table 63. Frequency of occurrence of buccal swab odor VOCs in total population and within subgroups (*Notes*: [a]n=70, [b]n=31, [c]n=19, [d]n=20)

Compound Name	Occurrence in Total Population[a]	Occurrence within Group		
		Healthy[b]	T2DB[c]	MDD[d]
Decanal	100%	31	19	20
Hexanoicacid	100%	31	19	20
Furan,2-pentyl-	91%	30	15	19
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	90%	28	16	19
Nonanoicacid,ethylester	87%	27	16	18
Nonanoicacid	84%	24	17	18
2,4-Nonadienal,(E,E)-	77%	28	10	16
Dodecanoicacid,ethylester	77%	24	14	16
6-Dodecanone	70%	24	14	11
2-Nonenal,(E)-	63%	25	6	13
Hexadecanoicacid,ethylester	61%	20	10	13
Tetradecanoicacid,ethylester	61%	20	11	12
1-Tetradecene	60%	17	13	12
1,1'-Biphenyl,2,2'-diethyl-	56%	13	11	15
Octanoicacid,ethylester	50%	19	7	9
6-Methyl-3,5-heptadiene-2-one	43%	10	10	10
Hexanoicacid,ethylester	41%	20	3	6
1-Dodecene	40%	13	6	9
1-Decene	39%	8	11	8
Tetradecane	39%	10	10	7
Benzaldehyde	36%	12	8	5
Hexanal	34%	18	1	5
Naphthalene,1-methyl-	31%	14	2	6
Hexanoicacid,pentylester	30%	13	2	6
Naphthalene,2-methyl-	29%	12	4	4
2-Tetradecene,(E)-	24%	7	4	6
Dodecanoicacid	24%	7	5	5
Heptanoicacid	24%	1	8	8
Heptanoicacid,ethylester	24%	8	3	6
1,1'-Biphenyl,4-methyl-	23%	2	6	8
1-Hexanol	23%	7	6	3
2-Octenoicacid	23%	9	4	3
Dibutanoylmorphine	21%	11	2	2
Pentanoicacid	21%	5	3	7

Decanoicacid,ethylester	20%	6	3	5
DiethylPhthalate	20%	9	3	2
Linalool Oxide	20%	11	1	2
Pentadecanoicacid,ethylester	20%	7	2	5
3-Nonen-2-one	19%	10	0	3
Cyclododecane	19%	8	5	0
Naphthalene,2,7-dimethyl-	19%	6	3	4
Furfural	17%	8	1	3
1-Octanol	16%	9	1	1
Hexadecane	16%	6	1	4
2(3H)-Furanone,5-ethyldihydro-	14%	3	5	2
2(3H)-Furanone,dihydro-5-pentyl-	14%	3	5	2
2-Octenal,(E)-	14%	7	0	3
Acetophenone	14%	3	3	4
Phenol	14%	2	4	4
Benzene,1-methyl-2-[(4-methylphenyl)methyl]-	13%	3	4	2
Benzothiazole	13%	3	4	2
Caryophyllene	13%	5	3	1
2,5-Cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)-	11%	4	1	3
E-11-Hexadecenoicacid,ethylester	11%	2	1	5
IsopropylMyristate	10%	5	2	0
Vanillin	10%	5	0	2
5,9-Undecadien-2-one,6,10-dimethyl-, (Z)-	9%	5	1	0
Benzoicacid,ethylester	9%	5	0	1
Galaxolide	7%	2	0	3
IsopropylPalmitate	7%	2	1	2
1-Pentadecene	6%	4	0	0
3,5,9-Undecatrien-2-one,6,10-dimethyl-, (E,Z)-	6%	3	1	0
3,7-Dimethyl-octa-1,6-diene	6%	3	0	1
5,9-Undecadien-2-one,6,10-dimethyl-	6%	4	0	0
Benzene,1,2,4,5-tetramethyl-	6%	0	1	3
Benzophenone	6%	4	0	0
ButylatedHydroxytoluene	6%	1	2	1
Cyclopropane,nonyl-	6%	4	0	0
Hexadecanoicacid,methylester	6%	1	2	1
Octadecanal	6%	3	0	1
Octadecane	6%	2	1	1
1-Hexadecene	4%	2	0	1
2(3H)-Furanone,dihydro-5-propyl-	4%	1	2	0

Benzene,1,1'-methylenebis[4-methyl-	4%	3	0	0
β-Bourbonene	4%	3	0	0
Calamenene	4%	3	0	0
Heptadecane	4%	3	0	0
Hexanoicacid,anhydride	4%	3	0	0
Homomenthylsalicylate	4%	0	0	3
OctanoicAcid	4%	3	0	0
Tributylphosphate	4%	1	1	1
1,1'-Biphenyl,3-methyl-	3%	1	1	0
1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-, (Z)-	3%	2	0	0
1-Heptadecene	3%	2	0	0
1-Pentanol	3%	2	0	0
Cyclodecane	3%	2	0	0
Cyclotetradecane	3%	2	0	0
Dodecanal	3%	1	0	1
Ethyl9-hexadecenoate	3%	2	0	0
Naphthalene,2,6-dimethyl-	3%	0	1	1
Tridecane	3%	0	2	0
γ-Cadinene	3%	1	0	1
1,1'-Biphenyl,2-ethyl-	1%	1	0	0
11-Octadecenoicacid,methylester	1%	0	0	1
2,4-Decadienal,(E,E)-	1%	1	0	0
2-Decenal,(E)-	1%	0	0	1
2-Dodecenal,(E)-	1%	1	0	0
2-Heptenoicacid	1%	1	0	0
3-Eicosene,(E)-	1%	1	0	0
3-Heptadecene,(Z)-	1%	1	0	0
4,8-Dimethyl-nona-3,8-dien-2-one	1%	1	0	0
7-Hexadecene,(Z)-	1%	1	0	0
9-Octadecenoicacid,(E)-	1%	1	0	0
Aceticacid	1%	1	0	0
Benzaldehyde,4-(1-methylethyl)-	1%	1	0	0
Benzene, 1-methyl-2-[(3-methylphenyl)methyl]-	1%	1	0	0
Benzene,1,2,3,5-tetramethyl-	1%	0	1	0
Benzene,1-ethyl-2,3-dimethyl-	1%	0	0	1
Benzene,1-methyl-4-nitro-	1%	0	1	0
Carvone	1%	0	1	0
Cyclododecanol,1-ethenyl-	1%	1	0	0
Cyclohexadecane	1%	0	1	0

trans-p-Menthan-3-one	1%	0	1	0
Δ-Cadinene	1%	1	0	0
Docosane	1%	1	0	0
Dodecanoicacid,methylester	1%	1	0	0
Eicosane	1%	1	0	0
Ethyltridecanoate	1%	1	0	0
Glycocyanidine	1%	1	0	0
Heneicosane	1%	1	0	0
Hexanoicacid,propylester	1%	0	1	0
Menthol	1%	0	1	0
Nonadecanoicacid,ethylester	1%	1	0	0
Pentadecane,7-methyl-	1%	1	0	0
Tetradecanoicacid,2-methyl-,methyl ester	1%	1	0	0

Table 64. Frequency of occurrence of breath odor VOCs in total population and within subgroups (*Notes*: [a]n=70, [b]n=31, [c]n=19, [d]n=20)

Compound Name	Occurrence in Total Population[a]	Occurrence within Group		
		Healthy[b]	T2DB[c]	MDD[d]
Decanal	99%	31	18	20
Nonanal	99%	30	19	20
Xylenes	76%	22	15	16
Benzophenone	76%	29	10	14
1-Hexanol,2-ethyl-	73%	25	11	15
Benzene,1,2-dichloro-	73%	25	10	16
Styrene	66%	29	6	11
ButylatedHydroxytoluene	60%	24	6	12
Dodecane	56%	13	13	13
Tetradecane	53%	11	13	13
Tridecane	50%	10	13	12
Undecane	50%	13	10	12
Phenol	47%	18	3	12
Toluene	30%	8	6	7
Undecanal	26%	9	4	5
Benzene,1,3,5-trimethyl-	24%	4	6	7
Dibutylphthalate	24%	16	1	0
2,6-Diisopropylnaphthalene	23%	13	2	1
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	20%	9	1	4
p-Benzoquinone	17%	1	6	5
Acetophenone	17%	10	0	2
Benzene,1-methyl-3-propyl-	17%	0	5	7
Benzene,4-ethyl-1,2-dimethyl-	17%	2	5	5
Dodecanal	17%	8	1	3
1-Dodecene	16%	10	0	1
Longifolene	14%	10	0	0
Naphthalene	14%	5	3	2
Benzene,1,2,3-trimethyl-	13%	2	2	5
Nonanoicacid	13%	5	0	4
Benzene,1-ethyl-2,4-dimethyl-	11%	1	2	5
Benzene,1-ethyl-2-methyl-	10%	1	2	4
Diisopropylnaphthalene	10%	4	2	1
Indole	10%	6	0	1
Phenol,2-(1,1-dimethylethyl)-	10%	7	0	0
Limonene	10%	2	2	3

Benzene,1-ethyl-2,3-dimethyl-	9%	0	4	2
Benzene,2-ethyl-1,4-dimethyl-	9%	0	5	1
Naphthalene,2-methyl-	9%	1	1	4
1,1'-Biphenyl,4-methyl-	7%	2	0	3
1,3,5,7-Cyclooctatetraene	7%	1	3	1
4-Cyanocyclohexene	7%	1	4	0
Benzene,1,4-dichloro-	7%	3	1	1
5-Hepten-2-one,6-methyl-	6%	2	0	2
Benzene,1,2,3,4-tetramethyl-	6%	0	1	3
Benzene,1,2,4,5-tetramethyl-	6%	0	0	4
DiethylPhthalate	6%	1	1	2
Hexadecane	6%	3	0	1
Menthol	6%	1	3	0
2-Dodecene,(Z)-	4%	0	2	1
2-Propanol,1-propoxy-	4%	1	1	1
Acetone	4%	0	2	1
Benzene,1-methyl-4-(1-methylpropyl)-	4%	1	1	1
Benzene,2-ethyl-1,3-dimethyl-	4%	0	2	1
Benzene,diethyl-	4%	1	0	2
Cyclododecane	4%	3	0	0
Hexanedioicacid,bis(1-methylethyl) ester	4%	0	2	1
Longicyclene	4%	3	0	0
n-Decanoicacid	4%	2	0	1
3-Heptanone	3%	0	1	1
Benzene,1,2,3,5-tetramethyl-	3%	0	0	2
Benzene,1-methyl-2-(1-methylethyl)-	3%	0	0	2
Benzene,propyl-	3%	1	0	1
β-Maaliene	3%	2	0	0
Galaxolide	3%	1	0	1
Heptadecane	3%	2	0	0
Hexanal	3%	1	0	1
Homomenthylsalicylate	3%	1	0	1
Triacetin	3%	0	0	2
Undecane,2,6-dimethyl-	3%	1	0	1
1S-α-Pinene	1%	1	0	0
3-Dodecene,(Z)-	1%	0	0	1
3-Octanol,3,7-dimethyl-,(.+/-.)-	1%	1	0	0
5-Dodecene,(E)-	1%	0	1	0
Aceticacid	1%	0	0	1
Benzaldehyde	1%	1	0	0

Benzaldehyde,4-methoxy-	1%	1	0	0
Benzene,(1-methylethyl)-	1%	0	0	1
Benzene,1,2,4-trimethyl-	1%	0	0	1
Benzene,1,3-dichloro-	1%	0	1	0
Benzene,1,3-diethyl-5-methyl-	1%	1	0	0
Benzene,1-ethyl-3,5-dimethyl-	1%	1	0	0
Benzene,1-ethyl-3-methyl-	1%	0	0	1
Benzene,1-methyl-3-(1-methylethyl)-	1%	1	0	0
Benzene,1-methyl-4-(1-methylethyl)-	1%	0	0	1
Benzene,1-methyl-4-propyl-	1%	0	0	1
Benzene,3-cyclohexen-1-yl-	1%	1	0	0
Benzene,pentamethyl-	1%	0	0	1
β-Gurjunene	1%	1	0	0
Caryophyllene	1%	1	0	0
Cyclohexane,methyl-	1%	1	0	0
Decane,2-methyl-	1%	0	0	1
Decane,3-methyl-	1%	1	0	0
Dodecanoicacid	1%	0	0	1
Dodecanoicacid,methylester	1%	1	0	0
Eicosane	1%	1	0	0
Ethanol,2-phenoxy-	1%	1	0	0
Indolizine	1%	1	0	0
Isobornylacetate	1%	0	1	0
IsopropylPalmitate	1%	1	0	0
Methyldihydrojasmonate	1%	0	0	1
MethylSalicylate	1%	1	0	0
n-Hexadecanoicacid	1%	0	0	1
Nonadecane	1%	1	0	0
Nonane,3-methyl-	1%	1	0	0
Octanal	1%	1	0	0
Tetrachloroethylene	1%	0	0	1
Tetradecanal	1%	1	0	0
Tetradecanoicacid	1%	0	0	1
Undecane,3,6-dimethyl-	1%	1	0	0

Table 65. Frequency of occurrence of breath odor VOCs in total population and within subgroups (*Notes*: [a]n=70, [b]n=31, [c]n=19, [d]n=20)

Compound Name	Occurrence in Total Population[a]	Occurrence within Group		
		Healthy[b]	T2DB[c]	MDD[d]
Hexadecane	99%	30	19	20
Nonanal	94%	28	19	19
2,6-Diisopropylnaphthalene	93%	30	15	20
Tetradecane	91%	30	16	18
Undecane	91%	29	16	19
Benzaldehyde,2,4,6-trimethyl-	89%	31	12	19
Tridecane	89%	29	14	19
Dodecane	86%	28	13	19
Hexanal	76%	26	11	16
Heptadecane	69%	22	12	14
BenzylAlcohol	60%	25	6	11
Diisopropylnaphthalene	57%	19	7	14
Furan,2-butyltetrahydro-	56%	23	9	7
Furan,2-pentyl-	47%	20	6	7
Decanal	44%	15	9	7
1-Dodecene	41%	18	6	5
Cyclohexanone	41%	19	3	7
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	37%	14	4	8
2-Dodecene,(Z)-	36%	12	6	7
Longifolene	33%	6	8	9
1-Decene	31%	7	8	7
1-Octen-3-ol	31%	8	9	5
2-Undecanone	30%	11	3	7
Benzene,1,2-dichloro-	29%	12	5	3
Octadecane	29%	6	6	8
1-Pentanol	26%	12	2	4
2-Heptanone	21%	8	2	5
Benzene,1,3,5-trimethyl-	20%	0	7	7
Heptanal	20%	5	4	5
Cyclododecane	19%	10	2	1
1-Dodecanol	17%	5	4	3
Naphthalene,2-methyl-	16%	0	5	6
Benzene,1,2,3,5-tetramethyl-	14%	0	3	7
Benzene,1,2,4,5-tetramethyl-	14%	0	2	8
Benzene,4-ethyl-1,2-dimethyl-	14%	1	1	8
Naphthalene	14%	1	6	3

Benzene,1-ethyl-2,4-dimethyl-	13%	0	5	4
Xylenes	13%	3	2	4
4-Cyanocyclohexene	11%	2	3	3
Cyclopropane,nonyl-	11%	7	1	0
Menthol	11%	4	4	0
Octanal	11%	6	0	2
1-Octanol	10%	5	2	0
2-Heptanone,6-methyl-	9%	5	1	0
2-Propanol,1-propoxy-	9%	1	3	2
Benzene,1,4-dichloro-	9%	4	1	1
Naphthalene,1-methyl-	9%	0	2	4
2,4-Diphenyl-4-methyl-1(E)-pentene	7%	3	0	2
Benzenemethanol,.alpha.,.alpha.-dimethyl-	7%	3	0	2
Ethanol,2-butoxy-	7%	3	1	1
1-Phenyl-2-butanone	6%	3	0	1
3-Heptanone	6%	0	3	1
Benzaldehyde,4-(methylthio)-	6%	2	0	2
Benzene,1,2,3,4-tetramethyl-	6%	0	1	3
Benzene,1,2,3-trimethyl-	6%	0	1	3
Benzene,1-ethyl-2-methyl-	6%	0	1	3
Benzene,2-ethyl-1,4-dimethyl-	6%	0	1	3
Heptanol	6%	3	1	0
1,1'-Biphenyl,2-methyl-	4%	1	1	1
2,5-Octanedione	4%	0	2	1
3-Dodecene,(Z)-	4%	0	2	1
Benzaldehyde	4%	2	0	1
Dodecane,1-chloro-	4%	0	0	3
Naphthalene,2,7-dimethyl-	4%	0	2	1
Phenol	4%	2	0	1
1,1'-Biphenyl,4-methyl-	3%	1	0	1
3,7-Dimethyl-octa-1,6-diene	3%	1	1	0
3-Isopropylbenzaldehyde	3%	1	0	1
4-Heptanone	3%	2	0	0
5-Hepten-2-one,6-methyl-	3%	2	0	0
Benzene,1,4-diethyl-2-methyl-	3%	0	1	1
Benzene,1-ethyl-3,5-dimethyl-	3%	0	2	0
Benzene,1-ethyl-3-methyl-	3%	0	1	1
Benzene,1-methyl-3-propyl-	3%	1	0	1
Benzene,1-methyl-4-(1-methylpropyl)-	3%	1	1	0
Decane	3%	0	1	1
Naphthalene,2,3,6-trimethyl-	3%	1	1	0
Phenol,2,4,6-trimethyl-	3%	2	0	0

Phenol,4-methoxy-2-nitro-	3%	2	0	0
Z-8-Hexadecene	3%	0	1	1
1,1'-Biphenyl,3-methyl-	1%	0	1	0
1,2-Benzenediol,3,5-bis(1,1-dimethylethyl)-	1%	1	0	0
β-Cyclocitral	1%	0	0	1
1-Hexadecene	1%	0	1	0
1-Tetradecene	1%	1	0	0
2-Decanone	1%	1	0	0
2-Propanol,1-butoxy-	1%	0	1	0
4-tert-Butylcyclohexylacetate	1%	0	1	0
Acetone	1%	1	0	0
Acetophenone	1%	1	0	0
Benzaldehyde,2-hydroxy-	1%	0	0	1
Benzaldehyde,3-hydroxy-	1%	1	0	0
Benzaldehyde,ethyl-	1%	0	0	1
Benzene,1,3-dichloro-	1%	1	0	0
Benzene,1,3-dimethyl-5-(1-methylethyl)-	1%	0	1	0
Benzene,1-ethyl-2,3-dimethyl-	1%	0	1	0
Benzene,1-methyl-4-(1-methylethyl)-	1%	0	1	0
Benzene,2,4-dimethyl-1-(1-methylethyl)-	1%	0	1	0
Benzene,diethyl-	1%	1	0	0
Benzene,ethyl-1,2,4-trimethyl-	1%	0	1	0
Cyclodecane	1%	1	0	0
Cyclooctane	1%	1	0	0
Cyclooctane,1,2-dimethyl-	1%	1	0	0
Cyclotetradecane	1%	0	1	0
Dimethylsulfone	1%	1	0	0
Limonene	1%	1	0	0
Dodecane,2,6,10-trimethyl-	1%	0	1	0
Heptadecane,9-octyl-	1%	1	0	0
Hexanedioicacid,bis(1-methylethyl) ester	1%	0	1	0
IsopropylMyristate	1%	1	0	0
Naphthalene,1,6-dimethyl-	1%	0	0	1
Naphthalene,1,7-dimethyl-	1%	0	1	0
Naphthalene,2,3-dimethyl-	1%	0	0	1
Naphthalene,2,6-dimethyl-	1%	0	0	1
Nonane	1%	0	0	1
Tetrachloroethylene	1%	0	0	1
Tetradecanal	1%	1	0	0

Table 66. Frequency of occurrence of breath odor VOCs in total population and within subgroups (*Notes*: [a]n=70, [b]n=31, [c]n=19, [d]n=20)

Compound Name	Occurrence in Total Population[a]	Occurrence within Group		
		Healthy[b]	T2DB[c]	MDD[d]
4-Heptanone	90%	30	14	19
Carvone	56%	21	7	11
Nonanal	51%	13	11	12
Cedrol	44%	6	11	14
Menthol	43%	12	8	10
4-Nonylphenol	37%	22	0	4
Pyrrole	31%	16	2	4
Benzene,1-methyl-4-(1-methylethenyl)-	27%	10	4	5
Benzene,1,4-dichloro-	26%	15	0	3
Phenol,4-(1,1,3,3-tetramethylbutyl)-	24%	15	0	2
4-Terpineol	23%	8	5	3
2-Isopropylbenzaldehyde	21%	7	3	5
.+/-.-4-Acetyl-1-methylcyclohexene	19%	10	0	3
Benzaldehyde,3,5-dimethyl-	19%	7	2	4
Decanal	19%	5	4	4
2-Pentanone	17%	3	4	5
p-Cresol	17%	5	2	5
3-Cyclohexen-1-one2-isopropyl-5-methyl-	16%	9	0	2
γ-Terpinene	16%	7	3	1
Benzaldehyde	14%	4	4	2
Benzene,1,2-dichloro-	14%	7	1	2
Benzophenone	14%	7	1	2
Benzene,1-methyl-4-(1-methylethyl)-	13%	5	3	1
p-Menthan-3-one	13%	6	0	3
ButylatedHydroxytoluene	11%	0	5	3
N,N-Diethylcarbanilide	11%	6	2	0
p-Menth-1-en-3-one	11%	4	2	2
α-Terpinene	11%	5	2	1
Butanoicacid,butylester	10%	1	5	1
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	9%	5	0	1
Dodecanoicacid	9%	4	0	2
Phenol,nonyl-	9%	6	0	0
3-Heptanone	7%	0	3	2
Benzene,1-methyl-3-(1-methylethyl)-	7%	2	1	2
Benzeneacetaldehyde,.alpha.-methyl-	7%	3	0	2
Cyclopropane,isothiocyanato-	7%	3	2	0
1,6-Octadien-3-ol,3,7-dimethyl-	6%	3	0	1
2-Methoxy-4-vinylphenol	6%	1	2	1

Benzene,1,3-dichloro-	6%	2	1	1
Dibutanoylmorphine	6%	2	1	1
ValproicAcid	6%	0	2	2
β-Damascenone	6%	3	0	1
2-Heptanone	4%	3	0	0
2-Propenal,3-phenyl-	4%	2	0	1
AllylIsothiocyanate	4%	3	0	0
Dibutylphthalate	4%	3	0	0
Dihydrocarvone	4%	2	0	1
Dimethyltrisulfide	4%	3	0	0
Ethanone,1-(3-methoxyphenyl)-	4%	3	0	0
Eucalyptol	4%	2	1	0
Eugenol	4%	2	1	0
α-Terpineol	4%	0	1	2
(E)-p-2-Menthen-1-ol	3%	2	0	0
1,3,8-p-Menthatriene	3%	2	0	0
1-Dodecanol	3%	2	0	0
2-Nonenal,(E)-	3%	2	0	0
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	3%	1	1	0
3-Ethylcyclopentanone	3%	2	0	0
Benzene,-(1-formylethyl)-	3%	0	1	1
Benzene,(3-methyl-2-butenyl)-	3%	2	0	0
Benzeneacetaldehyde	3%	1	0	1
Benzenemethanol,α,α,4-trimethyl-	3%	2	0	0
Ethanone,1-(2-hydroxyphenyl)-	3%	0	1	1
Ethanone,1-(4-methylphenyl)-	3%	2	0	0
Isobornylacetate	3%	0	1	1
p-Chloroaniline	3%	0	0	2
Phenol,2,5-dichloro-	3%	1	0	1
β-Thujene	3%	2	0	0
(3E,5Z)-1,3,5-Undecatriene	1%	1	0	0
1-Butene,4-isothiocyanato-	1%	0	1	0
p-Mentha-1,8-dien-7-ol	1%	1	0	0
1-Octanol	1%	1	0	0
1-Undecanol	1%	1	0	0
2,3-Dehydro-1,8-cineole	1%	1	0	0
2,3-Octanedione	1%	1	0	0
2,4-Cycloheptadien-1-one,2,6,6-trimethyl-	1%	0	0	1
3-Allyl-6-methoxyphenol	1%	1	0	0
3-Heptanone,6-methyl-	1%	1	0	0
3-Nonen-2-one	1%	0	1	0
3-Octen-2-one	1%	0	0	1
4'-(2-Methylpropyl)acetophenone	1%	1	0	0
4-Hepten-3-one,4-methyl-	1%	0	1	0

Acetone	1%	0	1	0
Benzaldehyde,3-chloro-	1%	0	0	1
Benzaldehyde,4-chloro-	1%	0	0	1
Benzenamine,2,6-dimethyl-	1%	0	0	1
Benzenamine,4-chloro-2-(trifluoromethyl)-	1%	0	0	1
Benzene,(2-isothiocyanatoethyl)-	1%	0	1	0
Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-	1%	0	1	0
Benzene,1-methyl-2-(1-methylethyl)-	1%	1	0	0
Benzene,1-pentenyl-	1%	1	0	0
Benzene,4-ethyl-1,2-dimethyl-	1%	0	0	1
BenzoicAcid	1%	1	0	0
Benzoicacid,2-amino-,methylester	1%	0	0	1
BenzylAlcohol	1%	0	1	0
cis-β-Terpineol	1%	1	0	0
Cyclodecane	1%	1	0	0
Cyclopentane,(methylthio)-	1%	1	0	0
Cyclotetradecane	1%	0	0	1
DiethylPhthalate	1%	1	0	0
Dimethylsulfone	1%	1	0	0
Diphenylamine	1%	1	0	0
E-2-Tetradecen-1-ol	1%	0	1	0
Ethanone,1-(4-chlorophenyl)-	1%	0	0	1
Heptadecane	1%	1	0	0
Hexadecane	1%	1	0	0
IsopropylPalmitate	1%	0	0	1
Naphthalene,2,3-dimethyl-	1%	0	1	0
Naphthalene,2-methyl-	1%	0	1	0
Nonanoicacid	1%	0	1	0
Phenol,2-methoxy-	1%	0	1	0
Phenol,2-methyl-5-(1-methylethyl)	1%	1	0	0
Phenol,4-ethyl-2-methoxy-	1%	1	0	0
Pulegone	1%	1	0	0
Thymol	1%	1	0	0
Toluene	1%	0	0	1
trans-p-Mentha-2,8-dienol	1%	1	0	0
α-Cedrene	1%	1	0	0
α-Cedreneoxide	1%	1	0	0
α-Cubebene	1%	0	1	0
β-Phellandrene	1%	1	0	0

The chi-square significance test was performed on the frequency of occurrences of each of the volatile compounds extracted from the headspace above the collected biological specimen samples across the three population subgroups of interest. The chi-square test was used to test whether the observed frequencies of occurrence (proportions of the compound occurrence within the subgroup) differ significantly from those which would be expected on the null hypothesis. The null hypothesis (H_0) in this case is that there is no significant difference in the proportion of the volatile compound subjected to the chi-square test between the compared population subgroups. The alternate hypothesis (H_a) is that the population subgroups do *not* have the same proportions of the volatile compounds subjected to the chi-square test (statistically significantly different). The chi-square equation for two-way tables is as follows:

$$\chi^2 = \sum \frac{(Observed - Expected)^2}{Expected} \qquad \textbf{Equation 6}$$

Table 67 to Table 71 list the compounds that were found to be different (p<0.05) between the healthy, diabetic, and depressed subgroups on the basis of results of chi-square tests. At a 95% confidence interval and two degrees of freedom, compounds with a chi-square value of greater than 5.99 was assumed to be significant in the proportion of that particular compound being present in a given specimen across the three subgroups. For the five biological specimens studied, the number of compounds that were assumed to be significantly different between three subgroups was as follows: fifteen for hand odor, fourty-seven for buccal swabs, twenty-nine for both breath and blood, and twenty-seven for urine. It should be noted that the frequency differences in majority of the compounds

listed in Table 62 through Table 66 are not statistically significant and therefore will not be used for future studies.

Performing the chi-square test to the data obtained through the present study is a way of data mining in order to reduce the data volume for further future statistical analyses. The greater the dataset size, the greater the probability of encountering errors, where a greater number of compounds will be found to be significantly different by chance. Therefore, by reducing the dataset size, the chances of error can thereby be reduced also. The reduced dataset obtained from the chi-square test will be utilized in the future statistical analyses using principal component analysis and discriminant analysis, in an attempt to identify any potential biomarker that would eventually help in identifying the difference in the VOC profiles of the samples collected from the different population subgroups.

Table 67. Occurrences of 15 VOCs with significant difference between population subgroups for hand odor through the chi-square test (p=0.05, dF=2)

Compound Name	Observed			Expected			χ^2 value
	Healthy	T2DB	MDD	Healthy	T2DB	MDD	
Undecanal	30	7	10	31	19	20	12.61
Octadecane	18	11	17	31	19	20	9.27
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	23	9	12	31	19	20	10.53
Tridecane	22	9	12	31	19	20	11.08
Undecane	15	11	12	31	19	20	14.83
Dodecane	14	5	7	31	19	20	28.09
BenzylAlcohol	13	2	5	31	19	20	36.91
Dodecanal	10	2	4	31	19	20	42.24
1,6-Octadien-3-ol,3,7-dimethyl-	7	1	3	31	19	20	50.08
1-Hexanol,2-ethyl-	6	2	3	31	19	20	49.82
Dodecanoicacid,methylester	3	4	3	31	19	20	51.58
2-Nonenal,(E)-	3	4	1	31	19	20	55.18
Acetophenone	3	3	2	31	19	20	54.96
Benzaldehyde	4	1	2	31	19	20	56.77
Octanoicacid,methylester	2	1	1	31	19	20	62.23

Table 68. Occurrences of 47 VOCs with significant difference between population subgroups for buccal swab through the chi-square test (p=0.05, dF=2)

Compound Name	Observed			Expected			χ^2 value
	Healthy	T2DB	MDD	Healthy	T2DB	MDD	
6-Dodecanone	24	14	11	30	19	20	6.57
2-Nonenal,(E)-	25	6	13	30	19	20	12.18
Hexadecanoicacid,ethylester	20	10	13	30	19	20	10.05
Tetradecanoicacid,ethylester	20	11	12	30	19	20	9.90
1-Tetradecene	17	13	12	30	19	20	10.73
1,1'-Biphenyl,2,2'-diethyl-	13	11	15	30	19	20	14.25
Octanoicacid,ethylester	19	7	9	30	19	20	17.66
6-Methyl-3,5-heptadiene-2-one	10	10	10	30	19	20	22.60
Hexanoicacid,ethylester	20	3	6	30	19	20	26.61
1-Dodecene	13	6	9	30	19	20	24.58
1-Decene	8	11	8	30	19	20	26.70
Tetradecane	10	10	7	30	19	20	26.05
Benzaldehyde	12	8	5	30	19	20	28.42
Hexanal	18	1	5	30	19	20	33.10
Naphthalene,1-methyl-	14	2	6	30	19	20	33.54
Hexanoicacid,pentylester	13	2	6	30	19	20	34.64
Naphthalene,2-methyl-	12	4	4	30	19	20	35.44
2-Tetradecene,(E)-	7	4	6	30	19	20	39.28
Dodecanoicacid	7	5	5	30	19	20	39.20
Heptanoicacid	1	8	8	30	19	20	41.60
Heptanoicacid,ethylester	8	3	6	30	19	20	39.41
1,1'-Biphenyl,4-methyl-	2	6	8	30	19	20	42.23
1-Hexanol	7	6	3	30	19	20	40.98
2-Octenoicacid	9	4	3	30	19	20	40.99
Dibutanoylmorphine	11	2	2	30	19	20	43.44
Pentanoicacid	5	3	7	30	19	20	42.76
Decanoicacid,ethylester	6	3	5	30	19	20	43.92
DiethylPhthalate	9	3	2	30	19	20	44.37
Linalool Oxide	11	1	2	30	19	20	45.29
Pentadecanoicacid,ethylester	7	2	5	30	19	20	44.09
Naphthalene,2,7-dimethyl-	6	3	4	30	19	20	45.47
Furfural	8	1	3	30	19	20	47.64
1-Octanol	9	1	1	30	19	20	49.80
Hexadecane	6	1	4	30	19	20	49.05
2(3H)-Furanone,5-ethyldihydro-	3	5	2	30	19	20	50.82
2(3H)-Furanone,dihydro-5-pentyl-	3	5	2	30	19	20	50.82
Acetophenone	3	3	4	30	19	20	50.57
Phenol	2	4	4	30	19	20	50.78
Benzene,1-methyl-2-[(4-methylphenyl)methyl]-	3	4	2	30	19	20	52.34
Benzothiazole	3	4	2	30	19	20	52.34
Caryophyllene	5	3	1	30	19	20	52.36
2,5-Cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)-	4	1	3	30	19	20	54.04
E-11-Hexadecenoicacid,ethylester	2	1	5	30	19	20	54.44
IsopropylPalmitate	2	1	2	30	19	20	59.39
ButylatedHydroxytoluene	1	2	1	30	19	20	61.29
Hexadecanoicacid,methylester	1	2	1	30	19	20	61.29
Tributylphosphate	1	1	1	30	19	20	63.14

Table 69. Occurrences of 29 VOCs with significant difference between population subgroups for breath through the chi-square test (p=0.05, dF=2)

Compound Name	Observed			Expected			χ^2 value
	Healthy	T2DB	MDD	Healthy	T2DB	MDD	
Benzophenone	29	10	14	31	19	20	6.19
Benzene,1,2-dichloro-	25	10	16	31	19	20	6.22
Styrene	29	6	11	31	19	20	13.07
ButylatedHydroxytoluene	24	6	12	31	19	20	13.68
Dodecane	13	13	13	31	19	20	14.80
Tetradecane	11	13	13	31	19	20	17.25
Tridecane	10	13	12	31	19	20	19.32
Undecane	13	10	12	31	19	20	17.91
Phenol	18	3	12	31	19	20	22.13
Toluene	8	6	7	31	19	20	34.41
Undecanal	9	4	5	31	19	20	38.71
Benzene,1,3,5-trimethyl-	4	6	7	31	19	20	40.86
2,6-Diisopropylnaphthalene	13	2	1	31	19	20	43.71
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	9	1	4	31	19	20	45.47
p-Benzoquinone	1	6	5	31	19	20	49.18
Benzene,4-ethyl-1,2-dimethyl-	2	5	5	31	19	20	48.69
Dodecanal	8	1	3	31	19	20	48.57
Naphthalene	5	3	2	31	19	20	51.48
Benzene,1,2,3-trimethyl-	2	2	5	31	19	20	53.59
Benzene,1-ethyl-2,4-dimethyl-	1	2	5	31	19	20	55.49
Benzene,1-ethyl-2-methyl-	1	2	4	31	19	20	57.04
Diisopropylnaphthalene	4	2	1	31	19	20	56.78
Limonene	1	2	3	31	19	20	58.69
Naphthalene,2-methyl-	1	1	4	31	19	20	58.88
1,3,5,7-Cyclooctatetraene	1	3	1	31	19	20	60.56
Benzene,1,4-dichloro-	3	1	1	31	19	20	60.39
DiethylPhthalate	1	1	2	31	19	20	62.28
2-Propanol,1-propoxy-	1	1	1	31	19	20	64.13
Benzene,1-methyl-4-(1-methylpropyl)-	1	1	1	31	19	20	64.13

Table 70. Occurrences of 29 VOCs with significant difference between population subgroups for blood through the chi-square test (p=0.05, dF=2)

Compound Name	Observed			Expected			χ^2 value
	Healthy	T2DB	MDD	Healthy	T2DB	MDD	
Heptadecane	22	12	14	31	19	20	6.99
BenzylAlcohol	25	6	11	31	19	20	14.11
Diisopropylnaphthalene	19	7	14	31	19	20	14.02
Furan,2-butyltetrahydro-	23	9	7	31	19	20	15.78
Furan,2-pentyl-	20	6	7	31	19	20	21.25
Decanal	15	9	7	31	19	20	21.97
1-Dodecene	18	6	5	31	19	20	25.60
Cyclohexanone	19	3	7	31	19	20	26.57
5,9-Undecadien-2-one,6,10-dimethyl-, (E)-	14	4	8	31	19	20	28.36
2-Dodecene,(Z)-	12	6	7	31	19	20	28.99
Longifolene	6	8	9	31	19	20	32.58
1-Decene	7	8	7	31	19	20	33.40
1-Octen-3-ol	8	9	5	31	19	20	33.58
2-Undecanone	11	3	7	31	19	20	34.83
Benzene,1,2-dichloro-	12	5	3	31	19	20	36.41
Octadecane	6	6	8	31	19	20	36.26
1-Pentanol	12	2	4	31	19	20	39.66
2-Heptanone	8	2	5	31	19	20	43.53
Heptanal	5	4	5	31	19	20	44.90
Cyclododecane	10	2	1	31	19	20	47.49
1-Dodecanol	5	4	3	31	19	20	48.10
Benzene,4-ethyl-1,2-dimethyl-	1	1	8	31	19	20	53.28
Naphthalene	1	6	3	31	19	20	52.38
Xylenes	3	2	4	31	19	20	53.30
4-Cyanocyclohexene	2	3	3	31	19	20	55.05
2-Propanol,1-propoxy-	1	3	2	31	19	20	58.71
Benzene,1,4-dichloro-	4	1	1	31	19	20	58.62
Ethanol,2-butoxy-	3	1	1	31	19	20	60.39
1,1'-Biphenyl,2-methyl-	1	1	1	31	19	20	64.13

Table 71. Occurrences of 27 VOCs with significant difference between population subgroups for urine through the chi-square test (p=0.05, dF=2)

Compound Name	Observed			Expected			χ^2 value
	Healthy	T2DB	MDD	Healthy	T2DB	MDD	
Carvone	21	7	11	31	19	20	14.85
Nonanal	13	11	12	31	19	20	17.02
Cedrol	6	11	14	31	19	20	25.33
Menthol	12	8	10	31	19	20	23.01
Pyrrole	16	2	4	31	19	20	35.27
Benzene,1-methyl-4-(1-methylethenyl)-	10	4	5	31	19	20	37.32
4-Terpineol	8	5	3	31	19	20	41.83
2-Isopropylbenzaldehyde	7	3	5	31	19	20	43.30
Benzaldehyde,3,5-dimethyl-	7	2	4	31	19	20	46.59
Decanal	5	4	4	31	19	20	46.45
2-Pentanone	3	4	5	31	19	20	48.38
p-Cresol	5	2	5	31	19	20	48.27
γ-Terpinene	7	3	1	31	19	20	50.10
Benzaldehyde	4	4	2	31	19	20	51.56
Benzene,1,2-dichloro-	7	1	2	31	19	20	51.83
Benzophenone	7	1	2	31	19	20	51.83
Benzene,1-methyl-4-(1-methylethyl)-	5	3	1	31	19	20	53.33
α-Terpinene	5	2	1	31	19	20	55.07
p-Menth-1-en-3-one	4	2	2	31	19	20	54.93
Butanoicacid,butylester	1	5	1	31	19	20	57.40
Benzene,1-methyl-3-(1-methylethyl)-	2	1	2	31	19	20	60.38
2-Methoxy-4-vinylphenol	1	2	1	31	19	20	62.29
Benzene,1,3-dichloro-	2	1	1	31	19	20	62.23
Dibutanoylmorphine	2	1	1	31	19	20	62.23

3.7. Human Biological Specimen Compound Database

Four hundred fifty three (453) compounds were extracted across all samples of hand odor, buccal swab, breath, blood, and urine from the 70 subjects (31 healthy, 19 diabetic, 20 depressed) taken during this study. Of these 453 compounds, 111 have been omitted from the biological specimen database for one or more of the following reasons: compound identity was questionable or completely unidentifiable, compound was a known background compound of environment and/or collection material, compound was a known laboratory solvent. The remaining 342 compounds extracted and identified from the headspace of five biological specimen samples from all 70 subjects of this study are presented in Table 72. Compounds were identified by the standard mass spectrum in the NIST library. Where standard reference compounds were available, the compounds were identified by using both the standard mass spectrum in the NIST library and the corresponding reference standard retention time and spectrum. As noted in Table 72, many of the identified compounds can be attributed to exogenous sources, as the majority of the identified compounds are used in the flavor and fragrance industries. Common names for some of the compounds are given in parentheses and italicized. Many of the presented compounds have been previously reported to be volatile emanation compounds of human skin[5,14,28,29,123], saliva[127], breath[44,48,97,128], volatile constituents of human blood[18,62,66,67], and urine[70-72]. Compounds denoted by "en" are possibly endogenous compounds, while compounds denoted by "ex" are possibly of exogenous origin. Compounds denoted by "food" are known to be present in food and beverages (vegetables, fruits, dairy, meat, alcohol, beverages, etc.). The compounds that are denoted with both "en" and "ex" are compounds used in flavor and/or fragrance industry, but can

also be found occurring naturally in the body. However, whether those compounds are actually occurring naturally in the body or seem to be occurring naturally due to food consumption or exposure to the exogenous-origin compounds is unclear.

Table 72 Biological specimen compound database (*Notes:Identity verified by standard comparison; en=possibly endogenous; ex=probably exogenous; food=food origin)**

Compound Name	Specimen					CAS #	Natural Occurrence	Use	Class
	HD	BS	BR	BL	UR				
(3E,5Z)-1,3,5-Undecatriene					x	51447-08-6			
.+/-.-4-Acetyl-1-methylcyclohexene					x	6090-09-1	natural		
1,1'-Biphenyl,2,2'-diethyl-		x							
1,1'-Biphenyl,2-ethyl-		x				1812-51-7			
1,1'-Biphenyl,2-methyl-	x			x		643-58-3			
1,1'-Biphenyl,3-methyl-		x		x		643-93-6			
1,1'-Biphenyl,4-methyl-	x	x	x	x		644-08-6	not found in nature	flavor	food
1,2-Benzenediol,3,5-bis(1,1-dimethylethyl)-	x			x		1020-31-1			
1,3,5,7-Cyclooctatetraene *(Annulene)*			x			629-20-9	fungi		
1,3,8-p-Menthatriene					x	21195-59-5		food flavor, chinese medicine	food/ex
1,3-Cyclohexadiene,1-methyl-4-(1-methylethyl)- *(α-Terpinene)*					x	99-86-5	plant	flavor/fragrance	food
1,4-Cyclohexadiene,1-methyl-4-(1-methylethyl)- *(γ-Terpinene)*					x	99-85-4	plant oil	flavor/fragrance	food/ex
1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-, (Z)- *((Z)-β-Farnesene)*		x				28973-97-9	plant oil		
1,6-Octadien-3-ol,3,7-dimethyl- *(Linalool; Linalool oxide)*	x	x			x	78-70-6	plant	flavor/fragrance	en/food/ex
11-Octadecenoicacid,methylester		x				52380-33-3			
1-Butene,4-isothiocyanato-					x	3386-97-8	natural, food	flavor	food
1-Cyclohexene-1-carboxaldehyde,2,6,6-trimethyl- *(ß -Cyclocitral)*				x		432-25-7	natural, food	flavor/fragrance	ex
1-Cyclohexene-1-methanol,4-(1-methylethenyl)- *(p-Mentha-1,8-dien-7-ol)*					x	536-59-4	plant oil	flavor/fragrance	ex
1-Decene*	x	x		x		872-05-9			
1-Dodecanol*	x			x	x	112-53-8	plant oil	flavor/fragrance; pharmaceuticals	food/ex
1-Dodecene*	x	x	x	x		112-41-4	plant	flavor; solvent; viscosity controlling agent	ex
1-Heptadecene		x				6765-39-5			
1-Hexadecene		x		x		629-73-2			
1-Hexanol*		x				111-27-3	plant oil, alcohol		
1-Hexanol,2-ethyl-*	x		x	x	x	104-76-7	plant		ex
1-Octanol*	x	x		x	x	111-87-5	plant oil	flavor/fragrance	ex
1-Octen-3-ol*	x			x		3391-86-4	plant oil, food		en/ex
1-Pentadecene*	x	x				13360-61-7			
1-Pentanol	x	x		x		71-41-0	plant oil, food		
1-Phenyl-2-butanone *(Benzyl ethyl ketone)*	x			x		1007-32-5			
1S-α-Pinene*			x			7785-26-4	turpentine	flavor/fragrance	
1-Tetradecene*	x	x		x		1120-36-1			
1-Undecanol					x	112-42-5	plant oil		
2(3H)-Furanone,5-ethyldihydro- *(γ-Caprolactone)*		x				695-06-7	natural, insect pheromone	flavor/fragrance	ex
2(3H)-Furanone,dihydro-5-pentyl- *(γ-Amylbutyrolactone)*		x				104-61-0	natural, food	flavor/fragrance	ex
2(3H)-Furanone,dihydro-5-propyl- *(γ-Heptanolactone)*		x				105-21-5	natural, food	flavor/fragrance	ex
2,3-Dehydro-1,8-cineole					x	66113-06-2		flavor	
2,3-Octanedione					x	585-25-1	fermentation byproduct	flavor	food
2,4-Cycloheptadien-1-one,2,6,6-trimethyl- *(Eucarvone)*					x	503-93-5	natural; pheromone		food

2,4-Nonadienal		x				6750-03-4	coffee		
2,4-Nonadienal,(E,E)-*		x				5910-87-2	food		
2,5-Cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)- *(p-Benzoquinone)*	x	x	x	x	x	719-22-2		pharmaceutical, herbicide, cosmetics	ex
2,5-Octanedione				x		3214-41-3			food
2,6-Diisopropylnaphthalene*	x		x	x		24157-81-1			
2-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl)-, trans- *((E)-p-2-Menthen-1-ol)*					x	29803-81-4			
2-Cyclohexen-1-one,2-methyl-5-(1-methylethenyl)- *(DL-Carvone)*					x	99-49-0	plant oil	flavor/fragrance	food/ex
2-Cyclohexen-1-one,2-methyl-5-(1-methylethenyl)-, (S)- *((+)-Carvone)*		x			x	2244-16-8	plant oil	flavor/fragrance	food/ex
2-Cyclohexen-1-one,3-methyl-6-(1-methylethyl)- *(p-Menth-1-en-3-one)*					x	89-81-6	plant oil	flavor/fragrance	food/ex
2-Decanone*	x			x		693-54-9	plant oil		
2-Decenal,(E)-		x				3913-81-3	plant oil, food	flavor/fragrance	food/ex
2-Dodecenal,(E)-		x				20407-84-5	plant oil, food	flavor/fragrance	food/ex
2-Dodecene,(Z)-	x		x	x		7206-26-0			
2-Heptanone*	x			x	x	110-43-0	plant oil, alcohol, cheese	flavor/fragrance	food/ex
2-Heptanone,6-methyl-	x			x		928-68-7	found in nature	flavor/fragrance	food/ex
2-Heptenoicacid		x				18999-28-5			
2-Isopropylbenzaldehyde					x	6502-22-3			
2-Methoxy-4-vinylphenol					x	7786-61-0	natural, food	flavor/fragrance	ex
2-Nonenal,(E)-*	x	x			x	18829-56-6	food	flavor/fragrance	food/ex
2-Octenal,(E)-*		x				2548-87-0	food	flavor/fragrance	food/ex
2-Octenoicacid		x				1470-50-4			
2-Pentanone*	x				x	107-87-9	food	flavor/fragrance	food/ex
2-Propanol,1-butoxy-				x		5131-66-8		fragrance	ex
2-Propanol,1-propoxy-	x		x	x		1569-01-3		fragrance	ex
2-Propenal,3-phenyl- *(Cinnamaldehyde)*					x	104-55-2	plant	flavor/fragrance	ex
2-Tetradecene,(E)-		x				35953-53-8			
2-Undecanone *(Methyl nonyl ketone)*	x			x		112-12-9	plant	flavor/fragrance; insect/animal repellent	food/ex
3,5,9-Undecatrien-2-one,6,10-dimethyl-, (E,Z)- *((E,Z)-Pseudoionone)*		x					plant	flavor	ex
3,5-di-tert-Butyl-4-hydroxybenzaldehyde					x	1620-98-0			
3,7-Dimethyl-octa-1,6-diene *(Dyhydromyrcene)*	x	x		x		2436-90-0	plant	fragrance	food/ex
3-Allyl-6-methoxyphenol					x	501-19-9			
3-Cyclohexen-1-ol,4-methyl-1-(1-methylethyl)- *(4-Terpineol)*					x	562-74-3	plant oil	flavor/fragrance	food/ex
3-Cyclohexen-1-ol,4-methyl-1-(1-methylethyl)-, (R)- *((-)-4-Terpineol)*					x	20126-76-5			
3-Cyclohexen-1-one2-isopropyl-5-methyl-					x			food additive	food
3-Cyclohexene-1-methanol,α,α,4-trimethyl-, (S)- *(α-Terpineol)*					x	98-55-5	plant oil/fruits	flavor/fragrance	en/food/ex
3-Dodecene,(Z)-			x	x		7239-23-8			
3-Eicosene,(E)-		x				74685-33-9			
3-Ethylcyclopentanone					x	10264-55-8	urine		
3-Heptadecene,(Z)-		x							
3-Heptanone*			x	x	x	106-35-4	natural	flavor/fragrance	
3-Heptanone,6-methyl-					x	624-42-0	pheromone, urine		
3-Isopropylbenzaldehyde	x			x		34246-57-6			
3-Nonen-2-one		x			x	14309-57-0	natural	flavor/fragrance	
3-Octanol,3,7-dimethyl-,(.+/-.)-			x			57706-88-4	synthetic	flavor/fragrance	ex

3-Octen-2-one					x	1669-44-9	natural	cigarette ingredient; flavor/fragrance	ex
4'-(2-Methylpropyl)acetophenone					x	38861-78-8		Ibuprofen related	food
4,8-Dimethyl-nona-3,8-dien-2-one *(Citrinone)*		x				817-88-9	not found in nature	flavor	
4-Cyanocyclohexene	x		x	x		100-45-8			en/ex
4-Heptanone *(GBL)**	x			x	x	123-19-3	food	flavor/fragrance	food/ex
4-Nonylphenol					x	104-40-5	synthetic, sewage		ex
4-tert-Butylcyclohexylacetate				x		32210-23-4	synthetic	fragrance	ex
5,9-Undecadien-2-one,6,10-dimethyl- *(Geranyl acetone)**		x			x	689-67-8	plant oil	flavor/fragrance	food/ex
5,9-Undecadien-2-one,6,10-dimethyl-, (E)- *(trans-Geranyl acetone)**	x	x	x	x	x	3796-70-1	plant	fragrance	food/ex
5,9-Undecadien-2-one,6,10-dimethyl-, (Z)- *(Neryl acetone)**		x				3879-26-3	coffee	flavor/fragrance	food
5-Dodecene,(E)-			x			7206-16-8			
5-Hepten-2-one,6-methyl- *(Sulcaatone)**	x		x	x		110-93-0	natural, pheromone		
6-Dodecanone		x				6064-27-3			
6-Methyl-3,5-heptadiene-2-one		x				1604-28-0	plant oil, plant	flavor/fragrance	en/food/ex
7-Hexadecene,(Z)-		x				35507-09-6			
9-Octadecenoicacid,(E)-		x				112-79-8			
Aceticacid		x	x			64-19-7	plant	flavor	en/food/ex
Aceticacid,butylester	x					123-86-4	food	flavor/fragrance (cosmetic solvent)	food/ex
Acetone	x		x	x	x	67-64-1	plant oil, food	flavor, extraction solvent	en/food/ex
Acetophenone*	x	x	x	x		98-86-2	plant oil, food	flavor/fragrance	food/ex
Allylisothiocyanate					x	57-06-7	plant oil		food
Azulene			x	x		275-51-4			
Benzaldehyde*	x	x	x	x	x	100-52-7	plant oil, food	flavor/fragrance	en/food/ex
Benzaldehyde,2,4,5-trimethyl- *(Duraldehyde)*	x			x		5779-72-6			
Benzaldehyde,2,4,6-trimethyl- *(Mesitaldehyde)*	x			x		487-68-3			
Benzaldehyde,2-hydroxy- *(Salicylaldehyde)*				x		90-02-8	plant oil	flavor/fragrance	ex
Benzaldehyde,3,5-dimethyl-					x	5779-95-3			
Benzaldehyde,3-chloro-					x	587-04-2			
Benzaldehyde,3-hydroxy-	x			x		100-83-4			
Benzaldehyde,4-(1-methylethyl)- *(p-Cumic aldehyde)*		x				122-03-2	plant oil	flavor/fragrance	ex
Benzaldehyde,4-(methylthio)-	x			x		3446-89-7		intermediate (pharmaceuticals, pesticides)	ex
Benzaldehyde,4-chloro-					x	104-88-1			
Benzaldehyde,4-methoxy-			x			123-11-5	plant oil, plant	flavor/fragrance	food/ex
Benzaldehyde,ethyl-				x		53951-50-1			
Benzenamine,2,6-dimethyl-					x	87-62-7		synthesis of lidocane	ex
Benzenamine,4-chloro-2-(trifluoromethyl)-					x	445-03-4		dye	ex
Benzene,(1-formylethyl)- *(Cumene aldehyde)*					x	93-53-8	not found in nature	flavor/fragrance	ex
Benzene,(1-methylethyl)- *(Cumene)*			x			98-82-8	crude oil; refined fuels	varnish, cement, primer, auto product	ex
Benzene,(2-isothiocyanatoethyl)-					x	2257-09-2	food	flavor	food
Benzene,(3-methyl-2-butenyl)-					x	4489-84-3			
Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-					x	644-30-4	plant oil	flavor/fragrance	
Benzene,1,1'-methylenebis[4-methyl- *(α-Curcumene)*		x				4957-14-6			
Benzene,1,2,3,4-tetramethyl- *(Prehnitene)*			x	x		488-23-3			
Benzene,1,2,3,5-tetramethyl- *(Isodurene)*		x	x	x		527-53-7			

Benzene,1,2,3-trimethyl-			x	x		526-73-8			
Benzene,1,2,4,5-tetramethyl- *(Durene)**		x	x	x		95-93-2	natural		ex
Benzene,1,2,4-trimethyl- *(ψ-Cumene)*			x			95-63-6		fragrance	
Benzene,1,2-dichloro-*	x		x	x	x	95-50-1			
Benzene,1,3,5-trimethyl- *(Mesitylene)*			x	x		108-67-8		fragrance	ex
Benzene,1,3-dichloro-	x		x	x	x	541-73-1			
Benzene,1,3-diethyl-5-methyl-			x			2050-24-0			
Benzene,1,3-dimethyl- *(m-Xylene)**	x		x	x		108-38-3			
Benzene,1,3-dimethyl-5-(1-methylethyl)-				x		4706-90-5			
Benzene,1,4-dichloro-	x		x	x	x	106-46-7			
Benzene,1-ethyl-2,3-dimethyl-		x	x	x		933-98-2			
Benzene,1-ethyl-2,4-dimethyl-			x	x		874-41-9			
Benzene,1-ethyl-2-methyl-			x	x		611-14-3			
Benzene,1-ethyl-3,5-dimethyl-			x	x		934-74-7			
Benzene,1-ethyl-3-methyl-			x	x		620-14-4			
Benzene,1-methyl-2-(1-methylethyl)-			x		x	527-84-4			
Benzene,1-methyl-2-[(3-methylphenyl)methyl]-		x				21895-13-6			
Benzene,1-methyl-2-[(4-methylphenyl)methyl]-		x				21895-17-0			
Benzene,1-methyl-3-(1-methylethyl)- *(β-Cymene)*			x		x	535-77-3	species pheromone		
Benzene,1-methyl-3-propyl-	x		x	x		1074-43-7			
Benzene,1-methyl-4-(1-methylethenyl)- *(α,p-Dimethylstyrene)*					x	1195-32-0	plant oil	flavor/fragrance	food/ex
Benzene,1-methyl-4-(1-methylethyl)- *(p-Cymene)*			x	x	x	99-87-6	plant oil	flavor/fragrance	ex
Benzene,1-methyl-4-(1-methylpropyl)-	x		x	x		1595-16-0			
Benzene,1-methyl-4-nitro-		x				99-99-0			
Benzene,1-methyl-4-propyl-			x			1074-55-1			
Benzene,1-pentenyl-					x	826-18-6			
Benzene,2,4-dimethyl-1-(1-methylethyl)-				x		4706-89-2			
Benzene,2-ethyl-1,3-dimethyl-			x			2870-04-4			
Benzene,2-ethyl-1,4-dimethyl-			x	x		1758-88-9			
Benzene,3-cyclohexen-1-yl-			x			4994-16-5			
Benzene,4-ethyl-1,2-dimethyl-	x		x	x	x	934-80-5			
Benzene,diethyl-	x		x	x		25340-17-4	not found in nature	fragrance	ex
Benzene,pentamethyl-			x			700-12-6			
Benzene,propyl-*			x			103-65-1			
Benzeneacetaldehyde					x	122-78-1	plant oil	flavor/fragrance	ex
Benzeneacetaldehyde,α-methyl- *(Cumene aldehyde)*					x	93-53-8	not found in nature	flavor/fragrance	ex
Benzeneethanol,2-methyl-						19819-98-8	not found in nature	fragrance	ex
Benzenemethanol,α,α,4-trimethyl- *(p-Cymenol)*					x	1197-01-9	plant oil	flavor/fragrance	food/ex
Benzenemethanol,α,α-dimethyl- *(α-Cumyl alcohol)*	x			x		617-94-7	cocoa	flavor/fragrance	ex
BenzoicAcid					x	65-85-0	plant	food preservative; antimicrobial	food
Benzoicacid,2-amino-,methylester					x	134-20-3	plant oil	flavor/fragrance, bird repellant for plants	ex
Benzophenone*	x	x	x	x	x	119-61-9	plant	photo-initiator, UV blocker	food/ex
Benzothiazole		x				95-16-9	plant oil	flavor/pharmaceuticals	ex
BenzylAlcohol*	x			x	x	100-51-6	plants, plant oil	flavor/fragrance, preservative, solvent	food/ex

Bicyclo[3.1.0]hex-2-ene,4-methyl-1-(1-methylethyl)- *(β-Thujene)*					x	28634-89-1	plant oil		food
Butanoicacid,butylester					x	109-21-7	fruits	flavor/fragrance	food/ex
ButylatedHydroxytoluene*		x	x		x	128-37-0		antioxidant additive, preservative	food/ex
Calamenene		x				483-77-2	plant oil		food
Caryophyllene*		x	x			87-44-5	plant oil	flavor/fragrance	food
Cedrol	x				x	77-53-2	plant oil	flavor/fragrance	en/ex
cis-β-Terpineol					x	7299-40-3			
Cyclodecane	x	x		x	x	293-96-9			
Cyclododecane	x	x	x	x		294-62-2	not found in nature	intermediate, volatile binding media	ex
Cyclohexadecane		x				295-65-8			
Cyclohexane,methyl-			x			108-87-2		organic solvent, jet fuel	ex
Cyclohexane,pentyl-			x			4292-92-6			
Cyclohexanol,5-methyl-2-(1-methylethyl)-, (1α,2β,5α)- *(DL-Menthol)**	x	x	x	x	x	89-78-1	not found in nature	flavor/fragrance	food/ex
Cyclohexanol,5-methyl-2-(1-methylethyl)-, (1α,2β,5α)-(.+/-.)- *(DL-Menthol)**	x		x	x	x	15356-70-4	not found in nature	flavor/fragrance	food/ex
Cyclohexanol,5-methyl-2-(1-methylethyl)-, [1R-(1α,2β,5α)]- *(-)-Menthol)*	x		x	x	x	2216-51-5	plant oil	flavor/fragrance	food/ex
Cyclohexanol,5-methyl-2-(1-methylethyl)-, [1S-(1α,2α,5β)]- *(Menthol)*	x		x	x		2216-52-6	not found in nature	flavor/fragrance	food/ex
Cyclohexanol,5-methyl-2-(1-methylethyl)-, [1S-(1α,2β,5α)]- *((+)-Menthol)*					x		not found in nature	flavor/fragrance	food/ex
Cyclohexanol,5-methyl-2-(1-methylethyl)-, [1S-(1α,2β,5β)]- *(Menthol)*					x	23283-97-8	not found in nature	flavor/fragrance	food/ex
Cyclohexanone*	x			x		108-94-1	plant oil	precursor to nylon	ex
Cyclohexanone,2-methyl-5-(1-methylethenyl)- *(Dihydrocarvone)*					x	7764-50-3	plant oil	flavor/fragrance	food/ex
Cyclohexanone,2-methyl-5-(1-methylethenyl)-, trans- *(trans-Dihydrocarvone)*					x	5948-04-9	plant oil		
Cyclohexanone,5-methyl-2-(1-methylethyl)- *(p-Menthan-3-one)*					x	10458-14-7	plant oil	fragrance	ex
Cyclohexanone,5-methyl-2-(1-methylethyl)-, cis- *(cis-p-Menthan-3-one)*					x	491-07-6	plant oil	fragrance	ex
Cyclohexanone,5-methyl-2-(1-methylethyl)-, trans- *(trans-p-Menthan-3-one)*		x			x	89-80-5	plant oil	fragrance	ex
Cyclohexene,1-methyl-4-(1-methylethenyl)-, (.+/-.)- *(DL-Limonene)**	x		x	x		138-86-3	plant oil/fruits	flavor/fragrance	food/ex
Cyclooctane	x			x		292-64-8			
Cyclopentane,(methylthio)-					x	7133-36-0			
Cyclopropane,isothiocyanato-					x	56601-42-4			
Cyclopropane,nonyl-	x	x		x		74663-85-7			
Cyclotetradecane		x		x	x	295-17-0			
Decanal*	x	x	x	x	x	112-31-2	plant oil, fruits	flavor/fragrance	en/food/ex
Decane				x		124-18-5	natural	solvent/diluent; fragrance	food/ex
Decane,2-methyl-			x			6975-98-0	plant species		
Decane,3-methyl-			x			13151-34-3			
Decanoicacid,ethylester*		x				110-38-3	plant oil	flavor/fragrance	ex
Dibutanoylmorphine		x			x	66641-03-0	synthetic diester of morphine		
Dibutylphthalate			x		x	84-74-2	plant oil	solvent/diluent for flavor/fragrance agents	ex
DiethylPhthalate		x	x		x	84-66-2	not found in nature	solvent (cosmetics); plasticizer	ex
Diisopropylnaphthalene	x		x	x		38640-62-9			
Dimethylsulfone	x			x	x	67-71-0	plant	food; solvent	en/food/ex
Dimethyltrisulfide*					x	3658-80-8	food	flavor/fragrance	food
Diphenylamine*					x	122-39-4	natural, species pheromone	scald-inhibitant for apples	
Docosane*		x				629-97-0	plant oil	fragrance	ex
Dodecanal*	x	x	x			112-54-9	plant oil	flavor/fragrance	ex

Dodecane*	x		x	x		112-40-3	plant oil, food	solvent/diluent; fragrance	ex
Dodecane,1-chloro-				x		112-52-7			
Dodecane,2,6,10-trimethyl-				x		3891-98-3			
Dodecanoicacid*		x	x		x	143-07-7	plant oil, human milk		en
Dodecanoicacid,ethylester		x				106-33-2	plant oil, alcohol	flavor/fragrance	ex
Dodecanoicacid,methylester*	x	x	x			111-82-0	natural; food	flavor/fragrance	ex
Eicosane*		x	x			112-95-8	plant oil	fragrance	ex
Ethanol,2-butoxy-	x			x		111-76-2	cheese	sanitizing agent, solvent (cosmetics)	ex
Ethanol,2-phenoxy-			x			122-99-6	plant	preservative (flavor/fragrance)	food/ex
Ethanone,1-(2-hydroxyphenyl)-					x	118-93-4	food	flavor/fragrance	food
Ethanone,1-(3-methoxyphenyl)-					x	586-37-8	plant oil	flavor/fragrance	food/ex
Ethanone,1-(4-chlorophenyl)-					x	99-91-2			
Ethanone,1-(4-methylphenyl)-					x	122-00-9	plant oil, food	flavor/fragrance	food/ex
Ethyl9-hexadecenoate		x				54546-22-4	species pheromone		
Ethyltridecanoate		x				28267-29-0			
Eucalyptol					x	470-82-6	plant oil	flavor/fragrance	ex
Eugenol					x	97-53-0	plant oil	flavor/fragrance	
Furan,2-butyltetrahydro-	x			x		1004-29-1	not found in nature	flavor	
Furan,2-pentyl-*	x	x		x		3777-69-3	natural; food	flavor/fragrance	ex
Furfural*		x				98-01-1	plant oil	flavor, solvent (cosmetics)	ex
Galaxolide		x	x			1222-05-5	not found in nature	fragrance	ex
Heneicosane*		x				629-94-7	plant oil	fragrance	ex
Heptadecane*	x	x	x	x	x	629-78-7	plant oil	fragrance	food/ex
Heptadecane,9-octyl-	x			x		7225-64-1	plant oil		food
Heptanal*	x			x		111-71-7	fruits, food	flavor/fragrance	food/ex
Heptanoicacid*		x				111-14-8	fruits, alcohol	flavor/fragrance, cigarette additive	food/ex
Heptanoicacid,ethylester*		x				106-30-9	food, alcohol	flavor/fragrance	food/ex
Heptanol*	x			x		111-70-6	food, alcohol	flavor/fragrance	food/ex
Hexadecane*	x	x	x	x	x	544-76-3	plant oil	fragrance	food/ex
Hexadecanoicacid,ethylester *(Ethyl palmitate)*		x				628-97-7	plant oil, rice, vanilla	flavor/fragrance	ex
Hexadecanoicacid,methylester *(Methyl palmitate)*		x				112-39-0	plant oil	flavor/fragrance	ex
Hexanal*	x	x	x	x		66-25-1	plant oil, food	flavor/fragrance	food/ex
Hexanedioicacid,bis(1-methylethyl) ester *(Diisopropyl adipate)*			x	x		230-072-0	not found in nature	solvent for flavor/fragrance agents	ex
Hexanoicacid*	x	x				142-62-1	plant oil, food	flavor/fragrance	food/ex
Hexanoicacid,anhydride		x				2051-49-2			
Hexanoicacid,ethylester		x				123-66-0	fruits, food	flavor/fragrance	food/ex
Hexanoicacid,pentylester		x				540-07-8	fruits, cheese	flavor/fragrance	food/ex
Hexanoicacid,propylester		x				626-77-7	food, alcohol	flavor/fragrance	food
Hexatriacontane				x		630-06-8			ex
Homomenthylsalicylate		x	x			52253-93-7	not found in nature	UV adsorbing agent (sunscreen)	ex
Indole			x			120-72-9	plant oil, food	flavor/fragrance	ex
Indolizine			x			274-40-8			
Isobornylacetate			x		x	125-12-2	plant oil	flavor/fragrance	food/ex
IsopropylMyristate	x	x		x		110-27-0	plant oil	solvent/diluent for flavor/fragrance agents	food/ex

IsopropylPalmitate		x	x		x	142-91-6	not found in nature	solvent/diluent for flavor/fragrance agents	ex
Lilial	x					80-54-6	not found in nature	fragrance	ex
Longicyclene			x			1137-12-8	plant oil		ex
Longifolene	x		x	x		475-20-7	plant oil	flavor/fragrance	ex
Methyldihydrojasmonate			x			24851-98-7	plant	flavor/fragrance	ex
Methyl Salicylate*			x		x	119-36-8	plant oil, fruits	flavor/fragrance, aspirin	food/ex
N,N-Diethylcarbanilide *(Ethyl centralite)*					x	85-98-3		celluloid plasticizer, explosives stabilizer	ex
Naphthalene*	x		x	x		91-20-3	natural		
Naphthalene,1,6-dimethyl-				x		575-43-9			
Naphthalene,1,7-dimethyl-				x		575-37-1			
Naphthalene,1-methyl-*		x		x		90-12-0	food	flavor	food
Naphthalene,2,3,6-trimethyl-	x			x		829-26-5			
Naphthalene,2,3-dimethyl-				x	x	581-40-8			
Naphthalene,2,6-dimethyl-		x		x		581-42-0			
Naphthalene,2,7-dimethyl-		x		x		582-16-1			
Naphthalene,2-methyl-		x	x	x	x	91-57-6	plant/fruits	flavor/fragrance	ex
n-Decanoicacid*			x			334-48-5	plant oil, food, mamalian milk	flavor/fragrance, food additive, phamaceuticals	food/ex
n-Hexadecanoicacid*			x			57-10-3	plant oil, food	thickener, emulsifier,stabilizer,pharmaceuticals	food/ex
Nonadecane			x			629-92-5	plant	fragrance	food/ex
Nonadecanoicacid,ethylester		x				18281-04-4	alcohol		food
Nonanal*	x		x	x	x	124-19-6	plant oil, food	flavor/fragrance	en/food/ex
Nonane*				x		111-84-2	plant oil	fragrance	ex
Nonane,3-methyl-			x			5911-04-6			
Nonanoicacid		x	x		x	112-05-0	plant oil, fruits	flavor, cosmetic (solvent, perfumery)	food/ex
Nonanoicacid,ethylester*		x				123-29-5	fruits, alcohol	flavor/fragrance	ex
Octadecanal		x				638-66-4	plant oil, insect pheromone	cosmetic	food
Octadecane	x	x		x		593-45-3	plant oil	fragrance	en/food/ex
Octanal	x		x	x		124-13-0	plant oil, food	flavor/fragrance, antimicrobial	food/ex
OctanoicAcid*		x				124-07-2	plant oil, fruits, mamalian milk	flavor/fragrance	food/ex
Octanoicacid,ethylester*		x				106-32-1	fruits, alcohol	flavor/fragrance	food/ex
Octanoicacid,methylester	x					111-11-5	plant/fruits, species pheromone	flavor/fragrance	food/ex
p-Chloroaniline					x	106-47-8		precursor to antiseptic	ex
Pentadecane*	x					629-62-9	plant oil, food	fragrance	food/ex
Pentadecane,7-methyl-		x				6165-40-8			
Pentadecanoicacid,ethylester		x				41114-00-5	not found in nature	flavor	ex
Pentanoicacid *(Valeric acid)**		x				109-52-4	fruits, food	flavor/fragrance	food/ex
Phenol*	x	x	x	x		108-95-2	plant oil	plastics production, pharmaceuticals	food
Phenol,2-(1,1-dimethylethyl)-			x			88-18-6		fragrance	ex
Phenol,2,4,6-trimethyl- *(Mesitol)*	x			x		527-60-6	not found in nature	flavor/fragrance	ex
Phenol,2,5-dichloro-					x	583-78-8	p-dichlorobenzene metabolite		
Phenol,2,6-bis(1,1-dimethylethyl)-4-ethyl-					x	4130-42-1			
Phenol,2-methoxy- *(o-guaiacol)*					x	90-05-1	food	flavor/fragrance	food/ex
Phenol,2-methyl-5-(1-methylethyl) *(p-Cymen-2-ol)*					x	499-75-2	plant oil	flavor/fragrance	
Phenol,4-(1,1,3,3-tetramethylbutyl)-*					x	140-66-9	not found in nature	adjuvant for pesticide	ex

Phenol,4-ethyl-2-methoxy-					x	2785-89-9	alcohol, produced by yeast	flavor/fragrance	ex
Phenol,4-methyl- *(p-Cresol)**					x	106-44-5	plant oil, food	flavor/fragrance (costmetics)	en/food
Phenol,nonyl-					x	25154-52-3			
Propanoicacid,2-methyl-,butylester					x	97-87-0	plant oil	flavor/fragrance	ex
Pulegone					x	15932-80-6	plant oil	flavor/fragrance	food/ex
p-Xylene *(Benzene,1,4-dimethyl-)**	x		x	x		106-42-3			
Pyrrole					x	109-97-7	plant	flavor	food
Styrene*			x			100-42-5	plant, food	cosmetics, plastics	food/ex
Tetrachloroethylene			x	x		127-18-4	environmental/soil contaminant	solvent; dry cleaning	ex
Tetradecanal *(Myristaldehyde)*	x		x	x		124-25-4	plant oil, food	flavor/fragrance	food/ex
Tetradecane*	x	x	x	x		629-59-4	plant, food	flavor/fragrance	en/food/ex
Tetradecanoicacid *(Myristic acid)*			x			544-63-8	plant oil, food	food additive; fragrance	ex
Tetradecanoicacid,2-methyl-,methyl ester		x				55554-09-1			
Tetradecanoicacid,ethylester		x				124-06-1	food	flavor/fragrance	food/ex
Thymol *(p-Cymen-3-ol)*					x	89-83-8	plant oil	flavor/fragrance; antiseptic , cigarette additive	ex
Toluene			x		x	108-88-3	plant, plant oil	solvent; antioxidant	food/ex
trans-p-Mentha-2,8-dienol					x				
Triacetin			x			102-76-1	not found in nature	solvent (flavor); food additive; cigarette additive	ex
Tridecane*	x	x	x	x		629-50-5	plant oil	fragrance	food/ex
Undecanal*	x		x			112-44-7	plant oil, food	flavor/fragrance	en/food/ex
Undecane*	x		x	x		1120-21-4	plant oil	fragrance	food
Undecane,2,6-dimethyl-			x			17301-23-4			
ValproicAcid					x	99-66-1	synthetic	pharmaceutical (anticonvulsant)	ex
Vanillin		x				148-53-8	plant	flavor/fragrance	
Z-8-Hexadecene				x			plant		
α-Cedrene					x	469-61-4	plant oil	flavor/fragrance	
α-Cedreneoxide					x	29597-36-2	not found in nature	fragrance	ex
α-Cubebene					x	17699-14-8	plant oil		food/ex
β-Bourbonene		x				5208-59-3	plant oil, species pheromone	flavor/fragrance	ex
β-Cadinene		x				523-47-7	plant oil, plant species		food
β-Damascenone					x	23696-85-7	plant	fragrance	ex
β-Damascenone					x	23726-93-4	plant/fruits	flavor	ex
β-Gurjunene			x			17334-55-3	plant oil	flavor/fragrance	
β-Maaliene			x			489-29-2	plant oil	flavor/fragrance	
β-Phellandrene					x	555-10-2	plant oil	fragrance	ex
γ-Cadinene		x				39029-41-9	fruits		food
Δ-Cadinene		x				483-76-1	plant oil		food

4. CONCLUSIONS

The SPME-GC/MS method developed and optimized has been demonstrated to be capable of sampling, identifying and differentiating the VOCs present in various biological specimens of forensic and medical importance. While previous studies have generally looked at individual or pairs of biological specimens for diagnostic purposes, the current method allows for the direct comparison between the major samples consisting of hand odor, buccal swabs, breath, blood, blood, and urine taken from the same individuals.

The pre-treatment method developed allowed the removal of the targeted VOCs from the sampling kits prior to sampling, extraction and analysis. Optimized SPME-GC/MS conditions yielded excellent detection limits for the VOCs from blood, breath, buccal cells, and urine with average limits of detection of 8.3 ng. The VOCs from breath were detected with the lowest limit of detection while urine samples showing the highest limit of detection which was four times higher than breath for breath samples. The data obtained using this optimized method yielded promising results as for each of the specimens investigated, the VOC profiles of different subjects are distinct and show reproducible ratios of characteristic VOCs present. Visual, spearman rank correlation, and PCA comparisons of the most abundant and frequent VOCs (from replicate samples of the same individuals) demonstrates that each specimen has characteristic VOCs and that within specimens there is correlation of VOCs for replicates from individuals and differentiation (lack of correlation) among individuals. The present method, for the first time, allows for a large scale study to be completed simultaneously comparing these five biological specimens from a larger population of individuals over an extended period of

time in order to evaluate the utility of these biological specimens for profiling (individualization) and diagnostic (disease state) purposes.

Prior to this study, no work had been done to investigate the potential for differentiating people in terms of odor components other than skin emanations (mainly hand, arm, and armpits) on gauze. Through the combined methods of chromatogram comparison, Spearman rank correlation comparison, and principal component analysis, it is possible to distinguish the VOC profiles of individuals for each of the specimens with high confidence. The data obtained from this study also revealed that VOC profiles of different biological specimens from the same individual are too different to be used for the purpose of individual profiling.

Comparison of VOC profiles of healthy individuals, patients with Type 2 diabetes, and patients with MDD revealed that it was possible to distinguish individuals even when they are diagnosed with the same physiological or psychological condition. Chi-square test on the preliminary comparison of VOCs present in odor profiles across the three populations revealed a group of volatile compounds that could be used for potential biomarkers to differentiate between the healthy, diabetic, and clinically depressed study groups.

Finally, a human biological specimen compound database has been created compiling the volatile compounds present in the emanations of human hand odor, oral fluids (buccal cells and saliva), breath, blood, and urine. Compounds were classified as possibly endogenous, exogenous, originating from food and beverage sources, or a combination of any of the three. Majority of the volatile compounds are of plant origin and are widely

used in the flavor and/or fragrance industries. Because the VOCs emanated from the human body may be the results of exogenous VOC inhalation and/or absorption or the production of endogenous VOCs by the body's metabolic processes, it is unwise to attempt to derive/determine the genetic basis of human odor compounds at the present time.

Results from the present study all further support the individual odor hypothesis in that each individual possesses a scent profile that is distinguishable from others. However, because many of the extracted VOCs are known to be exogenous and/or from food or food additives while the metabolic origins of the other extracted volatile compounds remain unclear, individual human odor should be considered as an "extended phenotype" as initially mentioned in the introduction of this research study. Extended phenotype is most suitable to describe human odor, as variations in the volatile compounds present in the human odor may be due to either genetic or environmental factors or a combination of both.

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APPENDICES

Appendix A

A1 Calibration curves for selected VOCs found in hand odor

Figure 91. Calibration curve for furfural

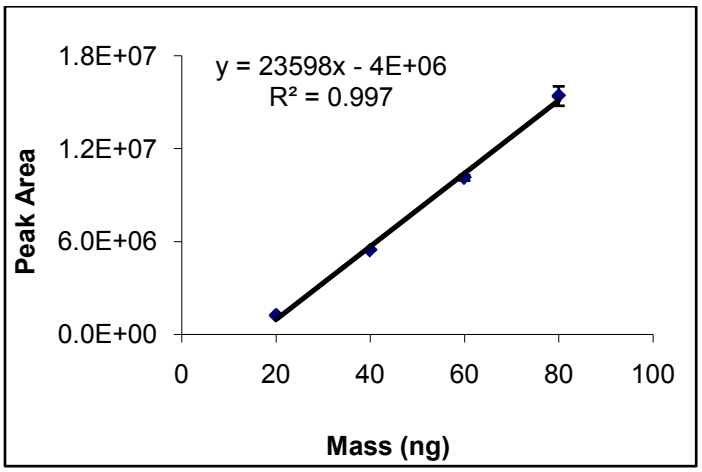


Figure 92. Calibration curve for 2-furanmethanol

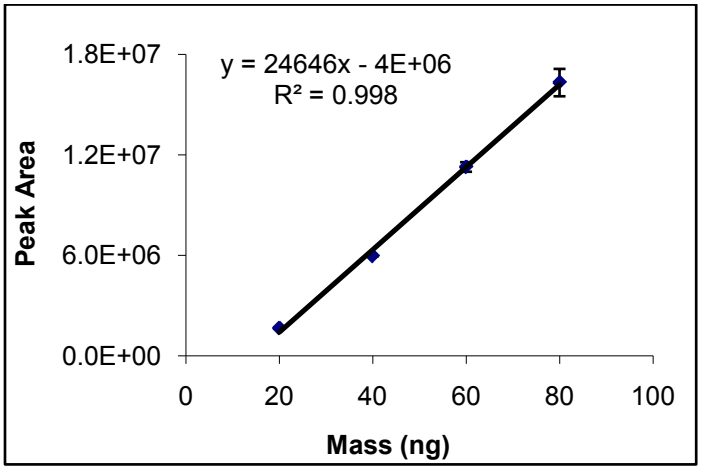


Figure 93. Calibration curve for dimethyl malonate

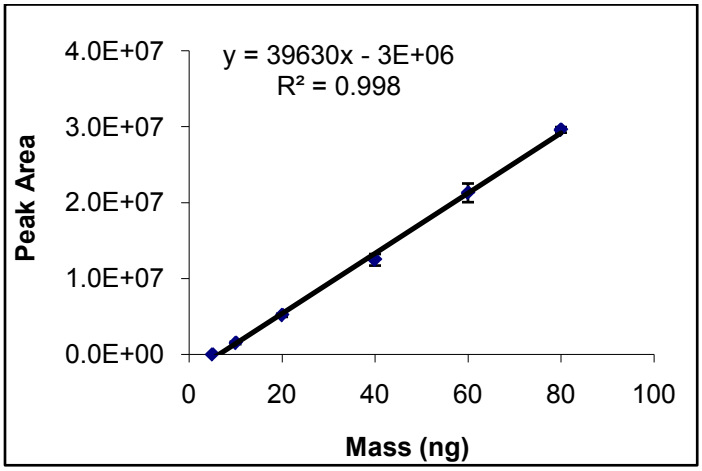


Figure 94. Calibration curve for undecane

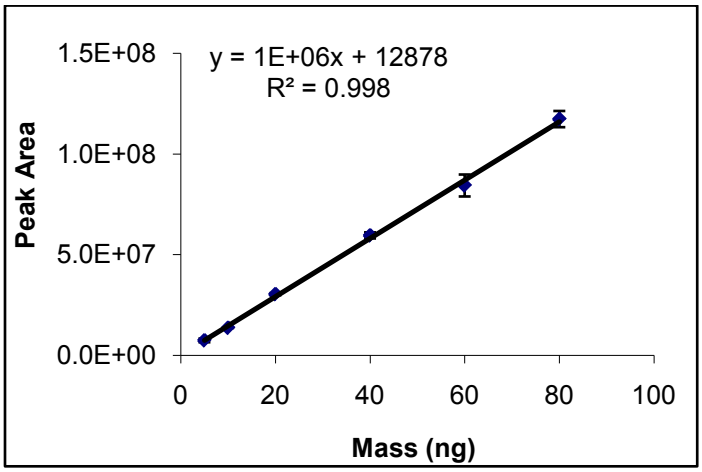


Figure 95. Calibration curve for (E)-6,10-Dimethyl-5,9-undecadien-2-one

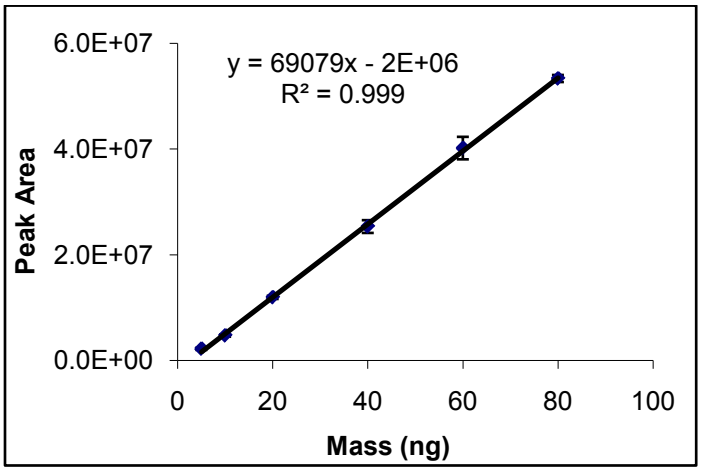
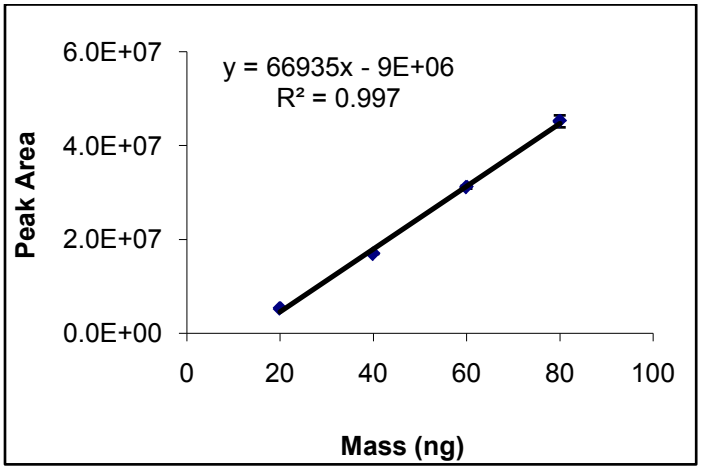


Figure 96. Calibration curve for dodecanoic acid



A2 Calibration curves for selected VOCs found in buccal swab

Figure 97. Calibration curve for hexanal

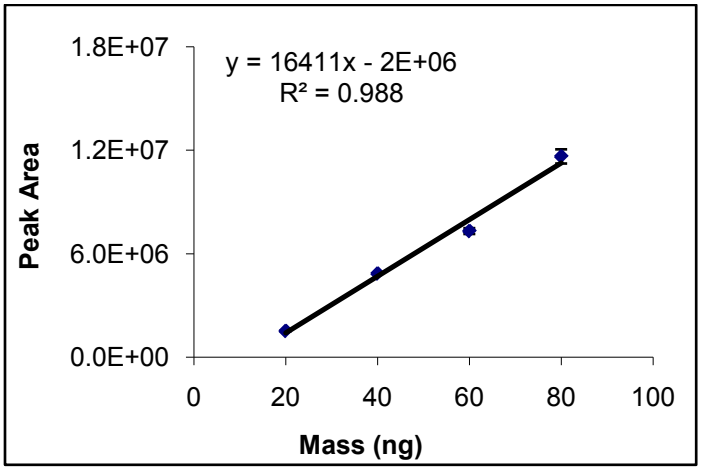


Figure 98. Calibration curve for hexanoic acid

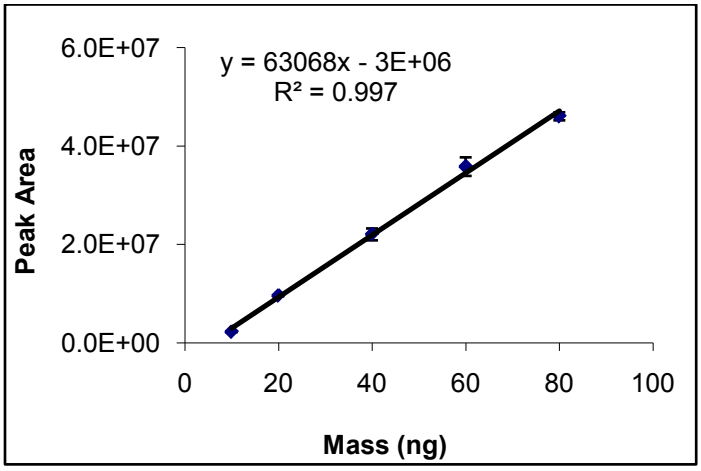


Figure 99. Calibration curve for acetophenone

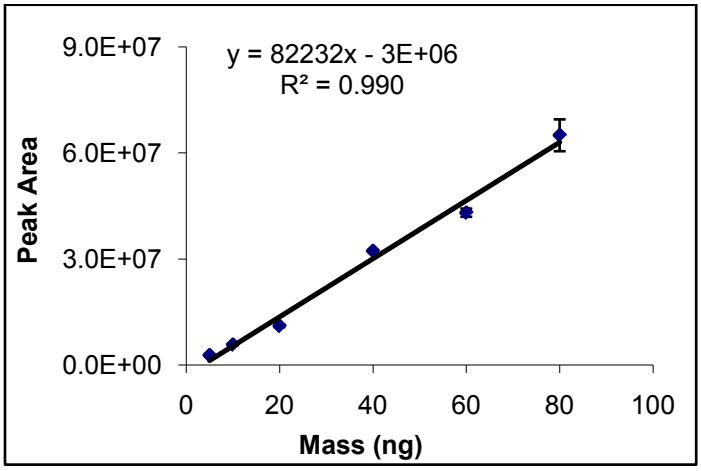


Figure 100. Calibration curve for 1-octanol

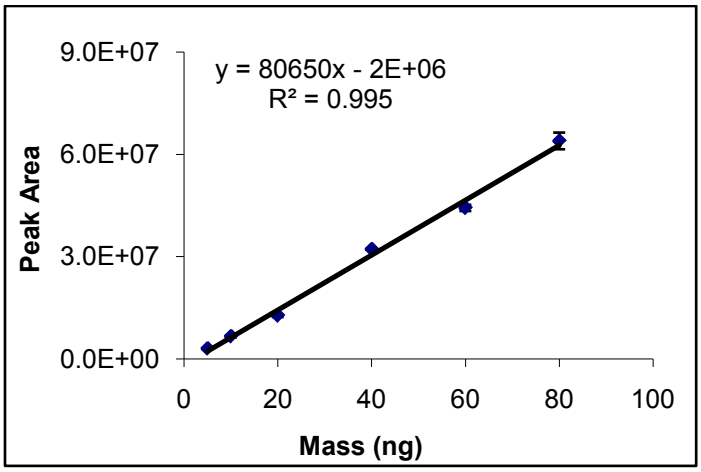


Figure 101. Calibration curve for octanoic acid, ethyl ester

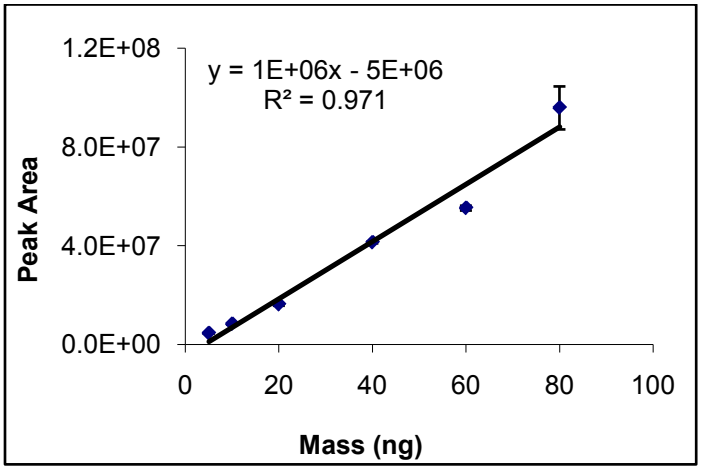


Figure 102. Calibration curve for 1-methylnaphthalene

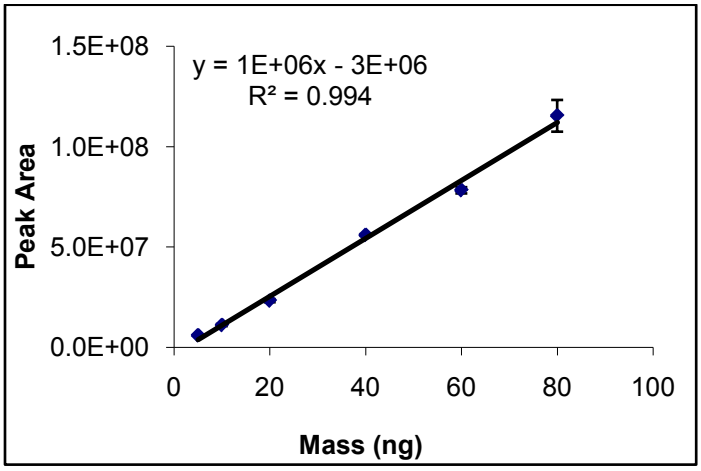
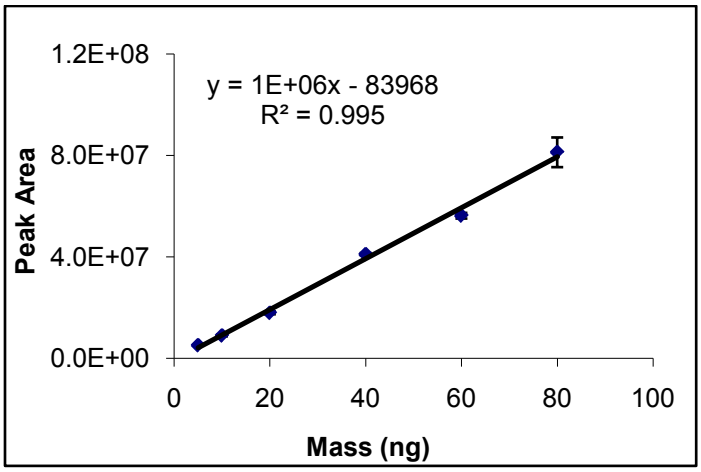


Figure 103. Calibration curve for tetradecane



A3 Calibration curves for selected VOCs found in breath

Figure 104. Calibration curve for 3-heptanone

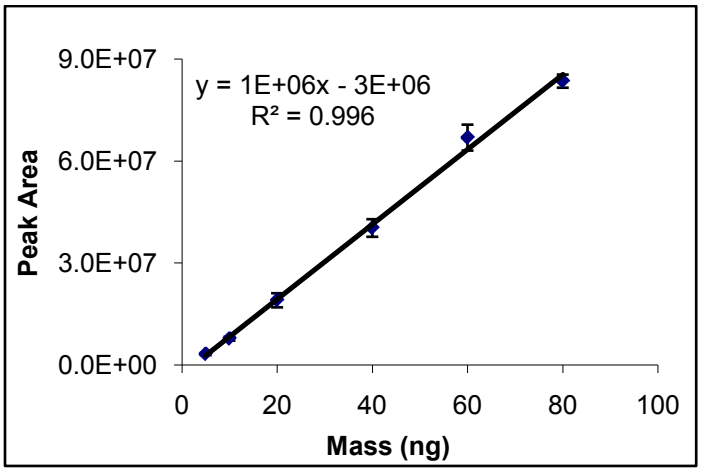


Figure 105. Calibration curve for styrene

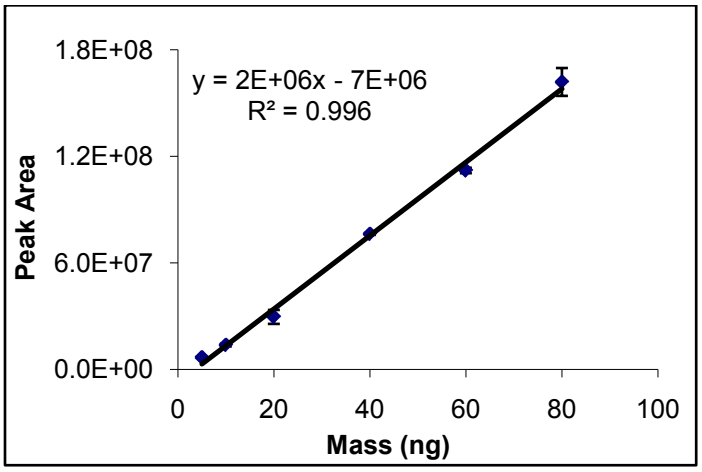


Figure 106. Calibration curve for phenol

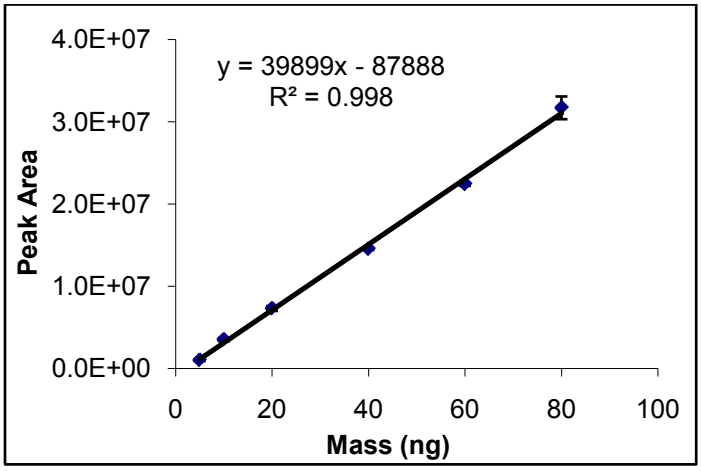


Figure 107. Calibration curve for nonanal

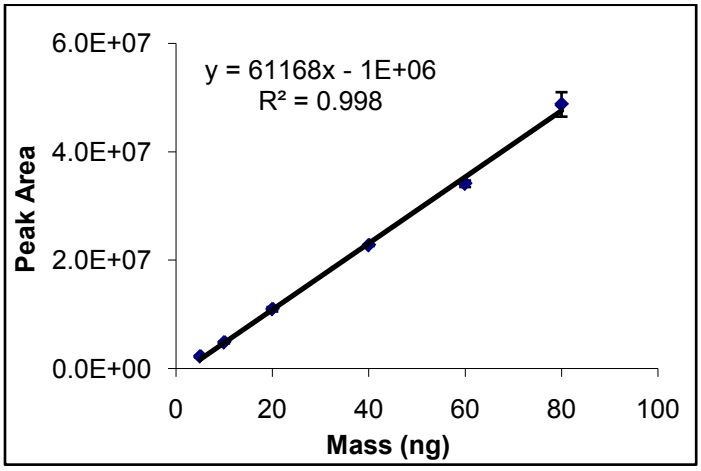


Figure 108. Calibration curve for 1-methylnaphthalene

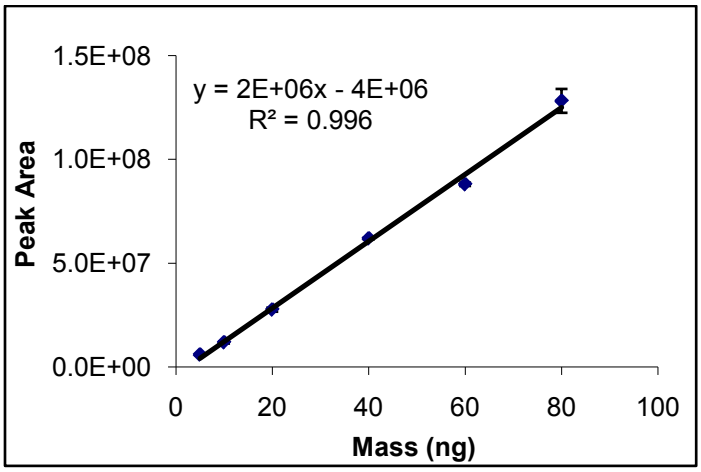
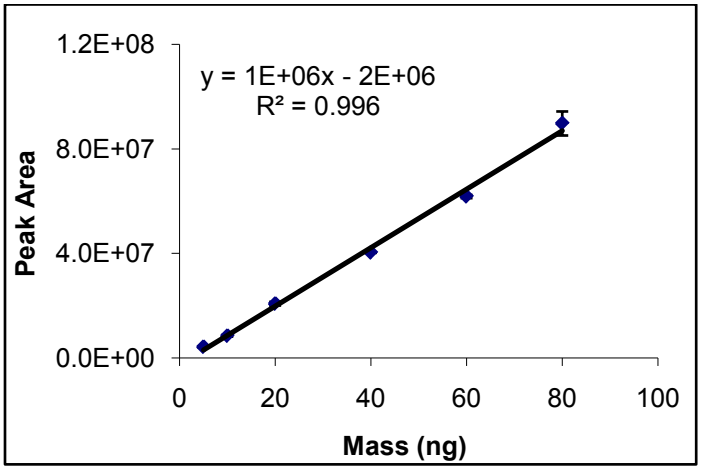


Figure 109. Calibration curve for caryophyllene



A4 Calibration curves for selected VOCs found in blood

Figure 110. Calibration curve for cyclohexanone

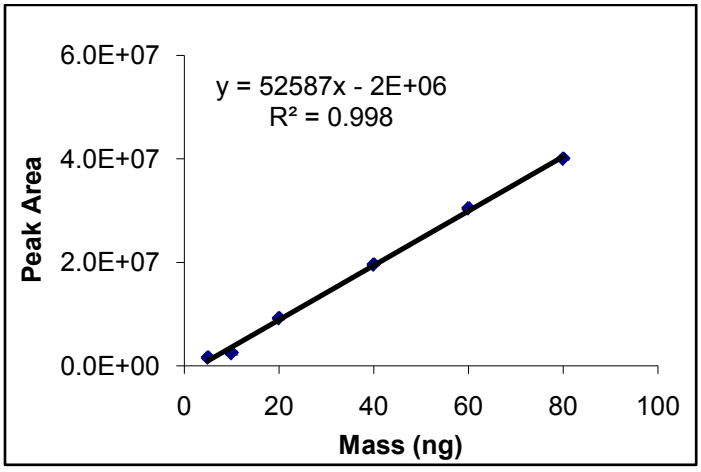


Figure 111. Calibration curve for heptanal

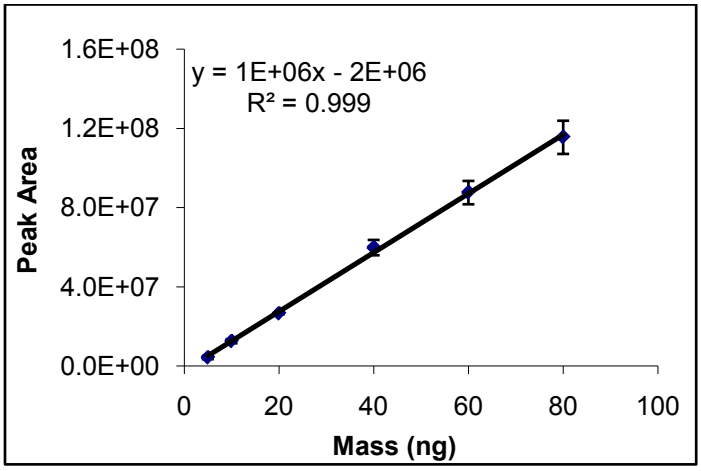


Figure 112. Calibration curve for 1-octen-3-ol

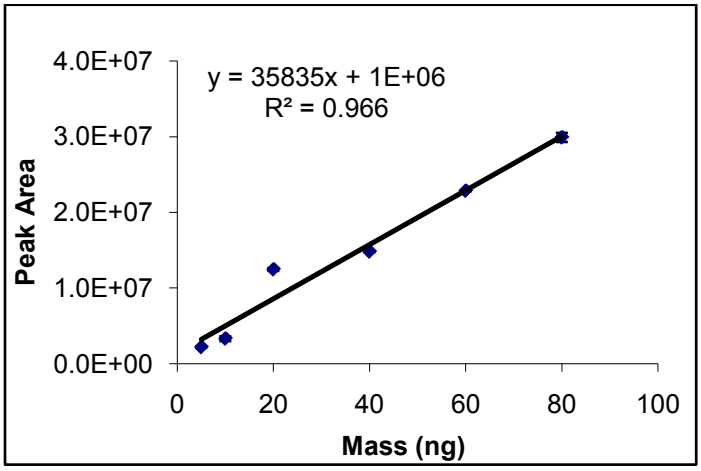


Figure 113. Calibration curve for 2-pentylfuran

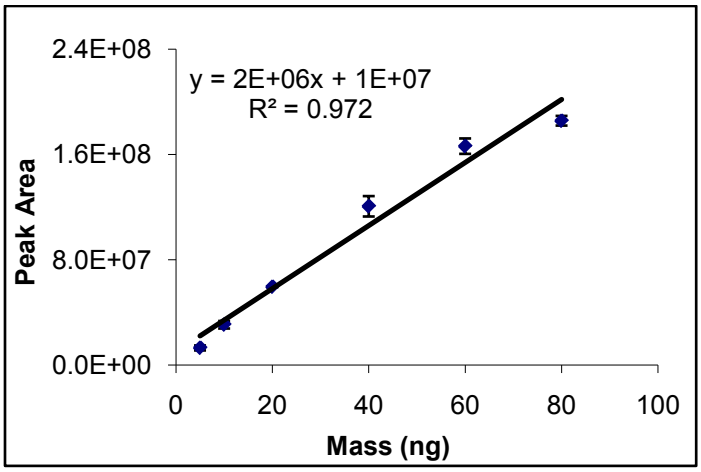


Figure 114. Calibration curve for 1-tetradecene

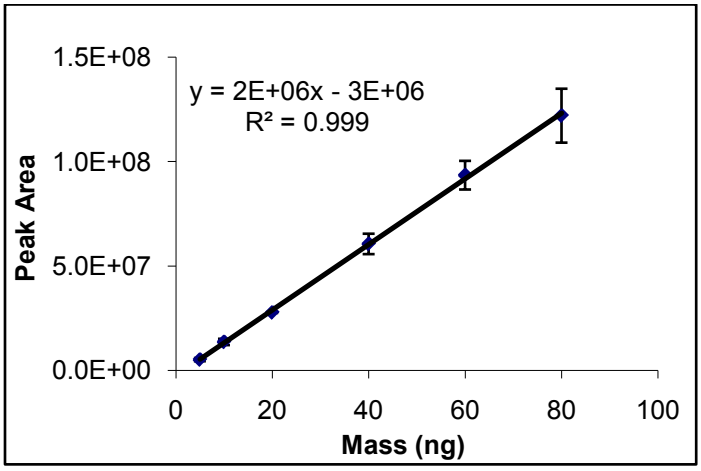
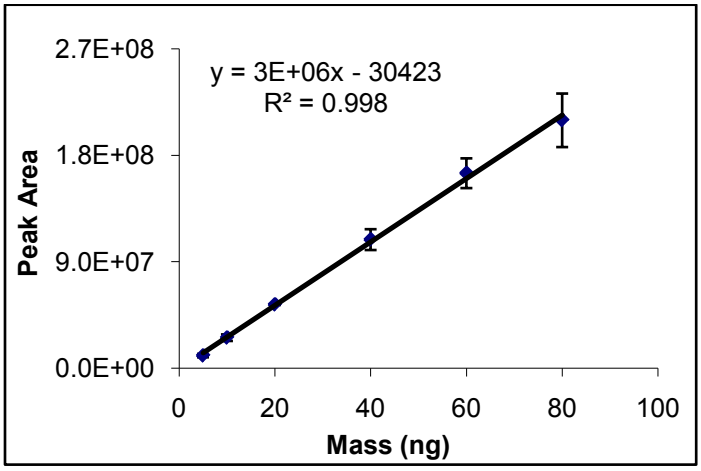


Figure 115. Calibration curve for 2,6-diisopropylnaphthalene



A5 Calibration curves for selected VOCs found in urine

Figure 116. Calibration curve for 4-heptanone

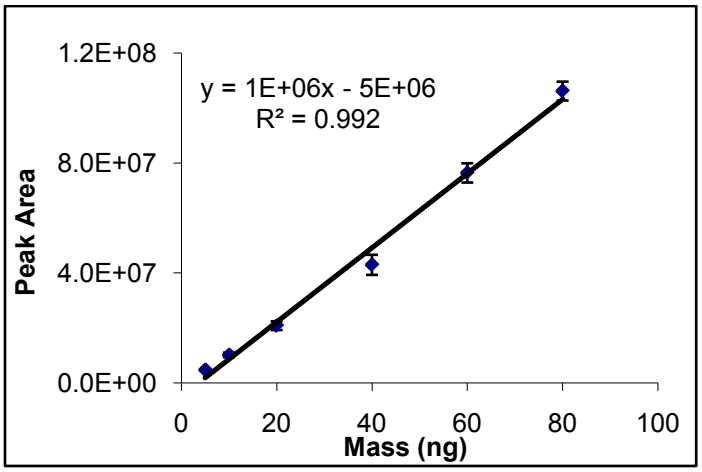


Figure 117. Calibration curve for dimethyl trisulfide

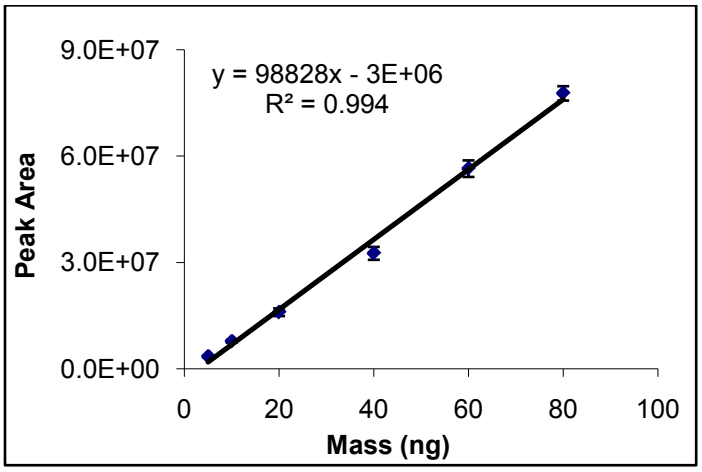


Figure 118. Calibration curve for 1,2-dichlorobenzene

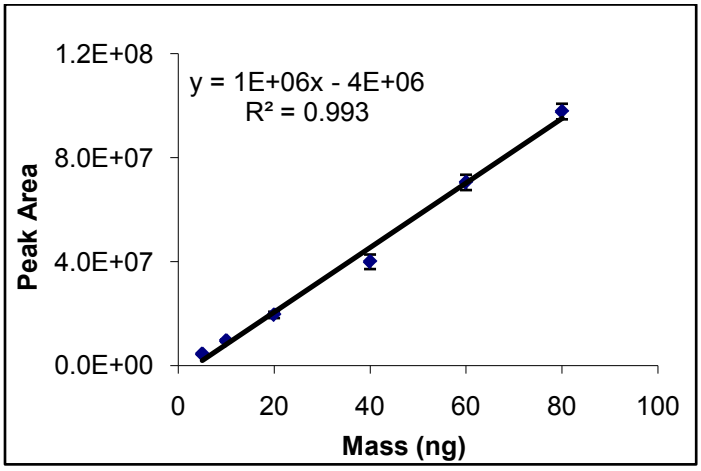


Figure 119. Calibration curve for linalool

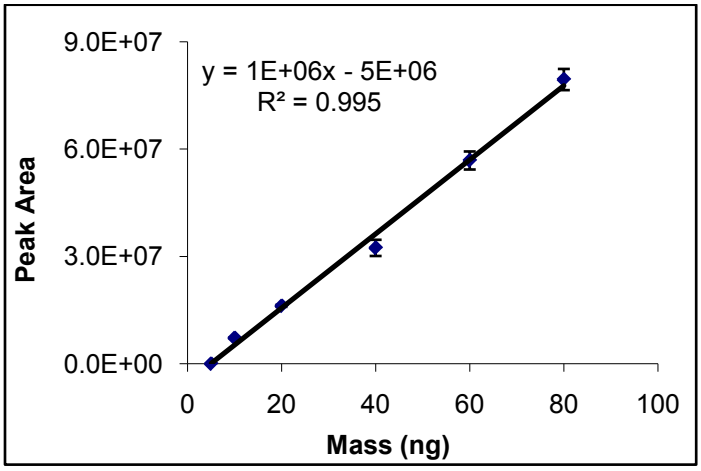


Figure 120. Calibration curve for (E)-2-nonenal

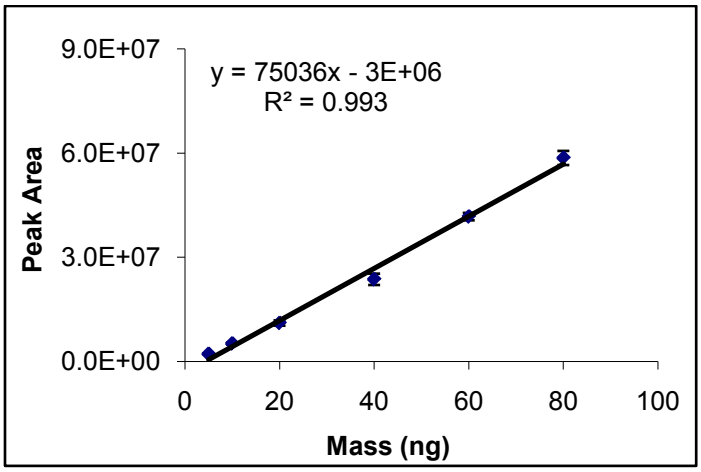
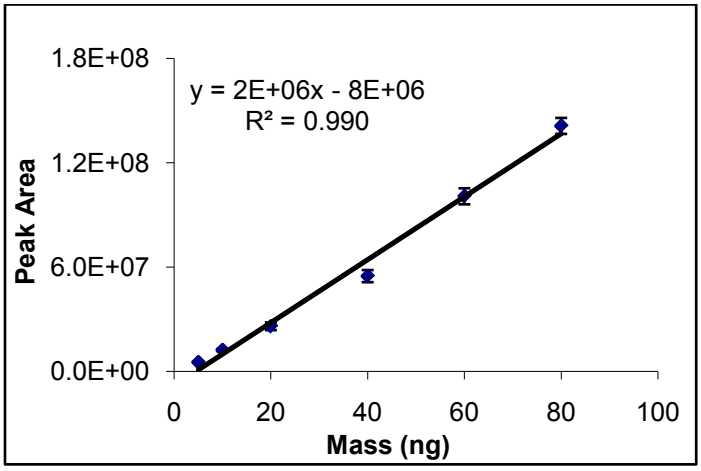


Figure 121. Calibration curve for diphenylamine



Appendix B

B1 Table 73. Target ion and qualifying ions for frequently occurring hand odor VOCs used for identification and quantitation

RT (min)	Compound Name	Target	Q1	Q2	Q3	MW (g/mol)
	2-Pentanone	43	86	41	58	86
6.25	Furfural	96	96	95	39	96
6.93	2-Furanmethanol	98	81	53	41	98
8.10	Heptanal	70	44	55	81	114
8.85	Propanedioicacid,dimethylester	101	59	74	42	132
9.44	Benzaldehyde	106	77	51	105	106
9.98	Phenol	94	66	65	39	94
10.06	6-Methyl-5-hepten-2-one	43	108	69	55	126
10.25	Hexanoicacid	60	73	41	87	116
10.36	Octanal	41	43	57	84	128
10.97	BenzylAlcohol	79	108	107	77	108
11.56	Acetophenone	105	77	120	51	120
12.14	Undecane	57	43	71	85	156
12.16	3,7-dimethyl-1,6-Octadien-3-ol	71	41	55	93	154
12.22	Nonanal	57	41	56	98	142
12.56	Octanoicacid,methylester	74	87	127	55	158
13.14	(E)-2-Nonenal	41	55	70	43	140
13.31	Nonanol	56	55	43	70	144
13.65	2-Decanone	58	43	71	59	156
13.75	Dodecane	57	43	71	41	170
13.85	Decanal	41	57	43	55	156
15.21	Tridecane	57	43	71	85	184
15.33	Undecanal	57	43	71	85	170
16.57	Tetradecane	57	43	71	85	198
16.70	Dodecanal	57	41	43	55	184
17.04	(Z)-6,10-dimethyl-5,9-Undecadien-2-one	43	69	41	151	194
17.27	(E)-6,10-dimethyl-5,9-Undecadien-2-one	43	69	41	151	194
17.74	1-Pentadecene	55	41	83	97	210
17.83	Pentadecane	57	43	71	85	212
18.65	Dodecanoic acid	73	60	43	129	200
19.02	Hexadecane	57	71	43	85	226
20.16	Heptadecane	57	43	71	85	240
23.23	Eicosane	57	71	43	85	282
24.17	Heneicosane	57	71	43	85	296
25.06	Docosane	57	71	43	281	310

B2 **Table 74. Target ion and qualifying ions for frequently occurring buccal swab VOCs for identification and quantitation**

RT (min)	Compound Name	Target	Q1	Q2	Q3	MW (g/mol)
	Aceticacid	43	45	60	42	60
	Nonanoicacid	60	73	57	115	158
	1-Pentanol	42	55	70	41	88
	Ethanol	31	29	45	46	46
5.24	Hexanal	44	56	41	57	100
6.25	Furfural	96	96	95	39	96
7.29	1-Hexanol	56	55	43	41	102
8.46	Pentanoicacid	60	73	41	45	
9.43	Benzaldehyde	106	105	77	51	106
9.95	Phenol	94	66	65	95	94
10.12	2-Pentylfuran	81	82	138	53	138
10.13	1-Decene	56	55	41	70	140
10.4	Hexanoicacid	60	73	41	87	116
11.41	(E)-2-Octenal	55	57	70	83	126
11.57	Acetophenone	105	77	120	51	120
11.66	1-Octanol	56	55	41	69	130
12.07	Heptanoicacid	60	73	43	41	130
12.13	Heptanoicacid,ethylester	88	43	113	60	158
13.14	(E)-2-Nonenal	41	55	70	43	140
13.41	Menthol	71	81	95	41	156
13.55	OctanoicAcid	60	73	55	144	144
13.63	1-Dodecene	55	41	69	56	168
13.72	Octanoicacid,ethylester	88	101	127	57	172
13.86	Decanal	41	57	43	55	156
13.99	(E,E)-2,4-Nonadienal	81	41	67	138	138
15.18	Nonanoicacid,ethylester	88	101	141	41	186
15.23	Tridecane	57	43	71	85	184
15.47	1-Methylnaphthalene	142	141	115	143	142
16.48	1-Tetradecene	55	41	83	69	196
16.52	Decanoicacid,ethylester	88	101	43	155	200
16.58	Tetradecane	57	43	71	85	198
16.72	Dodecanal	57	41	43	55	184
16.98	Caryophyllene	93	133	91	79	204
17.04	(Z)-6,10-dimethyl-5,9-Undecadien-2-one	43	69	41	151	194
17.28	(E)-6,10-dimethyl-5,9-Undecadien-2-one	43	69	41	151	194
17.75	1-Pentadecene	55	43	83	69	210
18.14	Dodecanoicacid,methylester	74	87	43	55	214
18.65	Dodecanoicacid	73	60	200	43	200
19.04	Hexadecane	57	71	43	85	226
19.49	Benzophenone	105	182	77	51	182
20.17	Heptadecane	57	71	43	85	240
23.25	Eicosane	57	71	43	85	282
24.18	Heneicosane	57	71	43	85	296
25.08	Docosane	57	71	43	85	310

B3 Table 75. Target ion and qualifying ions for frequently occurring breath VOCs for identification and quantitation

RT (min)	Compound Name	Target	Q1	Q2	Q3	MW (g/mol)
	Acetone	43	58	42		58
	Carbon dioxide	44	28	16		44
	Carbon disulfide	76	78	44	32	76
	Heptane	43	100	71	57	100
	Isoprene	67	68	53	39	68
	Pentane	43	72	57	42	72
	Toluene	91	92	65	63	92
4.16	Octane	43	85	41	57	114
4.28	Hexanal	44	56	41	57	100
5.28	Ethylbenzene	91	106	51		106
5.42	p-Xylene	91	106	105	77	106
5.75	3-Heptanone	57	85	114	72	114
5.79	Styrene	104	103	78	51	104
6.52	α-Pinene	93	91	92	77	136
6.89	Propylbenzene	91	120	92		120
7.01	Benzaldehyde	106	105	77	51	106
7.37	Phenol	94	66	65	39	94
7.48	6-Methyl-5-hepten-2-one	43	41	108	55	126
8.16	D-Limonene	68	93	67	79	136
8.26	1,2-Dichlorobenzene	147	111	112	75	146
8.80	Acetophenone	105	77	120	51	120
9.29	Undecane	57	43	71	41	156
9.37	Nonanal	57	41	56	55	142
9.59	1,2,4,5-Tetramethylbenzene	119	134	91	133	134
10.48	Menthol	71	81	95	41	156
10.65	Naphthalene	128	127	129	102	128
10.70	1-Dodecene	55	41	83	97	168
10.82	Dodecane	57	43	71	170	170
10.91	Decanal	41	57	43	55	156
12.25	Tridecane	57	43	71	85	184
12.36	Undecanal	43	57	41	55	170
12.50	1-Methylnaphthalene	142	141	115	143	142
13.26	n-Decanoicacid	73	60	129	41	172
13.58	Tetradecane	57	43	71	85	198
13.72	Dodecanal	41	55	43	57	184
13.99	Caryophyllene	93	133	91	41	204
14.04	(Z)-6,10-dimethyl-5,9-Undecadien-2-one	43	69	41	151	194
14.28	(E)-6,10-dimethyl-5,9-Undecadien-2-one	43	69	41	151	194
15.08	ButylatedHydroxytoluene	205	220	206	57	220
15.65	Dodecanoicacid	73	60	41	43	200
16.04	Hexadecane	57	71	43	85	226
16.50	Benzophenone	105	182	77	51	182
17.17	Heptadecane	57	71	43	85	240
17.59	2,6-Diisopropylnaphthalene	197	212	155	198	212
19.74	n-Hexadecanoicacid	73	60	43	41	256
20.03	Eicosane	57	71	43	85	282

B4 **Table 76. Target ion and qualifying ions for frequently occurring blood VOCs for identification and quantitation**

RT (min)	Compound Name	Target	Q1	Q2	Q3	MW (g/mol)
	Acetone	43	58	42		58
	Octanal	43	44	56	84	128
	Hexanal	44	41	56	57	100
	1-Pentanol	42	55	70	41	88
4.80	1-Hexanol	56	55	43	41	102
4.82	Benzene,1,3-dimethyl- [m-xylene]	91	106	105	77	106
4.83	p-Xylene	91	106	105	77	106
4.88	4-Heptanone	71	43	114	41	114
5.09	3-Heptanone	57	85	72	41	114
5.16	2-Heptanone	43	58	71	59	114
5.23	Cyclohexanone	55	42	98	69	98
5.24	Nonane	43	57	71	41	128
5.30	Heptanal	70	44	41	55	114
6.23	Benzaldehyde	106	105	77	51	106
6.35	1-Heptanol	70	56	55	43	116
6.51	1-Octen-3-ol	57	43	72	41	128
6.55	Phenol	94	66	65	55	94
6.66	6-Methyl-hepten-2-one	43	42	126	69	126
6.67	1-Decene	56	55	41	70	140
6.69	2-Pentylfuran	81	82	138	53	138
7.29	D-Limonene	68	93	67	79	136
7.39	BenzylAlcohol	79	77	108	107	108
7.39	1,2-Dichlorobenzene	146	148	111	113	146
7.89	Acetophenone	105	77	120	51	120
7.99	1-Octanol	55	41	69	70	130
8.36	Undecane	57	43	71	41	156
8.43	Nonanal	57	41	56	55	142
8.65	1,2,4,5-Tetramethylbenzene	119	134	91		134
9.52	Menthol	71	81	95	41	156
9.69	Naphthalene	128	127	129	102	128
9.73	1-Dodecene	55	43	41	69	168
9.76	2-Decanone	58	43	71	59	156
9.85	Dodecane	57	43	71	41	170
9.94	Decanal	57	43	41	55	156
11.26	Tridecane	57	43	71	85	184
11.51	1-Methylnaphthalene	142	141	115	143	142
12.49	1-Tetradecene	41	55	83	69	196
12.59	Tetradecane	57	43	71	85	198
12.72	Dodecanal	57	41	43	67	184
13.06	(Z)-6,10-dimethyl-5,9-Undecadien-2-one	43	69	41	151	194
13.29	(E)-6,10-dimethyl-5,9-Undecadien-2-one	43	69	41	151	194
13.53	1-Dodecanol	55	69	83	168	186
15.04	Hexadecane	57	71	43	85	226
16.17	Heptadecane	57	71	43	85	240
16.58	2,6-Diisopropylnaphthalene	197	212	155	198	212

B5 **Table 77. Target ion and qualifying ions for frequently occurring urine VOCs for identification and quantitation**

RT (min)	Compound Name	Target	Q1	Q2	Q3	MW (g/mol)
	Toluene	91	92	65		92
	2-Pentanone	43	86	41	58	86
	Pyrrole	67	39	41	28	67
	4-Nonylphenol	107	220	108		220
7.08	4-Heptanone	71	43	114	41	114
7.38	3-Heptanone	57	85	72	41	114
7.46	2-Heptanone	43	58	71	59	114
8.68	Benzaldehyde	106	105	77	51	106
8.80	Dimethyltrisulfide	126	79	45	64	126
9.15	1-Decene	41	55	56	70	140
9.77	1,2-Dichlorobenzene	146	148	111	75	146
10.24	1-Octanol	56	55	41	69	130
10.30	4-Methylphenol	107	108	77	79	108
10.62	3,7-dimethyl-,6-Octadien-3-ol	93	71	41	43	154
10.65	Nonanal	57	41	56	98	142
11.29	(E)-2-Nonenal	41	55	70	43	140
11.47	Menthol	71	81	95	41	156
11.71	MethylSalicylate	120	98	152	121	152
11.77	Decanal	57	43	41	55	156
12.67	5-(2-propenyl)-1,3-Benzodioxole	162	131	104	103	162
13.94	(E)-6,10-dimethyl-5,9-Undecadien-2-one	43	69	41	151	194
14.10	(Z)-6,10-dimethyl-5,9-Undecadien-2-one	43	69	41	151	194
14.64	ButylatedHydroxytoluene	205	220	206	57	220
14.99	Dodecanoicacid	73	60	200	43	200
15.26	Hexadecane	57	71	43	85	226
15.39	4-(1,1,3,3-Tetramethylbutyl)-phenol	135	107			206
15.55	Diphenylamine	169	168	167	170	169
15.63	Benzophenone	105	182	77	51	182
16.02	Heptadecane	57	43	71	85	240

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