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Method Development for the Detection and Quantitation of Illicit Drugs in Fingerprints Using Matrix-Assisted Laser Desorption Ionization-Mass Spectrometry

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METHOD DEVELOPMENT FOR THE DETECTION AND QUANTITATION OF ILLICIT DRUGS IN FINGERPRINTS USING MATRIX-ASSISTED LASER DESORPTION IONIZATION-MASS SPECTROMETRY

by
Madison Veronica Roussel

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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Approved by

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Reader: Dr. John Samonds
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ABSTRACT

The purpose of this research was to develop a method for the detection and quantitation of caffeine and 11-nor-9-carboxy-Δ9- tetrahydrocannabinol in fingerprints using matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). The matrix used was 2,5-dihydroxybenzoic acid (DHB). The presence of the analytes was confirmed by analysis using liquid chromatography-mass spectrometry (LC-MS). 11-nor-9-carboxy-Δ9- tetrahydrocannabinol was chosen as the drug of interest because it is the major metabolite of tetrahydrocannabinol, the main psychoactive component of marijuana. Marijuana’s popularity as a recreational drug continues to increase with its legalization and decriminalization across the country. It is critical to establish methods for the quantification of the drug in humans for the purpose of determining levels of intoxication, similar to measuring blood alcohol levels. This research shows that MALDI-MS is not capable of quantification at this time, only qualitation within the limits of detection of the instrument. Further studies will explore better methods to improve quantification and new matrices to improve the limit of detection.
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LIST OF ABBREVIATIONS

11-OH-THC 11-hydroxy-tetrahydrocannabinol
ACE-V Analyze Compare Evaluation Verification
AFIS Automated Fingerprint Identification System
CHCA α-cyano-4-hydroxycinnamic acid
CNS Central Nervous System
Da Dalton
DEA Drug Enforcement Administration
DHB 2,5-dihydroxybenzoic acid
DI Deionized
DNA Deoxyribonucleic Acid
GC-MS Gas Chromatography-Mass Spectrometry
HOMO Highest Occupied Molecular Orbital
HPLC High Performance Liquid Chromatography
LC-MS Liquid Chromatography-Mass Spectrometry
LUMO Lowest Occupied Molecular Orbital
m/z Mass-to-Charge Ratio
MALDI-MS Matrix-assisted Laser Desorption-Mass Spectrometry
mg Milligram
ml Milliliter
mm Millimeter
mol Mole
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>PCP</td>
<td>Phencyclidine</td>
</tr>
<tr>
<td>RF</td>
<td>Radio frequency</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SIM</td>
<td>Single ion monitoring</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THC</td>
<td>$\Delta^9$-tetrahydrocannibinol</td>
</tr>
<tr>
<td>THCCOOH</td>
<td>11-nor-9 carboxy- $\Delta^9$-tetrahydrocannibinol</td>
</tr>
<tr>
<td>TIC</td>
<td>Total ion chromatogram</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet-visible</td>
</tr>
<tr>
<td>$\mu$g</td>
<td>$10^{-6}$ gram</td>
</tr>
<tr>
<td>$\mu$l</td>
<td>$10^{-6}$ liter</td>
</tr>
<tr>
<td>$\mu$m</td>
<td>$10^{-6}$ meter</td>
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I. INTRODUCTION

1. Fingerprints as Forensic Evidence

The forensic value of fingerprints as evidence is based on the well-accepted theory that no two fingerprints have the same friction ridge pattern. Currently, fingerprints are used to identify an individual and place him or her at a crime scene. Latent fingerprints are those that cannot be seen with the naked eye. Enhancement techniques are used to visualize the fingerprints. The type of enhancer used depends on the surface the fingerprint is on. Prints on hard, nonporous surfaces, such as a window, can be visualized using magnetic or fluorescent powders. After dusting with powder, the print can be lifted using lifting tape and preserved for later examination. If a relatively small, nonporous object is suspected of containing latent prints, cyanoacrylate fuming, also known as superglue fuming, followed by powder dusting, can be used to attempt to visualize the prints. Prints on porous surfaces, such as paper, can be visualized using chemical enhancers like ninhydrin or iodine. Ninhydrin interacts with the amino acids in the fingerprint residue and turns the print purple (1, 2).

After the print is visualized, it is examined by a forensic scientist and entered into AFIS (the Automated Fingerprint Identification System), if it is of a high enough quality, to search for a probable match. The ACE-V (Analyze Compare Evaluation- Verification) method is a common way to analyze a print. Analysis includes determining the quality of the print and if it is suitable for entry into AFIS. The comparison step is when the examiner compares the print to possible matches, whether they are AFIS matches or other prints. Evaluation is when the examiner
comes to a conclusion about the print, whether it is a match, an exclusion, or inconclusive. The optional verification step is when a second examiner reviews the work of the first examiner and verifies their conclusion (1, 2).

Not all fingerprints found at a crime scene are usable; many do not contain enough individual detail, minutiae, to enter into AFIS or to confidently match them to a suspect’s prints. In cases such as these, it would be useful to have a method that could give some other information to forensic scientists and law enforcement. It would be helpful for law enforcement to know if the person had recently ingested any illicit drugs or medication or come into contact with other substances, such as explosives. This information could provide an investigative lead or narrow down the suspect pool. That is the basis for this project: to provide additional information about a person from their fingerprint. The technique used, MALDI-TOF-MS, will also provide an image of the print so that minutiae will not be lost as a result of the chemical testing. This study will focus on the carboxylic acid metabolite of tetrahydrocannabinol (THC), 11-nor-9-carboxy-Δ9- THC (THCCOOH). The presence of this compound in the fingerprint ridges indicates recent marijuana use by the individual. Continuing research on this project will focus on other illicit drugs of abuse and their metabolites. Hopefully, this technology will become less expensive and more user friendly so that it can be incorporated into crime lab procedures for routine casework.
2. Composition of Fingerprints

2.1. Endogenous Compounds

Fingerprint residue is composed of compounds from three major sources: the glands in the epidermis, the dermis, and outside contaminants. The epidermis is the outside layer of skin, which includes dead skin cells that are shed and replaced regularly. Specific proteins are involved in desquamation, the process of the skin renewing itself. Three desquamation proteins that have been identified in fingerprint residue are keratins 1 and 10 and cathepsin D. The epidermis also includes the horny layer, which protects the layers beneath it with a hydrolipidic film. The film is composed mostly of glycerides and fatty acids; it also contains some cholesterol and sterol esters. The film’s components are produced in the layer below the horny layer, the granular layer, from sebaceous glands (3).

The dermis is the layer of skin below the epidermis. It contains millions of secretory glands that contribute to the composition of fingerprints. The secretions of the glands travel through epidermal pores to the skin’s surface. There are three major types of glands, two of which are located on specific parts of the body. Apocrine gland secretions contribute little, if any, to fingerprint residue. These glands are only found in specific areas of the body, including the underarm, groin, and genital regions. Eccrine glands are found throughout the body. They are the key contributor to fingerprint residue. Their secretions are 99% water but include other compounds. Some proteins from the eccrine glands have been identified in fingerprint residue. These include albumin, keratins 1 and 10, cathepsin D, and dermcidin. Identifying the proteins is more difficult than detecting them. The matrix
of a fingerprint is complex; it contains many different classes of compounds. The concentrations of these compounds are also low in a single fingerprint, making detection more difficult. Amino acids, the building blocks of proteins, are easier to detect and identify than whole proteins. Common forensic fingerprint enhancement techniques, such as ninhydrin, work by reacting with the amino acids in fingerprints. The most abundant amino acid is serine, followed by glycine. Research has not yet quantified the amino acids found in fingerprints; their abundance is measured as a ratio to serine. B-group vitamins, including riboflavin, and choline have been identified in fingerprint residue. Inorganic components also come from the eccrine glands. These include chloride, sodium, potassium, ammonia, calcium, sulfide, and magnesium. Additional compounds identified from eccrine glands are urea, lactic acid, phenol, uric acid, and creatinine (3).

Sebaceous glands are located everywhere except the hands and feet; they make sebum, one of the most abundant compounds in fingerprints. Touching other body parts (e.g. the forehead) causes sebum to transfer to the fingertips and then to print residue. Lipid compounds in fingerprints come from sebaceous glands. The majority of the lipids in fingerprints are free fatty acids. Twenty-three saturated and unsaturated free fatty acids, ranging from 8-carbon units to 24-carbon units, have been identified. Wax esters, which are made of a fatty acid and fatty alcohol, have been found, but there is little data available on their contribution to the overall fingerprint composition. Triglycerides, three fatty acids bonded to a glycerol, have been found, but there is limited data on their identification. Squalene and its oxidized degradation products are easily identifiable. Cholesterol is not a secretion.
of the sebaceous gland, but it gets incorporated into sebaceous gland secretions through the blood (3).

2.2. Exogenous Compounds

The composition of fingerprint residue not only includes the endogenous compounds, those naturally secreted by glands in the skin, but also any contaminants that may be present on the skin or surface where the print was deposited. These exogenous compounds can include cosmetics, hair products, perfume, dirt, and food residues. Cosmetic products may contain compounds that are normally found in gland secretions, making it difficult to distinguish between naturally occurring compounds and contaminants. Exogenous compounds can also include drugs and their metabolites that are incorporated into the fingerprint residue (3). Detection of the parent drug, the unchanged form of the drug, can be caused by simply touching the compound, or it can be from ingestion and excretion from the body unchanged. Detection of a metabolite indicates drug use by the depositor of the fingerprint. When a drug enters the body, it is metabolized by enzymes in the body. A portion of the drug remains unchanged and is excreted as the parent drug while the rest of the drug is secreted as one or more metabolites of the drug. Nicotine, explosives, and caffeine are a few of the exogenous compounds that have been used to image fingerprints (3). The long-term focus of this study is on exogenous compounds, specifically illicit drugs.
2.3. *Composition of Aged Fingerprints*

The composition of a fingerprint after it is deposited on a surface changes over time. As stated before, eccrine gland secretions, which are mainly water, are the major contributor to the composition of fingerprint residue. Naturally over time, the water from the eccrine glands will evaporate. A study found that after two weeks, 85%, by weight, of the fingerprint had disappeared; it was hypothesized that this decrease in weight was caused by the evaporation of water. Amino acids and proteins in the residue are fairly stable, especially in fingerprints left on paper and other cellulose products. A small portion is lost over time, but there is enough left for detection using chemical fingerprint enhancers, such as ninhydrin and iodine. Because the amount of chloride decreases by an insignificant amount, it can be said that it remains constant (3). Chloride content would not be a good marker to study when attempting to determine the relative age of fingerprints. There is little data on the stability of other eccrine gland compounds (3).

Sebaceous compounds not only decrease in concentration but also are degraded into oxidized compounds. Squalene reacts with oxygen in the air and microbes to produce epoxides, ketones, and alcohols. Long fatty acids are common in new fingerprints while shorter fatty acids are found in older prints. A study determined that the most likely cause for this was that the long fatty acids are degraded into the smaller fatty acid chains. The degradation products of cholesterol have not been distinctly identified (3).
2.4. Composition Variability

The initial composition of fingerprints depends heavily on the individual. Some factors that influence the initial endogenous composition are the individual’s age, medications, diet, and overall health. The presence of exogenous compounds in the initial composition depends on what the individual has touched recently, such as food and cosmetics. The age of the individual also affects the initial composition of the print. Children, adolescents, and adults leave fingerprints with different ratios of endogenous components. Children’s prints contain less fatty acids and more water than adults. The ratio of eccrine to sebaceous compounds was highly variable in adolescents. A study suggested that this variability is caused by puberty because it starts at different ages among individuals. There is no concrete data on how gender affects the composition of fingerprints. There has not been enough research done to determine if the high variability of fingerprint composition is unique enough to determine a person’s identity from their fingerprint, compared to DNA analysis (3).

The quantitative composition of a fingerprint can be affected by how the fingerprint was deposited onto the surface. The less pressure that is applied when depositing a fingerprint, the fewer compounds are transferred, leaving less material to analyze. A study found that different fingers on the same individual contained different amounts of chloride. The right fingers had less chloride than the left fingers, and also the thumb, index, and middle fingers had less chloride than the other two fingers. They concluded that this is a result of most people being right-handed and that the ring and little finger have a thicker layer of secretions because they are used less often than the other fingers (3).
3. Overview of Marijuana

3.1. Background and Legal Consequences

Marijuana is one of the most used and abused drugs in the world, second only to alcohol (4, 5, 6, 7). It is produced from the plant Cannabis sativa and comes in a variety of forms (5, 7). There are strains of Cannabis sativa that contain different concentrations of its major psychoactive component, THC. There are sixty other cannabinoids in the plant that do not contribute to its physical effects. Marijuana typically consists of a mixture of dried leaves, stems, seeds, and flower buds of the plant (5). The ratio of the plant parts affects the potency of the mixture. The stems contain the least amount of THC, and the flower buds and leaves contain the most. Sinsemilla is a particularly potent preparation of marijuana that contains unfertilized flowering tops of female plants. Hashish, a preparation of the flower buds’ resin, and hash oil, made by soaking the plant in oil or another solvent, are other common marijuana preparations (4, 5). The most common method of marijuana use is inhalation, or smoking (5). Pyrolysis, or burning, of marijuana produces more than 2,000 chemicals, including the cannabinoids cannabinol and cannabidiol (4, 5). Cannabinol is about 10% as potent as THC; cannabidiol is not psychoactive, but it does have therapeutic properties (4, 7). Other drugs of abuse, such as cocaine, methamphetamines, and phencyclidine (PCP), can be incorporated into marijuana cigarettes to enhance the “high” of smoking. Marijuana can also be ingested orally, usually after cooking into food (4).

Certain countries do not criminalize marijuana use because it considered harmless by lawmakers. Across most of the world, however, marijuana use is
classified as illegal, and laws exist to control its use and distribution (5). Marijuana laws in the United States are complex. Federal law classifies marijuana as a Schedule I drug according to the Controlled Substances Act. Schedule I substances have no accepted medical use in the United States, have a high potential for abuse, and are not safe under medically unsupervised circumstances (8). Federal law prohibits marijuana possession, use, and distribution, but states have the ability to pass laws that decriminalize marijuana to varying degrees. The penalties, if any, for possession and distribution vary among the states. Currently, Colorado, Washington, Oregon, Alaska, and Washington, D.C., have legalized marijuana for personal use for persons 21 years and older. Twenty states allow use for medicinal purposes, and 4 states have decriminalized it. Marijuana is still illegal in the other 21 states. In states where medical use and decriminalization laws exist, possession of less than one ounce for personal use does not carry a penalty. Possession of more than one ounce or possession with the intent to sell does carry a penalty, which varies among state laws (9, 10).

3.2. Effects

Humans contain a natural cannabinoid system of neurotransmitters, receptors, enzymes, and transporters. The functions of this endogenous cannabinoid system include controlling emotions, cognition, movement, eating, behavior, and addiction patterns. It also influences the functions of the cardiovascular system, enzyme systems, hormones, and neurotransmitters. The introduction of outside sources of cannabinoids, such as those found in marijuana, interrupt the natural functions of the cannabinoid system. THC has a high affinity for the cannabinoid
receptor CB1 in the brain, and a lesser affinity for the CB2 receptors located in peripheral organs. CB1 receptors are primarily located in the cerebral cortex, hippocampus, amygdala, and cerebellum of the brain. The location of the CB receptors corresponds to the observed effects of marijuana use. The hippocampus controls long and short-term memory as well as spatial perception. The amygdala influences memory, decision-making processes, and emotions, especially fear. The cerebellum controls movement and motor learning function. As with other drugs of abuse, THC acts on the reward system in the brain, affecting dopamine release and uptake. This leads to the habit-forming behavior associated with drug abuse (4, 5).

Marijuana produces a variety of effects in the user. It cannot be classified as only a stimulant, depressant, or hallucinogen because it produces effects that can be found in all three of these classes (4). The most prominent behavioral effects include relaxation, a diminished capacity for learning and forming memories, lack of spontaneous recall, decrease in concentration, and altered perception of time. Panic reactions and paranoia are common changes in mood. Physical effects can include dry mouth and throat, rapid heart rate (tachycardia), increased hunger, hypotension (low blood pressure), and dizziness (4, 5). These effects can occur within minutes after exposure and typically disappear after 3-8 hours (4).

Marijuana can indirectly contribute to the user’s death. It has been attributed as the cause of many car accidents and other risky behaviors. Driving while intoxicated can result in increased reaction times, poor distance determination and control of the car, drowsiness, and lack of alertness and coordination. Cannabis use can affect decision making and inhibition and motor control, resulting in dangerous
behaviors that the user would not normally engage in. The cardiovascular and respiratory effects have not been shown to be a major factor in marijuana-related deaths. (4)

Maternal cannabis use can affect the fetus because cannabinoids can cross the placenta membrane (4). Fetal exposure to cannabinoids can disrupt implantation and cause infertility, low birth weight, and birth defects. Birth defects that have been linked to marijuana include those that affect the brain, cardiovascular system, gastrointestinal system, and the limbs (4, 6). Long-term effects of maternal cannabis use include problems during childhood development and an increase in susceptibility to substance abuse later in life (4). THC can be secreted into breast milk and passed to the baby. Infants who have ingested contaminated breast milk can show symptoms of sleepiness and lack of muscle tone (5). Studies have shown that prenatal exposure to marijuana can cause impaired mental function later in life. Children were shown to have lowered IQs, language comprehension, attention spans, and analytical abilities. Prenatal marijuana use by the mother has been linked to an increase in the probability of offspring to use marijuana (6).

3.2.1 Therapeutic and Medicinal Uses

The use of cannabis as a therapeutic and medicinal drug is controversial and still being researched. It can potentially be used as an analgesic (pain reliever), antiemetic (prevents nausea and vomiting), and antispasmodic (prevents muscle spasms) (4, 7). Epilepsy, asthma, and glaucoma are also medical conditions that are the subject of current research (5, 7). Synthetic cannabinoids have been made to
provide the medical benefits of marijuana without the psychoactive effects. A commercial example is Dronabinol, a synthetic analog of THC that is used as an antiemetic for chemotherapy patients. (4).

3.3 Pharmacokinetics

3.3.1 Absorption

Smoking cannabis results in faster absorption than oral ingestion. The central nervous system (CNS) is exposed to THC almost immediately after smoking. Cannabis does not actually contain THC; rather it contains a carboxylic acid of THC. The heat from smoking cannabis naturally decarboxylates nearly all the THC carboxylic acid to produce active THC (4). However, only about 70% of the total THC content is available for consumption because 30% of it is destroyed during pyrolysis. Other factors that influence the actual amount of THC consumed include the volume of inhaled smoke, the time the smoke is held in the lungs, and the time between puffs; this results in a variability in the amount of THC consumed, between 18-50% of the original amount in the cigarette (4, 5). Blood plasma concentration of THCCOOH reaches a maximum between 0.5 hours and 4 hours, with an average of 1.9 hours, after initial exposure (4).

Only 6-18% of the THC is available for absorption from marijuana products ingested orally. This decrease is a result of enzymes in the stomach that degrade THC and first-pass metabolism by the liver before any drug is available in the bloodstream. Blood plasma concentration of THCCOOH peaks at 2-8 hours after ingestion. Effects of orally ingested cannabis appear and disappear slower than those of smoked cannabis because of the longer and slower route of absorption (4).
3.3.2 Distribution

THC is distributed to the lungs, heart, brain, and liver faster than other organs. It is distributed throughout the body as it circulates in the blood. THC is lipophilic, meaning it is soluble in body fat. It is quickly absorbed by adipose tissue and then slowly released back into the blood (5). It is possible for THC to be stored in fat tissue and detected weeks after initial cannabis use. Its solubility in fat is one reason why THC has a relatively long half-life, about 4 days, in blood. THC can be detected in blood for up to 7 days and up to 24 days in urine (4).

3.3.3 Metabolism

Many metabolites of THC have been identified, but the major metabolic pathway is the transformation of THC to THCCOOH (Figure 1). First, cytochrome P450 enzymes hydroxylate THC, producing the psychoactive metabolite 11-hydroxy-THC (11-OH-THC). 11-OH-THC is oxidized to produce THCCOOH, which is not psychoactive (4, 5). The glucuronide conjugate of THCCOOH is also produced during this final step, though the ratio of glucuronide conjugate to THCCOOH is low. The glucuronide increases the solubility of THCCOOH in water, which is important for excretion (4).

3.3.4 Elimination

THCCOOH can be detected longer than either THC or 11-OH-THC in urine and blood. 30-65% of the dose is excreted in feces, and 20% is excreted in urine. Since only one metabolite, THCCOOH, is used for detection in urine, extremely low percentages of the original amount of THC are recovered. Five days after exposure, 80-90% of the urinary metabolites, 11-OH-THC and THCCOOH, are eliminated (4, 5).
The glucuronide THCCOOH conjugate has a urinary excretion half-life of 3-4 days. At first, concentrations of THCCOOH in urine decrease quickly, but once it reaches 20-50 μg/L, the concentration decreases much slower than before, a result of the release of stored THC in body tissues (4).

3.4. Chronic Effects

The most common long-term effects of consistent cannabis use are impaired memory, concentration, and learning abilities and an increased risk of anxiety. These conditions can result in poor work performance, low quality and quantity of work, and social isolation (6). Physical effects of chronic use include damage to the respiratory, reproductive, cardiovascular, and immune systems (4, 6). Lung conditions associated with marijuana use includes decreased lung density, cysts, bronchitis, emphysema, lung cancer, and general damage to lung tissue. Smoke from marijuana is different from cigarette smoke. It is also inhaled differently; cannabis users take deep puffs and hold the smoke deep in their lungs. The smoke causes the airways to become inflamed and can cause chronic bronchitis. One study showed that smoking one marijuana joint has the same effect on the lungs as smoking 2.5 to 5 tobacco cigarettes. Vasodilation, hypertension, and tachycardia are the most common cardiovascular effects of chronic cannabis use. During exercise, users may also experience increased heart rates, resulting in shorter tolerable periods of exercise. Less common effects include myocardial infarction (heart attacks) and cardiac arrhythmia (abnormal heart beat). Users with pre-existing heart conditions have a higher risk of cardiovascular complications (6).
Chronic marijuana use can increase the risk of developing cancer. Lung, head, neck, larynx, prostate, cervical, testicular, and brain cancer have all been associated with marijuana use. Maternal cannabis use increases the risk of inheritable cancers, such as neuroblastoma and leukemia, which can be passed to the fetus. Studies that have attempted to positively link heavy marijuana use with cancer development have not been conclusive, possibly as a result of small sample sizes. Cannabinoids have been shown to affect DNA and DNA repair mechanisms, but the full extent of their effect has not been studied in depth yet. Naturally occurring cannabinoids oxidize the DNA base guanine as part of their signaling. DNA repair mechanisms are then triggered to remove the oxidized base and replace it with a non-oxidized base. It is suggested that introducing more cannabinoids into the body, through smoking marijuana, can overload the normal DNA repair system and cause it to stop functioning. If the damaged DNA were not repaired, the damaged sections would be reflected in altered RNA and proteins. Cannabinoids are also known to block topoisomerase II function. Topoisomerase II is an enzyme that uncoils DNA for transcription and helps in DNA repair, replication during meiosis, and transcribing mRNA into proteins (6).

There is a distinct association with marijuana use and mental illnesses. Manic psychotic episodes can be caused by heavy marijuana use. Studies have shown that the cannabis use can intensify symptoms of depression, schizophrenia, psychosis, and bipolar disorder (5, 6). The reason why this occurs is unknown, but it has been proposed that the cannabinoids interact with environmental and genetic factors that contribute to these disorders (5). There is debate about whether chronic
cannabis use results in permanent changes in brain function. There are inconsistent results from studies that have attempted to determine the cognitive effects of long-term marijuana use. The varying results can be attributed to individual differences among subjects and experiment design, among other things (4). Chronic marijuana use among youths is associated with poor performance in school and increased dropout rates. Early marijuana abuse and dependence can be an indicator of future abuse of other illicit drugs (5).

Studies suggest that marijuana dependence can develop as a result of chronic abuse. Key factors that can be used to determine dependence are obsession and compulsion of using cannabis and the tendency to relapse after a period of abstinence. After stopping cannabis use, over half of users exhibited withdrawal symptoms of irritability, anxiety, craving, and interrupted sleep patterns (5).


Matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) is a technique used for determining the mass of a compound (Figure 2). It can identify an extremely wide range of masses, from 100 Daltons (Da) to 1 million Da. The sample compound is mixed with a suitable matrix, and then applied to a target plate and left to dry. The purpose of the matrix is to absorb the energy from the laser, transfer it to the sample compound to ionize it, and donate or accept a proton, depending on the ionization mode. MALDI is a soft ionization technique, meaning it causes minimal fragmentation of compounds. In the vacuum of the instrument, a laser of nitrogen gas (237nm) is continuously pulsed onto the
sample to ionize it. The sample and matrix become an ionized gas. The ions pass through a ground grid that gives every ion the same kinetic energy. Since kinetic energy is defined as \( \frac{1}{2} \times \text{mass} \times \text{velocity}^2 \), the speed that the ions move through the linear flight tube depends on their mass. Lighter ions reach the detector first and then the heavier ions reach it later (11, 12).

5. Liquid Chromatography-Mass Spectrometry

5.1. Liquid Chromatography

Liquid chromatography is a separation technique that uses a liquid mobile phase and solid stationary phase to separate components of a liquid mixture (Figure 3). The liquid analyte is injected into the system and carried through the chromatography column by one or more organic solvents. Gradients of solvents can be used to improve separation by changing the polarity of the liquid phase to flush the analyte out of the column. At the start of analysis, the gradient is at its most polar. It becomes more nonpolar as analysis time increases. The gradient used depends on the analyte and column chemistry. The mobile phases used in this research were water with 0.1% formic acid and acetonitrile with 0.1% formic acid. The gradient is stated in the Materials and Methods section and described in the Results and Discussion section. Liquid chromatography is useful for separating a variety of compounds because the analytes do not have to be volatile or thermodynamically stable since LC-MS does have a heated injection port. The LC-MS system used in this research has a packed column. A packed column is a steel tube that is packed with porous silica beads. The analytes interact with the beads; the
degree of interaction is based on the polarity of the analytes and the mobile phase gradient. Nonpolar analytes, THCCOOH in this case, interact more with the silica beads than polar analytes, such as caffeine, and require different gradients to be eluted from the column (13, 14, 15).

5.2. Mass Spectrometry

The mass analyzer in the LC-MS system used in this research has a single quadrupole mass analyzer. A quadrupole mass analyzer is four metal rods that have an oscillating radiofrequency (RF) voltage and constant direct current (DC) voltage across them (Figure 4). The ion beam passes in between the four rods. Ions of different mass-to-charge ratios (m/z) are separated by changing the electric potential of the RF and DC voltages. Ions that resonate with the RF voltage are sent to the detector. Ions that do not resonate collide with the rod or pass between the rods and go undetected (16).

6. Gas Chromatography-Mass Spectrometry

Gas chromatography is a separation technique that uses a gas mobile phase and solid stationary phase to separate components of a mixture (Figure 5). The liquid or gas analyte is injected into the system and carried through the separatory column by a carrier gas. The analyte must be volatile, able to be converted to a gas, in order to be analyzed by GC-MS. Additionally, the analyte should also be thermodynamically stable so that it is not fragmented or degraded when it is injected into the heated column. The most important part of GC instrumentation is the column. There are many different columns available that are designed for
different types of analytes. The type of column used in this research is a DB-1. The mass analyzer in the GC-MS was a quadrupole, the same as the LC-MS instrument (17).

7. Ultraviolet-Visible Light Spectroscopy

Ultraviolet – visible (UV-Vis) spectroscopy measures the absorbance of a compound at a particular wavelength in the ultraviolet (200-400 nm) or visible light (400-700 nm) spectrum. When light is absorbed, electrons go from a low energy ground state in the highest occupied molecular orbital (HOMO) to a high energy excited state in the lowest unoccupied molecular orbital (LUMO). In electronic transitions, electrons go from single bond sigma (σ) orbital, double or triple bond pi (π) orbital, or nonbonding (n) orbital in the HOMO to either sigma anti-bonding (σ*) or pi anti-bonding (π*) orbitals in the LUMO (Figure 6). Since sigma bonds have the lowest energy and are the most stable, they absorb less light than pi or nonbonding electrons. In order for a bond to absorb light, the energy of the wavelength of light must be equal to the energy of the HOMO-LUMO transition. The energy of the HOMO-LUMO transition is dependent on the type of bond and what elements are bonded. (18, 19, 20, 21)

The design of a UV-Vis spectrometer is rather simple, compared to the other instruments used in this research (Figure 7). A beam of UV and/or visible light is separated into its different wavelengths by either a prism or diffraction grating. Each wavelength is then split in two. One passes through the cuvette containing the sample; the other passes through a reference cuvette that contains the solvent used
for the sample. The detector measures the intensity (I) of the light that passes through the sample and reference cuvettes. Intensity can be converted to absorbance (A). Values for absorbance range from zero (no absorption) to 2 (about 99% absorption). The reference cuvette should not absorb much light and have an absorbance close to zero. The absorbance of the reference cuvette is subtracted from the absorbance of the sample cuvette to give the actual absorbance of the analyte. Absorbance can also be reported as transmittance; 0 absorbance equals 100% transmittance. (18, 20)

The spectrometer produces a spectrum of wavelength (x-axis) versus absorbance or transmittance (y-axis). The wavelength of the most absorption (λ max) can be characteristic of the analyte. The mathematical equation that governs the function of a UV-Vis spectrometer is the Beer-Lambert Law: A = cbe. It states that the absorptivity (A) of the solution is equal to its molar concentration (c) times the path length of the light through the sample (b) times the molar absorptivity (ε) of the analyte. Since the path length of most cuvettes is 1 cm, this term is generally omitted from calculations. This equation can be used to calculate the concentration or molar absorptivity of a sample from the measured absorbance or to calculate the theoretical absorbance of a compound with a known concentration and absorbance. (18, 19, 20, 21)
8. Specific Aims

The goal of this research is to develop a method to analyze fingerprints for the presence of legal and illegal drugs. First, a matrix had to be chosen, and the instrument parameters had to be optimized. Another goal of this project was to quantify the amount of drug in the sample. To do this, a calibration curve of standard concentration has to be created. One curve was created from the first analysis of THCCOOH standards. Ten subsequent analyses were run in an attempt to reproduce this curve; none were successful. The focus of the study then moved to imaging fingerprints since the imaging software was working again. The goal of the imaging studies was to detect caffeine in the fingerprints. The caffeine imaging was successful. Saliva and sweat samples were included in the final imaging study to verify the presence of caffeine in the fingerprints. The samples from the final study were also analyzed using LC-MS to further confirm the presence of caffeine. The final imaging and LC-MS studies were successful at proving the presence of caffeine in the samples.
II. Materials

1. Standards

1 ml of 1 mg/ml (-)-11-nor-9-carboxy-Δ9-THC in methanol was purchased from Cerilliant.

2. MALDI-TOF-MS

The MALDI-TOF-MS used in this research was an Autoflex II MALDI-TOF-MS made by Bruker Daltonics. The computer software used was flexControl, flexImaging, and flexAnalysis. The target plate was an MTP 384 massive target plate made by Bruker Daltonics. The MALDI matrix used was 2,5-dihydroxybenzoic acid, purchased from Sigma-Aldrich.

3. GC-MS

The GC-MS used in this research belongs to ElSohly Laboratories. The chromatography column was a capillary DB-1 column with a length of 30 meters, internal diameter of 0.25 mm, and film thickness of 0.25 μm.

4. LC-MS

The LC-MS used in this research was a 1260 Infinity Binary LC System coupled to 6100 Series Single Quadrupole MS (Aglient Technologies, Santa Clara, CA, USA) The chromatography column was a SB C18 with a particle diameter of 1.8 μm, internal diameter of 2.1mm, and length of 50mm.

5. UV-Vis

The UV-Vis spectrometer used in this research was a Carey [NUMBER] (Aglient Technologies, Santa Clara, CA, USA)
III. Methods

1. MALDI-TOF-MSI

Pipette 5 µl of deionized (DI) water onto plate dots, leaving 2-3 dots between each drop. Pipette 7.5 µl of calibration standards of caffeine and THCCOOH spiked with 10 mmol of caffeine onto each water drop. Let the plate sit until the drops are completely dry. While the plate is drying, prepare the matrix. Weigh 1.2 g of 2,5-dihydroxybenzoic acid (DHB) and mix with 40 ml methanol (0.2-0.3 g DHB per 1 ml methanol). Take a picture of the target plate to use later for the imaging software. Spray target plate with all 40 ml matrix using nebulizer. Wait for previous coat of matrix to dry (2-3 seconds between coats) before spraying next coat. Once final coat is completely dry, load plate into MALDI-TOF-MS. Set up imaging by entering teach points and selecting the regions of the plate where the calibrators are. Set the mass range to 280 – 600 Daltons (Da) and the laser to 100% strength.

2. LC-MS

The same calibrators and biological samples analyzed using MALDI-TOF-MS were analyzed by LC-MS. The mobile phases used were water, 0.1% formic acid (solution A) and acetonitrile, 0.1% formic acid (solution B). The mobile phase gradient, represented as percentage of solution A, used for the calibration standards and biological samples is shown below in Table 1. For both analyses, 1 µl of sample was injected and and the flow rate was 0.2 ml/min. Ions at 194 m/z and 344 m/z were monitored in single ion monitoring mode (SIM).
### Calibration Gradient | Biological Sample Gradient

<table>
<thead>
<tr>
<th>Time</th>
<th>% Solution A</th>
<th>Time</th>
<th>% Solution A</th>
</tr>
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<tbody>
<tr>
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<td>95</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
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<td>2.5</td>
<td>90</td>
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<td>10</td>
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<td>10</td>
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</tr>
<tr>
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<td>0</td>
<td>10.5</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>95</td>
<td>11</td>
<td>95</td>
</tr>
</tbody>
</table>

Table 1: Gradient for LC-MS analysis

3. GC-MS

The same calibrators and biological samples were analyzed using GC-MS. However, due to technical difficulties, the samples were not analyzed in time to be included in this discussion.

4. UV-Vis

Pipette 3 ml of methanol into both the reference quartz cuvette and the sample quartz cuvette. Pipette 20 μl of THC-COOH standard into the sample cuvette and measure the absorbance. This was repeated until 180 μl of standard was added; then, 40 μl was added at a time. Analysis stopped after the addition of a total of 340 μl of THC-COOH standard.
IV. RESULTS AND DISCUSSION

1. MALDI-TOF-MS

1.1 CHCA Matrix Testing

The development of the final method used to analyze the THCCOOH calibrators and samples using MALDI required trial and error using different matrices and sample preparations. Another focus of the method development was finding the m/z of the peak that corresponds to THCCOOH; the position of the peak for THCCOOH using MALDI-TOF-MS was not found in the literature. THCCOOH calibrators of a wide range of concentrations were prepared once for use throughout the research. The first matrix used was α-cyano-4-hydroxycinnamic acid (CHCA). Calibrators with an initial concentration of 500, 250, 125, 62.5, 6.25, 0.625, 0.125, 1x10⁻⁷, and 1x10⁻¹⁰ μg/ml were used. The concentration was further diluted with the addition of the matrix solution. The CHCA matrix was prepared by mixing 50:50 DI water/acetonitrile and 1% trifluoroacetic acid (TFA) with 10-20 mg CHCA. In new vials for each concentration, 80 μl of matrix was mixed with 20 μl of calibrator. Twenty μl of the sample and matrix mixture was pipetted onto a glass slide. The samples were left to dry on the glass slides for approximately two hours. The instrument settings were the same as described in Methods, except the laser strength was 74%. The laser strength was optimized before analysis by manually selecting sample spots and adjusting the laser to give the best spectrum.

The mass spectrum of the CHCA matrix (Figure 8) shows that there are peaks in the m/z range of interest, rendering it unusable for analysis of THCCOOH or any other low mass compound. This is not unexpected since MALDI-MS is commonly
used in protein analysis; the peaks in the low mass range would not affect the high mass range where proteins would be found.

1.2 DHB Matrix Testing

Next, DHB matrix was used. To make the DHB matrix, 25 mg of DHB and 1 ml methanol were vortexed for 1 minute, or until the DHB was completely dissolved. 80 μl of matrix and 20 μl of 500, 6.25, 0.625, and 0.125 μg/ml calibrators were mixed in separate vials. A limited range of concentrations was used since it was unknown if this method would work. 20 μl of the matrix and calibrator solution was pipetted onto glass slides. Since methanol is highly volatile, the spots dried within a minute, a timesaving advantage over the CHCA matrix. The instrument settings were the same as described in Methods.

The DHB matrix blank (Figure 9) shows high intensity matrix peaks between 100 and 200 m/z and around 270 m/z. There are some low intensity peaks between 300 and 400 m/z (Figure 10) and around 500 m/z (Figure 11). The mass spectrum of the 500 μg/ml calibrator (Figure 12) showed a peak at 335 m/z that did not appear in the matrix. Figure 13 shows a closer view of the peak at 335 m/z. The molecular mass of THCCOOH is 345 g/mol so this m/z is a probable match that is supported by the fact that there was no significant peak near 335 m/z in the matrix. There was also a slight increase in the intensity of the peak at 500 m/z (Figure 14). The significance of this peak is discussed later. The overall intensity of the spectra is relatively low, but that does not discount the fact that the peaks are there.
1.3 Calibration of THCOOH Using DHB Matrix

Since the probable location of the THCCOOH peak was known, two complete calibration sets were run in the same day using 500, 62.5, 6.25, 0.625, 0.125, 0.0025, 0.005, 0.001, and $1 \times 10^{-5}$ μg/ml calibrators. The samples were prepared the same as before and spotted onto glass slides. A blank matrix spot was also included. After set 1 was finished, the glass slides were cleaned with methanol and DI water and then spotted with the calibrators. The spectra showed peaks at 337-338 m/z, a slight shift from 335 m/z in the test run. For 0.125 μg/ml and concentrations below, there were no visible peaks at or around 337 m/z (Figures 15-17). As with the DHB test run, the intensity of the spectra was relatively low. The intensity of the second run was extremely low compared to the first run. The average peak intensity at 337 m/z for set 1 was 352 and 9 for set 2. This was most likely because there was less sample than in set 1. While preparing the slides, the matrix-sample spots began to spread, which had not occurred before. Instead of 20 μl spots, layers of 2 μl spots were used for set 2. Spreading is common for organic solvents, such as methanol. For set 1, there was good linearity ($r^2=0.99818$) of the average intensities for 337 m/z between 62.5, 6.25, and 0.625 μg/ml. However, there were only three points used to determine the linearity, and it was not reproducible in subsequent trials.

1.4 Problems with Calibration of THCCOOH

Figures 18 and 19 illustrate why the quantitation was neither successful nor reproducible. Figure 18 shows the distribution of caffeine when the matrix and sample are mixed and then pipetted onto the target plate. Figure 19 shows the distribution of caffeine when the sample is pipetted onto the target plate and then
sprayed with the matrix. Spraying the matrix leads to a more even, but not perfect, distribution of analyte. There is still a cluster of caffeine at the bottom of the spot. Each line of the mass spectrum is the average of 1000 spectra taken in the square area between each of the red dots. To create a calibration curve, the concentration of the sample is graphed against the average peak height. The uneven distribution of analyte results in peaks with extremely high intensities and peaks with extremely low intensities. This affects the average of the heights and causes the average of all the heights to be similar. This effect is seen in both methods of matrix application.

1.5 New Method for Calibration of THCCOOH

For the next set of calibrators, more concentrations between 62.5 and 0.625 were made, a different target plate (MTP 384) was used, and a faster analysis method was tested. Calibrators at concentrations of 62.5, 31.25, 15.625, 7.81, 6.25, 3.125, 1.56, 0.78, and 0.625 μg/ml were prepared in DHB matrix as before. The steel target plate contained a grid of etched dots. 10 layers of 2 μl of each calibrator were spotted approximately 3 dots apart because of spreading. The new analysis method included setting the mass range from 280-400. The new range eliminates matrix peaks that occur before 280 and allows the analyte peak to be seen more clearly. Spectra were collected for each grid dot that contained sample. The instrument software recognizes the grid, and the grid spots are manually selected. The spectrum for each spot was an average spectrum of 4999 positions on the spot. There were still matrix peaks around 335 m/z that made detection of the analyte difficult. On the third run, the mass range was extended to 600 m/z out of curiosity. A rather large peak around 500 m/z was found in all sample spots, with intensities
up to 5000. There was a peak around 500 m/z in the blank, but the intensity was lower than 300 and could be the result of contamination, possibly from not cleaning the plate thoroughly between runs. The peak at 500 m/z could be the actual analyte peak, not 337 m/z. This is plausible if the matrix is still bound to the analyte when it reaches the detector because 499 Da is the mass of THCCOOH (345 Da) plus the mass of DHB (154 Da). The analyte crystalizes in a cluster of matrix; it is plausible that not all of the matrix is desolvated before entering the flight tube. It is also possible that both peaks indicate THCCOOH is present; 337 m/z is THCCOOH alone and 500 m/z is THCCOOH plus DHB.

The intensity did not decrease steadily with concentration, but this is not unexpected. The laser randomly hits spots, and if the sample is not uniformly distributed in the dot, then it is up to chance whether the laser will actually hit any analyte. Some dots may have more clusters of sample and other dots may have more uniform distribution. Nothing was found in the literature to determine if THCCOOH would react with DHB in methanol. In an attempt to determine if it did react, the sample and matrix solution was analyzed using GC-MS but nothing was seen except methanol. The column was changed hoping to aid in analysis; there was an error when tuning the instrument, and no further analysis was performed at that time.

1.6 Calibration of THCCOOH with Caffeine as Internal Standard

The next set of calibrators was spiked with 10mmol of caffeine to normalize the peaks to help with quantitation. The m/z of caffeine on MALDI-TOF-MS is known. It was analyzed without matrix, just caffeine standard and methanol, to find its exact location without noise from matrix peaks. To reduce spreading, 5 μl of
water was spotted before adding 7.5 μl of calibrator. The spots were left to dry, sprayed with 40 ml of DHB matrix, and analyzed as before. The internal standard did not help with quantitation.

Figures 20-22 show the same target plate, prepared and run by Sarah Glenn, but with different mass filters. A variety of samples were tested on this plate. Caffeine in CHCA matrix, THCCOOH and caffeine with no matrix, DHB matrix, caffeine in DHB matrix, and THCCOOH spiked with caffeine in DHB matrix. The matrix was mixed with the sample prior to pipetting onto the target plate. THCCOOH and caffeine were analyzed with no matrix to show that there is no ionization without the matrix. Caffeine was run with CHCA to see if CHCA was a suitable matrix for caffeine analysis; it is not (Figure 20, red box). DHB matrix was run alone as a blank. It showed no interference at 190 m/z or 496 m/z (Figures 20, 22); the signal at 336 m/z is likely the result of contamination from previous analyses or sample spreading (Figure 21, blue box). Caffeine in DHB matrix showed high intensity signals at the two highest concentrations (10 mM and 1mM) and no signal in lower concentrations (Figure 20, yellow box). THCCOOH spiked with caffeine showed signals at all mass filters (Figures 20-22, purple box). Figure 20 shows caffeine, and figures 21 and 22 show THCCOOH.

1.7 Imaging of Caffeine in Fingerprints (Trial 1)

After the method and instrument parameters were fully tested and perfected with the calibration standards, the research shifted toward fingerprints. The first focus was to detect caffeine in fingerprints, to first prove that the method can be applied to fingerprints before moving on to illicit drugs. Illicit drugs were not used
because they require a license from the Drug Enforcement Administration (DEA); low concentrations of caffeine do not require a license. Caffeine was chosen because it has been thoroughly studied and can be found in a variety of food and drink products.

Four fingerprints from three volunteers were rolled onto the MTP 384 target plate. The volunteer touched their face to collect oil to ensure there was enough print residue transferred onto the plate. The plate was sprayed with 40 ml of DHB matrix and then imaged using flexImaging software. The center of the print was selected for analysis. Imaging the entire print was not time-effective, as it would have taken over two days for the instrument to complete the analysis. A mass filter of 190 Da was applied to the image (Figure 23). The red in the image shows each spot where the spectrum contained a peak at 190 m/z. The intensity of the color correlates to the intensity of the peak; brighter red indicates a higher concentration of caffeine. The print on the top right contains the highest amount of caffeine, corroborated by the volunteer’s recent caffeine intake. The print on the top left contains a medium amount of caffeine; the volunteer had a high, steady caffeine intake over the past 24 hours. The two prints on the bottom are from the same volunteer; the volunteer maintains a low, steady caffeine intake. The same plate was run a second time to study the effects of aging on the prints out of curiosity (Figure 24). The brightness of the red caffeine spots decreased for all the prints, indicating that there was some loss of caffeine. This may be caused by degradation of the matrix or loss of caffeine from the previous analysis; further research is needed to better understand the decrease in signal.
1.8 Imaging of Caffeine in Fingerprints (Trial 2)

Next, prints from volunteers who recently ingested caffeine (within 12 hours), have a constant caffeine intake, and have not knowingly had caffeine within the last 24 hours were pressed onto a target plate. Caffeine calibration standards were also spotted onto the plate in order to compare the spot brightness to estimate caffeine concentration in the fingerprints. Figure 25 is the image of the fingerprints with the calibration standards. The results from this test are inconclusive as the instrument aborted the run at the top of the second fingerprint. The analysis was set to run over the weekend, and the problem was not discovered until Monday morning. It was not set to run again because of sample degradation; the plate was ejected from the instrument and exposed to the air, which can degrade the matrix. As seen in the previous test, old matrix does not produce good results. The partial results showed a small intensity of caffeine in the fingerprint of the volunteer who did not have caffeine. This shows that caffeine is a common compound in a variety of foods and drinks and can go unnoticed by consumers.

1.9 Imaging of Caffeine in Fingerprints, Saliva, and Sweat

To further confirm that the fingerprints contain caffeine, sweat, saliva, and fingerprint residue from two volunteers were tested along with pressed fingerprints on MALDI-MS, GC-MS, and LC-MS. 1 ml of saliva was collected in microcentrifuge tubes and then centrifuged for 10 minutes at 10,000xg to precipitate any large molecules. 600 µl of the supernatant was filtered using HPLC filters and mixed with 1 ml of methanol. Sweat was collected by dabbing the volunteers’ foreheads and neck with a clean cotton swab. The cotton swabs were soaked in 1 ml of methanol
for approximately 30 minutes. To collect fingerprint residue, the volunteers washed their hands with water only, and let them air dry. Then, a cotton swab was passed over their hands for approximately 3 minutes. The cotton swabs were soaked in methanol for 30 minutes. The target plate was prepared by spotting caffeine calibrators, saliva, sweat, and fingerprint residue. The volunteers also pressed a fingerprint directly onto the plate. The plate was sprayed with 40 ml of DHB matrix and imaged.

All samples contained detectable levels of caffeine. The calibration standards were included as a reference to estimate the concentration of caffeine since it was previously determined that quantitation could not be accomplished using our methods. Figure 26 shows the intensity of the peak at 190 Da as a multi-colored gradient. Saliva showed the highest intensity, sweat showed a medium intensity, and the fingerprint residue showed the least. The saliva on the left contained more caffeine than the sample on the right; the volunteer had a few sips of a caffeinated beverage that morning, approximately 2 hours before sample collection. Volunteer 2 also had a caffeinated beverage that morning, but it was consumed 3-4 hours prior to analysis; this is why the intensity in the second saliva sample is lower than saliva sample 1. A probable hypothesis is that some caffeine was still in the mouth and was released in the saliva that was collected. The intensity of the caffeine signal in the pressed fingerprints was highest around the edges. It appears that the print on the left was not fully circled in preparing the picture for imaging; there is a bright spot on the top right that suggests that there is a cluster of caffeine on the section that was omitted. The print was difficult to see, and its location was estimated from
noting the columns and rows where it was pressed. It also appears that not all of the biological samples were selected for analysis. The samples spread when they were pipetted onto the plate, and they were difficult to see when dried.

The area in between the two pressed prints is a matrix blank; there is no sample there, only matrix. There is a very low intensity signal at 190 Da in the blank. This can be attributed to noise in the system or residual caffeine on the target plate from previous runs. Prior to testing any samples, the matrix alone was analyzed to determine where the matrix peaks are in the spectrum. There were no peaks at or around 190 Da that would cause interference with caffeine. Future testing would include a more thorough wash step after analysis to prevent contamination. The spot used as the matrix blank can also be swabbed with a cotton swab with methanol and then water immediately before spraying with matrix to ensure that no contamination occurred while preparing the samples on the target plate.

1.10 Limitations

Only a few prints were tested at one time because of instrument and time constraints as well as the fact that the purpose of this research is to develop a method and not test a large volume of samples. The MALDI-MS can only accommodate one target plate at a time, and each target plate can only fit approximately six medium fingerprints or three prints plus calibration standards. The analysis time for a set of four fingerprints and calibration standards can take as long as 30 hours, depending on the size of the prints.
2. LC-MS

The THCCOOH standards spiked with caffeine and saliva, sweat, and fingerprint residue were also analyzed using LC-MS to back-up the results obtained from MALDI-MS. First, the calibration standards were run using a general mobile phase gradient in order to get an idea of where the analytes elute from the column. After multiple adjustments to the gradient, a final method was selected and used to analyze the samples. The gradient for the calibration standards had a longer nonpolar section at the end to elute the THCCOOH from the column. This longer gradient was not necessary for the biological samples since they did not contain THCCOOH. The mass spectrometer was run in two modes: total ion chromatogram (TIC) and single ion monitoring (SIM). The ions monitored were 194.14 (caffeine) and 344.45 (THCCOOH).

Figure 27 shows the overlay of the SIM of 194 and 344 for the calibration standards. Figure 28 shows a close-up of the caffeine peak, with a retention time of 2.06. The blue peak is not aligned with the others because it was run by itself prior to the others to test the method. There is no decrease in the peak height because the concentration remained constant for all samples. Figure 29 shows a close-up of the THCCOOH peak with a retention time of 13.2. There is a decrease in peak area with concentration. A calibration curve of concentration versus peak area showed linearity of 0.98 for both the SIM and TIC (Figure 30). An additional calibration curve of THCCOOH peak area as a ratio to the peak area of caffeine also shows linearity of 0.99 for the SIM and 0.98 for the TIC (Figure 31). The chromatograms for the calibrators were much cleaner than the chromatograms of the biological fluids.
It was important to run the mass spectrometer in the SIM mode because there was no clear peak at the time when caffeine eluted from the column. Because of the complexity of the matrices and the low concentration of analyte relative to the other compounds, the caffeine peak was very low and indistinguishable from the baseline. The intensity of the peak of 194 in the SIM spectra is high; it is just extremely low compared to the other masses. Figure 34 shows the overlay of the SIM spectra of the saliva samples. The caffeine peak is distinct because the concentration is relatively high, as shown in the MALDI-MS image (Figure 26). Figure 33 shows the SIM overlay of the sweat samples. The caffeine is not as prominent because the concentration is lower. Figure 34 shows the SIM overlay of the fingerprint residue. The shape of the spectra of the sweat and fingerprint residue is very similar. This is because sweat is a major contributor to fingerprint residue, as discussed in the introduction. In future work, the analyte could be extracted from the biological matrix prior to analysis to remove the compounds that are masking the analyte peak.

One problem encountered during analysis was the presence of peaks at 344 in the samples, mostly in the fingerprint residue. The volunteers have never smoked marijuana. This peak can potentially be residual THCCOOH from the calibrators, a fragment of a larger molecule, or a naturally occurring compound. THCCOOH from the calibrators may have gotten stuck on the column and eluted with compounds from the biological samples. The samples were run twice, once with the calibrators and once without. The 344 Da peaks were still seen in the second run. If the 344 Da peak is THCCOOH eluting from previous runs or if it is a fragment from a larger
molecule, it would not be a problem for MALDI-MS analysis. Compounds do not fragment because the matrix absorbs the energy that would cause fragmentation; instead, the compounds are ionized either positively or negatively. The least favorable cause for a peak at 344 Da is that there is a compound in fingerprints with that mass. A naturally occurring compound in fingerprint residue with a mass of 344 Da would pose a problem when looking for THCCOOH since it is the same mass.

3. GC-MS

Due to technical difficulties, the samples were not run on GC-MS. The GC-MS on-site was still out of use. After changing the column, the instrument repeatedly failed to tune. The computer for the off-site GC-MS of ElSohly Laboratories crashed during the sequence in which the samples were set to run. The computer was repaired, but the GC-MS needed to be cleaned. The instrument was not ready in time to have the samples analyzed by the deadline.

4. UV-Vis

The purpose of analyzing the THCCOOH samples by UV-Vis spectroscopy was to find the wavelength at which THCCOOH absorbs light and attempt to quantify the amount of THCCOOH based on its absorbance. In the early stages of method development, HPLC with a UV-Vis detector was going to be used to support the results; an LC-MS system became available and was used instead. The wavelength of absorbance of THCCOOH was unknown so experimentation was done to determine it. It was found that THCCOOH absorbs light at 287 nm. The range of concentrations of THCCOOH that were measured was 75 μM to 32 mM. This corresponds to 78 μl of standard to 338 μl of standard in 3 ml of methanol. Measurements started by adding
2 µl of standard at a time; the volume was then increased to 10 µl and then to 20 µl. The plot of absorbance versus concentration showed linearity of 0.99 (Figure 35).

V. CONCLUSION

With the technology available, MALDI-TOF-MS is not suitable for quantitative analysis, only qualitative analysis. This research showed proof of the principle that MALDI-TOF-MS can be used to detect compounds in fingerprints. Caffeine was successfully found in fingerprints and other biological samples using MALDI-TOF-MS. These results were supported by analysis of the same samples using LC-MS. To further refine this method, more matrices need to be tested. Using a matrix with a higher molecular mass can potentially eliminate the low mass interferences. Additionally, more drugs, illicit and non illicit, should be tested to expand the applications of this method.

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Figure 1: Metabolism of tetrahydrocannabinol by cytochrome p450 enzymes. 11-OH-THC is excreted within hours of marijuana consumption. 11-nor-9-carboxy THC (THCCOOH) is excreted within days to weeks of marijuana consumption. This is the metabolite targeted by drug testing methods. The glucuronide group, which is added to a small portion of THCCOOH, aids in solubility in water for excretion through urine and sweat (21).
Figure 2: MALDI-TOF-MS schematic with a linear flight path. The laser ablates the sample. The ground grid gives all ions the same kinetic energy. The ions travel through the flight tube to the detector based on their mass. Light ions travel faster than heavier ions (23).
Figure 3: LC-MS Schematic of binary pump system with a mass detector. The solvents are pumped into the column according to the programmed gradient, which is based on the polarity of the sample. The sample is injected into the column. Analytes elute at characteristic times and enter the detector. A quadrupole mass analyzer was used as the detector in this study (24).
Figure 4: Quadrupole mass analyzer schematic. Four metal rods have an oscillating radiofrequency (RF) voltage and constant direct current (DC) voltage across them. The ion beam passes in between the four rods. Ions of different mass-to-charge ratios (m/z) are separated by changing the electric potential of the RF and DC voltages. Ions that resonate with the RF voltage are sent to the detector. Ions that do not resonate collide with the rod or pass between the rods and go undetected (16).
Figure 5: GC-MS schematic with quadrupole mass analyzer. The sample is injected into the heated column through the heated injection port. The analytes interact with the column and elute at characteristic times into the quadrupole mass analyzer (25).
Figure 6: Electronic transitions during absorption of UV-Vis light. The blue arrows represent the increase in energy level(s) when a bond absorbs ultraviolet or visible light energy (20).
Figure 7: UV-Vis spectrometer schematic with reference and sample cuvettes. The light beam originates at the top of the figure. It passes through a filter that selects the wavelength. The beam is split into two; one beam passes through the reference cuvette, and the other passes through the sample cuvette. The signal from the reference cuvette is subtracted from the signal from the sample cuvette to give the net absorbance or intensity (18).
Figure 8: CHCA mass spectrum contains peaks in the mass range of THCCOOH. There are high intensity matrix peaks in the range of 300 – 400 m/z. Since the mass of THCCOOH is 345, CHCA is not a suitable matrix because of the interfering masses.
Figure 9: Mass spectrum of DHB shows matrix peaks from 120-200 m/z and at 280 m/z. There is some noise in parts of the spectrum, but it is not intense enough to interfere with sample analysis. Because of the lack of interference between 300 and 400 m/z, DHB was used for all subsequent analyses.
Figure 10: Magnification of DHB mass spectrum from 300-400 m/z shows very low noise (intensity below 10). The boxed area is where the analyte peak was seen in subsequent analyses of THCCOOH.
Figure 11: Magnification of DHB mass spectrum from 400-500 m/z shows very low noise (intensity below 3). The boxed area is where another possible analyte peak was seen in later analyses. This peak is not confirmed to be THCCOOH and needs further examination.
Figure 12: Mass spectrum of 500 µg/ml THCCOOH with DHB with a prominent peak at 336 m/z and a slight increase at 496 m/z. The peak at 336 m/z is THCCOOH. The significance of the peak at 496 m/z was discovered in later runs when the mass range was limited to 280 – 600 m/z. Since the high intensity matrix peaks between 100 and 200 m/z were removed, it allowed the peak at 496 m/z to be seen more clearly. It was seen in the DHB blank at a low intensity, but it was present in the samples of THCCOOH with a much higher intensity. It is suspected to be THCCOOH and DHB that are being detected together.
Figure 13: Magnification of 300-400 m/z of mass spectrum of 500 μg/ml THCCOOH with DHB matrix. Each colored line represents the average of 1000 mass spectra of a spot with an area of 200 microns. Each calibration standard sample contains about 200 measurement spots. This spectrum shows all 200 measurements for 500 μg/ml THCCOOH. The wide range of intensity is caused by the uneven distribution of analyte in the measurement spots.
Figure 14: Magnification of 400-500 m/z of mass spectrum of 500 μg/ml THCCOOH with DHB. Each colored line represents the average of 1000 mass spectra of a spot with an area of 200 microns. Each calibration standard sample contains about 200 measurement spots. This spectrum shows all 200 measurements for 500 μg/ml THCCOOH. The wide range of intensity is caused by the uneven distribution of analyte in the measurement spots.
Figure 15: Mass spectrum of 0.125 μg/ml THCCOOH with DHB matrix with decreased peaks at 336 m/z and 496 m/z. The concentration of THCCOOH is below the limit of detection for the instrument. Calibration standards below this concentration were not used in further runs.
Figure 16: Magnification of 300-400 m/z of mass spectrum of 0.125 μg/ml THCCOOH with DHB. There is no analyte peak at 336 m/z. The amount of THCCOOH in the sample is too low for the instrument to measure.
Figure 17: Magnification of 400-500 m/z of mass spectrum of 0.125 μg/ml THCCOOH with DHB. The intensity of the peak at 496 m/z is similar to that of the blank.
Figure 18: 10 mM caffeine with laser grid. This illustrates the uneven distribution of caffeine caused by spotting matrix with sample. This method of mixing the matrix with the sample and then pipetting it onto the plate was used in all attempts of calibration. The average intensities at 336 m/z of all of the concentrations used to create the calibration curve were around the same. This is caused by the extreme high concentrations (red and pink spots) and extreme low concentrations (dark blue). The grey spots contain no sample; it is the metal target plate. This phenomenon was not seen until focus of the research had moved to fingerprints and caffeine. The imaging software was not working for the month and a half during which the calibration was being done.
Figure 19: 10 mM caffeine with laser grid. There distribution of caffeine with spray matrix is much more even than the distribution for the spotting method. Due to time, calibration and quantitation of THCCOOH was not attempted with this method. This will be studied more thoroughly in future work. Only two of the ten caffeine standards used were above the limit of detection; this is not enough to make a calibration curve.
Figure 20: 190 m/z filter of target plate spotted with a variety of samples. This plate was run immediately after the imaging software was fixed. It was run to get an image of everything together. The run stopped by itself after the first four THCCOOH samples. The sequence order was: caffeine in CHCA, THCCOOH and caffeine with no matrix, DHB matrix, caffeine in DHB, and THCCOOH in DHB. There is no ionization of the samples without matrix (green box). Only two concentrations of caffeine (10 mmol and 1 mmol) are above the limit of detection. This is when the uneven distribution caused by spotting samples was discovered.
Figure 21: 336 m/z filter of the same target plate from Figure 20. This plate was run immediately after the imaging software was fixed. It was run to get an image of everything together. The run stopped by itself after the first four THCCOOH samples. The sequence order was: caffeine in CHCA, THCCOOH and caffeine with no matrix, DHB matrix, caffeine in DHB, and THCCOOH in DHB. There is no ionization of the samples without matrix (green box). THCCOOH can be seen in the four spots that were analyzed in the purple box. By visual comparison, it appears that the intensity of the THCCOOH peak decreases with concentration. There is less and less red and yellow in the lower concentrations.
Figure 22: 496 m/z filter of the same target plate from Figures 20 and 21. This plate was run immediately after the imaging software was fixed. It was run to get an image of everything together. The run stopped by itself after the first four THCCOOH samples. The sequence order was: caffeine in CHCA, THCCOOH and caffeine with no matrix, DHB matrix, caffeine in DHB, and THCCOOH in DHB. The potential significance of the peak at 496 m/z had been discovered prior to this run. There is some signal in the THCCOOH purple box at this mass.
Figure 23: Image of fingerprints with varying caffeine levels and 10 mM caffeine standard with 190 m/z filter. The four fingerprints are from three volunteers (both low, steady caffeine prints are from two fingers of one volunteer). The brightness of the red corresponds to the intensity of caffeine in the print. The brightest red of the recent caffeine spike means that that print contained the most caffeine. The low, steady caffeine prints contained the least amount of caffeine. The intensity of the caffeine standard is between the intensity of the high, steady and the low, steady prints.
Figure 24: Image of second run (two days later) of samples from Figure 22 with 190 m/z filter. There is an overall decrease in the intensity of caffeine. There are two probable explanations for this. First, the first analysis destroyed some of the sample when the laser ablated it; there was a less analyte to work with for the second analysis. Second, the matrix degraded and resulted in a weaker signal. For all other analyses, the matrix was made just before application to the plate; fresh matrix equals good signals.
Figure 25: Image of incomplete analysis of caffeine calibration standards and fingerprints with 190 m/z filter. An unknown instrumental error caused the run to abort. The plate was set to run on a Friday afternoon, and the error was not discovered until Monday morning. The plate had been sitting in the open air for two days, and it was not set to run again because of the degradation of the matrix. Consistent with previous data, only the two highest caffeine standards are measurable. The print of the volunteer who claimed to not have knowingly ingested caffeine shows a caffeine signal because caffeine is a natural ingredient in a variety of products. The partial image of the low, steady caffeine print shows intensity higher than the print that was thought to contain no caffeine.
Figure 26: Image of caffeine calibration standards, saliva, sweat, fingerprint residue, latent fingerprint, and blank matrix spot with 190 m/z filter. There is a high intensity of caffeine in the saliva due to residual caffeine from recent caffeinated beverage consumption. Volunteer 1 consumed caffeine 2 hours prior to sample collection, and volunteer 2 consumed caffeine 4 hours prior to sample collection. Volunteer 2 had begun to metabolize the caffeine; there is a higher caffeine signal in the fingerprint and swear of volunteer 2 than in those of volunteer 1.
Figure 27: Overlay of the LC-MS chromatogram of THCCOOH spiked with caffeine calibrators. Caffeine has a retention time of 2.09 min. THCCOOH has a retention time of 13.2 min. Because THCCOOH is extremely nonpolar, it required a long nonpolar gradient to elute from the column that was determined by many trial-and-error analyses.
Figure 28: Zoom overlay of caffeine. There is a shift in RT of the blue peak because that sample, the highest concentration of THCCOOH, was run separately. It was being used to test the gradient, and because of limited sample volume, it was not run again with the other samples once the gradient was determined. The shift is not significant; the time is still characteristic of caffeine. There is no change in peak height because the concentration of caffeine was kept constant, 10 mmol, for each sample.
Figure 29: Zoom overlay of THCCOOH. There is a shift in RT of the blue peak because that sample, the highest concentration of THCCOOH, was run separately. It was being used to test the gradient, and because of limited sample volume, it was not run again with the other samples once the gradient was determined. The shift is not significant; the time is still characteristic of THCCOOH. The peak area decreased consistently with THCCOOH concentration.
Figure 30: Calibration curve of THCCOOH peak height vs. concentration in both SIM and TIC modes. The peak area for SIM mode is higher because the mass analyzer is only looking for that specific mass; it is not scanning for all masses like in TIC mode.
Figure 31: Calibration curve of THCCOOH with caffeine internal standard for both SIM and TIC modes. There is better precision when using an internal standard to normalize the analyte peak area. The smallest concentration of THCCOOH, 1.72 μM, was excluded because it was out of the range of linearity. The peak had to be manually integrated during data analysis because it was so small; it was near the noise range for the instrument.
Figure 32: Overlay of saliva samples. Volunteer 1 is the blue line, and volunteer 2 is the red line. This is the SIM chromatogram, monitoring for 195 Da and 344 Da. The multiple peaks in the chromatogram illustrate the complex matrix of biological samples. In future work with biological samples, the drug can be extracted from the matrix or derivatized.
Figure 33: Overlay of sweat samples. Volunteer 1 is the blue line, and volunteer 2 is the red line. This is the SIM chromatogram, monitoring for 195 Da and 344 Da. The multiple peaks in the chromatogram illustrate the complex matrix of biological samples. In future work with biological samples, the drug can be extracted from the matrix or derivatized. The shape of the sweat and fingerprint residue chromatograms are extremely similar because fingerprints are mostly sweat.
Figure 34: Overlay of fingerprint residue samples. Volunteer 1 is the blue line, and volunteer 2 is the red line. This is the SIM chromatogram, monitoring for 195 Da and 344 Da. The multiple peaks in the chromatogram illustrate the complex matrix of biological samples. In future work with biological samples, the drug can be extracted from the matrix or derivatized. The shape of the sweat and fingerprint residue chromatograms are extremely similar because fingerprints are mostly sweat.
Figure 35: Calibration curve of THCCOOH for UV-Vis spectroscopy. This calibration curve was created early in this project when the plan was to use HPLC with a UV-Vis detector. However, an LC-MS was made available and used instead.