CRITICAL COMPARISON OF TOTAL VAPORIZATION- SOLID PHASE MICROEXTRACTION VS HEADSPACE- SOLID PHASE MICROEXTRACTION

by

Alexandra Train

A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Forensic and Investigative Sciences at IUPUI

Indianapolis, Indiana

May 2021

THE PURDUE UNIVERSITY GRADUATE SCHOOL

STATEMENT OF COMMITTEE APPROVAL

Dr. John Goodpaster, Chair

Department of Chemistry and Chemical Biology

Dr. Nicholas Manicke

Department of Chemistry and Chemical Biology

Dr. Christine Picard

Department of Biology

Approved by:

Dr. John Goodpaster

For my family, Jacob and myself.

ACKNOWLEDGMENTS

I would like to thank my advisor, John Goodpaster, for his support and guidance throughout my time here at IUPUI and for sharing his expertise with me. I would also like to thank my friends and classmates, Janean Fabino, Caitlyn Estanique, Natalie Sellnau, Tracy Nguyen, Courtney Cruse, and Madi Reavis for their help and friendship on this journey. I lastly would like to thank Kymeri Davis for guiding me in the lab and always being there for any questions I had.

TABLE OF CONTENTS

LIST OF TABLES 7
LIST OF FIGURES 8
ABSTRACT 10
CHAPTER 1. COMPARISON OF TV-SPME VS HS-SPME 11
Introduction 11
Experimental 15
Materials 15
Sample Preparation 15
Essential Oils 15
Marijuana 16
Cannabidiol Oil 17
GC-MS Parameters 17
Results and Discussion 18
Essential Oils 18
Marijuana 27
Cannabidiol Oil 38
Conclusion 42
Essential Oils 42
Marijuana 42
Cannabidiol Oil 43
CHAPTER 2. ANALYSIS OF SYNTHETIC CANNABINOIDS 45
Introduction 45
Methods 47
Materials 47
Sample Preparation 47
GC-MS Parameters 47
Results 48
Conclusions 49

CHAPTER 3. ANALYSIS OF SALIVA 51
Introduction 51
Methods 52
Materials 52
Sample Preparation 53
GC-MS Parameters 53
Results 53
Conclusions 58
REFERENCES 60

LIST OF TABLES

Table 1. Names and structures of compounds found in Essential Oil samples. 22

Table 2. Names and structures of compounds found in marijuana plant samples. 29

Table 3. Names, structures, and purpose of compounds found in CBD Oil samples. 41

Table 4. Names, structures, and purpose of compounds found in Real Saliva samples using TV-SPME GC-MS. 56

LIST OF FIGURES

Figure 1. Depiction of headspace SPME of a liquid sample (A) and of total vaporization SPME of a liquid sample after heating (B). 11

Figure 2. Stacked total ion chromatogram (TIC) of Spearmint Oil (red), Lemongrass Oil (yellow), and Thieves Oil (blue) ran with HS-SPME. 19

Figure 3. Stacked TIC of Spearmint Oil (red), Lemongrass Oil (yellow), and Thieves Oil (blue) ran with TV-SPME. 20

Figure 4. Stacked TIC of Lemongrass Oil ran with HS-SPME (red) and TV-SPME (yellow). 21

Figure 5. Peak area comparison of three main compounds found in lemongrass essential oil with standard deviation error bars. 22

Figure 6. Stacked TIC of marijuana plant material ran at 100 °C (red), 120 °C (yellow) and 140 °C (yellow). 28

Figure 7. Peak Area vs. Extraction Temperature of marijuana plant material to compare CBD, THC, and CBN. 29

Figure 8. Stacked TIC of plant material ran with HS-SPME (red), extracted plant material ran with HS-SPME (yellow) and extracted plant material ran with TV-SPME (blue). 34

Figure 9. Peak area comparison of THC and CBN found in marijuana with standard deviation error bars. 34

Figure 10. Stacked TIC of fresh marijuana plant material ran at 100 °C (red), 120 °C (yellow) and 140 °C (yellow). 35

Figure 11. Peak Area vs. Extraction Temperature of fresh marijuana plant material utilizing the TV-SPME method to compare THC and CBN. 35

Figure 12. Stacked TIC of THC Standard utilizing the HS-SPME method ran at 100 °C (red), 120 °C (yellow) and 140 °C (yellow). 36

Figure 13. Peak Area vs. Extraction Temperature of THC standard utilizing the HS-SPME method to compare THC and CBN. 36

Figure 14. Stacked TIC of THC Standard utilizing the TV-SPME method ran at 100 °C (red), 120 °C (yellow) and 140 °C (yellow). 37

Figure 15. Peak Area vs. Extraction Temperature of THC standard utilizing the TV-SPME method to compare THC and CBN. 37

Figure 16. CBN:THC ratio comparison of fresh marijuana plant material, HS-SPME of THC standard and TV-SPME of THC standard. 38

Figure 17. Stacked TIC of CBD Oil ran with HS-SPME (red), extracted CBD Oil ran with HS-SPME (yellow) and extracted CBD Oil ran with TV-SPME (blue). 39

Figure 18. Stacked extracted ion chromatogram (EIC) of CBD Oil ran with HS-SPME (red) and CBD Oil ran with TV-SPME (yellow). 40

Figure 19. Peak area comparison of CBD found in CBD Oil with standard deviation error bars.40

Figure 20. Molecular structure for 1. FUB-AMB (383.4 g/mol) and 2. 5-fluoro-MDMB-PICA (376.5 g/mol)..... 46

Figure 21. Stacked TIC of FUB-AMB standard (red) and FUB-AMB on potpourri (yellow)..... 48

Figure 22. Stacked TIC of 5-fluoro-MDMB-PICA standard (red) and 5-fluoro-MDMB-PICA on potpourri (yellow). 49

Figure 23. Stacked TIC of real saliva ran with TV-SPME underivatized(red) and real saliva ran with TV-SPME derivatized (yellow). 54

Figure 24. TIC of artificial saliva ran with HS-SPME. 55

Figure 25. Stacked TIC of artificial saliva ran with TV-SPME underivatized(red) and artificial saliva ran with TV-SPME derivatized (yellow)..... 56

ABSTRACT

Solid Phase Microextraction (SPME) is a popular sampling technique that can be paired with Gas Chromatography/Mass Spectrometry (GC-MS). SPME-GC-MS is used in forensic chemistry due to its simplification of the sample preparation process. Headspace-Solid Phase Microextraction (HS-SPME) is a technique where the sample is heated to generate volatiles in the headspace of the vial. A SPME fiber is then inserted into the vial and the compounds in the headspace will bind to the fiber. Total Vaporization- Solid Phase Microextraction (TV-SPME) is a technique that is derived from the HS-SPME technique.

In Chapter 1, the critical comparison of HS-SPME and TV-SPME is discussed. Samples including marijuana, essential oils, and CBD oil were utilized to compare the two techniques. The compounds of interest in marijuana are the three main cannabinoids: cannabinol (CBN), cannabidiol (CBD), and tetrahydrocannabinol (THC). The sample preparation and GC-MS parameters were kept the same for all samples to determine which SPME technique works best for these sample types and yielded the greatest sensitivity. It was found that HS-SPME shows greater sensitivity with CBN and equivalent sensitivity with essential oils, THC and CBD.

In Chapter 2, the detection of synthetic cannabinoids utilizing liquid-liquid injection as well as HS-SPME and TV-SPME is discussed. The detection of these compounds is important because this type of drug has become more prevalent in the United States because they can be chemically altered slightly so they still have the effects of a drug but can evade drug legislation. The detection of synthetic cannabinoids using liquid injection was found to be successful but detection using HS-SPME and TV-SPME was found to be unsuccessful.

In Chapter 3, the analyses of real and artificial saliva utilizing HS-SPME and TV-SPME is discussed. Determining the compounds present in real saliva and artificial saliva will be of importance for future research into determining if the presence of drugs in saliva can be analyzed with these techniques. The analyses of real and artificial saliva were found to be successful using HS-SPME, without derivatization, and TV-SPME, with and without derivatization. Many of the compounds present in the real saliva were detected and were confirmed to be compounds regularly found in saliva by other scientific literature.

CHAPTER 1. COMPARISON OF TV-SPME VS HS-SPME

Introduction

Gas Chromatography-Mass Spectrometry (GC-MS) is considered the "gold standard" of analytical instrumentation when it comes to analysis of controlled substances in forensic science laboratories.[1] However, certain substances can require extended and difficult sample preparation before being able to be analyzed using GC-MS. Headspace- Solid Phase Microextraction (HS-SPME) is a technique where a sample is heated to generate volatiles in the headspace of the vial. A SPME fiber which is coated with a polymeric compound, for example polydimethylsiloxane-divinylbenzene (PDMS/DVB), and then inserted into the headspace of the sample vial and the analytes will bind to the fiber. The fiber will then be placed inside the GC inlet for desorption. In TV-SPME, the same technique is utilized but the volume of the sample is much less so that when the sample is heated, it is completely vaporized before the fiber is introduced into the vial for adsorption onto the fiber.

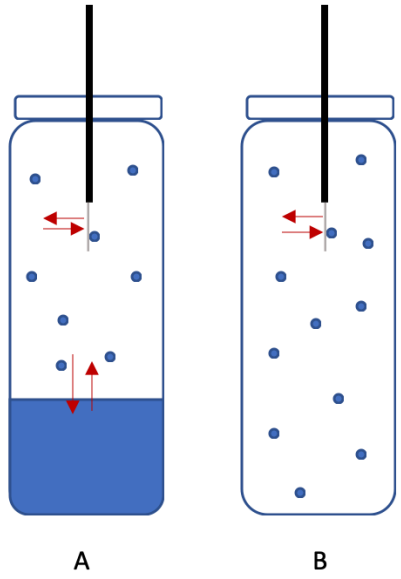


Figure 1. Depiction of headspace SPME of a liquid sample (A) and of total vaporization SPME of a liquid sample after heating (B).

Despite HS-SPME being a popular sampling technique, there is a lack of research investigating the analysis of marijuana plant materials with this technique. Rather *et al.* was the first study to utilizing the rapid analysis of marijuana plant materials to determine the volatile constituents that were present. After the analysis, 17 volatile compounds were identified, making up 94.8% of the total identified compounds in the marijuana samples.[2] Pellati *et al.* also studied volatile compounds in hemp samples using HS-SPME paired with GC-MS. The analysis of the hemp material was able to detect 43 different volatile compounds, most of which were determined to be terpenes.[3] Calvi *et al.* validated and applied a technique using HS-SPME coupled with GC-MS to determine an in-depth profile of all terpenes detected in two varieties of medical grade marijuana and its oil. The study began with an optimization study of SPME fibers, where it was determined that DVB/CAR/PDMS or PDMS/DVB extracted a higher number of terpenes and an optimization study on extraction time, where it was determined that at 60 and 120 minutes had the highest extraction yields. The analysis of these marijuana samples leads to the detection of 109 different terpene compounds. The accurate analysis of terpenes in marijuana is important to determine the terpen profile for a better understanding of the synergistic effect the terpenes will have with cannabinoids.[4] Lachenmeier *et al.* analyzed a variety of hemp food products for the presence of cannabinoids utilizing this technique. They were able to detect THC, CBD, and CBN along with other cannabinoids in all of the tested samples. This group was also able to determine a limit of detection of 0.01 and 0.17 mg/kg and a precision of 0.4 and 11.8% depending on the characteristics of the matrix of the sample. Overall, it was determined that in comparison with liquid injection, HS-SPME is substantially faster and easier to perform while maintaining the same sensitivity and reproducibility.[5]

TV-SPME is a relatively new technique that is being studied and compared with HS-SPME to determine the superior technique. Due to TV-SPME completely vaporizing the sample, there will be a partitioning of the analyte between only the fiber and the vapor, which will force more sample to be adsorbed onto the fiber and relatively large sample volumes can be used (1 µl - 100 µl).[6] There will also be fewer matrix effects when utilizing this technique due to the change from a three phase system, which includes the sample, the vapor, and the fiber (HS-SPME), to a two phase system, which includes the vapor and the fiber.[7] TV-SPME has also been determined to have a greater sensitivity for nicotine and cotinine by Rainey *et al.* Rainey *et al.* also determined an

equation to calculate the amount of sample volume that should be used to ensure total vaporization of the sample.

$$V_0 = \frac{(10^{A-\frac{B}{T}+C})V_V}{\mathrm{RT}}\left(\frac{M}{p}\right) \quad (1)$$

This equation will determine the volume of sample (V_0) that can be completely vaporized where A, B, and C are Antoine constants for the specific solvent that is being used, T is the temperature of extraction, V_v is the volume of the vial, R is the ideal gas constant, M is the molar mass of the solvent, and p is the density of the solvent.[8] Some studies that have used TV-SPME as their extraction technique to pair with GC-MS was Kranz *et al.* to determine thee lipid profiles of blow flies, Sauzier *et al.* to analyze double-based smokeless powder residues, and Bors *et al.* to assist in the mapping of smokeless powder residue on PVC pipe bombs.[9,10,11] TV-SPME has also been used in studies for the detection of illicit drugs in a variety of matrices, such as soft drinks, alcoholic drinks and human urine.[7]

Essential Oils are mixtures of naturally occurring chemical compounds found in plants that are believed to have many health benefits. These compounds are extracted from plants by distillation or solvent extraction, separating these lipophilic compounds from the plant components.[12] Essential Oils have become increasingly popular over the years as people begin searching for natural alternatives for things such as pharmaceuticals, cosmetic/hygiene ingredients and artificial flavorings.[13] Although the most popular uses of essential oils are as alternative medicines and flavorings, another use that has been showing promise is the use of essential oils as natural pesticides and food preservatives to decrease the harmful effects of the non-natural/unhealthy pesticides and preservatives that are being used now.[14,15] A major ingredient of essential oils are the terpene/terpenoid compounds which are the compounds that give the essential oils their fragrance and flavors. Terpenes are relatively simple hydrocarbons and terpenoids are more complex hydrocarbons containing functional groups.[16,17] GC-MS is a commonly used method for the analysis of essential oils for the presence of the terpene/terpenoid compounds present due to their volatility.

Marijuana is the dried leaves, stems, and seeds from the *Cannabis indica* or *Cannabis sativa* plant. It is a Schedule I drug and the most commonly used illicit drug in the United States.[18] People can take marijuana by smoking, eating, or drinking it. Marijuana is made up of about 544

different compounds, 113 of which are cannabinoids and 120 are terpenes.[19,20] Cannabinoids are a specific class of compounds that have only been found in these cannabis plants. They are the compounds that are responsible for the biological activities of the cannabis plant.[21] The three most abundant cannabinoids in marijuana are cannabinol (CBN), cannabidiol (CBD), and tetrahydrocannabinol (THC).[22] THC is the main psychoactive substance found in marijuana, which can have many effects on the human body with analgesic, anti-inflammatory, appetite stimulant, and antiemetic properties.[23] CBN has been found to have a sedative effect and CBD has been found to balance out the euphoric effects of THC, as well as having its own effects on the human body such as anxiety reduction, anti-cancer, anti-diabetic, and antipsychotic effects.[24,25] The terpenes found in marijuana give it a distinct fragrance, but they have also been found to have a synergistic effect with the cannabinoids in marijuana to increase its original effects. [26] The common sample preparation of marijuana plant material is liquid-liquid extraction and GC-MS is the commonly used method in forensic laboratories for the analysis of marijuana plant material for the presence of THC.

Cannabidiol Oil, also known as CBD Oil, is a product that has become popular throughout the world in the past few years. CBD Oil is sold to treat/manage symptoms of many different medical conditions, from anxiety to cancer.[27] CBD oil is similar to essential oils, in that it is a concentrated extract of the cannabis leaves and/or flowers mixed with some type of edible oil.[28] CBD oils can also contain other cannabinoids such as THC or cannabigerol (CBG) as well as terpenes depending on the plant it is derived from.[29] The legal status of CBD Oil in the United States is complicated due to the fact that THC, which an illegal substance, can be extracted into the CBD oil due to its presence in the plant. CBD oil is sold legally in all 50 states as long as it has less than 0.3% THC.[30] GC-MS is a commonly used method for the analysis of CBD oils for the presence of CBD, THC, and other cannabinoids.

The critical comparison of HS-SPME and TV-SPME is important to determine if there is a superior method or to see which method works best with specific samples. Both of these methods require little to no sample preparation in comparison to liquid injection, the sample can be put straight into the headspace vial rather than having to do a separate extraction technique to the sample before being injected into the GC. TV-SPME will have little matrix effects as a result of fully vaporizing the analyte and its matrix when compared to HS-SPME where matrix effects will result between the two phases.[8] TV-SPME also utilizes smaller sample sizes than HS-SPME which

can be beneficial when a laboratory only receives a small amount of the sample. TV-SPME will utilize about 1 µl - 100 µl of sample whereas HS-SPME utilizes at least 1mL. Using a quantitative comparison will help determine which of these techniques is recommended for testing essential oil, marijuana, and CBD oil samples.

Experimental

Materials

Peppermint Jim Spearmint essential oil, dōTERRA lemongrass essential oil, and young living thieves' essential oil were obtained from Norma Keywan (Healthy Living Massage, Zionsville IN). Methanol was purchased from Sigma-Aldrich (Saint Louis, Missouri). Marijuana was obtained from The Indiana State Police (DEA License Number: RI0320262, State of Indiana License Number: 61100499B) Tetrahydrocannabidiol was purchased from Cayman Chemical (Ann Arbor, Michigan). Acetonitrile was purchased from Thermo Fisher Scientific (Waltham, Massachusetts). Charlotte's Web Chocolate Mint CBD Oil was obtained from Donna Roskowski, Academic Specialist at IUPUI. Dichloromethane was purchased from Sigma-Aldrich (Saint Louis, Missouri). Polydimethylsiloxane- divinylbenzene (PDMS-DVB) SPME fibers, 65 µm film thickness, were purchased from Supelco (Bellefonte, Pennsylvania).

Sample Preparation

Essential Oils

Qualitative analysis was carried out by utilizing empty essential oil bottles. These essential oils were analyzed by HS-SPME by using a cotton swab to swab the inside of each bottle, lemongrass, thieves, or spearmint respectively. The swabs were then placed into 20 mL headspace vials. These essential oils were analyzed by TV-SPME by adding 1 mL of methanol to each of the empty vials of essential oil, the bottle was vortexed for 1 minute and then extracting the solution form the bottle. 86 µl of each of these solutions were added to 20 mL headspace vials. All samples were heated to 60 °C inside of a Gerstel agitator. The fiber was exposed to the sample vial for 10

minutes before being inserted into the GC inlet for desorption at 250 °C. The samples were analyzed with a 15:1 split.

The lemongrass essential oil was diluted in methanol with a concentration of 1mg/ml. This solution was analyzed using TV-SPME by placing 86 µl of the sample into three 20 mL headspace vials. This solution was analyzed using HS-SPME by placing 86 µl of the sample into three 20 mL headspace vials and allowing the methanol to evaporate off in the hood for 60 minutes before closing the vial. All samples were heated to 60 °C inside of a Gerstel agitator. The fiber was exposed to the sample vial for 10 minutes before being inserted into the GC inlet for desorption at 250 °C. The lemongrass was analyzed with a 15:1 split.

Marijuana

Marijuana plant material was grinded using a mortar and pestle and then 3 mg of the marijuana was placed into five 20 mL headspace vials. Each sample was heated to 100 °C, 110 °C, 120 °C, 130 °C, and 140 °C, respectively, inside of a Gerstel agitator. The fiber was exposed to the sample for 30 minutes before being inserted into the GC inlet for desorption at 250 °C. The plant material was analyzed with a splitless injection. A second 3 mg sample was analyzed at 140 °C using the same sample preparation and analysis except this sample was analyzed with a 34.9:1 split.

Three milligrams of marijuana plant material were put into a scintillation vial with 3 mL of methanol, which was then vortexed for 1 minute. The liquid solution was then extracted to be separated from any plant material. This solution was analyzed using TV-SPME by placing 86 µl of the sample into three 20 mL headspace vials. This solution was analyzed using HS-SPME by placing 86 µl of the sample into three 20 mL headspace vials and allowing the methanol to evaporate off in the hood for 60 minutes before closing the vial. Both samples were heated to 140 °C inside of a Gerstel agitator. The fiber was exposed to the sample vial for 30 minutes before being inserted into the GC inlet for desorption at 250 °C. The extracted marijuana was analyzed with a splitless injection.

The THC standard was diluted in acetonitrile with a concentration of 1mg/mL. This solution was analyzed using TV-SPME by placing 57 ul, 73 ul, 92 ul, 114 ul, or 140 ul into 20 mL headspace vials. This solution was analyzed using HS-SPME by placing 57 ul, 73 ul, 92 ul, 114 ul, or 140 ul into 20 mL headspace vials and allowing the acetonitrile to evaporate off in the hood

for 120 minutes before closing the vial. Each sample was heated to 100 °C, 110 °C, 120°C, 130°C, and 140°C respectively inside of a Gerstel agitator. The fiber was exposed to the sample vial for 30 minutes before being inserted into the GC inlet for desorption at 250 °C. The THC standard samples were analyzed with a splitless injection.

Fresher marijuana plant material was obtained, and samples were prepared and analyzed the same way as the other plant material analyzed in this chapter.

Cannabidiol Oil

One milliliter of CBD Oil was put into a 20 mL headspace vial. The sample was heated to 140 °C inside of a Gerstel agitator. The fiber was exposed to the sample vial for 30 minutes before being inserted into the GC inlet for desorption at 250 °C. The CBD Oil was analyzed with a splitless injection.

CBD oil was diluted in dichloromethane with a concentration of 1mg/ml. This solution was analyzed using TV-SPME by placing 200 µl of the sample into three 20 mL headspace vials. This solution was analyzed using HS-SPME by placing 200 µl of the sample into three 20 mL headspace vials and allowing the dichloromethane to evaporate off in the hood for 60 minutes before closing the vial. All samples were heated to 60 °C inside of a Gerstel agitator. The fiber was exposed to the sample vial for 30 minutes before being inserted into the GC inlet for desorption at 250 °C. The CBD oil samples were analyzed with a splitless injection.

GC-MS Parameters

An Agilent 6890N gas chromatograph with a Gerstel MPS autosampler coupled to an Agilent 5975 mass spectrometer was used for all experiments. The column was an Agilent Technologies DB-5MS column that was 30 m long with a 250 µm inner diameter of and a film thickness of 0.25 µm.

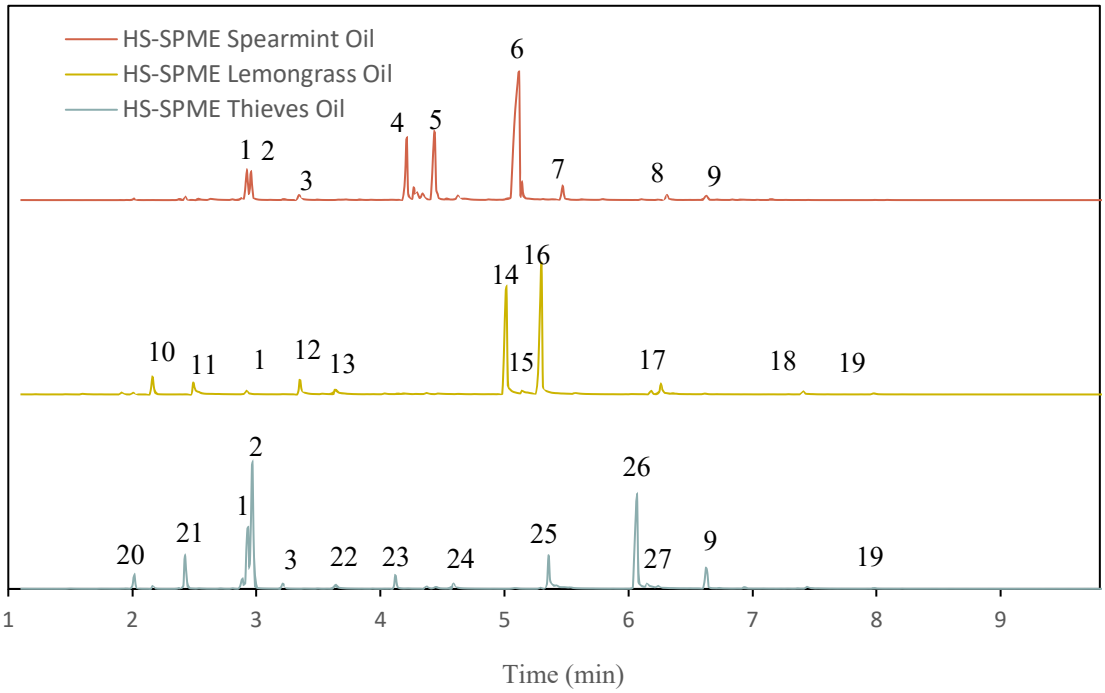
The initial oven temperature was 60 °C, held for 1 minute. The temperature then ramped to 250 °C at a 15 °C /min ramp and then held for 1 minute at the final temperature for all essential oil samples and ramped to 300 °C at a 15 °C /min ramp and then held for 1 minute at the final temperature for all the marijuana and CBD oil samples. The mass transfer line was set to 250 °C and the flow rate was 2.5 mL/min for all samples except the HS-SPME essential oil samples in the

qualitative analysis which had a flow rate of 3.4 mL/min. The source was held at 230 °C and the quadrupoles were held at 150 °C. The mass range scanned was 40 – 550 m/z. Total ion chromatograms (TIC) were produced, and all compounds were identified using the NIST and/or SWGDRUG libraries. Extracted Ion Chromatograms (EIC) were produced at m/z 231.1 for the CBD oil samples to identify cannabidiol.

Results and Discussion

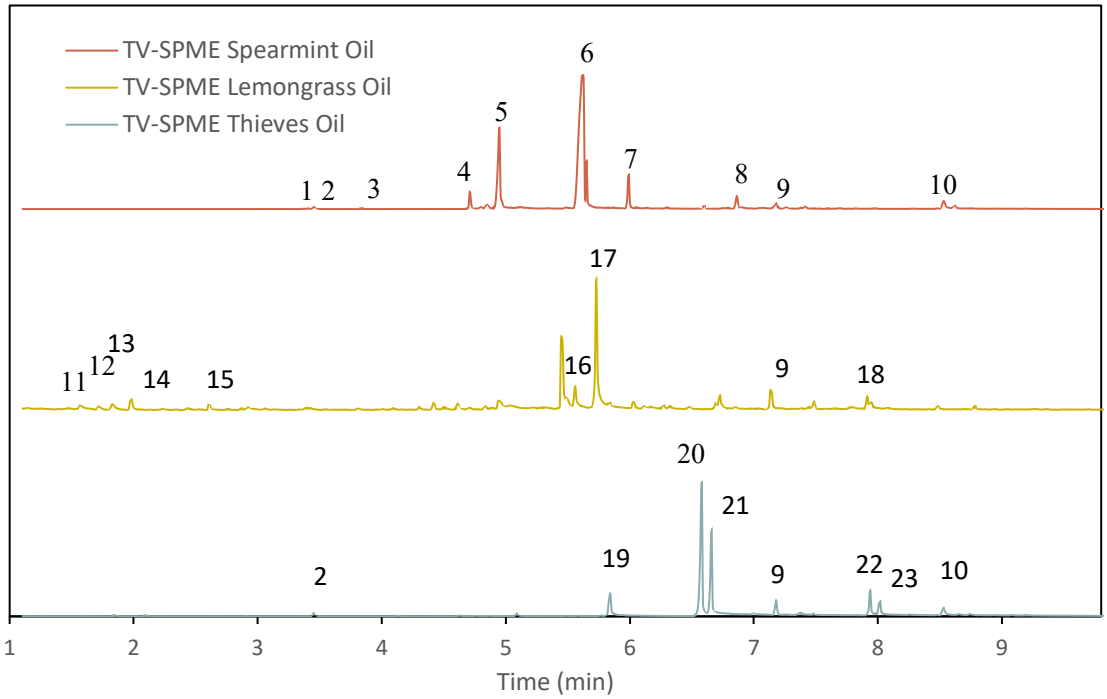
Essential Oils

The stacked HS-SPME chromatograms for Spearmint oil, Lemongrass oil, and Thieves oil are shown in Figure 2 and the stacked TV-SPME chromatograms for these oils are shown in Figure 3. The quantitative comparison of HS-SPME and TV-SPME for Lemongrass oil chromatograms are shown in Figure 4. The corresponding peak area comparison bar graph is shown in Figure 5.



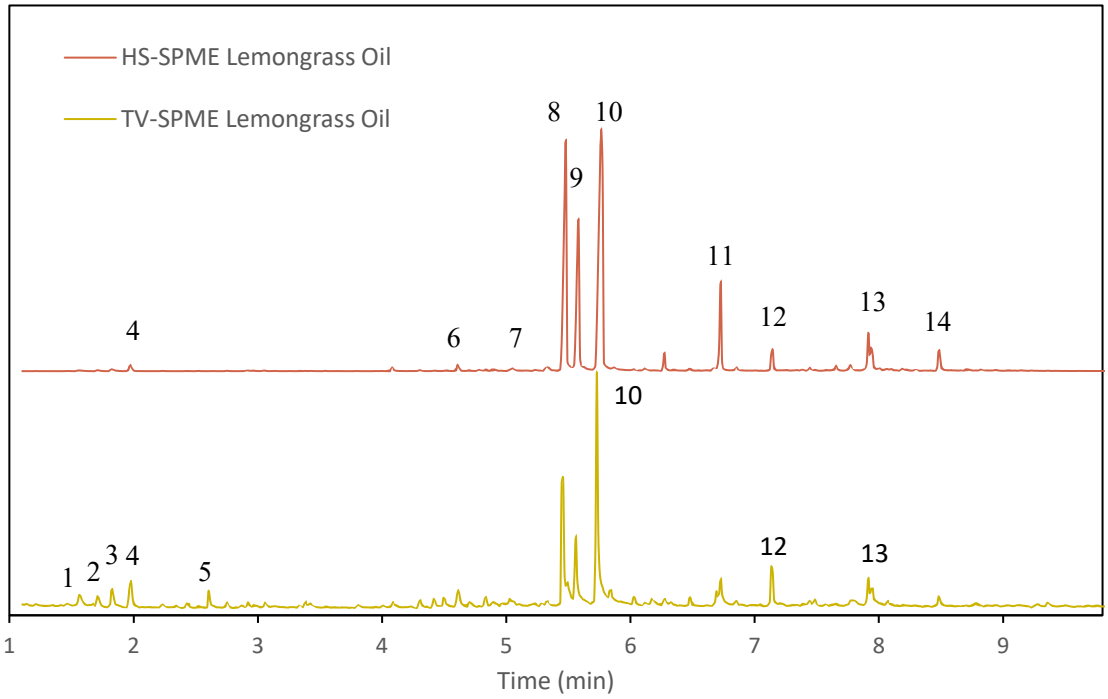
1. D-limonene
2. Eucalyptol
3. Gamma terpinene
4. L-menthone
5. Menthol
6. D-Carvone
7. 3-p-menthene
8. Beta bourbonene
9. Caryophyllene
10. Camphene
11. Sulcatone
12. 4-nonanone
13. Linalool
14. Beta-citral
15. Beta-myrcene
16. Alpha-Citral
17. Neryl-2-methylbutanoate
18. Gamma muurolene
19. Alloaromadendrene
20. Alpha Pinene
21. Beta pinene
22. Geranyl isobutyrate
23. Camphor
24. Alpha-terpineol
25. Cinnamaldehyde
26. Eugenol
27. Dihydroeugenol

Figure 2. Stacked total ion chromatogram (TIC) of Spearmint Oil (red), Lemongrass Oil (yellow), and Thieves Oil (blue) ran with HS-SPME.



1. D-limonene
2. Eucalyptol
3. Gamma terpinene
4. L-menthone
5. Menthol
6. D-Carvone
7. 3-p-menthene
8. Beta bourbonene
9. Alpha-Citral
10. Gamma muurolene
11. Cinnamaldehyde
12. Eugenol
13. Dihydroeugenol
14. Eugenol Acetate
15. Ethylvanillin
16. Caryophyllene
17. Alloaromadendrene
18. 2,6-dimethyl heptane
19. 2,6-dimethyl-1-heptene
20. 2,6-dimethyl-2-heptene
21. 2,6-dimethyl-1,5-heptadiene
22. Camphene
23. Citronellol

Figure 3. Stacked TIC of Spearmint Oil (red), Lemongrass Oil (yellow), and Thieves Oil (blue) ran with TV-SPME.



1. 2,6-dimethyl heptane
2. 2,6-dimethyl-1-heptene
3. 2,6-dimethyl-2-heptene
4. 2,6-dimethyl-1,5-heptadiene
5. Camphene
6. Citronellal
7. Alpha terpineol
8. Beta citral
9. Geraniol
10. Alpha-Citral
11. Geranyl Acetate
12. Caryophyllene
13. Gamma muurolene
14. Alloaromadendrene

Figure 4. Stacked TIC of Lemongrass Oil ran with HS-SPME (red) and TV-SPME (yellow).

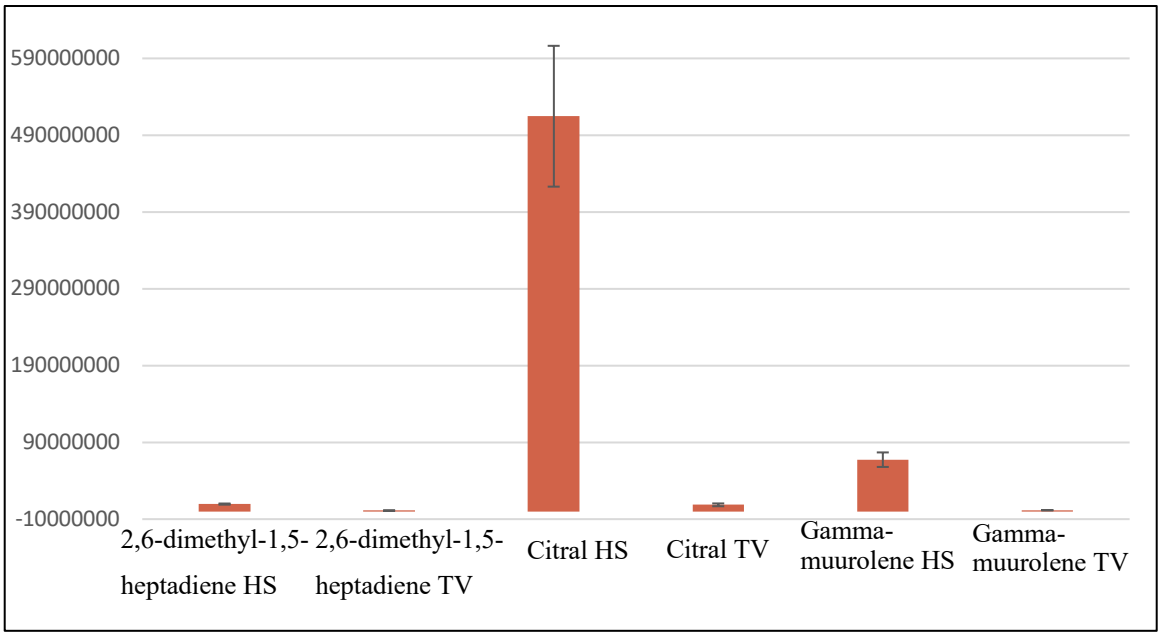


Figure 5. Peak area comparison of three main compounds found in lemongrass essential oil with standard deviation error bars.

A statistical analysis was done utilizing the data from Figure 5 to determine the statistical significance of the data. The p-value calculated for 2,6-dimethyl-1,5-heptadiene was 0.00003, the p-value for citral was calculated to be 0.01, and the p-value for gamma-muurlonene was calculated to be 0.006.

Table 1. Names and structures of compounds found in Essential Oil samples.

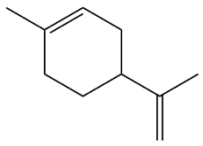
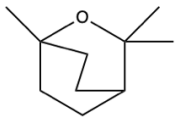
Compound	Structure
D-limonene	
Eucalyptol	

Table 1 continued

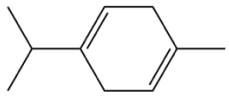
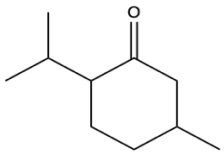
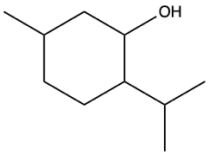
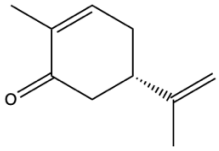
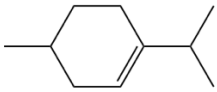
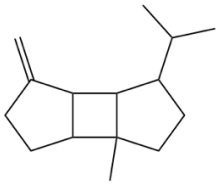
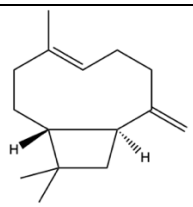
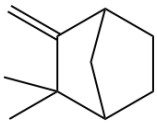
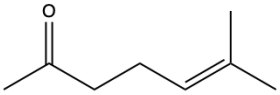
Gamma terpinene	
L-menthone	
Menthol	
D-Carvone	
3-p-menthene	
Beta bourbonene	
Caryophyllene	
Camphene	
Sulcatone	

Table 1 continued

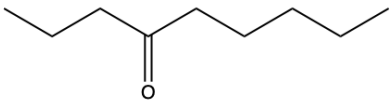
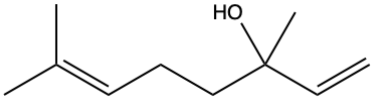
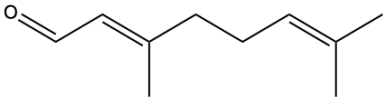
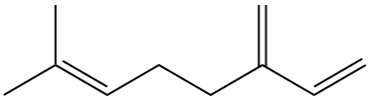
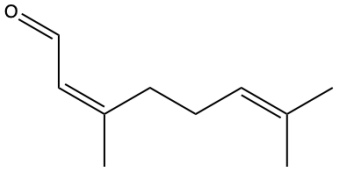
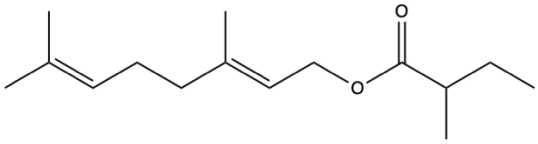
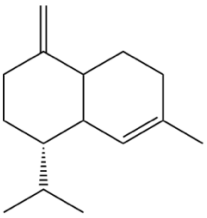
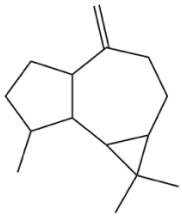
4-nonanone	
Linalool	
Beta-Citral	
Beta-myrcene	
Alpha-Citral	
Neryl-2-methylbutanoate	
Gamma muurolene	
Alloaromadendrene	

Table 1 continued

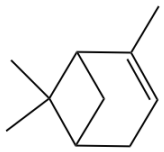
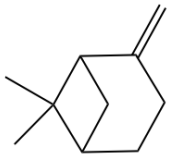
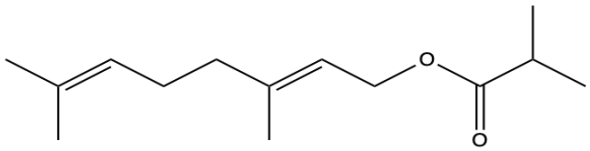
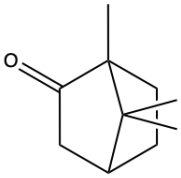
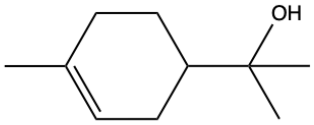
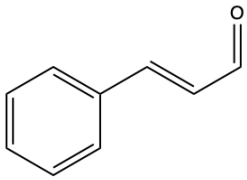
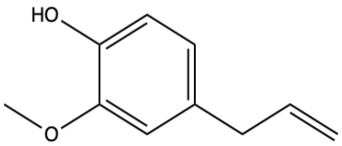
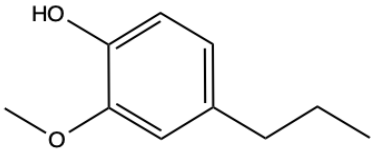
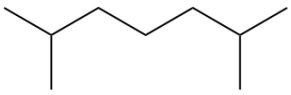
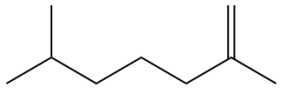
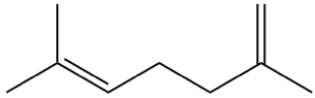
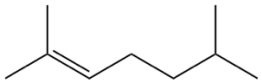
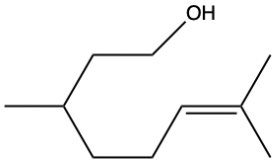
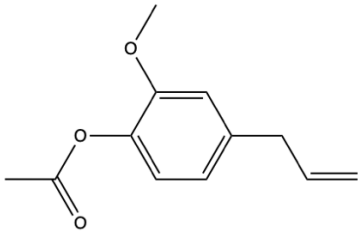
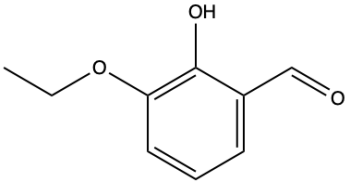
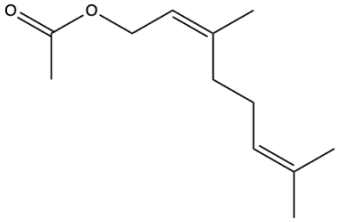
Alpha pinene	
Beta pinene	
Geranyl isobutyrate	
Camphor	
Alpha-terpineol	
Cinnamaldehyde	
Eugenol	
Dihydroeugenol	
2,6-dimethyl heptane	

Table 1 continued

2,6-dimethyl-1-heptene	
2,6-dimethyl-1,5-heptadiene	
2,6-dimethyl-2-heptene	
Citronellol	
Eugenol Acetate	
Ethylvanillin	
Geranyl Acetate	

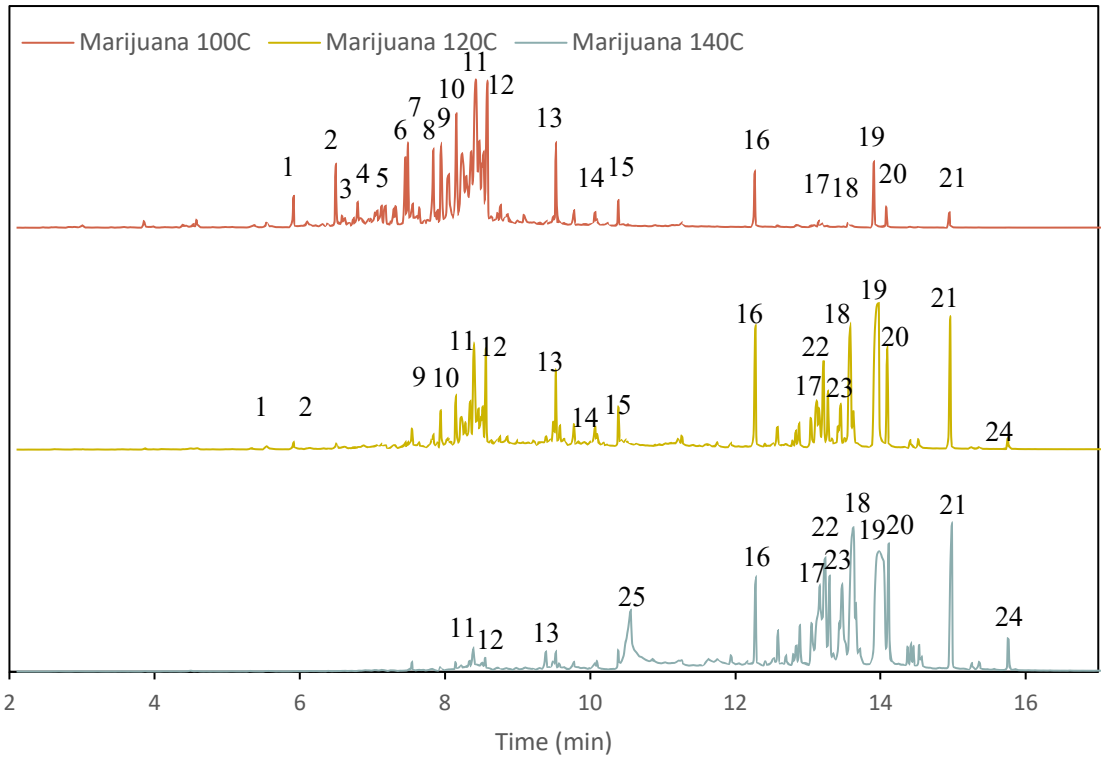
D-limonene, eucalyptol, gamma terpinene, L-menthone, menthol, D-carvone, 3-p-menthene, beta bourbonene, caryophyllene, camphene, sulcatone, linalool, beta-citral, beta-myrcene, alpha-

citral, Neryl-2-methylbutanoate, gamma murolene, alloaromadendrene, alpha pinene, beta pinene, Geranyl isobutyrate, camphor, alpha terpineol, cinnamaldehyde, eugenol, citronellol, and geranyl acetate are compounds that are classified as terpenes/terpenoids and found in essential oils due to their flavor and/or scent.[31,32,33] These compounds are also specifically added to the respective oil due to the health claim that is associated with each one. 4-nonanone is a flavoring agent that can be found in essential oils because it gives the oil a sweet/earthy taste and aroma.[34] Dihyrdoeugenol is a naturally occurring methoxyphenol compound that is found in flowers or spices, such as carnations and clove.[35] This compound can be found in essential oils because it gives the oil a sweet aroma and/or taste.[36] 2,6-dimethylheptane, 2,6-dimethyl-1-heptene, 2,6-dimethyl-2-heptene, and 2,6-dimethyl-1,5-heptadiene are naturally occurring compounds found in plants. These compounds can be found in essential oils due to the extraction process of the desired terpenes.[37] Eugenyl acetate is a naturally occurring benzoate ester compound that is found in some spices, such as allspice and anise. This compound can be found in essential oils because it gives the oil a specific aroma and/or taste.[38] Ethyl Vanillin is a naturally occurring hydroxybenzaldehyde compound. This compound can be found in essential oils because it gives the oil a sweet and caramel flavor.[39]

Marijuana

The stacked HS-SPME chromatograms for marijuana plant material at an extraction temperature of 100 °C,120 °C, and 140 °C is shown in Figure 6. The corresponding comparison of peak area vs extraction temperature of the three main components CBD, THC, and CBN is shown in Figure 7. The stacked chromatograms for HS-SPME of marijuana plant material, HS-SPME of extracted plant material, and TV-SPME of extracted plant material are shown in Figure 8. The corresponding peak area comparison bar graph is shown in Figure 9. The stacked HS-SPME chromatograms for fresh marijuana plant material at an extraction temperature of 100 °C,120 °C, and 140 °C is shown in Figure 10. The corresponding comparison of peak area v. extraction temperature of THC and CBN found in the fresh marijuana is shown in Figure 11. The stacked HS-SPME chromatograms for the THC standard at an extraction temperature of 100 °C,120 °C, and 140 °C is shown in Figure 12. The corresponding comparison of peak area v. extraction temperature of THC and CBN found in the THC standard is shown in Figure 13. The stacked TV-SPME chromatograms for the THC standard at an extraction temperature of 100 °C,120 °C, and

140 °C is shown in Figure 14. The corresponding comparison of peak area v. extraction temperature of THC and CBN found in the THC standard is shown in Figure 15. A comparison of CBN to THC ratios of the THC standard ran with HS-SPME and TV-SPME as well as the fresh marijuana is shown in Figure 16.



1. Piperitone
2. Caryophyllene
3. Alpha-guaiene
4. Humulene
5. Beta-bisabolene
6. Beta-panasinene
7. Selina-3,7(11)-diene
8. Caryophyllene oxide
9. Guaiol
10. 8-epi-gama-eudesmol
11. Alpha-eudesmol
12. Alpha-bisabolol
13. 6,10,14-trimethyl-2-pentadecanone
14. Methyl ester hexadecenoic acid
15. Dibutyl phthalate
16. Cannabichromene
17. Cannabidiol (CBD)
18. Tetrahydrocannabinol (THC)
19. Cannabinol (CBN)
20. Hexacosane
21. Nonacosane
22. Hexahydrocannabinol
23. Delta 8-tetrahydrocannabinol
24. Tricosane
25. Palmitic acid

Figure 6. Stacked TIC of marijuana plant material ran at 100 °C (red), 120 °C (yellow) and 140 °C (yellow).

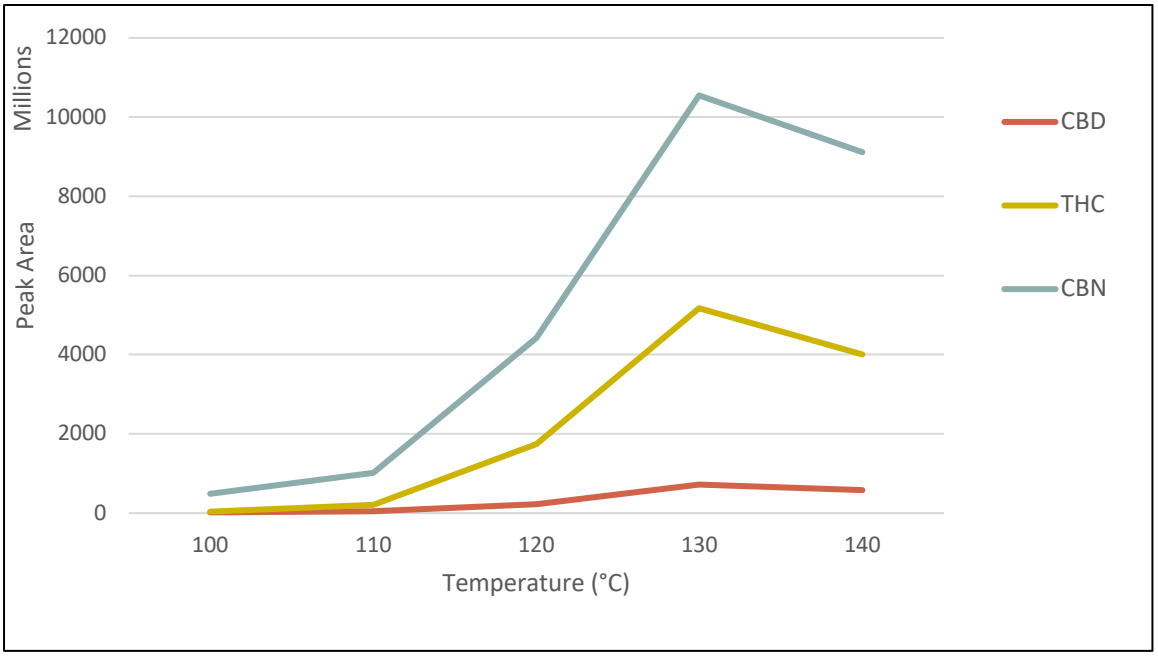


Figure 7. Peak Area vs. Extraction Temperature of marijuana plant material to compare CBD, THC, and CBN.

Table 2. Names and structures of compounds found in marijuana plant samples.

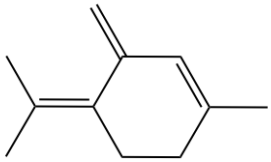
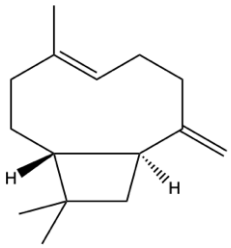
Compound	Structure
Piperitone	
Caryophyllene	

Table 2 continued

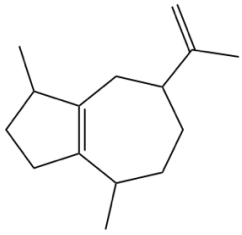
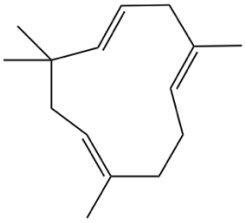
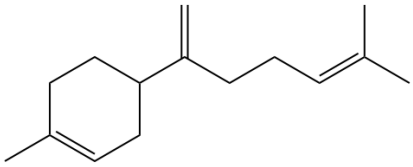
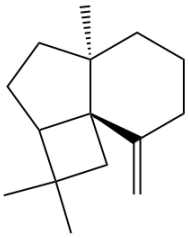
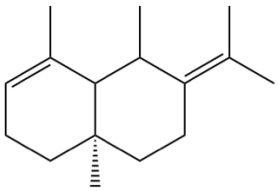
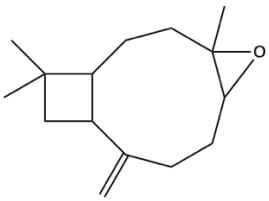
Alpha-guaiene	
Humulene	
Beta-bisabolene	
Beta-panasinene	
Selina-3,7(11)-diene	
Caryophyllene oxide	

Table 2 continued

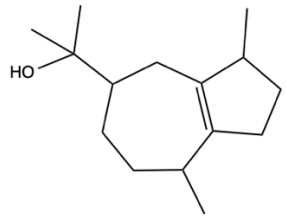
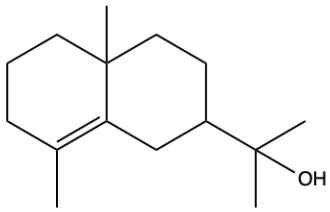
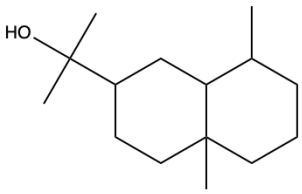
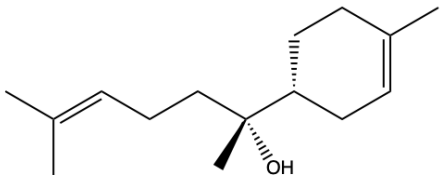
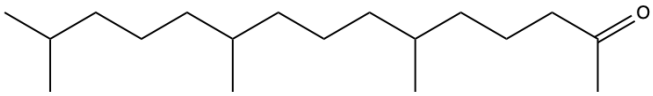
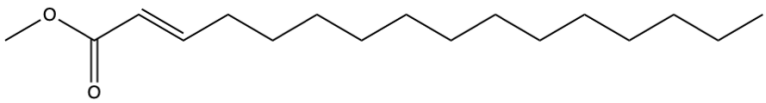
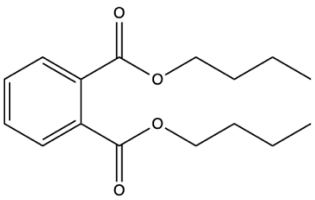
Guaiol	
8-epi-gamma-eudesmol	
Alpha-eudesmol	
Alpha-bisabolol	
6,10,14-trimethyl-2-pentadecanone	
Methyl-ester hexadecenoic acid	
Dibutyl phthalate	

Table 2 continued

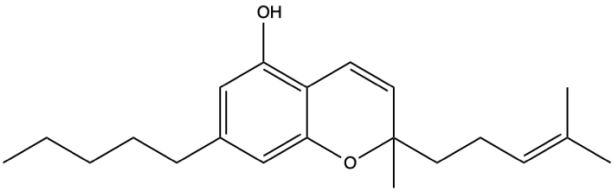
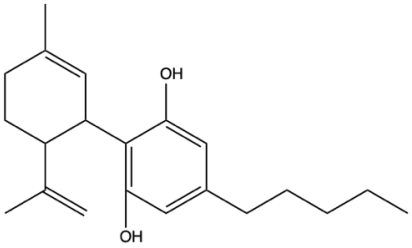
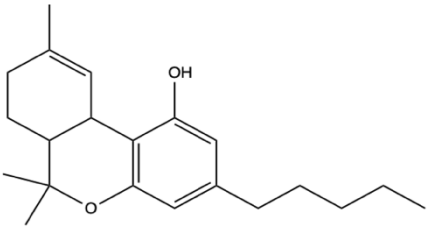
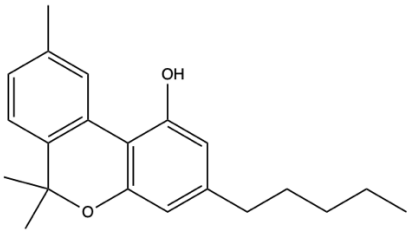
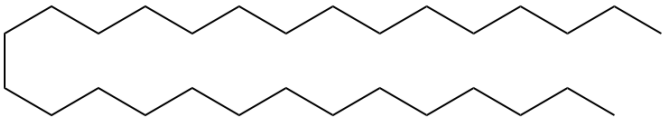
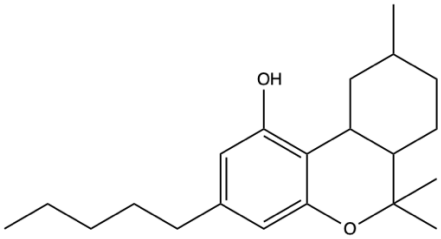
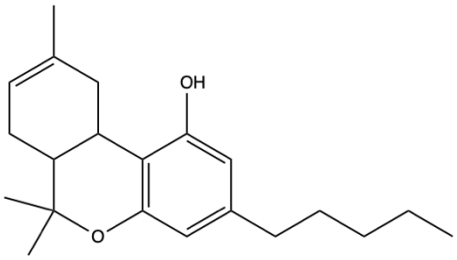
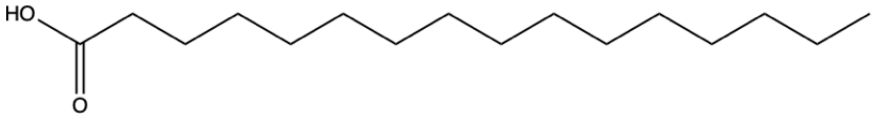
Cannabichromene	
Cannabidiol (CBD)	
Tetrahydrocannabinol (THC)	
Cannabinol (CBN)	
Hexacosane	
Nonacosane	
Hexahydrocannabinol	

Table 2 continued

Delta-8-tetrahydrocannabinol	
Tricosane	
Palmitic acid	

Piperitone, caryophyllene, alpha-guaiene, humulene, beta-bisabolene, beta-panasinene, seline-3,7(11)-diene, caryophyllene oxide, guaiol, 8-epi-gamma-eudesmol, alpha-eudesmol, alpha-bisabolol, and 6,10,14-trimethyl-2-pentadecanone are compounds that are classified as terpenes/terpenoids and found in marijuana.[40,41] The methyl ester of hexadecenoic acid was identified in marijuana but there is no known reason why it is present. Dibutyl phthalate is a compound that is commonly used as a plasticizer.[42] This compound could be found in these samples due to the marijuana previously being packaged in a plastic, Ziploc type bag. Cannabichromene, cannabidiol, tetrahydrocannabinol, cannabinol, hexahydrocannabinol, and delta-8-tetrohydrocannabinol are compounds that are classified as cannabinoids and found in the cannabis plant.[43,44] Hexacosane, Nonacosane, and tricosane are all straight chain alkanes that are plant metabolites which is why it can be found in the marijuana plant material.[45] Palmitic Acid is a naturally occurring long-chain fatty acid that can be found in plants.[46]

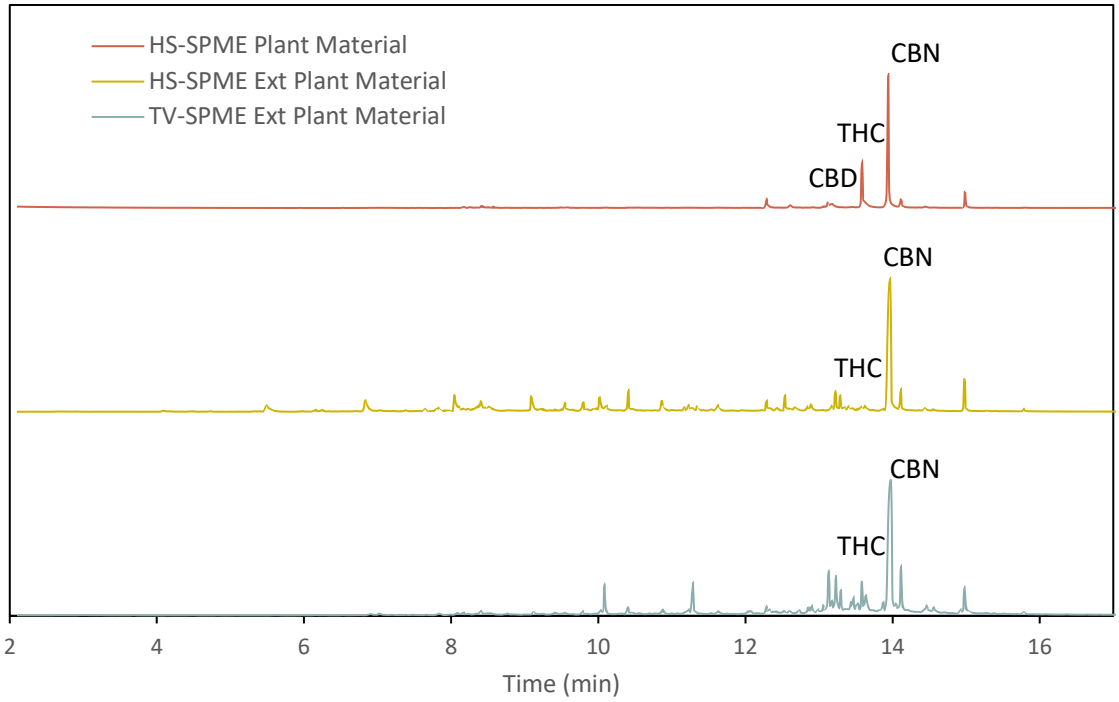


Figure 8. Stacked TIC of plant material ran with HS-SPME (red), extracted plant material ran with HS-SPME (yellow) and extracted plant material ran with TV-SPME (blue).

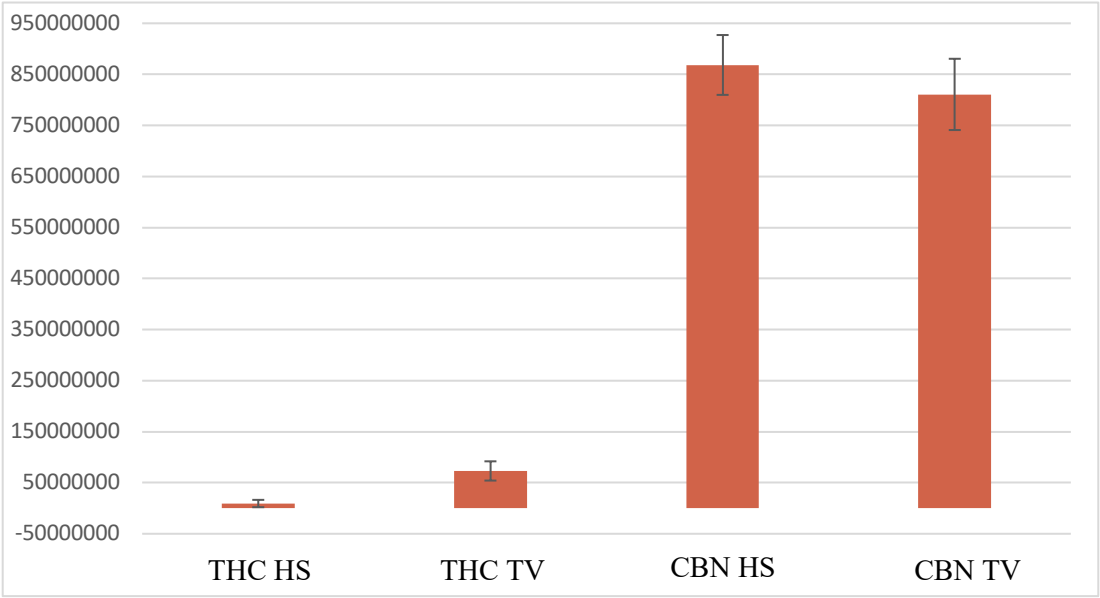


Figure 9. Peak area comparison of THC and CBN found in marijuana with standard deviation error bars.

A statistical analysis was done utilizing the data from Figure 9 to determine the statistical significance of the data. The p-value calculated for THC was 0.018 and the p-value for CBN was calculated to be 0.34.

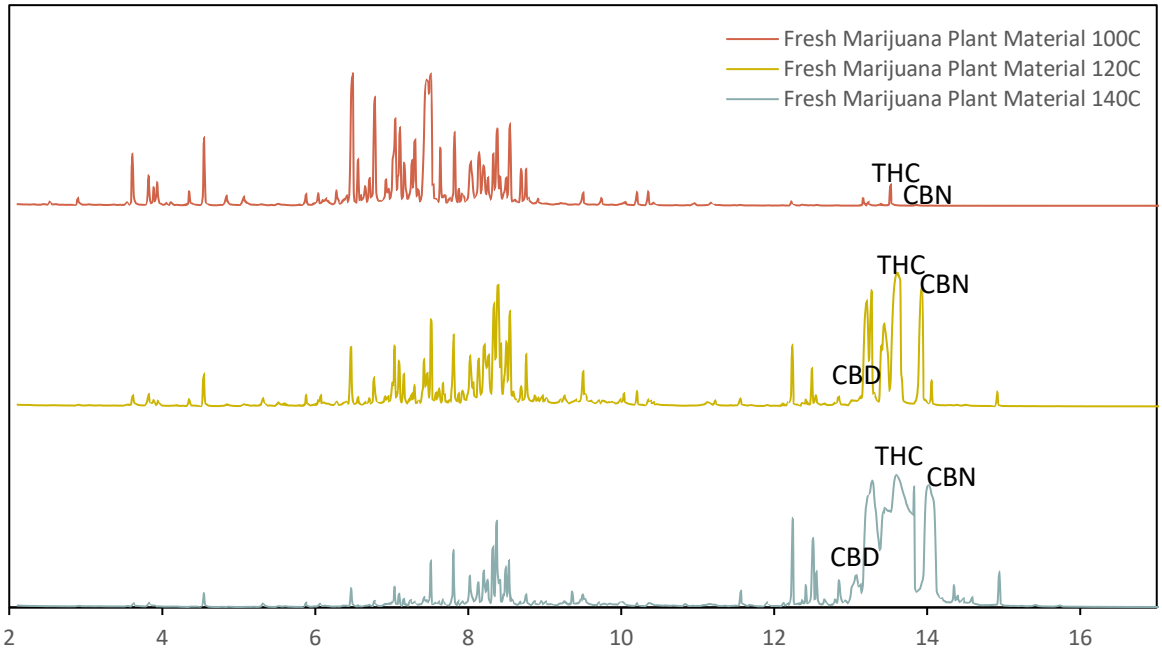


Figure 10. Stacked TIC of fresh marijuana plant material ran at 100 °C (red), 120 °C (yellow) and 140 °C (yellow).

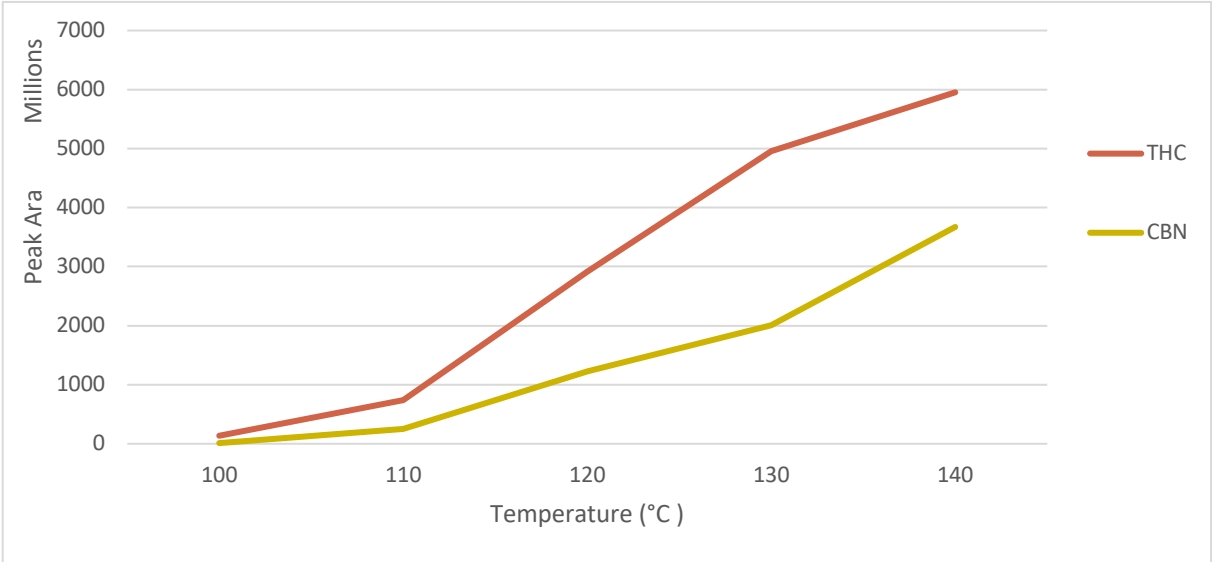


Figure 11. Peak Area vs. Extraction Temperature of fresh marijuana plant material utilizing the TV-SPME method to compare THC and CBN.

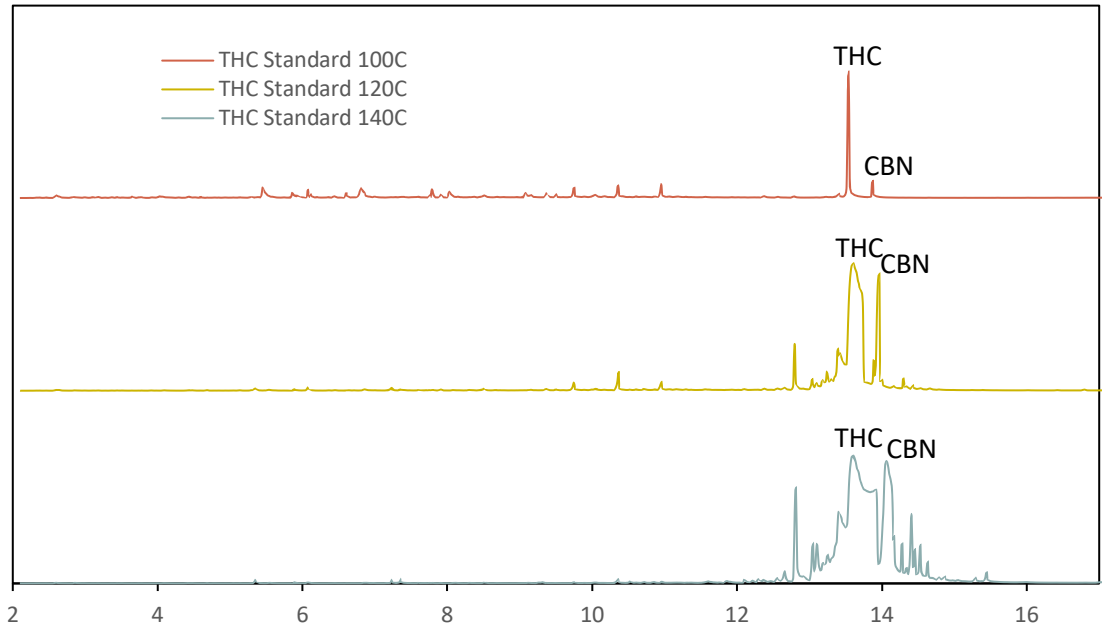


Figure 12. Stacked TIC of THC Standard utilizing the HS-SPME method ran at 100 °C (red), 120 °C (yellow) and 140 °C (yellow).

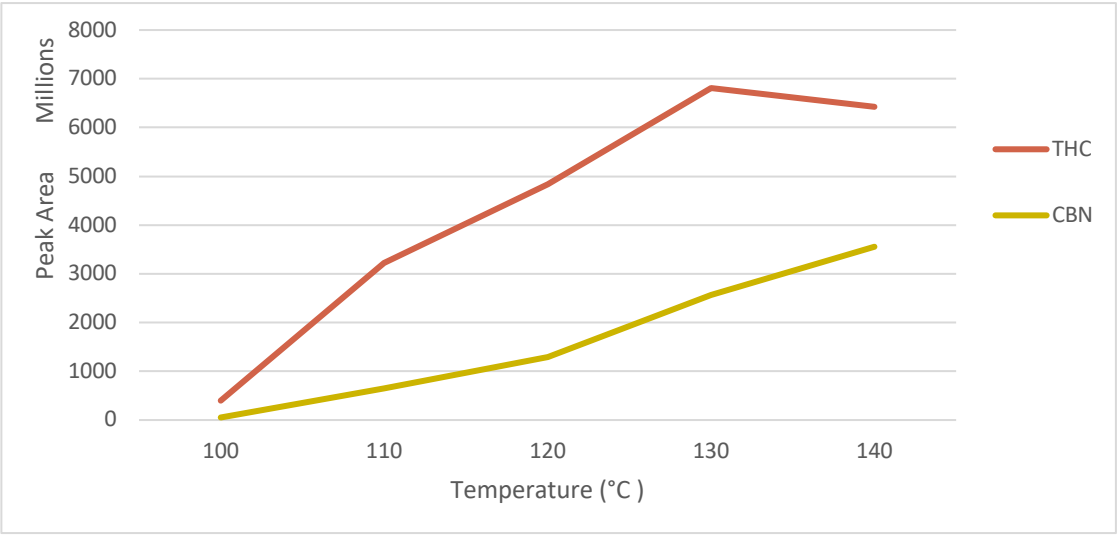


Figure 13. Peak Area vs. Extraction Temperature of THC standard utilizing the HS-SPME method to compare THC and CBN.

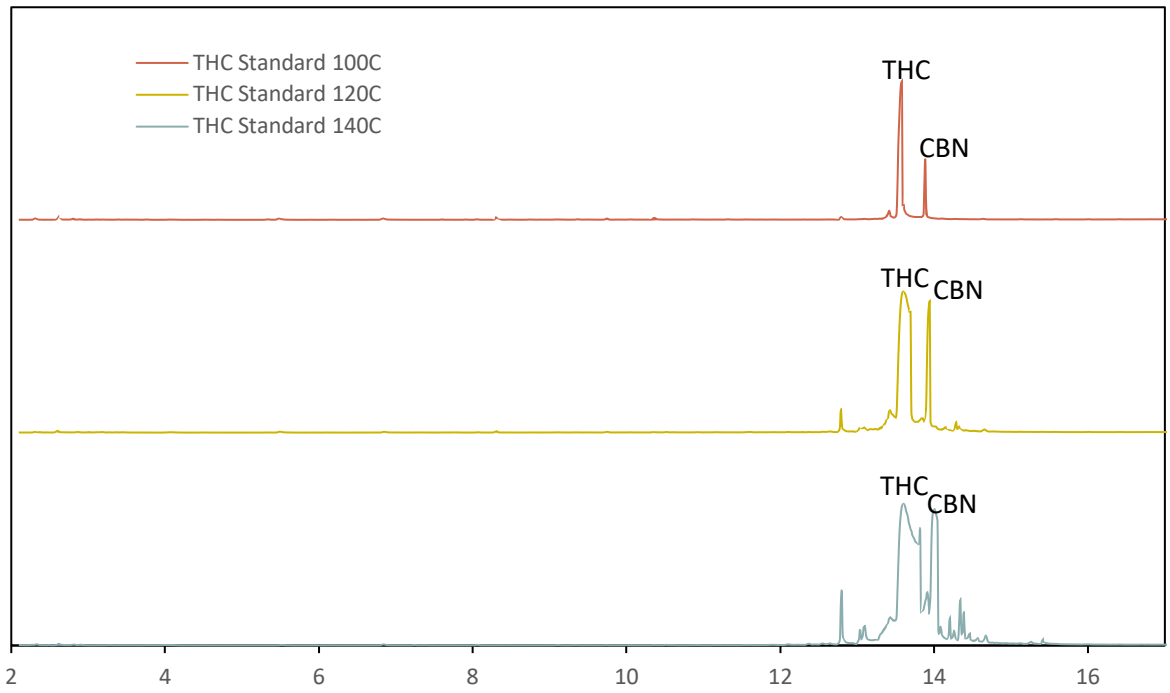


Figure 14. Stacked TIC of THC Standard utilizing the TV-SPME method ran at 100 °C (red), 120 °C (yellow) and 140 °C (yellow).

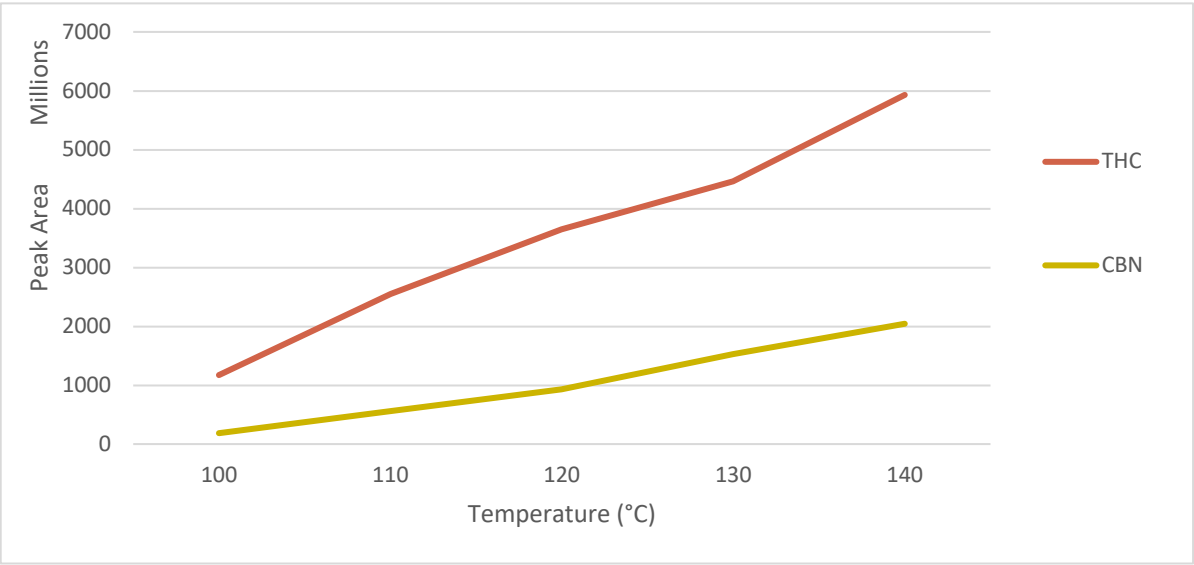


Figure 15. Peak Area vs. Extraction Temperature of THC standard utilizing the TV-SPME method to compare THC and CBN.

This THC standard should only have one detected peak, THC, but in all five samples there is more than one peak detected. For the sample with a 100 °C extraction temperature only one other peak was detected, which was identified as CBN. For the samples ran with 110 °C, 120 °C, 130 °C, and 140 °C, a second large peak was detected to be CBN, but multiple smaller peaks were also detected and identified as other common cannabinoids.

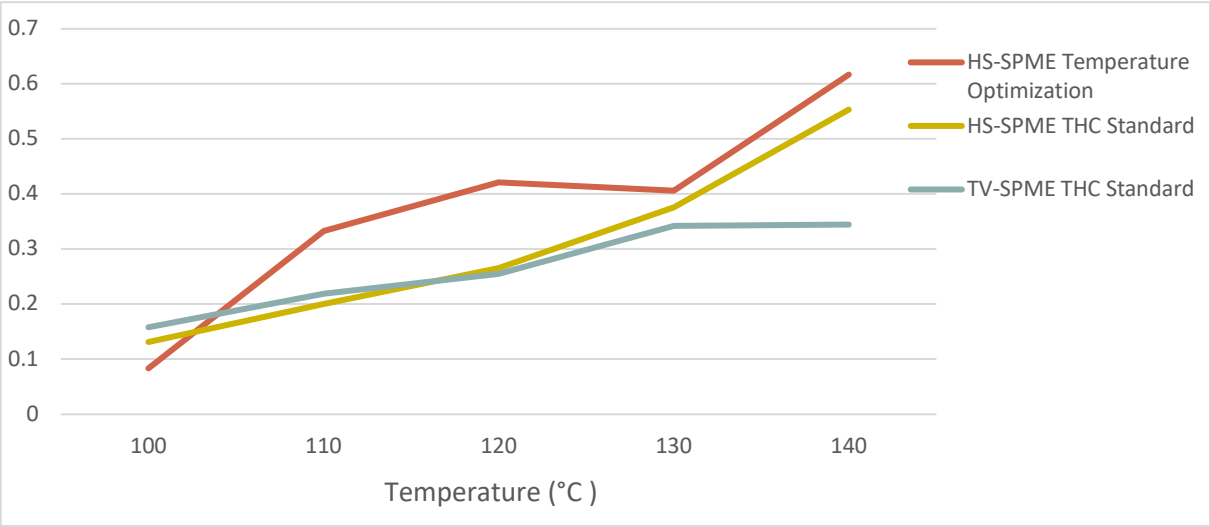


Figure 16. CBN:THC ratio comparison of fresh marijuana plant material, HS-SPME of THC standard and TV-SPME of THC standard.

Cannabidiol Oil

The stacked TIC chromatograms for HS-SPME of CBD Oil, HS-SPME of extracted CBD Oil, and TV-SPME of extracted CBD Oil are shown in Figure 17. The stacked EIC chromatograms for HS-SPME of extracted CBD Oil, and TV-SPME of extracted CBD Oil are shown in Figure 18. The corresponding peak area comparison bar graph is shown in Figure 19.

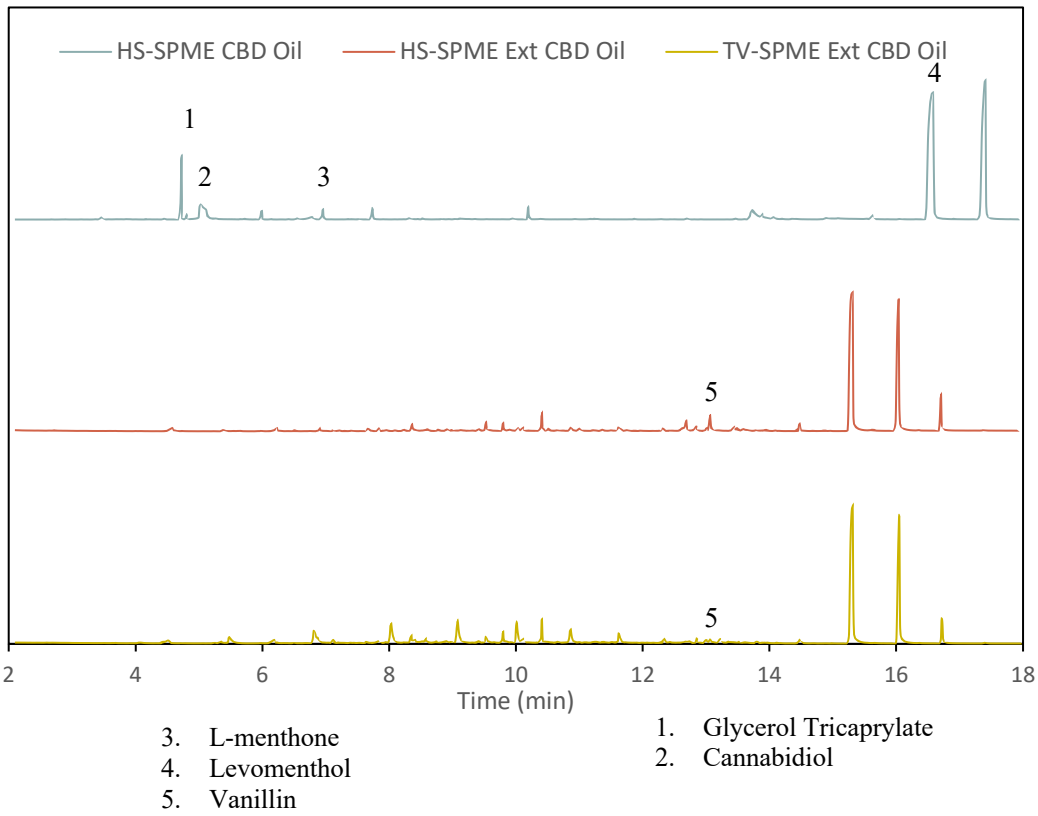


Figure 17. Stacked TIC of CBD Oil ran with HS-SPME (red), extracted CBD Oil ran with HS-SPME (yellow) and extracted CBD Oil ran with TV-SPME (blue).

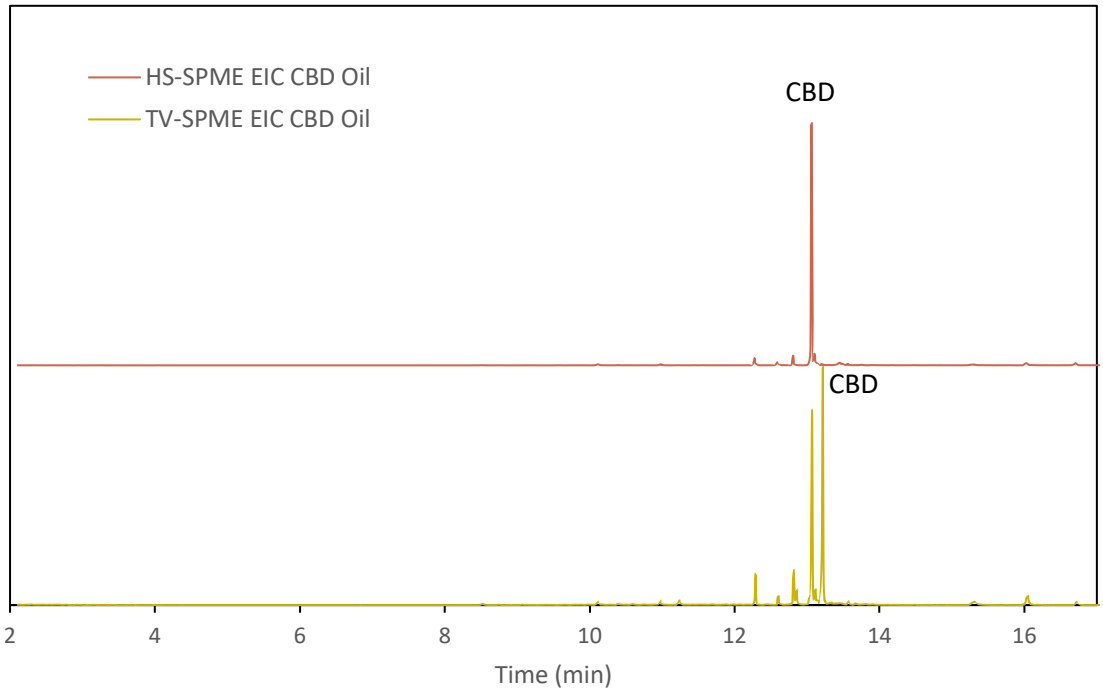


Figure 18. Stacked extracted ion chromatogram (EIC) of CBD Oil ran with HS-SPME (red) and CBD Oil ran with TV-SPME (yellow).

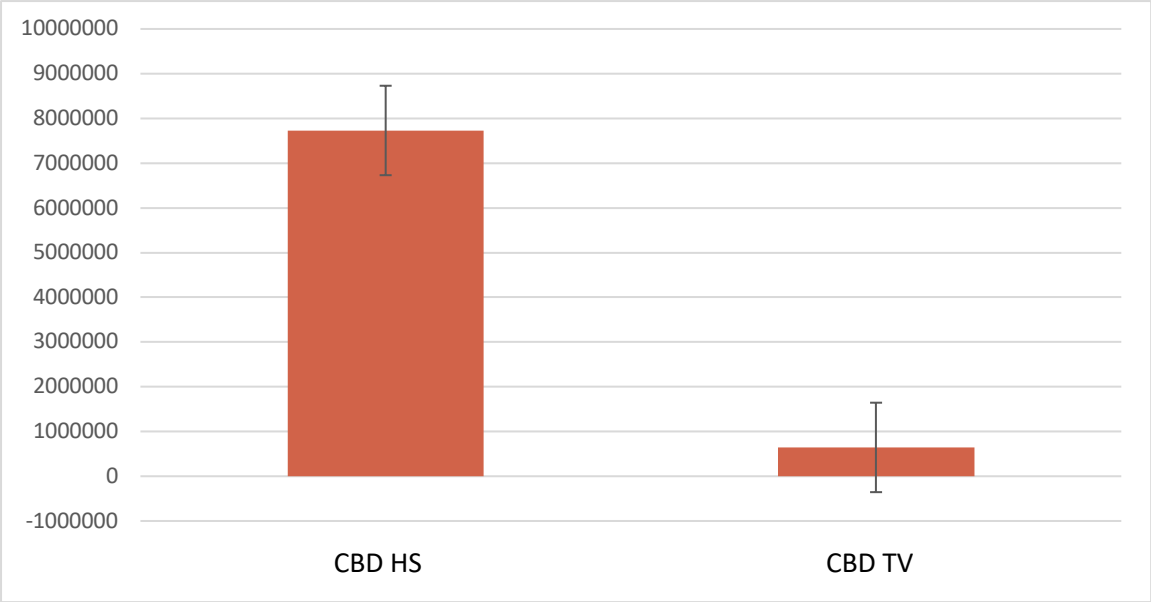
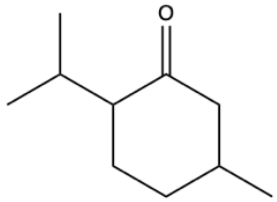
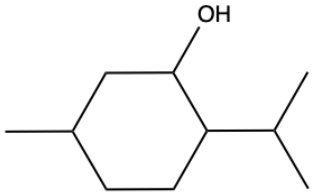
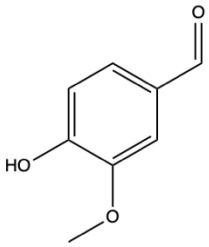
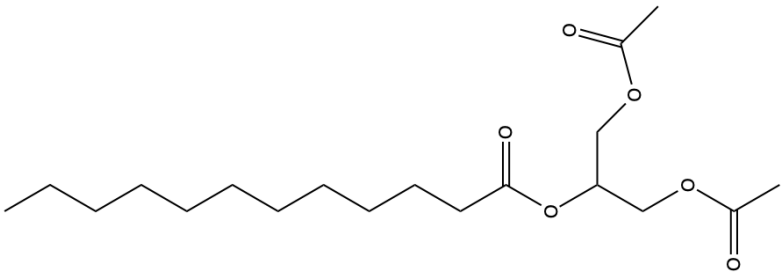
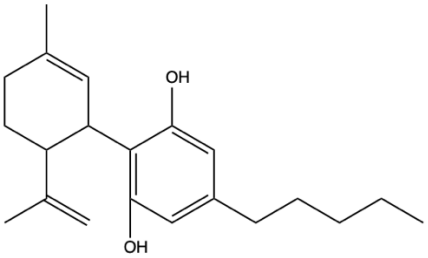


Figure 19. Peak area comparison of CBD found in CBD Oil with standard deviation error bars.

A statistical analysis was done utilizing the data from Figure 12 to determine the statistical significance of the data. The p-value calculated for CBD was 0.017.

Table 3. Names, structures, and purpose of compounds found in CBD Oil samples.

Compound	Structure
L-menthone	
Levomenthol	
Vanillin	
Glycerol Tricaprylate	
Cannabidiol	

L-menthone, levomenthol, and Vanillin are compounds that are classified as terpenes and found in essential oils. These compounds give this oil the flavor and aroma it has which is

chocolate mint.[47] Glycerol tricaprylate is a triglyceride that is used as the oil base for this CBD oil.[48] Cannabidiol is a cannabinoid that is the main ingredient of this oil. This compound is put into this oil to be used to treat certain conditions such as pain, anxiety, and insomnia.[49]

Conclusion

Essential Oils

All three essential oils were successfully analyzed with both HS-SPME and TV-SPME paired with GC-MS. During the qualitative comparison of spearmint, thieves, and lemongrass oils it was determined that either method is suitable for analyzing these samples. In all three oils, the main compounds that makeup the essential oils are seen in both HS-SPME and TV-SPME. There are slight differences between HS-SPME and TV-SPME that can be noted for each oil, where some compounds are only present in one or the other. Specifically, some compounds that eluted in the first few minutes were only present in the HS-SPME samples. Some compounds that eluted in the last few minutes were only present in the TV-SPME samples due to their low volatility.

During the quantitative comparison of the lemongrass oil, it was determined that there is no statistical significance of the differences in peak area between the HS-SPME method and TV-SPME method. This would conclude that neither technique is more sensitive than the other when it comes to the analysis of essential oils.

Marijuana

Marijuana plant material was successfully analyzed with HS-SPME when ran “as is”, showing the three main cannabinoids, CBD, THC, and CBN. The marijuana extraction temperature study showed that 130 °C was the optimal extraction temperature for marijuana.

Marijuana plant material was successfully analyzed with both HS-SPME and TV-SPME when ran after being extracted using methanol, showing two of the three main cannabinoids THC and CBN. During the quantitative comparison of the extracted marijuana, it was determined that there is no statistical significance of the difference in peak area of THC between the HS-SPME method and TV-SPME method. It was also determined that there is a statistical significance of the

difference in peak areas of CBN. This would conclude that neither technique is more sensitive than the other when it comes to the analysis of THC but HS-SPME is more sensitive for the analysis of CBN. CBD, although an important cannabinoid, was not considered in this critical comparison due to the compound not being present after extraction using methanol.

Fresh marijuana plant material was successfully analyzed with HS-SPME when ran "as is", detecting the two main cannabinoids, THC, and CBN in all samples at all five temperatures and detecting CBD at 120 °C, 130 °C, and 140°C. It can be seen that in the fresh marijuana there is more THC than CBN unlike the older marijuana plant material. This could be due to the fact that THC metabolizes into CBN so in older marijuana there will be a greater concentration of CBN than in fresh marijuana. It can be concluded that HS-SPME of marijuana is a technique that can be used for a qualitative analysis of marijuana.

The THC standard was successfully analyzed with both HS-SPME and TV-SPME. THC and CBN were identified in all HS-SPME and TV-SPME samples that were analyzed. The CBN to THC ratio was then calculated and compared for the HS-SPME of fresh marijuana plant material, the HS-SPME of the THC standard and the TV-SPME of the THC standard to see if THC is being oxidized during the SPME method. As the temperature increases, the ratio of CBN to THC also increases. This would conclude that heating THC will cause it to oxidize and some of it will form CBN. This oxidation is shown to occur in this standard as well as in the fresh marijuana plant material. This oxidation of THC needs to be taken into consideration when analyzing marijuana plant material with these HS-SPME and TV-SPME methods. It will not affect the qualitative analysis of marijuana plant material when just looking to determine the presence of THC, but this will affect the quantitative analysis of THC in the marijuana plant material. This would conclude that these HS-SPME and TV-SPME methods should only be used for a qualitative analysis of marijuana plant material.

Cannabidiol Oil

CBD Oil was successfully analyzed with HS-SPME when ran "as is", showing the presence of CBD. CBD Oil was successfully analyzed with both HS-SPME and TV-SPME when ran after being extracted using dichloromethane, showing the presence of CBD, but the extracted ion profile

had to be used to identify CBD in the sample due to its low abundance. During the quantitative comparison of the extracted CBD oil, it was determined that there is no statistical significance of the differences in peak area between the HS-SPME method and TV-SPME method. This would conclude that neither technique is more sensitive than the other when it comes to the analysis of CBD.

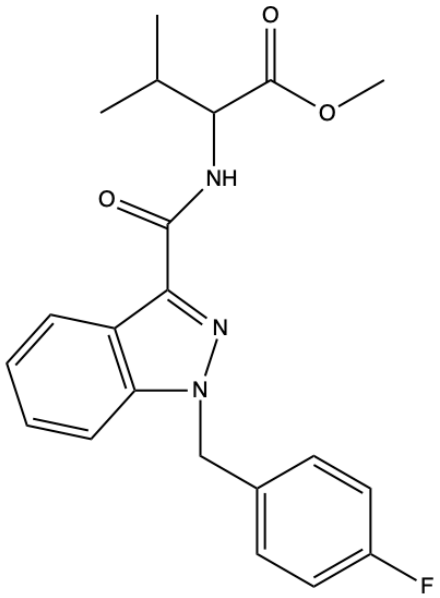
CHAPTER 2. ANALYSIS OF SYNTHETIC CANNABINOIDS

Introduction

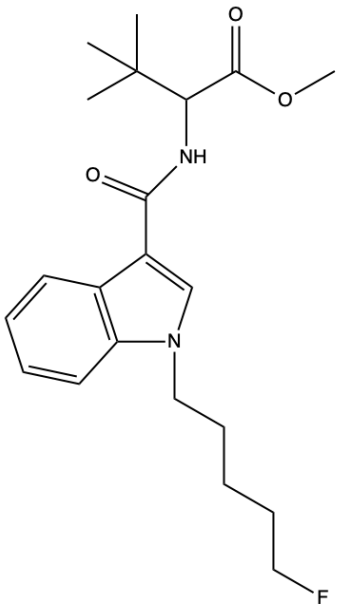
Synthetic cannabinoids are designer drugs that have been synthesized since the 1960's. These compounds were initially synthesized to study the pharmacology of cannabinoids and determine any therapeutic effects from these types of drugs. In the past 20 years, synthetic cannabinoids have started to be commercially produced and abused.[40] Synthetic cannabinoids have come into the drug market as packets of herbal mixtures, with many different names such as Spice, K2, herbal incense, and Cloud 9. They have been sold over the internet as well as in some retail shops under misleading labels. [51] The packets will hold around 3 g of plant material that are laced with a synthetic cannabinoid. To make this laced plant material, the synthetic cannabinoid will be dissolved in some kind of solvent and then the plant material will be saturated with this drug solution and the solvent will be allowed to evaporate off, leaving the synthetic cannabinoid on the plant material. It has been found that the amount of synthetic cannabinoid found on this plant material is highly variable, which leads to variable potencies.[52]

These herbal mixtures will then be smoked, and they will produce marijuana-like effects. Synthetic cannabinoids will also have the same or very similar physiological effect in the human body as natural cannabinoids such as THC. They both bind to the cannabinoid receptor type 1 (CB_1) and cannabinoid receptor type 2 (CB_2) that are mostly found in the central nervous system but can also be found in the brain, lungs, and liver. The difference between synthetic cannabinoids and THC is that the synthetic cannabinoids have a higher affinity for the CB_1 receptor, and their metabolites retain a high affinity for the receptor which leads to the conclusion that they are more toxic than natural cannabinoids.[53] Most synthetic cannabinoids are placed on the Schedule I list once they are determined to be these designer drugs. The issue that has arose is that these banned drugs will be replaced with another compound that is structurally similar with the same pharmacological effects that avoid the regulation of the banned drugs. This has become an unending circle of these designer drugs being banned by the drug enforcement administration (DEA) and then a new group of them will come out that will then have to be tested and go through legislation to also become a banned compound. This has been a constant challenge in the forensic toxicology field when an identification needs to be done of new synthetic cannabinoids so that law

enforcement and public health agencies can then assess the risks.[54] FUB-AMB and 5-fluoro-MDMB-PICA, seen in Figure 17, are two synthetic cannabinoids that have been recently encountered in Indiana. Both of these synthetic cannabinoids are on the Schedule I list. FUB-AMB was identified as the second-most common and 5-fluoro-MDMB-PICA was identified as the fifth-most common synthetic cannabinoid seized synthetic cannabinoids by the DEA in 2018.[55] GC-MS and LC-MS are the commonly used methods for the analysis of the herbal mixtures for the presence of synthetic cannabinoids, which normally include an extraction step to separate the synthetic cannabinoids of interest from the plant material.[56] Using the TV-SPME and HS-SPME methods paired with GC-MS could develop a faster method of testing for these types of drugs which could benefit the forensic toxicology field.



1)



2)

Figure 20. Molecular structure for 1. FUB-AMB (383.4 g/mol) and 2. 5-fluoro-MDMB-PICA (376.5 g/mol)

Methods

Materials

FUB-AMB was purchased from Cayman Chemical (Ann Arbor, Michigan). 5-fluoro-MDMB-PICA was purchased from Cayman Chemical (Ann Arbor, Michigan). Dichloromethane was purchased from Sigma-Aldrich (Saint Louis, Missouri).

Sample Preparation

1 mg of FUB-AMB was diluted in dichloromethane with a concentration of 1 mg/ml and 1 mg of 5-fluoro- MDMB-PICA was diluted in dichloromethane with a concentration of 1 mg/mL. These solutions were analyzed using liquid-liquid injection by placing 1 mL of the sample into the respective GC vial. These samples were analyzed with a 50:1 split.

10 mg of potpourri was placed into three 20 mL headspace vial with 100 ul of dichloromethane or 100 ul of the 1mg/mL solution of FUB-AMB or 5-fluoro-MDMB-PICA and the dichloromethane was then allowed to evaporate off in the hood for 60 minutes. 1 mL of methanol was then added to the vials, vortexed for 1 minute and the solvent was extracted from the vial and placed into a GC vial for analysis by liquid-liquid injection. These samples were ran using a splitless injection.

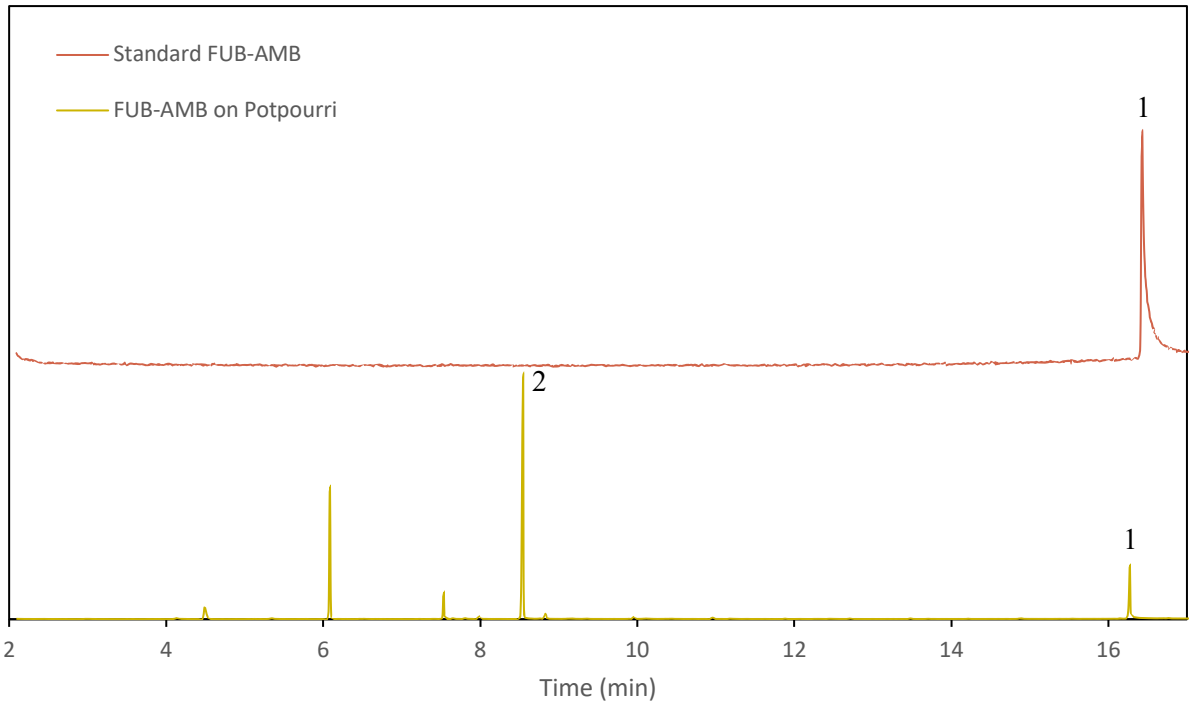
GC-MS Parameters

The same instrumentation and column that were used in Chapter one was also used for these experiments.

The initial oven temperature was 60 °C, held for 1 minute. The temperature then ramped to 300 °C at a 15 °C /min ramp and then held for 1 minute at the final temperature. The mass transfer line was set to 250 °C and the flow rate was 2.5 mL/min. The source was held at 230 °C and the quadrupoles were held at 150 °C. The mass range scanned was 40 – 550 m/z. Total ion chromatograms (TIC) were produced, and all compounds were identified using the NIST and/or SWGDRUG libraries.

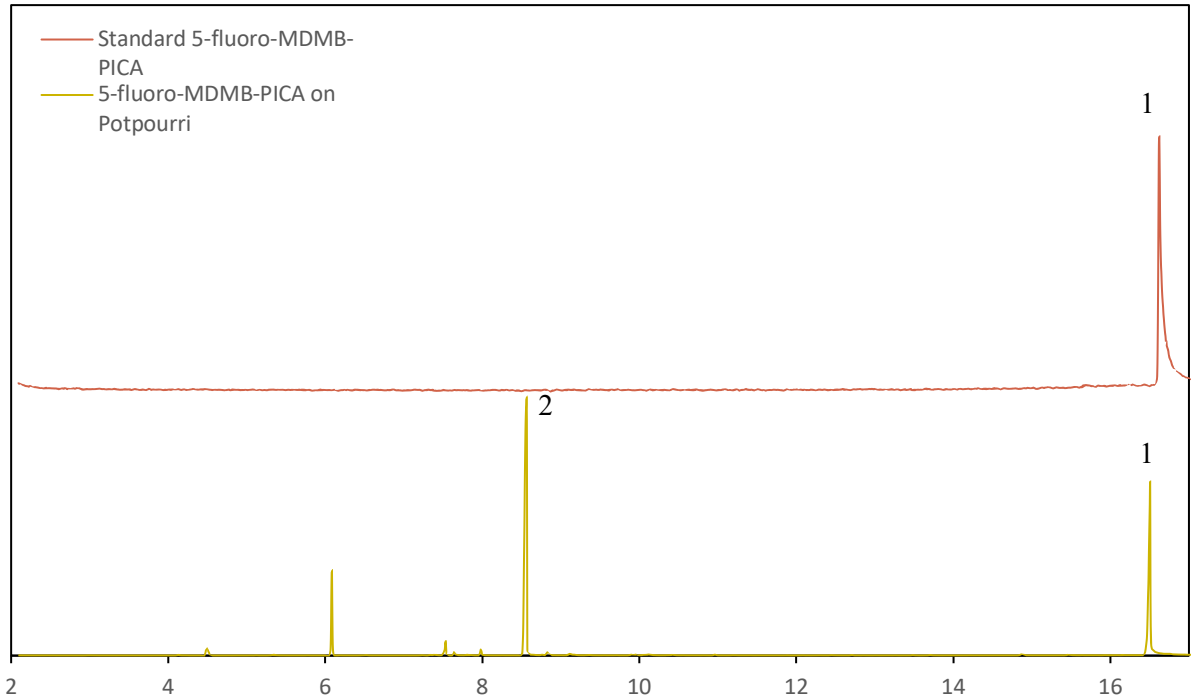
Results

The stacked TIC chromatograms for the FUB-AMB standard and FUB-AMB on potpourri are shown in Figure 21. The stacked TIC chromatograms for the 5-fluoro-MDMB-PICA standard and 5-fluoro-MDMB-PICA on potpourri are shown in Figure 22. These cannabinoids standards were analyzed with HS-SPME on potpourri at 100 °C and analyzed with TV-SPME on potpourri at 100 °C, 120 °C, 140 °C, 160 °C, and 180 °C but neither of the drugs were detected using either method.



1. FUB-AMB
2. Diethyl Phthalate

Figure 21. Stacked TIC of FUB-AMB standard (red) and FUB-AMB on potpourri (yellow).



1. 5-fluoro-MDMB-PICA
2. Diethyl Phthalate

Figure 22. Stacked TIC of 5-fluoro-MDMB-PICA standard (red) and 5-fluoro-MDMB-PICA on potpourri (yellow).

Conclusions

FUB-AMB and 5-fluoro-MDMB-PICA standards were analyzed using a liquid injection GC-MS method to determine the elution time of the synthetic cannabinoids. FUB-AMB and 5-fluoro-MDMB-PICA were spiked onto potpourri and analyzed using a liquid injection GC-MS method. The synthetic cannabinoid samples had a final concentration of 100 ppm, which are realistic concentrations for synthetic cannabinoids found on potpourri. The compounds detected in the FUB-AMB sample were diethyl phthalate, which is a plasticizer that could have come from the bag the potpourri was held in, and FUB-AMB, the synthetic cannabinoid of interest. The compounds detected in the 5-fluoro-MDMB-PICA sample were diethyl phthalate and 5-fluoro-MDMB-PICA, the synthetic cannabinoid of interest.

FUB-AMB and 5-fluoro-MDMB-PICA were spiked onto potpourri and analyzed using TV-SPME GC-MS methods and a HS-SPME GC-MS method. Both methods were unable to detect

FUB-AMB or 5-fluoro-MDMB-PICA, this is likely due to their high molecular weight making it difficult to vaporize these compounds.

CHAPTER 3. ANALYSIS OF SALIVA

Introduction

Saliva is an oral fluid that is produced by the discharge of fluids from many different glands in the mouth of a human or animal. Saliva is about 99% water and 1% all other compounds such as electrolytes, proteins, enzymes, mucus, and antibacterial compounds.[57] A typical human can produce between 500 and 1500 mL of saliva every day, and the flow rate can vary from 0 to several mL per minute. This flow rate will vary depending on specific conditions of the individual, including emotional state, health, and hunger.[58,59] In recent year, saliva has become a popularly researched alternative for drug testing. Some potential applications for this type of testing would include, on site testing of suspected drivers under the influence, forensic investigations, and monitoring patients that are going through drug detoxification. Saliva is an advantageous testing matrix because it is readily available due to the large production of it in the human body, it is a non-invasive collection technique, and it can be collected under supervision, if necessary, and is comparatively free of interfering substances with a much lower protein content than other bodily fluids.[60,61]

Saliva is a blood filtrate, so drugs are transferred into the saliva from blood by passive diffusion and active transport. Being a blood filtrate would mean that the concentration of drug in saliva should reflect the concentration of drug in the blood. There are some factors that could affect this salivary concentration, contamination of the mouth from smoking or ingesting the drug will increase the concentration of the drug in the saliva, and changes in the pH or flow rate of the saliva could either increase or decrease the drugs concentration.[62] Even if these factors apply, the presence of the drug can still be detected to indicate that there was recent use of the drug. The drugs that will be present in saliva are going to be the "parent drug", not a metabolite since it will not have gone through the metabolism phase before being introduced to the saliva. This may cause problems during the analysis with GC-MS because many drugs of abuse are nonvolatile.[63] When the analysis of these kinds of drugs needs to be done, the drug can be derivatized to increase the thermal stability and make the compound more volatile.

Derivatization is the process in which a derivatization agent is introduced to the compound of interest and a reaction will occur that will chemically change the compound of interest making

it more volatile. Specifically, an active hydrogen on the analyte will be replaced by a functional group that will allow better separation in the column.[64] There are three different forms of derivatization, alkylation, silylation, and acylation, only silylation will be performed in these saliva analyses.[65] The silylation derivatization agent used in this experimentation was N,O-bis (trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS). For TV-SPME and HS-SPME methods, an on-fiber derivatization was done. This is performed by exposing the SPME fiber to a vial containing the derivatization agent for a fixed amount of time, determined depending on the analyte. In these experiments, direct immersion derivatization was used so the SPME fiber was fully immersed in the derivatization agent. The fiber was then exposed to the vial containing the analyte of interest. The normal process of SPME will then occur so the vial is heated inside the agitator, so the analyte vaporizes and is adsorbed onto the fiber with the derivatization agent. The derivatization will then take place on the fiber until the fiber is moved to the inlet for desorption. This type of on-fiber derivatization is advantageous because this will reduce sample analysis time.[7]

GC-MS and LC-MS are the commonly used methods for the analysis of saliva for the presence of drugs as well as the volatile organic compounds that are naturally present in salive.[66,58] These analyses normally include a detailed sample preparation, including an extraction to remove the saliva components as well as a derivatization process but there have been some studies utilizing HS-SPME paired with GC-MS to reduce the sample preparation time.[61,62] Using the TV-SPME method paired with GC-MS and utilizing an on-fiber derivatization could develop a faster method with less matrix effects of testing for these types of drugs.

Methods

Materials

Saliva was collected from myself, Alexandra Train. Artificial Saliva was obtained from Dr. Frederique Deiss's Lab, Department of Chemistry at IUPUI. BSTFA +1% TMCS was purchased from Thermo Fisher Scientific (Waltham, Massachusetts).

Sample Preparation

Saliva was analyzed using TV-SPME by placing 2.6 ul of saliva into all 20 mL headspace vials. For the derivatized samples the PDMS/DVB fiber was first immersed in BSTFA + 1% TMCS for 10 minutes before being inserted into the sample vials. All samples were heated to 60 °C inside of a Gerstel agitator. The fiber was exposed to the sample vial for 10 minutes before being inserted into the GC inlet for desorption at 250 °C. All saliva samples were analyzed using a splitless injection.

Artificial saliva was analyzed using HS-SPME by placing 1 mL of the artificial saliva into a 20 mL headspace vial. Artificial saliva was analyzed using TV-SPME by placing 2.6 ul of the artificial saliva into each 20 mL headspace vial. For the derivatized samples the PDMS/DVB fiber was first exposed to the vial containing BSTFA + 1% TMCS for 10 minutes before being inserted in the sample vials. The fiber was exposed to the sample vial for 10 minutes before being inserted into the GC inlet for desorption at 250 °C. The saliva was analyzed using a splitless injection.

GC-MS Parameters

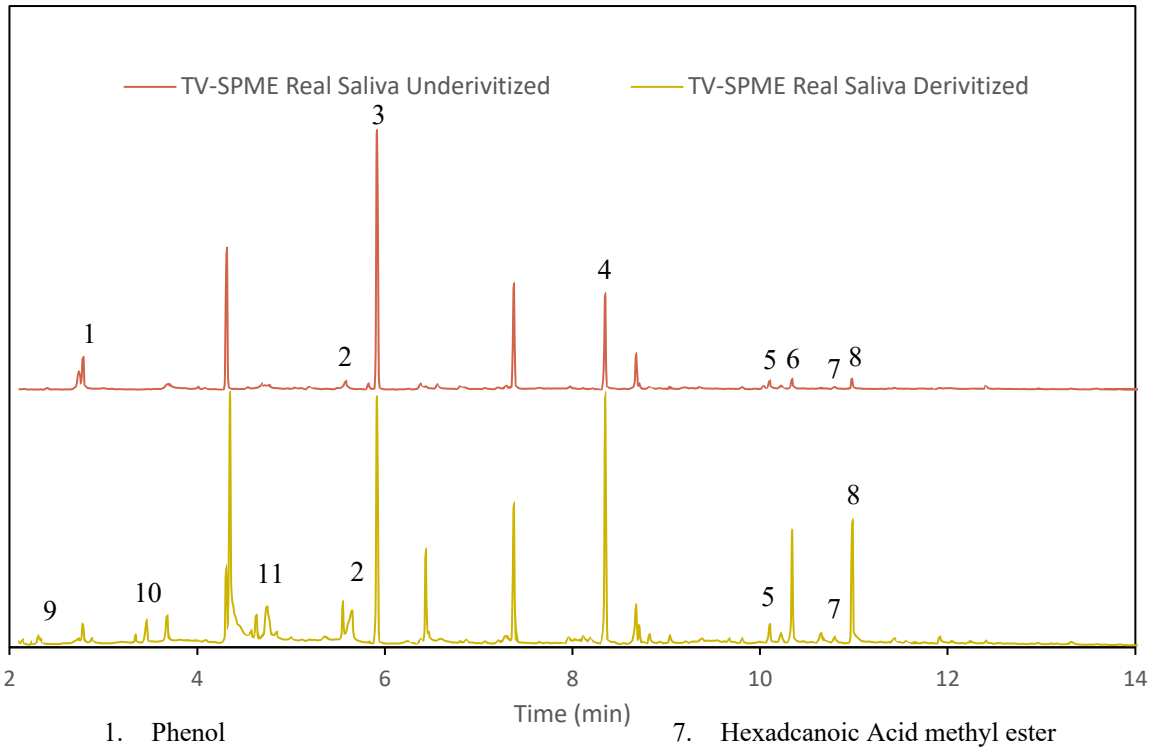
The same instrumentation and column that were used in Chapter one was also used for these experiments.

The initial oven temperature was 60 °C, held for 1 minute. The temperature then ramped to 250 °C at a 15 °C /min ramp and then held for 1 minute at the final temperature. The mass transfer line was set to 250 °C and the flow rate was 3.4 mL/min. The source was held at 230 °C and the quadrupoles were held at 150 °C. The mass range scanned was 40 – 550 m/z. Total ion chromatograms (TIC) were produced, and all compounds were identified using the NIST and/or SWGDRUG libraries.

Results

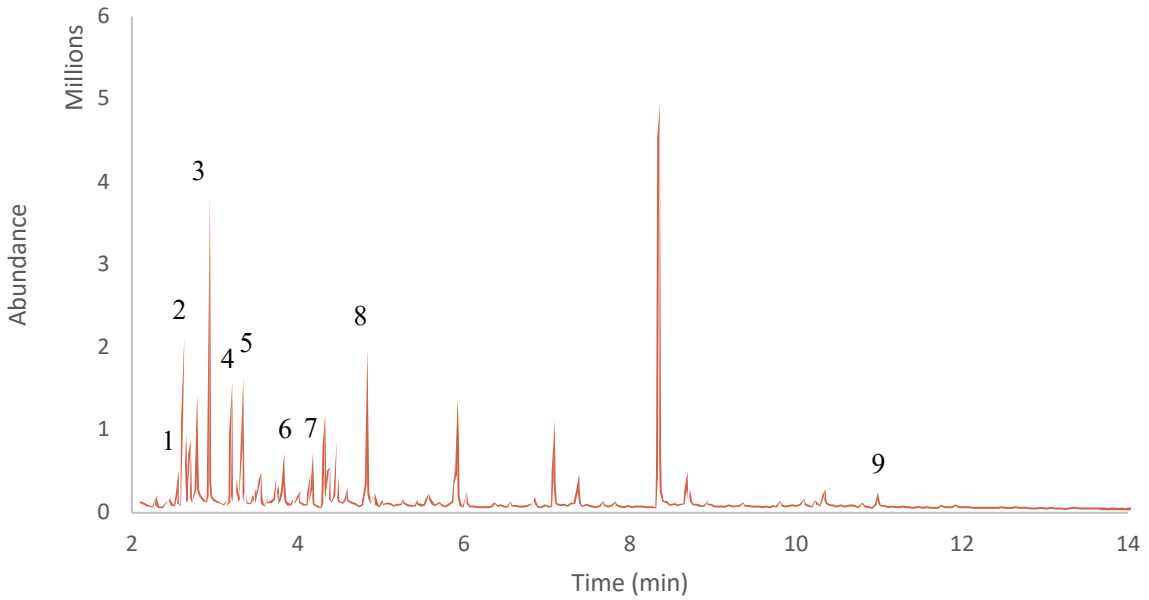
The stacked TIC chromatograms for TV-SPME of saliva underivatized and derivatized are shown in Figure 23. The TIC chromatogram for HS-SPME of artificial saliva is shown in Figure 24. The stacked TIC chromatograms for TV-SPME of artificial saliva underivatized and derivatized are shown in Figure 25. Saliva was spiked with a standard solution of

methamphetamine and THC and analyzed with TV-SPME but neither of the drugs were detected using this method.



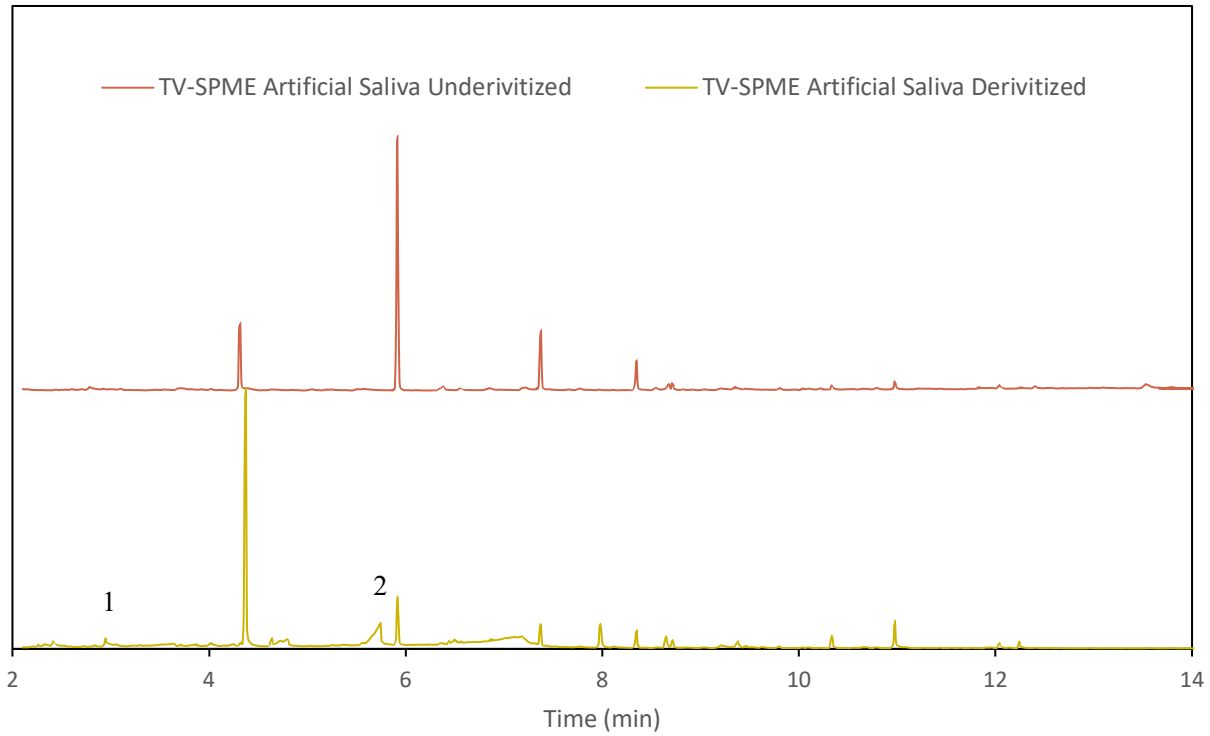
1. Phenol
2. Nonanoic Acid
3. Indole
4. Pentanoic Acid
5. Isopropyl Myristate
6. Phthalic Acid
7. Hexadcanoic Acid methyl ester
8. Dibutyl Phthalate
9. 2,4-dimethyl-pyridine
10. 4-methylvaleric acid trimethylsilyl ester
11. 2-pipridinone

Figure 23. Stacked TIC of real saliva ran with TV-SPME underivatized(red) and real saliva ran with TV-SPME derivatized (yellow).



1. Propyl-benzene
2. 1-ethyl-3-methyl-benzene
3. 1,2,3-trimethyl-benzene
4. Mesitylene
5. Indane
6. O-cymene
7. 1,2,3,4,5-tetramethyl benzene
8. Napthalene
9. Dibutyl phthalate

Figure 24. TIC of artificial saliva ran with HS-SPME.



1. 2,4,6-trimethyl-pyridine
2. Nonanoic acid

Figure 25. Stacked TIC of artificial saliva ran with TV-SPME underivatized(red) and artificial saliva ran with TV-SPME derivatized (yellow).

Table 4. Names, structures, and purpose of compounds found in Real Saliva samples using TV-SPME GC-MS.

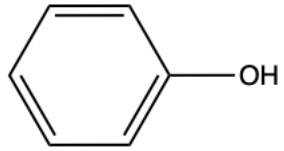
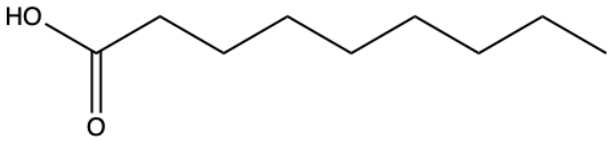
Compound	Structure
Phenol	
Nonanoic Acid	

Table 4 continued

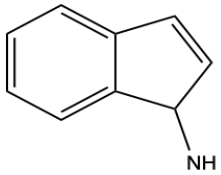
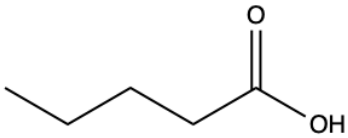
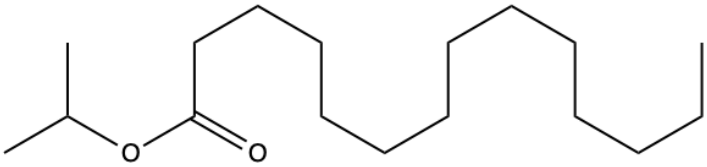
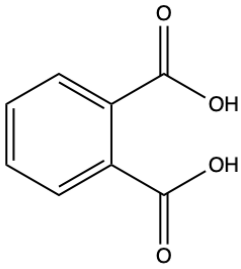
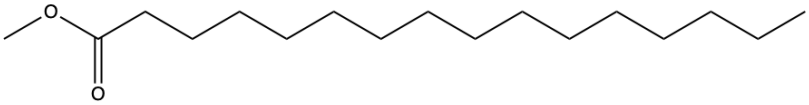
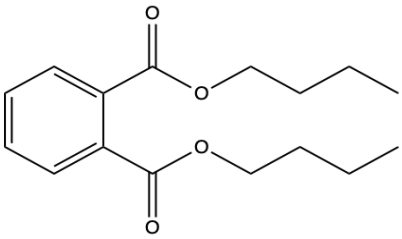
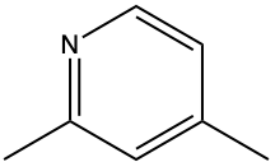
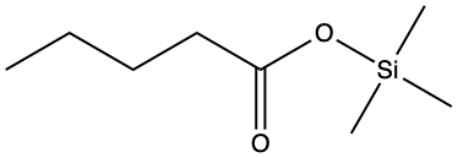
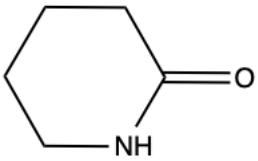
Indole	
Pentanoic Acid	
Isopropyl Myristate	
Phthalic Acid	
Hexadcanoic Acid methyl ester	
Dibutyl Phthalate	
2,4-dimethyl-pyridine	

Table 4 continued

4-methylvaleric acid trimethylsilyl ester	
2-piperidinone	

Phenol and indole are both naturally occurring compounds that can be found in saliva due to the metabolism of tyrosine, phenylalanine and tryptophan by bacteria.[67] Nonanoic acid, pentanoic acid, Hexadcanoic Acid methyl ester, 4-methylvaleric acid trimethylsilyl ester are naturally occurring fatty acids that can be found in saliva due to eating foods that contain these compounds.[68] Isopropyl Myristate is composed of isopropyl alcohol and myristic acid that can be found in saliva due to its presence in mouth wash.[69] Phthalic acid and dibutyl phthalate are metabolites of phthalate, which is a plasticizer. Phthalic acid and dibutyl phthalate can be found in saliva due to environmental contaminations such as plastic toothbrushes and/or straws being inside the mouth or personal care products getting into the mouth.[70] 2,4-dimethyl-pyridine is an organic compound that has not been identified in saliva before but can be found in soil or coal so could be present due to environmental pollution.[71] 2-piperidinone is an organic compound which has been found in saliva but the reason has not been determined. It is speculated that it is due to the metabolism of cadaverine which is a diamine formed by the degradation of lysine in the body from exercise.[72]

Conclusions

Saliva was successfully analyzed using TV-SPME with and without derivatization, showing the presence of phenol, nonanoic acid, indole, pentanoic acid, isopropyl myristate, phthalic acid, dibutyl phthalate, and 2-piperidinone which corresponds with the literature on saliva. Artificial

saliva was successfully analyzed using HS-SPME without derivatization and successfully analyzed using TV-SPME with and without derivatization.

REFERENCES

1. Hickey, L. Automated Derivatization and Identification of Controlled Substances Via Total Vaporization Solid Phase Microextraction (TV-SPME) and Gas Chromatography-Mass Spectrometry (GC-MS). IUPUI, Indianapolis, 2018.

2. Rather, M. A., Dar, B. A., Sofi, S. N., Tauheeda, H., Nasir, A., Lone, A. H. Headspace solid phase microextraction (HS SPME) gas chromatography mass spectrometric analysis of the volatile constituents of Cannabis sativa L. from Kashmir. *J Pharm Res*, **2011,** *4*(8), 2651-2653.

3. Pellati, F., Brighenti, V., Sperlea, J., Marchetti, L., Bertelli, D., Benvenuti, S. New methods for the comprehensive analysis of bioactive compounds in Cannabis sativa L.(hemp). *Molecules*, **2018,** *23*(10), 2639.

4. Calvi, L., Pentimalli, D., Panseri, S., Giupponi, L., Gelmini, F., Beretta, G., Giorgi, A. Comprehensive quality evaluation of medical Cannabis sativa L. inflorescence and macerated oils based on HS-SPME coupled to GC–MS and LC-HRMS (q-exactive orbitrap®) approach. *Journal of pharmaceutical and biomedical analysis*, **2018,** *150*, 208-219.

5. Lachenmeier, D. W., Kroener, L., Musshoff, F., Madea, B. Determination of cannabinoids in hemp food products by use of headspace solid-phase microextraction and gas chromatography–mass spectrometry. *Analytical and bioanalytical chemistry*, **2004,** *378*(1), 183-189.

6. Davis, K. E., Hickey, L. D., Goodpaster, J. V. Detection of ɣ-hydroxybutyric acid (GHB) and ɣ-butyrolactone (GBL) in alcoholic beverages via total vaporization solid-phase microextraction (TV-SPME) and gas chromatography–mass spectrometry. *Journal of Forensic Sciences*.

7. Davis, K. Detection of Illicit Drugs in Various Matrices Via Total Vaporization Solid-Phase Microextraction (TV-SPME). IUPUI, Indianapolis, 2019.

8. Rainey, C. L., Bors, D. E., Goodpaster, J. V. Design and optimization of a total vaporization technique coupled to solid-phase microextraction. *Analytical chemistry*, **2014,** *86*(22), 11319-11325.

9. Kranz, W., Carroll, C., Dixon, D., Picard, C., Goodpaster, J. Optimization of total vaporization solid-phase microextraction (TV-SPME) for the determination of lipid profiles of *Phormia regina*, a forensically important blow fly species. *Analytical and bioanalytical chemistry*, **2017,** *409*(27), 6349-6357.

10. Sauzier, G., Bors, D., Ash, J., Goodpaster, J. V., Lewis, S. W. Optimisation of recovery protocols for double-base smokeless powder residues analysed by total vaporisation (TV) SPME/GC-MS. *Talanta*, **2016,** *158*, 368-374.

11. Bors, D., Goodpaster, J. Mapping explosive residues on galvanized pipe bomb fragments using total vaporization solid phase microextraction (TV-SPME). *Analytical Methods*, **2015,** *7*(23), 9756-9762.

12. Charles, D. J., and Simon, J.E. Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. *Journal of the American Society for Horticultural Science* **1990,** *115.3*, 458-462.

13. Sangwan, N., Farooqi, A., Shabih, F. *et al.* Regulation of essential oil production in plants. *Plant Growth Regulation* **2001,** *34*, 3–21.

14. Nerio, L.S., Olivero-Verbel, J. and Stashenko, E., Repellent activity of essential oils: a review. *Bioresource technology* **2010,** *101*(1), 372-378.

15. Preedy, Victor R., ed. *Essential oils in food preservation, flavor and safety*. Academic Press, 2015.

16. Baron, E. P. Medicinal properties of cannabinoids, terpenes, and flavonoids in cannabis, and benefits in migraine, headache, and pain: an update on current evidence and cannabis science. *Headache: The Journal of Head and Face Pain*, **2018,** *58*(7), 1139-1186.

17. Turek, C., Stintzing, F. C. Stability of essential oils: a review. *Comprehensive reviews in food science and food safety*, **2013,** *12*(1), 40-53.

18. Substance Abuse Center for Behavioral Health Statistics and Quality. Results from the 2015 National Survey on Drug Use and Health: Detailed Tables. SAMHSA. https://www.samhsa.gov/data/sites/default/files/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015.pdf. Published September 8, 2016.

19. Pertwee R, ed. *Handbook of Cannabis*. Oxford University Press; 2014. https://oxford.universitypressscholarship.com/view/10.1093/acprof:oso/9780199662685.001.0001/acprof-9780199662685. Accessed March 12, 2021.

20. ElSohly, M. A., Slade, D. Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life sciences*, **2005,** *78*(5), 539-548.

21. Ahmed, S. A., Ross, S. A., Slade, D., Radwan, M. M., Khan, I. A., ElSohly, M. A. Minor oxygenated cannabinoids from high potency Cannabis sativa L. *Phytochemistry*, **2015,** *117*, 194-199.

22. Burgdorf, J. R., Kilmer, B., Pacula, R. L. Heterogeneity in the composition of marijuana seized in California. *Drug and alcohol dependence*, **2011,** *117*(1), 59-61.

23. Grotenhermen, F., Russo, E. *Cannabis and cannabinoids: pharmacology, toxicology, and therapeutic potential*. Psychology Press, **2002.**

24. Aizpurua-Olaizola, O., Omar, J., Navarro, P., Olivares, M., Etxebarria, N., Usobiaga, A. Identification and quantification of cannabinoids in Cannabis sativa L. plants by high performance liquid chromatography-mass spectrometry. *Analytical and bioanalytical chemistry*, **2014,** *406*(29), 7549-7560.

25. McPartland, J. M., Russo, E. B. (2001). Cannabis and cannabis extracts: greater than the sum of their parts? *Journal of Cannabis Therapeutics*, **2001,** *1*(3-4), 103-132.

26. Russo, E. B. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *British journal of pharmacology*, **2011,** *163*(7), 1344-1364.

27. White, C. M. A review of human studies assessing cannabidiol's (CBD) therapeutic actions and potential. *The Journal of Clinical Pharmacology*, **2019,** *59*(7), 923-934.

28. Hazekamp, A. The trouble with CBD oil. *Medical cannabis and cannabinoids*, **2018,** *1*(1), 65-72.

29. Romano, L. L., Hazekamp, A. Cannabis oil: chemical evaluation of an upcoming cannabis-based medicine. *Cannabinoids*, **2013,** *1*(1), 1-11.

30. Mead, A. The legal status of cannabis (marijuana) and cannabidiol (CBD) under US law. *Epilepsy & Behavior*, **2017,** *70*, 288-291.

31. Telci, I., Bayram, E., Yılmaz, G., Avcı, B. Variability in essential oil composition of Turkish basils (*Ocimum basilicum L.*). *Biochemical Systematics and Ecology*, **2006,** *34*(6), 489-497.

32. Marotti, M., Piccaglia, R., Giovanelli, E. Differences in essential oil composition of basil (Ocimum basilicum L.) Italian cultivars related to morphological characteristics. *Journal of Agricultural and Food Chemistry*, **1996,** *44*(12), 3926-3929.

33. Noriega, P. Terpenes in Essential Oils: Bioactivity and Applications. In *Terpenes and Terpenoids*. IntechOpen. **2020.**

34. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids, 2005. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to Flavouring Group Evaluation 7 (FGE.07): Saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids from chemical group 5. *EFSA Journal*, **2005,** *3*(2),164, 62.

35. Molina-Gutiérrez, S., Ladamiral, V., Bongiovanni, R., Caillol, S., Lacroix-Desmazes, P. Emulsion Polymerization of Dihydroeugenol-, Eugenol-, and Isoeugenol-Derived Methacrylates. *Industrial & Engineering Chemistry Research,* **2019,** *58*(46), 21155-21164.

36. Good Scents Company, Dihydroeugenol, In *Fragrance Demo Formulas*. http://www.thegoodscentscompany.com/demos

37. Zhang, D., Wang, P., Mao, Z. *et al.* Stable carbon isotope composition of monoterpenes in essential oils and crude oils. *Sci. China Earth Sci.* **2010,** *53*, 522-528.

38. Acetyleugenol. PubChem: U.S. National Library of Medicine.

39. Ethyl Vanillin. PubChem: U.S. National Library of Medicine.

40. Elzinga, S., Fischedick, J., Podkolinski, R., Raber, J. C. Cannabinoids and terpenes as chemotaxonomic markers in cannabis. *Nat. Prod. Chem. Res*, **2015,** *3*(81), 10-4172.

41. Hanuš, L. O., Hod, Y. Terpenes/Terpenoids in Cannabis: Are They Important? *Medical Cannabis and Cannabinoids*, **2020,** *3*(1), 25-60.

42. Dibutyl phthalate. PubChem: U.S. National Library of Medicine.

43. Mudge, E. M., Murch, S. J., Brown, P. N. Leaner and greener analysis of cannabinoids. *Analytical and bioanalytical chemistry*, **2017,** *409*(12), 3153-3163.

44. Lee, Y. R., Xia, L. Efficient one-pot synthetic approaches for cannabinoid analogues and their application to biologically interesting (−)-hexahydrocannabinol and (+)-hexahydrocannabinol. *Tetrahedron Letters*, **2008,** *49*(20), 3283-3287.

45. De Zeeuw, R. A., Wijsbeek, J., Malingre, T. M. Interference of alkanes in the gas chromatographic analysis of Cannabis products. *Journal of Pharmacy and Pharmacology*, **1973,** *25*(1), 21-26.

46. Palmitic Acid. PubChem. U.S. National Library of Medicine.

47. Verma, R. S., Rahman, L., Verma, R. K., Chauhan, A., Yadav, A. K., Singh, A. Essential oil composition of menthol mint (Mentha arvensis) and peppermint (Mentha piperita) cultivars at different stages of plant growth from Kumaon region of Western Himalaya. *Open Access Journal of Medicinal and Aromatic Plants*, **2010,** *1*(1), 13.

48. Glycerol Tricaprylate. PubChem. U.S. National Library of Medicine.

49. Shannon, S., Opila-Lehman, J. Effectiveness of cannabidiol oil for pediatric anxiety and insomnia as part of posttraumatic stress disorder: a case report. *The Permanente Journal*, **2016,** *20*(4).

50. World Drug Report 2019-Booklet 5 Cannabis and Hallucinogens. United Nations Office on Drugs and Crime, 2019, p https://wdr.unodc.org

51. Camp, N. E. Synthetic cannabinoids. *Journal of Emergency Nursing*, **2011,** *37*(3), 292-293.

52. Zuba, D., Byrska, B., Maciow, M. Comparison of "herbal highs" composition. *Analytical and bioanalytical chemistry,* **2011,** *400*(1), 119–126.

53. Mills, B., Yepes, A., Nugent, K. Synthetic cannabinoids. *The American journal of the medical sciences*, **2015,** *350*(1), 59-62.

54. Cox, A. O., Daw, R. C., Mason, M. D., Grabenauer, M., Pande, P. G., Davis, K. H., Huffman, J. W. Use of SPME-HS-GC–MS for the analysis of herbal products containing synthetic cannabinoids. *Journal of analytical toxicology*, **2012,** *36*(5), 293-302.

55. Emerging Threat Report: Annual 2018. Drug Enforcement Administration Special Testing and Research Laboratory: U.S. Department of Justice, 2018, p https://www.dea.gov

56. Uchiyama, N., Kikura-Hanajiri, R., Ogata, J., Goda, Y. Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. *Forensic science international*, **2010,** *198*(1-3), 31-38.

57. Hold, K. M., de Boer, D., Zuidema, J., Maes, R. A. Saliva as an analytical tool in toxicology. *Int J Drug Test*, **1996,** *1*(1), 1-36.

58. Huda, A. K., de Lacy Costello, B., Ratcliffe, N. An investigation of volatile organic compounds from the saliva of healthy individuals using headspace-trap/GC-MS. *Journal of breath research*, **2013,** *7*(3), 036004.

59. Drummer, O. H. Drug testing in oral fluid. *Clinical Biochemist Reviews*, **2006,** *27*(3), 147.

60. Fucci, N., De Giovanni, N., Chiarotti, M. Simultaneous detection of some drugs of abuse in saliva samples by SPME technique. *Forensic science international*, **2003,** *134*(1), 40-45.

61. Hall, B. J., Satterfield-Doerr, M., Parikh, A. R., Brodbelt, J. S. Determination of cannabinoids in water and human saliva by solid-phase microextraction and quadrupole ion trap gas chromatography/mass spectrometry. *Analytical chemistry*, **1998,** *70*(9), 1788-1796.

62. Yonamine, M., Tawil, N., de Moraes Moreau, R. L., Silva, O. A. Solid-phase micro-extraction–gas chromatography–mass spectrometry and headspace-gas chromatography of tetrahydrocannabinol, amphetamine, methamphetamine, cocaine and ethanol in saliva samples. *Journal of Chromatography B*, **2003,** *789*(1), 73-78.

63. Segura, J., Ventura, R., Jurado, C. Derivatization procedures for gas chromatographic–mass spectrometric determination of xenobiotics in biological samples, with special attention to drugs of abuse and doping agents. *Journal of Chromatography B: Biomedical Sciences and Applications*, **1998,** *713*(1), 61-90.

64. Danielson, N. D., Gallagher, P. A., Bao, J. J. Chemical reagents and derivatization procedures in drug analysis. *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation*. **2006.**

65. Lin, D. L., Wang, S. M., Wu, C. H., Chen, B. G., Liu, R. H. Chemical derivatization for the analysis of drugs by GC-MS--A conceptual review. *Journal of Food and Drug Analysis*, **2008,** *16*(1).

66. Moore, C., Coulter, C., Uges, D., Tuyay, J., Van der Linde, S., Van Leeuwen, A., Orbita Jr, J. Cannabinoids in oral fluid following passive exposure to marijuana smoke. *Forensic Science International*, **2011,** *212*(1-3), 227-230.

67. Smith, E. A, Macfarlane, G. T. Formation of phenolic and indolic compounds by anaerobic bacteria in the human large intestine *Microb. Ecol.,* ***1997,*** 33, 180–188

68. Actis, A. B., Perovic, N. R., Defagó, D., Beccacece, C., Eynard, A. R. Fatty acid profile of human saliva: a possible indicator of dietary fat intake. *Archives of oral biology*, **2005,** *50*(1), 1-6.

69. Isopropyl Myristate. PubChem. U.S. National Library of Medicine.

70. Silva, M. J., Reidy, J. A., Samandar, E., Herbert, A. R., Needham, L. L., Calafat, A. M. Detection of phthalate metabolites in human saliva. *Archives of toxicology*, **2005,** *79*(11), 647-652.

71. Sims, G. K., Sommers, L.E. "Degradation of pyridine derivatives in soil". *Journal of Environmental Quality*. **1985,** *14*(4), 580–584.

72. Gardner, A., Carpenter, G., So, P. W. Salivary metabolomics: From diagnostic biomarker discovery to investigating biological function. *Metabolites*, **2020,** *10*(2), 47.