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Experimental Challenges in Chemical Analysis of CBD and THC in Hemp Oil via Gas Chromatography and Mass Spectrometry

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by

Emily Hsu

An Abstract of a Project
in
Forensic Science

Submitted in Partial Fulfillment
of the Requirements
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Department of Chemistry
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State University of New York

ABSTRACT

Recently, there has been a sharp rise in the use of cannabis products in the United States of America. This is largely due to decriminalization and legalization of marijuana across many states. However, marijuana remains illegal on the federal level because it contains the psychoactive component, Δ^9-tetrahydrocannabinol (THC). THC is currently listed as a schedule I drug by Drug Enforcement Agent (DEA), meaning there is no accepted medical use, but it has a high potential for abuse. Therefore, cannabis products such as hemp oil sold in the United States cannot a concentration greater than 0.3% THC.

The goal of this research project is to examine whether 5 commercial hemp oil products have less than the allowed THC concentration and determine the concentration of cannabidiol (CBD) via gas chromatography (GC) with flame ionization detector (FID) and mass selective detector (MSD). This research project described several experimental challenges of chemical analysis of CBD and THC in hemp oil via GC and development of experimental methods to quantify target compounds. Some experimental challenges described in this project are septum bleeding, degradation of target compounds, and decarboxylation of precursors. By the use of improved analytical method, CBD and THC in five hemp oil products were analyzed. It was found that CBD concentrations were significantly lower than advertised on the product label, and no traces of THC were detected from all samples.

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List of Abbreviations

AC = Alternate Current

CBD = Cannabidiol

CBN = Cannabinol

CNS = Central Nervous System

CSA = Controlled Substance Act

DC = Direct Charge

DEA = Drug Enforcement Agent

FDA = United States Food and Drug Administration

GC-FID = Gas Chromatography- Flame Ionization Detector

GC-MS = Gas Chromatography- Mass Spectrometry

GC-MSD = Gas Chromatography – Mass Selective Detector

ISD = Internal Standard

MSD = Mass Selective Detector

m/z = Mass to Charge Ratio

ng = Nanogram

PA = Peak Area

PTSD = Post Traumatic Stress Disorder

RPM = Revolutions Per Minute

R^2 = Linear Regression

RT = Retention Time

VOC = Volatile Organic Compound

μg = Microgram

°/min = Degree per Minute

Δ^9-THC = Delta Nine Tetrahydrocannabinol

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I. Introduction and Background

1.1 Motivation and Objective

The use of hemp and marijuana in the United States can be traced back to the 1600's. Hemp in particular was a highly coveted agriculture because of its versatility. It was often used to make clothing, rope and sails during those times. Marijuana was more widely used for medicinal purposes, such as pain relief, appetite stimulation, and counter opioid withdrawal. For nearly a century, marijuana was an ingredient used in a variety of medicine until the early 20th century. In 1910, Mexican immigrants sought refuge from the Mexican revolution and introduced recreational marijuana to the United States. As a result, Americans began to associate marijuana with the influx of Mexican immigrants. This led to growing racism in the United States and eventually the illegalization of cannabis.

The Marijuana Tax Laws of 1937 was the first step to criminalizing cannabis nationwide. It placed taxes on the possession, sale and trading of any cannabis products in the United States; this included both hemp and marijuana. During this time, fear of drugs was becoming more prevalent in the United States, and President Nixon declared a "war on drugs." The declaration imposed harsher drug laws, such as mandatory prison sentences, to mitigate public fear. He also created the Controlled Substance Act (CSA) of 1970, which allowed the federal government to regulate controlled substances [1]. The CSA separated known drugs into five different classification schedules. Depending on which classification schedule the drug was placed under, it would regulate how the substance was distributed, manufactured, and used. The CSA categorized the drugs based on their potential for abuse and addiction and whether they have legitimate medical use. Drugs, such as heroin, that have a high potential for abuse with no accepted medical use would be classified as a schedule I drugs. Whereas, Robutussin, a common cough syrup, would be classified as a schedule IV drug with low potential for abuse and accepted medical usage.

Following the development of the CSA, cannabis, hemp and marijuana, were immediately deemed as a schedule I drug. This made it difficult for scientists and doctors to study the plant for medicinal purposes. It was not until 1996 when California became the first state to legalize marijuana for medical use and shortly afterwards, Colorado became the first state to legalize recreational use of marijuana. Finally, in 2018, hemp was officially removed from the CSA via the Hemp Farming Act of 2018. However, to this day, marijuana remains listed as a schedule I drug.

As of 2021, 14 states have legalized the recreational use of marijuana and 16 states have decriminalized its use (Figure 1). Over 20 states allow for the sale of cannabis for medicinal use, whether through injection, inhalation or prescription medicine. Marijuana remains illegal under the federal law, but state marijuana laws are continuously changing.

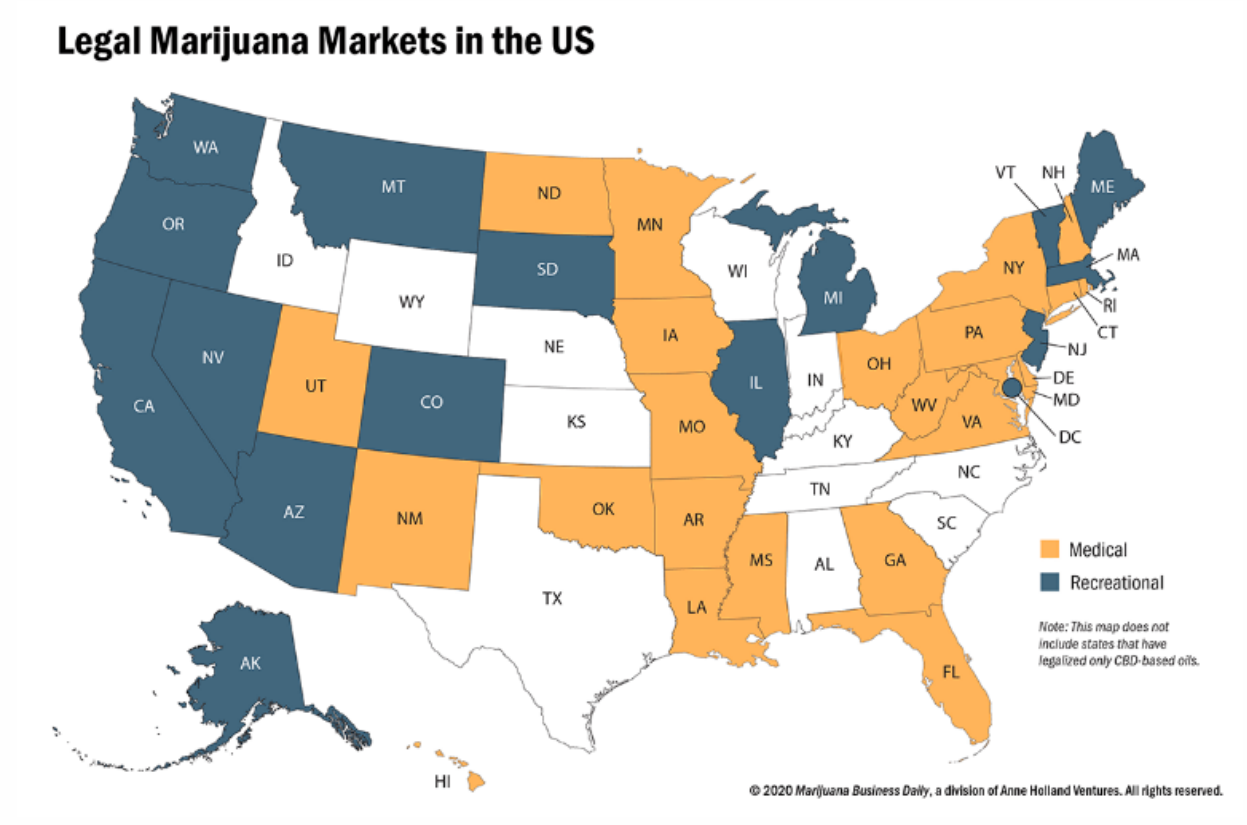

Figure 1. Legal status for marijuana across the United States. Obtained from Marijuana Policy Project.

It is a common misconception that marijuana and hemp are two different species of plant. However, they are in fact just two different names for *Cannabis sativa L.*, a flowering plant in the *Cannabaceae* family. Although science does not differentiate between "marijuana" and "hemp," the law separates the two based on the level of tetrahydrocannabinol (THC) present. Federal law defines hemp as "the plant *Cannabis sativa L.* that contains 0.3% or less THC content by dry weight," [2] whereas marijuana is any cannabis that contains over 0.3% THC content.

The main reason for the two plants to be differentiated legally, is because consumption of THC to a certain amount can cause psychological effects. *Cannabis sativa L.* is comprised of over 100 cannabinoids, but THC is the primary psychoactive component. Routine marijuana smokers have shown signs of "subtle working memory impairment,"

mood swings and an altered sense of time [3]. Working memory refers to the ability to store and manipulate the information to produce a response. For instance, an individual under the influence of marijuana would have more difficulty reciting a particular sentence backwards compared to a sober individual.

As more states legalize and decriminalize marijuana, many companies are eager to introduce new innovational CBD and hemp products into the market. Since Colorado legalized recreational marijuana in 2012, CBD products have saturated the market in various forms including oils, edibles, vaporizers, creams etc. It is now very common to see these products sold all across the U.S. and even for online purchase. Currently, in New York, CBD products derived from marijuana are considered illegal. However, CBD products derived from hemp can be sold as long as they follow state regulations. The Department of Health allows for the "intermediate sales of hemp extract containing up to 3.0% THC…provided that the sale is between licensed processors in New York State" [10]. However, hemp extract products are not required to be labeled with the concentration of CBD in the product. In comparison, products extracted from marijuana are legally obligated to disclose the amount of milligrams of THC and CBD per serving [10].

The main focus of this study was to determine if five commercial hemp oil follow federal and New York state regulations; in addition to whether the amount of CBD was reflective of the amount printed on the product label. This study also addressed the challenges faced when performing chemical analysis of CBD and THC via gas chromatography.

In the current study, both GC-FID and GC-MSD were utilized to analyze CBD compound in hemp oil samples. The reasoning behind using both GC-FID and GC-MSD was because the GC-FID produces a stronger signal and is more sensitive for quantitative analysis. While the GC-MSD provides qualitative information, such as chemical structure of the compound [11]. The GC-MSD is also useful in identifying any unknown compound that

may be present in the hemp oil sample matrix. With the allowance of up to 0.3% THC, this quantitative experiment served to determine if there were any evidence of THC violation and whether the concentration printed on the label refers to CBD concentration.

1.2 Endocannabinoid and Phytocannabinoid

In the past, cannabinoids were thought to be naturally occurring compounds derived solely from the *Cannabis sativa L.* plant. However, in 1990, the cannabinoid 1 (CB1) receptor in the human body was discovered by Allyn Howlett and William Devane. Shortly after, the endocannabinoid system was discovered in 1992. The finding of the Endocannabinoid system was significant because it implied that the human body produces its own cannabinoids similar to the ones produced by cannabis. Cannabinoids produced by plants are called phytocannibinoids and cannabinoids produced by mammals are called endocannabinoids. Phytocannabinoids react to the endocannabinoid receptors in the body [4] and could be the reasoning behind why THC and CBD derived from marijuana have such a strong effect on humans. The main purpose of the endocannabinoid system is to help the body maintain homeostasis. There are two main cannabinoid receptors, CB1 and cannabinoid receptor 2 (CB2). CB1 is most abundant in the Central Nervous System (CNS), whereas CB2 is primarily located on immunological tissues. Since these cannabinoid receptors are ubiquitous throughout the human body, it explains why smoking marijuana can affect cognition, mood, pain, appetite, and nausea. Since the human body already utilizes endocannabinoids to maintain different bodily functions, it naturally follows that phytocannabinoid will also affect the same bodily functions. Although there are many other cannabinoid receptors, CB1 and CB2 receptors are the most studied.

1.3 Mechanism of THC and CBD

CBD is an isomer of THC, with both sharing the same molecular weight of 314

g/mol. THC is the primary psychoactive component in *Cannabis sativa L.* and is responsible for the 'high' people experience when smoking marijuana. In comparison, CBD does not produce any psychoactive effect. This could be due to the differences in chemical structure and how they interact with the receptors in the body. Although they are both comprised of the same molecular formula, CBD has a hydroxyl group and THC has a cyclic ring (Figure 2). The broken ring in CBD allows it to bend in the 3-dimension, whereas THC has a more rigid and flat structure.

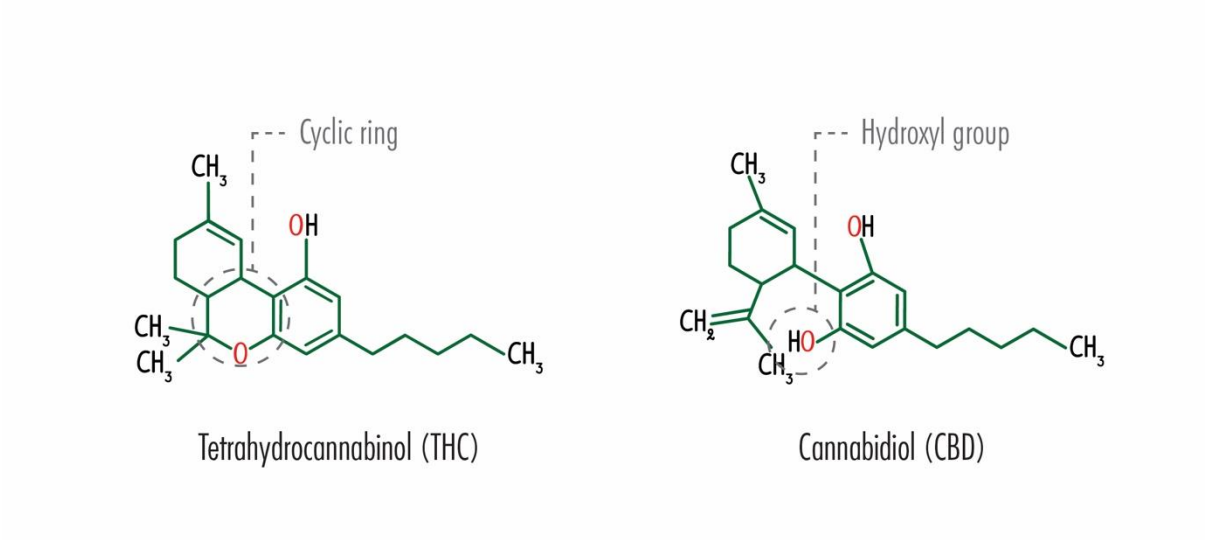

Figure 2. Chemical structure of THC and CBD.

There are currently 113 known phytocannabinoids, but CBD and THC are by far the two most studied phytocannabinoid. Cannabinoids are composed of a phenol group and a 5-carbon chain. When cannabis is consumed, CBD and THC binds to either CB1 or CB2. However, THC has a higher affinity to bind to CB1, which is located primarily in the central nervous system. Since THC is a partial agonist, it stimulates the CB1 receptors to produce psychological effects. This response includes that overwhelming euphoric feeling marijuana users get addicted to. One possible explanation why CBD does not exhibit the same psychotropic effect as THC is that CBD is a negative allosteric modulator [4]. A negative

allosteric modulator alters the shape of the receptor by binding to a secondary site on the receptor, thus making it difficult for CB1 agonist, such as THC, to produce a psychotropic response. Since CBD does not actually bind or stimulate the CB1 receptor, it could explain why cannabis users tend to not experience the same 'high' when consuming CBD-only products. The exact mechanism for how THC and CBD interacts with the body is still the subject of ongoing research.

1.4 Analysis of Cannabis Products

Before cannabis products are allowed to enter the market, they must go through a series of tests prior to approval. Cannabis product testing differs from state to state, but the four most common tests are potency testing, residual solvent testing, heavy metal testing and lastly, pesticide testing. Potency testing is the reporting of the dry weight of THC and CBD in the product. This is typically done using high performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS) [6]. Residual solvent tests for any residual solvent that may have been left over in the product during the extraction process. Ethanol, methanol and other organic solvents are the most commonly used solvents in the extraction process for CBD. Testing for these residual solvents is critical in preventing risk of alcohol toxicity and death. Presently, the U.S. Food and Drug Administration (FDA) has established a limit of no more than 200 ppm of ethanol or methanol to be present in any consumable product [7].

In the same sense, cannabis products also need to be tested for any heavy as exposure to high concentration of heavy metals can be lethal. Cannabis products may contain traces of heavy metal because the plant could have taken it in from the soil or fertilizers. Lastly, pesticide testing screens for the presence of any pesticides that may have ended up in the products. But which pesticides to screen for varies greatly across the country. For example, in

California, CBD products must test for 55 different types of pesticides, whereas in Colorado, it is only required to test for 13 types of pesticides.

1.5 Gas Chromatography

Gas chromatography can be coupled with several detectors, but typically organic compounds are analyzed using either flame ionization detector (FID) or mass spectrometry detector (MSD). GC technology started in the 1950's by Anthony T. James and Archer J. P. Martin of the National Institute for Medical Research. The technique was later built upon to accommodate other analytical techniques, such as the mass spectrometry (MS), and eventually evolved into the GC-MSD. Now, the GC-MSD is commonly used for quick chemical analysis in forensics, drug analysis, medical labs, and many more.

In general, chromatography is a laboratory technique used to separate different components of a solution. Once the components are separated, they can be analyzed individually. Typically, for GC, a sample is injected by a syringe through a septum into a heated chamber. The septum serves to seal the injection port and prevent any compounds from leaking out. The heat vaporizes the sample, and the carrier gas pushes the sample into a capillary column. The analytes are then separated in the capillary column. Separation is dependent on the size of the analytes and its affinity to the stationary phase. Analytes that are smaller and have less affinity to the stationary phase will elute faster. Larger analytes with a higher affinity to the stationary phase, meaning it will interact more with the column, will elute slower. As the compounds elute from the column, it is detected by the detector.

1.5.1 *GC-FID Instrumental Operation*

For FID, it detects ions formed through combustion in a hydrogen flame (Figure 3). As compounds elute from the column, hydrogen mixes with the carrier gas containing the compounds. A flame is ignited by burning hydrogen, air and the carrier gas. As the analytes

are burnt by the flame, hydrocarbons will produce ions. These ions are then detected by a collector that sends signals to be converted into peaks.

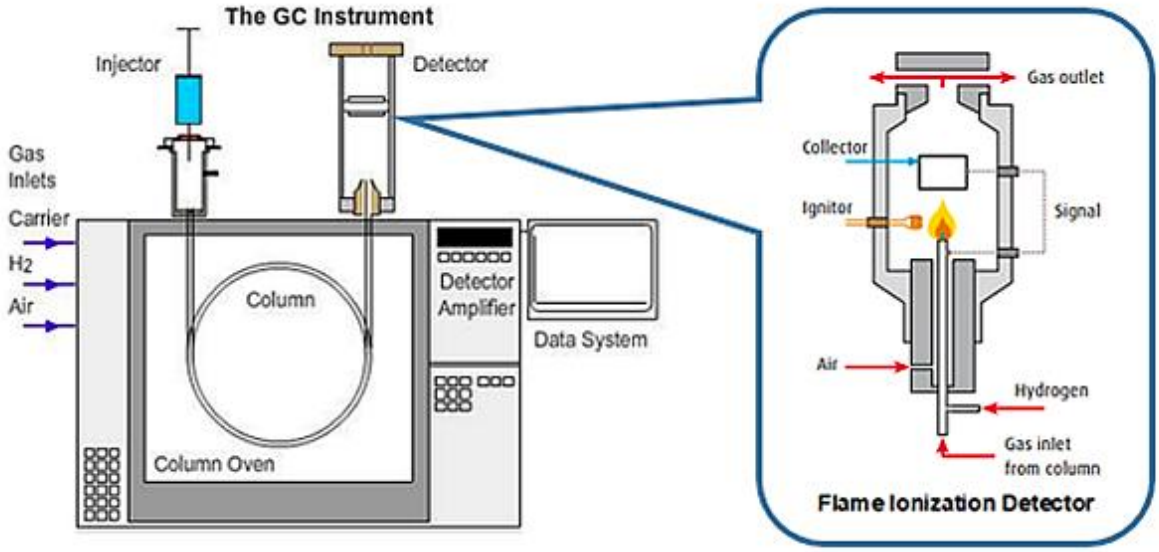

Figure 3. Overview of GC-FID analytical instrument.

1.5.2 *GC-MSD Instrumental Operation*

Similar to the FID, the MSD evaluates the individual compounds that elute out of the GC column. However, instead of passing through a flame, the analytes are bombarded with electrons to break them into molecular ions and fragments of these molecular ions (Figure 4). These ions then travel through a quadruple mass analyzer that filters them based on their size. A quadruple mass analyzer is made of four parallel rods with a space for the central axis to allow ions to travel through [8]. Superimposed direct current (DC) and alternate current (AC) voltages are applied at each opposing pair of electrodes. The DC and AC voltages are varied linearly while keeping the ratio constant. As ions pass through the oscillating electric field, they are filtered based on their mass-to-charge ratio (*m/z*) and only ions with a certain *m/z* will pass through the quadruple to be detected. Ions with a *m/z* outside of the specified

voltage will not be able to pass through the quadruple, and thus will not be detected [9]. After passing through the quadruple, the MSD calculates how many of each ion with a particular mass was present in the sample. This information is presented as mass spectra and compounds can be identified based on the mass spectra produced.

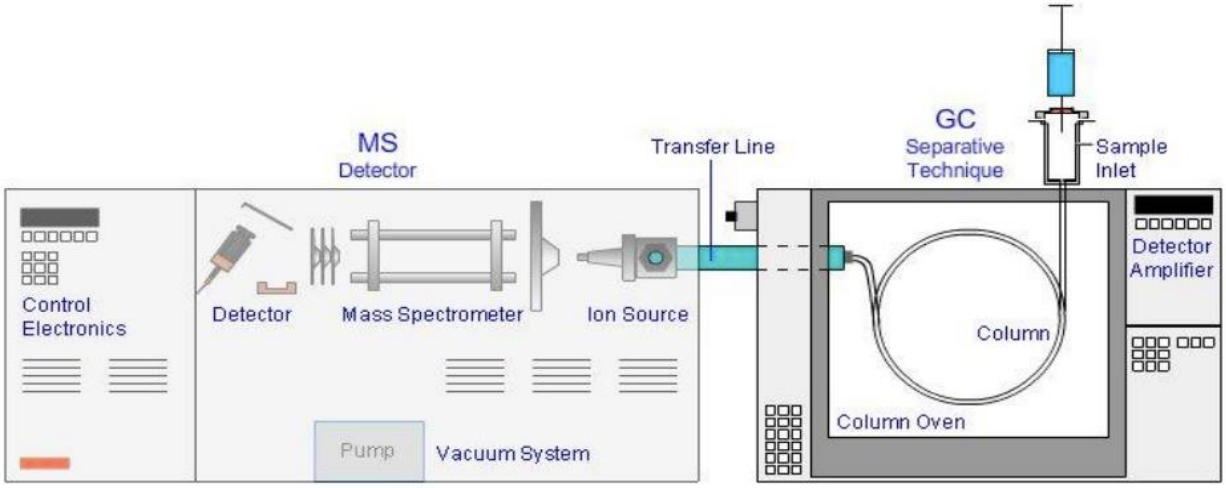

Figure 4. Overview of GC-MSD analytical instrument.

II. Materials and Methods

2.1 Reagents and Standards

THC and CBD were purchased from Cerilliant Corporation (Round Rock, Texas USA) with a 1.000 ± 0.005 mg/mL concentration in methanol (Figure 5). The five commercial hemp oil samples were purchased on Amazon. Most of the hemp oils came in 30 mL amber bottles and were immediately refrigerated upon receiving. Methanol and chloroform were purchased from Thermo Fisher. All solvents are HPLC grade.

Figure 5. Photograph of (a) commercial hemp oil samples and (b) CBD and THC standards in methanol.

2.2 Collection of Samples

In this study, five hempseed oils samples were purchased and tested. The five brands of hemp oil being tested were O'rmeas, Hempio, Hemp Techniques, Greenive, and Zatural. All five hemp oils were sourced from the United States of America. O'rmeas, Hemp Technique, Greenive and Zatural listed hemp seed oil as their sole ingredient in their product. Hempio's ingredient list included hemp seed oil, vitamin A, vitamin E, and vitamin C. The samples varied from 300 mg to 35,000 mg per bottle according to the label.

2.3 Sample Preparation and Extraction

An external calibration curve was created for both CBD and THC. The stock solutions were purchased at a concentration of 1.000 ± 0.005 mg/mL (~1000 ppm). The solutions were then further diluted to 5 ppm, 10 ppm, 50 ppm, 100 ppm, and 250 ppm and these became the calibration standards. The calibration standards were used to generate the calibration curve for the GC-FID. The standards were diluted with HPLC-grade methanol. After the dilutions, 1 µL was injected into the analytical system. Each sample was analyzed in quadruplicate.

The hemp oil samples were diluted with chloroform because they were not miscible with methanol. Initially, the hemp oil samples were diluted by a factor of 5; however, two of

the five samples did not fall within the calibration range. Therefore, they were further diluted by a factor of 50 as shown in Table 1. Lastly, 1 μL aliquot was injected into the GC-FID for analysis. All solutions were kept in amber bottles and stored in a refrigerator set to - 20 °C.

Table 1. Dilution factors of hemp oil samples.

Sample	Dilution
Hempio	1:5
Hemp Technique	1:5
O'Rmeas	1:5
Greenive	1:50
Zatural	1:50

2.4 Instrumental Parameters

Two distinct gas chromatography (Agilent Technologies 7890A) instruments were used, one was coupled with the internal FID and the other was coupled with the MSD (Agilent Technologies 5975C). Both used a 5% phenyl methyl siloxane capillary (HP-5MS) column and helium was the carrier gas. The GC-FID used a HP-5MS, 30 m × 0.25 mm × 0.25 μm column and the GC-MS used a HP-5MS, 30 m × 0.25 mm × 1 μm column. Each sample was introduced into a heated inlet set to 260 °C to ensure the sample was completely vaporized prior to entering the column. For the GC-MSD, the final temperature of 300 °C was held for 2 min longer than the GC-FID because the GC-MSD had a slightly thicker stationary phase compared to the one installed in the GC-FID. The GC's oven temperatures were optimized for each instrument to achieve complete separation during the elution phase

(Table 2-3). Since the GC-MSD had a larger column compared to the GC-FID, the GC-MSD had a higher flow rate of 2.5 mL/min and a split ratio of 10:1; whereas the GC-FID had a flow rate of 1.5 mL/min and a split ratio of 25:1. The injection volume for both instruments were 1 μL aliquots. After compounds eluted from the GC column, they were bombarded with a 70-eV electron bean that fragmented the compounds in the MSD. The quadrupole was set to 150 °C while it sorted out ions before detection. The ions monitored for the GC-MSD were at m/z 299 and 314 for THC, and m/z 231 and 246 for CBD [12].

Table 2. GC-FID oven temperature.

Temperature (°C)	Rate of Increase (°C/min.)	Hold time (min.)
160 °C	-	1
190 °C	15	1
300°C	20	2

Table 3. GC-MSD oven temperature.

Temperature (°C)	Rate of Increase (°C/min.)	Hold time (min.)
160 °C	-	1
190 °C	15	1
300°C	20	4

2.5 Septa Experimental Details

Three different septa (Figure 6) were tested at three different inlet temperatures to determine which septum showed the least amount of bleeding and interference. The three septa tested were a red septum from Agilent Technologies (inlet septa, general purpose, red 5mm), a teal septum from Restek (Thermolite® septa), and a gray septum from Agilent Technologies (inlet septa, general purpose, gray 5mm). The experiment was conducted on the GC-FID (Agilent Technologies 7890A) under the same parameters presented in Table 2. Each septum was tested at in inlet temperature of 220 °C, 260 °C, and 300 °C. Once a septum was installed, the method was immediately run to examine septum bleeding effect before conditioning. This was done to gauge how effective conditioning a septum was in preventing septum bleeding. All GC measurements were conducted at each of the three inlet temperature and conditioned for 30 hrs ± 5 min at 300 °C. The start and end times for conditioning are shown in Table 4. Following conditioning, each septum was rerun at the three different inlet temperatures using the same method as before conditioning.

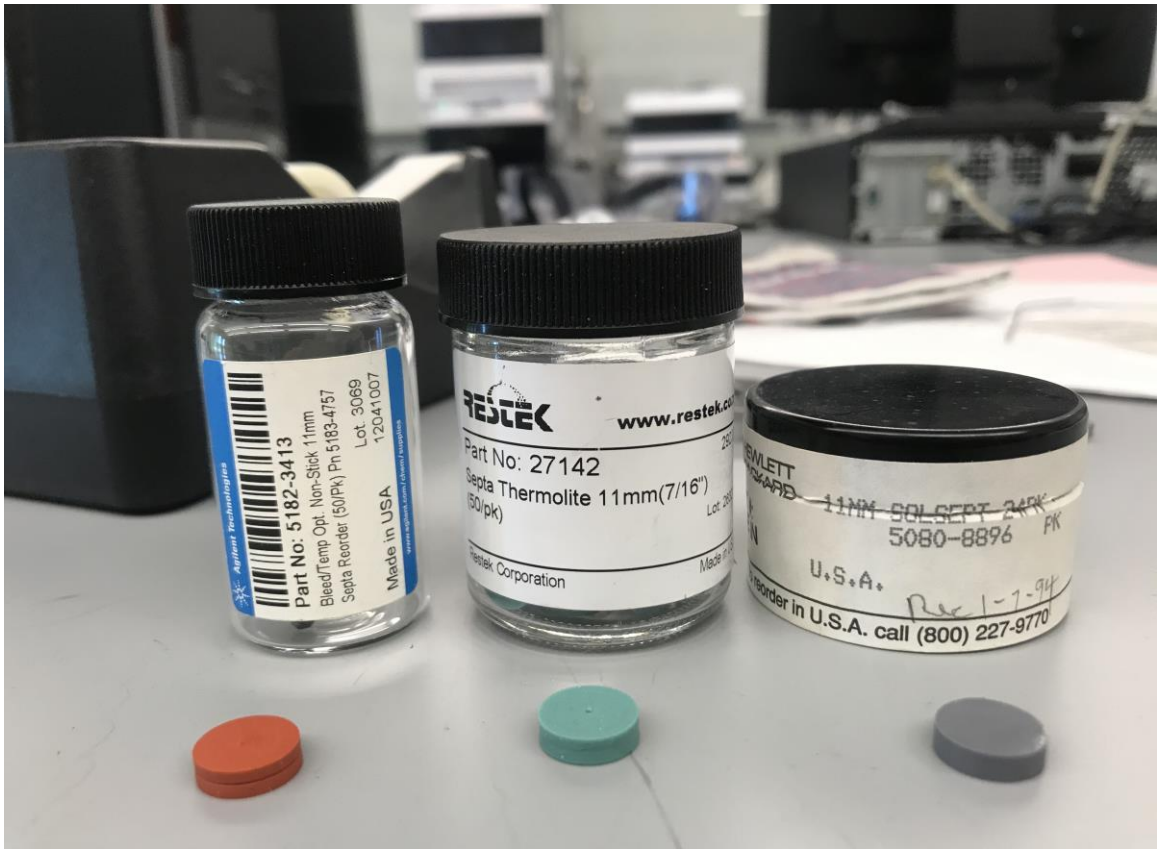

Figure 6. Photographs of the three septa used in this study.

Table 4. Conditioning timetable for each septum.

Septum	Date and Time Started	Date and Time Ended
Teal	02/10/21 – 6:12 PM	02/11/21 – 12:12 PM
Gray	02/11/21 – 6:05 PM	02/12/21 – 12:06 PM
Red	02/16/21 – 6:41 PM	02/17/21 – 12:46 PM

III. Results and Discussion

3.1 Septum Interference

One challenge faced during the analysis of hemp oil via gas chromatography is the possibility of septum interference or septum bleeding. At high temperatures, the septum could bleed into the GC column and result in ghost peaks. These peaks on the chromatogram, but the corresponding compound may not necessarily be present in the sample. These ghost peaks cause interference during detection and quantification of compounds. Ghost peaks could also hinder reproducibility of results. One example of how septum bleeding occurs is volatile organic compounds (VOC) could be trapped in the septum. When running the GC instrument, the high inlet temperature causes the trapped VOCs to be released from the septum and bleed into the column, resulting in ghost peaks. Particularly, in the GC-FID, the capillary columns are very narrow with a typical flow rate of less than 2 mL/min, which could result in VOCs becoming very concentrated and cause bleeding to be more pronounced [13]. Another source for ghost peaks is the bleeding of the actual septum material itself. All septa are comprised of multiple compounds such as silicone oils, long hydrocarbons, phthalates, etc. At high temperature, these septum material can be released and result in septum interference [13].

3.1.1 Teal Septum

In order to minimize the effects of septum bleeding, three different septa were tested to see which septum was most suited for hemp oil analysis. For the teal septum, there were peaks observed at all three injection port temperatures, regardless of conditioning. Their respective chromatograms are shown in Figure 7-12. These peaks started to appear around the 7.5-min mark and persisted until the end of the run time, around 11.5 min. At 220 °C, there was a significant amount of bleeding prior to conditioning (Figure 7). Figure 8 shows the chromatogram at 220 °C after conditioning and there were visibly less crowding of peaks.

This pattern remained constant for the remaining injection port temperatures as well. (Figures 9-12). Comparing the post-conditioning chromatograms, there were the least amount of peak interference at 260 °C and 300 °C showed the most amount of peak interference.

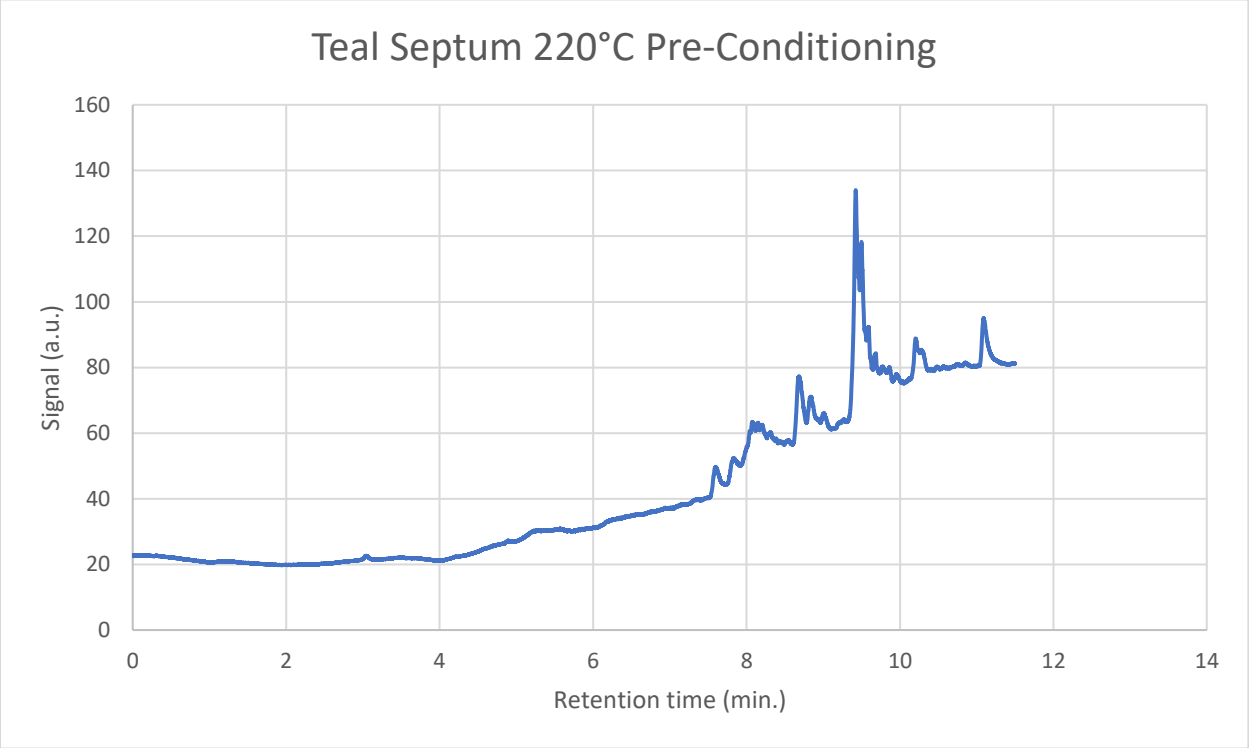

Figure 7. GC chromatograms of teal septum observed at 220 °C before conditioning.

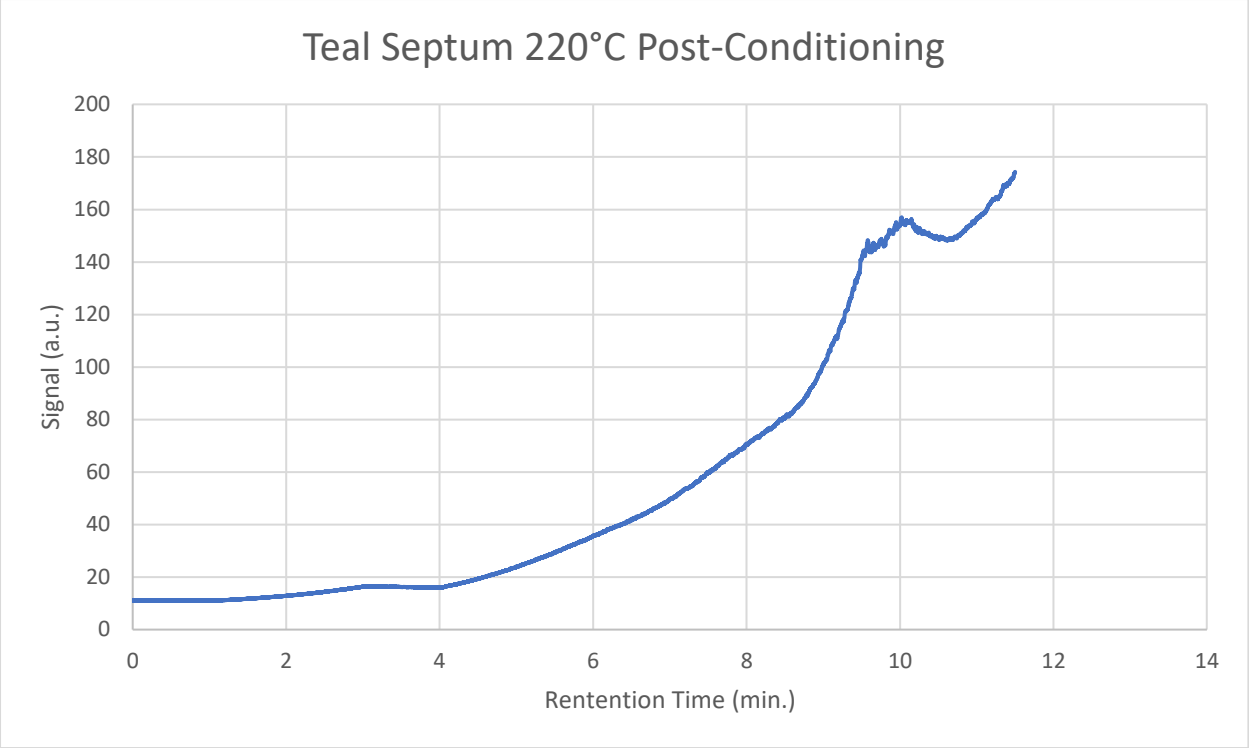

Figure 8. GC chromatograms of teal septum observed at 220 °C after conditioning.

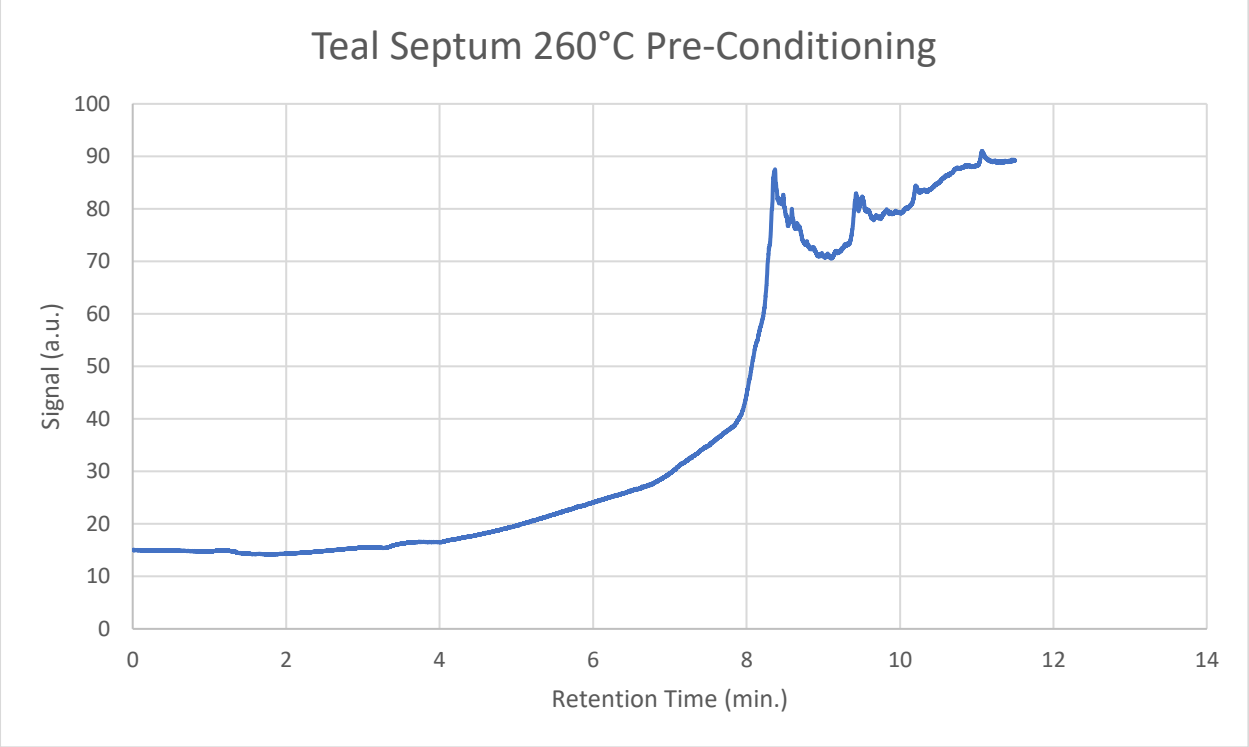

Figure 9. GC chromatograms of teal septum observed at 260 °C before conditioning.

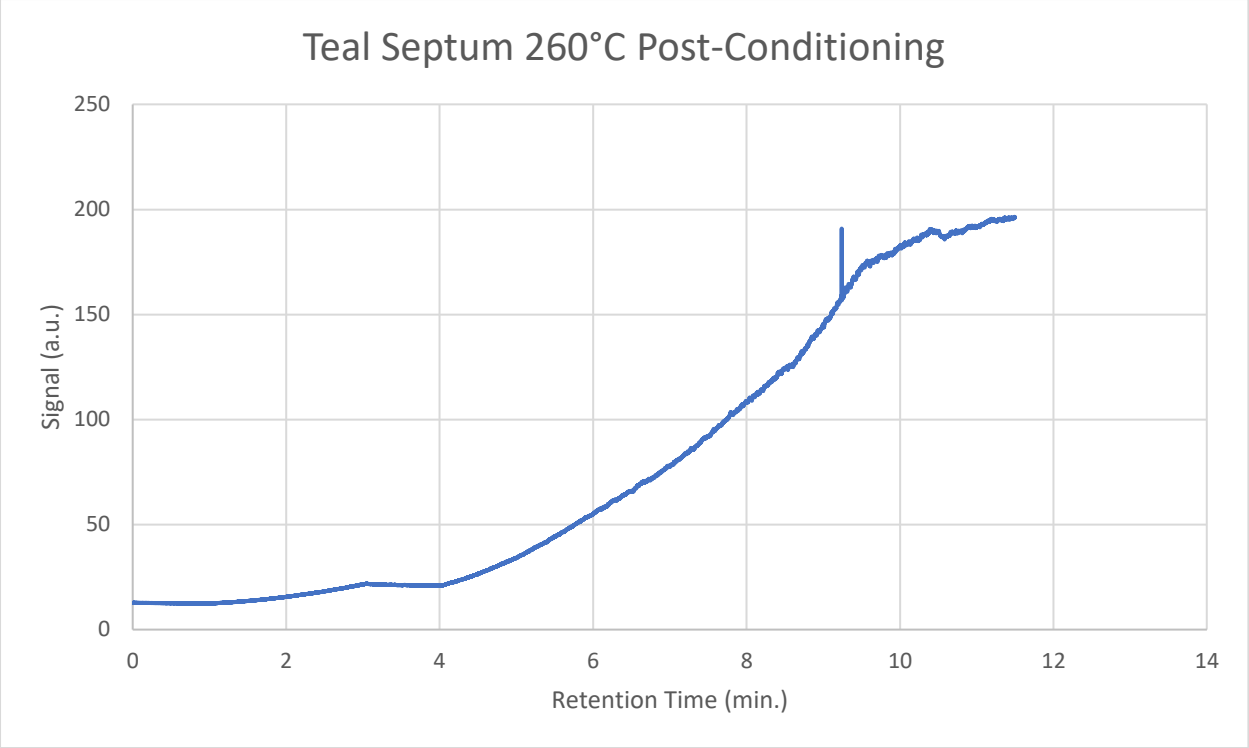

Figure 10. GC chromatograms of teal septum observed at 260 °C after conditioning.

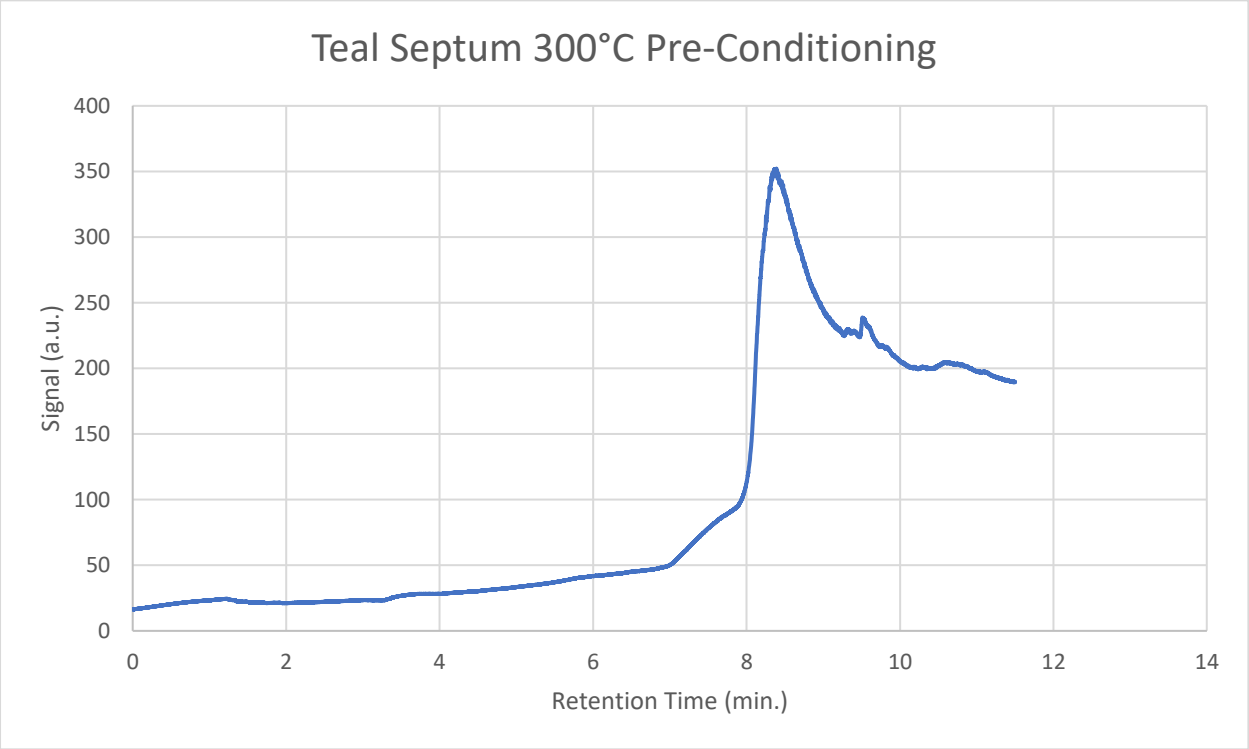

Figure 11. GC chromatograms of teal septum observed at 300 °C before conditioning.

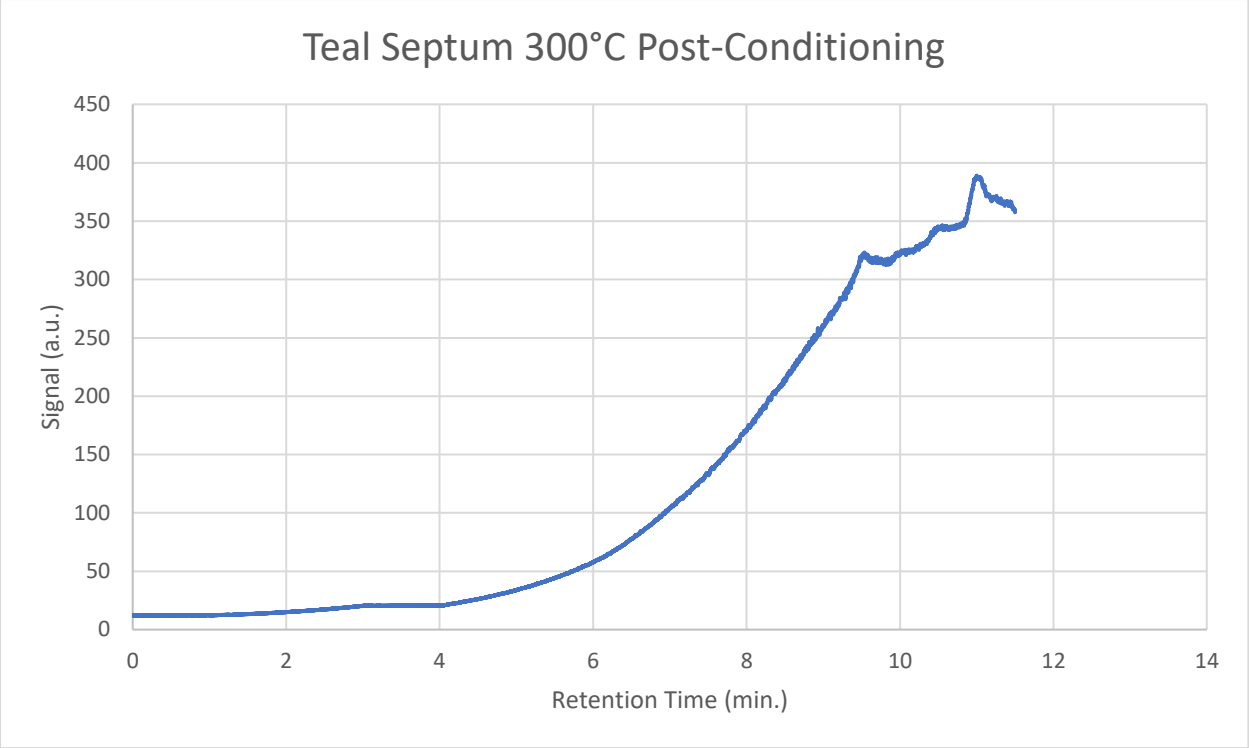

Figure 12. GC chromatograms of teal septum observed at 300 °C after conditioning.

3.1.2 Gray Septum

In case of the gray septum, there were ghost peaks observed at all three injection port temperatures, but considerably less compared to the teal septum. The chromatograms for the gray septum at each inlet temperatures are shown in Figures 13-18. Comparing the pre-conditioning graphs to its corresponding post-conditioning graphs, there were visibly less peaks after conditioning at each inlet temperature. After conditioning, septum performance seems to be equal, where chromatograms had only some small peaks around retention time at 9.5 min.

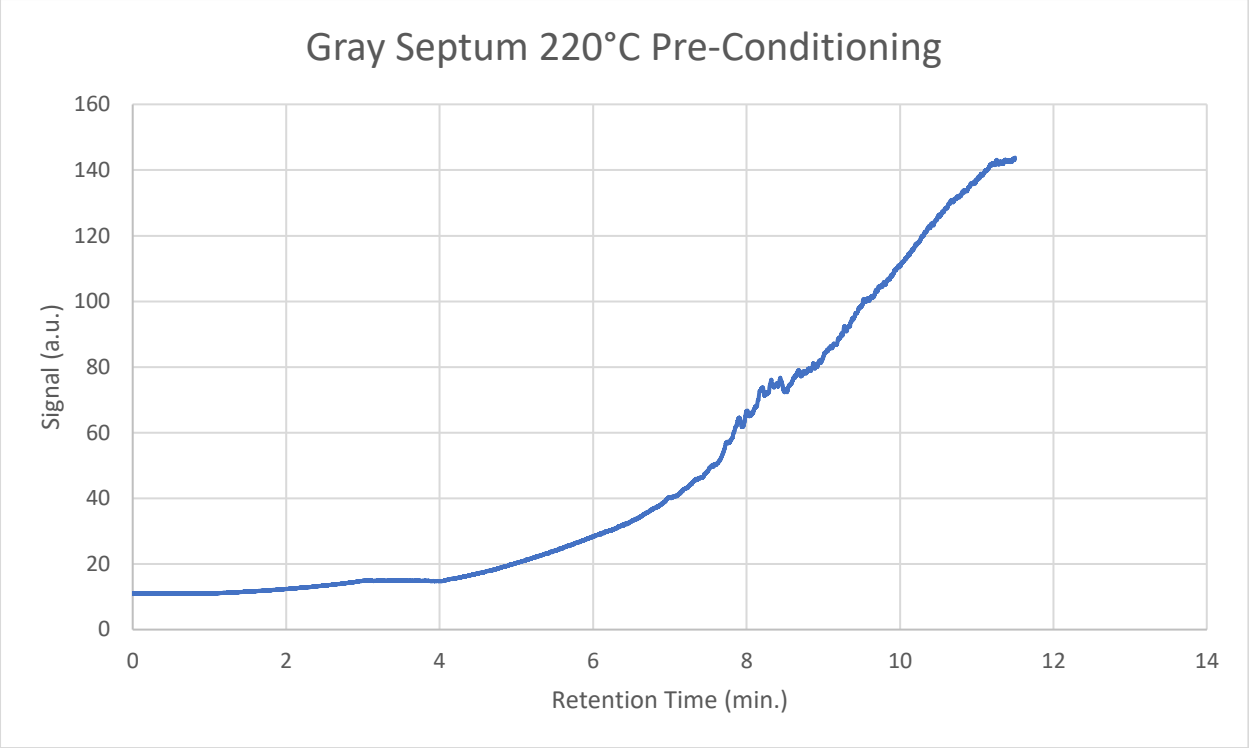

Figure 13. GC chromatograms of gray septum observed at 220 °C before conditioning.

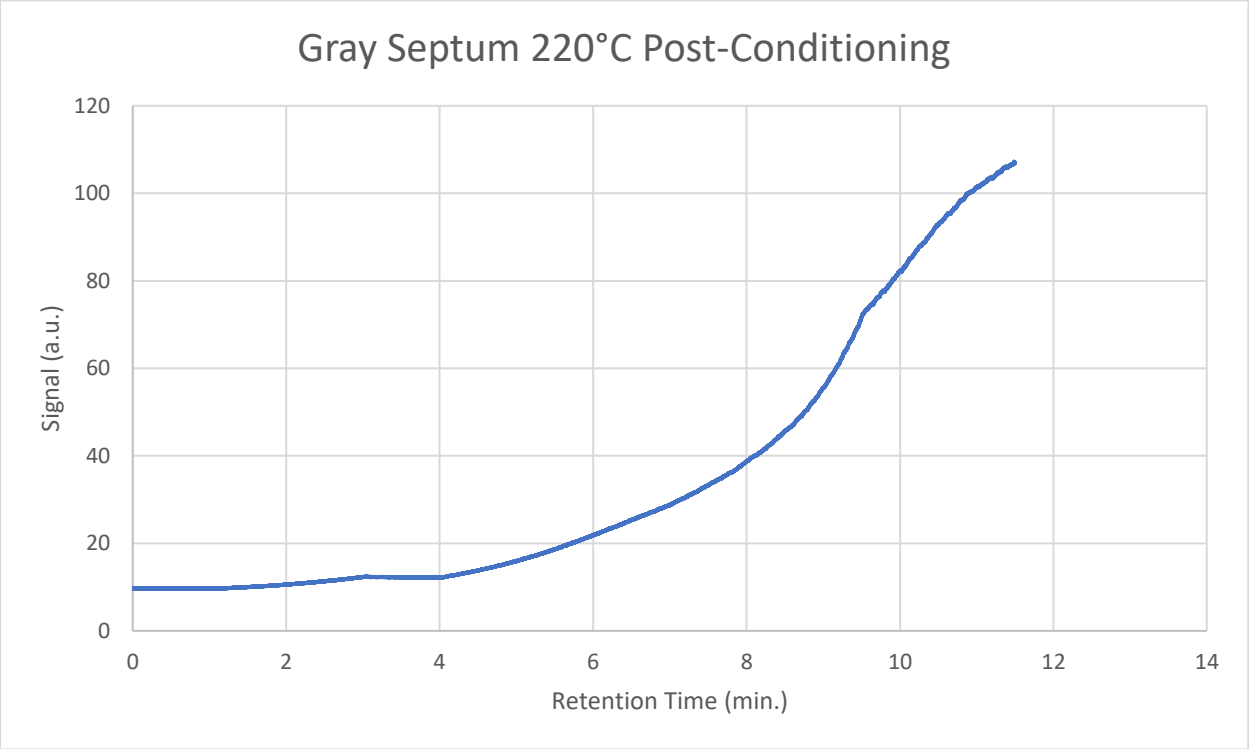

Figure 14. GC chromatograms of gray septum observed at 220 °C after conditioning.

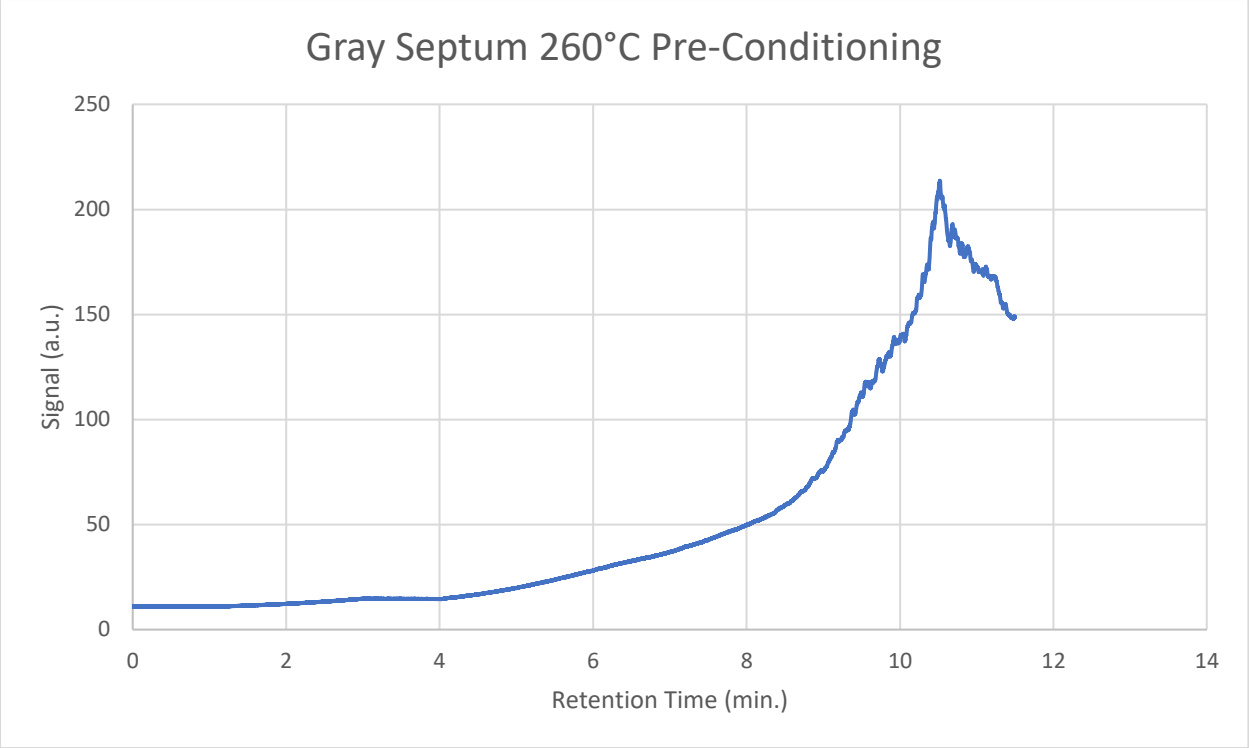

Figure 15. GC chromatograms of gray septum observed at 260 °C before conditioning.

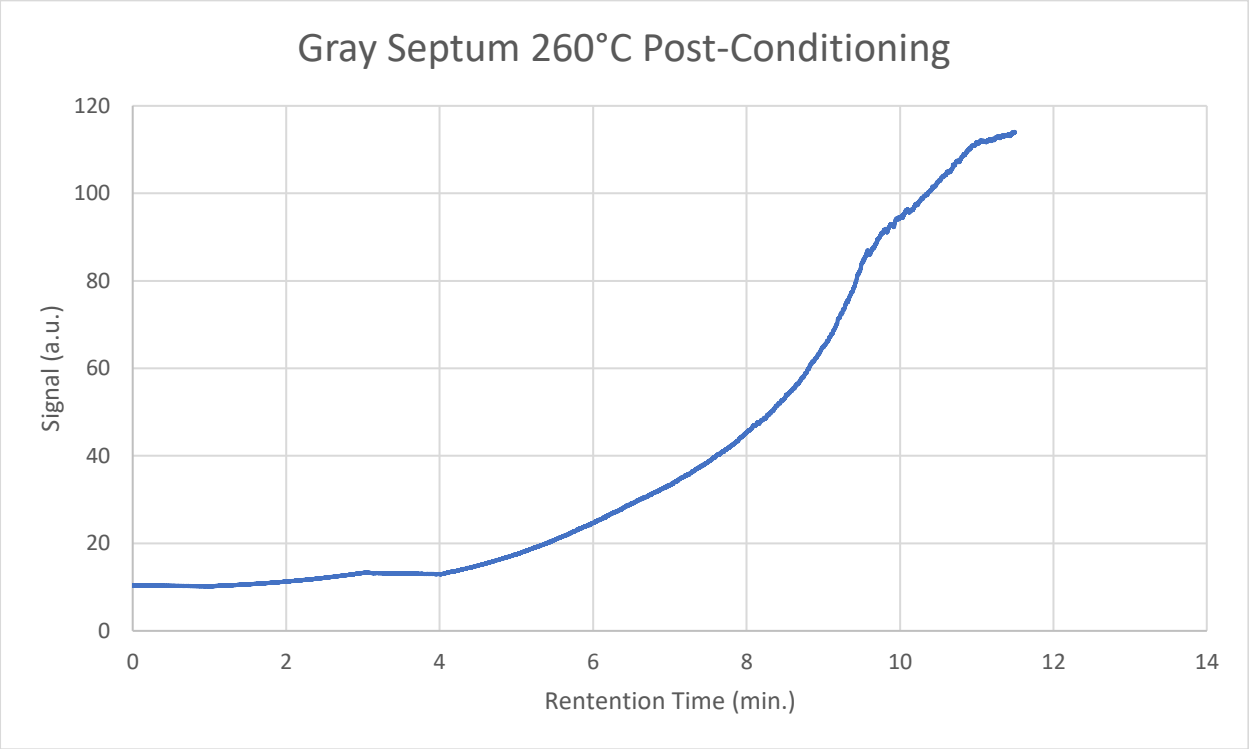

Figure 16. GC chromatograms of gray septum observed at 260 °C after conditioning.

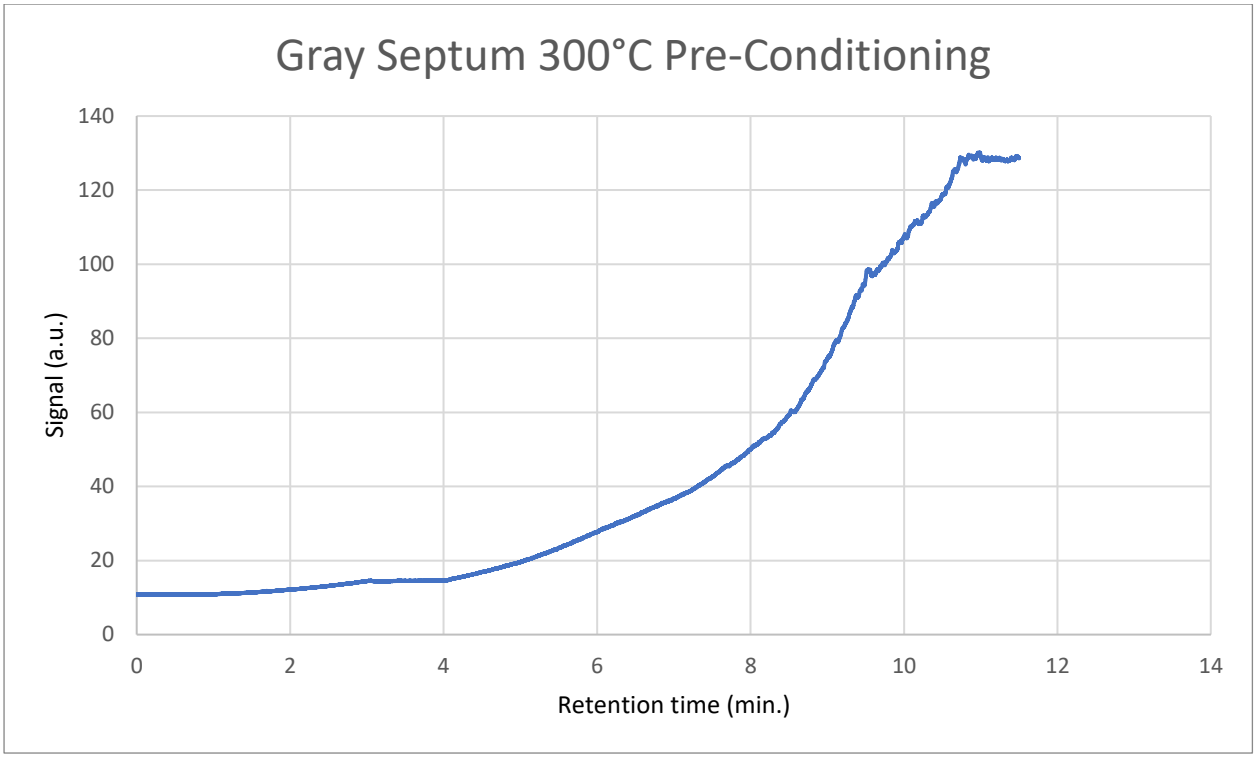

Figure 17. GC chromatograms of gray septum observed at 300 °C before conditioning.

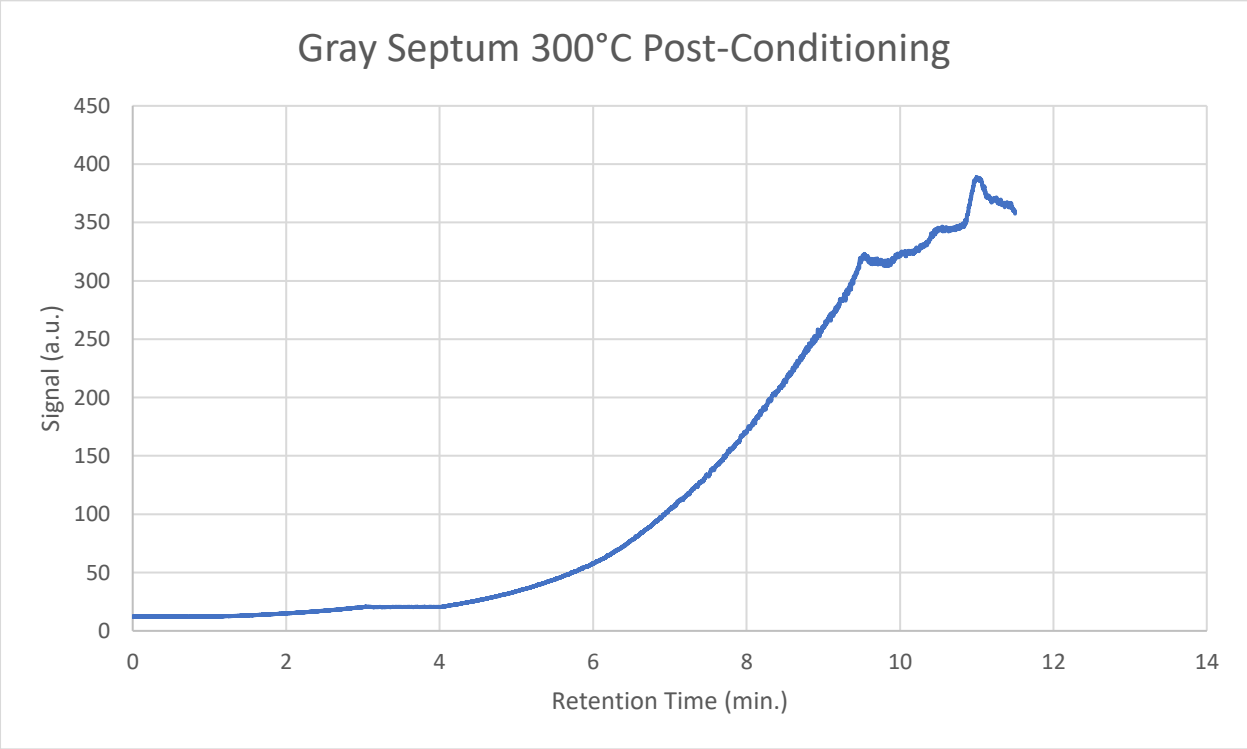

Figure 18. GC chromatograms of gray septum observed at 300 °C after conditioning.

3.1.3 Red Septum

Lastly, for the red septum there was not as a significant change between pre-conditioning and post-conditioning chromatogram at each inlet temperatures. The chromatograms for the red septum at each of the inlet temperatures are shown in Figures 19-24.

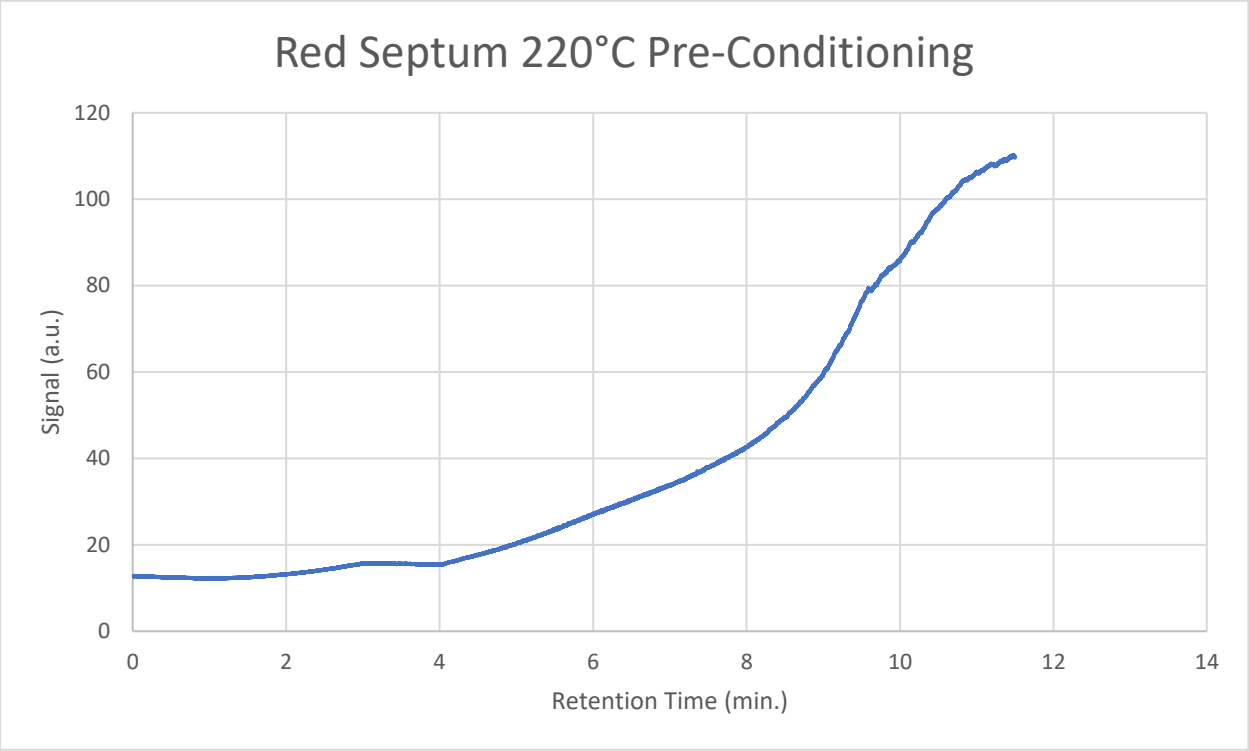

Figure 19. GC chromatograms of red septum observed at 220 °C before conditioning.

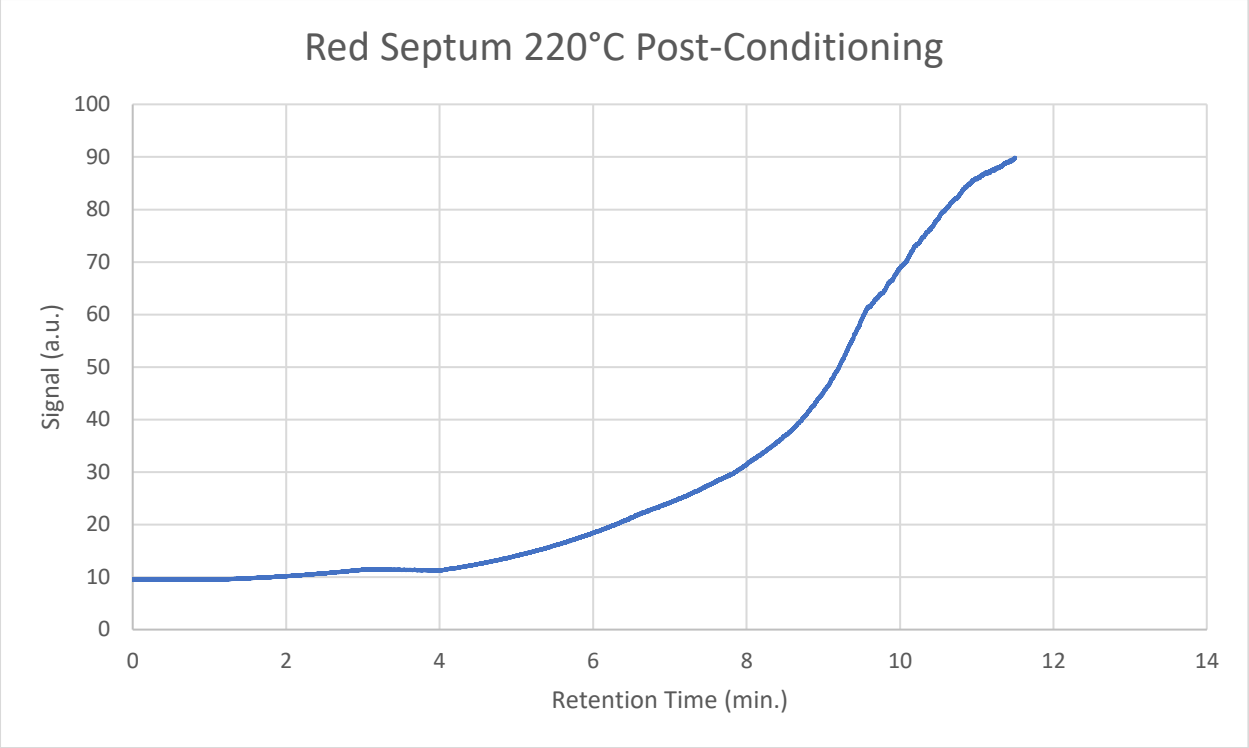

Figure 20. GC chromatograms of red septum observed at 220 °C after conditioning.

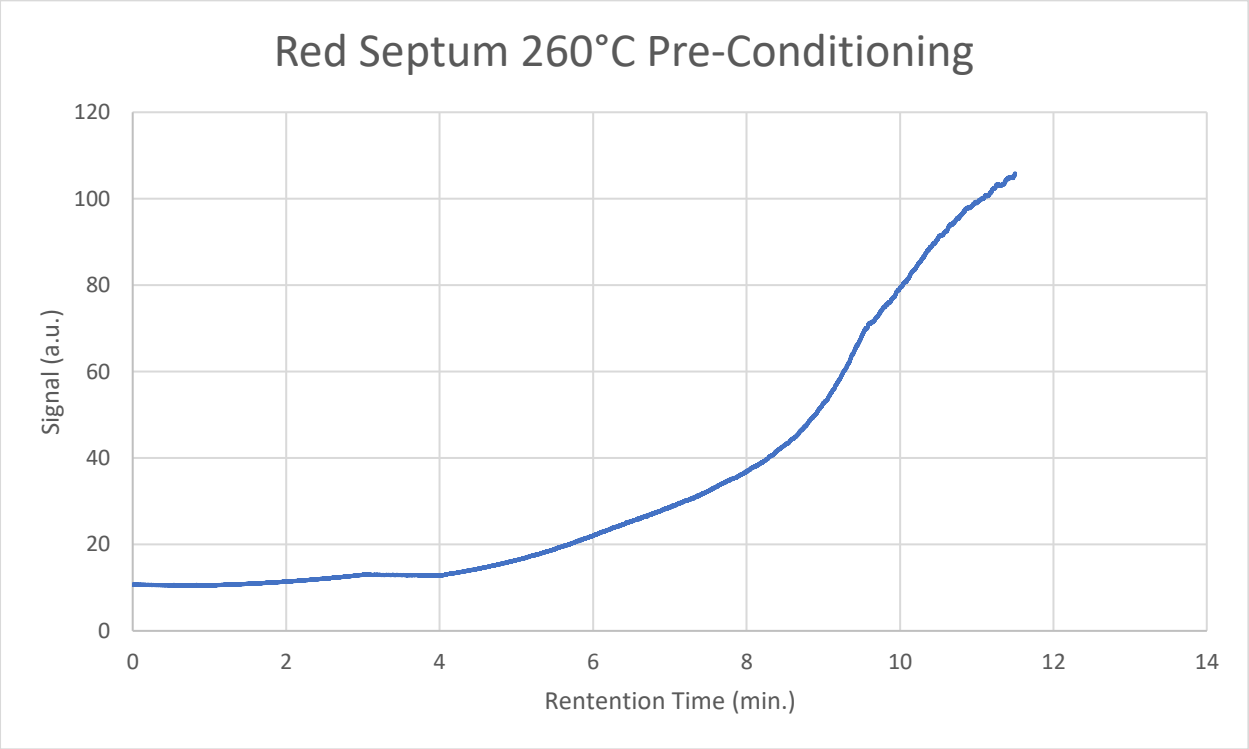

Figure 21. GC chromatograms of red septum observed at 260 °C before conditioning.

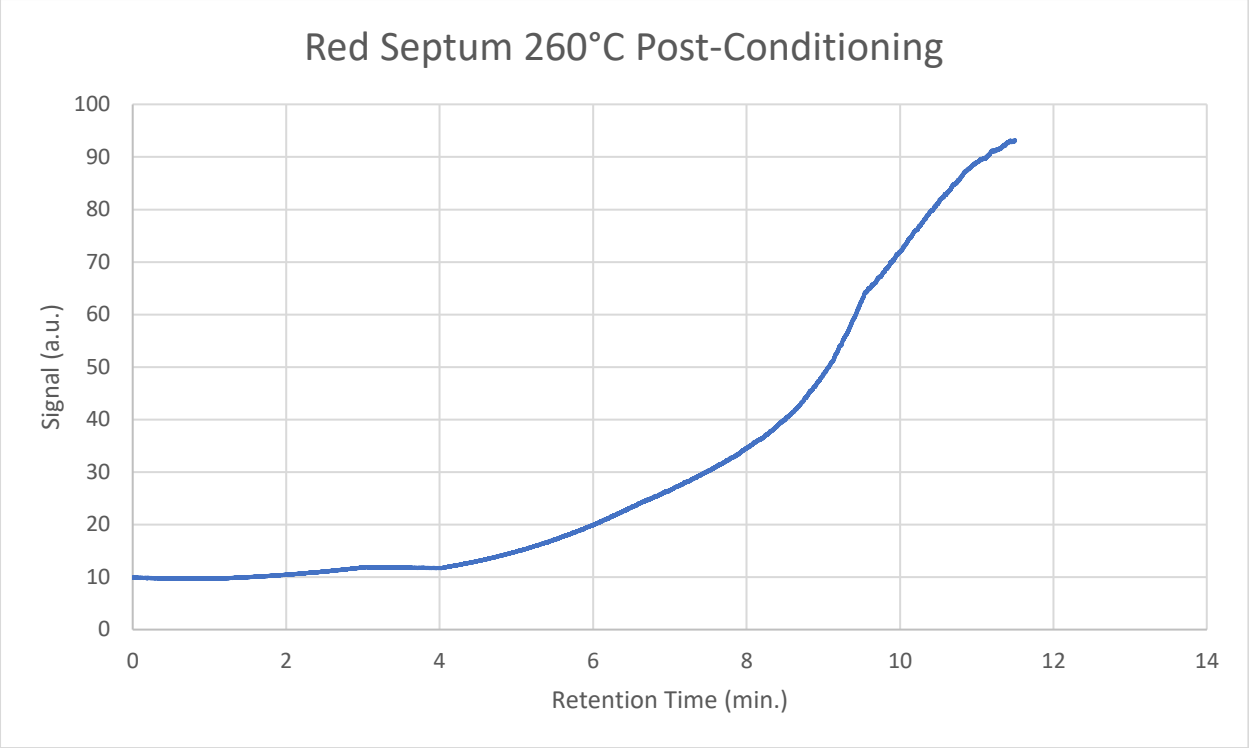

Figure 22. GC chromatograms of red septum observed at 260 °C after conditioning.

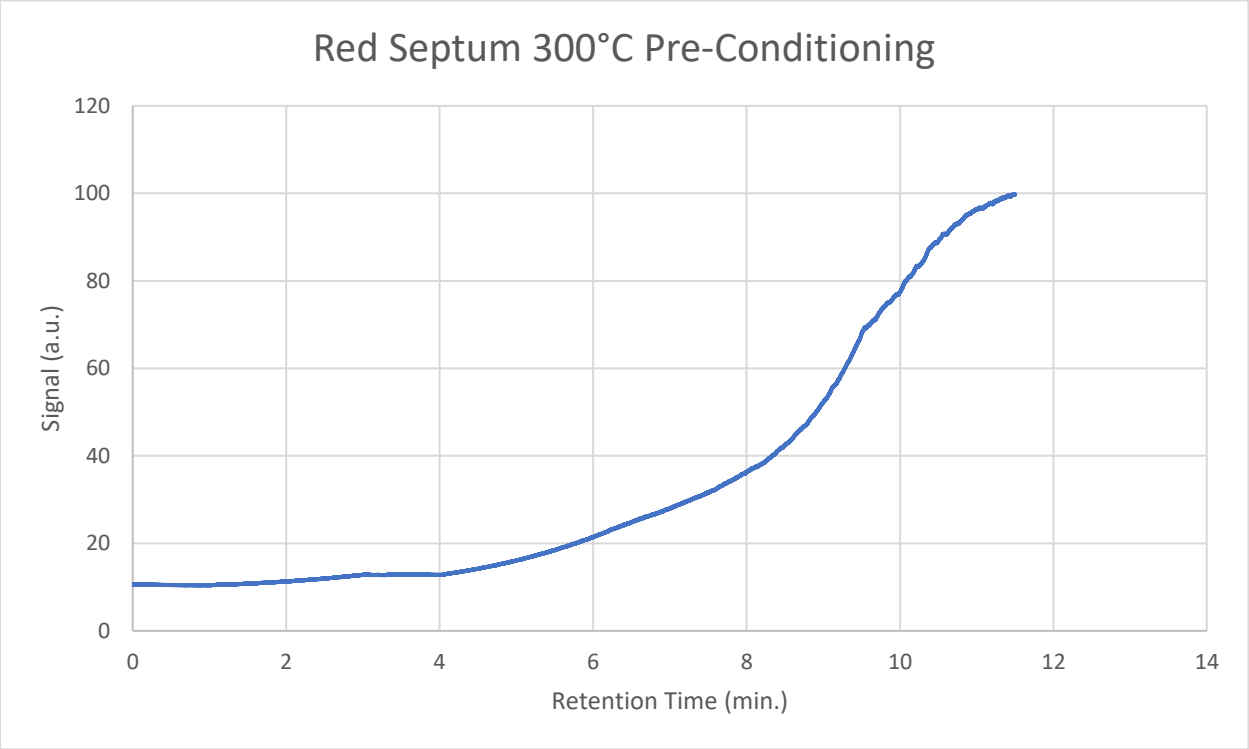

Figure 23. GC chromatograms of red septum observed at 300 °C before conditioning.

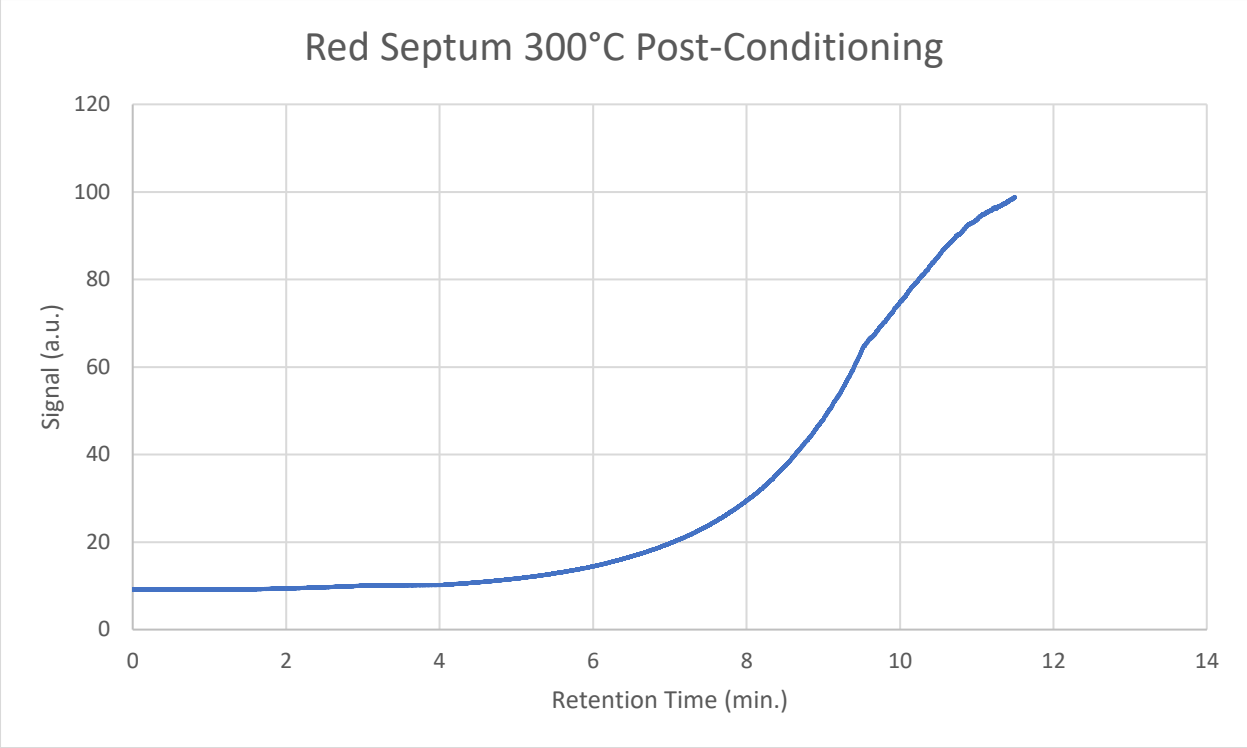

Figure 24. GC chromatograms of red septum observed at 300 °C after conditioning.

3.1.4 Septum Bleeding Results and Discussion

As shown in Figures 7-24, chromatograms remained free from any ghost peaks in all three septa when oven temperatures were held at 160 °C and 190 °C. However, when oven temperatures ramped up to > 200 °C, multiple peaks started to appear in the teal and gray septum before conditioning. Since GC experimental parameters, such as the oven-heating temperature and carrier gas remained constant for the entirety of the septum test, the observed peaks were ascribed to septum bleeding. Out of the three septa, the red septum had the least number of observed peaks at temperatures above 200 °C and the teal septum had the highest amount. This could be due to the fact Restek Thermolite® septum, has a maximum temperature of 340 °C, whereas the Agilent general purpose septum has a maximum temperature of 400 °C. All three septa showed visibly less bleeding after conditioning for 30 hrs. As a result of this test, the most suitable septum for testing hemp oil samples was determined to be the red septum and needed to be conditioned prior to running the samples.

In all of the chromatograms, there was a rise in baseline after the GC oven temperature began to ramp up to 300 °C around the 6 min mark. The rise in baseline could be attributed to possible column bleeding of cyclic siloxane or any oxygen trapped in the phase that was not fully purged during conditioning. As temperatures ramp up, the stationary phase can be susceptible to bleeding and detected by the GC-FID. Another possible explanation for the rising baseline is that oxygen was not fully purged during conditioning, meaning the carrier gas was not allowed to flow through the column long enough prior to running the samples. This could result in further phase oxidation as the oven temperature ramps up and contribute in rising baseline [14]. Nonetheless, the rise in baseline does not interfere with the results of the septa experiment because column bleeding is different from septum bleeding. Septum bleeding results in distinct peaks as seen in Figure 15 rather than a steady incline as seen in Figure 23.

3.2 Test for Decarboxylation of CBDA

Another issue researchers must keep in mind when quantifying CBD via GC, is decarboxylation [15]. Cannabidiolic acid (CBDA) and tetrahydrocannabinolic acid (THCA) are the most abundant cannabinoids found in the cannabis plants. Hence, it would not be surprising to find traces of CBDA and THCA in cannabis products such as CBD and hemp oil. When CBDA and THCA are exposed to high heat, it undergoes decarboxylation and the compounds are converted to CBD and THC, respectively. Decarboxylation is a chemical reaction that removes a carboxyl group and produces carbon dioxide as a biproduct. When samples are injected into the GC instrument, the high inlet temperature causes CBDA and THCA in the sample to decarboxylate and their products are detected in the chromatogram. Therefore, when quantifying CBD in the hemp oil samples, it includes the total concentration of CBD, including any CBDA that was decarboxylated from the high injection temperature.

For the purpose of proving decarboxylation occurred, salicylic acid was injected into the GC instrument. At the time of the experiment, CBDA and THCA were unavailable for purchase, subsequently salicylic acid was used instead to mimic the decarboxylation of CBDA. Similar to CBDA, salicylic acid also contains a carboxyl group that can be decarboxylated. When salicylic acid is decarboxylated, it produces phenol and carbon dioxide. Looking at the chromatogram of salicylic acid (Figure 25), there are two significant peaks observed at 6.9 and 11.8 min. The peak with the stronger signal at 11.8 min was identified to be salicylic acid and the peak at 6.9 min was suspected to be phenol. In order to confirm the peak corresponded to phenol, phenol was injected into the GC-FID using the same method to test salicylic acid (Figure 26). Since GC-FID only generates retention time, a positive identification of a compound is only possible by confirming the known retention time of the desired compound.

The chromatogram for phenol revealed a strong peak at 6.9 min, which matches the same retention time of the unknown peak in salicylic acid. Comparing the two chromatograms, it proved salicylic acid underwent decarboxylation because when only salicylic acid was injected, there was a positive confirmation for the presence of phenol in the sample. This experiment confirmed that decarboxylation can occur due to the high temperatures required for GC analysis.

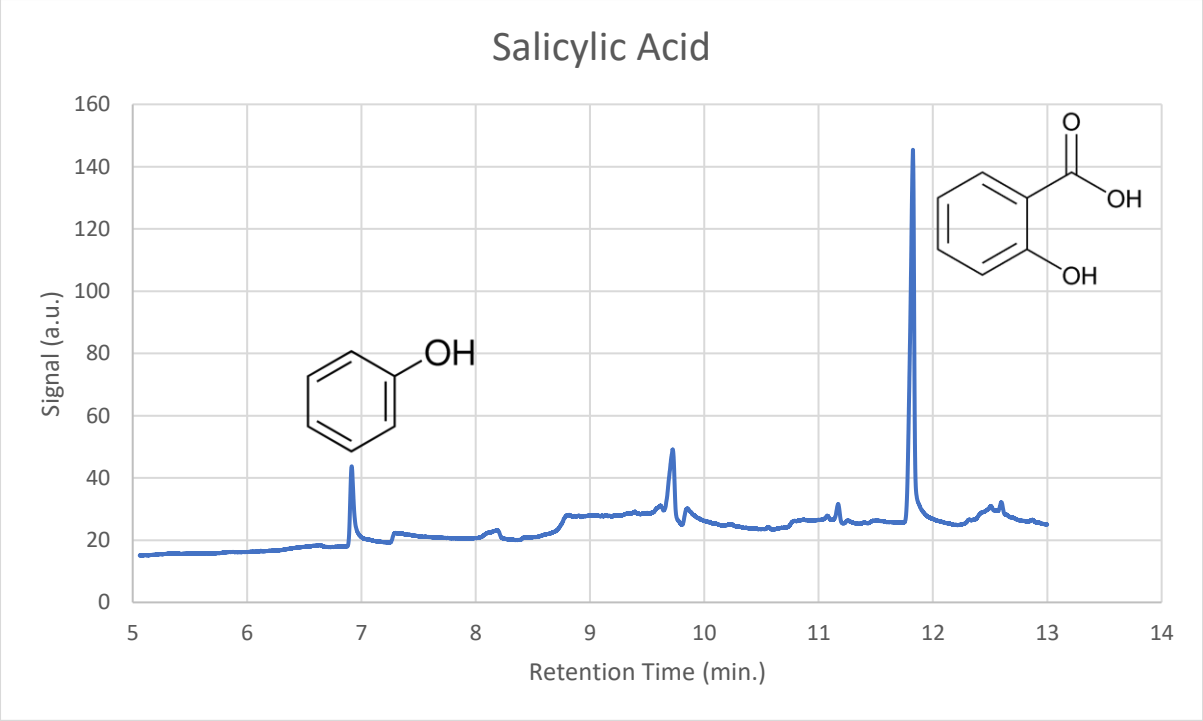

Figure 25. GC-FID for salicylic acid.

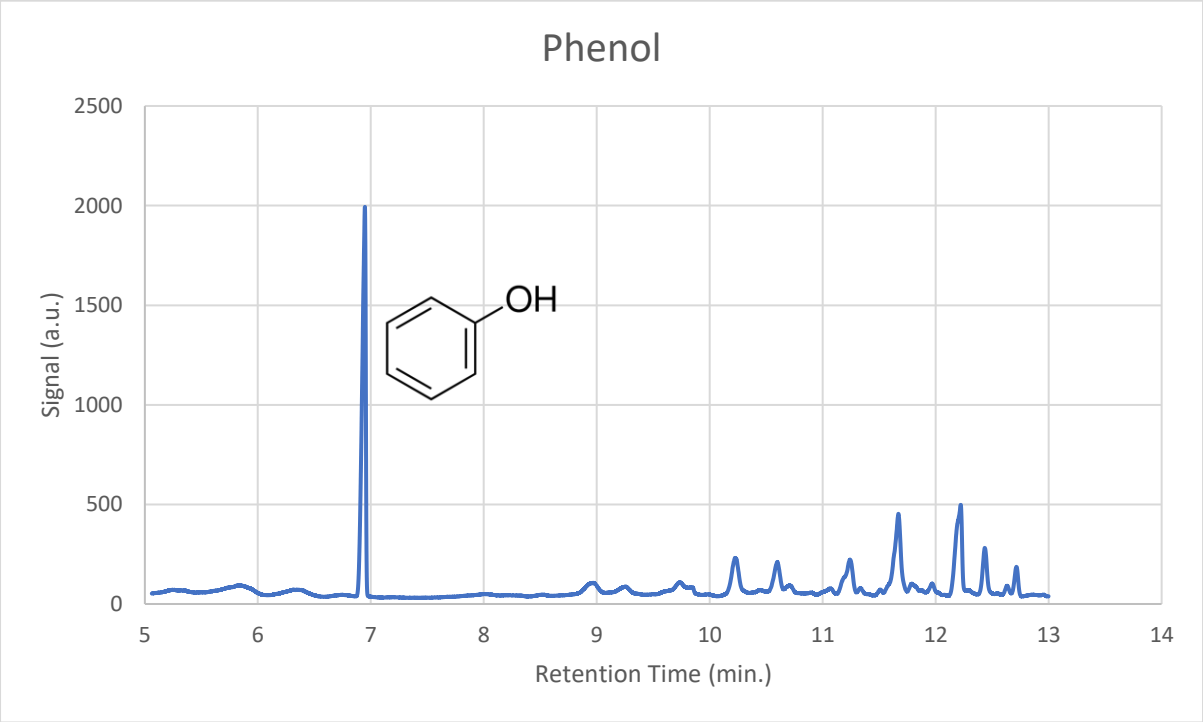

Figure 26. GC-FID for phenol.

3.3 Derivatization of Cannabinoids

A common method to protect compounds from decarboxylation is to derivatize them. Derivatization is the process by which a compound is chemically changed, to produce a new compound that makes it more suitable for an analytical instrument, or in this case the GC. Derivatization is useful for research because it prevents decarboxylation, protects hydrophilic ends, creates better separation and resolution, and helps with the reproducibility of results. For cannabinoids, silylation is the most common derivatization technique. In silylation, the silylation reagents react with compounds containing hydroxyl groups by replacing it with an alkyl silyl group, typically trimethylsilyl. For example, in Figure 27, CBDA is coupled with bis(trimethylsilyl) acetamide to protect the hydrophilic ends. This resulted in a derivatized CBDA where the hydroxyl groups were replaced with trimethylsilyl groups.

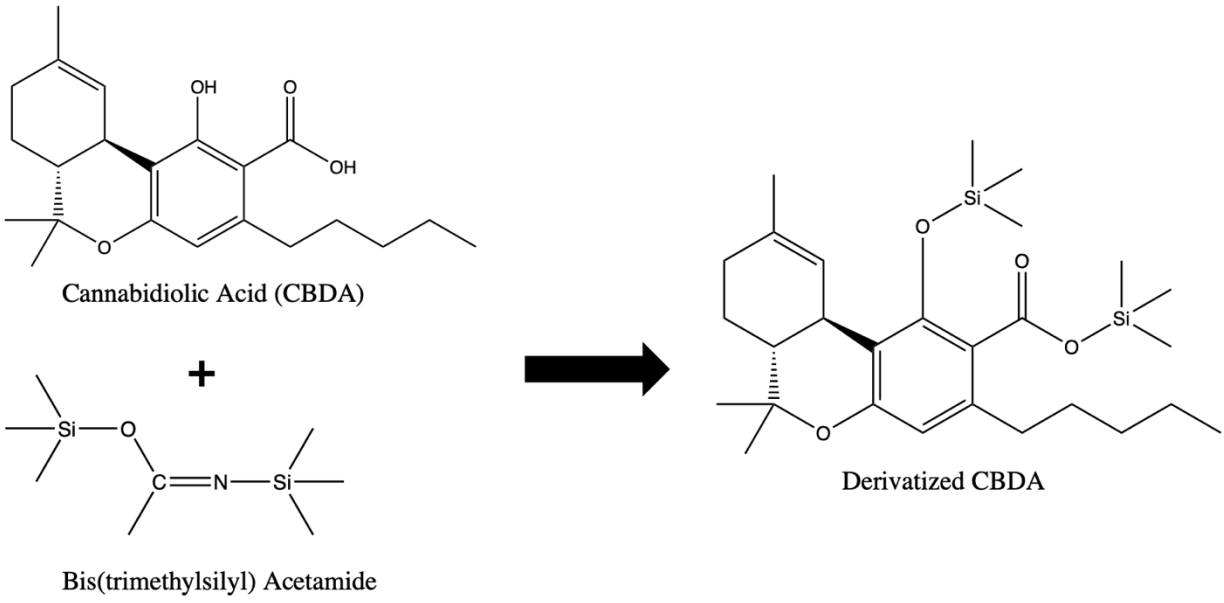

Figure 27. Derivatization of CBDA using silylation.

Due to lack of time and resources, CBDA was not derivatized before testing the hemp oil samples. Therefore, the concentration of CBD detected in the GC included the total amount of CBD, including those that may have come from CBDA.

3.4 Photodegradation of CBD

Lastly, photodegradation is a prevailing challenge when analyzing CBD via GC [16]. Photodegradation is the alteration of compounds through light. Previous research had shown CBD to degrade over time due to light and heat [16-19]. Depending on the temperature and the amount of light CBD is exposed to, it can degrade by 15% in a month [17] and sometimes even by 50% over a period of two months [19]. This research utilized the GC-MSD to conduct a degradation study over a period of 5 months on the CBD standard. The CBD standard (1.000 ± 0.005 mg/mL) was first tested on the GC-MSD in September 2020 and again in February 2021 (Figure 28).

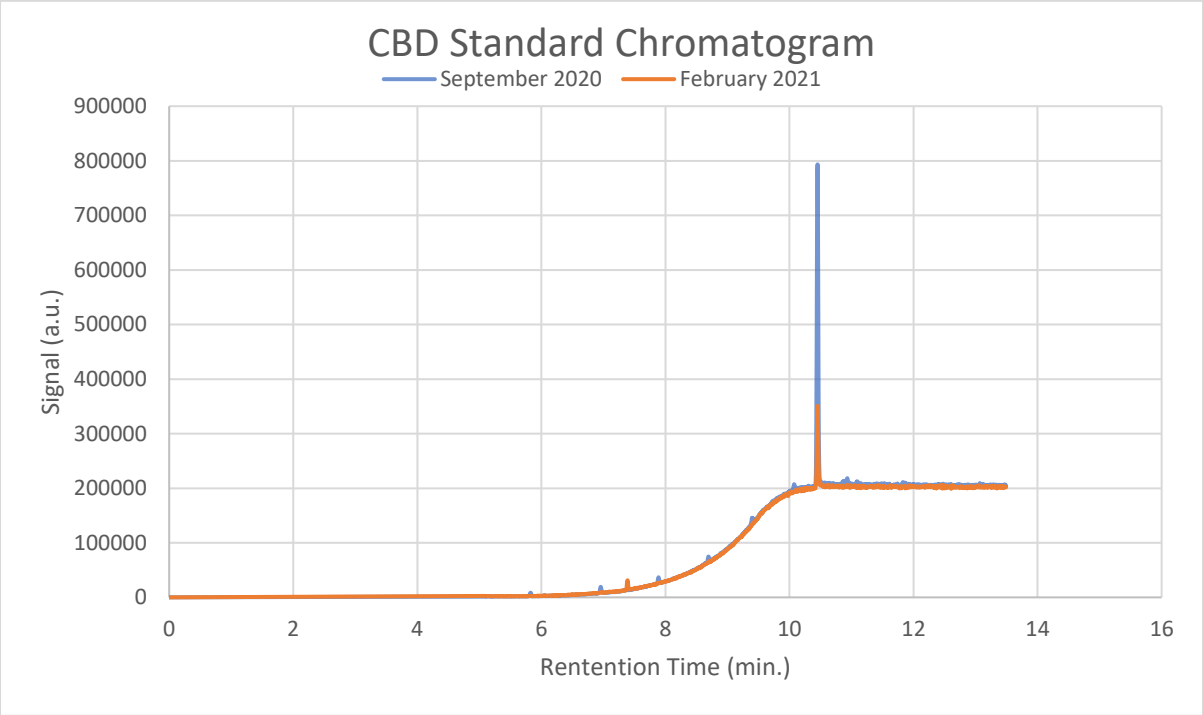

Figure 28. Comparison of CBD chromatograms taken in September 2020 and February 2021.

Examining the two chromatograms, both detected CBD and produced a corresponding CBD peak at a retention time of 10.5 min. This peak was confirmed to be CBD by analyzing the mass spectra (Figure 30) of the peak and identifying it based on the library search function on the GC-MSD. The GC-MSD library search function matched the fragmentation pattern of each analyte and correlate it to a known database in the software for an identification. Not only was it confirmed using the internal database, but it was also further confirmed to be CBD by comparing the fragmentation pattern to the literature value found on the NIST Chemistry WebBook. The CBD mass spectra (Figure 29) matched the spectra provided by NIST Chemistry WebBook (Figure 30) with both spectra having peaks at *m/z* = 174, 193, 231, 246, and 314.

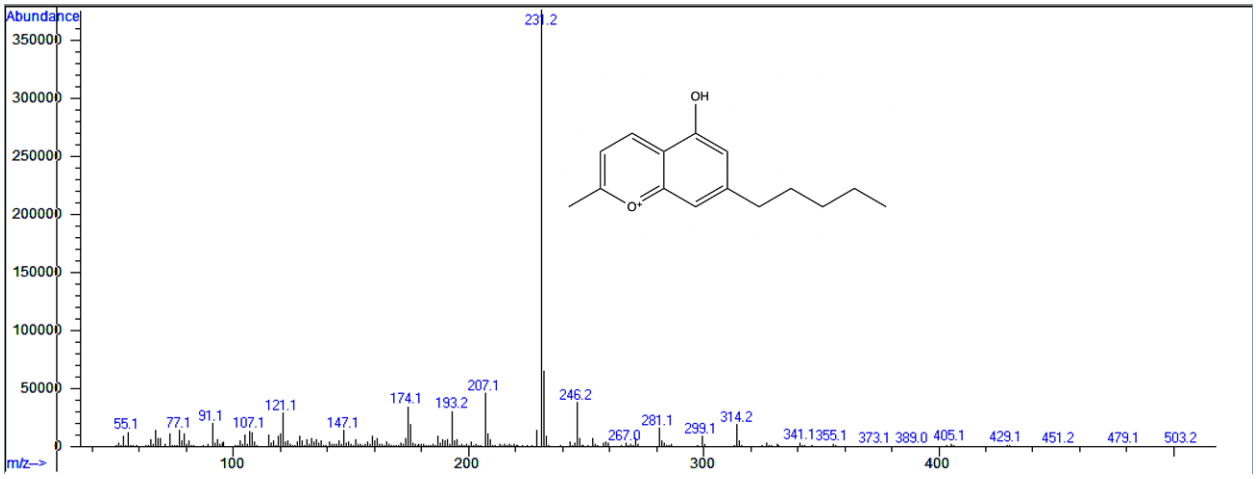

Figure 29. Mass Spectra of CBD standard from September 2020.

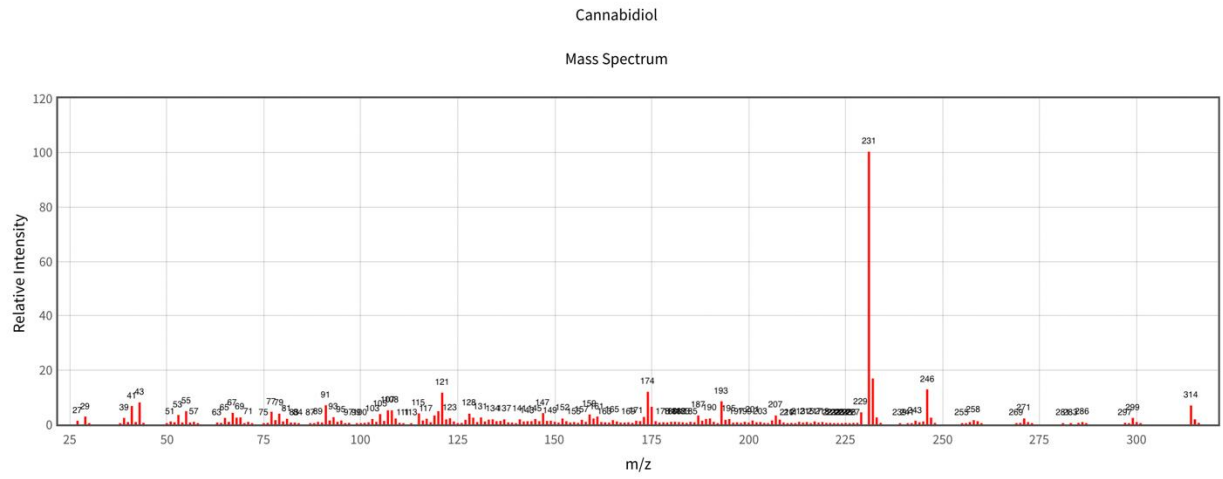

Figure 30. Mass Spectra of CBD standard. Obtained from NIST WebBook.

Comparing the CBD peaks taken in September and February, the signal intensity was much stronger in September compared to February, where the signal intensity was about 800,000 au versus 350,000 au, respectively. Furthermore, upon closer examination, there was a second peak around the 7-min mark in the chromatogram taken in February. This peak indicated the presence of another compound in the sample and most likely resulted from the photodegradation of CBD over time. Unfortunately, there was no positive identification of for compound peak using the library search function in the GC-MSD. Regardless, after this experiment, it was confirmed that the CBD standard had degraded to some degree. This led to extra measures taken to ensure the hemp oils to be analyzed were relatively new and had not been sitting on the shelf for a long period of time.

3.5 Cannabinoid Elution Order

When running the GC, the chromatograms provide two crucial pieces of information: peak area and retention time. The retention time indicated when the compounds are detected during the method and the peak area correlates with its concentration. The peak area can vary

depending on the amount of compounds in the sample, human errors, or instrumental errors. However, the retention time should remain constant for identical compounds.

Prior to starting the research, it was important to establish the elution order of CBD and THC in the optimized method. This was done by injecting 1µL of each cannabinoid at 1000 ppm into the GC-FID to determine the time frame in which these compounds would elute. Figure 31 shows the elution order of CBD and THC using the optimized method previously discussed. The total run time on the GC-FID was 11.5 min and two peaks were observed at 9.6 and 10.1 min. CBD was identified to elute from the column at 9.6 min and THC eluted out shortly after at 10.1 min.

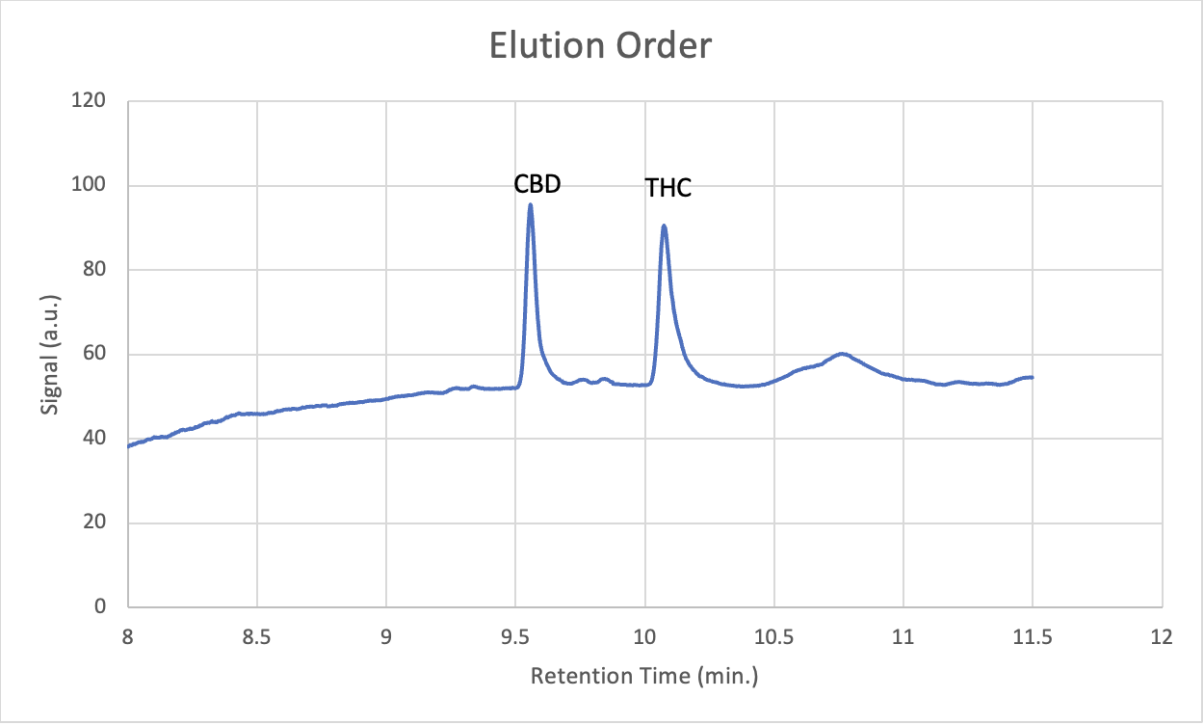

Figure 31. The chromatogram containing CBD (9.6 min) and THC (10.1min) on the GC-FID.

3.6 Calibration Curves

An external calibration curve was created for both CBD and THC by creating a series of dilution at 5 ppm, 10 ppm, 50 ppm, 100 ppm, and 250 ppm for each compound. There were no interfering peaks observed around the retention time of cannabinoids. The signal intensities of cannaboids changed between the varying concentrations. However, the retention time remained consistent. The peak area was calculated by integrating the area under the peak using the Agilent CDS ChemStation software. Figures 32 and 33 show the calibration curve for each cannabinoid created with data from the GC-FID.

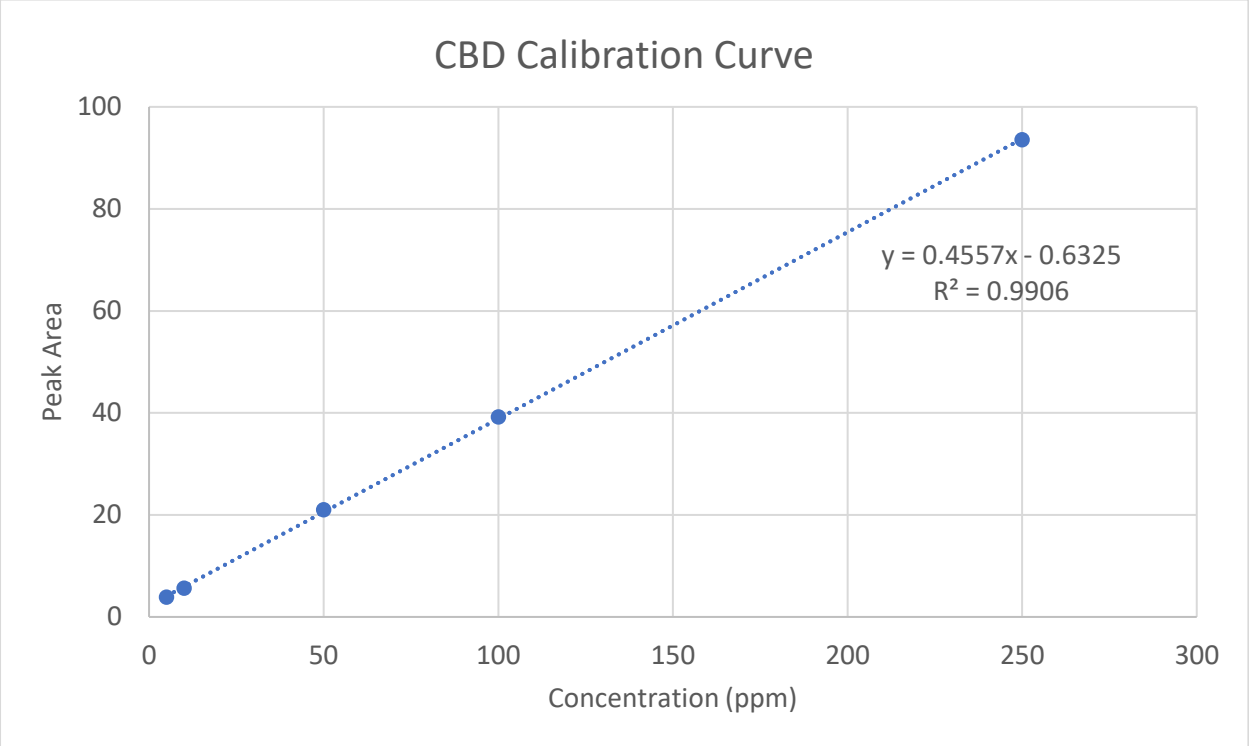

Figure 32. The external calibration curve for CBD using GC-FID.

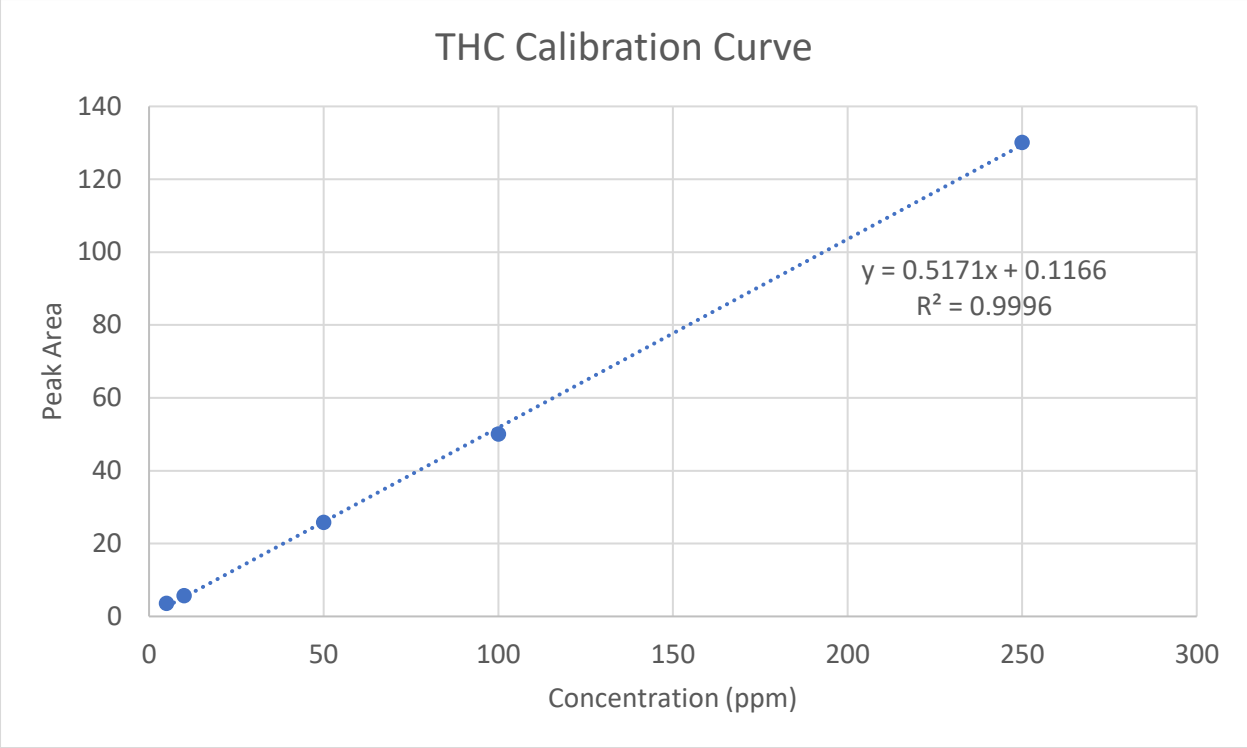

Figure 33. The external calibration curve for THC using GC-FID.

A comparison between the linearity of the two compounds are shown in Table 5. THC produced more linear data compared to CBD in the FID. The correlation coefficient (R^2) value for both were above 0.99, which indicates the curve to be well-fitted. These were the calibration curves used to quantify the total amount of CBD and THC in hemp oil samples.

Table 5. Comparison between the linearity of the calibration curves for CBD and THC.

Cannabinoid	**Equation of Line**	**R^2 (FID)**
CBD	y=0.011x-0.0123	0.9906
THC	y=0.01x-0.0085	0.9996

3.7 Hemp Oil Quantitative Results

The main purpose of this experiment was to determine if there were any quantifiable CBD and THC in five brands of hemp oil while being mindful of all the potential challenges that are commonly associated with GC analysis.

The O'Rmeas hemp oil had a peak around 9.5 min, which has previously been identified as CBD (Figure 34). There were no visible peaks at 10 min retention time, which suggested there were no detectable THC in this hemp oil sample. This was not surprising since hemp plants typically contain low levels of THC compared to marijuana plants. Hemp Technique did not show any distinct peaks at both retention time 9.5 and 10 min (Figure 35). Thus, there were no detectable amount CBD or THC in this hemp oil. Figures 36-38 are the chromatograms for Hempio, Greenive, and Zatural, respectively. They all had a visible CBD peak at around 9.5 min and had no detectable THC. In Hempio, the chromatogram was crowded with significantly more peaks compared to the other hemp oil brands. This could be due to the fact that Hempio has other ingredients aside from hemp seed oil.

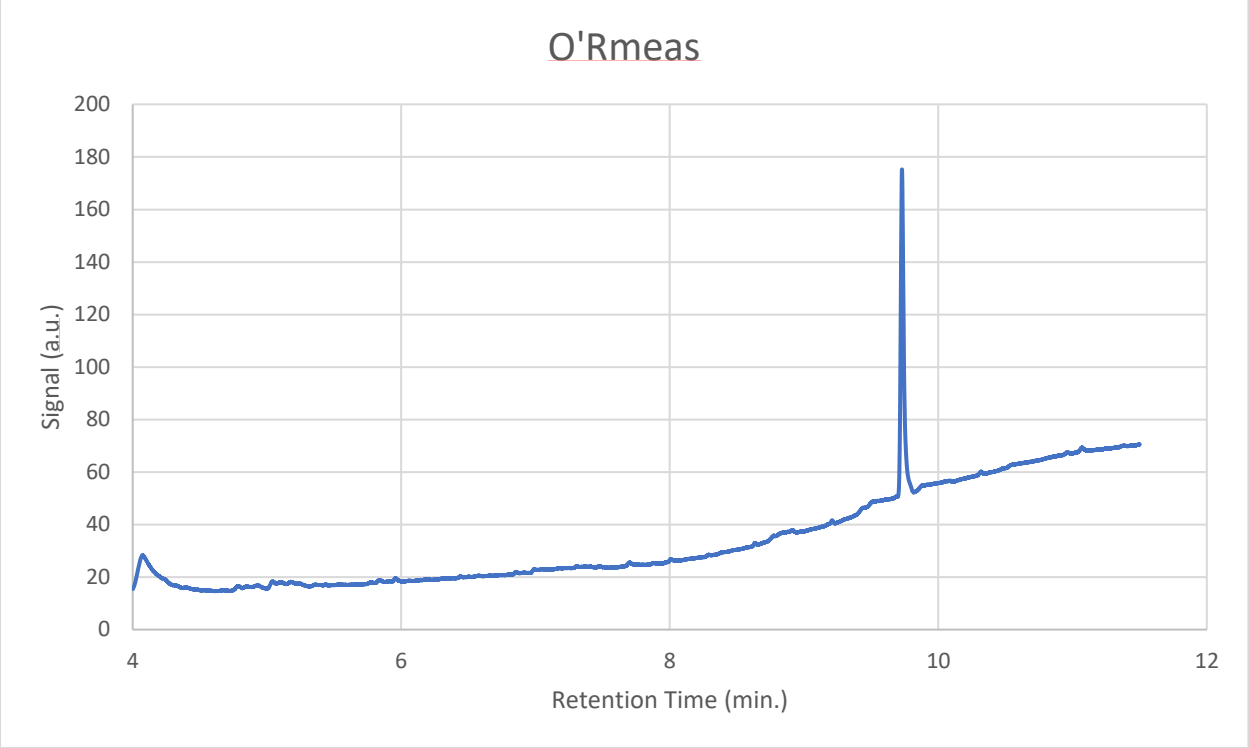

Figure 34. GC-FID chromatogram of O'Rmeas hemp oil.

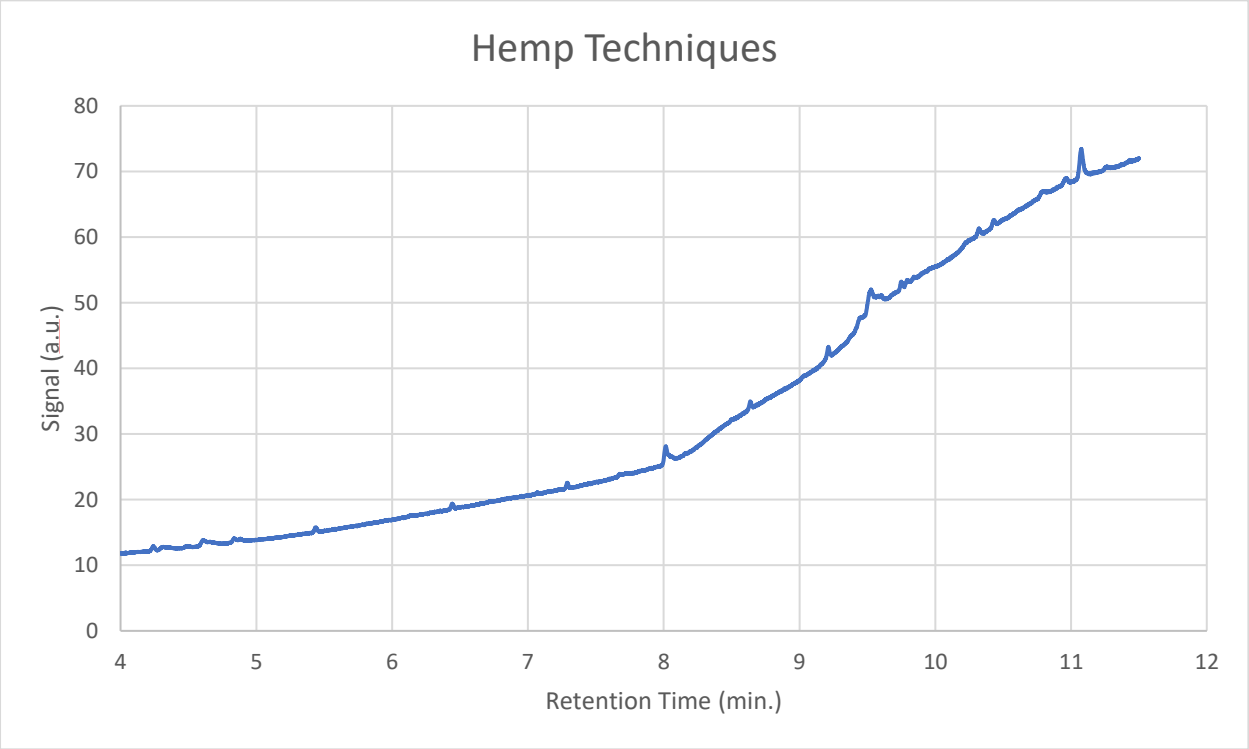

Figure 35. GC-FID chromatogram of Hemp Techniques hemp oil.

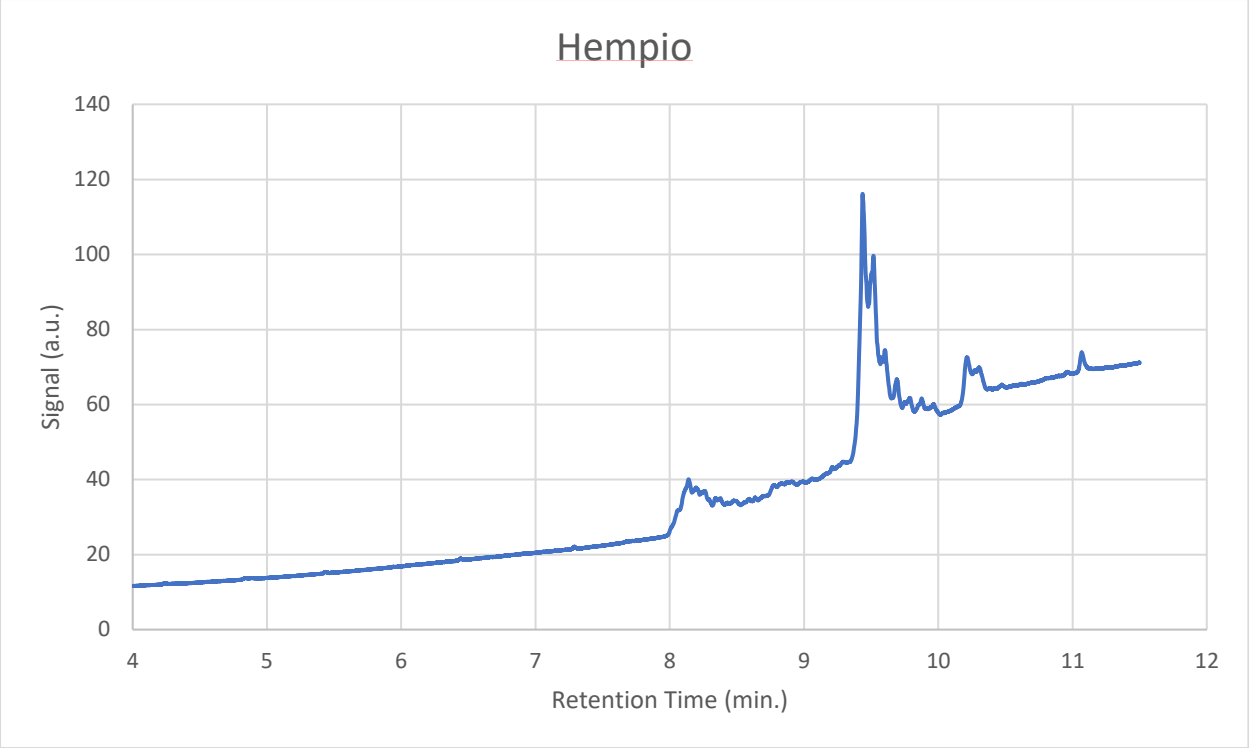

Figure 36. GC-FID chromatogram of Hempio hemp oil.

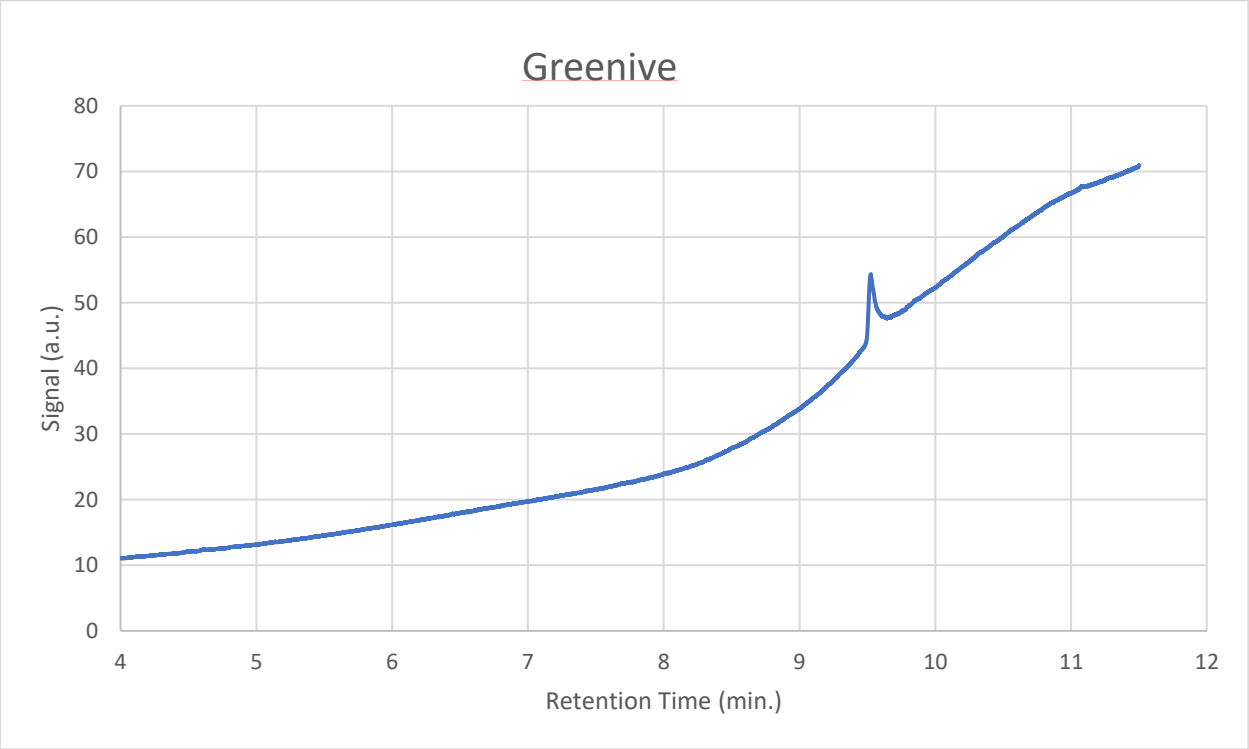

Figure 37. GC-FID chromatogram of Greenive hemp oil.

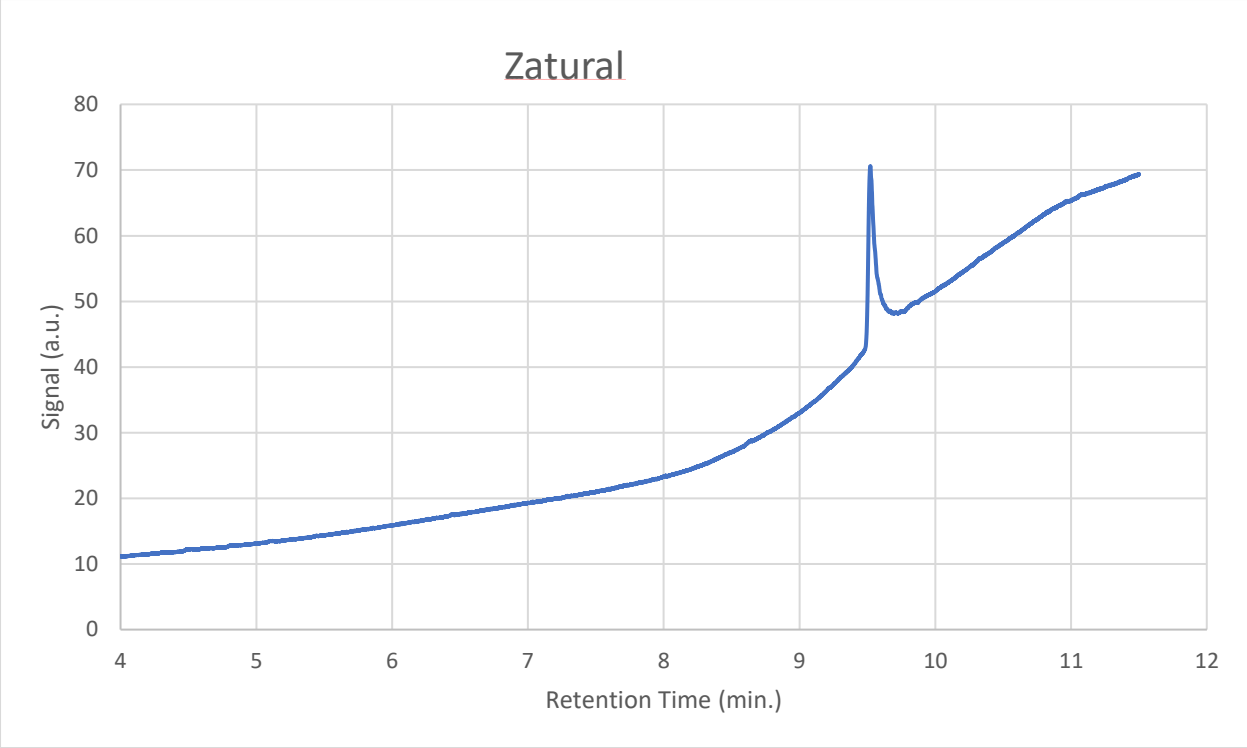

Figure 38. GC-FID chromatogram of Zatural hemp oil.

Overall, four out of the five brands of hemp oils had a detectable amount of CBD. None of the hemp oil had any detectable levels of THC present. Each of the hemp oil had a concentration printed on their products but did not specify what it accounted for. Table 6 shows the calculated CBD amount detected by the GC-FID from the brand with the lowest quantity to the highest quantity of CBD as well as the quantity printed on the product label. Zatural had the highest amount of CBD at 13.83 mg, followed by Greenive at 4.39 mg, Hempio at 0.49 mg, O'Rmeas at 0.14 mg and Hemp Techniques had no detectable CBD. As shown, the actual CBD amount for every hemp oil sample were significantly lower than what was printed on the product label. This indicated the amount on the label was not intended to reflect the CBD amount. The number on the label is most likely indicative of the amount of hemp oil extract in the bottle, however it does not say specifically.

Table 6. Calculated CBD amount for each hemp oil sample and the amount printed on their product label. Amount advertised does not say specify what it alludes to.

Brand Name	Amount Advertised (mg)	Calculated CBD Amount (mg)
Hemp Techniques	30,000	0
O'Rmeas	35,000	0.14
Hempio	35,000	0.49
Greenive	28,000	4.39
Zatural	300	13.83

3.8 GC-MSD of Hemp Oil

Since Zatural was determined to have the highest concentration of CBD, it was ran through the GC-MSD for further chemical analysis. The GC chromatogram of Zatural showed one visible peak (Figure 39). which was suspected to be CBD. The mass spectra of the peak were analyzed and is shown in Figure 40. The parent peak was at *m/z* 231.2 and a second peak observed at m/z 207; its proposed molecular structure is shown on Figure 40.

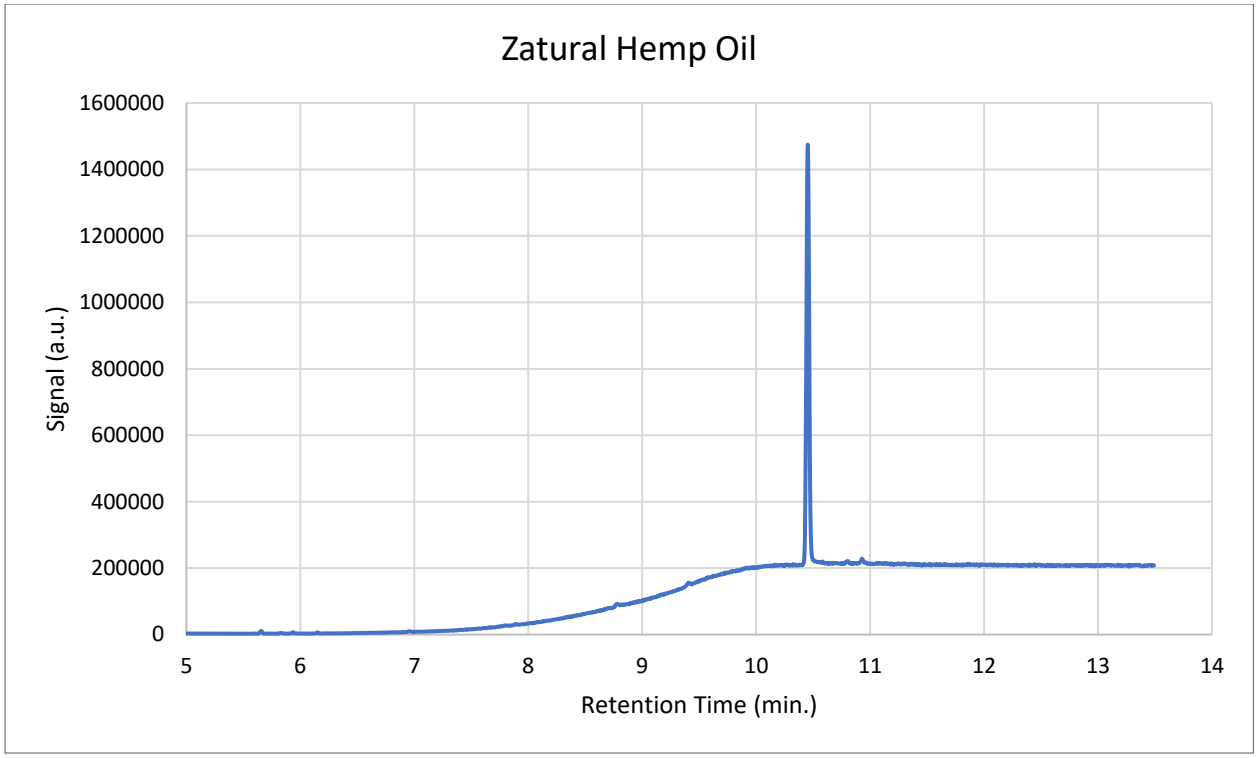

Figure 39. GC-MSD chromatogram of Zatural hemp oil.

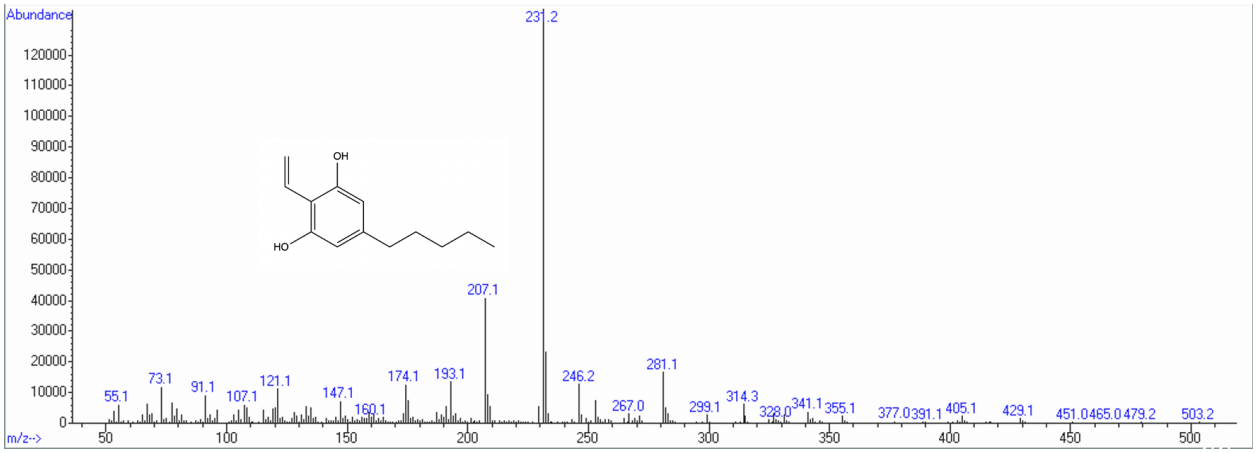

Figure 40. Mass spectrum of the compound at retention time 10.6 min peak (Figure 39). The suspected major fragment ion at the peak at *m/z* = 207.1 is shown above.

IV. Summary and Conclusions

4.1 Challenges of GC Analysis

The basic principle of gas chromatography is to separate organic compounds in a solution by injecting it into a sample port to be vaporized at noticeably high heat. CBD and THC have a relatively high boiling point of 180 °C and 157 °C respectively [20,21]. Subsequently, a high operating temperature is required to separate the cannabinoids from the sample matrix. Due to the harsh conditions and high temperatures of the GC, it could lead to a couple potential challenges researchers need to keep in mind when quantifying and examining CBD. First of all, at high temperatures, septum bleeding can produce ghost peaks in the chromatogram. Second, it is difficult to quantify CBD without taking into account the CBD produced through decarboxylation of its precursor, CBDA. Lastly, CBD is susceptible to degradation and does not have an extensive shelf life. Hence, experiments involving CBD need to be conducted in a timely manner.

4.2 Examination of CBD and Hemp Oils

All things considered, the GC parameters optimized for this research were able to detect and identify CBD in commercial hemp oil products. There were no detectable traces of THC in any of the five hemp oils. This proves the products were in compliance with New York state and federal regulations. Additionally, hemp products are not legally required to explicitly state the concentration of CBD. This provides a loophole for companies to print product labels with a large concentration without specifying what it actually accounts for. The amount printed on the label most likely referred to the total amount of hemp oil extract in the bottle. However, to an uninformed consumer, the vagueness the vagueness of the product label could mislead them into thinking they are buying products containing a generous amount of CBD. This research supports the previous sentiment as four out of the five products tested had significantly lower CBD than what was printed on the label. One of them,

Hemp Techniques, did not even contain any detectable CBD despite the printed label reading 30,000 mg.

Despite the numerous challenges faced in chemical analysis of CBD and THC, it is paramount to continue developing a reliable GC method to quantify cannabinoids, especially in the forensic field. Today, marijuana remains illegal on the federal level, however hemp has been removed from the Controlled Substance Act (CSA). However, law enforcement finds it difficult to differentiate between the two cannabis plants by sight alone since they are remarkably similar in appearance. As a result, forensic scientists rely on differentiating the two plants through chemical analysis. Not only is the GC useful in identifying various cannabinoids, but it can also accurately quantify how much is present in a sample. This is crucial since, legally, hemp and marijuana only differ by their THC concentration. Products containing CBD, such as hempseed oil, are becoming increasingly popular among consumers to help with anxiety, Post Traumatic Stress Disorder (PTSD), fatigue, muscle aches and many more. The result of the present study provides a scientific basis for low levels of CBD in hemp oil products that might suggest otherwise on their product label. By continuing to develop a dependable protocol for CBD analysis can companies be held accountable for product transparency, allowing consumers to truly make the most of the numerous health benefits CBD can provide.

V. References

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