CHARACTERIZATION OF CANNABINOIDS

IN CANNABIDIOL (CBD) PRODUCTS

By

MCCAYLA LOPEZ

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IN CANNABIDIOL (CBD) PRODUCTS

Thesis Approved:

Dr. Jarrad Wagner

Thesis Adviser

Dr. Russell Lewis

Dr. Mark Payton

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Abstract: The purpose of this research was to determine what cannabinoids other than cannabidiol (CBD) can be found in various CBD products, as well as determine their legality. CBD is federally a Schedule I drug, although CBD products are legal for sale in the State of Oklahoma if they contain less than 0.3% tetrahydrocannabinol (THC). Many CBD products sold do not have all the cannabinoids in the product included on the label, which could be a health risk. The samples in this study were purchased from CBD shops in Oklahoma and provided to Oklahoma State University (OSU) for analysis. Liquid chromatography with ultra-violet detection and liquid chromatography with mass spectrometry were used to separate 12 different cannabinoids commonly found in the *Cannabis* plant. The results showed that all five products had a higher amount of CBD than any other cannabinoid. It was also found that when CBD was present, cannabidivarin (CBDV) was also present, although most packages did not indicate the presence of cannabinoids other than CBD. All products contained at least two cannabinoids, with the highest concentration being CBD. All products tested were determined to have false information on their packaging, although they are legal in the State of Oklahoma since the detectable level of THC was less than 0.3%.

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CHAPTER I

INTRODUCTION

Cannabidiol (CBD) has become a well-known alternative to pain relief over the past few years, not only in the United States, but world-wide. It is classified as a cannabinoid, which comes from the *Cannabis* plant, mainly known as marijuana. It has been found to counteract the effects of tetrahydrocannabinol (THC), which is the main psychoactive component in *Cannabis*. (Zuardi et al. 2006) CBD was originally legalized in Oklahoma for children with epilepsy. In 2017, it became legal for any adult over the age of 18 to buy CBD products.

While THC has been extensively researched, little is known about the exact mechanism of CBD and other cannabinoids, including their drug-drug interactions. Because CBD products have not been used for long, there is not much data on what cannabinoids can be found in them. This has caused a gap in research that needs to be conducted for public health, as well as forensic purposes. Many states are experiencing issues with marijuana prosecutions because CBD and hemp is legalized, and the main defense to these prosecutions is CBD or hemp products.

Most studies testing CBD products only determined the concentrations of THC, CBD, and their acidic forms. This study will look for 12 different cannabinoids. These cannabinoids were determined by looking at guidelines for labs testing these products in states with legalized *Cannabis*. Both liquid chromatography with ultra-violet detection and liquid chromatography tandem mass spectrometry were used for confirmation. Both methods are utilized to determine which method could provide more accurate results.

CHAPTER II

REVIEW OF LITERATURE

2.1 Introduction

Marijuana is frequently used across the world. Although illegal in many countries, it has begun to be legalized in many areas due to the possible medicinal properties of some of the compounds found in *Cannabis*. In the United States, every state has a different stance, while the federal government has stayed the same. Marijuana is still classified federally as a Schedule I drug, meaning it has a high potential for abuse, no accepted medical use, and lacks accepted safety for use. This research is being conducted because some states have only legalized high cannabidiol (CBD) and low tetrahydrocannabinol (THC) products. These products are currently unregulated.

2.2 Laws Related to CBD

California was the first state to legalize the medical use of marijuana in 1996. (Mead 2017) As of June 2018, 31 states, the District of Columbia, Guam, and Puerto Rico have implemented these laws. (NCSL 2018) An additional 15 states allow high CBD, low THC products to be sold for medicinal use. (NCSL 2018) Only 4 states do not allow the use of any products made from *Cannabis*. The federal government still views any product that comes from *Cannabis* as illegal. In 1970 the Controlled Substances Act was formed. All drugs were placed

in one of five schedules depending on their abuse potential, accepted medical use in the US, and its safety and potential for addiction. (Anderson 2018) *Cannabis*, and its components, were placed in Schedule I. There have been many petitions to move CBD further down the list because there has been research to show it possesses some medicinal properties.

In May 2014, legislation was passed by the House of Representatives to stop the Drug Enforcement Administration (DEA) from targeting legal medicinal marijuana operations. (Fasinu et al. 2016) Under the Obama Administration, the U.S. Department of Justice issued a memorandum stating it would not prosecute medical marijuana patients and caregivers in states where it is legal. (Gostin et al. 2018) However, in January 2018, Attorney General Jeff Sessions issued a memorandum rescinding the previous guidance. (Gostin et al. 2018) The goal of the new guidance is to prevent minors' access to marijuana. This guidance has caused major controversy with many prosecutors stating they will continue to use the guidelines provided by the Obama Administration. (Gostin et al. 2018)

In April 2015, Oklahoma Governor Mary Fallin signed HB 2154. This bill, also known as Katie's Law, helped children with epilepsy obtain high CBD, low THC oils and products to help with treatment. (procon.org 2018) In 2016, the bill was amended to include adults. It also added other conditions to the list, including spasticity due to multiple sclerosis or paraplegia, intractable nausea and vomiting, and appetite stimulation with chronic wasting diseases, as long as they have written certification from a doctor. In 2017, the bill was amended again, there could be no more than 0.3% THC in the products, and almost anyone could obtain them. (Echols and Yen 2017)

In August 2018, medicinal marijuana became legal in Oklahoma. Title 310, Chapter 681 sets the rules for medicinal marijuana. Its definition of marijuana states, "all parts of a plant of the genus cannabis, whether growing or not; the seeds…the resin…and every compound, manufacture, salt, derivative, mixture or preparation…" (Bailey 2018) Medicinal marijuana

products have a slightly different definition, "a product that contains cannabinoids that have been extracted from plant material or resin…and is intended for administration to a qualified patient…" (Bailey 2018) Standards have been created for medicinal marijuana by 12 Oklahoma residents that have unique qualifications related to food safety and are experts in the marijuana industry. (Bailey 2018)

2.3 Compounds found in *Cannabis*

More than 500 compounds have been identified in cannabis. (Lafaye et al. 2017) They fall into three main groups: cannabinoids, terpenes, and phenolic compounds. Cannabinoids are the major group of compounds found and have been the most researched with about 104 identified. (Lafaye et al. 2017) Cannabinoid concentration varies with the species of *Cannabis*, as well as with the part of the plant used and the time of the harvest. The endocannabinoid system was discovered in 1990. It has two receptors, CB_1 and CB_2. CB_1 receptors are found mainly in the brain, while CB_2 receptors are found in immune and hematopoietic cells. (Fasinu et al. 2016) CB_1 receptors are found at the terminals of the neurons. They affect cognition, memory, motor movements, and pain perception. (Atakan 2012) CB_2 receptors will increase in the nervous system in response to peripheral nerve damage. (Hill et al. 2017)

2.3.1 Cannabinoids

There is a lot of debate about the health benefits of cannabis and its compounds. The main benefit of marijuana is in the treatment of chronic pain. (Hill et al. 2017) There have been many studies indicating cannabis can produce acute pain-inhibitory effects, but more research needs to be done to understand the effects of using cannabis for pain. There have also been some reports about the decrease of opioid dependence and overdoses in states with medical marijuana. (Hill et al. 2017) THC exhibits anti-inflammatory, anti-cancer, analgesic, muscle relaxants, neuro-antioxidative, and anti-spasmodic activities. (Andre et al. 2016) Δ^9-THC is the major

psychoactive component of cannabis and is the most studied of the compounds. Δ^8-THC does not significantly contribute to any activity of the plant and is the isomerization of Δ^9-THC. (Izzo et al. 2009) Δ^9-THC is a partial agonist of CB_1 and inhibits neurotransmitter release. (Atakan 2012)

Cannabinol (CBN) was the first cannabinoid isolated and was originally thought of as the active component. (Izzo et al. 2009) It is a product of Δ^9-THC oxidation, so as Δ^9-THC degrades, CBN increases. It has a lower affinity for CB_1 and a higher affinity for CB_2 compared to THC, affecting the immune system more than the nervous system. (Andre et al. 2016) Cannabichromene (CBC) is one of the major cannabinoids found in fresh cannabis. (Izzo et al. 2009) CBC exhibits some anti-inflammatory, sedative, analgesic, antibacterial and antifungal properties. (Andre et al. 2016) Another major cannabinoid is cannabigerol (CBG). It also exhibits antibacterial and anti-proliferative activity. (Izzo et al. 2009) Both CBC and CBG have low affinity for both CB receptors. (Izzo et al. 2009)

Tetrahydrocannabivarin (THCV) and cannabicarin (CBDV) are found at higher concentrations in *Cannabis indica*, where CBDV is the precursor to THCV. (Rock et al. 2013). *In vivo* work has shown CBDV's potential in anti-inflammatory effects and its effectiveness as an anti-convulsant in animal models. It acts as an agonist at human transient receptor potential (TRP) channels. (Rock et al. 2013) THCV acts as a receptor antagonist for CB_1 and CB_2 receptors, and it activates the CB_2 receptors. It can reduce food intake and body weight, reduce seizures in animal models, reduce inflammation and inflammatory pain, and can reduce Parkinson' s disease symptoms. (Rock et al. 2013)

Cannabinoids are accumulated as cannabinoid acids in the plant. As the plant is dried, stored, or heated they decarboxylize into their neutral forms. Δ^9-tetrahydrocannabinolic acid (THCA) exerts anti-proliferative and anti-spasmodic actions. (Izzo et al. 2009) Cannabidiol acid (CBDA) also exerts anti-proliferative actions. CBDA is 95% of CBD in fresh plant material.

(Izzo et al. 2009) In some cases, there are still concentrations of the acids. When smoking occurs, they become the decarboxylated forms, and are not seen at high concentrations. (Fasinu et al. 2016)

There are many health risks associated with marijuana use: cardiovascular effects, respiratory problems, endocrine effects, birth defects, and cognitive function. Acute marijuana use can cause an increase in heart rate and blood pressure. (Khalsa 2007) Higher doses can cause increased cardiac output. (Khalsa 2007) Bronchitis, coughing, and wheezing are associated with chronic heavy marijuana smoking. In marijuana-only smokers, impairment of pulmonary function, pulmonary responsiveness, and bronchial cell characteristics are found. (Khalsa 2007) Smoking marijuana could also lead to histopathological changes that precede lung cancer and increase the risk of respiratory cancer. (Khalsa 2007) Many studies indicate marijuana affects endocrine and reproductive functions, from hormone secretion to the birth of children. (Khalsa 2007) Chronic, high doses of THC can lower testosterone secretion, impair semen and sperm, and disrupt the ovulatory cycle. (Khalsa 2007) There is some evidence that suggests prenatal exposure of marijuana can lead to postnatal developmental deficits. (Khalsa 2007)

There is also evidence to suggest that chronic use is associated with "…impairment of cognition, particularly affecting short-term memory and executive functioning in humans…". (Khalsa 2007) Patients that were abstinent for 28 days or longer recovered their cognitive function. (Khalsa 2007) This indicates that marijuana use causes only short-term memory function, and not long-term function. Daily marijuana smokers demonstrated withdrawal symptoms, proving that marijuana does produce dependence. (Khalsa 2007)

2.3.2 Terpenes

Terpenes are the largest group of compounds found in cannabis and are responsible for the odor and flavor of cannabis strains. (Andre et al. 2016) They are classified into families by

their number of repeating units of 5-carbon building blocks. (Andre et al. 2016) Terpenes vary similarly to cannabinoids based on the part of the plant harvested and the species. There is a positive correlation between the amounts of terpenes and cannabinoids found in the plant. Terpenes are lipophilic and easily cross membranes. There are many terpenes that are also found in different plants. They have an array of health benefits similar to those found in cannabinoids. (Andre et al. 2016)

2.3.3 Phenolic Compounds

Phenolic compounds make up the last group identified in cannabis. They are one of the most widely distributed groups in plants. (Andre et al. 2016) About 20 flavonoids, a type of phenolic compound, in cannabis have been identified. They also exhibit many of the health benefits found in both cannabinoids and terpenes, and have mainly presented anti-inflammatory, anti-cancer, and neuroprotective properties. (Andre et al. 2016)

2.3.4 Synthetic Cannabinoids

In recent years, a number of synthetic cannabinoids have been created. Only a few have been approved by the Food and Drug Administration (FDA). Dronabinol is a synthetic THC approved for treating anorexia in patients suffering from AIDS. It can also be used to treat nausea and vomiting in patients undergoing chemotherapy. (Lafaye et al. 2017) Nabilone is another synthetic THC also used to help with chemotherapy nausea and vomiting. (Porter 2017) Nabixomols is a combination of synthetic THC and CBD equally used to treat spastic pain in patients with neurological disorders. It has not been approved by the FDA but has approval in several other countries. (Lafaye et al. 2017) In June 2018, the FDA approved the first drug with an active ingredient from cannabis. Epidiolex is an oral solution with CBD to treat seizures associated with Lennox-Gastaut syndrome and Dravet syndrome. (FDA 2018)

2.4 Cannabidiol

CBD is thought to counteract the effects of THC and could be up to 40% of the extract in cannabis. (Zuardi et al. 2006) Over the past few years, the ratio of THC:CBD has changed. THC has increased while CBD decreased. From 1995 to 2014, the potency of 38,681 seized samples of marijuana were tested. There was an 8% increase in THC and a 0.5% decrease in CBD. (Lafaye et al. 2017) This increase in the ratio could lead to higher risks of psychotic effects. (Lafaye et al. 2017)

Because CBD's effects are different than THC's, it might have some significant health benefits. The main medicinal purpose is to help control seizures in people with epilepsy. However, there is not enough proof to definitively state CBD has antiepileptic activity. (Lafaye et al. 2017) There are many uses for CBD products as it has been shown to display some anxiolytic effects, as well as antipsychotic and anti-inflammatory affects. Grotenhermen, *et al.*, found that high doses of CBD alone do not produce THC like effects, meaning CBD is not psychoactive. (Grotenhermen et al. 2017) This is one of the many reasons it has become marketable and been given legal status in many states. Many people turn to these products even though they are not under the guidance of good manufacturing practices and do not have regulations placed on them. (Iffland and Grotenhermen 2017)

CBD is a pleiotropic drug, meaning it produces multiple effects in different pathways. This can lead to a multitude of potential uses. As an antipsychotic, it has been associated with fewer adverse effects than typical antipsychotics. (Izzo et al. 2009) CBD has also been shown to produce anxiolytic actions, possibly due to its activity with serotonin receptors. (Izzo et al. 2009) CBD is also an antioxidant and can exert neuroprotective actions that could be used to treat neurodegenerative diseases. (Izzo et al. 2009)

CBD can be delivered in many different forms, similarly to THC. When taken orally, it has a small bioavailability, about 6%. It is highly affected by the first pass effect. (Fasinu et al. 2016) CBD is highly lipophilic, so it accumulates quickly into adipose tissues and is highly bound to proteins and blood cells. (Fasinu et al. 2016) Smoking, as with most xenobiotics, has the highest bioavailability at around 31%. It is mainly excreted in feces, and a small amount is excreted in urine. CBD has a half-life of 18-32 hours. (Fasinu et al. 2016)

There are not many studies on drug-drug interactions with CBD. There have been a few studies on the effects CBD has with cytochrome P450-enzymes. CBD is partially metabolized by CYP3A4, which metabolizes about 60% of clinically prescribed drugs. (Iffland and Grotenhermen 2017) Some studies have shown that CBD can inactivate CYP450 isozymes for a short time but will induce them after continued administration in mice. (Iffland and Grotenhermen 2017) It is also a potent inhibitor of CYP2C, CYP2D6, an CYP3A isoforms. (Fasinu et al. 2016)

2.5 *Cannabis* Products

Cannabinoids can be provided in many different products. The most common and pure samples are tinctures, which are oils that can be placed under the tongue. (Ministry of Hemp 2016) Concentrates usually have the strongest dosage and are consumed the same as tinctures. (Ministry of Hemp 2016) The most popular type of concentrate is shatter. (Ministry of Hemp 2016) Topicals have also become popular. Similar to lotion, the topical is rubbed into the skin to theoretically help treat chronic pain, inflammation, acne, psoriasis, and anti-aging. (Ministry of Hemp 2016) Vapes are also popular because these oils can be placed in a vape pen and can be consumed at the user's leisure. (Ministry of Hemp 2016) Edibles have also become very popular, especially in places with legalized marijuana. In 2014, 4.81 million units of *Cannabis* edibles

were purchased in Colorado. (Wiley et al. 2016) Edibles can be anything from gummy candies to brownies and cookies.

There have been many challenges associated with *Cannabis* products. In states where edibles are legal, there has been an increase in emergency room visits of children because the packaging can be appealing. (Wiley et al. 2016) Another issue is these products are unregulated, meaning there is a lack of standardization in the formulation of the products, so two formulations of the same edible made by the same company could have very different potencies from each other. (Wiley et al. 2016) Even when there is mandated threshold testing, variations could still occur. This means that most labels are inaccurate.

From 2015-2017, the FDA has tested many products claiming to contain CBD. Many of the products tested did not contain the levels of CBD that the package claimed. (FDA 2017) A study conducted by Bonn-Miller *et al.*, analyzed 84 CBD products from 31 companies sold online to determine the accuracy of labeling. (Bonn-Miller et al. 2017) Thirty-six of the products were under-labeled, 22, were over-labeled, and 26 were labeled accurately. (Bonn-Miller et al. 2017) The concentration of unlabeled cannabinoids in these products was low, with THC detectable in 18 of the samples. (Bonn-Miller et al. 2017) Vandrey *el al.*, also conducted a labeling study for products containing cannabinoids. (Vandrey et al. 2015) They purchased 75 products in three of the biggest cannabinoid industries: San Francisco, Los Angeles, and Seattle. They found that 17% were labeled correctly, 23% were under-labeled, and 60% were over-labeled. (Vandrey et al. 2015) These results show that the labels on cannabinoid product packaging is not always reliable, and it does not always represent everything in these products.

2.6 Previous Analyses

When a substance thought to be marijuana is brought into a lab for analysis, the first step is usually the Duquenois-Levine test. This test has been used for over 80 years in forensic labs.

(Jacobs and Steiner 2014) It consists of three parts and will react with THC to produce a purple color. (Jacobs and Steiner 2014) The Duquenois reagent can cross react with many different substances, leading to many false positives. When the Levine reagent is added, it eliminates the potential of false positives due to the addition of chloroform. Molecules with long aliphatic chains can cross the chloroform layer, which includes THC. (Jacobs and Steiner 2014) Another preliminary test for marijuana is observation under a microscope. Cystolithic hairs are good indicators of plant material because no other plants have these hairs. (NCSCL 2016) According to SWGDRUG, there should be at least two tests performed to determine the identity of the substance. (SWGDRUG 2016)

However, it is difficult to run a preliminary test on a cannabis product. Since they come in many different forms, it can be complicated to determine if there is anything in these products. A few methods have been implemented in forensic drug labs, but they are not perfect. The most common confirmatory test in these labs is gas chromatography with either a mass spectrometer or flame-ionization detector. Both of these tests could be problematic if the scientist is unsure what they are looking for.

Terpenes are chemically unstable in many extraction techniques. Like the cannabinoid acids, they are thermally liable. (Omar et al. 2013) Omar *et al.* focused on finding an extraction method that would assure they could be seen. Supercritical fluid extraction (SFE) can be used because it requires low temperature and pressure during extractions. They were able to see both cannabinoids and terpenes in different extractions using different extraction parameters. (Omar et al. 2013) They proved that terpenes could be seen in GC-MS despite the high temperatures used. Fischedick *et al.*, also found a way to view terpenes using GC-FID. (Fischedick et al. 2010) However, neither study looked at cannabis products, but at various species of *Cannabis sativa*.

Pellegrini *et al.* created a GC/MS procedure to determine cannabinoids in hemp food products. (Pellegrini et al. 2005) They tested three products found in Italy: liqueur, pastilles, and seeds. They found using the European Union standard of 0.2% THC that none of the products were considered illegal. (Pellegrini et al. 2005) They also found that the concentrations of CBD and CBN were lower than THC in all products tested. (Pellegrini et al. 2005) None of the studies conducted using GC in combination with FID or MS could identify the cannabinoid acids because they are thermally unstable and decarboxylize to their neutral forms under high heat. Therefore, liquid chromatography with either an ultraviolet or diode-array detector or an MS detector would be better to find the acids. LC does not need to super heat samples like GC does.

Brighenti *et al.*, determined dynamic maceration was the best extraction method for identifying cannabinoids in hemp using both LC-UV and LC-MS/MS. (Brighenti et al. 2017) Their results show CBDA as the most abundant compound, followed by CBD, which was 4-10 times lower than CBDA. (Brighenti et al. 2017) Peace *et al.*, evaluated two CBD formulations used in electronic cigarettes. Both formulations were from the same manufacturer and claimed to be produced with a hemp strain with the highest CBD potency allowed. (Peace et al. 2016) Both contained at least twice the amount of CBD than what the manufacturer claimed, and the only cannabinoid found was CBD. (Peace et al. 2016) This could be potentially harmful to those using these products because they could be ingesting more than is needed.

More research needs to be conducted to determine not only what is in CBD products, but also at what concentrations these compounds are found. It is dangerous for consumers to conclude that the concentration stated on a product is correct because the literature so far shows the majority are wrong. It is also dangerous for consumers to use these products when there is little data to show what compounds are found in them. This research will help consumers be more aware of the issue that without regulation, no one can be sure what exactly they are using.

CHAPTER III

METHODOLOGY

3.1 Introduction

Cannabinoids are one of the largest groups of compounds found in *Cannabis sativa L.* plants. THC, the main psychoactive substance in cannabis is classified as a cannabinoid. Some people claim cannabis has medicinal purposes; however, because it is illicit, cannabidiol, or CBD, is used instead. Many products have been created with CBD in them and these products are legal across many states. Some of these products state CBD and THC potencies, but not what else might be in them. This project is to develop a method to characterize some of the most common cannabinoids in these products.

3.2 Materials

Methanol (Fisher Scientific, Hampton, NH) was HPLC grade. 98% formic acid (EDM Millipore Corp, Billerica, MA) was ACS grade. Cannabichromene, cannabidiol, cannabidicarin, cannabigerol acid, cannabinolic acid, tetrahyrocannabinolic acid, tetrahydrocannabivarin, and androstenedione were ordered from Cerilliant (Cerilliant Corporation, Round Rock, TX). Cannabidolic acid, cannabigerol, cannabinol, Δ9-tetrahydrocannabinol, and Δ8-tetrahydrocannabinol were ordered from Cayman Chemical (Cayman Chemical, Ann Arbor, MI). **Error! Reference source not found.** below shows the standard concentrations and solvents.

Table 1. Standard concentrations and solvents.

Standard	Concentration	Solvent
Cannabichromene (CBC)	1.0 mg/mL	1 mL Methanol
Cannabidiol (CBD)	1.0 mg/mL	1 mL Methanol
Cannabidolic Acid (CBDA)	1.0 mg/mL	1 mL Methanol
Cannabidivarin (CBDV)	1.0 mg/mL	1 mL Methanol
Cannabigerol (CBG)	1.0 mg/mL	1 mL Methanol
Cannabigerol Acid (CBGA)	1.0 mg/mL	1 mL Acetonitrile
Cannabinol (CBN)	1.0 mg/mL	1 mL Methanol
Cannabinolic Acid (CBNA)	1.0 mg/mL	1 mL Methanol
D8-Tetrahydrocannbinol (THC)	1.0 mg/mL	1 mL Methanol
D9-Tetrahydrocannabinol (THC)	1.0 mg/mL	1 mL Methanol
Tetrahydrocannabinolic Acid (THCA-A)	1.0 mg/mL	1 mL Methanol
Tetrahydrocannabivarin (THCV)	1.0 mg/mL	1 mL Methanol
Androstenedione	1.0 mg/mL	1 mL Acetonitrile

3.3 Instrumentation

All samples were analyzed with a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) consisting of a system controller, CBM-20A, a solvent delivery unit, LC-20AD, an auto-sampler, SIL-20AC, a column over, CTO-20AC, and a UV-vis detector, SPD-20AV. An Agilent Poroshell 120, EC-C18, 3.0 x 50 mm, 2.7 μm column was used for LC separation (Agilent Technologies, Santa Clara, CA).

The Shimadzu HPLC system was attached to an Applied Biosystems 4000 Q-Trap LC-MS/MS System (Applied Biosystems, Foster City, CA). The mass spectrometer was equipped with a Turbo V™ electrospray ionization source, a Harvard Apparatus syringe pump (Holliston, MA) and a Genius 3020 nitrogen generator as the source of gases for the instrument (Peak Scientific Instruments Ltd, Paisley, United Kingdom). Analyst® 1.6.2 Software was used to control the instrument and for data processing.

3.4 Methods

3.4.1 Standard Preparation

A curve was prepared by adding 100 µL of all 12 cannabinoid standards to methanol to create 2 mL of the highest point on the curve, at 50 µg/mL. Curve concentrations of 25 µg/mL, 10 µg/mL, 5 µg/mL, and 1 µg/mL were created using serial dilution. Three quality controls were used at levels of 25 µg/mL, 5 µg/mL, and 0.5 µg/mL. All standards were kept in a freezer between -15° and -20° C. The internal standard solution was prepared at 10 µg/mL by adding 50 µL of androstenedione to methanol to create 5 mL of solution.

3.4.2 Sample Preparation

Solid samples were extracted by weighing 100 mg of dry product. Liquid samples were extracted by removing 100 µL. The samples were placed in a clean, labeled microcentrifuge tube, 100 µL of internal standard solution and 900 µL of methanol was added. The tubes were vortexed for 10 seconds, then placed on a shaker at 2000 RPM for 10 minutes. The tubes are then centrifuged for 6 minutes at 13000 RPM. Supernatant was removed at a volume of 900 µL and placed in a clean tube. Nitrogen was applied to the supernatant until dryness was complete or until only the oil remained. The samples were resuspended in 200 µL of running buffer, 40% mobile phase A/60% mobile phase B, vortexed, and centrifuged for 6 minutes at 13000 RPM. The buffer was removed and placed in vials for analysis.

3.4.3 Analytical Procedure

All unknowns were extracted in triplicate. Each run included a set of calibrators from high to low, a set of quality controls high to low, a blank, the unknowns, a blank, the quality controls high to low, and the calibrators from low to high. There are two methods used to determine the potency, as well as, the cannabinoids present.

3.4.4 Liquid Chromatography Parameters

The analytes were separated using an Agilent Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm column. Mobile phase A is 0.1% formic acid in water. Mobile phase B is 0.05% formic acid in methanol. A flow rate of 1 mL/min was used for a run time of 11 minutes. The column temperature is 50° C. The injection volume is 5 µL. The mobile phase gradient begins at 60% mobile phase B, increases to 77% from 1 minute to 6 minutes, increases to 85% from 6 minutes to 7.75 minutes, and then increases to 95% from 7.75 minutes to 8.75 minutes. It holds at 95% mobile phase B until the end of the run at 9.5 minutes. An equilibration time of 1.5 minutes was utilized to return to starting conditions.

3.4.5 Ultra Violet Detection Parameters

Both the tungsten and deuterium lamps were used. The wavelength detector was set to 230 nm and ran for 9.5 minutes.

3.4.6 Mass Spectrometry Parameters

The mass spectrometer is used to identify the cannabinoids by their masses. The MS was run in both positive and negative mode. Positive mode is used to identify androstenedione. Negative mode is used to identify the 12 cannabinoids. **Error! Reference source not found.** shows the optimized parameters for each analyte.

Table 2. Mass Spectrometer Parameters for each analyte.

Polarity	Q1 Mass	Q3 Mass	Retention Time	Analyte	DP	CE	CXP
Negative	313.081	190.996	8.24	CBC	-70	-30	-9
Negative	313.081	178.916	8.24	CBC 2	-70	-26	-13
Negative	313.092	244.997	6.32	CBD	-100	-32	-13
Negative	313.092	178.967	6.32	CBD 2	-100	-28	-9
Negative	356.785	244.952	6.61	CBDA	-60	-40	-15
Negative	356.785	178.967	6.61	CBDA 2	-60	-28	-9
Negative	285.068	217.026	4.75	CBDV	-65	-32	-11
Negative	285.068	150.937	4.75	CBDV 2	-65	-26	-9

Polarity	Q1 Mass	Q3 Mass	Retention Time	Analyte	DP	CE	CXP
Negative	315.062	191.987	6.39	CBG	-100	-30	-11
Negative	315.062	136.032	6.39	CBG 2	-100	-38	-7
Negative	359.018	315.193	7.07	CBGA	-50	-38	-9
Negative	359.018	148.984	7.07	CBGA 2	-50	-22	-13
Negative	309.037	279.057	7.28	CBN	-105	-44	-15
Negative	309.037	221.889	7.28	CBN 2	-105	-60	-13
Negative	353.931	310.077	8.45	CBNA	-5	-32	-17
Negative	353.931	279.926	8.45	CBNA 2	-5	-50	-15
Negative	313.096	244.993	7.83	D8-THC	-70	-40	-5
Negative	313.096	191.052	7.83	D8-THC 2	-70	-40	-13
Negative	313.046	244.949	7.69	D9-THC	-95	-40	-3
Negative	313.046	191.112	7.69	D9-THC 2	-95	-40	-17
Negative	357.308	313.064	8.79	THCA-A	-110	-50	-15
Negative	357.308	245.005	8.79	THCA-A 2	-110	-44	-13
Negative	285.107	216.937	6.18	THCV	-80	-34	-7
Negative	285.107	162.959	6.18	THCV 2	-80	-40	-9
Positive	287.14	109.056	1.7	Androstenedione	71	27	6
Positive	287.14	97.105	1.7	Androstenedione 2	71	31	2

3.4.7 Percentage Determination

Because most packaging shows the percentage of cannabinoids in the products, determining their percentage was necessary. The equation: %CB = [CB] x (DIL) x (VOL/MG) x 100 is used. [CB] is the concentration of the cannabinoid in µg/mL. (DIL) is the dilution factor used. VOL is the external volume of methanol added to the vial. MG is the sample weight used in mg. The whole equation is multiplied by 100 to get the percent.

CHAPTER IV

RESULTS

4.1 LC/UV Results

Concentrations and percentages of the cannabinoids were determined using the UV method described above. The Ultra-Premium Hemp Oil Tincture (Gold Tincture), Gold Spectrum Full Spectrum Hemp Oil (Gold FSHO), and Bee's Knee's CBD's (Bee's Knees) were oils. The SAT-A-VET Chewable CBD for pets (SAT-A-VET) was a soft pet treat. The Blue Dream Kalm Concentrate (Kalm Concentrate) was a shatter. All five products were purchased at a CBD store in Oklahoma City. **Error! Reference source not found.** displays the concentrations. The products had high levels of CBD and CBG, as well as low levels of CBDV. Four of the products had some detectable level of THC. The percentages of CBD ranged from 0.1% to 2.7%. **Error! Reference source not found.** shows the percentages of each cannabinoid in all five products. Peaks representing the cannabinoids were determined by relative retention time. The relative retention time was determined by subtracting the retention time of the analyte and the retention time of the internal standard. The peak was determined positive if it was within plus or minus 7.5% of the average for the curve.

Table 3. Concentrations of cannabinoids found in LC/UV analysis.

Cannabinoids Concentrations (μg/mL)	Gold Tincture	SAT-A-VET	Kalm Concentrate	Gold FSHO	Bee's Knees
CBC	N/D	N/D	2.6	N/D	1.5
CBD	357.0	145.3	5215.0	1343.3	135.0
CBDA	4.2	N/D	N/D	109.2	N/D
CBDV	8.1	4.0	24.6	93.8	5.7
CBG	23.3	13.6	328.5	23.2	7.9
CBGA	2.0	N/D	4.1	N/D	6.9
CBN	N/D	N/D	2.2	2.7	N/D
CBNA	N/D	N/D	N/D	N/D	3.1
Δ8-THC	N/D	N/D	N/D	2.4	1.8
Δ9-THC	3.1	N/D	3.2	N/D	4.1
THCA	N/D	N/D	1.2	N/D	N/D
THCV	8.3	6.1	3.8	N/D	N/D

N/D denotes the cannabinoid was not detected in this sample.

Table 4. Percentages of cannabinoids found in LC/UV analysis.

Cannabinoids Percentages	Gold Tincture	SAT-A-VET	Kalm Concentrate	Gold FSHO	Bee's Knees
CBC	N/D	N/D	0.0%	N/D	N/D
CBD	0.2%	0.1%	2.7%	0.7%	0.1%
CBDA	0.0%	N/D	N/D	0.1%	N/D
CBDV	0.0%	0.0%	0.0%	0.0%	0.0%
CBG	0.0%	0.0%	0.2%	0.0%	0.0%
CBGA	0.0%	N/D	0.0%	N/D	N/D
CBN	N/D	N/D	0.0%	0.0%	N/D
CBNA	N/D	N/D	N/D	N/D	0.0%
Δ8-THC	N/D	N/D	N/D	0.0%	0.0%
Δ9-THC	0.0%	N/D	0.0%	N/D	0.0%
THCA	N/D	N/D	0.0%	N/D	N/D
THCV	0.0%	0.0%	0.0%	N/D	N/D

N/D denotes the cannabinoid was not detected in this sample.

Example chromatograms of the LC/UV methods are shown in Figure 1, Figure 6, Figure 3, Figure 4, Figure 5, and Figure 6. Figure 1 displays an example chromatogram of the highest

calibrator at 50 µg/mL. Figures 2 through 6 display examples of the unknown samples chromatograms.

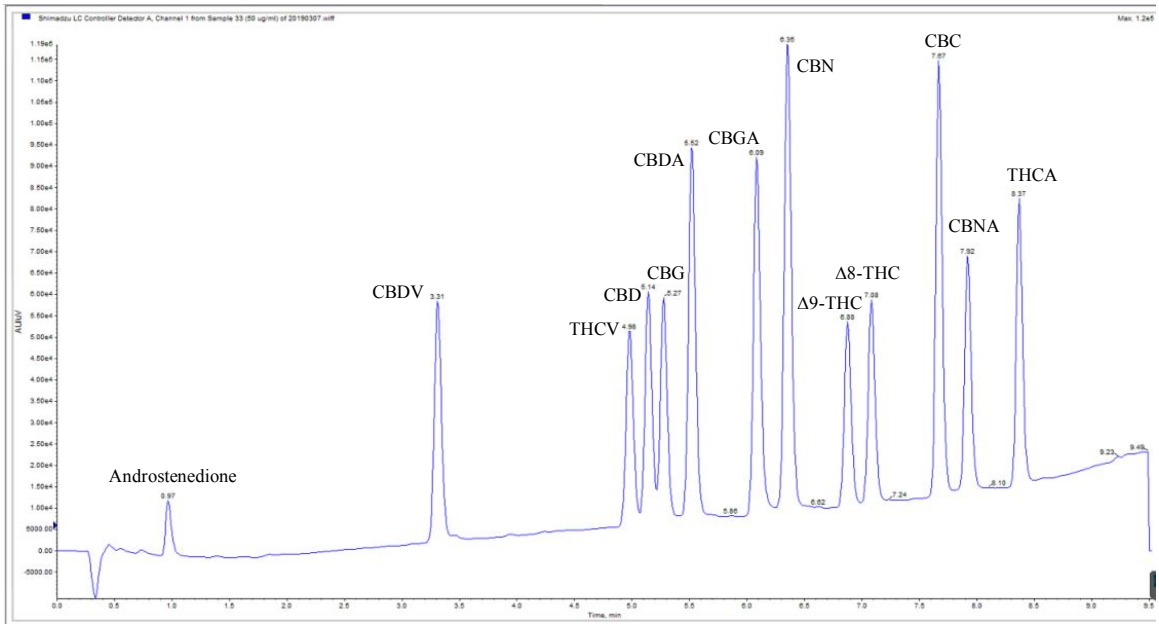

Figure 1. Sample chromatogram of the LC/UV 50 µg/mL calibrator.

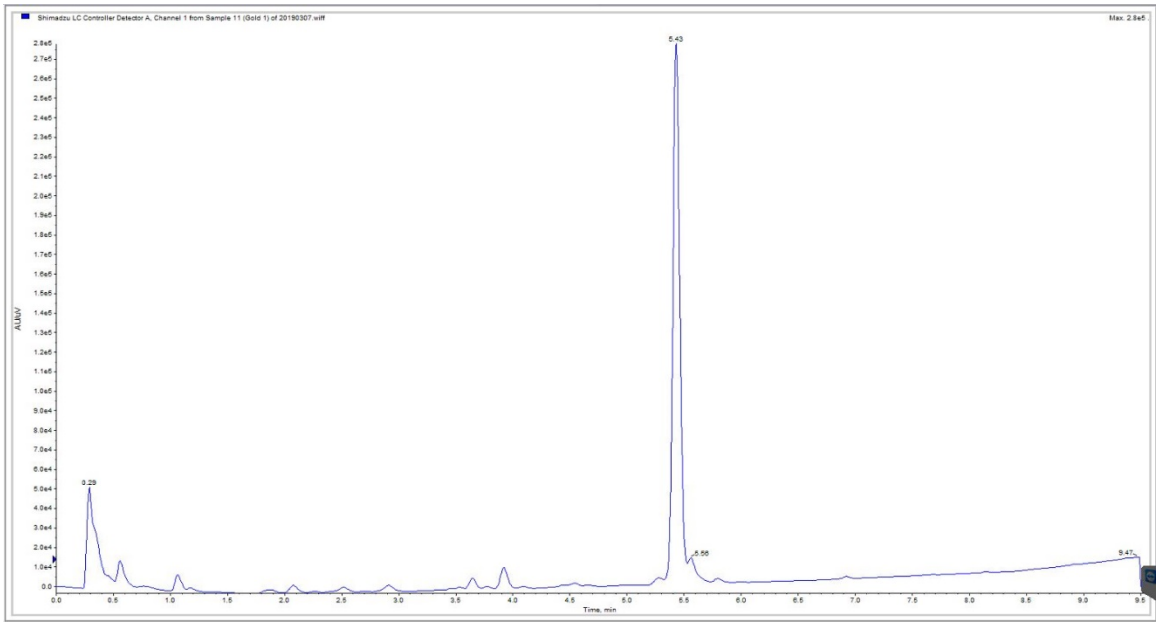

Figure 2. Sample chromatogram of the LC/UV Gold Tincture extract.

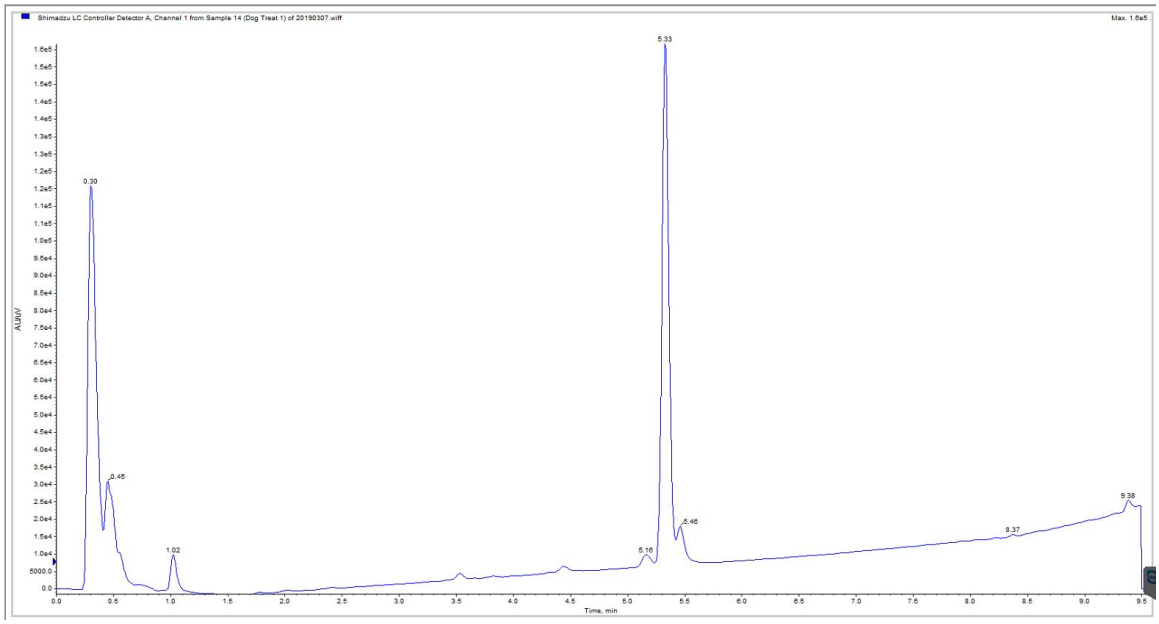

Figure 3. Sample chromatogram of the LC/UV SAT-A-VET extract.

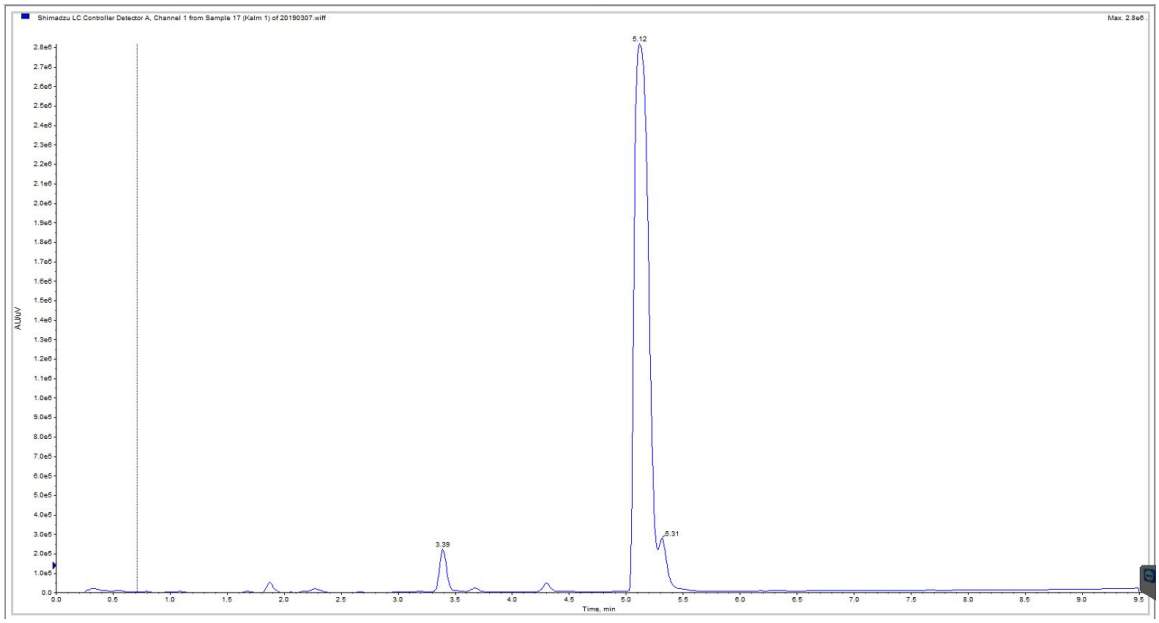

Figure 4. Sample chromatogram of the LC/UV Kalm Concentrate extract.

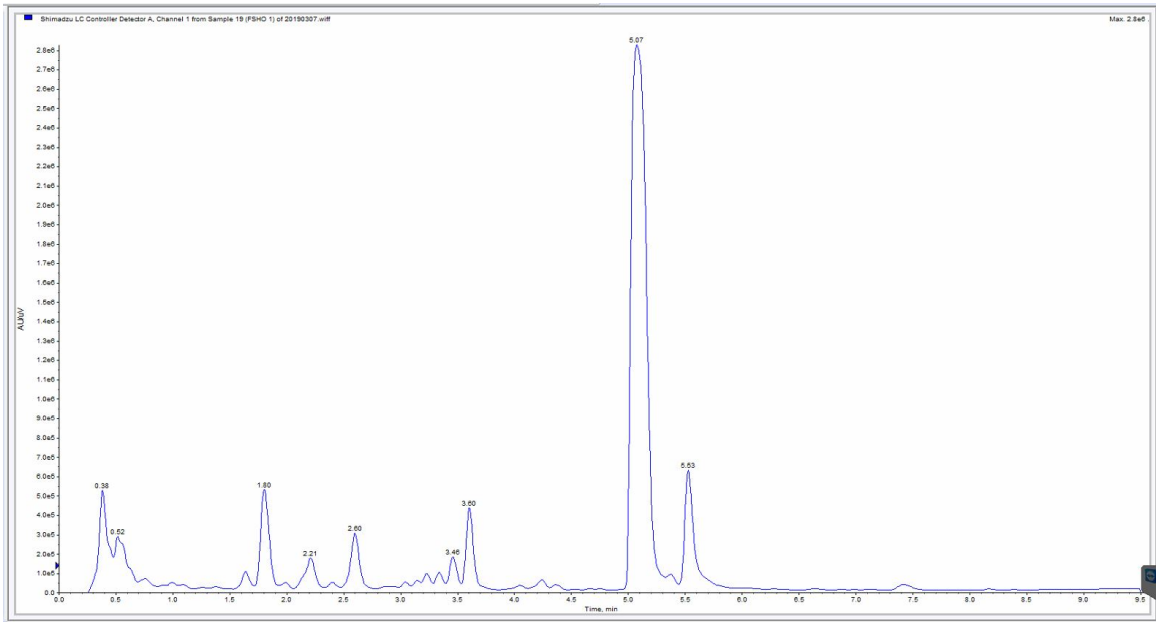

Figure 5. Sample chromatogram of the LC/UV Gold FSHO extract.

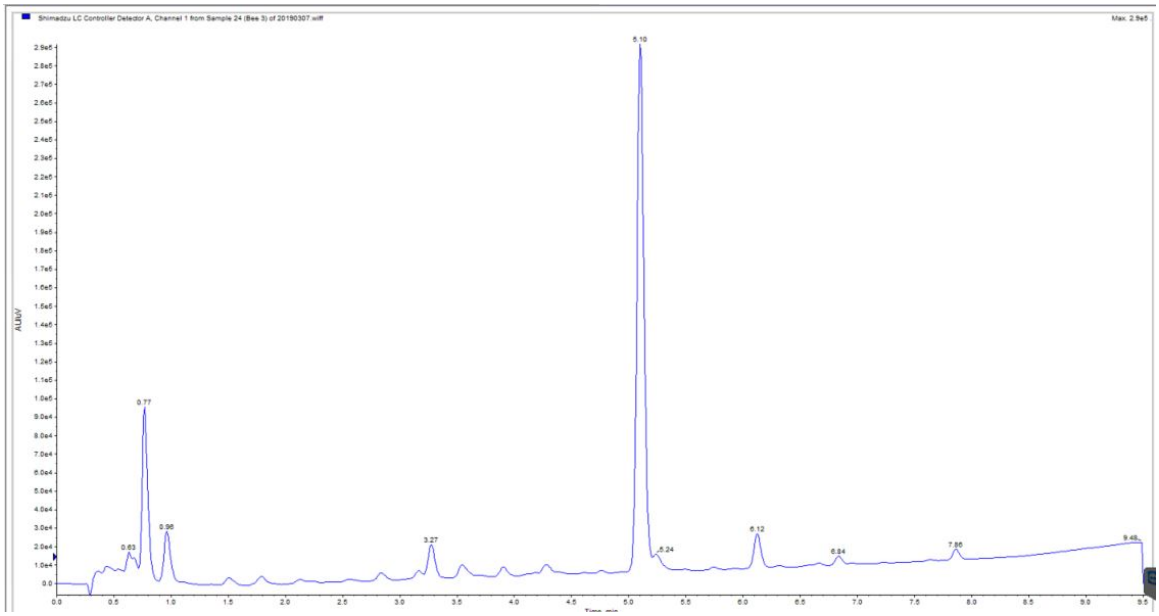

Figure 6. Sample chromatogram of the LC/UV Bee's Knees extract.

4.1.1 LC/UV Statistical Analysis

GraphPad Prism® version 7.04 was used for statistical analysis. CBDV, CBD, and CBG were found in all five products. They were compared using ANOVA. The Kalm Concentrate was not used in the ANOVA because it was an outlier. The values determined for CBDV, CBD, and CBG were determined to be outliers using Grubbs' Test and therefore was not was not used in the ANOVA post-test. THCV, CBGA, and Δ9-THC were found in three of the five products and were also compared using ANOVA. THCV, CBGA, and Δ9-THC include the Kalm Concentrate although it only has two replicates. Table 5 shows the comparisons. In the table below, there exist significant differences among the means as tested with ANOVA. Within a response variable, two means with the same letter are not significantly different using a Fisher-type pairwise comparison at a 0.05 level. The cannabinoid was not detected in the product if N/D is denoted.

Table 5. Means and standard deviations of cannabinoids in each product using LC/UV with ANOVA post-test denotations.

Cannabinoids	Gold Tincture	SAT-A-VET	Gold FSHO	Bee's Knees	Kalm Concentrate
CBDV	8.1^{b}±0.5	4.0^{c}±0.0	19.3^{a}±2.1	10.0^{b}±0.9	
CBD	357.0^{b}±39.0	145.3^{c}±5.7	1343.3^{a}±105.0	135.0^{b}±36.3	
CBG	23.3^{a}±7.9	13.6^{b}±0.1	23.2^{a}±6.3	7.9^{c}±4.7	
CBGA	2.0^{c}±0.0	N/D	N/D	6.9^{a}±0.9	4.1^{b}±0.2
Δ9-THC	3.1^{a}±0.2	N/D	N/D	4.1^{a}±0.9	3.2^{a}±0.4
THCV	8.3^{a}±0.5	6.1^{b}±0.3	N/D	N/D	3.8^{c}±0.3

Two means with the same letters are not significantly different from each other at a 0.05 level. Comparisons are done across the rows.

4.2 LC-MS/MS Results

Concentrations and percentages of the cannabinoids were determined using the MS method described above. Table 6 displays the concentrations. All products were positive for CBD and CBDV. Two of the products had some detectable level of THC. The percentages of CBD ranged from 0% to 1.4%. Table 7 shows the percentages of each cannabinoid in all five products.

The Gold FSHO saturated the detector for CBDA, therefore there is not an accurate concentration and greater than upper limit of quantitation (> ULQ) is provided for the concentration and percentage.

Table 6. Concentrations of cannabinoids found in LC-MS/MS analysis.

Cannabinoids Concentrations (µg/mL)	Gold Tincture	SAT-A-VET	Kalm Concentrate	Gold FSHO	Bee's Knees
CBC	N/D	N/D	N/D	N/D	N/D
CBD	133.0	86.5	2660.0	2450.0	200.7
CBDA	2.6	N/D	26.3	> ULQ	N/D
CBDV	10.4	2.1	986.0	392.0	39.6
CBG	N/D	N/D	N/D	138.3	N/D
CBGA	N/D	N/D	N/D	249.3	N/D
CBN	N/D	N/D	11.6	29.7	2.1
CBNA	N/D	N/D	N/D	2.2	N/D
Δ8-THC	N/D	N/D	N/D	N/D	N/D
Δ9-THC	N/D	N/D	N/D	N/D	11.8
THCA	N/D	N/D	N/D	16.0	N/D
THCV	N/D	N/D	N/D	N/D	19.3

N/D denotes the cannabinoid was not detected in this sample.

Table 7. Percentages of cannabinoids found in LC-MS/MS analysis.

Cannabinoids Percentages	Gold Tincture	SAT-A-VET	Kalm Concentrate	Gold FSHO	Bee's Knees
CBC	N/D	N/D	N/D	N/D	N/D
CBD	0.1%	0.0%	1.4%	1.2%	0.1%
CBDA	0.0%	N/D	0.0%	> ULQ	N/D
CBDV	0.0%	0.0%	0.5%	0.2%	0.0%
CBG	N/D	N/D	N/D	0.1%	N/D
CBGA	N/D	N/D	N/D	0.1%	N/D
CBN	N/D	N/D	0.0%	0.0%	0.0%
CBNA	N/D	N/D	N/D	0.0%	N/D
Δ8-THC	N/D	N/D	N/D	N/D	N/D
Δ9-THC	N/D	N/D	N/D	N/D	0.0%
THCA	N/D	N/D	N/D	0.0%	N/D
THCV	N/D	N/D	N/D	N/D	0.0%

N/D denotes the cannabinoid was not detected in this sample.

Example chromatograms of the LC-MS/MS method are shown in Figure 7, Figure 8, Figure 9, Figure 10, Figure 11, Figure 12, and Figure 13. Figure 7 displays an example chromatogram of the highest calibrator at 50 µg/mL. Figure 8 shows the same chromatogram with the ion transitions for CBG and CBGA extracted. CBG and CBGA had the same retention times, therefore they were not seen as individual peaks in the total ion chromatogram. Figures 8 through 13 are representative chromatograms for the unknown samples used in the study. Some of the peaks are not shown in the total ion chromatogram because the peaks for CBD have a larger height. CBDA saturated the detector in all three extractions of the Gold FSHO.

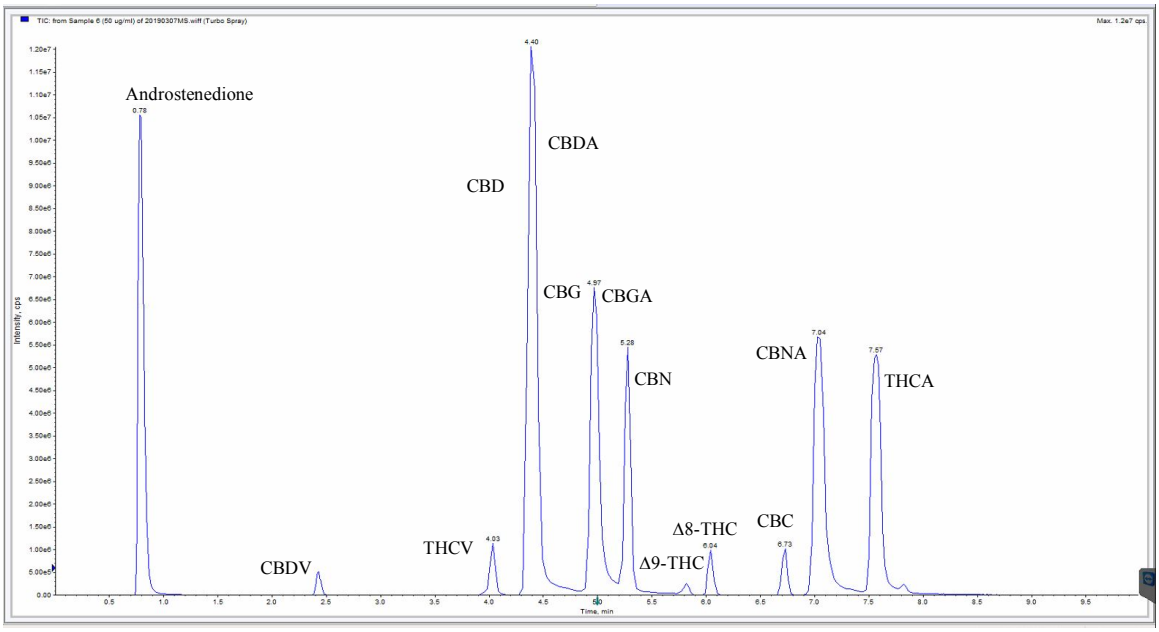

Figure 7. Sample chromatogram of the LC-MS/MS 50 µg/mL calibrator.

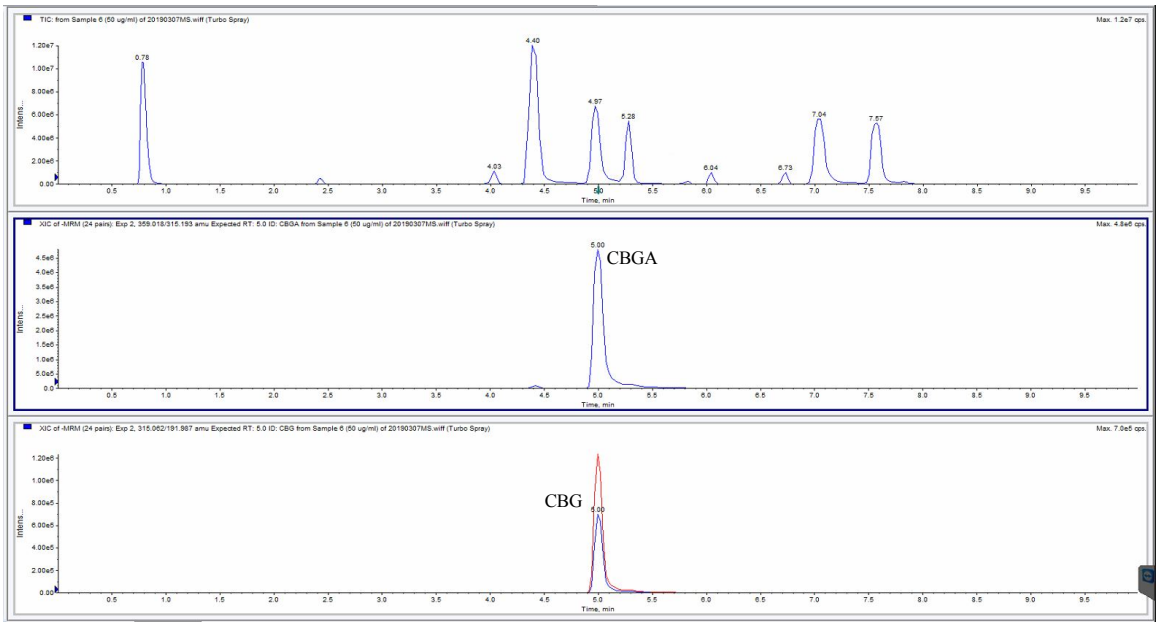

Figure 8. 50 µg/mL calibrator chromatogram showing the extracted ion transitions of CBG and CBGA.

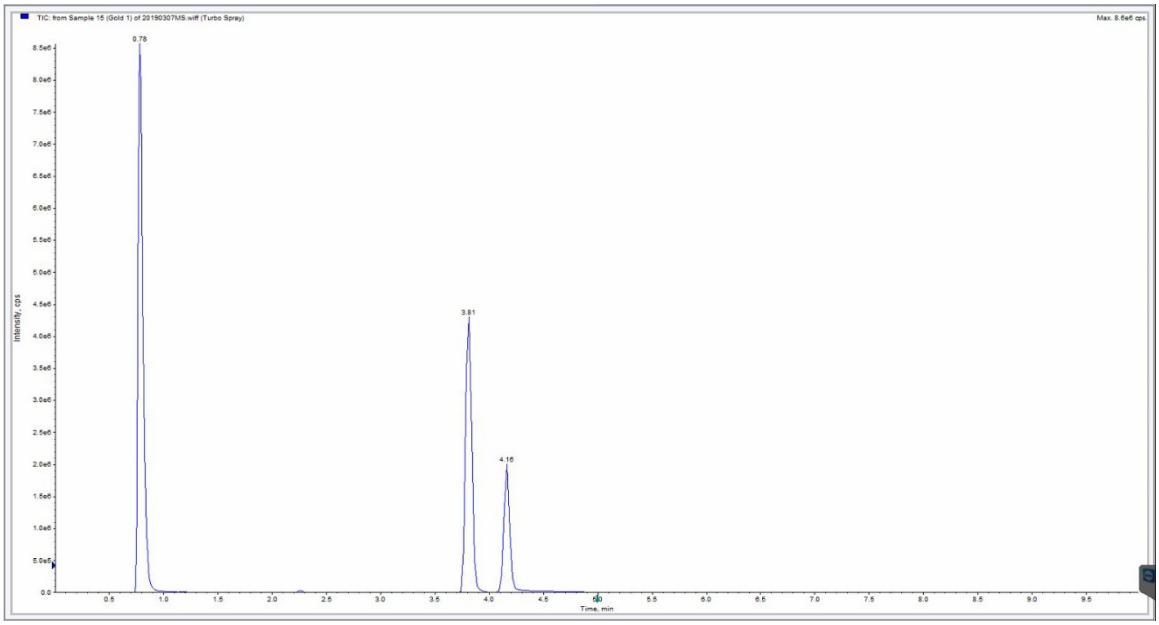

Figure 9. Sample chromatogram of the LC-MS/MS Gold Tincture extract.

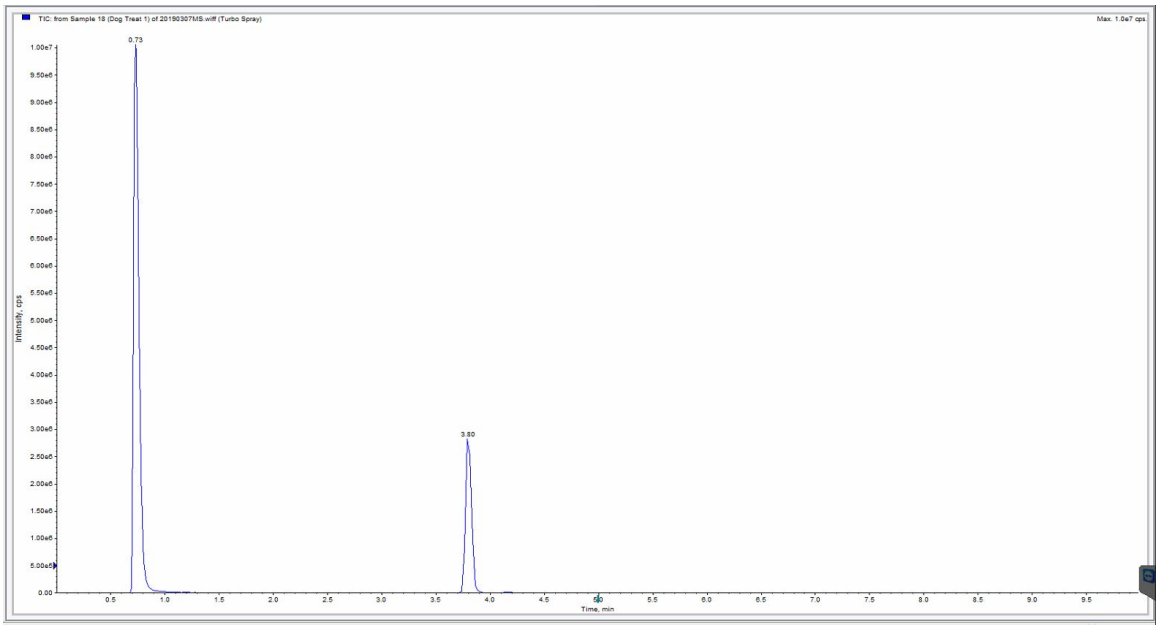

Figure 10. Sample chromatogram of the LC-MS/MS SAT-A-VET extract.

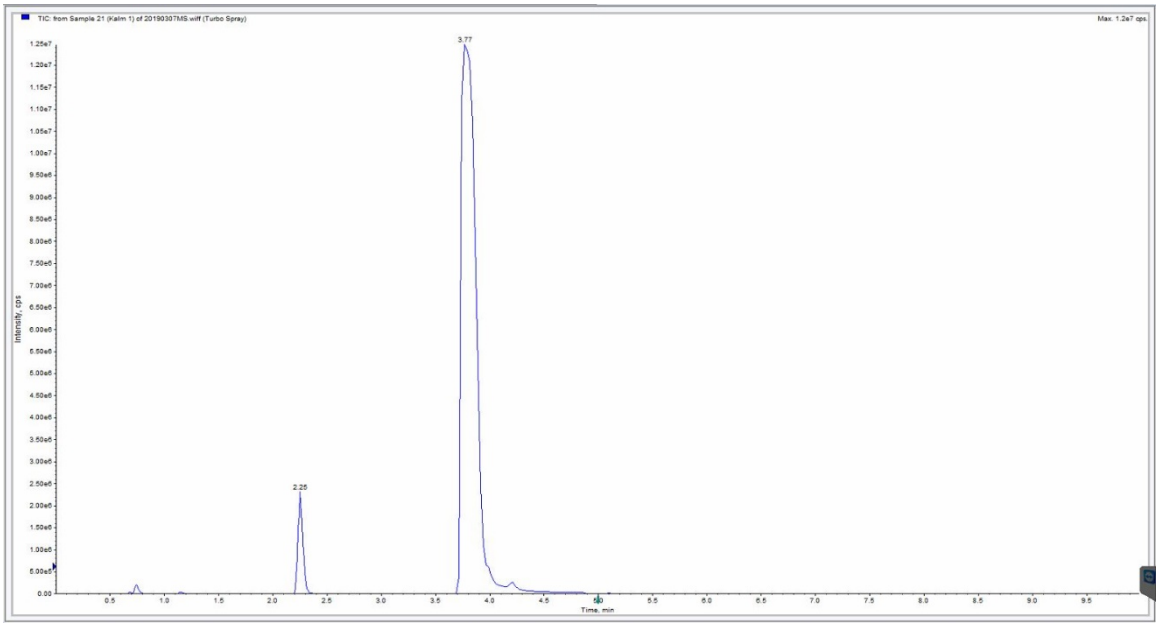

Figure 11. Sample chromatogram of the LC-MS/MS Kalm Concentrate extract.

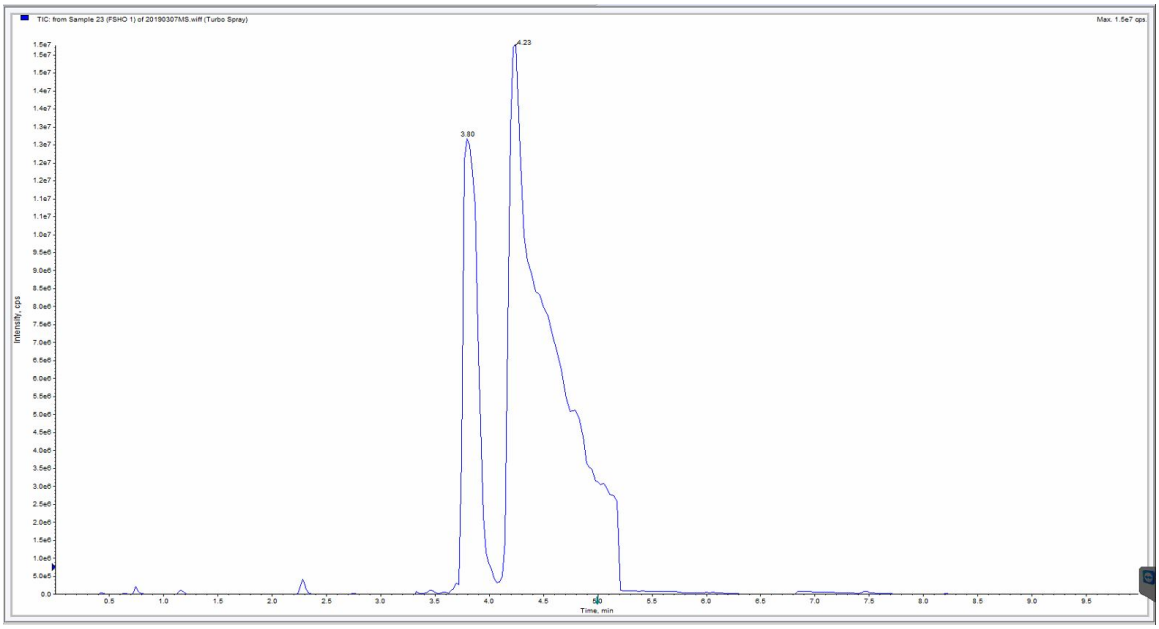

Figure 12. Sample chromatogram of the LC-MS/MS Gold FSHO extract.

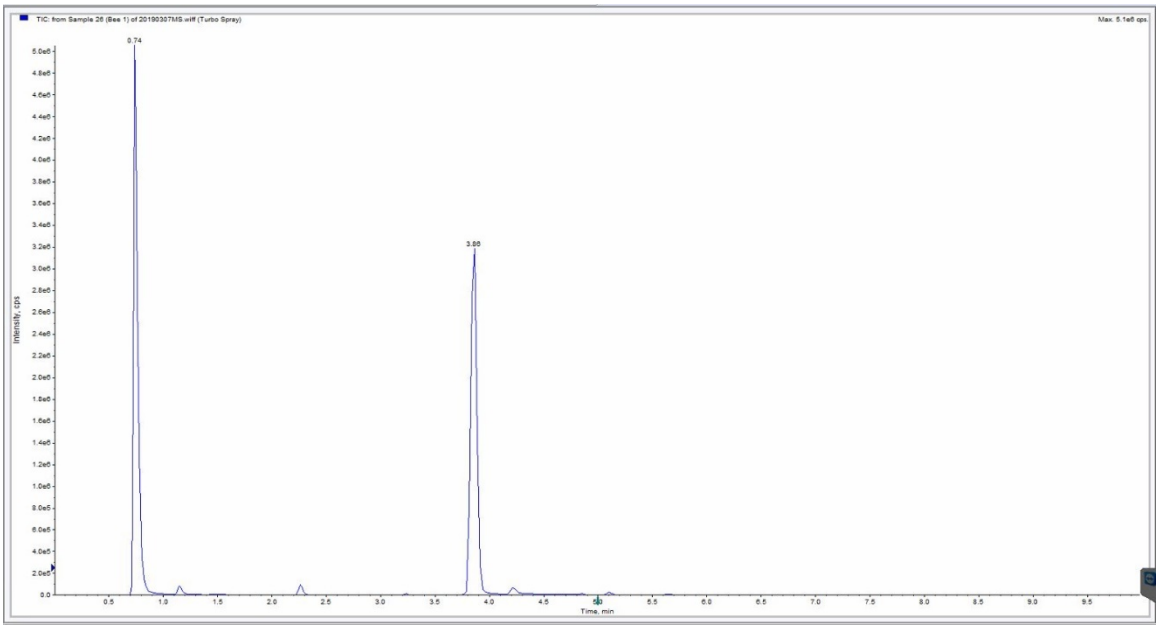

Figure 13. Sample chromatogram of the LC-MS/MS Bee's Knees extract.

4.2.1 LC-MS/MS Statistical Analysis

CBD and CBDV was found in all five products using the MS analysis. These cannabinoids were compared using the same ANOVA test as above. The Kalm Concentrate was not used in the ANOVA because it was an outlier. The values determined for CBD and CBDV were determined to be outliers using Grubbs' Test and therefore were not was not used in the ANOVA post-test. CBN and CBDA was positive in three of the products tested. CBDA does not have an ANOVA test because Gold FSHO was saturated. CBN does include Kalm Concentrate. show the results. In the table below, there exist significant differences among the means as tested with ANOVA. Within a response variable, two means with the same letter are not significantly different using a Fisher-type pairwise comparison at a 0.05 level. The cannabinoid was not detected in the product if N/D is denoted.

Table 8. Means and standard deviations of cannabinoids in each product using LC-MS/MS with ANOVA post-test denotations.

Cannabinoid	Gold Tincture	SAT-A-VET	Gold FSHO	Bee's Knees	Kalm Concentrate
CBD	133.0^{b}±1.0	86.5^{b}±3.7	2450.0^{a}±182.5	200.7^{b}±38.1	
CBDV	10.4^{c}±1.1	2.1^{c}±0.1	392.0^{a}±15.6	39.6^{b}±8.8	
CBN	N/D	N/D	29.7^{a}±7.8	2.1^{b}±1.5	11.6^{b}±0.5

Two means with the same letters are not significantly different from each other at a 0.05 level. Comparisons are done across the rows.

4.3 Method and Packages Comparison

An issue with CBD products is misinformation on the packaging. The five samples used in this study ranged from having no information about the amount of CBD to having the amount of CBD per serving. For comparison purposes, the concentrations obtained for each sample were calculated to equal 1 mL. Table 9, Table 10, Table 11, Table 12, and Table 13 Table *9.* Gold Tincture Comparison.show the concentrations and percentages of each cannabinoid found in each product. The asterisk designates that the concentrations of the cannabinoids was statistically

different between the two analyses according to an unpaired t-test using a confidence interval of 0.05. If the cannabinoid was not detected during the analysis, N/D was used in place of a number.

Table 9. Gold Tincture Comparison.

Cannabinoid	LC/UV Concentration	LC/UV Percentages	LC-MS/MS Concentration	LC-MS/MS Percentages	Packaging Concentration	Packaging Percentages
CBD	3570*	1.8%	1330*	0.7%	2000	1%
CBDA	41.6*	0.0%	26.1*	0.0%	N/D	N/D
CBDV	80.8*	0.0%	103.7*	0.1%	N/D	N/D
CBG	233.3	0.1%	N/D	N/D	10	0.50%
CBGA	19.7	0.0%	N/D	N/D	N/D	N/D
Δ9-THC	31.3	0.0%	N/D	N/D	N/D	N/D
THCV	83.4	0.0%	N/D	N/D	N/D	N/D

*designates the concentrations are statistically different between the two methods

Table 10. SAT-A-VET Comparison.

Cannabinoid	LC/UV Concentration	LC/UV Percentages	LC-MS/MS Concentration	LC-MS/MS Percentages	Packaging Concentration	Packaging Percentages
CBD	1453.3*	0.7%	865*	0.4%	6000	3%
CBDV	39.8*	0.0%	21.4*	0.0%	N/D	N/D
CBG	136	0.1%	N/D	N/D	N/D	N/D
THCV	60.8	0.0%	N/D	N/D	N/D	N/D

*designates the concentrations are statistically different between the two methods

Table 11. Kalm Concentrate Comparison.

Cannabinoid	LC/UV Concentration	LC/UV Percentages	LC-MS/MS Concentration	LC-MS/MS Percentages	Packaging Concentration	Packaging Percentages
CBC	26.1	0.0%	N/D	N/D	N/D	N/D
CBD	52150	27.4%	26600	14.1%	180000	90%
CBDA	N/D	N/D	262.5	0.1%	N/D	N/D
CBDV	246*	0.1%	9860*	5.2%	N/D	N/D
CBG	3285	1.7%	N/D	N/D	4000	2%
CBGA	40.5	0.0%	N/D	N/D	N/D	N/D
CBN	21.8*	0.0%	115.5*	0.1%	N/D	N/D
Δ9-THC	31.6	0.0%	N/D	N/D	N/D	N/D
THCA	12.4	0.0%	N/D	N/D	N/D	N/D
THCV	37.9	0.0%	N/D	N/D	N/D	N/D

*designates the concentrations are statistically different between the two methods

Table 12. Gold FSHO Comparison.

Cannabinoid	LC/UV Concentration	LC/UV Percentages	LC-MS/MS Concentration	LC-MS/MS Percentages	Packaging Concentration	Packaging Percentages
CBD	13433.3*	6.7%	24500*	12.2%	60000	30%
CBDA	1091.7	0.5%	>ULD	N/D	N/D	N/D
CBDV	938*	4.7%	3920*	2.0%	N/D	N/D
CBG	232*	0.1%	1383.3*	0.7%	N/D	N/D
CBGA	N/D	N/D	2493.3	1.2%	N/D	N/D
CBN	26.5*	0.0%	297.3*	0.2%	N/D	N/D
CBNA	N/D	N/D	22.1	0.0%	N/D	N/D
Δ8-THC	24.5	0.0%	N/D	N/D	N/D	N/D
THCA	N/D	N/D	159.7	0.1%	N/D	N/D

*designates the concentrations are statistically different between the two methods

Table 13. Bee's Knees Comparison

Cannabinoid	LC/UV Concentration	LC/UV Percentages	LC-MS/MS Concentration	LC-MS/MS Percentages	Packaging Concentration	Packaging Percentages
CBC	14.6	0.0%	N/D	N/D	N/D	N/D
CBD	1350	0.6%	2006.7	1.0%	N/D	N/D
CBDV	57.5*	0.0%	396*	0.2%	N/D	N/D
CBG	79.4	0.0%	N/D	N/D	N/D	N/D
CBGA	68.5	0.0%	N/D	N/D	N/D	N/D
CBN	N/D	N/D	21.1	0.0%	N/D	N/D
CBNA	30.7	0.0%	N/D	N/D	N/D	N/D
Δ8-THC	17.5	0.0%	N/D	N/D	N/D	N/D
Δ9-THC	40.7*	0.0%	117.7*	0.1%	N/D	N/D
THCV	N/D	N/D	193	0.1%	N/D	N/D

*designates the concentrations are statistically different between the two methods

CHAPTER V

DISCUSSION

5.1 Product Comparison

5.1.1 LC/UV Comparison

All five products had detectable amounts of CBD, CBG, and CBDV. Four of the products had a detectable amount of THC or THCA. All products had at least four cannabinoids, with the Kalm Concentrate and Bee's Knees having the most cannabinoids at eight each. The highest concentration of the standards was 50 μg/mL. Any concentration above that number is an estimation because it is above the quantitation level. Other cannabinoids were detected in each product below the lower limit of 1 μg/mL and were therefore not included in the study.

The Gold Tincture and Bee's Knees were not significantly different when comparing CBDV concentrations. The Kalm Concentrate had the highest overall concentration but could not be used due to its insufficient number of replicates. The concentration of CBD was higher than all the other cannabinoids, which was expected. SAT-A-VET and Bee's Knees were not significantly different when comparing CBD. They had the lowest concentrations. Gold FSHO was only significantly different from Bee's Knees when comparing CBG. Most of the concentrations found were around 20 μg/mL, except the Kalm Concentrate.

THCV was found in three of the products. Gold Tincture, SAT-A-VET, and Kalm Concentrate were all significantly different from each other. CBGA was also found in three products, Gold Tincture, Kalm Concentrate, and Bee's Knees. They were also significantly different from each other. Δ9-THC was found in Gold Tincture, Bee's Knees, and Kalm

Concentrate, and were not significantly different from each other.

CBDA was found at a low concentration in the Gold Tincture and at a high concentration in Gold FSHO. CBN was also found at low concentrations in Kalm Concentrate and Gold FSHO. Δ8-THC was found in Gold FSHO and Bee's Knees. CBC was found in Kalm Concentrate and Bee's Knees. Only Bee's Knees contained CBNA and THCA was only found in Kalm Concentrate.

Overall, the Kalm Concentrate had the highest concentrations of cannabinoids present. Since concentrates are known for having the highest potency on the market, this makes sense. The SAT-A-VET pet treats had the lowest concentration of cannabinoids present.

5.1.2 LC-MS/MS Comparison

All five products had detectable amounts of CBD and CBDV. Two of the products had a detectable amount of THC or THCA. All products had at least two cannabinoids, with Gold FSHO having the most cannabinoids at eight. The highest concentration of the standards was 50 µg/mL. Any concentration above that number is an estimation because it is above the quantitation level. Other cannabinoids were detected in each product below the lower limit of 1 µg/mL and were therefore not included in the study. One product, Gold FSHO, saturated the MS detector. Therefore, there is not a concentration provided for CBDA for this product.

Only Gold FSHO was significantly different from all other products when comparing CBD. The other products were not significantly different from each other. Gold Tincture and SAT-A-VET were not significantly different from each other when comparing CBDV. All other products were significantly different. CBN and CBDA were positive in three of the products. Due to the saturation of Gold FSHO, CBDA was not compared statistically. For CBN, Bee's Knees and Gold FSHO were significantly different, as were Gold FSHO and Kalm Concentrate.

Only Gold FSHO contained CBG, CBGA, CBNA, and THCA. Bee's Knees was the only product that contained Δ9-THC and THCV. However, in the last replicate run, THCV was not detected. CBC and Δ8-THC were not found in any of the products.

5.1.3 Comparison of Methods

The results obtained between the two analyses were different. A main reason for this could be the specificity obtained with the LC-MS/MS method versus the LC/UV method. The LC-MS/MS looks for specific mass transitions, while the LC/UV looks for an absorbance that is common to cannabinoids but not necessarily specific. Both methods utilized chromatography, and it was found that the relative retention times varied by about 7.5% across all the calibrators, quality controls, and unknown samples. This is slightly more variation than is usually acceptable in a liquid chromatography method, and it is apparent that the wider variation depended on the specimen type. For instance, it was observed early in method development that oil-based products caused a shift to the left in the chromatogram (eluting earlier), and therefore the approach of extraction into solvents, followed by dry down and resuspension in running buffer, was utilized. It is felt that future work should attempt to measure actual extraction efficiencies from each product type, and somehow normalize results to account for the variations in extraction efficiency. It was more difficult to discern peaks on the LC/UV than the LC-MS/MS, as there were far more peaks in a given retention time window via LC/UV than LC/MS/MS. The analyst determined if the cannabinoid was present by verifying the peak was within 7.5% of expected.

The methods agreed that CBD and CBDV were found in all products. However, CBG was found in all products using the LC/UV but was only found in Gold FSHO using the LC/MSMS. There were also less positives for THC in the LC/MSMS than were found in the LC/UV. With the exception of Gold FSHO and Bee's Knees, the total concentrations and

percentages of cannabinoids in the products was higher with the LC/UV. Most of the products had a higher number of cannabinoids identified in the LC/UV method.

5.2 Actual v. Expected Percentages

It is important to point out that the products themselves presented a variety of matrices that created challenges in complete and consistent cannabinoid extraction. While internal standard was used to control for extraction efficiency somewhat, the studies did not discern between potential matrix effects and actual differences in extraction efficiencies.

The Gold Tincture had a serving size of 1 mL on the packaging. In the 1 mL serving, there should be 10 mg, or 1%, CBD and 0.5 mg, or 0.05%, CBG. The percent of CBD determined for 1 mL in the LC/UV method was 1.8% and 0.7% in the LC-MS/MS method. For CBG, 0.1% was detected in the LC/UV method and there was no detectable amount in the LC-MS/MS method. The total cannabinoid percent found in the LC/UV method was 2% and, in the LC-MS/MS method, was 0.7%. The SAT-A-VET treats claimed 1.5 mg of the 50 mg treat, or 3%, was CBD and an additional 5 mg, or 10%, was hemp extract. The percent determined per treat was 0.7% CBD and 0.8% total cannabinoids in the LC/UV method. In the LC-MS/MS method, 0.4% CBD and 0.4% total cannabinoids.

The Gold FSHO packaging stated 30% Active CBD and no other cannabinoids were represented on the packaging. Since this was not given as a serving, it was assumed that this number was for the total volume of 1 mL. The percent determined for 1 mL was 6.7% in the LC/UV method. The total percent of cannabinoids present is 7.9%. The LC-MS/MS method determined 12.2% CBD and 16.3% overall cannabinoids. The Kalm Concentrate stated 90% CBD and 2% CBG. Because there is no serving size provided on the packaging, 1 mL was assumed. The actual percent calculated for 1 mL was 27.4% CBD and 1.7% CBG. The total

cannabinoids present was 29% in the LC/UV method. The total percent of cannabinoids found in the LC-MS/MS method was 19.6%, with 14.1% CBD and no CBG found.

The Bee's Knees packaging contained no information about serving size or the cannabinoids present in the product. There were three syringes with 50 mg each in the packaging. Since there are three syringes, it was assumed one syringe is one serving. There was 0.3% CBD in each serving, with total cannabinoids determined as 0.4% in the LC/UV method. The LC-MS/MS method found 0.5% CBD and 0.6% total cannabinoids.

All five products have incorrect labeling. Three of the five products had concentrations lower than stated on the package using both the LC/UV and the LC-MS/MS for comparison. Gold Tincture was higher when using the LC/UV method for comparison, but lower when using the LC-MS/MS method for comparison. The fifth, Bee's Knees, had no information about concentrations of cannabinoids. All products had more cannabinoids than were stated on the labels.

5.3 Conclusions

The results found in this study were consistent with results found in other studies. All CBD products tested were determined to be legal because they had low detectable amounts of THC. None of the packaging on the products tested were correct, due to a lack of regulations in Oklahoma. Most CBD products are created using *Cannabis indica*. This seems to be true of the products used in this study. *Cannabis indica* has a lower concentration of THC, and has higher concentrations of the other, non-psychoactive cannabinoids.

The LC-MS/MS method may be more reliable than the LC/UV method because mass spectrometry is more sensitive and more reliable with retention time identification. While the LC/UV method did work, there was more noise and because you cannot create a retention time window, it was harder to identify for certain which peaks were cannabinoids due to the peak

shifting in the samples. The noise is most likely due to the many other compounds that are present in these products. Terpenes are common in CBD products because they help with flavor and odor. Artificial flavors may have also been added to help with the taste. There are also many more cannabinoids than those looked for in this research. This can also lead to an increase in peaks in the LC/UV chromatogram.

There are many issues with products containing phytocannabinoids. Most have inaccurate data on their labels and do not provide serving sizes. This is a challenge to novel consumers, as well as experienced users, which can lead to adverse reactions. Regulations are needed to solve this problem. Another issue with these products is the lack of research on the non-psychoactive cannabinoids. While they do not cause the "high" associated with products containing THC, most of their mechanisms of action are not known. There is also a lack of drug-drug interaction studies, which could cause major problems for many people. The current study points to the presence of a multitude of cannabinoids, and shows the need for future research to characterize CBD products and determine their effects on users.

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VITA

McCayla Lopez

Candidate for the Degree of

Master of Science

Thesis: CHARACTERIZATION OF CANNABINOIDS IN CANNABIDIOL PRODUCTS

Major Field: Forensic Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in Forensic Sciences at Oklahoma State University, Stillwater, Oklahoma in May 2019.

Completed the requirements for the Bachelor of Science in Biology at University of North Texas, Denton, Texas in May 2017.

Experience:

Oklahoma State University Clinical Laboratory Services August 2017

Professional Memberships: Society of Forensic Toxicologists