THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

DEVELOPMENT AND APPLICATION OF AN ANALYTICAL METHOD FOR CANNABIS

REBECCA PLESSEL
Spring 2017

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biochemistry and Molecular Biology
with honors in Biochemistry and Molecular Biology

Reviewed and approved* by the following:

Frank Dorman
Associate Professor of Biochemistry and Molecular Biology
Thesis Supervisor

Lorraine Santy
Associate Professor of Biochemistry and Molecular Biology
Honors Adviser

Scott Selleck
Department Head for Biochemistry and Molecular Biology

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Throughout history, cannabis has been used as a panacea, an herbal remedy for nearly all medical concerns from simple headaches to severe seizures. Now that many states have legalized medical cannabis, it is important to have analytical methods to study the compounds that the patients will be ingesting or inhaling. Our lab focused especially on the common five most concentrated cannabinoids: Δ-9-tertahydrocannabinol, cannabidiol, cannabinol, cannabigerol, and cannabichromene. The cannabinoids themselves have medical relevance, and are helpful in management of symptoms in patients with seizures and pain.

With the increase in usage of cannabis for medical ailments, creating a method for analyzing these compounds is necessary for the regulation of the industry and safety of distribution. The cannabinoid analytical method was developed on a GC-FID using liquid injection, following sample extraction. Additional experiments using this method were performed to assess cannabinoid variance, as consistent dosing is difficult natural products in general, and cannabis, specifically. If the cannabis is not homogenized before administration, the patient could be underdosing or overdosing due to natural variance. To test this, we determined the concentrations of the five major cannabinoids in homogenized and non-homogenized cannabis samples. We found that homogenized cannabis has a lower variance than unhomogenized samples of the same plant. This suggests that the medical marijuana industry should be more tightly regulated, especially with regards to use of the actual natural product or plant tissue directly, as a simple change in sample preparation makes the drug dose much more consistent.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to thank the following people and companies for their assistance in this project. Thank you Dr. Frank Dorman, my research advisor, for all the opportunities he's given me to grow as scientist, improve my problem solving skills, and learn about all the aspects that go into research. Thank you to Maura McGonigal, our previous research assistant, who taught me almost all the techniques I know. Thank you to the Dorman lab for all the help and support. Restek Corp. collaborated with our group to provide us with standards. Julie Kowalski is our main contact at Restek and has been especially helpful. Gerstel USA provides us with instrumentation. Thanks to Gerstel's president Robert Collins for all his support. Jackie Whitecavage and Heather Beiter have provided assistance in trouble shooting with experiments and malfunctioning instrumentation. Thanks to Agilent for providing us with the instruments to conduct these experiments. Additionally, without the help of Officer Randy Hoffman and Lieutenant Stephanie Brooks from the University Park Police Department, and Chief Diane Conrad from the Ferguson Township Police Department we wouldn't have had any samples to test.

Chapter 1

Background Information

What is Medical Cannabis

It is estimated that over 8% of the United States population have used, or currently use cannabis.[1] This puts the current total at roughly 25.5 cannabis users within America alone. While this number encompasses all the self-reported recreational users, nearly 1.5 million people are registered as medical cannabis patients.[2] The NIH as defines medical cannabis, "using the whole, unprocessed marijuana plant or its basic extracts to treat disease or symptom."[3]

History of Cannabis

As early as 104 BC, the cannabis plant was used for several purposes, including textiles, paper, rope, and food in China during the Han dynasty. Later in Chinese history, cannabis was no longer used for food, but for medicinal purposes. The Chinese used this plant to treat "rheumatic pain, intestinal constipation, disorders of the female reproductive system, malaria, and others."[4] Even today, cannabis seeds are still used as a laxative by Chinese physicians. In the history of India, cannabis was widely used as a medication, and also a psychoactive drug, being described as one of the sacred plants: the source of happiness, joy, and freedom. Here it was used as an analgesic, anticonvulsant, hypnotic, tranquilizer, anesthetic, anti-inflammatory, antibiotic, anti-parasite, antispasmodic, digestive and appetite stimulant, diuretic, and expectorant.

From the beginning of the Common Era (0 AD) to the 18th century, cannabis use remained heavy in India and spread to the Middle East and Africa. In Africa, cannabis use has been traced back to the 15th century at the latest. During this time, cannabis also spread to the Americas in roughly the 16th century, starting in South America. It likely started in Brazil where the seeds of the plant were carried over by African slaves. Here it was used as part of a religious ritual and in the treatment of diseases and ailments including toothaches and menstrual cramps. Around this time, cannabis was used exclusively for fibers and rope until its potential as a paper product was introduced by Mu

It's use in the West extended into the 19th century where the seeds were used as homeopathic medications until an Irish physician, Willian B. O'Shaughnessy, expanded its use. He tested its toxicity in animals, studied literature on the plant, described various preparations, and tested its effects on patients with different pathologies. He published his work and describes successful human experiments where the plant was used to alleviate symptoms of rheumatism, convulsions, and most importantly muscular spasms of tetanus and rabies. After this, the research into cannabis grew exponentially, and out of all the data, a review was written that summarized the many findings. First, cannabis is a sedative and hypnotic able to be used for various mental ailments from rabies to insomnia. Second, it is an analgesic, able to relieve pain from diseases, or cure a headache. Finally, it had various other uses affecting all parts of the body including the stomach, lungs, intestinal tract, and female reproductive system.[4]

In part due to its various effects that were unpredictable for each patient, Western medicine reduced and nearly abolished its use as a legitimate medicine in the 20th century. Not long after, the drug was illegalized and experimentation with cannabis came to a nearly complete halt.[4]

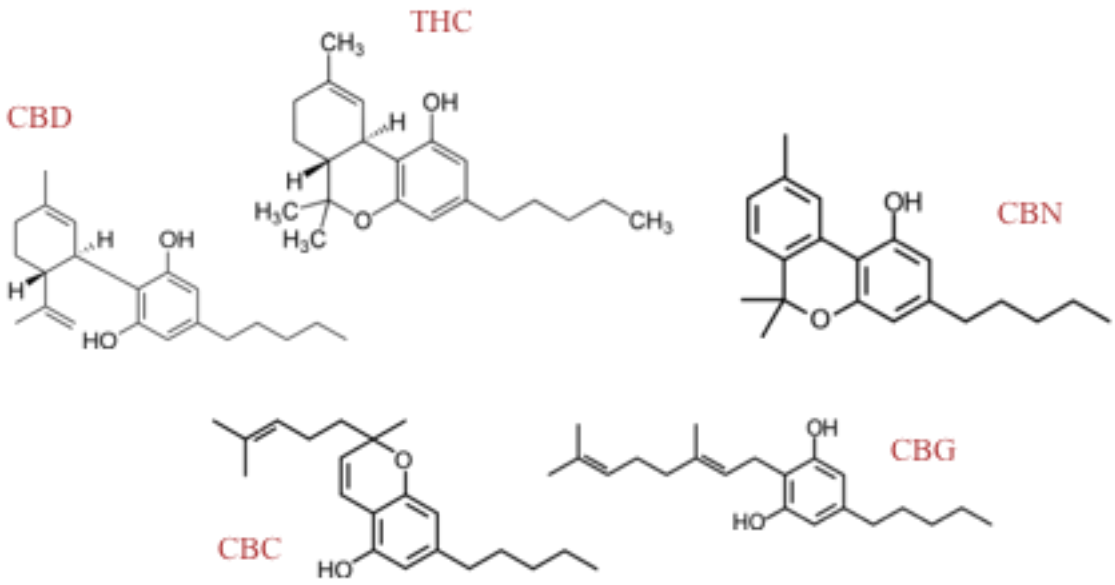
In the US, cannabis is currently a "Schedule I" substance[5], which is reserved for substances that are dangerously addictive and have no medical properties as determined by the federal government. Other Schedule I drugs include heroin, MDMA, and cathinones (aka "bath salts"). "Schedule II" drugs are those that the Federal government has acknowledged to have some medicinal properties, while also being highly addictive. This class of drugs includes cocaine, oxycodone, vicodin, and methamphedamine.[6] This is one of the many reasons that the FDA has not done any clinical trials. If the DEA deems cannabis medically irrelevant, placing it in the Schedule I category, it serves no purpose to spend millions of dollars on clinical studies.[7] In addition, this makes it incredibly difficult for researchers to obtain the substance legally, as the researcher needs a DEA registraion and cooperation from law enforcement in order to source and hold material. While obtaining this registration is not onerous, it is the sourcing of the material that can be much more problematic as only law enforcement has historically been able to obtain the plant materials in the process of investigation of criminal activity. As a result, comparatively little is known about the plants actual detailed chemical composition, and how these compounds act for potential therapeutic benefit.

Relevance of Medical Cannabis Now

Medically speaking, cannabis is known to treat symptoms effectively. The cannabinoids being studied are Tetrahydracannabinol (THC), cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), and cannabinol (CBN) (Figure 1). THC is the psychoactive component in the plant. It has also been shown to reduce pain, ease anxiety, increase hunger, and subside nausea.[8] CBD is the compound suspected of reducing the severity and frequency of seizures in

children.[9] CBG shows antibiotic activity against certain types of bacteria.[10] CBC has analgesic effects and the potential to stimulate cell growth. CBN is not only produced by the plant, but is also a product of THC broken down through the body's biochemical pathway.[11] These are not the only active compounds in the cannabis plant. There are between 80-100 total cannabinoids within the cannabis plant, and another class of compounds called terpenes that also likely have biological activity. It is also not as simple as identifying what each compound does individually, there are several theories and some evidence to suggest that the compounds are not as effective when working alone, and the medicinal properties are a result of the collective effects of all the compounds present. Still, the compounds with the highest concentrations within the plant are the five cannabinoids listed above, and therefore they were the focus of this project.

Figure 1: Structures of Relevant Cannabinoids



Biology of Cannabinoids

Much about cannabis is not known, including how exactly the drug affects so many diseases and parts of the body. What is known is that the cannabinoids bind to Cannabinoid Receptor 1 (CNR1) and Cannabinoid Receptor 2 (CNR2) in many mammals, although this abbreviation denotes human orthologs. These are G-protein coupled receptors (GPCR) and are a part of the endocannabinoid system, which also includes endocannabinoids and the enzymes that synthesize and degrade these endocannabinoids (eCBs). The cannabinoids in the cannabis plant take advantage of this system, binding to CNR1 and CNR2.

The CNR1 is expressed mostly in the brain, more specifically in the basal ganglia and the limbic system. Patients with limbic system issues can have a wide range of symptoms including fatigue, insomnia, depressed appetite, constipation, pain, and low blood pressure.[12] CNR1 polymorphisms have been associated with a variety of diseases including Alzheimer's disease, Parkinson's disease, and Obsessive Compulsive disorder.[13] It is likely because of the receptor's presence in the brain that cannabis is able to affect these diseases.

CNR2 is also present in the brain, but more commonly found in the immune system and tissues, such as the spleen. Its presence in these tissues may explain the anti-inflammatory effects of cannabis when used.

Not much is known beyond the binding of the cannabinoids to the receptors. Even individual cannabinoids, when studied, have only been proven to bind to these receptors. Interestingly enough, one study found that the eCB system becomes desensitized. Instead of downregulating the expression and production of receptors, the binding of the compounds decreases. This occurs when the receptor is phosphorylated and an inwardly rectifying potassium channel is activated. After this, the CNR is internalized, and cannot be accessed.[14] Similar to

having a high alcohol tolerance, this could be part of the reason that frequent users report needing to smoke/ingest more to obtain a similar euphoria.

Preparations of Cannabis for Use

There are many different ways of using cannabis. The plant material can be smoked and inhaled similar to cigarettes. The plant material can also be heated in what's known as a vaporizer, which simply evaporates the cannabinoids out of the plant material without the smoke. Placing some plant material in a specially formed glass container, colloquially known as a "bong," and heating it with a lighter can also evaporate it. These are various ways to inhale the substance. This is typically the quickest way to obtain a high, although the feeling is not as long lasting as when ingested. The plant material, or more commonly the oil extract of the plant material, can be baked into foods. Most commonly baked goods are used such as cookies, brownies, or the like. The oils and basic extracts can be ingested directly by dropping some onto the tongue. This method of ingestion usually results in a slower peaking, but longer lasting, euphoria.

Note that in all these scenarios the substance is heated in some fashion. Even the oil extraction process usually requires a heating step. This is because there are two forms to the cannabinoids, the acid form (containing a carboxylic acid) and the active form (shown in figure 1). Each cannabinoid has an acid form that is not biologically active. In order to become active, they need to be decarboxylated, which can be accomplished easily through heating or burning. This is why swallowing the substance raw (unheated or extracted) usually does not result in a high.

Chapter 2

Introduction

Cannabis has been related to easing the symptoms listed above, oftentimes better than the currently leading drugs. While that may be due, in part, to the euphoria often experienced through THC or through actual interaction with those biochemical pathways is unknown. So, with many states legalizing medical marijuana, and in some cases for recreational use as well, many have taken it upon themselves to self-medicate. Others have moved to one of the 28 states with laws that permit medical marijuana, commonly Colorado or California.[15] While some of these states limit the way one can ingest the material, one common way to do this is to smoke the plant material. This poses some problems with variance between doses. Considering only THC, there is commonly anywhere between 10-20% THC by weight in the plant material for a plant bred for high THC. While smoking, 20-70% of the THC will enter the lungs, based on how much and how often you inhale. Following inhalation, 5-24% of the THC that enters the lungs reaches the brain. This produces an incredible amount of variance within the body. Based on these numbers alone, a person may receive as little as 1% or as high as 17% of the initial concentration of THC within the plant. Because controlling body operation is not possible, the goal of this work was to determine the variance within the distribution of the plant material itself.

The goal of this experiment is to identify the variance of the cannabinoids within the cannabis plant material using gas chromatography coupled to flame ionization detection (GC-FID). GC can be used to determine the concentrations of compounds within samples, by measuring the response factor or the peak area. The area is proportional to the original concentration of the sample. Using this principle, a calibration curve was created. Several mixtures of cannabinoid standards with known concentrations were run. A graph was created

that plotted peak area vs. concentration. From there, the equation for the line that represents the change in peak area for the change in concentration was obtained, and applied to the samples that were later run.

Four experiments were run. The first was methanol spiked with known-concentration, cannabinoid standards to test the variance of the instrumental analysis itself. In the second test, dried parsley was spiked with standards to determine possible matrix effects using a surrogate for the cannabis that did not contain the cannabinoids of interest. Non-homogenized cannabis was analyzed to reflect the variance in the plant material as it's normally distributed. Homogenized cannabis was run to determine if this improves upon the variance between samples. A method was created for the extraction of the cannabis, as there is no certified method available. As mentioned before, cannabis research is limited by source, registration, and often the law. Because this limits the research that can be done, at the time, there was no public extraction method. Crime labs may have had their own methods for extraction, but these were not open to the public. Two different GC columns were tested Rtx-5 and the Rtx-200 (Restek Corporation, Bellefonte, PA). After evaluating the columns, a surrogate needed to be chosen to mimic the general structure of the cannabinoids to measure recovery. JWH 007 (a synthetic cannabinoid), 4-hydroxycoumarin, and 1-Naphthol were evaluated.

Chapter 3

Materials and Methods

Plant Material

The cannabis used was provided to us by the University Park Police Department through cooperation with Lt. Stephanie Brooks and Srgt. Monica Himes.

Instrument

The gas chromatograph used was an Agilent Technologies (Wilmington, DE) 6890 GC-dual FID. Chromatographic gasses (zero air and hydrogen) were supplied using gas generators (Peak Scientific, Inchinnan, UK) or through standard gas cyliners for nitrogen and helium. The GC was equipped with a Gerstel MPS 2, autosampler, capable of liquid, solidphase microextraction (SPME) and headspace injections.

GC Parameters

Oven Program: 80°C (2 min hold) at 12°C/min to 300°C (2 min hold), Rtx-200, 30m x 0.25mm ID x 0.5um df. Total run time: 23 min and 7 min cooldown. Inlet: 250°C, 19.2 psi, Split injection 10:1, helium carrier gas. Front detector (FID): 350°C, H2 flow 40ml/min, Air Flow 450ml/min, Makeup flow 48.4ml/min.

Experimental

Cannabis plant material (nominally 100 mg) was measured out and spiked with 1-Naphthol (50 uL, 25ng/uL) to act as a surrogate and allow for sample extraction efficiency control. The sample was extracted in methanol (2.0 mL). The mixture was sonicated (15 min, 23°C), and then syringe filtered through a PTFE filter (0.45nm) to yield a green (homogenized) or yellow-brown (unhomogenized) extract. The extract (100 uL) was spiked with anthracene (20 uL, 2ng/ul) as the internal standard, and analyzed using the GC-FID under the conditions above.

Table 1: Retention Times for Analytes

Analyte	1-Na	Anthracene	CBC	CBD	THC	CBG	CBN
RT (min)	10.930	13.173	16.503	16.640	17.159	17.483	17.862

Table 1: The analytes that were targets in the project are listed in the top row. 1-Naphthol (1-Na) was the surrogate and Anthracene was the internal standard. The remaining are the target cannabinoid compounds. The retention times (RT) at which the analytes appear on the chromatogram are listed in the bottom row in minutes.

Chapter 4

Results and Discussion

Columns and Surrogates

Initial method development began using an Rtx–5 (30m, 0.25mm, 0.5um), which is 5% diphenyl, 95% dimethylsiloxane. This is a very common GC column, which exhibits high efficiency or a large number of theoretical plates. As all five the cannabinoids are fairly similar in structure and polarity, this column was chosen to evaluate the use of theoretical plates, rather than selectivity, to separate these compounds.

Figure 2: Co-Eluting Peaks

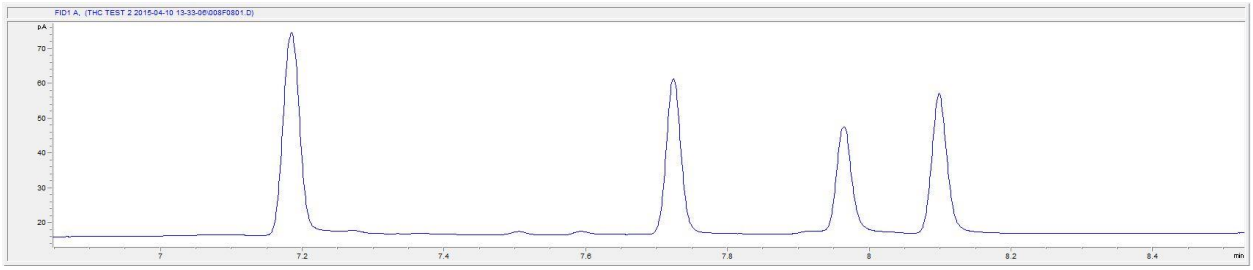


Figure 2: Each of the cannabinoid standards were spiked into solution at a concentration of 200ng/ul on the Rtx-5. Instead of 5 individual peaks, only four appeared. This was the case despite all the oven temperature program changes listed below. The left-most peak is the co-elution of CBC and CBD. The remaining three peaks in order of elution are THC, CBG, and CBN.

As seen in figure 2 above, with this column, CBC and CBD were co-eluting. Individual standards were run separately to the co-elution. Various parameters were evaluated including the oven temperature program in an attempt to separate the compounds, although this was unsuccessful (Table 2).

Table 2: Attempted Temperature Programs

Attempt	Initial Temp	Hold Time	Ramp	Final Temp	Hold Time

	(°C)	(min)	(°C/min)	(°C)	(min)
Original	150	2	25	325	2
1	150	2	25	275	2
	275	0	10	325	2
2	150	2	50	275	2
	275	0	10	325	2
3	175	2	50	275	2
	275	0	10	325	2
4	200	2	50	275	2
	275	0	10	325	2
5	175	2	60	275	2
	275	0	10	325	2

Table 2: These listed are oven temperature programs that were used in an attempt to separate the co-eluting compounds, but were unsuccessful. Those attempts with two rows indicate 2 different ramps in the same temperature program. For the final successful program used, see Chapter 3: Materials and Methods.

The next column tested was an Rtx–200 (30m, 0.25mm, 0.5um). It is 100% crossbond trifluoropropylmethyl polysiloxane, which is mid-polar and selective for lone electron pairs.[16] The cannabinoids have lone electron pairs on the oxygen atoms, which would allow for greater selectivity with the Rtx–200 phase and improved separation. This new column solved the co-elution problem as five peaks were observed in the cannabinoid mixture and verified by retention time match with individual standards (Figure 3). This column was used for the remainder of the experimental work.

Figure 3: Chromatogram of Cannabinoids

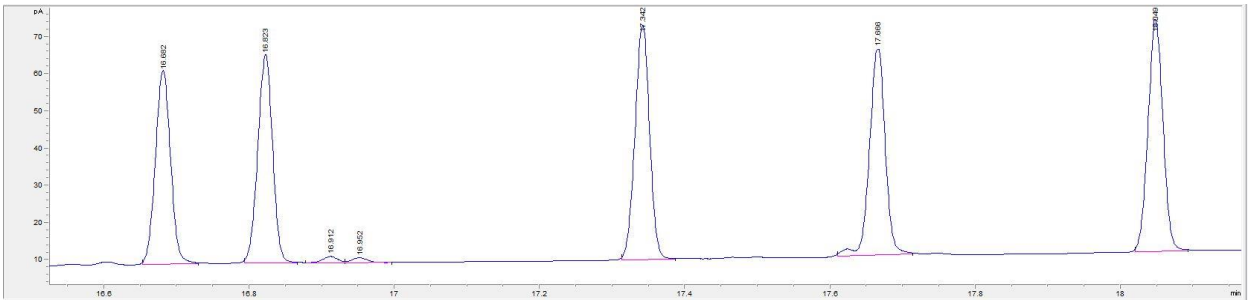
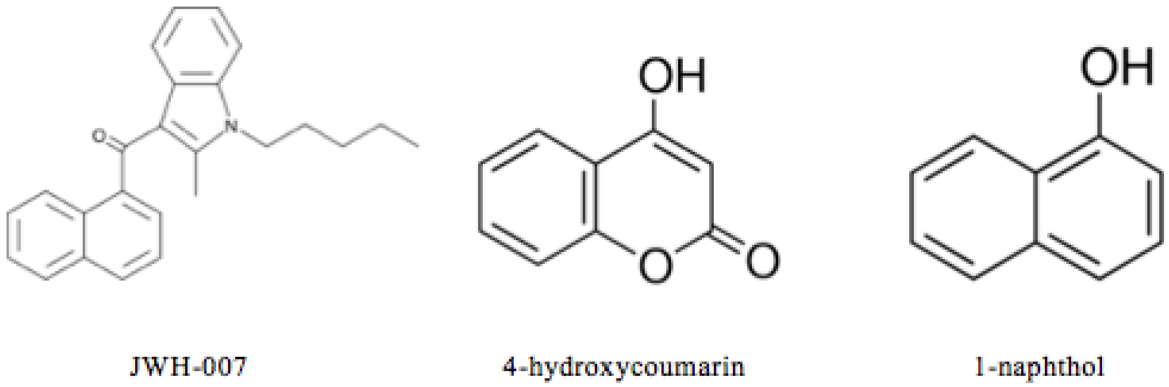


Figure 3: Standards of the five cannabinoids were combined and injected into the GC using the method described in Chapter 3. The order of elution is as follows: CBC, CBD, THC, CBG, and CBN.

A surrogate was needed to mimic the reactivity and the structure of the cannabinoids to use as a known reference for recovery in order to maintain the integrity through the extraction

process. The first compound chosen was a synthetic cannabinoid, JWH 007 (Figure 4). This molecule was chosen for its similarity in structure to the cannabinoids and similar binding capacity to the cannabinoid receptor.[17] Unfortunately, the basic nitrogen on the JWH-007 proved to be reacting likely with the acidic chromatographic surfaces (inlet liner, for example), which is not an issue for the acidic target cannabinoids. Additionally, the compound was expensive making adaptation for future studies unlikely. 4-hydroxycoumarin was evaluated second (Figure 4). While similar in structure, the compound's high pKa resulted in degradation within the inlet. Finally, 1-naphthol was chosen, for its similar structure and similar pKa to the cannabinoids (Figure 4). It has proven a reliable surrogate and was utilized for all remaining studies.

Figure 4: Surrogate Structures



JWH-007 4-hydroxycoumarin 1-naphthol

Instrument Variance

In order to measure the variance associated within the cannabinoids in the cannabis plant, the instrumental variance of the GC-FID was determined. Finding the error within the instrument is important in determining that it is working properly and in later experiments to know how much of the deviation is a result of the plant and how much is a result of the analytical method.

Methanol blanks were spiked with cannabinoid standards at 25ng/ul final concentration. Ten replicates were created in this manner. Table 3 shows the recovery and the variance within the extraction and instrument without plant material.

Table 3: Variance within the Agilent 6890 GC-FID using Spiked Methanol Blanks

	1-Naphthol	CBC	CBD	THC	CBG	CBN
Mean:	23.26	21.99	21.76	21.58	21.40	22.09
%RSD:	10.63%	11.34%	11.81%	13.18%	11.73%	11.56%

Table 3: All compounds were spiked in at 25ng/ul. The mean is the average recovered concentration of the samples as determined by external calibration. The Percent Relative Standard Deviation (%RSD) was determined by dividing the standard deviation by the mean.

The recovery of the cannabinoids is no less than 80%. The variance in the spiked methanol blank repeats was between 10-13%. Because of this, our method was acceptable to move forward and begin conducting control tests on plant material to test the difference in error when a plant matrix is introduced.

Effect of Plant Matrix on Instrument Variance

To determine the change in variance when a plant matrix is present, the target cannabinoids were spiked onto to dry, ground parsley leaves. This was chosen to mimic the cannabis plant material, as most of the sample is dried. It was believed that the cannabinoids would adsorb to the parsley as they would to the cannabis, which could alter the variance between samples. Table 4 shows the recovery and variance within the plant matrix spiked with cannabinoids.

Table 4: Variance of Instrument with Addition of Spiked Parsley Plant Material

	1-Naphthol	CBC	CBD	THC	CBG	CBN

Mean:	13.62	13.08	12.91	12.42	13.42	13.42
%RSD:	7.88%	7.92%	8.51%	8.19%	7.78%	7.25%

Table 4: All compounds were spiked in at 25ng/ul. The mean is the average recovered concentration of the samples as determined by external calibration. The Percent Relative Standard Deviation (%RSD) was determined by dividing the standard deviation by the mean.

As observed in Table 4, the recovery is less than that of the pure solvent at roughly 50%. This data indicates that some of the analytes adsorbed onto the parsley matrix. More surprisingly, the data demonstrates that the %RSD decreased relative to the solvent control experiment, being no greater than 8.51%. In theory, the introduction of an additional variable, plant matrix in this case, should have increased the variance by some degree, although the opposite is seen here. This data was promising with respect to analysis with the cannabis plant.

Variance within Unhomogenized Cannabis

To mimic the way medical cannabis is sometimes distributed, in plant form to be smoked, the cannabis was left whole for analysis. Buds and leaves were picked off stems to be roughly 100mg in weight and the cannabinoids were then extracted and analyzed using the method described in Chapter 3.

Table 5: Variance in Non-Homogenized Cannabis

	CBC	**CBD**	**THC**	**CBG**	**CBN**
Mean	0.21%	0.04%	8.77%	0.78%	4.18%

%RSD	9.33%	5.92%	8.56%	9.89%	7.44%

Table 5: 1-Naphthol was spiked in at 25ng/ul. The mean is the average recovered (weight percent) of the samples as determined by external calibration. Percent Relative Standard Deviation (%RSD) was determined by dividing the standard deviation by the mean.

The variance observed in non-homogenized cannabis material was determined to be as high as 10% (Table 5). This data represents the inhomogeneity that any one plant can have. If distributed to patients in this fashion, it could result in underdosing or overdosing, which can effect therapeutic results. If underdosed, patients do not get the pain relief they need or will experience the seizures they are trying to prevent. Overdosing is a problem in the long term, as there is data that suggests that brains will develop more slowly as a result of constant exposure to THC in chronic cannabis users.[18] While this hasn't been proven irreversible, it poses a concern for children with severe epilepsy who respond well to cannabis treatment. Currently the medical marijuana industry is unregulated as the federal government does not recognize its capacity as a medicinal substance. Because of this, patients are at a higher risk for not getting the relief they need or for long-term impairment after years of use.

Variance in Homogenized Cannabis

Seeing the large variance within these small samples of 100mg, it was hypothesized that simply homogenizing the cannabis by grinding it to a fine powder prior to sampling would likely result in less variance between samples. The theory is that a more uniform sample would result in more uniform replicates. This also would be an easy adjustment for the medical marijuana industry that could be implemented. Table 6 shows the results of the variance of homogenized cannabis.

Table 6: Variance in Homogenized Cannabis

	CBC	CBD	THC	CBG	CBN
Mean	0.18%	0.04%	6.66%	0.43%	4.70%
%RSD	5.99%	5.05%	6.16%	4.29%	4.98%

Table 6: 1-Naphthol was spiked in at 25ng/ul. The mean is the average recovered percent weight of the samples as determined by external calibration. The Percent Relative Standard Deviation (%RSD) was determined by dividing the standard deviation by the mean.

Between Table 5 and Table 6, the average variance in homogenized cannabis is between one third and one half less than that of unhomogenized. As predicted the variance is reduced when the cannabis is ground into a uniform powder.

This is not a permanent solution. If cannabis is to be considered a medication as most pharmaceuticals, it should follow those set by the FDA. If this material were to be regulated by the FDA, it would be complicated because of the complexities of the The way drug concentrations and deviations are assessed by FDA are through confidence intervals (CI) and how likely the concentration of multiple samples is to fall within the confidence interval. This is especially important for Highly Variable Drugs (HVDs) and Highly Variable Drug Products (HVDPs). HVDs are formulations that have high within-subject variabilities, largely due to differences in biochemistry in patients. In other words, the drug will have different potencies with different patients. HVDPs are products that are “pharmaceutically poor” and exhibit a range of potencies prior to patient use. Cannabis would fall under both of these categories, as the plant is variable and patients will respond to it differently. The FDA requires the substance to fall within a 90% CI, but the width of this interval is also variable and depends on the within-subject variability (WSV) and the number of subjects in a clinical study. The wider the WSV, the less likely the substance will fall within the 90% CI, and the narrower the WSV, the more reliable the drug. Currently, there are no specific acceptance criteria for HVDs and HVDPs.[19] Some of the

cannbinoids do fall within this 90% CI, but with the additional variance of WSV, cannabis as a drug would likely not obtain the FDA's approval as a smoked natural product. With simple techniques, such as the grinding of the plant material, a more representative evaluation of the cannabinoid content within the plant. Greater confidence in the cannabinoid content would benefit the cannabis industry.

Chapter 5

Future Studies

In an attempt to further characterize the medical properties of cannabis, it is important to have a method to separate and analyze the other compounds within the plant. As mentioned above, there are many cannabinoids and other compounds within the cannabis plant that have medicinal properties. The five most concentrated cannabinoids do not fully characterize this material. Terpenes are another class of compounds found within cannabis, but also many other plants. They are volatile hydrocarbons that produce a scent. From studies with other plants, terpenes have shown some medicinal properties in combination with other compounds and with each other.[20] A method in being developed by which terpenes can be analyzed. The sample preparation for terpenes is different as the analytes are more volatile and therefore liquid extraction could result in bias toward the heavier molecules that stay in solution more readily than some of the lighter ones. Two other extraction methods include Headspace (HS) and Headspace Solid Phase Microextraction (HS-SPME) that utilize the volatile properties of the analytes and extract them from the vapor above the dried leaves. No liquid is used.

Additionally, the extraction of the cannabinoids can be further simplified if the concept applied to terpenes is used. Cannabinoids have a lower vapor pressure than terpenes, so they do not volatilize as easily, and therefore, Headspace and Headspace Solid Phase Microextraction are not reasonable to use. However, in theory, a Thermal Desportion Unit (TDU) could be used. The TDU would volatilize the analytes off the cannabis material through heating and inject the vapor into the inlet. This method would require no extraction. Only minimal sample preparation is

needed to place the sample into the TDU-specific tubes. In this case, this extraction method could save time.

BIBLIOGRAPHY

[1] Hall et al, The Lancet 2014, 374 (9698), 1383-1391
[2] Marijuana Policy Project Patient Numbers. https://www.mpp.org/issues/medical-marijuana/state-by-state-medical-marijuana-laws/medical-marijuana-patient-numbers/ (accessed 23 March 2017)
[3] National Institute of Health, "Marijuana as Medicine" https://www.drugabuse.gov/publications/drugfacts/marijuana-medicine (accessed 23 March 2017)
[4] Zuardi, Antonio Waldo. "History of cannabis as a medicine: a review" *Rev Bras Psiquiatr.* 2006; 28(2): 153-7
[5] Drug Enforcement Agency, "DEA Announces Actions Related to Marijuana and Industrial Hemp" https://www.dea.gov/divisions/hq/2016/hq081116.shtml (accessed 23 March 2017)
[6] Drug Enforcement Agency. "Drug Scheduling". https://www.dea.gov/druginfo/ds.shtml
[7] Forbes. "Many States Have Legalized Medical Marijuana, So Why Does DEA Still Say It Has No Therapeutic Use?" http://www.forbes.com/sites/ritarubin/2016/11/16/many-states-have-legalized-medical-marijuana-so-why-does-dea-still-say-it-has-no-therapeutic-use/#6f8922f635a1 (accessed 23 March 2017)
[8] Strouse, Cannabis and Cannabinoid Research 2016, 1(1), 38-43
[9] "Is Marijuana Medicine?" NIH, July 2015.
[10] Elsohly, Mahmoud A., Slade, Desmond. "Constituents of Marijuana: The Complex Mixture of Natural Cannabinoids" *Life Sciences* 78 (2005) 539-548
[11] Oier Aizpurua-Olaizola†‡, Umut Soydaner†, Ekin Öztürk†, Daniele Schibano†, Yilmaz Simsir†, Patricia Navarro‡,Nestor Etxebarria‡, and Aresatz Usobiaga*‡ "Evolution of the Cannabinoid and Terpene Content during the Growth of Cannabis sativa Plants from Different Chemotypes". *J. Nat. Prod*., 2016, 79 (2), pp 324–331
[12] Self Hacked "All About Cannabinoid System" https://selfhacked.com/2016/01/14/the-lectin-sensitive-gene-cannabanoid-receptor-1-genes-cnr1-rs1049353/ (accessed 28 March 2017)
[13] D. Gadzicki, K. Muller-Vahl, M. Stuhrmann. "A frequent polymorphism in the coding exon of the human cannabinoid receptor (CNR1) gene." *Molecular and Cellular Probes*. 13(1999), 321-323
[14] Luk, T., Jin, W., Zvonok, A., Lu, D., Lin, X.Z., Chavkin, C., Makriyannis, A., Mackie, K. "Identification of a potent and highly efficacious, yet slowly desensitizing CB1 receptor antagonist." *British Journal of Pharmacology.* 142(2004) 495-500
[15]ProCon.org, Medical Marijuana. http://medicalmarijuana.procon.org/view.resource.php?resourceID=000881 (accessed 23 March 2017)
[16] Restek Co. Rtx-200 Columns. http://www.restek.com/catalog/view/1338 (accessed 23 March 2017)

[17] Huffman, J.W. "3-Indolyl-1-naphthylmethanes: New Cannabimimetic Indoles Provide Evidence for Aromatic StackingInteractions with the CB1 Cannabinoid Receptor" *Bioorganic & Medicinal Chemistry* 11 (2003) 539–549

[18] Iversen, L. "Cannabis and the brain". *Brain* 2003; 126 (6): 1252-1270. doi: 10.1093/brain/awg143

[19] Midha, K.K. "Highly Variable Drugs & Drug Products-A Rationale for Solution of a Persistent Problem" *University of Saskatchewan & Pharmalytics, Inc., Saskatoon Canada.* 2006 (Accessed 2 April 2017)

[20] Strouse, T.B. "Cannabinoids in Medical Practice," *Cannabis and Cannabinoid Research* 1(2016) 38-43

Academic Vita of Rebecca Plessel

rxp5172@psu.edu

Education

Penn State University – University Park *May 2017*

- B.S. Biochemistry and Molecular Biology with a Minor in Chemistry
- Millennium Scholar
- Schreyer Honors College

Research and Externship Experience

Analytical Chemistry Laboratory. Dr. Emmanuel Hatzakis | Penn State *Oct 2013 – Dec 2014*
Determined the structure of four pentacyclic acids using an 850 MHz NMR instrument to pave the way for further research into their potential use as medications.

NCIS Externship *Jul 2014*
Shadowed an NCIS forensic scientist at NCIS headquaters in Washington, DC

Analytical Chemistry Laboratory | Dr. Frank Dorman | Penn State *Nov 2014 – Present*
Method development for GC-FID quantification of medically relevant components in cannabis, including the cannabinoids and terpenes
This work will be the basis of my Honors Thesis for the Schreyer Honors College and Millennium Scholars Program

Molecular Pharmacology Laboratory | Dr. Erika Bach | NYU *Jun – Jul 2015*
Determined the effects of the RNAi mediated knockdown of genes on germline stem cells in the testes of fruit flies in order to evaluate potential therapy for cancers that originate in stem cell populations.

Technical Skills

- PCR
- Gel Electrophoresis
- NMR Spectroscopy
- Confocal Microscopy
- Immunostaining
- DNA and Protein Isolation and Purification

Work Experience

Tutor | Millennium Science Program — *Aug 2015 – May 2016*

Grader | Chemistry Department — *Jan – May 2016*

Note-Taker | Nittany Notes — *Aug – Dec 2016*

Leadership Experience

Treasurer | Club Science LionPride — *Apr 2014 – 2016*

Founder and Student Representative | Cohort Council of MSP — *Sept 2014 – Present*

Honors and Awards

Local 1262 Scholarship — *Jun 2013 – Jun 2015*
Merit-based scholarship

Schreyer Honors College — *Aug 2014 – May 2017*
Gateway entrance into the honors college between freshman and sophomore year

Millennium Scholars Program — *Jun 2013 – May 2017*
A merit-based scholarship program designed to prepare students for the pursuit of doctoral degrees in STEM disciplines

The Society of Applied Spectroscopy Undergraduate Student Grant — *Oct 2014*
Award granted to support undergraduates doing research in spectroscopy

Travel Grant — *Mar 2016*
Awarded to present research at Pittcon in Atlanta, Georgia

Erickson Discovery Grant — *Apr 2016*
Awarded to fund research at Penn State over the summer of 2016

Presentations

Leadership National Symposium — *July 2015*
NYU Summer Undergraduate Research Program — *July 2015*
Pittcon Conference and Exposition — *March 2016*
Undergraduate Research Conference at Penn State — *Aug 2016*
ACS Undergraduate Poster Symposium — *Sept 2016*
Webinar hosted by Gerstel GmbH and Co. — *Oct 2016*