5-14-2022

Statistical analyses of hemp cannabinoid test results

Rachel J. Stegmeier

Statistical Analyses of Hemp Cannabinoid Test Results

An Honors College Project Presented to

the Faculty of the Undergraduate

College of Science and Mathematics, James Madison University

James Madison University

By Rachel Jane Stegmeier

May 2022

Accepted by the faculty of the Department of Chemistry and Biochemistry, James Madison University, in partial fulfillment of the requirements for the Honors College.

FACULTY COMMITTEE:

Project Advisor: Daniel M. Downey, Ph.D.
Professor, Chemistry

Reader: Debbie L. Mohler, Ph.D.
Professor, Chemistry

Reader: Chrisi A. Hughey, Ph.D.
Professor, Chemistry

HONORS COLLEGE APPROVAL:

Fletcher Linder, Ph.D.,
Dean, Honors College

PUBLIC PRESENTATION

This work was presented at the National Conference on Undergraduate Research on April 7th, 2022.

TABLE OF CONTENTS

ACKNOWLEDGMENTS

I would like to express my appreciation to Dr. Downey for his valuable and constructive guidance during this research work. His willingness to give his support and time has been very much appreciated. I would also like to thank Dr. Mohler and Dr. Hughey for providing thoughtful feedback regarding my work. The Department of Chemistry and Biochemistry as well as the James Madison University Honors College has allowed me to explore and excel in my passion for chemistry research. Thanks are extended to my lab research collaborators Dawson Bowles and Samantha Forbes. Appreciation is extended to Brandon McKearney, his parents Rob and Peggy McKearney and their associate Shedric Porte who are the owners of Trium Botanical and Fowl Ball Farm. I would also like to express gratitude to Daniel Musikanth and my father, John Stegmeier for assisting in hemp sample collection. Special thanks to my mother, Cynthia Waters for her support. This project was supported by NSF-REU Grant No. CHE-1757874 and the Jeffrey E. Tickle ’90 Family Endowment in Science and Mathematics Award.

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Cannabis sativa L. is a flowering plant used for recreational and industrial purposes that produces a class of compounds called cannabinoids. Industrial hemp is a strain of *Cannabis sativa L.* that has been propagated to have a low Δ^9 tetrahydrocannabinol (Δ^9THC) and a high cannabidiol (CBD) content. With recent advancements in legislation, farms are now growing hemp for fiber, CBD production and other hemp derived product purposes but crops risk being destroyed if THC content levels exceed the current maximum legal limit of 0.3%. For the present study hemp samples were dried, ground, extracted with various alcohols, filtered, and assayed by ultra-High Pressure Liquid Chromatography with ultraviolet detection (uHPLC-UV) and Gas Chromatography with flame ionization detection (GC-FID) for ten cannabinoids, with primary focus on THC and CBD. Three replicates were done for each sample strictly following published protocols. We have found that the results of analyses vary considerably due to variations in cannabinoid content in plant biomass, different hemp varieties, growing location and before/after drying. The random sampling procedure, the heterogeneity of the crop and large standard deviations for analyses affected results. We have statistically evaluated data and conclude that large sample standard deviations are intrinsic with the protocols. These may lead to crops that are actually within the legal limit being destroyed by regulators. As a result, it was concluded that either 0.3% THC should not be applied as an absolute value for legality but should be associated with sample standard deviation for replicates of analyses, or the absolute criterion be raised to 0.5%.

Keywords: hemp, cannabinoids, analysis, THC, CBD, chromatography

INTRODUCTION

1.1 General

Cannabis (*Cannabis sativa* L.) is a rapidly growing and flowering plant that has been important in textiles and folk medicines since its first use in Central Asia 10,000 years before present (Andre et al. 2016). It is best known for the psychoactive response of some varieties when consumed (eaten, smoked) by humans. Many ancient cultures including Egyptians, Chinese, Greeks and Romans have used the domesticated crop (Li et al. 1974; Russo et al. 2007). Despite thousands of years of propagation and widespread adaptation for food, fiber and medicine, cannabis remains insufficiently understood (Schluttenhofer et al. 2017). Linnaeus established the taxonomy of cannabis in 1753 (Watts 2006) (**Table I**). Three unique strains are generally recognized: sativa, indica and ruderalis (Schwabe 2019). Sativa is the most common strain while indica is second. Ruderalis can survive and grow in conditions that are unacceptable for the first two strains, such as in extreme cold and poor farmland. While the ruderalis strain is not popular among consumers and commercial cannabis growers, it is useful in developing hybrid strains with more favorable traits and resistance to environmental conditions (White 2022). Sativa and indica strains can be considered as unseparated due to the amount of crossbreeding and hybridization that has occurred throughout history. There are disagreements among taxonomists as to whether these strains remain separate or are a variation of the same species (Russo 2019). Many people today refer to cannabis as sativa for marijuana (drug) varieties and hemp for plants that have no psychoactive effects.

Regardless of strain, cannabis can be separated into two categories: “non-drug” strains (hemp-types) and “drug” strains (marijuana-types) with the difference being the concentration of

the naturally produced psychoactive compound, Δ^9tetrahydrocannabinol (Δ^9THC) (Johnson 2018; Small et al. 1973). Hemp has been used primarily for agricultural and manufacturing purposes, such as food, personal care products, nutritional supplements, textiles, paper, construction materials and other manufactured goods until recent years (Johnson 2018). The seed is edible, and the oil can be used in topical salves, capsules, and other consumer friendly goods; the fibrous stem can be used for paper or rope; the flowering part of the plant produces terpenes that provide scent and flavor to hemp products. Hemp is currently referenced as "industrial" to emphasize plant parts including stems, curt, leaves, roots and seeds that are harvested for non-medicinal uses. In the past several decades, hemp has also been bred and propagated for therapeutic uses. Cannabis inflorescences, which are the complete flowering head including flowers, petals, etc., produce many kinds of chemical compounds which may be extracted, concentrated, and purified to make products for human consumption. Included in the list of chemicals produced by cannabis are a class of compounds specific to this genus known as cannabinoids. For non-drug strains, the most important cannabinoid is cannabidiol (CBD). For drug strains, CBD is a minor chemical in cannabis flowers replaced by Δ^9THC in high concentration. The legal definition of drug versus non-drug cannabis is that Δ^9THC does not exceed 0.3 % (weight percent) total THC content (Agriculture Improvement Act 2018), a value that was first suggested by Small and Becksted (1973).

Federal legislation passed in 2018 (Department of Agriculture 2019) has allowed for private cultivation of industrial hemp as an agricultural and research focused crop, resulting in the advancement of knowledge and discussion in the field. Since this legislation, the plant has been portrayed as a possible multi-million-dollar industry for both farmers and business interests. However, numerous people in the industry have been concerned about complying with

government standards. In addition, there are limitations for profitable products due to regulations that are still not well understood or established (Nyce 2020). Many of the issues stem from the analytical characterization of cannabis that is the focus of this study.

1.2 Description of Plant/Taxonomy/Parts of Plant

Cannabis is a member of the Cannabaceae family in which the hemp-type form belongs to the *Cannabis sativa* L. species (Schwabe 2021). Over time, the Cannabis genus has developed to consist of many different strains, hybrids, and varieties. All variations are a result of evolution and selective breeding or propagation of desired characteristics in the plant. The variable THC content has been selected for or against for the two "hemp-type" and "drug-type" forms of cannabis. Artificially selected traits, such THC content, and industrial use purposes are directly related to genetic data (Schwabe 2021). Variations in colloquial usage of the forms, strains, and varieties has caused debate about correct terminology, sometimes resulting in inconsistencies in discussion (Schwabe 2021).

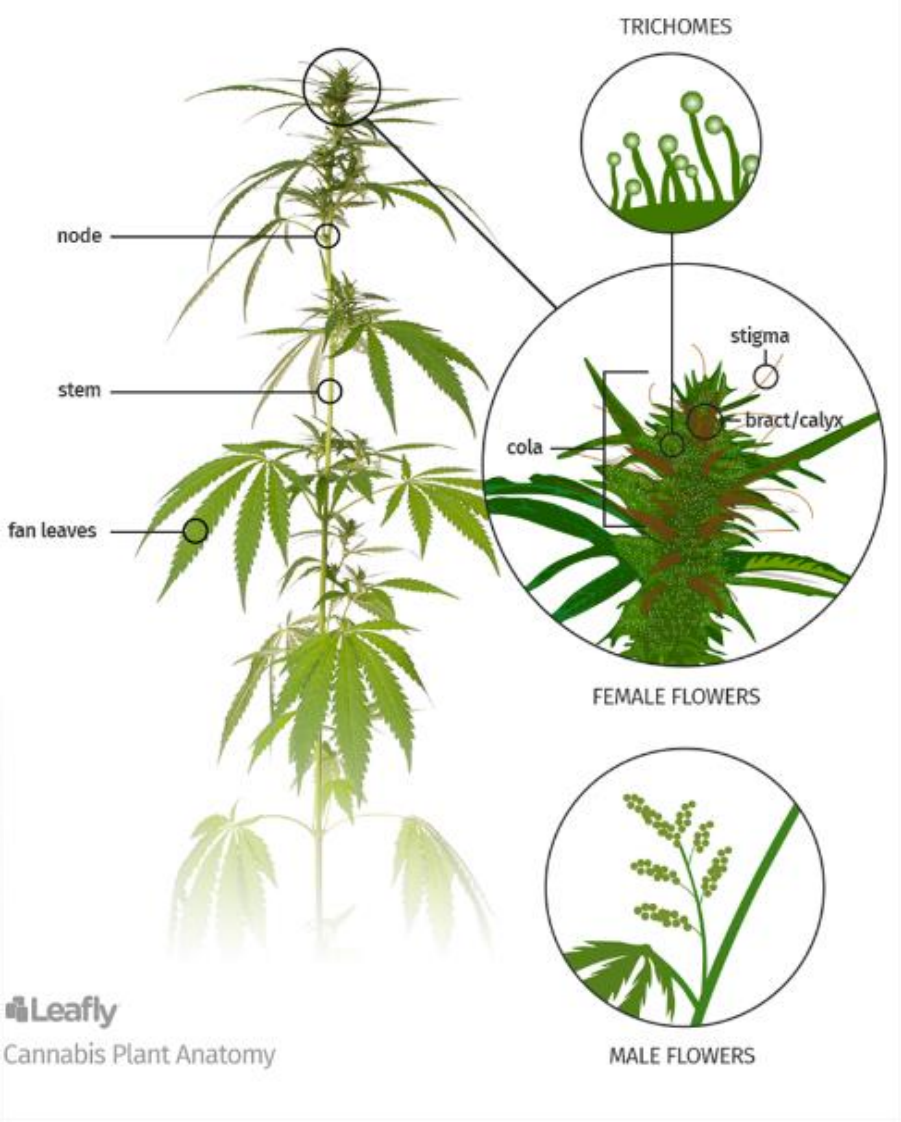

Figure 1. Cannabis plant anatomy (Leafly, Hennings and Rahn 2022).

Cannabis is an annual dioecious flowering plant that contains both female (pistillate) and male (staminate) parts (**Figure 1**). It can grow up to heights of one to three meters with the stem part of the crop making up between 0.2 and 0.6 meters. Although, like other plant species, this can vary depending on hereditary factors and growing conditions. Many species resemble hemp structurally such as *Hibiscus cannabinus, Acer palmatum, Urtica cannabina, and Dizygotheca elegantissima* (United Nations Office on Drugs and Crime 2009). As the hemp plant begins to

develop flowers, trichomes begin forming along with transports and cells within the crop. Trichomes are specialized structures that are found on the surfaces of hemp flowers that produce cannabinoids (Engene et al. 2012). This flowering and development process typically takes a hemp plant between four and twelve weeks when beginning with a seed and an additional week when grown from cuttings (United Nations Office on Drugs and Crime 2009). Trichomes can exist in three forms: bulbous trichomes, capitate sessile trichomes, and capitate-stalked trichomes. Capitate-stalked trichomes exist at the largest scale between 50-100 micrometers wide and can be visually seen as a waxy cuticle layer (Bennett 2016). In addition to producing cannabinoids in the flowers on trichomes, Cannabis has also been reported to have over 140 identified terpenoids (Giese et al 2015). Cannabinoids are produced for protection of the plant from sunlight, wind damage and insects while terpenoids and flavonoids contribute to the overall flavor and smell of hemp (Bennett 2016). The pungent trichome taste deters possible harm created by insects and animals. Aside from the flowering part of hemp, the cell wall consists of both bast fibers and woody fibers that are rich in cellulose, creating strength for the plant and increasing its use in textiles (Andre et al. 2016). Selective propagation has allowed for these desired qualities and characteristics.

1.3 Agriculture of Cannabis

Cannabis may be grown either outdoors or indoors with both approaches having advantages and disadvantages (Buchi 2020). Traditional farming practices include growing a conventional field crop taking advantage of natural sunlight, rainfall, and available soil. Typical fields range in size from one to twenty acres in Virginia. Rain may be supplemented with irrigation during drought periods. Fertilizers may be added to enhance soil derived nutrients. Weed control is generally accomplished by cultivation or manual methods; herbicides are

avoided especially for cannabis crops that will be ingested by humans. Outdoor growing is limited to the frost-free period of the year. Indoor farming is done in a structure built for the purpose or an existing building modified into a greenhouse. Structures may be brick and mortar, wooden, glass or plastic canopies. Supplemental heating may be available to extend the growing season beyond that available outdoors. The main advantage of outdoor cannabis is that there are many acres of fields with adequate soils for growing hemp in Virginia. Fields in use for commonly grown crops such as corn, alfalfa or pasture may be readily converted to grow cannabis. The outdoor approach potentially allows for copious amounts of hemp biomass being produced at lower costs than indoor hemp. The primary advantage of indoor farming is that a potentially longer growing season is possible since the plants are protected from outdoor weather conditions and control over the amount/type of light the plants experience. Indoor farming, usually less than one acre, provides protection from the variability of the natural elements (wind, hail, floods, and extreme temperatures) and controlling wind, insects, and other predators. Main problems for outdoor growing include little protection against the weather and about a five-month season in Virginia from May to September. Outdoor settings risk destruction of the crop by animals such as deer, groundhogs, and cattle. Outdoor hemp is also vulnerable to insect infestations such as aphids, mites, borers and cornworms and to airborne drift of pesticides from neighboring properties. The obvious disadvantage for indoor crop production in greenhouses is the expense of building, maintaining, and providing power. Greenhouses use either natural sunlight filtered through a glass or plastic canopies and/or artificial light fixtures to grow cannabis, which are expensive.

Either seeds or clippings (clones) may be used to start cannabis plants, with genetics chosen by the desired use for the plants. Seeds may be sown directly into the soil for outside

fields. However, most farmers start the plants in potting soil, then transplant to rows for field crops or large pots for indoor crops. Trade brands such as Rocket Fuel® or other high nitrogen greenhouse blends and biochar blends contain a typical mix of 80% compost and 20% perlite that may be used to provide nutrients, structure, and water to the root systems. When production demands a large biomass of hemp for fiber or bedding material outdoor field crops are the norm. For medical marijuana and CBD hemp, the industry in gravitating toward indoor production, to control light, moisture and insects. Indoor growing also provides protection from criminal acts.

Environmental and growing factors such as UV and visible light, watering systems, soil nutrients and temperature of surroundings have a direct effect on the concentration of cannabinoids produced by cannabis. Greenhouses are enclosed for climate control but come with the cost of reduction of natural sunlight. Greenhouses that use natural sunlight have glass or plastic roofs. UV and Visible solar radiation are reduced compared to outside crops due to absorption, reflection, and angle of the roof. Solid roofing with interior growing lights that may controlled by the grower offers better control than either outdoor or solar roofing but comes with a considerable financial cost. As a result, most medical marijuana is currently being grown indoors with growing lights, while CBD hemp is best produced indoors with solar roofing due to the financial aspects of sales versus production costs.

The present research project is directed toward CBD hemp grown both indoors and outdoors. Three to five months of growth are required from seed or clone to mature cannabis plant harvest. Farmers growing CBD hemp want to produce high CBD-yielding plants while keeping Δ^9THC < 0.3%. The greater the CBD content (and other cannabinoids) at harvest time, the lower the biomass that needs to be extracted for the same amount of product. Typically, CBD > 10% is desired, with >15% preferable (Denver Post 2018). The metabolic pathway for CBD

production (**Figure 2**) reveals that the pathway includes CBGA, then branches to either CBDA or THCA, which are both decarboxylated to CBD and THC, respectively. Hemp has been propagated to primarily follow the CBDA production pathway, but there is some THC produced in all hemp plants. When the CBD concentration exceeds 7.5%, the ratio of CBD to THC in hemp is near 25:1 and the THC will concurrently exceed 0.3% rendering the plant illegal by currently laws. Such a low legal limit seems extreme given that marijuana plants typically contain 14% THC and less than 0.3% CBD (ElSohly et al. 2016).

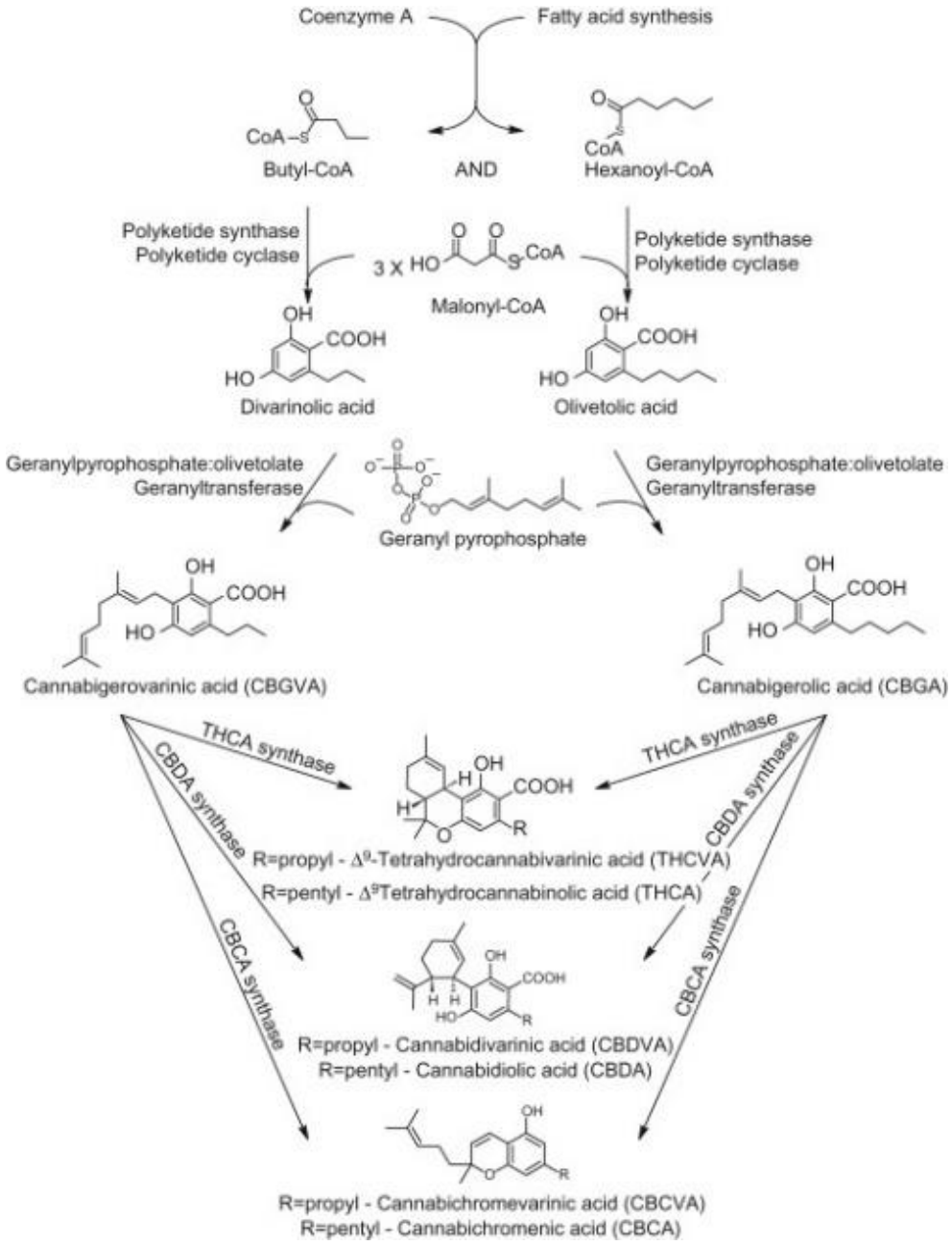

Figure 2. Metabolic Pathway for the Biosynthesis of Cannabinoids (Thomas and ElSohly 2016).

Hemp and marijuana cannabis cannot be visually distinguished from each other. The only way to determine with certainty if a plant is hemp or marijuana is with the advanced analytical methods and instrumentation described below. The Swiss Typification test (Department of Forensic Science 2019), a simple test to distinguish drug and non-drug cannabis, was developed

for screening by police officers when confronting suspects transporting cannabis. But that test is highly inaccurate and cannot distinguish reliably at 0.3% THC. So, farmers must rely on laboratory testing, experience, or intuition to keep their plants within legal limits and harvest their crop before THC exceeds > 0.3%. Cannabinoid concentrations increase rapidly as the plants mature, so it is likely that samples below < 0.3% will exceed the limit in the one week required to obtain laboratory results (Schmidt 2020).

Determining the best time to harvest the crop can be difficult as there are minimal visual indicators of the plant at peak maturity. Sometimes, a color change seen in trichomes can be used to make this distinction. Maturity in trichomes is displayed in a parabola-like apex of coloration with a transition from a clear translucent state to a cloudy white and later, amber hue (Bennett 2016). However, this can vary between different strains. Studies have supported that observation of full flower ripeness can be seen when about 75 percent of the stigmas are brown (United Nations Office for Drugs and Crime 2009). Variation in inter-laboratory results of cannabinoid content also contributes to the dilemma that farmers face when trying to grow hemp legally (Smith 2019).

1.4 Industrial/Therapeutic Uses

Humans have used hemp for thousands of years. It has been found to have many beneficial applications; it is a renewable resource, has antibacterial properties, and many applications for fiber (Andre et al. 2016). As a plant, it is stronger than polypropylene plastic and lighter in weight, allowing for a stronger alternative to plastic that is also more suitable for respective processes (Marsh 2003). The United States Department of Agriculture recognized hemp as a “commodity that can be used for numerous industrial and horticultural purposes

including fabric, paper, construction materials, food products, cosmetics, production of cannabinoids, and other products," (United States Department of Agriculture 2018).

The plant has also shown to be important to medicine cannabinoids interact with endocannabinoids in humans (Andre et al. 2016). The National Academy of Sciences' Institute of Medicine claimed that cannabinoids play a role in "treating pain, nausea, AIDS-related weight loss or "wasting," [and] muscle spasms from multiple sclerosis," (Webster et al. 2004).

1.5 Cannabinoids

Cannabis produces more than 400 different chemical compounds. These compounds are subdivided into various classes including cannabinoids, terpenes, oils, fats, and lipids. Included in this list are compounds that have therapeutic, medicinal, and other value. Cannabinoids are a group of chemicals exclusive to the cannabis plant that are biosynthesized in the trichomes (Figure 2) via the polyketide pathway and the olivetolic acid and the plastidal 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways (Andre et al. 2016). More than 60 cannabinoids have been identified in cannabis. The ten most frequently found in highest concentration are listed in Table II. Most of the cannabinoids are formed as carboxylic acids (Pellati et al. 2018) and converted by decarboxylation to CBD, Δ^9THC, etc. or degradation products such as CBN (United Nations Office for Drugs and Crime 2009). CBDA is the predominant cannabinoid in fiber-type hemps (Andre et al. 2016).

1.6 Legal framework

Production of hemp in the United States goes as far back as the 17th century when the Virginia Assembly passed legislation in 1619 that required all farmers to grow hemp for purposes of rope, sail, clothing, and for exchange as a legal tender between colonies. Towards

the end of the nineteenth century, the marijuana form of cannabis was openly sold in public pharmacies for its medicinal properties while hemp was replaced by other domestic materials after the U.S. Civil War. In 1906, the Pure Food and Drug Act required all cannabis sold at pharmacies to be labeled. Marijuana leaf was not introduced recreationally until 1910 during the Mexican Revolution. With time and during the Great Depression, a fear was established relating marijuana to violence and crime among communities resulting in twenty-nine states to outlaw marijuana by 1931, which was shortly followed by the creation of the Federal Bureau of Narcotics (FBN). In 1937, the Marijuana Tax Act was established nationally to criminalize marijuana by restricting possession of the drug to specific individuals. During World War II, the use of hemp was reintroduced in military materials resulting in 375,000 acres of hemp harvested by farmers in America during the year 1943 (PBS 2014). The Agricultural Marketing Act of 1946 approved plans for the domestic production of hemp by states and Indian tribes (Department of Agriculture 2019). A back-and-forth legal battle followed for years that persists to the present day.

The USDA Farm Bill of 2018 made the growing, manufacturing and use of hemp legal in the United States. This bill implemented regulations and guidelines for the establishment and administration of a hemp production program in the United States (United States Department of Agriculture 2018). Four universities in Virginia, including James Madison, were charged to aid the development of the hemp industry by the Virginia Department of Agriculture and Consumer Services (VDACS). For farmers, the legalization of hemp came with a restriction that a hemp crop would not exceed 0.3% THC otherwise complete destruction of the crop was required. Since both Δ^9THC and Δ^9THCA are present in the living hemp plant, the limit is defined as <u>total</u> THC. The low legal THC concentration level was initially established with intentions of

preventing people from getting “high” off the consumption of hemp (Smith 2019). While requirements have been established to grow and process hemp, there is still difficulty in achieving legality for the farmers and others involved in this business activity due to both the growth cycle and inaccuracies in testing.

1.7 General Overview of Analytical Methods for Hemp Cannabinoids

In general, analytical methods consist of sample collection, processing, quantitation, and data interpretation. Field sample collection of inflorescences and other plant material is followed by drying, grinding, and mixing. Portions of the processed samples are then extracted to isolate and concentrate the cannabinoids before chromatographic separation and determination. Certified reference materials (CRM) standards are used in calibration of methods to find concentration levels, reported as a dry weight percent and peak identification in gas (**Figure 3)** and liquid **(Figure 4**) chromatography. Potency is a term used in the cannabis industry that

describes the amount of a specific cannabinoid, i.e., either % CBD or % THC content, with higher concentrations being "more potent." Averages, standard deviations, and statistical interpretations are used for evaluation the analytical data.

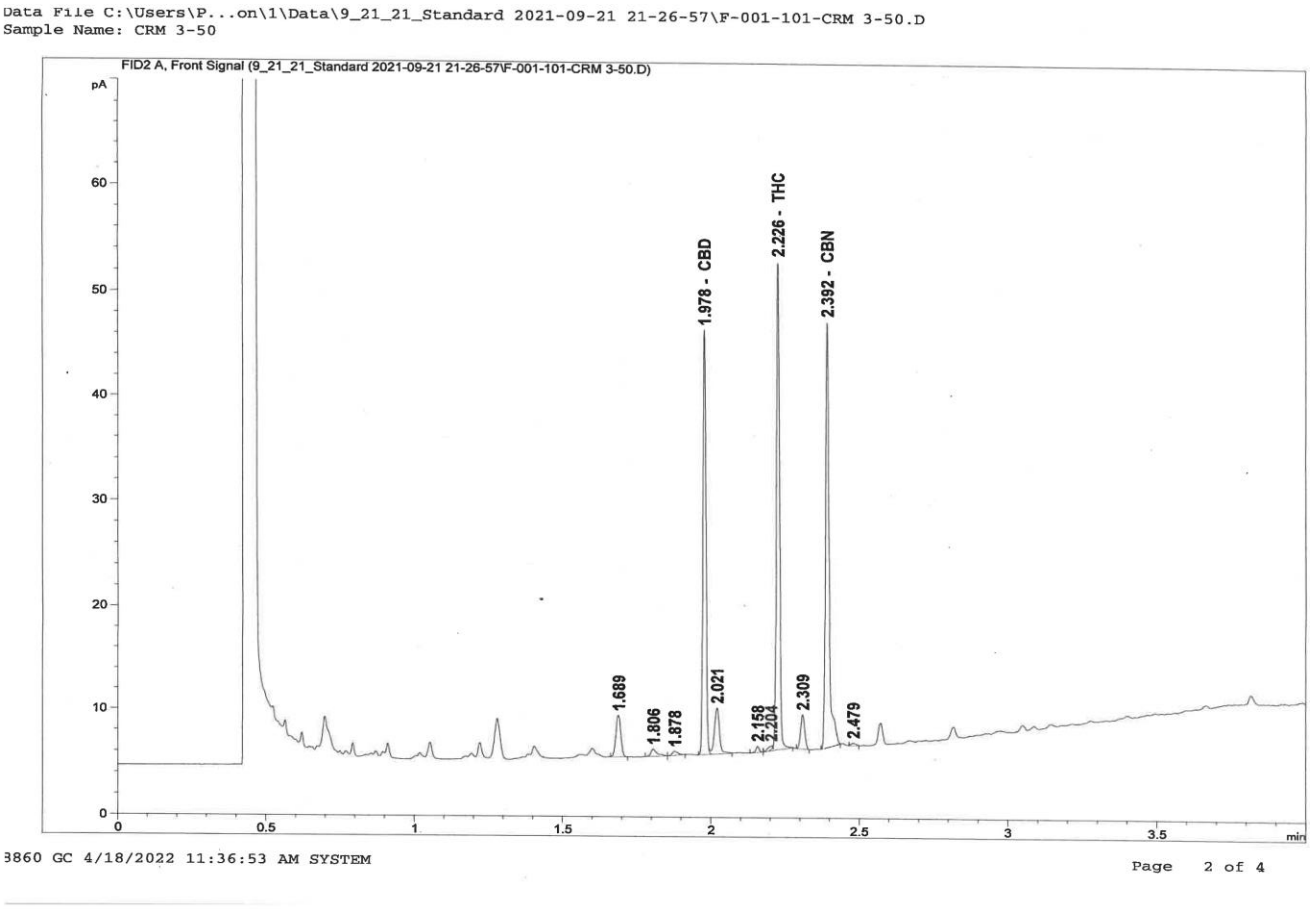

Figure 3. Gas Chromatogram – Flame Ionization Detection (GC-FID) of CRM 3 (100 ppm) collected using the operational details provided in Section 2.3.

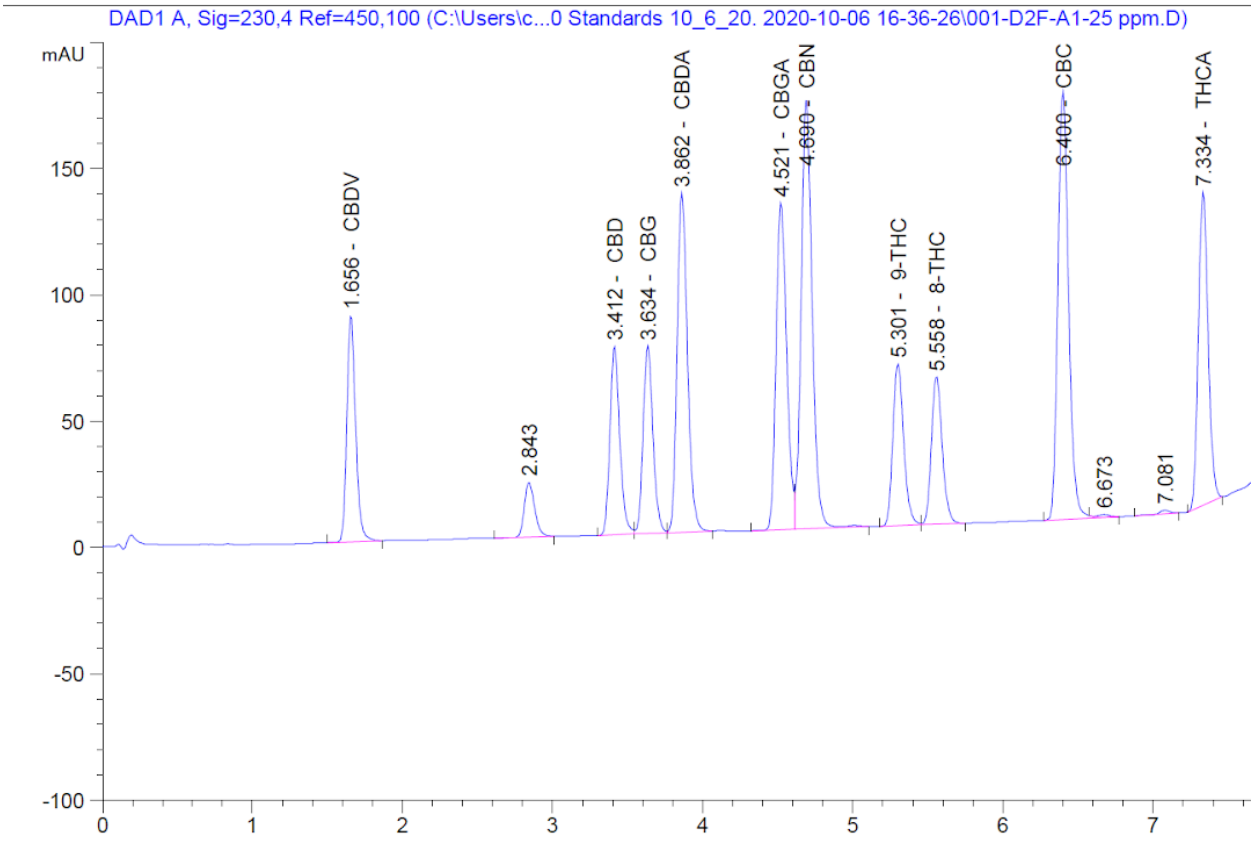

Figure 4. Liquid Chromatogram (uHPLC-DAD) of CRM 10 (100 ppm) collected using the operational details provided in Section 2.3.

The most sophisticated part of the analytical procedure for cannabinoid determination is the use of column chromatographic separation coupled with instrumental detection. Chromatography is a separation approach for mixtures of chemical compounds that partitions vapor or liquid phases with a solid phase in a column. First articulated in 1903 by Michael Tswett, separation was established and achieved by solutes differentially migrating at different rates. The speed by which solutes exit from a chromatography column depends on the degree of attraction of the solute between the stationary and the mobile phases. The mobile phase may be either gaseous (GC) or liquid (LC). For both GC and LC separation is achieved due to differences in solubility of the solutes for the stationary phase. In addition, the relative volatility of solutes also contributes to separation in GC. For both categories of chromatography, suitable

detectors are placed at the exit of columns for analysis so that separation and detection are accomplished in a single injection operation. Cannabinoids may be determined by either GC or LC, but either way, expensive instruments and skilled operators are required to give reliable results.

Numerous methods have been published for cannabinoid analysis by GC. Virginia Department of Consolidated Laboratory Services (DCLS 2019) uses GC with flame ionization detection (FID) for THC potency and compliance. Since the heated injection port of GC decarboxylates the carboxylic acid forms, many of the contract laboratories are employing the alternative - high performance liquid chromatographic (HPLC) – to obtain values for CBDA, THCA, etc. Many HPLC methods, including methods employing ultra-high-pressure columns (uHPLC) for more rapid determinations, have also been published (Agilent 2018). Both methods utilize standards known as Calibrated Reference Materials (CRMs) for and developing standard calibration graphs, used to find weight percent values.

1.8 Statement of Problem

Smith (2019) has described an industry wide issue of variability of cannabis testing results that have caused problems for both regulators and farmers. When farmers send the same sample to multiple laboratories and receive different answers for potency testing frustration occurs. The two most common methods of analysis may fundamentally result in different answers, even though the industry requires ISO laboratory testing (May 2021). Furthermore, cannabis plants are heterogeneous in cannabinoid production within the same plant or from plant to plant. There is a lack of standardized methodology and no standard reference material for method calibration (Smith 2022). In analytical procedures multiple samples are tested and

averages and standard deviations are calculated. The accuracy of a test is how close the average value is to the true value (Smith 2018), the average coming from calibration of instrumentation with standards provided by various commercial suppliers. There are CRM standards, but no standard plant material that can be extracted a measured accurately. Therefore, the only estimate of the accuracy of the result is from the precision of the data set, which is limited in that potential systematic errors are unknown.

In addressing the variability issues, the Limit of Detection (LOD) and Limit of Quantification (LOQ) were first evaluated for both methods used for cannabinoid determination to begin this study. The LOD and LOQ values for each cannabinoid of interest were compared to judge differences and sensitivity. Once these values were obtained, the same variety of cannabis was analyzed by standard procedures for establishing difference in cannabinoid content from location in mature plants. Six varieties of cannabis grown both indoors and outdoors were tested by both analytical procedures with samples taken from the same relative position in mature plants and statistically evaluated for comparison. Finally, the indoor plants were analyzed after a period of storage and air drying by both methods to determine any changes in cannabinoid content. The data generated from these experiments should prove beneficial for both regulators and farmers in understanding the limitations of current methods of analysis.

EXPERIMENTAL METHODS

2.1 Sample preparation

The analytical plan (**Figure 5**) for hemp cannabinoid determination began with sample collection. Live hemp biomass samples were collected according to the Virginia Department of Agricultural and Consumer Services (VDACS) sampling protocol procedure (VDACS 2022) as follows. The respective average height, appearance, approximate density, condition of the plant and degree of maturity of the inflorescence were recorded for each hemp sample. Samples were collected at the end of the growing season in 2020 and 2021 when the plants had matured just before harvest. Inflorescences were cut wearing disposable gloves and clean scissors. About three to five inches of the tip of the plant at each sampling location were cut and placed in a clean quart zip-loc bag.

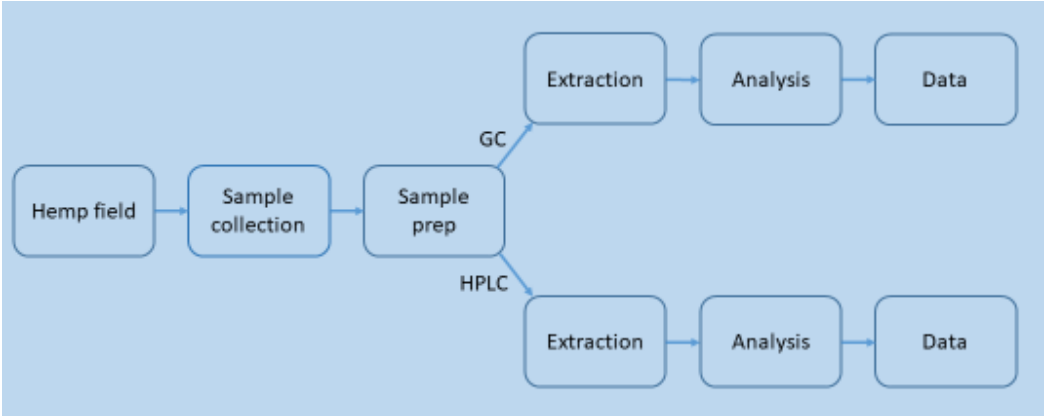

Figure 5: Analytical Plan for Hemp Analysis.

Hemp samples were dried in clean aluminum pans in a convection oven at 90 ± 5 °C for two hours. Additional intervals of drying were done until constant weight was achieved. The difference in original wet weight and final dry weight was used to calculate the water content. All analytical results were based on dry weight. Samples were stored in desiccators until analysis. The first step following drying was removal of leaves, stems, debris and foreign material manually

from each sample. Samples were then placed in a food processor (West Bend) and ground at high speed for one minute to produce a powder which was then passed through a U.S. number eight mesh (2.380 mm) brass sieve (Fisher Scientific, Massachusetts). The fine powder was then weighed to 500.0 ± 0.1 mg and placed into a dry, clean, tared 100 mL volumetric flask. The flask was filled to volume with methanol (a weighted ring was placed around the neck of the flask) and inserted into an ultrasonic bath for ten minutes. Upon removal the hemp was allowed to settle to the bottom of the flask after which a portion of the methanol extract was decanted into a clean, dry beaker. A 0.2 µm Fisher brand PTFE syringe filter was used to place the extract into a two mL screw capped vial (Agilent Technologies) for analysis by either gas or liquid chromatography.

2.2 Chemicals and Instrumentation

Methanol, water, and isopropanol (all HPLC grade, Fisher Scientific) were used as solvents. Formic acid (ACS certified, 88%, Fisher Scientific) was used as a modifying reagent in liquid chromatography. Either a gas chromatograph (GC, Agilent 8860) with flame ionization detector (GC-FID) or an ultra-high pressure liquid chromatograph (uHPLC, **Agilent Model 1290 Infinity II)** with diode array detector (LC-DAD) was used to separate and determine the cannabinoids of interest in this work (**Figures 3 & 4**). GC specifications (DCLS 2019): 0.25 x 15000 mm Restek Rxi-35Sil MS fused silica mid polarity column coated with 0.25 µm stationary phase and UHP helium carrier gas at 2.50 mL/min. Injection volumes were 1.00 µL with a 25:1 split ratio. The column oven was set at 225°C and raised to 330°C for a rapid 4.1-minute total analysis time. The injector and detector temperatures were 250°C and 350°C, respectively. The flame ionization detector gases were ultra-high purity hydrogen flow rate of 40 mL/min and ultra-high purity compressed air flow rate 450 mL/min. LC specifications (modified, Storm et al. 2020) 2.1 x 50 mm Agilent 120 ec-C18 column with a mobile phase comprised of 0.1% formic

acid in water (A) and 0.05% in methanol (B). Eluant flow rate was 1 mL/min and began as 40% A / 60% B for 1 minute then 23% A / 77% B (gradient) 6 minutes then 5% A / 95% B (gradient) for 2.2 minutes. The total run time was 9.5 minutes. The detector wavelength was 230 nm. The column compartment and detector were set to 40°C. Cayman Chemical Certified Reference Materials (CRM 3 and CRM 10) (**Table III**) were used for calibration.

2.3 Calibration

Standard calibration mixes solutions of 0 (blank) – 150.0 ppm (mg/L) of each cannabinoid were made based on anticipated concentration levels for samples extracted by the above procedure. X-Y calibration curves were plotted as concentration (ppm) vs. response (peak area) for each cannabinoid. Straight line linear regression was used to find the cannabinoid concentration each extract sample by the formula:

$$Y = mX + b \qquad \text{Equation 1}$$

The weight percent values were found by the formula:

$$\text{Cannabinoid \%} = \frac{\text{cannabinoid concentration (ppm)} * (100\ \text{mL})}{500\ \text{mg}} * 0.1 \qquad \text{Equation 2}$$

The heated GC injection port decarboxylates CBDA and Δ^9THCA to CBD and Δ^9THC, thus respectively reporting total CBD (CBD_T) and total Δ^9THC (Δ^9THC_T). For LC the following equations were used, with 0.877 accounting for decarboyxlation:

$$CBD_T\ (ppm) = CBD\ (ppm) + 0.877 * CBDA\ (ppm) \qquad \text{Equation 3}$$

**ref needed

$$\Delta^9THC_T\ (ppm) = \Delta^9THC\ (ppm) + 0.877 * \Delta^9THCA\ (ppm) \qquad \text{Equation 4}$$

2.4 Data Analysis of Methods

Limits of detection (LOD) were calculated to establish the lowest analyte concentration that can be statistically distinguished from baseline noise (Armbruster and Terry 2008). The two lowest concentrations of standard were injected repetitively seven times each. The resulting peak area values were used to find the average, sample standard deviation, relative standard deviation, % relative standard deviation, signal to noise ratio (reciprocal relative standard deviation) and variance for the two standards using an excel spreadsheet. An F test was conducted by taking the ratio of the variances of the two concentration peak area values (F, exp) for comparison to the F critical value at 98% confidence (Duncan 1955). A pooled standard deviation (s_{pooled}) was calculated by averaging the two sample standard deviations and combined with student t (t) at 99% confidence one tail. The minimum detectable signal (S_{min}) was calculated from the product of t and s_{pooled}. Calibration curve was used to convert S_{min} to concentration. The limit of detection (LOD, ppm) was calculated from the slope and the S_{min}. The limit of quantification (LOQ, ppm) was calculated as 10 times s_{pooled} / slope.

2.5 Sample Identification and Purpose of Sample Collection

Just prior to harvest on September 18th, 2020, three indoor plants were sampled randomly at Fowl Ball Farm in Augusta County, Virginia. These plants were all the variety T2 also known as Trump x Trump grown from feminized seed provided by Boring Hemp Company (Boring, Oregon). Product literature indicated the plants were expected to be 11-16% CBD by maturity at 8-10 weeks (Gu 2021). Each selected plant was sampled from three locations: top, middle, and bottom and analyzed in triplicate for a total of nine determinations per plant. The purpose of this sample collection was to determine the statistical variation for cannabinoid content at various

regions of the plant (Set 1). This was done to explore the heterogeneity of the plant and the effect of light on production of light sensitive cannabinoids. Just prior to harvest on October 2nd, 2021, 18 indoor and 18 outdoor plants were sampled at Fowl Ball Farm, three from each variety (Set 2), all samples being collected from the top portion of the plant. Varieties sampled were Hawaiian Haze, Special Sauce, Sour Space Candy, Elektra, Lifter, and Suver Haze (**Table IV**) that were advertised as 15-20 % CBD at maturity. The purpose of this sample collection set was evaluation of cannabinoid content by variety indoor and outdoor. A final set of 18 samples was collected from indoor air-dried plants on December 17th, 2021, three each from all six varieties to assess any change in cannabinoid content that had taken place following three months after harvest (Set 3).

2.6 Statistical Evaluation

Averages of weight percentages (Equation 2) for each cannabinoid with standard deviations were calculated for each location of the plant (top, middle and bottom). This was done to compare cannabinoid percentages throughout the plant. CBD, CBN and THC concentrations were measured by GC. Ten cannabinoids (**Table II**) were measured by LC. The two methods were statistically compared using a t-test and an established rejected or accepted null hypothesis. The null hypothesis stated of there is no difference between the mean for both analytical methods of the cannabinoid percentages at 95% confidence. This determined if there was a significant difference between the two methods (Wadhwa et al. 2021). Analysis was also done on hemp in indoor and outdoor growing locations to determine if variation existed based on environmental factors. Similar calculations were done on hemp varieties both indoor in outdoor to compare the effects of different strains on varieties as well as in varying environments.

RESULTS

3.1 Limit of Detection and Limit of Quantification for Cannabinoids by GC and LC

The limit of detection (LOD) values and limit of quantification (LOQ) values were found for GC (**Table V**) and LC (**Table VI**). The detection limit values were similar for all the cannabinoids for both GC and LC and considerably lower than the smallest calibration concentration (2 ppm). Quantification limits for 0.5 gram samples were also low values. The LOQ values for Δ^9THC were 0.0104 % and 0.0170 % for GC and LC, respectively, and are about twenty-thirty times lower than the compliance value of 0.3% Δ^9THC_T.

3.2 Variation of Δ^9THC_T and CBD_T in Top, Middle and Bottom of Hemp Plants

After establishing the LOD and LOQ values, the full analysis procedure was used to examine variation of production of major cannabinoids within hemp plants (Set 1); that is, heterogeneity assessment. A set of live indoor grown hemp plants were sampled and measured at three relative locations (top, middle and bottom) for Δ^9THC_T and CBD_T by GC and LC (**Table VII**). The probability that an observed difference could have occurred just by random chance was used for statistical analyses. The lower the P-value, the greater the statistical significance of the observed difference (Beers 2022). The null hypothesis states "there is no difference between average values being compared to one another." Specifically, any P-value less than 0.05 is statistically significant (95% confidence), indicating that the null hypothesis is rejected (McLeod 2019). In this study, we used a two-tailed t test to determine change. For all plants, the largest concentrations of cannabinoids were found in the top of the plant, which is the least shaded location. The middle and bottom locations were similar in concentration to each other. The average Δ^9THC_T and CBD_T values for the locations showed greater values for the top of the

plants than middle or bottom, but the null hypothesis was not rejected (**Table VIII**) regardless of whether GC or LC was used for the analyses. The LC data showed slightly greater Δ^9THC_T values than GC. The GC values were higher for CBD_T than the LC values. However, statistically (**Table IX),** there was no difference ($P<0.05$) between the GC and LC averages due to the large sample standard deviations (**Table VII**).

3.3 Variation of Δ^9THC_T and CBD_T between Varieties (Indoor & Outdoor)

Six varieties of live hemp (**Table IV**) grown indoors and outdoors simultaneously were sampled from the top location only for cannabinoids just before harvest (Set 2). In general, the indoor varieties ranked from highest to lowest Δ^9THC_T and CBD_T (**Table XI**) were Suver Haze (SH) > Special Sauce (SS) > Lifter (L) > Electra (E) > Hawaiian Haze (HH) > Sour Space Candy (SSC), while the outdoor varieties ranked as Lifter > Special Sauce > Hawaiian Haze > Electra > Sour Space Candy > Suver Haze. As found in the previous section, the replicate result averages generally showed large sample standard deviations (**Table X**), which led to acceptance of the null hypothesis in the comparison of indoor versus outdoor values for both GC and LC (**Table XII**) with one exception. It can be stated with statistical confidence (~99%) that Suver Haze produced more Δ^9THC_T and CBD_T when grown outdoors than when grown indoors for this crop. Four other varieties also produced more cannabinoids, but at lower confidence values (~80%). One variety (Special Sauce) was highly scattered in values (**Table XIII**).

3.4 Variation of Δ^9THC_T and CBD_T for Pre and Post Dried Indoor Hemp

The same hemp plants sampled before harvest were air dried in a barn for three months down to ~10% water weight. Unfortunately, the outdoor hemp was destroyed by mold, but the indoor hemp was separate and not affected. Samples were collected from indoor dried hemp (Set

3) for GC and LC analyses to assess any changes in Δ^9THC_T and CBD_T that might have occurred from drying and aging (**Table XIV**). There was no consistency to the changes before and following drying: some varieties increased, some decreased and for some there was little or no change (**Table XVI**). The P-values (**Table XV**) demonstrated that some of the changes were statistically significant. For example, there was >97% confidence that Δ^9THC_T increased in Sour Space Candy, but >91% confidence it decreased in Suver Haze.

3.5 Variation of Cannabinoids for Six Varieties Grown Indoors & Outdoors

In addition to Δ^9THC_T and CBD_T the LC method provided quantitative results (**Table XVII**) for eight other cannabinoids for the six varieties (Set 2) for the top region of the plants. Three of the cannabinoids, CBDV, Δ^8THC and CBN, were not detected (ND) in any of the samples. In general, the highest to lowest weight percent values were CBDA > CBD > Δ^9THC > CBC > Δ^9THCA > CBGA > CBG (not including ND results). As found in previous data sets some cannabinoids increased and some decreased when the indoor and outdoor results were compared (**Table XIX**). The P-values (**Table XVIII**) demonstrated that some of the changes were statistically significant.

3.6 Variation of Cannabinoids for Pre and Post Dried Indoor Hemp (Set 3)

In addition to Δ^9THC_T and CBD_T the LC method provided quantitative results (**Table XX**) for eight other cannabinoids for the six varieties (Set 3). Three of the cannabinoids, CBDV, Δ^8THC and CBN, were not found in any of the samples. In general, the highest to lowest weight percent values were CBDA > CBD > Δ^9THC > CBC > Δ^9THCA > CBGA > CBG (not including ND results). As found in previous data sets some cannabinoids increased and some decreased

when the indoor and outdoor results were compared (**Table XXII**). The P-values (**Table XXI**) demonstrated that some of the changes were statistically significant.

DISCUSSION

During 2019 Nelson (2020) developed analytical methodology for the separation and determination of cannabinoids. Building upon her work, Bowles (2021) observed notable differences in analytical results for hemp samples that he attributed to plant heterogeneity and other factors. Data sets were limited so this study was conducted to establish if differences were statistically significant and attempt to understand why these differences were occurring. In a typical analytical quantification there is sample collection, sample processing and analytical finish (**Figure 5**). The analytical finish for cannabinoid determination is chromatographic separation (GC or LC) followed by detection, either GC or LC. Both analytical finish methods were evaluated by repetitive analyses of calibration standards to ascertain any differences that were due to the intrinsic methodology. It was found that LOD values for both methods were similar and the values themselves low. Calculated LOQ values were also similar and low. R^2 values > 0.998 were observed for both GC and LC calibration lines except for Δ^9THCA (LC) which was 0.994. These observations support that there is high precision and accuracy in both instrumental methods with capability of analyses of low detection values. Of greatest importance is that the quantification variation in analytical results was not due to the analytical finish, either GC or LC. Based on LOD and LOQ data either GC or LC should provide trustworthy data of compliance of THC limits for industrial hemp.

Once the analytical finish methods were established statistically then hemp plant biomass was collected and processed (Set 1). The regulatory protocol for sampling hemp required

collection from the top one-third of plants. Intuitively it was suspected that this area of plant would contain greater amounts of cannabinoids than portions of the plant less exposed to sunlight. Hemp farmers have argued that this could represent a bias toward higher THC and is not in keeping with the fact that the whole plant is processed for CBD production. In fact, Δ^9THC_T and CBD_T were found to decrease from the top to the bottom of the plant. This may be due to the bottom and middle parts of the plant being equally covered by shade. Even though there seemed to be a trend, statistically the null hypothesis was not rejected due to the large sample standard deviations observed for most samples. It is important to note that each average was from three replicate measurements. Extraction recovery of cannabinoids was verified by repetitive analysis of previously extracted hemp samples as near 100%. Thus, these standard deviations are indicative of heterogeneity of cannabinoid production in the plant biomass and not extract. Variability in the standard protocol for compliance analysis includes grinding the dried sample, sieving and filtering. Parts of the plant that generally have low cannabinoid content such as stems, and leaves are included with parts such as inflorescence that have high cannabinoid content. Even after mixing, it is inherent to the protocol that some samples may contain larger portions of low THC content than others.

Another important observation of these initial plant biomass results was that Δ^9THC_T values appeared higher when using LC and CBD_T appeared higher when using GC. Again, there were large standard deviations for all results, supporting the indication of heterogeneity in plant biomass, but the null hypothesis was not rejected. Even though it cannot be conclusively stated that the two methods are giving different results it is a cause for concern. The Δ^9THC_T and CBD_T values are obtained from GC by decarboxylation that occurs in the injection port, but are calculated for LC from Δ^9THC, Δ^9THCA, CBD and CBDA, respectively. It is possible that

decarboxylation is incomplete for Δ^9THCA in the GC injection port. Whether or not this is the case, GC remains the method used by regulators for compliance.

The large sample standard deviations for repetitive testing of the same sample with relative standard deviations ranging as high as 35% demonstrate a fundamental problem with using an absolute value of 0.3% Δ^9THC_T as a regulatory value. For example, it is illegal if a hemp sample was found to be 0.45 ± 0.10% is it in excess of 0.3% and therefore illegal? Using the absolute criterion only the answer is "yes". However, a statistical evaluation and a null hypothesis can be used in which "there is no difference between the regulatory value and the average over than that which can be attributed to random variation." The lower the probability that the observed difference occurs by chance, the less likely it is that the null hypothesis is true. Usually, the null hypothesis is rejected if the probability of the observed difference occurring by chance is less than 1 in 20 (i.e., 0.05 or 5%) and in such a case the difference is said to be significant at the 0.05 (or 5%) level (this is a confidence level of 95%). Using this level of significance there is, on average, a 1 in 20 chance that we shall reject the null hypothesis when it is in fact true. In order to be more certain that we make the correct decision a higher level of significance can be used, usually 0.01 or 0.001 (1% or 0.1%). The significance level is indicated by writing, for example, P (i.e., probability) = 0.05, and gives the probability of rejecting a true null hypothesis. It is important to appreciate that if the null hypothesis is retained it has not been proved that it is true, only that it has not been demonstrated to be false. The following demonstrates this concept applied to our proposed 0.45% ± 0.10% using a student t value approach. A value of *t* is calculated by substituting the experimental results in this question. If |*t*| (i.e., the calculated value of *t* without regard to sign) *exceeds* a certain critical value then the null hypothesis is rejected. The critical value of |*t*| for a particular significance level is found in

Student's *t* tables. For example, for a sample size of 3 (i.e., 2 degrees of freedom) and a significance level of 0.05, the critical value of $|t|$ is 4.30.

An absolute t value may be calculated as follows:

$$| t |_{calculated} = | (\bar{x} - \mu)\sqrt{n}/s |$$

When $| t |_{calculated} > | t |_{critical}$ the null hypothesis is rejected.

For this example, at 95% confidence ($P < 0.05$) the $| t |_{calculated} = 2.60 < | t |_{critical} = 4.30$, so the null hypothesis is accepted and there is no difference between 0.3% and 0.45%.

These data also show that depending on instrumentation method used, results can vary but not significantly. Oftentimes be above or below the legal compliance limit of 0.3 % $THC_{T,}$ having a large effect on decision making as to whether a crop will be destroyed. This is supported by the statistical evaluation of GC and LC in which all p-values were above 0.05, indicating that there is strong evidence to support the null hypothesis and that observed difference is due to sampling or experimental error. Similar statistical results were displayed for locations within the live plant, showing that variation is not due to random error. This supports bias in random sampling procedures towards the more highly concentrated cannabinoids portions of the plant even when the entirety of the live hemp plant is harvested and used in production of various products.

For all varieties, THC_T and CBD_T values varied greatly. The only variety that was close to matching the CBD value advertised by Oregon CBD was Lifter (16 %), having values of 15.38 % (GC) and 14.17 % (LC) for outdoor hemp. All other varieties were below advertised values. This may be due to harvesting of samples before peak maturity, the random sampling

procedure, or the heterogeneity of the plant. When ranking varieties, all followed a trend in which: Suver Haze > Special Sauce > Lifter > Elektra > Hawaiian Haze > Sour Space Candy. All varieties exhibited an increase from indoor to outdoor grown hemp while Special Sauce and Suver Haze decreased from indoor to outdoor hemp. Additionally, most of the varieties displayed a p-value above 0.05, showing that there is a likely chance that differences in indoor or outdoor hemp are due to sampling or experimental error. In another interpretation method, it can be stated that for the Suver Haze variety that there is a 99% chance that THC_T will decrease from indoor to outdoor hemp for both methods.

Live and dried hemp samples were analyzed to learn whether drying changed cannabinoid concentrations. There was no consistency in the changes before and after drying with some cannabinoid concentrations increasing while others decreased or showed no change. Statistical p-values showed that at greater than 95% confidence, some but not all changes were significantly different. In addition to these two we examined all other eight cannabinoids that exhibited similar to trends for CBD_T and THC_T, Hawaiian Haze and Sour Space Candy showed increases for all cannabinoids except for CBG. This may be due to the very low amounts of CBG present in all samples. When looking at differences between growing locations, trends varied across varieties. CBGA and Δ^9 THCA showed increases for all varieties except for Elektra and Suver Haze. Δ^9 THC increased for all varieties but Special Sauce and Suver Haze. This variation can be attributed to the heterogeneity of the crop. P-values varied still throughout varieties, with split results of being above and below 0.05. All variations are most likely due to systematic errors in statewide established methods in Virginia, the heterogeneity of the hemp crop, and differences between varieties.

CONCLUSION

In summary, it was observed that LOD and LOQ values very low and similar for both GC and LC. THC and CBD concentrations trended top > middle > bottom of the plant but were not statistically different at 95% confidence. LC was greater than GC for THC while GC was greater LC for CBD, but data were not statistically different (95% confidence)**.** For indoor hemp both THC and CBD concentrations followed the order by variety SH > SS > L > E > HH > SSC**.** However, for outdoor hemp there was no trend in THC and CBD concentrations by variety. Fresh hemp at harvest and air-dried hemp showed no statistical differences (95% confidence) in THC and CBD concentration. Large sample standard deviations were the reason statistical results were not different (i.e., null hypotheses were not rejected).

In conclusion, it was found that the instrumental methods (GC vs. LC) made a difference in both compliance and production estimates of cannabinoids in hemp. Additionally, both GC and LC methodology have low detection limits and are well suited for cannabinoid analysis. The primary variable for cannabinoid concentrations is heterogeneity of the hemp plant. Sample collection protocols are biased towards high THC locations in the plant while all variations are most likely due to systematic errors in statewide-established methods in Virginia, the heterogeneity of the hemp crop, and differences between hemp varieties. An absolute value of 0.3% THC should not be applied for legality but should be associated with sample standard deviation. Based on data variation, values up to 0.5% THC should be considered as meeting the legislative requirement.

REFERENCES

Agilent. Dedicated Cannabinoid Potency Testing In Cannabis or Hemp Products Using the Agilent 1220 Infinity II LC System, 1-10, **2018.**

Andre, C. M.; Hausman, J.; Guerriero, G. *Cannabis sativa*: The Plant of the Thousand and One Molecules. *Frontiers in Plant Science* **2016**, 7, 1-17.

Armbruster, D. A., Pry, T. Limit of Blank, Limit of Detection and Limit of Quantitation. *Clin. Biochem Rev.* **2008**, 1, 49-52, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2556583/ (accessed February 17th, 2022).

Atkins, P. Right From the Grow: A Look at Sampling and Sample Preparation Methods of Solid Cannabis for Analysis. *Cannabis Science and Technology* **2019**, 2, 26-34.

Atakan, Z. Cannabis, a Complex Plant: Different Compounds and Different Effects on Individuals. *The Advanced Psychopharmacology* **2012**, 6, 241-254.

Beers, B. What P-value Tells Us. https://www.investopedia.com/terms/p/p-value.asp (accessed Mar 25, 2022).

Bennett, P. What are Trichomes and Why Do They Exist? https://www.leafly.com/news/cannabis-101/what-are-trichomes-on-cannabis (accessed January 19, 2022).

Bowles, D, Nelson, V., Stegmeier, R., Downey, D. Why Do My Hemp Cannabinoid Test Results Vary? National Conference on Undergraduate Research, NCUR 2021. Oral 9, Session 911, April 14, 2021.

Buchi. The Right-hand Book for Cannabis Scientists. BÜCHI Labortechnik AG, Flawil, Switzerland, 2020.

Citti, C., Braghiroli, D., Vandelli, M. A., Cannazza, G. Pharmaceutical and Biomedical Analysis of Cannabinoids: A Critical Review. *Pharmaceutical and Biomedical Analysis* **2018**, 147, 565-579.

Department of Consolidated Laboratory Services (DCLS). The Determination of Delta-9-THC and Related Compounds in Hemp Using Gas Chromatography. Commonwealth of Virginia, Department of General Services, Richmond, Virginia. Document 16612, Revision 3, January 22, **2019**.

Department of Agriculture. Establishment of a Domestic Hemp Production Program. *Federal Register* **2019**, 64, 58522-58564.

Department of Forensic Science, Virginia. Cannabis Typification Field Test ("4-AP or "Swiss" Test). https://www.dfs.virginia.gov/wp-content/uploads/2019/11/Cannabis-Typification-Field-Test-Instructions-V2-1.pdf (accessed February 22nd, 2022).

Duncan, D. B. Multiple Range and Multiple F Tests. *Biometrics* **1955**, 11, 1-42.

Giese, M.W.; Lewis, M. A.; Giese, L.; Smith, K. M. Development and Validation of a Reliable and Robust Method for the Analysis of Cannabinoids and Terpenes in Cannabis. *Journal of AOAC International* **2015**, 98, 1503-1522.

Gu. Fortuna Hemp, T2 Hemp Seeds. https://fortunahemp.com/t2-hemp-seeds/# (accessed February 24th, 2022).

Integrated Taxonomic Information System. Taxonomic Hierarchy for Cannabis. https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=19108#null (accessed January 31st, 2022).

Johnson, R. Hemp as an Agricultural Commodity. *Congressional Research Service* **2018**, 1-44.

Li, H. An Archeological and Historical Account of Cannabis in China. *Economic Botany* **1974**, 28, 437-438.

Marsh, G. Next Step for Automotive Materials. *Mater. Today* **2003**, 6. 36-43.

May, K. ISO/IEC 17025 Accreditation Falls Short for Cannabis Testing Laboratories. *Cannabis Industry Journal* **2021**, https://cannabisindustryjournal.com/feature_article/iso-iec-17025-accreditation-falls-short-for-cannabis-testing-laboratories/ (accessed February 24th, 2022).

McLeod, S. What a P-value Tells You About Statistical Significance. https://www.simplypsychology.org/p-value.html#:~:text=A%20p%2Dvalue%20less%20than,and%20accept%20the%20alternative%20hypothesis (accessed Mar 25, 2022).

Nelson, V., Downey, D. Quantification of Cannabinoids in Hemp. National Conference on Undergraduate Research, NCUR 2020, (conference cancelled); National Conference on Undergraduate Research, NCUR 2021. Poster 4, Session 3618, April 13, 2021.

PBS. Marijuana Timeline. https://www.pbs.org/wgbh/pages/frontline/shows/dope/etc/cron.html (accessed February 7th, 2022).

Pellati, F., Borgonetti, V., Brighenti, V., Biagi, M., Benvenuti, S., Corsi, L. *Cannabis sativa L.* and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer. *Biomed Res Int.* **2018**,

Rahn, B., Hennings, T. Marijuana Plant Anatomy, 2022. Leafly. https://www.leafly.com/learn/growing/marijuana-plant-anatomy (accessed Jan., 2021).

Russo, E. B. The Case for the Entourage Effect and Conventional Breeding of Clinical Cannabis: No "Strain," No Gain. *Front. Plant Sci.* **2019**, *9*, 1-8.

Schluttenhofer, C.; Yuan, L. Challenges towards Revitalizing Hemp: A Multifaceted Crop. *Trends in Plant Science* **2017**, *22*, 917-928.

Schmidt, E. How and When to Harvest Hemp, 2020. ACS Laboratory, Beyond Compliance. https://acslabcannabis.com/blog/harvesting/how-and-when-to-harvest-hemp/ (accessed March 12th, 2022).

Schwabe, A. L., Hansen, C. J., Hyslop, R. M., McGlaughlin, M. E. Comparative Genetic Structure of Cannabis sativa Including Federally Produced, Wild Collected, and Cultivated Samples. *Front. Plant Sci.* **2021**, 12, 1-10.

Small, E., Beckstead, H. Cannabinoid Phenotypes in *Cannabis sativa. Nature* **1973**, 245, 147-148.

Smith, B. C. Error, Accuracy, and Precision. *Cannabis Science and Technology* **2018,** 1, 12-16.

Smith, B. C. Hemp Testing Insanity. *Cannabis Science and Technology* **2019**, 2, 10-13.

Smith, B. C. How Standardized Sample Preparation Can Solve the Inter-Laboratory Variation Problem. *Cannabis Science and Technology* **2020**, 3, 10.

Storm, C., Zumwalt, M., Macherone, A. Dedicated Cannabinoid Potency Testing in Cannabis or Hemp Products Using the Agilent 1220 Infinity II LC System. Application Note, Food Testing and Agriculture. www.agilent.com/chem (accessed February 24, 2020).

The Denver Post. Best Hemp CBD Flower Strains. https://www.denverpost.com/2021/06/11/best-hemp-cbd-flower-strains-of-2021/ (accessed February 21, 2022).

Tswett, M. On a New Category of Adsorption Phenomena and their Application to Biochemical Analysis. *Warsaw Society of Nat. Sciences* **1903**, 1-18.

United Nations Office on Drugs and Crime. Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products. *Manual for National Drug Analysis Laboratories* **2009**, 1-47.

Virginia Department of Agriculture and Consumer Sciences. Industrial Hemp. https://www.vdacs.virginia.gov/plant-industry-services-hemp.shtml (accessed July 22nd, 2022).

Wadhwa, R., Marappa-Ganesha, R. T Test. *StatPearls* **2021**, https://www.ncbi.nlm.nih.gov/books/NBK553048/ (accessed February 20th, 2022).

Watts, G. Cannabis Confusion. *Science Commentary in the National Library of Medicine Journal* **2006**, 332, 175-176.

Webster, G. B.; Sarna, L. P.; Mechoulam, R. Conversion of CBD to Delta8-THC and Delta9-THC. *United States Patent Application Publication* **2004**.

White, J. Cannabis Ruderalis. https://www.cnbs.org/cannabis-types/cannabis-ruderalis/ (accessed January 15th, 2022).

APPENDIX

Table I: Integrated Taxonomic Information System Report: Taxonomic Hierarchy for Cannabis (Integrated Taxonomic Information System, 2022)

Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Superdivision	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Rosanae
Order	Rosales
Family	Cannabaceae
Genus	Cannabis L.
Species	Cannabis sativa L.

Table II: Primary cannabinoids assayed for the present study

Common Name	Symbol	IUPAC Name	Molar Mass (grams/mol)	Reference Standard
Cannabichromene	CBC	2-Methyl-2-(4-methylpent-3-enyl)-7-pentyl-5-chromenol	314.46	CRM 10
Cannabidiol	CBD	2-Methyl-2-(4-methylpent-3-enyl)-7-pentyl-5-chromenol	314.47	CRM 3, CRM 10
Cannabidiolic Acid	CBDA	(1'R,2'R)-2,6-Dihydroxy-5'-methyl-4-pentyl-2'-(prop-1-en-2-yl)-1',2',3',4'-tetrahydro[1,1'-biphenyl]-3-carboxylic acid	314.47	CRM 10
Cannabidivarin	CBDV	2-((1S,6S)-3-methyl-6-(prop-1-en-2-yl) cyclohex-2-enyl)-5-propylbenzene-1,3-diol	286.41	CRM 10
Cannabigerol	CBG	2-[(2E)-3,7-Dimethylocta-2,6-dienyl]-5-penyl-benzene-1,3-diol	316.48	CRM 10
Cannabigerolic Acid	CBGA	3-[(2E)-3,7-Dimethylocta-2,6-dien-1-yl]-2,4-dihydroxy-6-pentylbenzoic acid	360.50	CRM 10
Cannabinol	CBN	6,6,9-Trimethyl-3-pentyl-benzo[c]chromen-1-ol	310.43	CRM 3, CRM 10
Δ^8 Tetrahydrocannabinol	Δ^8THC	6,6,9-Trimethyl-3-pentyl-6a,7,10,10a-tetrahydrobenzo[c]chromen-1-ol	314.45	CRM 10
Δ^9Tetrahydrocannabinol	Δ^9THC	(6aR,10aR)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol	314.45	CRM 3, CRM 10
Δ^9 Tetrahydrocannabinolic Acid	Δ^9THCA	(6aR,10aR)-1-Hydroxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-2-carboxylic acid	358.50	CRM 10

Table III: Calibration data for CRM standards: slope (m), y intercept (b) and correlation coefficient (goodness of straight line fit)

	uHPLC CRM 10			GC CRM 3		
Cannabinoid	**Slope**	**Intercept**	**R^2**	**Slope**	**Intercept**	**R^2**
CBC	31.4453	-39.505	0.99988	-	-	-
CBD	12.1386	-12.645	0.99994	0.5900	-1.3373	0.99851
CBDA	24.0364	-25.4165	0.99993	-	-	-
CBDV	13.8423	-16.2744	0.99992	-	-	-
CBG	12.4101	-13.3871	0.99993	-	-	-
CBGA	21.5675	-16.5742	0.99997	-	-	-
CBN	31.9769	-36.7705	0.99991	0.6359	-0.8050	0.99890
Δ^8THC	11.1945	-15.2483	0.99998	-	-	-
Δ^9THC	11.8722	-15.0213	0.99990	0.6522	0.5383	0.99870
Δ^9THCA	20.6368	+67.4802	0.99370	-	-	-

Table IV: Hemp varieties (Oregon CBD Co., Independence, Oregon) grown for Sets 2 & 3 of this study with expected characteristics from marketing literature

Variety	**Abbreviation**	**Genetic Mixture**	**Flavor Profile**	**Terpenes**	**CBD %**
Elektra	E	ACDC x Early Resign Berry	Diesel Citrus Berry	Caryophyllene Pinene Humulene	15
Hawaiian Haze	HH	DC Haze “CC” x Early Resign Berry	Orange Fruity Floral	Caryophyllene Pinene Myrcene	17 - 20
Lifter	L	Suver Haze x Early Resign Berry	Diesel Lavender Blueberries Mint	Limonene Linalool Humulene	16
Sour Space Candy	SSC	Sour Tsunami x Early Resin Berry	Sour lemon Pine	Limonene Pinene	16
Special Sauce	SS	Original Special Sauce Strain x Early Resign Berry	Berry Earthy	Caryophyllene Pinene Myrcene	16
Suver Haze	SH	Suver #8 strain x Early Resin Berry	Peppery Citrus Fresh pine	Caryophyllene Farnesene Myrcene	18

Table V: LOD and LOQ for CRM 3 Standard (GC)

Cannabinoid	LOD (ppm)	LOQ (ppm)	LOD (%)	LOQ (%)
CBD_T	0.141	0.526	0.0028	0.0105
CBN	0.147	0.550	0.0029	0.0110
Δ^9THC_T	0.139	0.518	0.0028	0.0104

Table VI: LOD and LOQ for CRM 10 Standard (LC)

Cannabinoid	LOD (ppm)	LOQ (ppm)	LOD (%)	LOQ (%)
CBC	0.126	0.470	0.0025	0.0094
CBD	0.141	0.526	0.0028	0.0105
CBDA	0.145	0.540	0.0029	0.0108
CBDV	0.142	0.530	0.0028	0.0106
CBG	0.149	0.555	0.0030	0.0111
CBGA	0.239	0.891	0.0048	0.0178
CBN	0.147	0.550	0.0029	0.0110
Δ^8THC_T	0.139	0.518	0.0028	0.0104
Δ^9THC	0.143	0.532	0.0029	0.0106
Δ^9THCA	0.098	0.366	0.0020	0.0073
CBD_T	0.268	0.999	0.0053	0.0200
Δ^9THC_T	0.229	0.853	0.0046	0.0170

Table VII: Variation of Δ^9THC$_T$ and CBD$_T$ (Top, Middle and Bottom) for GC and LC

ID	GC THC$_T$	GC CBD$_T$	LC THC$_T$	LC CBD$_T$
Plant 1 Top	0.39 ± 0.02	9.34 ± 0.43	0.49 ± 0.02	8.38 ± 0.29
Plant 2 Top	0.18 ± 0.03	4.50 ± 0.72	0.23 ± 0.03	4.05 ± 0.57
Plant 3 Top	0.18 ± 0.03	4.34 ± 0.37	0.22 ± 0.03	3.76 ± 0.62
Top Ave.	**0.25 ± 0.05**	**6.06 ± 0.92**	**0.31 ± 0.01**	**5.40 ± 0.89**
Plant 1 Middle	0.25 ± 0.03	5.93 ± 0.84	0.27 ± 0.06	5.22 ± 0.70
Plant 2 Middle	0.16 ± 0.04	4.02 ± 0.83	0.21 ± 0.03	3.62 ± 0.72
Plant 3 Middle	0.20 ± 0.05	4.90 ± 1.29	0.26 ± 0.05	4.24 ± 1.06
Middle Ave.	**0.20 ± 0.11**	**4.95 ± 1.75**	**0.25 ± 0.31**	**4.36 ± 1.46**
Plant 1 Bottom	0.23 ± 0.05	5.52 ± 1.13	0.27 ± 0.05	4.88 ± 0.96
Plant 2 Bottom	0.15 ± 0.04	3.81 ± 0.95	0.20 ± 0.04	3.41 ± 0.83
Plant 3 Bottom	0.30 ± 0.04	7.05 ± 0.89	0.37 ± 0.04	6.07 ± 0.72
Bottom Ave.	**0.23 ± 0.08**	**5.46 ± 1.72**	**0.28 ± 0.08**	**4.78 ± 1.46**

Table VIII: Statistical Comparison of Δ^9THC$_T$ and CBD$_T$ for Plant Locations for Table VII (P <0.05 indicates 95% difference confidence)

	GC THC$_T$	GC CBD$_T$	LC THC$_T$	LC CBD$_T$
ID	**P value**			
Top vs Middle	0.51	0.39	0.75	0.35
Top vs Bottom	0.72	0.62	0.53	0.57

Table IX: Statistical Comparison of Δ^9THC$_T$ and CBD$_T$ for GC/LC Results forTable VII

	GC vs LC THC$_T$	GC vs LC CBD$_T$
ID	**P value**	
Top	0.11	0.42
Middle	0.81	0.68
Bottom	0.46	0.63
Whole Plant	0.82	0.76

Table X: Weight Percent of Δ^9THC_T and CBD_T for Hemp Grown Indoors and Outdoors

	GC Δ^9THC_T	GC CBD_T	GC Δ^9THC_T	GC CBD_T	LC Δ^9THC_T	LC CBD_T	LC Δ^9THC_T	LC CBD_T
Variety	**Indoors**		**Outdoors**		**Indoors**		**Outdoors**	
Elektra	0.41	12.11	0.47	12.52	0.66	11.73	0.54	11.98
	0.32	10.72	0.43	11.84	0.53	10.57	0.54	11.89
	0.21	8.30	0.39	11.22	0.42	8.28	0.49	11.06
Average	**0.31 ± 0.10**	**10.38 ± 1.93**	**0.43 ± 0.04**	**11.68 ± 0.85**	**0.54 ± 0.12**	**10.20 ± 1.76**	**0.52 ± 0.03**	**11.06 ± 0.51**
Hawaiian Haze	0.24	9.25	0.45	13.41	0.50	9.44	0.69	13.18
	0.25	9.23	0.48	13.34	0.45	9.37	0.66	13.10
	0.15	7.24	0.23	9.22	0.39	7.84	0.51	9.34
Average	**0.21 ± 0.06**	**8.58 ± 1.56**	**0.38 ± 0.14**	**11.99 ± 2.40**	**0.45 ± 0.06**	**8.89 ± 0.90**	**0.62 ± 0.10**	**11.87 ± 2.19**
Lifter	0.48	13.39	0.64	16.80	0.53	12.45	0.72	15.08
	0.46	13.27	0.60	15.63	0.64	12.59	0.74	14.59
	0.47	12.13	0.46	13.72	0.53	11.30	0.73	12.85
Average	**0.47 ± 0.01**	**12.93 ± 0.70**	**0.57 ± 0.09**	**15.38 ± 1.56**	**0.57 ± 0.06**	**12.12 ± 0.71**	**0.73 ± 0.01**	**14.17 ± 1.17**
Sour Space Candy	0.24	9.13	0.42	12.22	0.54	8.87	0.58	12.07
	0.20	8.07	0.28	10.04	0.44	8.04	0.48	8.64
	0.06	5.10	0.23	9.10	0.29	5.01	0.47	9.38
Average	**0.17 ± 0.10**	**7.43 ± 2.09**	**0.31 ± 0.10**	**10.45 ± 1.60**	**0.43 ± 0.16**	**7.31 ± 2.04**	**0.51 ± 0.06**	**10.03 ± 1.80**
Special Sauce	0.52	14.68	0.56	15.83	0.74	14.58	0.86	15.48
	0.49	13.73	0.36	11.64	0.75	13.70	0.62	11.48
	0.44	13.41	0.29	9.99	0.71	13.01	0.54	9.75
Average	**0.48 ± 0.04**	**13.94 ± 0.66**	**0.40 ± 0.14**	**12.49 ± 3.01**	**0.73 ± 0.03**	**14.58 ± 0.79**	**0.68 ± 0.17**	**12.23 ± 2.94**
Suver Haze	0.71	18.29	0.45	13.39	0.94	16.99	0.60	12.45
	0.74	17.36	0.27	9.73	0.82	15.57	0.44	9.11
	0.62	15.68	0.22	8.55	0.75	15.04	0.46	8.14
Average	**0.69 ± 0.07**	**17.11 ± 1.32**	**0.31 ± 0.12**	**10.55 ± 2.53**	**0.84 ± 0.10**	**15.87 ± 1.01**	**0.50 ± 0.09**	**9.90 ± 2.26**

Table XI: Varieties Ranked Highest to Lowest for Table X

Indoors				Outdoors			
GC THC	**GC CBD**	**LC THC**	**LC CBD**	**GC THC**	**GC CBD**	**LC THC**	**LC CBD**
SH	SH	SH	SH	L	L	L	L
SS	SS	SS	SS	E	SS	SS	SS
L	L	L	L	SS	HH	HH	HH
E	E	E	E	HH	E	E	E
HH	HH	HH	HH	SSC	SH	SSC	SSC
SSC	SSC	SSC	SSC	SH	SSC	SH	SH

Table XII: Statistical Evaluation for Δ^9THC_T and CBD_T Hemp Grown Indoors and Outdoors for Table X (P <0.05 indicates 95% difference confidence)

	GC Indoor vs. Outdoor Δ^9THC_T	**GC Indoor vs. Outdoor CBD_T**	**LC Indoor vs. Outdoor Δ^9THC_T**	**LC Indoor vs. Outdoor CBD_T**
ID	**P value**		**P value**	
Elektra	0.13	0.35	0.08	0.46
Hawaiian Haze	0.13	0.11	0.07	0.09
Lifter	0.13	0.07	0.01	0.06
Sour Space Candy	0.16	0.12	0.46	0.16
Special Sauce	0.40	0.46	0.64	0.25
Suver Haze	0.01	0.02	0.01	0.01

Table XIII: Differences in Δ^9THC_T and CBD_T for Hemp Grown Indoors and Outdoors

ID	**GC Indoor vs Outdoor Δ^9THC_T**	**GC Indoor vs Outdoor CBD_T**	**LC Indoor vs Outdoor Δ^9THC_T**	**LC Indoor vs Outdoor CBD_T**
Elektra	No change	No change	No change	No change
Hawaiian Haze	No change	No change	No change	No change
Lifter	No change	No change	Increase	No change
Sour Space Candy	No change	No change	No change	No change
Special Sauce	No change	No change	No change	No change
Suver Haze	Decrease	Decrease	Decrease	Decrease

Table XIV: Weight Percent of Δ^9THC_T and CBD_T for Pre/Post Dried Hemp

	GC Δ^9THC_T	GC CBD_T	GC Δ^9THC_T	GC CBD_T	LC Δ^9THC_T	LC CBD_T	LC Δ^9THC_T	LC CBD_T
Variety	**Harvest**		**Air Dried**		**Harvest**		**Air Dried**	
Elektra	0.41	12.11	0.50	11.94	0.66	11.73	0.75	10.07
	0.32	10.72	0.42	10.83	0.53	10.57	0.79	9.84
	0.21	8.30	0.37	9.74	0.42	8.28	0.67	8.38
Average	**0.31 ± 0.10**	**10.38 ± 1.93**	**0.43 ± 0.07**	**10.84 ± 1.10**	**0.54 ± 0.12**	**10.20 ± 1.76**	**0.74 ± 0.06**	**9.43 ± 0.92**
Hawaiian Haze	0.24	9.25	0.43	10.67	0.50	9.44	0.75	10.03
	0.25	9.23	0.41	10.03	0.45	9.37	10.03	0.41
	0.15	7.24	0.37	9.36	0.39	7.84	9.36	0.37
Average	**0.21 ± 0.06**	**8.58 ± 1.56**	**0.40 ± 0.03**	**10.02 ± 0.65**	**0.45 ± 0.06**	**8.89 ± 0.90**	**0.72 ± 0.06**	**9.41 ± 0.61**
Lifter	0.48	13.39	0.49	11.56	0.53	12.45	0.87	11.06
	0.46	13.27	0.46	11.13	0.64	12.59	0.83	10.85
	0.47	12.13	0.42	10.76	0.53	11.30	0.64	10.28
Average	**0.47 ± 0.01**	**12.93 ± 0.70**	**0.46 ± 0.04**	**11.15 ± 0.40**	**0.57 ± 0.06**	**12.12 ± 0.71**	**0.78 ± 0.12**	**10.73 ± 0.40**
Sour Space Candy	0.24	9.13	0.47	11.98	0.54	8.87	0.78	11.10
	0.20	8.07	0.42	10.93	0.44	8.04	0.72	10.59
	0.06	5.10	0.38	9.36	0.29	5.01	0.72	8.91
Average	**0.17 ± 0.10**	**7.43 ± 2.09**	**0.42 ± 0.05**	**10.76 ± 1.32**	**0.43 ± 0.16**	**7.31 ± 2.04**	**0.74 ± 0.04**	**10.20 ± 1.15**
Special Sauce	0.52	14.68	0.52	12.77	0.74	14.58	0.91	12.50
	0.49	13.73	0.48	11.61	0.75	13.70	0.87	11.11
	0.44	13.41	0.38	9.87	0.71	13.01	9.87	0.38
Average	**0.48 ± 0.04**	**13.94 ± 0.66**	**0.46 ± 0.07**	**11.42 ± 1.46**	**0.73 ± 0.03**	**14.58 ± 0.79**	**0.80 ± 0.16**	**11.06 ± 1.47**
Suver Haze	0.71	18.29	0.37	9.60	0.94	16.99	0.75	8.97
	0.74	17.36	0.36	8.97	0.82	15.57	0.66	8.56
	0.62	15.68	0.35	8.97	0.75	15.04	0.69	8.77
Average	**0.69 ± 0.07**	**17.11 ± 1.32**	**0.36 ± 0.01**	**9.18 ± 0.36**	**0.84 ± 0.10**	**15.87 ± 1.01**	**0.70 ± 0.05**	**8.77 ± 0.21**

Table XV: Statistical Evaluation for Δ^9THC_T and CBD_T Hemp at Harvest and After Three Months Air Dried for Table XIV (P <0.05 indicates 95% difference confidence)

	GC Harvest vs. Air Dried THC_T	GC Harvest vs. Air Dried CBD_T	LC Harvest vs. Air Dried THC_T	LC Harvest vs Air Dried CBD_T
ID	**P value**		**P value**	
Elektra	0.16	0.74	0.06	0.54
Hawaiian Haze	0.01	0.21	0.01	0.45
Lifter	0.66	0.02	0.05	0.04
Sour Space Candy	0.02	0.08	0.03	0.10
Special Sauce	0.70	0.05	0.49	0.02
Suver Haze	0.01	0.01	0.09	0.01

Table XVI: Changes in THC_T and CBD_T at Harvest and After Three Months Air Dried

ID	GC Pre vs. Post Drying THC_T	GC Pre vs. Post Drying CBD_T	LC Pre vs. Post Drying THC_T	LC Pre vs. Post Drying CBD_T
Elektra	No change	No Change	No change	No change
Hawaiian Haze	Increase	No change	Increase	No change
Lifter	No Change	Decrease	No change	Decrease
Sour Space Candy	Increase	No change	Increase	Increase
Special Sauce	No Change	No change	No change	Decrease
Suver Haze	Decrease	Decrease	No change	Decrease

Table XVII: Weight Percent of Ten Cannabinoids for Hemp Grown Indoors and Outdoors

		Variety					
Growing Condition	**Cannabinoid**	**Elektra**	**Hawaiian Haze**	**Lifter**	**Sour Space Candy**	**Special Sauce**	**Suver Haze**
Indoor	CBC	0.26 ±0.03	0.15 ±0.01	0.47 ±0.07	0.11 ±0.04	0.24 ±0.06	0.45 ±0.06
Outdoor		0.41 ±0.07	0.19 ±0.01	0.30 ±0.04	0.20 ±0.12	0.16 ±0.03	0.21 ±0.04
Indoor	CBD	4.73 ±0.28	2.58 ±0.33	8.49 ±1.25	1.10 ±0.42	3.93 ±0.94	8.79 ±1.84
Outdoor		7.95 ±1.29	3.05 ±0.31	5.15 ±0.83	3.05 ±2.65	2.58 ±0.66	3.97 ±0.82
Indoor	CBDA	6.23 ±1.68	7.19 ±0.68	4.13 ±0.66	7.07 ±2.09	11.21 ±0.58	8.07 ±2.74
Outdoor		4.21 ±1.07	10.05 ±2.59	10.28 ±0.45	7.95 ±1.18	11.00 ±2.83	6.77 ±1.64
Indoor	CBDV	ND	ND	ND	ND	ND	ND
Outdoor							
Indoor	CBG	0.06 ±0.05	0.01 ±0.00	0.15 ±0.02	0.01 ±0.00	0.01 ±0.00	0.17 ±0.02
Outdoor		0.18 ±0.09	0.01 ±0.00	0.07 ±0.07	0.01 ±0.02	0.01 ±0.00	0.07 ±0.02
Indoor	CBGA	0.13 ±0.06	0.22 ±0.10	0.12 ±0.04	0.10 ±0.01	0.35 ±0.10	0.43 ±0.24
Outdoor		0.11 ±0.06	0.35 ±0.15	0.20 ±0.03	0.18 ±0.01	0.43 ±0.24	0.10 ±0.02
Indoor	CBN	ND	ND	ND	ND	ND	ND
Outdoor							
Indoor	Δ^8THC	ND	ND	ND	ND	ND	ND
Outdoor							
Indoor	Δ^9THC	0.42 ±0.10	0.28 ±0.04	0.51 ±0.05	0.21 ±0.06	0.42 ±0.06	0.66 ±0.09
Outdoor		0.47 ±0.04	0.35 ±0.01	0.52 ±0.01	0.28 ±0.12	0.35 ±0.09	0.38 ±0.06
Indoor	Δ^9THCA	0.13 ±0.02	0.19 ±0.02	0.07 ±0.02	0.25 ±0.10	0.36 ±0.04	0.20 ±0.08
Outdoor		0.06 ±0.02	0.31 ±0.10	0.25 ±0.02	0.26 ±0.07	0.38 ±0.11	0.14 ±0.04

Table XVIII: Statistical Evaluation of Cannabinoids in Hemp Grown Indoors and Outdoors for Table XVII (P <0.05 indicates 95% difference confidence)

ID	CBC	CBD	CBDA	CBG	CBGA	Δ^9 THC	Δ^9THCA
Elektra	0.03	0.01	0.15	0.11	0.70	0.47	0.01
Hawaiian Haze	0.01	0.15	0.14	1.00	0.28	0.04	0.11
Lifter	0.02	0.02	0.01	0.13	0.05	0.75	0.01
Sour Space Candy	0.28	0.28	0.56	1.00	0.01	0.42	0.89
Special Sauce	0.11	0.11	0.91	1.00	0.62	0.33	0.78
Suver Haze	0.01	0.01	0.52	0.01	0.08	0.01	0.31

Table XIX: Changes in Cannabinoids for Indoor versus Outdoor Hemp

ID	CBC	CBD	CBDA	CBG	CBGA	Δ^9 THC	Δ^9 THCA
Elektra	Increase	Increase	No change	No change	No change	No change	Decrease
Hawaiian Haze	Increase	No change	No change	No Change	No change	Increase	No change
Lifter	Decrease	Decrease	Increase	No change	No change	No change	Increase
Sour Space Candy	No change	No change	No change	No Change	Increase	No change	No change
Special Sauce	No change	No change	No change	No Change	No change	No change	No change
Suver Haze	Decrease	Decrease	No change	Decrease	No change	Decrease	No change

Table XX: Pre and Post Dried Indoor Hemp

		Variety					
Drying Process	**Cannabinoid**	**Elektra**	**Hawaiian Haze**	**Lifter**	**Sour Space Candy**	**Special Sauce**	**Suver Haze**
Harvest	CBC	0.26 ±0.03	0.15 ±0.01	0.47 ±0.07	0.11 ±0.04	0.24 ±0.06	0.45 ±0.06
Air Dried		0.39 ±0.03	0.40 ±0.02	0.48 ±0.06	0.51 ±0.06	0.46 ±0.06	0.35 ±0.03
Harvest	CBD	4.73 ±0.28	2.58 ±0.33	8.49 ±1.25	1.10 ±0.42	3.93 ±0.94	8.79 ±1.84
Air Dried		4.62 ±0.57	4.98 ±0.27	5.81 ±0.87	6.98 ±0.67	5.18 ±0.97	3.80 ±0.39
Harvest	CBDA	6.23 ±1.68	7.19 ±0.68	4.13 ±0.66	7.07 ±2.09	11.21 ±0.58	8.07 ±2.74
Air Dried		5.49 ±0.40	5.05 ±0.64	5.61 ±1.41	3.67 ±0.64	6.70 ±0.57	5.66 ±0.68
Harvest	CBDV	ND	ND	ND	ND	ND	ND
Air Dried							
Harvest	CBG	0.06 ±0.05	0.00 ±0.00	0.15 ±0.02	0.00 ±0.00	0.00 ±0.00	0.17 ±0.02
Air Dried		0.14 ±0.12	0.13 ±0.11	0.14 ±0.12	0.06 ±0.11	0.21 ±0.01	0.07 ±0.12
Harvest	CBGA	0.13 ±0.06	0.22 ±0.10	0.12 ±0.04	0.10 ±0.01	0.35 ±0.10	0.43 ±0.24
Air Dried		0.19 ±0.02	0.17 ±0.02	0.18 ±0.02	0.00 ±0.00	0.19 ±0.03	0.05 ±0.09
Harvest	CBN	ND	ND	ND	ND	ND	ND
Air Dried							
Harvest	Δ^8 *THC*	ND	ND	ND	ND	ND	ND
Air Dried							
Harvest	Δ^9 *THC*	0.42 ±0.10	0.28 ±0.04	0.51 ±0.05	0.21 ±0.06	0.42 ±0.06	0.66 ±0.09
Air Dried		0.51 ±0.05	0.51 ±0.05	0.62 ±0.04	0.55 ±0.03	0.56 ±0.15	0.47 ±0.03
Harvest	Δ^9 *THCA*	0.13 ±0.02	0.19 ±0.02	0.07 ±0.02	0.25 ±0.10	0.36 ±0.04	0.20 ±0.08
Air Dried		0.26 ±0.02	0.24 ±0.02	0.19 ±0.16	0.21 ±0.00	0.28 ±0.00	0.26 ±0.03

Table XXI: Statistical Evaluation of Cannabinoids in Hemp at Harvest and After Three Months Air Dried for Table X (P <0.05 indicates 95% difference confidence)

ID	Harvest vs Air Dried CBC	Harvest vs Air Dried CBD	Harvest vs Air Dried CBDA	Harvest vs Air Dried CBG	Harvest vs Air Dried CBGA	Harvest vs Air Dried Δ^9 THC	Harvest vs Air Dried Δ^9 THCA
Elektra	0.01	0.54	0.50	0.35	0.18	0.06	0.01
Hawaiian Haze	0.01	0.45	0.02	1.00	1.00	0.01	0.04
Lifter	0.86	0.04	0.18	0.89	0.08	0.05	0.27
Sour Space Candy	0.01	0.10	0.05	1.00	1.00	0.03	0.53
Special Sauce	0.01	0.02	0.01	1.00	0.06	0.49	0.03
Suver Haze	0.06	0.01	0.21	0.23	0.06	0.09	0.29

Table XXII: Changes in Cannabinoids for Pre and Post Dried Hemp Indoor Hemp

ID	CBC	CBD	CBDA	CBG	CBGA	Δ^9THC	Δ^9THCA
Elektra	Increase	No change	No change	No change	No change	No change	Increase
Hawaiian Haze	Increase	No change	Decrease	No change	No change	Increase	Increase
Lifter	No Change	Decrease	No change	No Change	No change	No change	No change
Sour Space Candy	Increase	No change	No change	No change	No change	Increase	Decrease
Special Sauce	Increase	Increase	Decrease	No change	No change	No change	Decrease
Suver Haze	No change	Decrease	No change	No change	No change	No change	No change