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Quantitative Analysis of Tetrahydrocannabinol For Forensic Applications In Seized Drugs

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QUANTITATIVE ANALYSIS OF TETRAHYDROCANNABINOL FOR FORENSIC APPLICATIONS IN SEIZED DRUGS

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DEDICATION

This thesis is dedicated to my family Justice, Jaiden, Wyatt, and Luke Gannaway. Thank you for being supportive and patient so I could pursue my dreams. I love you and try to be my best for you.

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QUANTITATIVE ANALYSIS OF TETRAHYDROCANNABINOL FOR FORENSIC APPLICATIONS IN SEIZED DRUGS

An Abstract of the Thesis by
Jana Jo Gannaway

Cannabis and its' associated psychoactive cannabinoid THC have become more popular within the public community. And with popularity comes the political, social, medical, and fiscal concerns associated with cannabis demands not only governmental scrutiny, but also rigorous research and scientific examination. The forensic community needs to be prepared and competent in every aspect. In order to demonstrate an exhaustive research and scientific analysis, a quantitative method was developed using an Agilent GCMS quadrupole as this is the main workhorse in many forensic drug laboratories. The internal standard tribenzylamine was initially chosen along with the drug standard THC. The calibration curve was linear with correlation a coefficient of 0.98 – 0.99, however the internal standard and drug standard began interacting with each other and degrading after approximately 2 weeks. A new internal standard, tetradecane, was chosen for its non-polar properties. Results with tetradecane proved to be very unreproducible. Quality control samples regularly did not pass their ± 20% accuracy requirement. Relative standard deviation of the internal standard ranged from 6.10-25.77%. The limit of detection for both Agilent GCMS instruments was 0.1% THC on a total dry weight basis while the limit of quantitation was 0.4%. Relative standard deviation of the seized THC samples ranged 0-3.13%. Next, a quantitative method was developed using a Waters LC-UV-MS single quadrupole. A 5 point calibration curve was used each day. Calibration curves were run on 3 different days. Standard calibration curves were linear with a correlation coefficient of 0.99 or above each day. The limit of detection was 0.0002% THC on a total dry weight basis while the limit of quantitation was 0.00085%. The GCMS detectors were not sensitive enough to quantitate the wide dynamic range of THC needed. Clearly the LCMS with a UV detector is more than sensitive

enough to be able to quantify the range of THC concentrations that are routinely seen in the forensic laboratory.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

CHAPTER I

INTRODUCTION

With the rise in public interest, it is vital for forensic laboratories to develop a method to quantify THC in raw cannabis vegetation. Depending on the application, cannabis can have a wide range of THC content. This requires a tremendous amount of work as quantifying THC is time and labor-intensive. It is important to be able to detect and distinguish a large THC concentration range. THC is the major psychoactive cannabinoid produced in varying parts of the plant. Most forensic laboratories struggle with a way to decrease or eliminate their backlog. An efficient and validated method for quantifying THC over a wide dynamic range is needed while keeping the maintenance on the instrument to a minimum. This will allow for the method to be thoroughly tested and meet SWGDRUG guidelines and standup within the criminal court systems. It is imperative the method be meticulously examined before any casework be conducted.

CHAPTER II

CANNABIS OVERVIEW

HISTORY

Cannabis and its products are the most widely consumed illicit drug in the United States as well as worldwide (UNODC 2009; Ruppel . 2009; Mehmedic 2010). The psychoactive compound within the *Cannabis* species is called Δ9-tetrahydrocannabinol, commonly referred to as THC. It has been used for its euphoric effects on the central nervous system (CNS). There are various phenotypes of *Cannabis*. Phenotype I (drug type) has THC weight percent's ranging from 0.5-15%, phenotype II (intermediate type) has THC weight percent's ranging from 0.5-5%, and phenotype III (fiber type) has THC weight percent's ranging from 0.05-0.70% (Upton 2014, Galal 2009). From these various phenotypes have arisen much scientific and legal debate.

Within the forensic community there are two distinctive ideologies of the classification of the *Cannabis* genus, monotypic vs. polytypic (Hillig 2004, Hillig 2005). In 1753 Carrolous Linneaus purported the *Cannabis* species was composed of only one species (monotypic) *Cannabis sativa* L (Linneaus). Jean-Baptiste Lamarck published his findings in 1785 stating there were different species (polytypic) *cannabis sativa* and *cannabis indica*. Where sativa was composed of mainly the fiber type and indica was predominantly the drug type. In 1974 Schultes took the classification one step further and recognized three phenotypes and gave them each their own name. Cannabis indica classified as having high THC content and thus being the drug type, cannabis ruderalis as being the fiber type, and cannabis sativa being the intermediate (Shultes 1974). Even today, there are inconsistencies in how cannabis is classified. In the United States,

there is only one species cannabis sativa l. These distinctions become even more convoluted as the strains have been selectively interbred to produce various products with very different purposes.

TAXONOMY

Cannabis belongs to the *Cannabaceae* family along with another widely known plant *Humulus* (Hops). Cannabis is an annual plant where male and female flowers are found on separate plants (dioecious). Although in rare circumstances, monoecious examples of cannabis have been observed. Staminate (male) plants are in general taller and less robust than their pistillate (female) counterparts (UNODC 2009). The method of cultivation, the environment, and genetic factors all effect the height and branching of the plant. Most cannabis plants are erect and can grow anywhere from 1-3 meters high with their stem diameter ranging from 0.2-0.6 m (Figure 1) (UNODC 2009). The taproots are laterally branched 0.3-2.5 m deep and can have horizontal growth as much as 0.8m wide (Upton 2014). Cannabis leaves are palmately compound with odd numbered leaflets ranging from 3-11 leaflets. Leaflets have serrated edges with a lanceolate shape. The middle or apex leaflet is typically the longest (Potter 2004, 2009, 2014). The cannabis leaf has many trichomes or hair-like structures. On the upper portion of the leaf, there are cystolithic “bear claw” shaped non-glandular trichomes (Figure 2). A whitish appearance can be observed at the base of the cystolith. These are calcium carbonate crystals (Figure 3). On the lower surface of the cannabis leaf, there are profuse and slender covering trichomes (Figure 4). Many glandular trichomes can also be observed on the lower surface where the resin or cannabinoids are secreted (Figure 5). Seeds (achene) from the hops plant and cannabis plant can be confused by a novice. However, the reticulate or tortoise shell pattern (Figure 6) is present on the cannabis seed and absent from the hops (UNODC 2009).

CULTIVATION

Cannabis can be selectively bred to produce desirable traits. The drug type often has high levels of THC and low levels of CBD. This is attained by removing the staminate plants and only growing female cannabis. This growing and cultivation technique is called sinsemilla or "no seed". The female plants will produce high levels of sticky resin, increasing the chances of encountering pollen and producing seeds. Without the male plants, the pistillate high THC cannabis flowers or "buds" can then be harvested. The main type of cultivation is still outdoors. Sensemilla is achieved by removing or cutting down the male plants as soon as they can be identified. Indoor cultivation can induce sensemilla by propagating cuttings of the female plants (Cervantes 2006, Mills 2011). The cuttings then grow and are identical to the female mother plant. This technique is sometimes referred to as cloning (Bosca 1997). All of the desired female plants are grown and not the male plants. This is the preferred indoor propagating method as it ensures efficiency.

Harvest time can vary depending on the type and desired cannabinoid. The primary cannabinoid the cannabis plant makes is called delta 9-tetrahydrocanabinolic acid or THCA. The THCA is a very unstable compound and is naturally decarboxylated to THC when introduced to air, light, and heat (Harvey 1976, McPartland 1996, 2000, 2001, 2008, Upton 2014, Jones 2010). THC is at its peak potency when three-fourths of the stigmas have turned brown (Figure 7). The resin will also be clear at this time (Figure 8). Once the resin on the bud turns to an orange or brown, it is over ripe and the THC content has degraded into CBN (Figure 9A, 9B). Peak clandestine harvest in Kansas is during the month of September. Typically cannabis plants are sold as dried and mature female buds that have been manicured with their seeds and stems removed (Figure 10). Cannabis can be found growing wild throughout the state of Kansas. This is referred to as ditchweed. It typically has low levels of THC and high levels of CBD (Figure 11).

FEDERAL STATUS

Under the federal regulations in the United States, the Controlled Substances Act (CSA) regulates compounds that have a psychoactive effect on the CNS and have the potential for abuse. The Controlled Substances Act regulates the authority to control schedules, manufacturers, distributors, dispensers, offenses, penalties, enforcement provisions, import, and export of each controlled substance. Each compound listed under the CSA are provided a classification that is called a schedule. In total there are five schedules with Schedule I substances having the highest probability of becoming addictive and Schedule V being the least likely to be used for abusive purposes.

Schedule I is any compound that has a high probability for abuse, has no current medical use within the United States (U.S.), and there is a lack of safety reported even under medical supervision. Schedule II is any compound that has a high probability of abuse, has current medical applications with severe restrictions within the U.S., and may cause severe dependency. Schedule III has some possibility of abuse although to a lesser degree than Schedule I and II, has current medical applications within the U.S., and may cause moderate to low dependency. Schedule IV has a lower probability of abuse when compared to Schedules I, II, and III, has current medical applications within the U.S., and cause very limited dependence possibilities (DEA 2011a, Mead 2017).

Cannabis is still considered a Schedule I controlled substance under the federal CSA. Examples of drugs that fall into the same category are: methamphetamine, phencyclidine (PCP), and heroin. Since cannabis is a Schedule I drug The Drug Enforcement Agency (DEA) has strict control over the possession and transportation (DEA 2011b). The DEA stipulates cannabis must travel from one DEA licensed facility to another DEA licensed facility. As of 2017, the only federally recognized facility that is able to cultivate cannabis for medical and research purposes is the University of Mississippi. With growing public and private interest, the DEA began allowing

applications to manufacture marijuana for research purposes in August of 2016. The program registrants would operate under the CSA. To date, the DEA has not accepted nor rejected any candidate. Likewise, the Agricultural Act of 2014 Section 7606 "Farm Bill" has made an allowance for the term "industrial hemp". Industrial hemp is defined by the National Institute of Food and Agriculture (NIFA) section of the United States Department of Agriculture (USDA) as "the plant *cannabis sativa* L. and any part or derivative of such plant, including seeds of such plant, whether growing or not, that is used exclusively for industrial purposes (fiber and seed) with a tetrahydrocannabinols concentration of not more than 0.3 percent on a dry weight basis". The Farm Bill gives federal direction as how to legally participate in hemp research, in states where such activity is legal (USDA 2016). Additionally, each (NIFA) program applicant must be an institution of higher education or state department of agriculture (or designee) and grow industrial hemp under the umbrella of a state agriculture pilot program. Each state where industrial hemp is legal interprets the Farm Bill differently and who the state's Department of Agriculture may authorize to participate in the program.

Health care providers in all 50 states can prescribe a drug called Dronabinol (Bolognini 2010). It is a synthetic and FDA approved form of THC. It is specifically produced in sesame oil and encapsulated in a soft gelatin capsule. Dronabinol, also known as Marinol is a Schedule III controlled substance that is used to treat nausea and vomiting caused by cancer treatments or weight loss in AIDS patients (Neff 2002, Nelson 1994).

State laws on CBD vary widely as currently seventeen states allow products high in CBD and low in THC (Mead 2017). Although many CBD products are available on the internet and dispensaries however they are neither approved nor regulated by the FDA. Quality control measures on CBD are at best inconsistent and sometimes nonexistent. New and recent clinical trials have begun to focus on the potential for use of CBD to treat childhood seizure disorders (Patel 2017).

As of September 2017, twenty-nine states and the District of Columbia currently have vague laws legalizing marijuana in some form (Figure 12). Seven states in addition to the District of Columbia have adopted laws legalizing marijuana for recreational use (NCSL 2017).

KANSAS STATUS

Kanas state law defines marijuana as, "meaning all parts of all varieties of the plant Cannabis whether growing or not, the seeds thereof, the resin extracted from any part of the plant and every compound, manufacture, salt, derivative, mixture or preparation of the plant, its seeds or resin. "Marijuana" does not include the mature stalks of the plant, fiber produced from the stalks, oil or cake made from the seeds of the plant, any other compound, manufacture, salt, derivative, mixture or preparation of the mature stalks, except the resin extracted therefrom, fiber, oil or cake or the sterilized seed of the plant which is incapable of germination." Similar to federal regulations, cannabis and THC are considered a Schedule I controlled substance under the CSA. Unlike federal regulations, there are no current provisions to grow cannabis for research, medical considerations, or for industrial hemp. All types of cannabis within the state are treated the same according to Kansas law (Kansas Legislature 2014).

PHARMACOLOGY

Merriam Webster defines Pharmacology as the science of drugs including their origin, composition, pharmacokinetics, therapeutic use, and toxicology. As stated, cannabis is considered a Schedule I drug: however there is a lot of research that still needs to be conducted as to its pharmacology (Dewey1986). The cannabis plant has over 400 compounds and out of those there are over 60 cannabinoids (Figure 13) (Mechoulam 1990, 2002). To date THC and CBD are the two most widely studied cannabinoids. THC is readily absorbed through the lungs via inhalation more rapidly than through oral ingestion at a ratio of approximately 1:12 (Holler 2008). Once

ingested the body converts THC into 11-hydroxy THC which is then further degraded into its non-psychoactive form 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (Sharma 2012). THC is lipophilic and gets deposited in the adipose, lung, liver, and spleen tissues. For the infrequent user the half-life for THC is 1.3 days and for frequent users is anywhere from 5-13 days with the longest being in the adipose tissues (Sharma 2012).

There are also psychological and physiological symptoms reported with cannabis use. Physiological effects have been reported as dry mouth, increased appetite, vasodilation, decreased respiratory rate, rapid changes in heart rate, and redness of the conjunctiva. Abuse of cannabis is also known to damage the lungs and affect neonatal child development (Huestis 2002). Psychological effects have been described as a feeling of euphoria and relaxation, altered time perception, lack of concentration, impaired learning and memory, mood changes, feeling of panic and paranoia, and impaired motor coordination (Izo 2002, EMCDDA 2006, EMCDDA 2008, EMCDDA 2012).

THC acts as a dopamine agonist by stimulating the electrical brain reward system (Huestis 2002). As common with most drugs of abuse research has shown that THC induces stimulation of the brain rewards systems that are common with opioids, cocaine, and alcohol. A dependence of cannabis are characterized by preoccupation with its use, relapse or recurrent use, and impaired control of their use (Sharma 2012). Cessation syptoms from frequent users are described as irritability, anxiety, disrupted sleep, and cravings.

CHAPTER III

INSTRUMENTATION

GAS CHROMATOGRAPHY MASS SPECTROMETRY

Gas chromatography mass spectrometry or (GCMS) is used in virtually every forensic laboratory. It is also considered the "workhorse" of detecting the presence of controlled substances. GCMS is also considered the "gold standard" for drug detection. It is desirable to be able to utilize the GCMS technology because of its robustness and availability with in the forensic laboratory.

The retention time of the obtained peak and the mass spectrum can provide an absolute recognition of the tested compound. The GCMS combines two instruments, the gas chromatograph and the mass spectrometer. The gas chromatograph uses a capillary column and a variety of stationary phases. A carrier gas is used as the mobile phase. When a compound or analyte is injected onto the column, it will interact with the column based on certain properties such as polarity, shape, molecular weight, and viscosity. How long the analyte stays in the column is referred to as its retention time. In theory each analyte will have a specific and unique retention time. Thus it has the ability to separate a mixture of compounds. Once the analyte moves through the column it then enters the mass spectrometer. The mass spectrometer fragments each anaylte into particular fragments into a unique pattern which can be used for the identification of a compound. The fragments or puzzle pieces break apart in a very particular and predictable way. The fragments are then sent to a detector and data is collected. Based on the SWGRUG recommendations, the GCMS meeting the testing requirements for the identification

of marijuana as the mass spectrometer is considered a Category A test and the gas chromatograph is a Category B test. Both tests together produce a high level of selectivity and specificity.

GC technology is always evolving. Selected ion monitoring (SIM) increases sensitivity for target analytes through selective detection of ions most indicative of the compounds of interest. GCMS selected ion monitoring methods are useful and widely applied although they are somewhat complicated to develop depending on the number of ions used. SIM is most often used for target compound analysis. Recent changes to the GCMS methods allow for SIM and Scan modes to be carried out simultaneously. SIM methods can lock in the retention times of interest. Scan mode can verify each analyte by matching the corresponding retention times from the chromatogram and their spectrum. Running SCAN mode in addition to SIM mode also ensures there are no analytes within a mixture that elute at different times than the compounds of interest. Hence the SCAN mode mass spectral information provides additional confirmation to detecting a compound. This can allow for presumptive and confirmatory runs to also be conducted simultaneously.

LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

Liquid chromatography mass spectrometry or (LCMS) is similar to GCMS. It is a technique used to identify substances. LCMS uses a liquid solvent for the mobile phase similar to how the GCMS uses a gas. A LCMS also has high sensitivity and selectivity. One difference between LCMS and GCMS is that in LCMS a substance does not have to become volatile in order for it to be put onto the column. Heat labile analytes can be ran through the column without being degraded before they go into the mass spectrometer. Sample types can range from low molecular weight compounds to complex protein matrices. This makes the LCMS suitable for ionic, polar, and thermally unstable non-volatile compounds. This also eliminates the need for derivitization that is sometimes needed in GCMS. Another advantage to LCMS is shorter run times thus they are ideal for high throughput. An average drug screen run on the GCMS is around

20 minutes while the LCMS is 5-7 minutes. A couple of drawbacks to using the LCMS is they are generally less robust than the GCMS, require more maintenance, and are more costly.

Using both a UV detector and a mass selective detector increases sensitivity and selectivity. A diode array detector acquires data for a range of wavelengths producing spectra. There are analytes or compounds that absorb at a similar wavelength making it difficult for the UV detector to distinguish between. It is also possible to have analytes or impurities with the same mass. However, it is rare for similar analytes to have the same UV-Vis spectra as well as the same mass spectra. The data for a range of wavelengths can be collected simultaneously. Specific wavelengths can be selected post run and chosen to analyze mass spectrum to determine molecular weights as well as structural information.

CHAPTER IV

METHODS AND MATERIALS

GCMS- Gas chromatographic analyses was performed on two different instruments and were comprised of the following components: Agilent GC 7820A, Agilent 5977B MSD single quadrupole detector, GC column Phenomenex, Zebron Capillary Column ZB-5MSi 12m x 200µm ID, 0.33 µm film thickness, Agilent 7890B GC, 5977A MSD single quadrupole detector, GC column Agilent, Capillary Column J&W HP-5 30m x 250µm ID, 0.1 µm film thickness. The injector port temperature was set at 280°C with a 5.2 mm split/splitless deactivated glass wool inlet liner. A 10:1 injection ratio was used and helium was the carrier gas. The carrier gas flow was maintained at 1.5005 mL/min. The initial oven temperature was set at 65°C and held for 0.2 min, then ramped 25 °C/min to 335 °C. The transfer line was set at 285 °C. The mass spectrometer operated in selected ion monitoring (SIM)/Scan acquisition mode. For the quantitation two ions were selected. The ion 299 was selected as it is the base peak for THC. The second ion selected was 57 as it was the base peak for tetradecane. When processing data using tribenzylamine, the ion 91 was used as it is the base peak. Mass Hunter Quant Analysis software was used process data.

HPLC- Liquid chromatographic analyses performed were comprised of the following components: Waters Acquity H-class UPLC Quaternary Pump, Waters Acquity H-class Sample Manager – FTN (flow-through needle), Waters Acquity PDA (photodiode array detector), Waters Acquity QDa (electrospray ionization single quadrupole mass spectrometer), Waters Acquity UPLC CSH C18 1.7 µm particle, 2.1 mm x 100 mm column. The run time was 6.50 minutes with a 90:10 H2O:ACN wash solvent and purge solvent. The target column temperature was 50 °C,

resolution was 3.6nm with a range of 210-350nm. Mobile phase solvents were (A) water, (B) acetonitrile, (C) 125mM Formic Acid, and (D) 125mM Ammonium Hydroxide with gradient concentrations of 0.6mL/min. The separation was performed with the following gradients: 0-3.75 min, (20%-0% A, 70%-90% B, 10% D), 3.75-5.00 min (90%-70% B, 10% D), 5.01- 6.49 min (20%-0% A, 70%-90% B, 10% D). The injection volume was 1µL and detection was at 214 nm. MassLynx and OpenLynx software was used to process the data. Electronspray interface was operated in positive mode.

REAGENTS AND STANDARDS

ACS grade ammonium hydroxide was purchased from VWR International, Radnor, PA. OPTIMA-grade acetoniltrile and OPTIMA grade formic acid, tetradecane 98% pure, Pharmco-Aaper ethyl alcohol 200 proof was purchased from Fisher Scientific, Pittsburg,PA. Deonized water was purified by an in-house 18mΩ gradient filtration system. Standards were purchased from Cayman Chemical, Ann Arbor, MI. All standards were certified reference materials and were in solution at 1.0 mg/mL. Fresh ampules were used to ensure consistent and accurate data.

GCMS DECARBOXYLATION

All vegetation was analyzed via the microscope. Vegetation samples had to have the characteristic cystolithic trichomes or hairs and the covering hairs to be considered cannabis (Figure 4). Then vegetation samples were examined for approximate cannabinoid content. If large amounts of resin was observed (Figure 9A, 9B), it was notated and dilutions were anticipated. Approximately five grams of vegetation were ground for ten seconds, then ten one second pulses. The ground powder was dried at 35-40^{o}C for one hour. From the dried and ground samples, one hundred milligrams were weighed out on an analytical balance. Ten milliliters of ethanol spiked with tributylamine (0.153 mg/mL) were added to the dried vegetation and vortexed for 5 seconds. The solution was filtered with a 0.45 µl pore polytetrafluoroethylene (PFTE) filter and a 20

milliliter syringe. This was followed by pipetting 500 µl of the extract into a GC vial. It was then diluted with 1 mL of ethanol spiked with tributylamine to a total volume of 1.5mL.

A THCA curve was run along with ten evidence sample extracts (Table THCA Curve). Then a THC calibration curve was run along with ten evidence sample extracts (Table THC Curve). Blanks were run between each extract sample to monitor carryover (Figure TBA RT, Figure TBA Spectrum). Drug standards were made from 1.0 mg/mL certified reference materials at concentrations ranging from 0.16 mg/mL -0.54mg/ml. Stock solutions of individual THC standards were prepared in 25 mL Class A volumetric flasks. Stock solutions of individual THCA standards were prepared by pipetting various concentrations into 2 mL GC vials. Tributylamine was used at the internal standard at a concentration of 0.153 mg/mL. The filtered extracts and standards were capped after runs and stored at 20 ^{o}C. After approximately 10 days the TBA, THCA, and THC started to degrade and another internal standard was needed.

GCMS QUANT 100 SAMPLES

One hundred seized cannabis evidence samples were examined. All vegetation was analyzed via the microscope. Vegetation samples had to have the characteristic cystolithic trichomes and the covering trichomes to be considered cannabis (Figure 4). Then vegetation samples were examined for approximate cannabinoid content. If large amounts of resin was observed (Figure 9A, 9B), it was notated and dilutions were anticipated. Approximately five grams of vegetation were ground for ten seconds, then ten one second pulses. For treatment one the ground powder was dried at 35-40^{o}C overnight. For treatment two and three they were dried 35-40^{o}C for 1 hour. From the dried and ground samples, three hundred milligrams were weighed out on an analytical balance. Five milliliters of 100% ethanol spiked with 0.153mg/mL tetradecane were added to the dried vegetation and vortexed for five seconds. Treatment two was filtered with a 0.45 µl filter and a 20 milliliter syringe and treatment 3 was filtered with a cotton plugged pipette. Blanks were ran between each extract sample to monitor carryover. Drug

standards were made from 1.0 mg/mL certified reference materials at concentrations ranging from 0.09 mg/mL -0.53mg/mL. Tetradecane was used at the internal standard and was at a concentration of 0.153 mg/mL. The filtered extracts and standards were capped after runs and stored at 20 °C. A retention time of THC was determined to be 9.4 min ± 1%. Septum and liner were changed after every 100 runs. A series of four solvents (methanol, acetone, chloroform, and ethanol) were run between every five samples to keep the internal standard response to the detector more stable.

LCMS CANNABINOID QUALITATIVE ANALYSIS

A series of CBN, CBD, THC, and THCA were added to a GC vial and qualitatively run on the LCMS (Figure 20). It is important for the LCMS to be able to differentiate between the commonly occurring cannabinoids with adequate resolution. Certified reference materials were used from an accredited ISO/IEC 17025 and ISO Guide 34:2009 supplier with certificate of analysis provided. The cannabinoid test mix was made from 1.0 mg/mL certified reference materials. The standards were capped after runs and stored at 4 °C. Blanks were ran between each evidence extract sample to monitor carryover. Each cannabinoid was confirmed by referencing their mass spectrum and, retention times, and their parent ion fragment.

LCMS QUANTITATIVE ANALYSIS

A series of 15 evidence samples were examined. Then vegetation samples were examined for approximate cannabinoid content. If large amounts of resin was observed, it was notated and dilutions were anticipated. Approximately five grams of vegetation were dried for 3 hours at 35-40°C. Vegetation was dried to maintain minimal levels of water within the samples. The vegetation was then ground for ten seconds, then ten one second pulses. Two hundred milligrams were weighed out on an analytical balance and placed into a glass shell vial. Twenty-five milliliters of ethanol were added to the dried vegetation and sonicated for fifteen minutes with vortexing for thirty seconds after minute five, ten, and fifteen. The extract was filtered with a 0.45

µl filter and a 20 milliliter syringe. From the filtered extract, 100 µl were added to a two mL GC vial and placed in an oven at 150^{o}C for ten minutes to ensure complete decarboxylation of THC. The dried extract was reconstituted in 1mL of ethanol. Blanks were run between each evidence extract sample to monitor carryover. Retention times of THC was determined to be 2.32 min ± 7%.

CHAPTER V.

RESULTS AND DISCUSSION

In the decarboxylation trial, an inlet temperature of 280ºC was tested to monitor the decarboxylation of THCA. A THC curve and a THCA curve were run in the same batch along with the 15 evidence samples. Then each curve was used to quantitate the evidence samples. The inlet temperature was set at 280 ºC based on the validated method of (UNODC 2009). The calibration curves were both linear and concentrations of THC were calculated. The concentrations of the evidence samples were not the same when the THCA curve was used as when the THC curve. When the THC curve was used to quantify samples, all of the THCA samples were consistently low (Table III). When the THCA curve was used to quantify samples, all of the THC samples were consistently high (Table II). This was due to a calculation error. The conversion factor of 0.877 should have been used in order to account for the different molar mass of THCA after decarboxylation when mixing the THCA standards. When the 0.877 calculation conversion to THCA is applied, the THC concentrations appear to be fully decarboxylated in the inlet.

The THC curve was linear with a correlation coefficient (r^2) of 0.998 (Figure 14B). The THCA curve was linear with a correlation coefficient (r^2) of 0.989 (Figure 14A). All of the quantitations (mg/mL) were calculated in MassHunter software and the total percent THC was calculated by the following: THC%= (mg/mL cannabinoid in sample X volume of sample (mL) X dilution factor) / (mass of sample X 10^6) *100%. Each blank ran before the evidence sample was checked for contamination and none was present. Retention times for TBA and THC were

confirmed in the chromatograms as the peaks were fully resolved and no co-elution was observed. Each analyte within the sample extracts were identified by their retention times as well as their mass spectrum. The method was derived from the validated general drug screen that is typically used in casework so selectivity had already been determined. After approximately 10 days, interactions with the internal standard and THC were observed as both revealed degradation as well as new compounds formed in the chromatograms. This required the development of a new internal standard and tetradecane was chosen because of its non-polarity.

In the 100 evidence sample trial, all evidence samples were run on the Agilent GC 7820 and various samples were run on the Agilent GC 7890 (Table IV. A., B., C., D.,). The samples were also prepared by more than one forensic scientist. The average percent of THC in the 100 evidence samples was 10.29%, the maximum was 23.25%, and the minimum was 0.29%. The quality control samples as well as the standards routinely did not meet the +/- 20% accuracy requirement (Figure 19). A linear calibration curve was produced by plotting the response of STD/ISTD (y) against concentration (x). The standard curves were also not consistent. Standard curves had to be re-run as well as fresh batches had to be made while still failing to meet +/- 20% accuracy requirement. The relative standard deviation for all of the samples ranged from 0.00-3.13%, while the relative standard deviation for the internal standard ranged from ranged from 6.10-25.77%. All of the quantitations (mg/mL) were calculated in MassHunter software and the total percent THC was calculated by the following: THC%= (mg/mL cannabinoid in sample X volume of sample (mL) X dilution factor) / (mass of sample X 10^6) *100%. The limit of detection (LOD) and limit of quantitation (LOQ) was calculated using the standard error (produced in excel) of the y intercept (Table IX). The LOD was 0.09 mg/mL and the LOQ was 0.28 mg/mL. These numbers correspond with a 0.1% and 0.4% THC respectively on a dry weight basis.

The qualitative cannabinoid study evaluated the selectivity by looking at the commonly occurring cannabinoids in a cannabis sample. First a blank sample of ethanol was run to ensure to

interfering peaks were detected at the target retention times. Then the cannabinoid mix was run and the chromatographic peaks were monitored (Figure 20). Low and high cone voltage spectra were produced at 1.54 minutes, 1.96 minutes, and 2.32 minutes. The spectra at 1.54 minutes was indicative of CBD, the spectra at 1.96 minutes was indicative of CBN, and the spectra at 2.32 minutes was indicative of THC. It also appears THCA co-elutes at 2.28 minutes however since the method allows for decarboxylation in the oven before samples were placed on the instrument was not an issue.

For the quantitative THC study, a five point calibration curve was performed on each day for a total of 3 non-consecutive days. The five prepared standards were analyzed with every batch and used to determine the linearity of the instrument response. The calibration standard solutions were made with calibrated mechanical pipettes. A serial dilution of drug standards were made from 1.0 mg/mL certified reference materials at concentrations ranging from 0.00097 mg/mL - 0.5 mg/ml. A series of 15 evidence samples were ran along with the curves each day for a total of 3 non-consecutive days (Table VI. A., B., VII A., B., VIII. A., B.). As previously stated, the samples were run on non-consecutive days as well as by more than one forensic scientist. All seized evidence samples were within the calibration range.

Acceptance criteria stated the linear correlation coefficient could not be below 0.99 and the accuracy had to be within ± 20% of the THC target. Peak shape was excellent with no signs of chromatographic abnormalities. The r^2 for all of the batches were greater than the 0.99 for the THC target compound which meets the acceptance criteria of ≥ 0.99 and also met the ± 20% of the THC target acceptance criteria. For repeatability the percent relative standard deviation (RSD) of the peak area from the 15 samples analyzed was calculated. The batches ran showed an RSD ≤ 0.0798 %. The low RSD demonstrates that the method was precise in terms of needle injections and response to the detector during each days run. The intermediate precision showed an RSD $\leq 0.0.1466$ %. The limit of detection (LOD) and limit of quantitation (LOQ) was

calculated using the standard error (produced in excel) of the y intercept (Table X). The LOD was 0.000002 mg/mL and the LOQ was 0.000006 mg/mL. These numbers correspond with a 0.0002% and 0.00085% THC respectively on a dry weight basis.

Focus on cannabis has increased as states legalize varying degrees of usage. Whether it be extracts, edibles, or new and improved strains, the application and testing vary widely. Testing methods can be simple and efficient or long and laborious. In the quantitative methods created, procedures were long, labor intensive, as well as drastically increasing laboratory supplies needed.

Ideally a robust GCMS method was hoped to be developed as they are the workhorse of most drug chemistry laboratories. The difficulty with the internal standard interactions with the THC standard was unanticipated. Tribenzylamine was first chosen as it had been successfully been used to quantitate THC. The stability of THC was also overestimated. In the UNDOC 2009 reference, TBA was used along with CBN instead of THC. CBN is inherently more stable than THC. Since we never see degradation of our THC standards in our routine qualitative analysis, the stability of THC was not thought to be an issue. This was not the case. During the study, keeping standards and evidence samples at refrigerated or freezing temperatures was important although not always practical. In regular GCMS casework a forensic scientist may start a sequence that lasts several days. The autosamplers were not temperature or light controlled and visible degradation started even at room temperature. This also caused a lot of waste as each standard was prepared in a 25 mL Class A volumetric flask.

At one point in the study sample extracts were kept in the refrigerator as space in the freezers were very limited. This particular refrigerator had a glass door and would catch the Eastern morning sun. In just a few short days, the sample extracts went from a chlorophyll green to an amber brown. The refrigerator was in good working condition however THC is also

unstable in the presence of light. This further supports the information presented on cultivation and the conversion to CBN depicted in Figures 9A. and 9B. If the issue of temperature and light control can be addressed, using TBA as an internal standard may be revisited.

At the beginning of the study, it became apparent that the grinders purchased were not adequate (Figure 27). There would be some loss of resin in the grinding step and recovery would not be 100%. Grinding before drying the cannabis and grinding after drying the cannabis in the oven was observed. There was considerably more resin left in the grinders than predicted as most of the evidence samples were either several years old or came from other states and were from dried retail product. The size of the ground product was also questioned. The idea of using cryogenic grinders or liquid nitrogen with mortar and pestle were explored as they would additionally address the size of the ground product and homogeneity. More research needs to be conducted as to the best method for recovery as the grinders we have would be deemed unacceptable in casework.

In the decarboxylation trial, complete decarboxylation of THCA in the GCMS inlet was desired as derivitization would make the method more problematic. In order to work efficiently and effectively, it is better to calculate and quantitate the neutral form of THC instead of the neutral and acidic forms. A prior heating step was also tested however it was deemed unnecessary because the conversion of THCA to THC was complete upon injection into the GCMS. The concept of total THC content was applied and any deviation from this would result in increased labor and supplies. Another thing to note is the r^2 for the THCA curve was lower than the THC curve. This was most likely due to the amount of standard prepared. The cost for 1mg/mL of THC is significantly lower than the same quantity of THCA. As such, the THC standards were prepared in 25 mL Class A volumetric flasks while the THCA standards were 1mL total. Pipetting small amounts can greatly increase your error.

Initially we couldn't determine why when using the THCA curve, all of the THC standards were quantified in unusually low concentrations and the opposite was true when using the THC curve. This was eventually solved as we did not use the 0.877 conversion factor in order to account for the different molar mass of THCA after decarboxylation when mixing the THCA standards. It was assumed the starting concentration was 1 mg/mL stock solution of THCA to make the standards. Once injected into the GCMS, the acid portion of the THCA was cleaved and no longer were working with a stock solution of 1 mg/mL. Once the 0.877 conversion factor was used, the concentrations of the evidence samples and standards were more consistent.

The GCMS instruments available to the drug chemistry section only had one quadrupole rather than a triple quadrupole. This severely limited the ability to quantitate at low concentrations. While the samples were ran in SIM/Scan mode, the detector was still not sufficiently sensitive to detect concentrations that are indicative of hemp. It was difficult to maximize the instrument so the detector would detect the 0.09 mg/mL standards (0.1% THC). With the difficulty of the instrument, it is not surprising that most of the QC samples did not meet the acceptance criteria of ± 20%.

The internal standard tetradecane was chosen as it was a non-polar compound and thought to have less interactions with THC than the TBA internal standard. Tetradecane did show to be more stable than TBA however it had challenges as well. The internal standard response to the detector would drastically decrease with no apparent reason. Septum and liner were replaced after every 100 runs. This irregular detector response was irrespective as to when the septum and liner was changed. In order to alleviate the irregularity, a series of four different solvents were run between every 5 samples. This drastically increased batch sequences and runtimes. It appeared cleaning the source once a week was also necessary. This is less than desirable as cleaning the source is time intensive and greatly decreases the number of samples that can be ran during a forensic scientist's work week.

Since the data from the GCMS was unreliable, the possibility of developing a quantitative method on the LC-UV-MS was explored. Since resolution and reproducibility of the LCMS is much greater, only external standards were used. The cannabinoid test mix was run to determine selectivity. All cannabinoids were fully resolved except THCA. THCA co-eluted with THC based on the information provided by the total ion chromatogram. This was not considered an issue as the method developed had incorporated a decarboxylation step that would fully convert THCA to THC prior to injecting the extracts onto the column. Further controlled substances still need to be researched in order to be validated for casework. CBN, CBD, THC, and THCA was used based off of experience in casework as to the most common compounds found within a cannabis sample.

The wavelength 214 was initially used to quantify the evidence samples as it is a common wavelength for controlled substances to absorb. The method was developed based off of a validated methamphetamine quantitation method already used in casework at our laboratory. Through testing, THC and the other major cannabinoids frequently encountered in casework did in fact have an absorbance at 214nm. With the confirmation of the absorbance peak at 214nm, THC was deemed acceptable to quantitate THC samples. This allows uniformity in placing various controlled substances under one method. The mass spectrometer was used to detect ions within the same analytical run so a presumptive and confirmatory test can be conducted simultaneously. The LCMS method demonstrated a wide dynamic range as well as excellent reproducibility. The LCMS itself has the capability to yield high throughput and with a runtime of 6.5 minutes could help reduce backlog in casework. Although to achieve this, changes and advances to the method need to be investigated as well as slightly different columns.

Overall this project demonstrated scientifically relevant data in regards to forensic applications. The goal of the project of determining which method would best quantitate THC with the most consistent data was achieved. Previously published LCMS methods were less

suitable for the high sample volume seen in forensic casework as their average runtimes were over 30 minutes. The LC-UV-MS is more than capable of quantitating a large range of THC concentrations observed in routine cannabis samples. Despite the fact that the LCMS method was suitable for casework more additional research should be concentrated on finding more cost effective solvents and supplies since ethanol is expensive and evaporates very quickly. Further work should be focused on easier, faster, grinding and extractions as this will also decrease turnaround time.

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APPENDIX

TABLE 1. SWGDRUG

Category A	Category B	Category C
Infrared Spectrophotometry Mass Spectrometry	Gas Chromatography Thin-Layer Chromatography Liquid Chromatography Pharmaceutical Identifiers Microscopic Examination (Marijuana only)	Color Tests Ultraviolet Spectrophotometry

Marijuana identification requires a Category A test and a Category B (microscopic) test

TABLE 2. THCA Curve

Sample Name	Final Conc	Exp Conc	Accuracy
THC Std 1 - 0.167 mg/mL	0.2172		
THC Std 2 - 0.25 mg/mL	0.3009		
THC Std 3 - 0.35 mg/mL	0.4010		
THC Std 4 - 0.53 mg/mL	0.6533		
THC-A Std 1 - .016 mg/mL	0.1776	0.1600	111.01
THC-A Std 2 - 0.24 mg/mL	0.2252	0.2400	93.83
THC-A Std 3 - 0.34 mg/mL	0.3349	0.3500	95.68
THC-A Std 4 - 0.54 mg/mL	0.5802	0.5400	107.44
Sample 341-1	0.1294		
Sample 341-2	0.3127		
Sample 341-3	0.1485		
Sample 341-4	0.1149		
Sample 341-5	0.1803		
Sample 341-6	0.1623		
Sample 341-7	0.4093		
Sample 341-8	0.2315		
Sample 341-9	0.4800		
Sample 341-10	0.4936		
THC-A Std 3 - 0.34 mg/mL	0.3334	0.3500	95.24
THC Std 3 - 0.35 mg/mL	0.4348		

TABLE 3. THC Curve

Sample Name	Final Conc	Exp Conc	Accuracy
THC Std 1 - 0.167 mg/mL	0.1738	0.167	104.0966
THC Std 2 - 0.25 mg/mL	0.2454	0.25	98.17835
THC Std 3 - 0.35 mg/mL	0.3310	0.35	94.57224
THC Std 4 - 0.53 mg/mL	0.5467	0.53	103.1528
THC-A Std 1 - .016 mg/mL	0.1400		
THC-A Std 2 - 0.24 mg/mL	0.1807		
THC-A Std 3 - 0.34 mg/mL	0.2745		
THC-A Std 4 - 0.54 mg/mL	0.4842		
Sample 341-1	0.0988		
Sample 341-2	0.2555		
Sample 341-3	0.1151		
Sample 341-4	0.0864		
Sample 341-5	0.1423		
Sample 341-6	0.1269		
Sample 341-7	0.3381		
Sample 341-8	0.1861		
Sample 341-9	0.3985		
Sample 341-10	0.4102		
THC-A Std 3 - 0.34 mg/mL	0.2732		
THC Std 3 - 0.35 mg/mL	0.3599	0.3500	101.69

TABLE 4A. 1-100

Sample	Treatment 1	Treatment 2	Treatment 3	
1	0.29%			
2	7.50%			
3	0.96%			
4	2.80%			
5	4.46%			
6	1.73%			
7	6.89%			
8	6.95%			
9	7.42%	11.01%	9.03%	
	1.23%	1.31%	0.09%	STD DEV
10	7.17%	8.17%	8.32%	
	0.50%	0.20%	0.31%	STD DEV
11	7.20%			
12	7.42%			
13	9.21%			
14	9.07%			
15	8.71%			
16	8.11%	7.59%	7.68%	
	0.37%	0.14%	0.08%	STD DEV
17	7.99%	8.74%	6.32%	
	0.22%	0.75%	0.96%	STD DEV
18	9.50%	8.34%	7.20%	
	0.81%	0.00%	0.81%	STD DEV
19	8.95%	9.42%	10.83%	
	0.55%	0.22%	0.78%	STD DEV
20	8.11%	14.25%	14.81%	
	3.03%	1.32%	1.71%	STD DEV
21	7.48%	8.14%	8.50%	
	0.40%	0.07%	0.33%	STD DEV
22	12.42%	9.35%	11.91%	
	0.84%	1.33%	0.48%	STD DEV
23	12.54%	10.03%	11.04%	
	0.95%	0.83%	0.12%	STD DEV
24	7.94%	9.44%	9.87%	
	0.81%	0.25%	0.56%	STD DEV

TABLE 4B. 1-100

25	11.39%	11.81%	11.53%	
	0.13%	0.16%	0.03%	STD DEV
26	13.27%	12.06%	17.38%	
	0.68%	1.54%	2.22%	STD DEV
27	10.36%	10.99%	10.40%	
	0.16%	0.29%	0.13%	STD DEV
28	9.95%	8.93%	9.67%	
	0.31%	0.41%	0.11%	STD DEV
29	9.55%	13.04%	9.57%	
	0.83%	1.64%	0.81%	STD DEV
30	17.60%	15.74%	14.35%	
	1.21%	0.11%	1.09%	STD DEV
31	14.36%	12.67%	10.99%	
	1.19%	0.00%	1.19%	STD DEV
32	9.80%	13.55%	10.47%	
	1.04%	1.61%	0.57%	STD DEV
33	10.85%	13.40%	11.25%	
	0.69%	1.11%	0.41%	STD DEV
34	9.87%	10.43%	10.92%	
	0.38%	0.02%	0.36%	STD DEV
35	9.04%	8.21%	9.15%	
	0.17%	0.42%	0.25%	STD DEV
36	8.40%	9.84%	9.34%	
	0.56%	0.46%	0.10%	STD DEV
37	14.11%	14.22%	15.25%	
	0.29%	0.22%	0.51%	STD DEV
38	16.82%	15.16%	13.50%	
	1.17%	0.00%	1.17%	STD DEV
39	14.52%	13.83%	17.16%	
	0.46%	0.95%	1.41%	STD DEV
40	13.96%	17.16%	19.30%	
	2.01%	0.25%	1.76%	STD DEV

TABLE 4C. 1-100

41	9.45%	9.34%	8.51%	
	0.25%	0.17%	0.42%	STD DEV
42	9.57%	8.57%	10.31%	
	0.06%	0.65%	0.58%	STD DEV
43	9.58%	9.99%	10.08%	
	0.22%	0.08%	0.14%	STD DEV
44	8.75%	14.99%	11.39%	
	2.09%	2.32%	0.23%	STD DEV
45	9.08%	9.21%	9.27%	
	0.08%	0.02%	0.06%	STD DEV
46	10.69%	11.43%	10.60%	
	0.16%	0.37%	0.22%	STD DEV
47	17.90%	15.71%	16.51%	
	0.84%	0.70%	0.14%	STD DEV
48	14.16%	12.21%	11.83%	
	1.01%	0.37%	0.64%	STD DEV
49	20.56%		11.70%	
	3.13%		3.13%	STD DEV
50	14.35%	15.02%	11.79%	
	0.45%	0.92%	1.36%	STD DEV
51	19.62%			
52	21.12%	16.39%	16.78%	
	2.14%	1.21%	0.93%	STD DEV
53	17.13%	17.90%	15.69%	
	0.16%	0.70%	0.86%	STD DEV
54	15.58%	15.13%	13.65%	
	0.56%	0.24%	0.80%	STD DEV
55	8.38%	8.31%	7.66%	
	0.19%	0.14%	0.32%	STD DEV
56	8.71%	8.69%	8.39%	
	0.08%	0.07%	0.15%	STD DEV
57	8.87%	8.48%	9.81%	
	0.13%	0.41%	0.53%	STD DEV
58	14.01%			
59	13.89%	16.22%	14.48%	
	0.69%	0.96%	0.27%	STD DEV
60	16.59%	16.43%		
	0.06%	0.06%		STD DEV

TABLE 4D. 1-100

61	15.68%
62	17.62%
63	16.24%
64	23.25%
65	17.74%
66	19.00%
67	6.44%
68	12.36%
69	16.95%
70	15.64%
71	10.36%
72	9.29%
73	11.87%
74	13.70%
75	10.69%
76	10.83%
77	7.87%
78	7.29%
79	7.41%
80	7.43%
81	8.43%
82	8.05%
83	7.67%
84	6.93%
85	6.76%
86	7.43%
87	6.76%
88	6.45%
89	7.39%
90	7.70%
91	6.71%
92	6.40%
93	5.55%
94	6.55%
95	7.40%
96	7.90%
97	6.33%
98	7.21%
99	5.53%
100	6.08%

TABLE 5. GCMS DATA

Sample Name	Final Conc	Exp Conc	Accuracy
THC Std 1 - 0.09 mg/mL	0.0874	0.09	97.0643693
THC Std 2 - 0.167 mg/mL	0.1331	0.167	79.71044816
THC Std 3 - 0.25 mg/mL	0.1568	0.25	62.71320717
THC Std 4 - 0.53 mg/mL	0.5851	0.53	110.3953666
314-15	0.1844		
314-16	0.1685		
314-17	0.1648		
314-18	0.2078		
314-19	0.1914		
314-20	0.1680		
STD #2 0.25mg/mL	0.1350	0.2500	54.01

TABLE 6A. LCMS Day 1

Sample	RT	Height	Response	mg/mL	Area	weight	dilution	THC % Conc.
STD #1 0.001	2.323	5.81E+03	180	0.00097	180			
STD #2 0.016	2.316	8.58E+04	2691	0.0164	2691			
STD# 3 0.031	2.301	1.69E+05	5297	0.03242	5297			
STD# 4 0.25	2.29	1.28E+06	40591	0.2493	40591			
STD# 5 0.5	2.288	2.19E+06	74853	0.45984	74853			
40 10X	2.283	7.02E+05	22242	0.13654	22242	199.7	10	17.09313971
41 10X	2.285	3.41E+05	10902	0.06686	10902	199.9	10	8.36168084
42 10X	2.286	3.93E+05	12586	0.07721	12586	199.3	10	9.685148018
43 10X	2.307	4.01E+05	12838	0.07875	12838	200	10	9.84375
44 10X	2.287	4.42E+05	14099	0.08651	14099	200.7	10	10.77603388
45 10X	2.271	3.46E+05	10998	0.06745	10998	199.1	10	8.46936213
46 10X	2.274	5.90E+05	18794	0.11536	18794	199.6	10	14.4488978
47 10X	2.285	5.26E+05	16583	0.10177	16583	199.3	10	12.76593076
48 10X	2.267	6.16E+05	19566	0.1201	19566	199.6	10	15.04258517
49 10X	2.256	7.25E+05	23170	0.14225	23170	200.4	10	17.74575848
PRP1	2.26	4.69E+05	15087	0.09258	150871	199.1	10	11.62481165
PRP2	2.269	1.19E+05	3827	0.02338	3827	200.3	10	2.918122816
PRP3	2.266	2.13E+05	6799	0.04165	6799	200.3	10	5.198452322
PRP4	2.262	1.45E+05	4577	0.02799	4577	200.9	10	3.483076157
PRP5	2.254	3.57E+05	11457	0.07027	11457	199.4	10	8.810180542

TABLE 6B. LCMS Day 1

Sample	RT	Height	Response	mg/mL	Area	weight	dilution	THC % Conc.
STD #1 0.001	2.29	5.57E+03	178	0.00097	178			
STD #2 0.016	2.323	8.20E+04	2620	0.0161	2620			
STD# 3 0.031	2.285	1.64E+05	5336	0.03294	5336			
STD# 4 0.25	2.286	1.22E+06	40207	0.24905	40207			
STD# 5 0.5	2.285	2.12E+06	74431	0.46114	74431			
40 10X	2.283	6.83E+05	21464	0.13289	21464	199.7	10	16.63620431
41 10X	2.338	3.37E+05	10361	0.06408	10361	199.9	10	8.014007004
42 10X	2.324	4.11E+05	12739	0.07882	12739	199.3	10	9.887104867
43 10X	2.32	3.86E+05	12017	0.07434	12017	200	10	9.2925
44 10X	2.379	4.11E+05	12847	0.07949	12847	200.7	10	9.90159442
45 10X	2.356	3.22E+05	10286	0.06361	10286	199.1	10	7.987192366
46 10X	2.359	5.52E+05	17706	0.1096	17706	199.6	10	13.72745491
47 10X	2.349	4.76E+05	15589	0.09648	15589	199.3	10	12.10235825
48 10X	2.323	5.67E+05	18532	0.11472	18532	199.6	10	14.36873747
49 10X	2.391	5.88E+05	19038	0.11786	19038	200.4	10	14.70309381
PRP1	2.382	3.96E+05	12919	0.07993	12919	199.1	10	10.03641386
PRP2	2.325	1.08E+05	3654	0.02252	3654	200.3	10	2.810783824
PRP3	2.311	1.84E+05	6315	0.039	6315	200.3	10	4.867698452
PRP4	2.345	1.29E+05	4453	0.02747	4453	200.9	10	3.418367347
PRP5	2.342	2.91E+05	10044	0.06212	10044	199.4	10	7.788365095

TABLE 7A. LCMS Day 2

Sample	RT	Height	Response	mg/mL	Area	weight	dilution	THC % Conc.
STD #1 0.001	2.238	5.38E+03	180	0.00097	180			
STD #2 0.016	2.232	7.88E+04	2656	0.01631	2656			
STD# 3 0.031	2.237	1.56E+05	5289	0.03261	5289			
STD# 4 0.25	2.234	1.17E+06	39919	0.24706	39919			
STD# 5 0.5	2.244	2.06E+06	74971	0.46412	74971			
40 10X	2.299	6.39E+05	21889	0.1354	21889	199.7	10	16.9504256
41 10X	2.306	2.98E+05	10320	0.06376	10320	199.9	10	7.97398699
42 10X	2.292	3.44E+05	12130	0.07498	12130	199.3	10	9.40541897
43 10X	2.37	3.36E+05	11509	0.07113	11509	200	10	8.89125
44 10X	2.314	3.81E+05	13608	0.08413	13608	200.7	10	10.4795715
45 10X	2.293	2.82E+05	10337	0.06387	10337	199.1	10	8.01983928
46 10X	2.336	4.35E+05	15691	0.09703	15691	199.6	10	12.1530561
47 10X	2.334	3.84E+05	14174	0.08763	14174	199.3	10	10.9922228
48 10X	2.382	4.89E+05	18087	0.11186	18087	199.6	10	14.010521
49 10X	2.319	5.31E+05	19684	0.12175	19684	200.4	10	15.1883733
PRP1	2.338	3.58E+05	13436	0.08188	13436	199.1	10	10.2812657
PRP2	2.345	9.18E+04	3495	0.02121	3495	200.3	10	2.64727908
PRP3	2.383	1.43E+05	5487	0.03337	5487	200.3	10	4.1650025
PRP4	2.426	7.66E+04	2913	0.01766	2913	200.9	10	2.19761075
PRP5	2.297	2.37E+05	11500	0.07007	11500	199.4	10	8.78510532

TABLE 7B. LCMS DAY 2

Sample	RT	Height	Response	mg/mL	Area	weight	dilution	THC % Conc.
STD #1 0.001	2.301	3.83E+03	178	0.00097	178			
STD #2 0.016	2.293	5.72E+04	2710	0.01642	2710			
STD# 3 0.031	2.292	1.12E+05	5321	0.03235	5321			
STD# 4 0.25	2.295	8.59E+05	41039	0.25035	41039			
STD# 5 0.5	2.29	1.62E+06	79529	0.48526	79529			
40 10X	2.286	4.65E+05	22358	0.13633	22358	199.7	10	17.0668503
41 10X	2.291	2.28E+05	10985	0.06693	10985	199.9	10	8.37043522
42 10X	2.291	2.64E+05	12730	0.07757	12730	199.3	10	9.73030607
43 10X	2.29	2.68E+05	12998	0.07921	12998	200	10	9.90125
44 10X	2.282	2.97E+05	14401	0.08777	14401	200.7	10	10.9329846
45 10X	2.282	2.29E+05	11106	0.06766	11106	199.1	10	8.49573079
46 10X	2.283	3.93E+05	19207	0.1171	19207	199.6	10	14.6668337
47 10X	2.278	3.49E+05	16912	0.1031	16912	199.3	10	12.9327647
48 10X	2.278	4.09E+05	19929	0.12151	19929	199.6	10	15.2191884
49 10X	2.277	4.82E+05	23500	0.14331	23500	200.4	10	17.877994
PRP1	2.279	3.17E+05	15395	0.09384	15395	199.1	10	11.7830236
PRP2	2.275	8.07E+04	3903	0.0237	3903	200.3	10	2.95806291
PRP3	2.28	1.42E+05	6915	0.04208	6915	200.3	10	5.25212182
PRP4	2.281	9.62E+04	4665	0.02835	4665	200.9	10	3.52787456
PRP5	2.275	2.41E+05	11692	0.07124	11692	199.4	10	8.93179539

TABLE 8A. LCMS DAY 3

Sample	RT	Height	Response	mg/mL	Area	weight	dilution	THC % Conc.
STD #1 0.001	2.3	4.16E+03	120	0.0008	120			
STD #2 0.016	2.293	6.15E+04	2705	0.0165	2705			
STD# 3 0.031	2.293	1.19E+05	5306	0.0323	5306			
STD# 4 0.25	2.292	8.90E+05	40451	0.24583	40451			
STD# 5 0.5	2.293	1.66E+06	78060	0.47431	78060			
40 10X	2.293	4.80E+05	22027	0.13389	22027	199.7	10	16.76139209
41 10X	2.286	2.37E+05	10854	0.06601	10854	199.9	10	8.255377689
42 10X	2.286	2.74E+05	12592	0.07657	12592	199.3	10	9.604867035
43 10X	2.292	2.76E+05	12611	0.07669	12611	200	10	9.58625
44 10X	2.291	3.02E+05	13860	0.08427	13860	200.7	10	10.49701046
45 10X	2.292	2.32E+05	10669	0.06489	10669	199.1	10	8.14791562
46 10X	2.29	3.97E+05	18299	0.11124	18299	199.6	10	13.93286573
47 10X	2.288	3.49E+05	16163	0.09827	16163	199.3	10	12.32689413
48 10X	2.273	4.09E+05	19054	0.11583	19054	199.6	10	14.50776553
49 10X	2.273	4.77E+05	22219	0.13506	22219	200.4	10	16.8488024
PRP1	2.273	3.16E+05	14587	0.08869	14587	199.1	10	11.13636364
PRP2	2.259	8.04E+04	3732	0.02275	3732	200.3	10	2.839490764
PRP3	2.259	1.44E+05	6703	0.04079	6703	200.3	10	5.09111333
PRP4	2.266	9.89E+04	4583	0.02791	4583	200.9	10	3.473120956
PRP5	2.262	2.46E+05	11538	0.07017	11538	199.4	10	8.797642929

TABLE 8B. LCMS DAY 3

Sample	RT	Height	Response	mg/mL	Area	weight	dilution	THC % Conc.
STD #1 0.001	2.263	3.84E+03	177	0.00114	177			
STD #2 0.016	2.258	5.66E+04	2653	0.01619	2653			
STD# 3 0.031	2.261	1.12E+05	5222	0.0318	5222			
STD# 4 0.25	2.258	8.35E+05	39783	0.24176	39783			
STD# 5 0.5	2.253	1.61E+06	79317	0.48195	79317			
40 10X	2.253	4.76E+05	22649	0.13767	22649	199.7	10	17.2346019
41 10X	2.264	2.32E+05	11049	0.0672	11049	199.9	10	8.404202101
42 10X	2.265	2.68E+05	12833	0.07804	12833	199.3	10	9.789262418
43 10X	2.251	2.70E+05	12861	0.0782	12861	200	10	9.775
44 10X	2.249	2.99E+05	14280	0.08682	14280	200.7	10	10.81464873
45 10X	2.25	2.27E+05	10892	0.06624	10892	199.1	10	8.317428428
46 10X	2.253	4.00E+05	19050	0.1158	19050	199.6	10	14.50400802
47 10X	2.247	3.52E+05	16809	0.10219	16809	199.3	10	12.81861515
48 10X	2.238	4.14E+05	19918	0.12108	19918	199.6	10	15.16533066
49 10X	2.237	4.83E+05	23512	0.14291	23512	200.4	10	17.82809381
PRP1	2.242	3.12E+05	15103	0.09183	15103	199.1	10	11.53063787
PRP2	2.245	8.07E+04	3846	0.02344	3846	200.3	10	2.925611583
PRP3	2.249	1.41E+05	6723	0.04091	6723	200.3	10	5.106090864
PRP4	2.243	9.58E+04	4607	0.02806	4607	200.9	10	3.491786959
PRP5	2.239	2.42E+05	11713	0.07123	11713	199.4	10	8.930541625

TABLE 9. LOD & LOQ 1-100 SAMPLES

SE of intercept	216063.6451
SD of intercept	96626.59956
LOD	0.093
LOQ	0.283
Slope	3,419,082.56
√N	2.236067977

SUMMARY OUTPUT

Regression Statistics

Multiple R	0.945184871
R Square	0.89337444
Adjusted R Square	0.857832586
Standard Error	233403.0752
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.36933E+12	1.36933E+12	25.1358428	0.015278512
Residual	3	1.63431E+11	54476995496		
Total	4	1.53276E+12			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-362083.638	216063.6451	-1.675819354	0.19236697	-1049694.587	325527.311	-1049694.587	325527.3109
X Variable 1	3419082.561	681966.2182	5.013565878	0.01527851	1248761.69	5589403.43	1248761.69	5589403.433

TABLE 10. LOD & LOQ LCMS

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.9999999999							
R Square	0.9999999998							
Adjusted R Square	0.9999999998							
Standard Error	0.464331573							
Observations	5							
ANOVA								
	df	*SS*	*MS*	*F*	*gnificance F*			
Regression	1	4229947671	4229947671	19619076650	8.03E-16			
Residual	3	0.646811429	0.21560381					
Total	4	4229947671						
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*.ower 95%*	*Jpper 95%*	*ower 95.0%*	*pper 95.0%*
Intercept	21.83503384	0.272431567	80.1486922	4.28093E-06	20.96804	22.70203	20.96804	22.70203
X Variable 1	162732.8276	1.161812082	140068.114	8.02515E-16	162729.1	162736.5	162729.1	162736.5
SE of intercept		0.272431567						
SD of intercept		0.111219722						
LOD		0.0000022554	mg/mL					
LOQ		0.0000068345	mg/mL					
Slope		162733						
√N		2.449489743						

TABLE 11. LCMS RSD of 15 Samples

Sample ID	Concentration (% w/w)	Repeatability (% RSD)	Intermediate precision (%RSD)
40	16.96	0.0043	0.0120
41	8.23	0.0181	0.0211
42	9.68	0.0120	0.0156
43	9.55	0.0308	0.0373
44	10.57	0.0261	0.0322
45	8.24	0.0159	0.0245
46	13.91	0.0331	0.0611
47	12.32	0.0294	0.0538
48	14.72	0.0140	0.0307
49	16.70	0.0140	0.0307
PRP 1	11.07	0.0414	0.0609
PRP 2	2.85	0.0198	0.0365
PRP 3	4.95	0.0490	0.0747
PRP 4	3.27	0.0798	0.1466
PRP 5	8.67	0.0208	0.0462

FIGURE 1. 5ft. Tall Cannabis Plant

FIGURE 2. Cystolithic Hair “Bear Claw”

FIGURE 3. Calcium Carbonate Crystals

FIGURE 4. Covering Hairs

FIGURE 5. Glandular Hairs and Resin From Green and Purple Strains

FIGURE 6. Tortise Shell Appearance in Cannabis Seeds

FIGURE 7. Brown Stigmas

FIGURE 8. Clear Resin

FIGURE 9A. Brown Resin Converted to CBN

FIGURE 9B. Brown Resin Converted to CBN

FIGURE 10. Cannabis Buds Prior to Being Manicured and Trimmed

FIGURE 11. Ditchweed

FIGURE 12. Map of US and Marijuana Laws

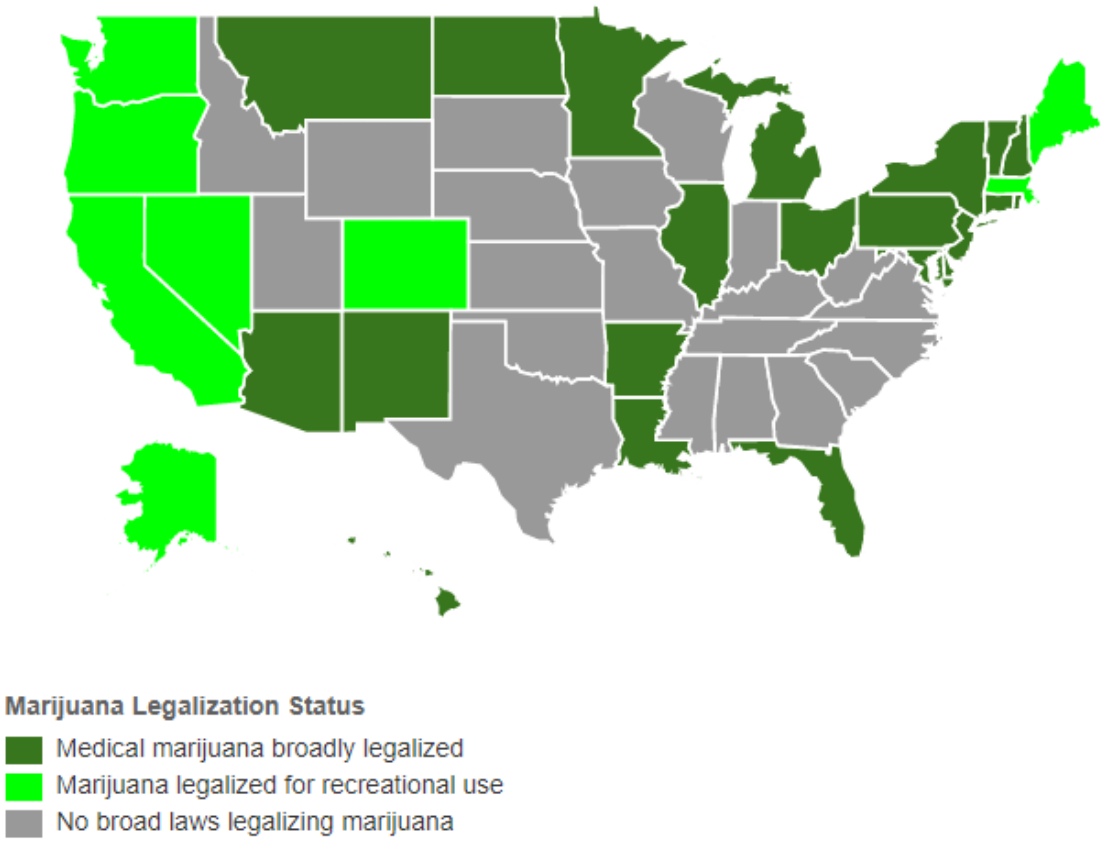
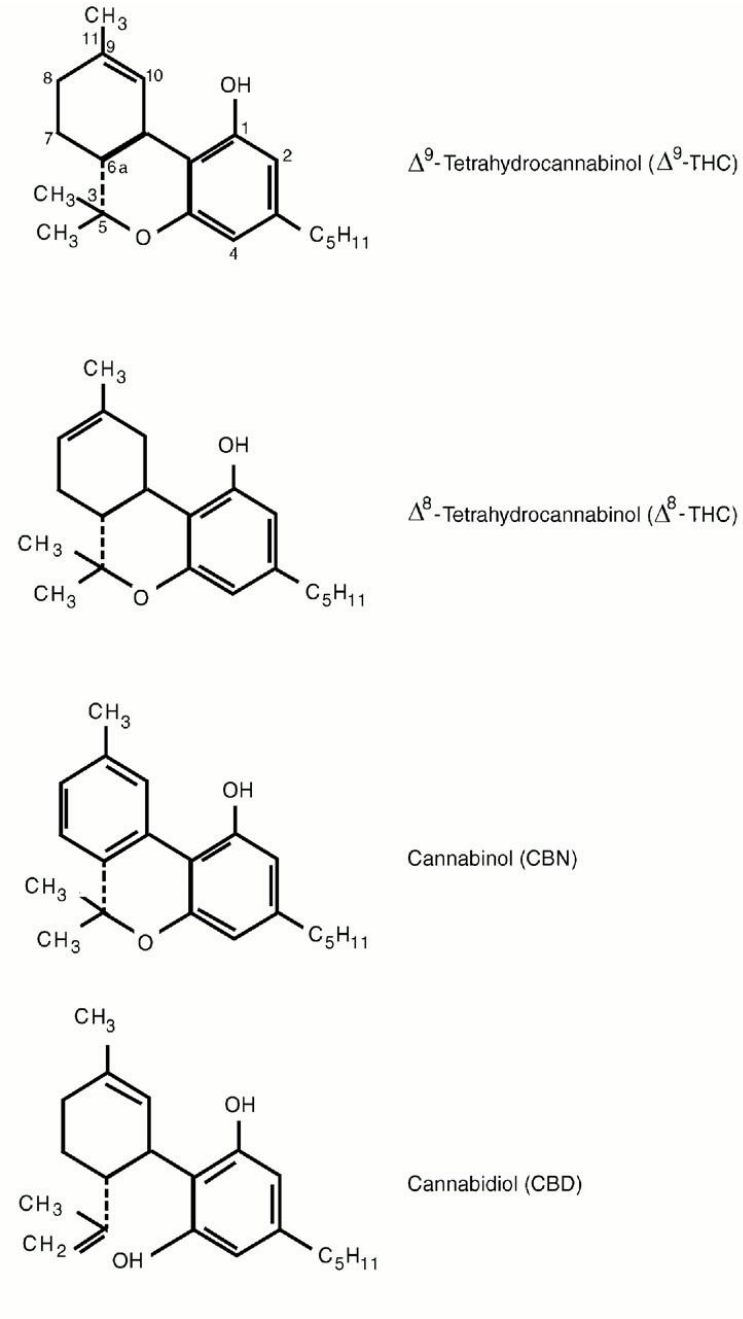


FIGURE 13 Structures of Common Canabinoids



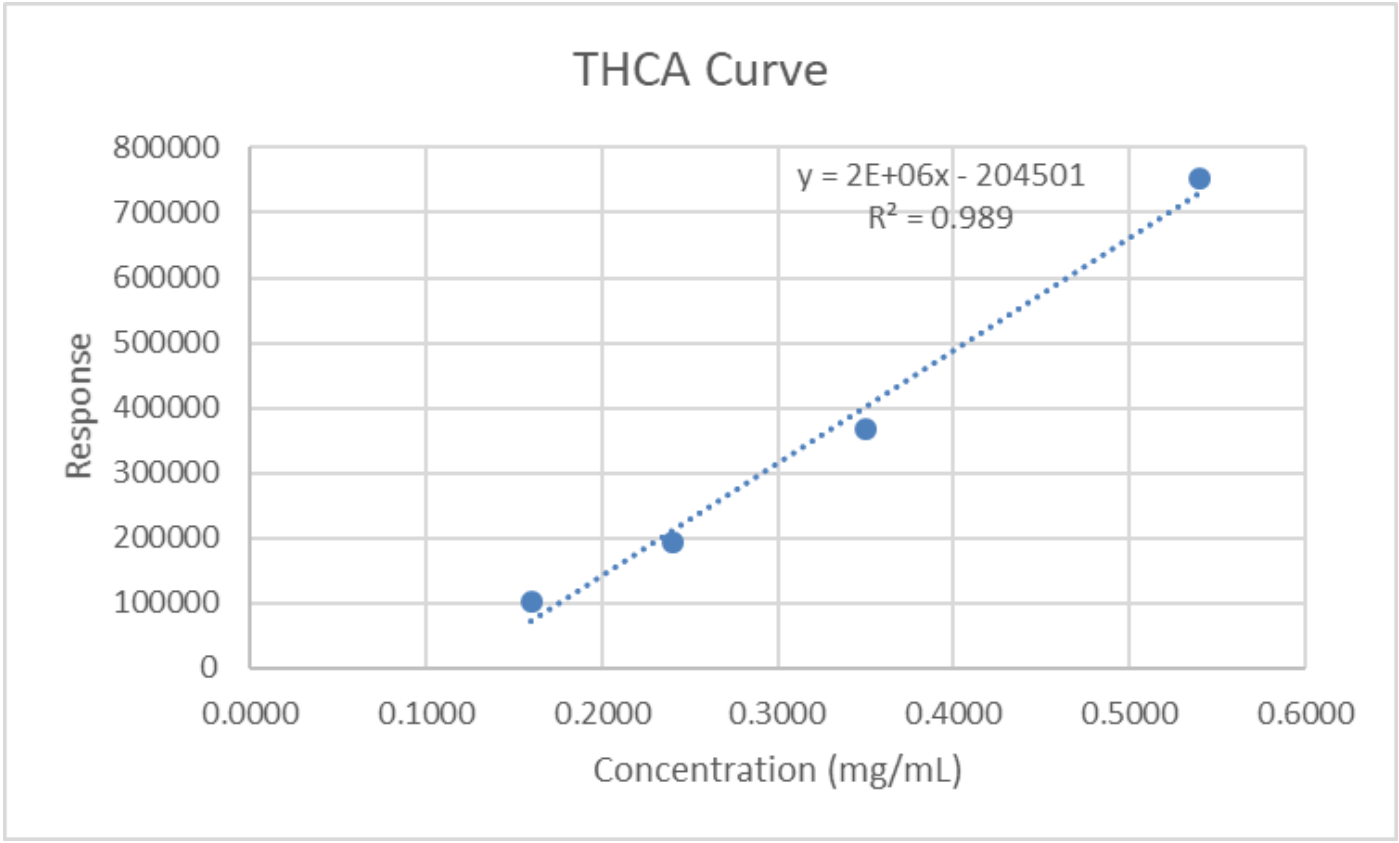


FIGURE 14A. Curve Using THCA Standards

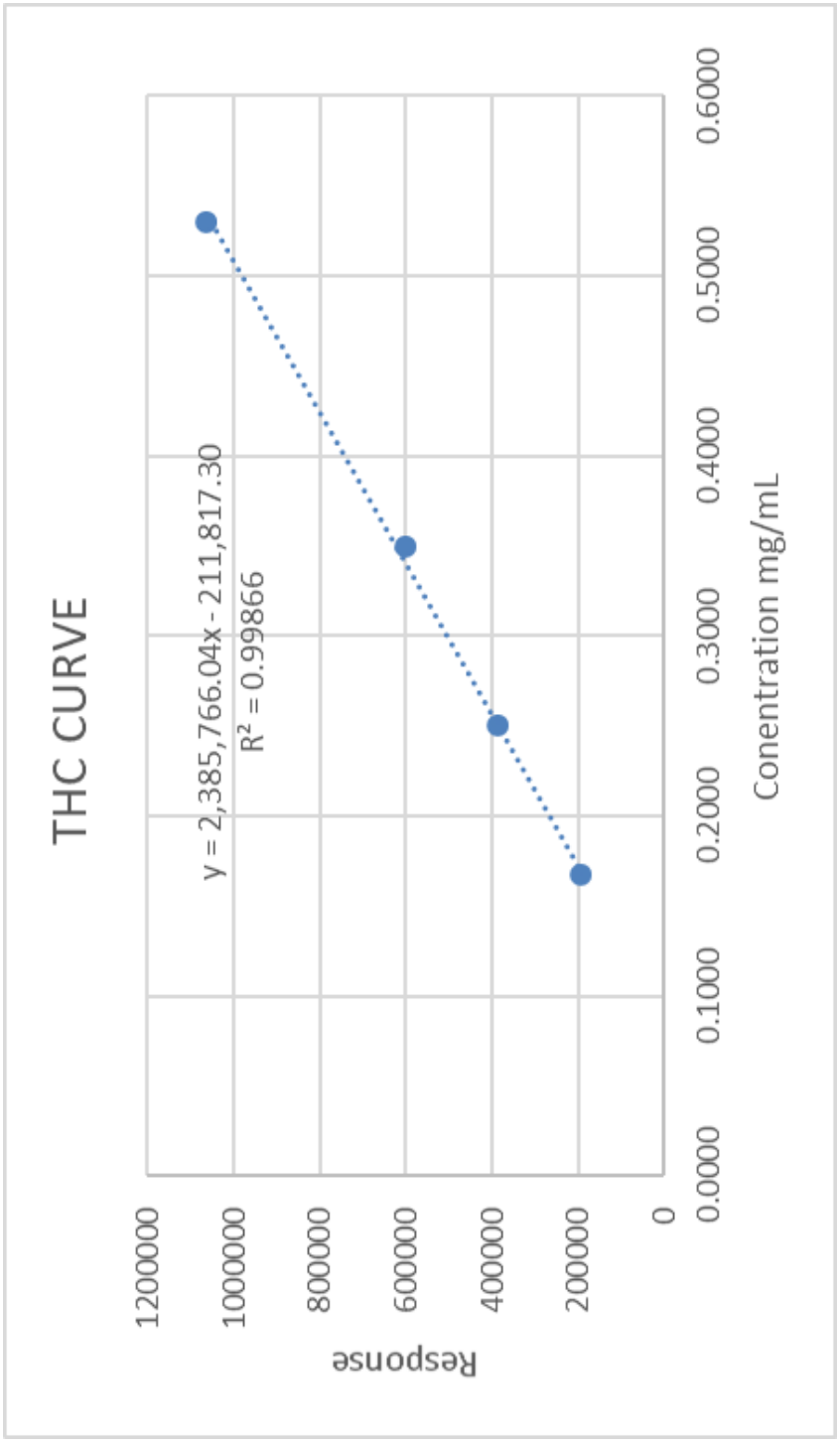
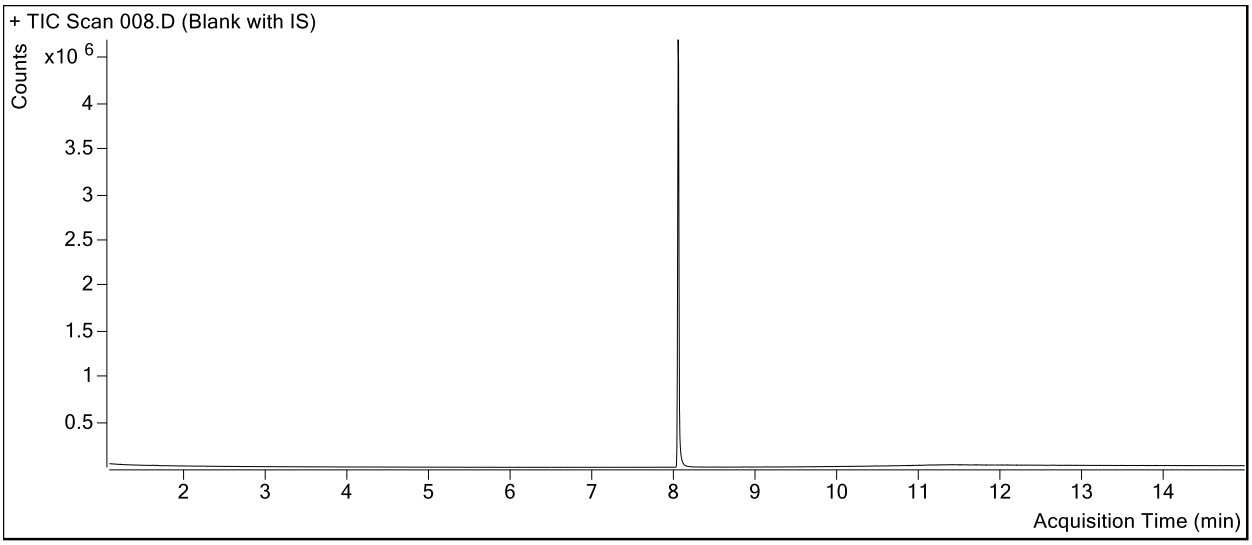


FIGURE 14B. Curve Using THC Standards

FIGURE 15. TBA Positive Identification



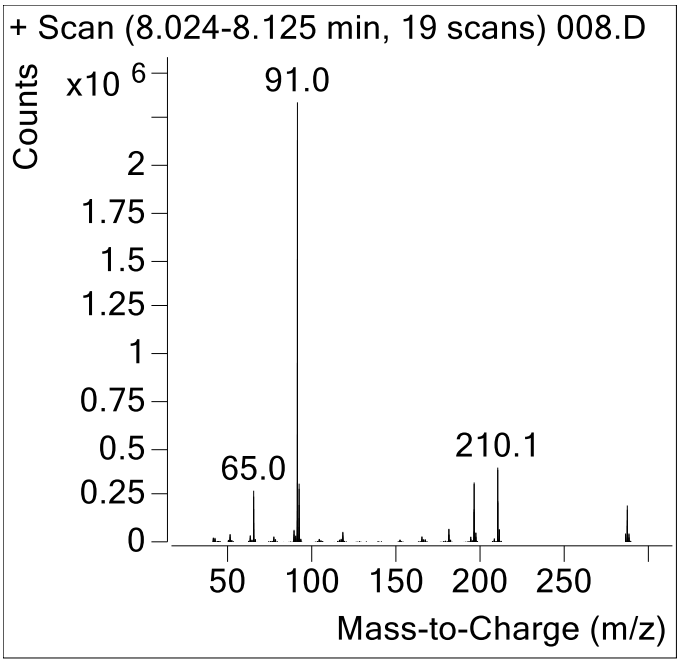
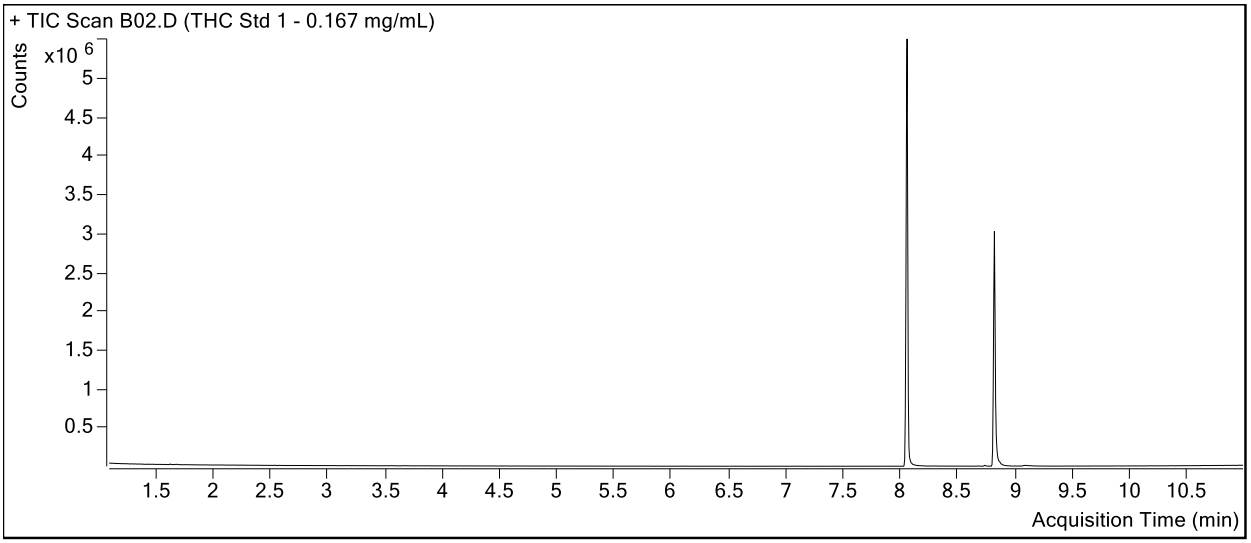


FIGURE 16. THC Standard Positive Identification



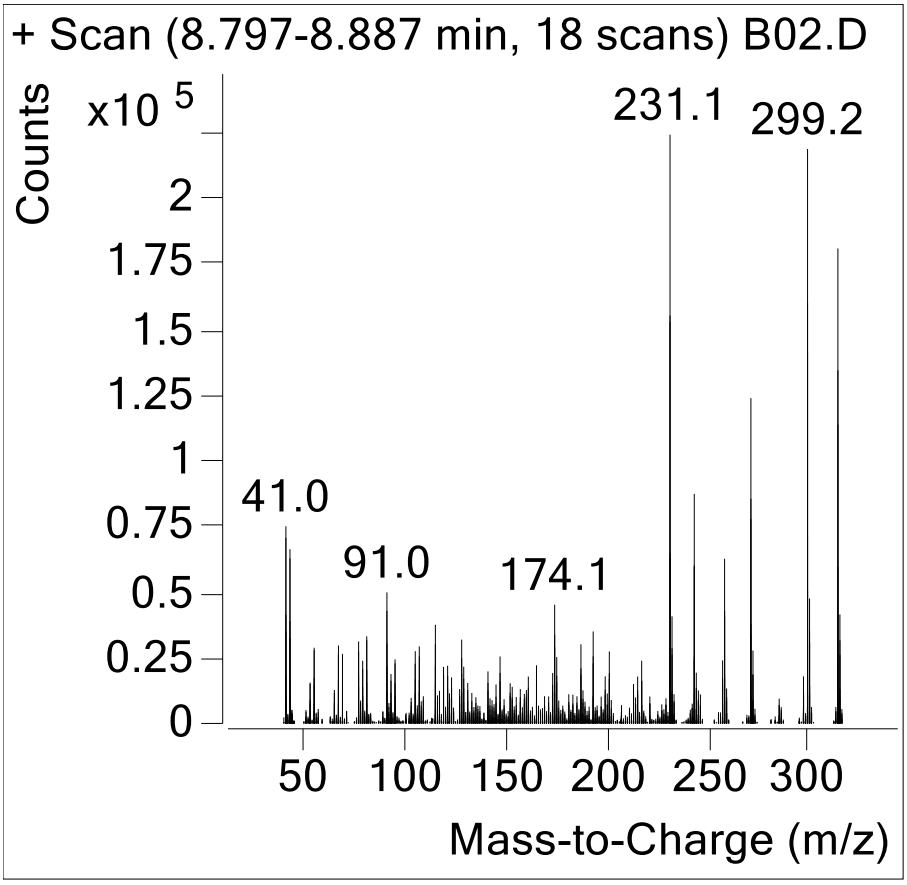
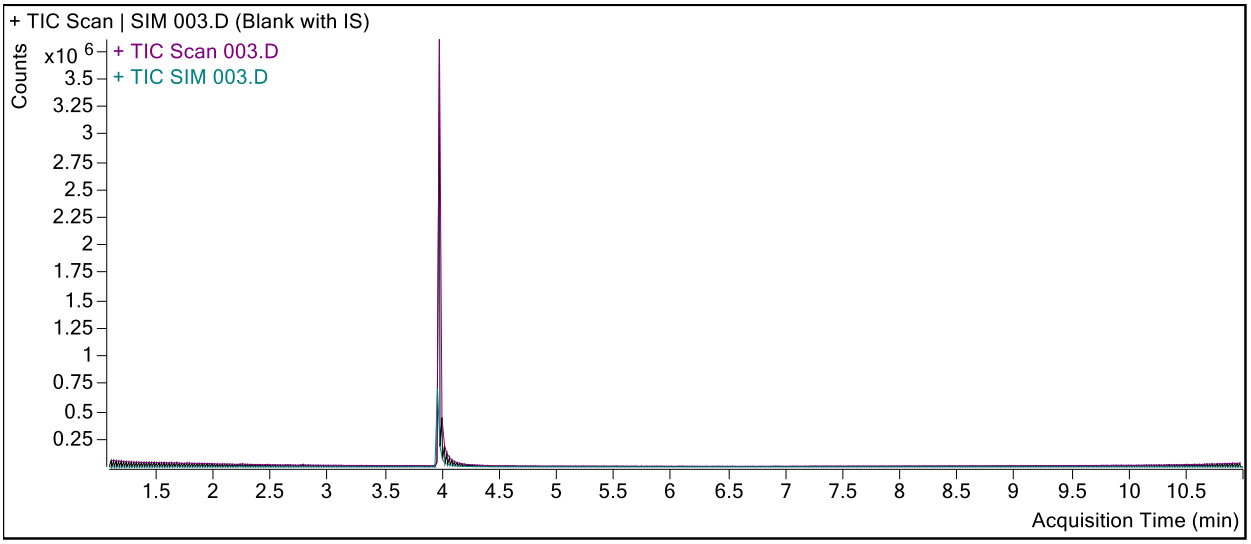


FIGURE 17. Tetradecane Positive Identification


+ TIC Scan | SIM 003.D (Blank with IS)
Counts
x10 6
+ TIC Scan 003.D
+ TIC SIM 003.D
3.5
3.25
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5
Acquisition Time (min)

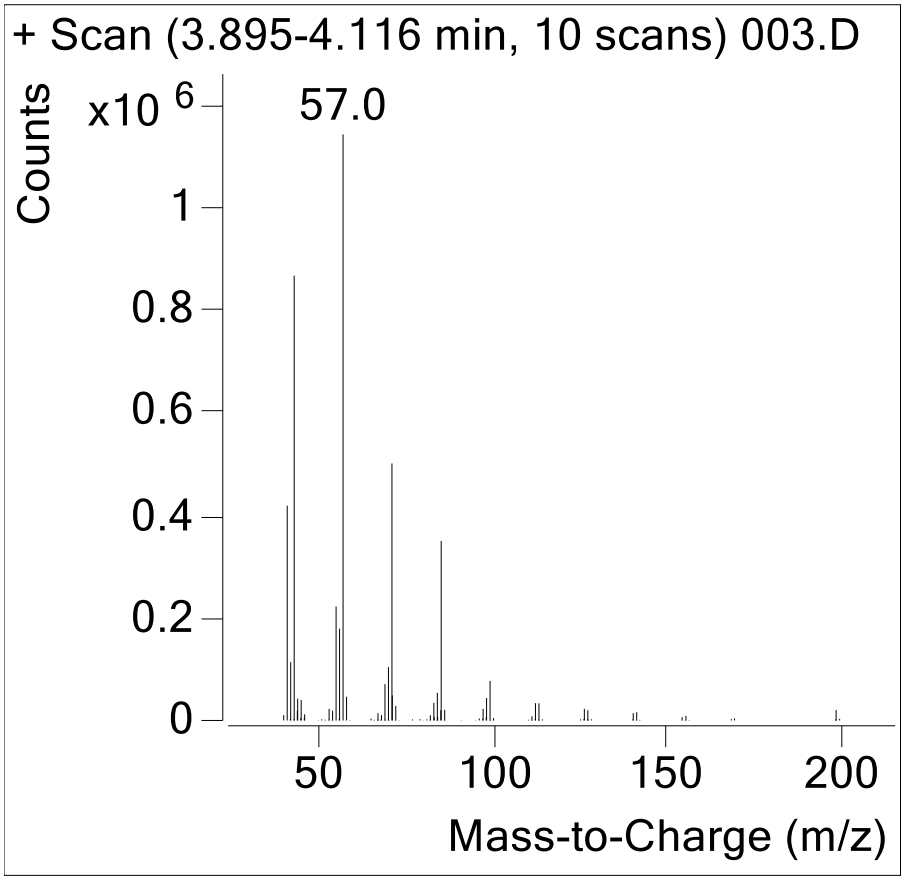
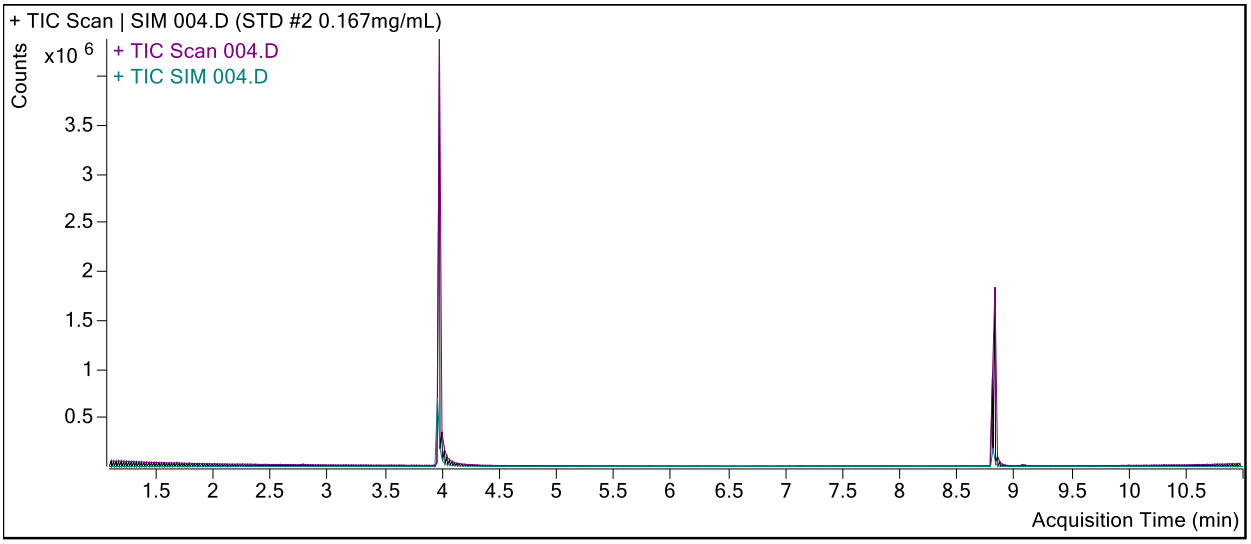
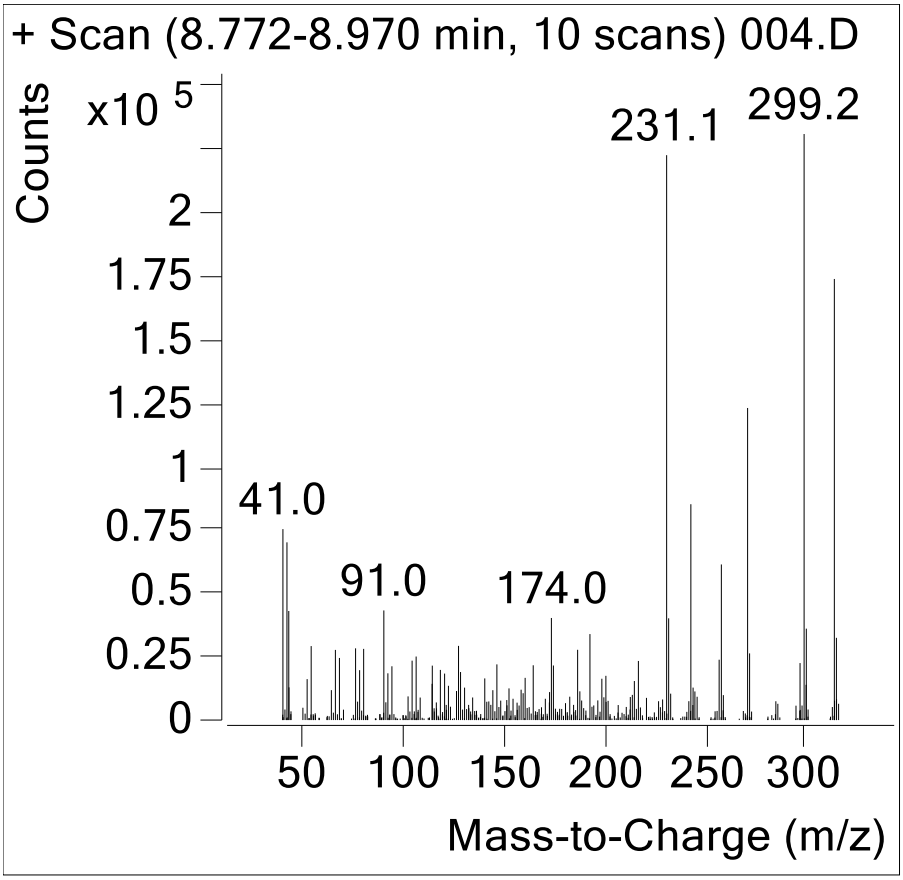

+ Scan (3.895-4.116 min, 10 scans) 003.D
Counts
x10 6
57.0
1
0.8
0.6
0.4
0.2
0
50 100 150 200
Mass-to-Charge (m/z)

FIGURE 18. Tetradecane Internal Standard and THC





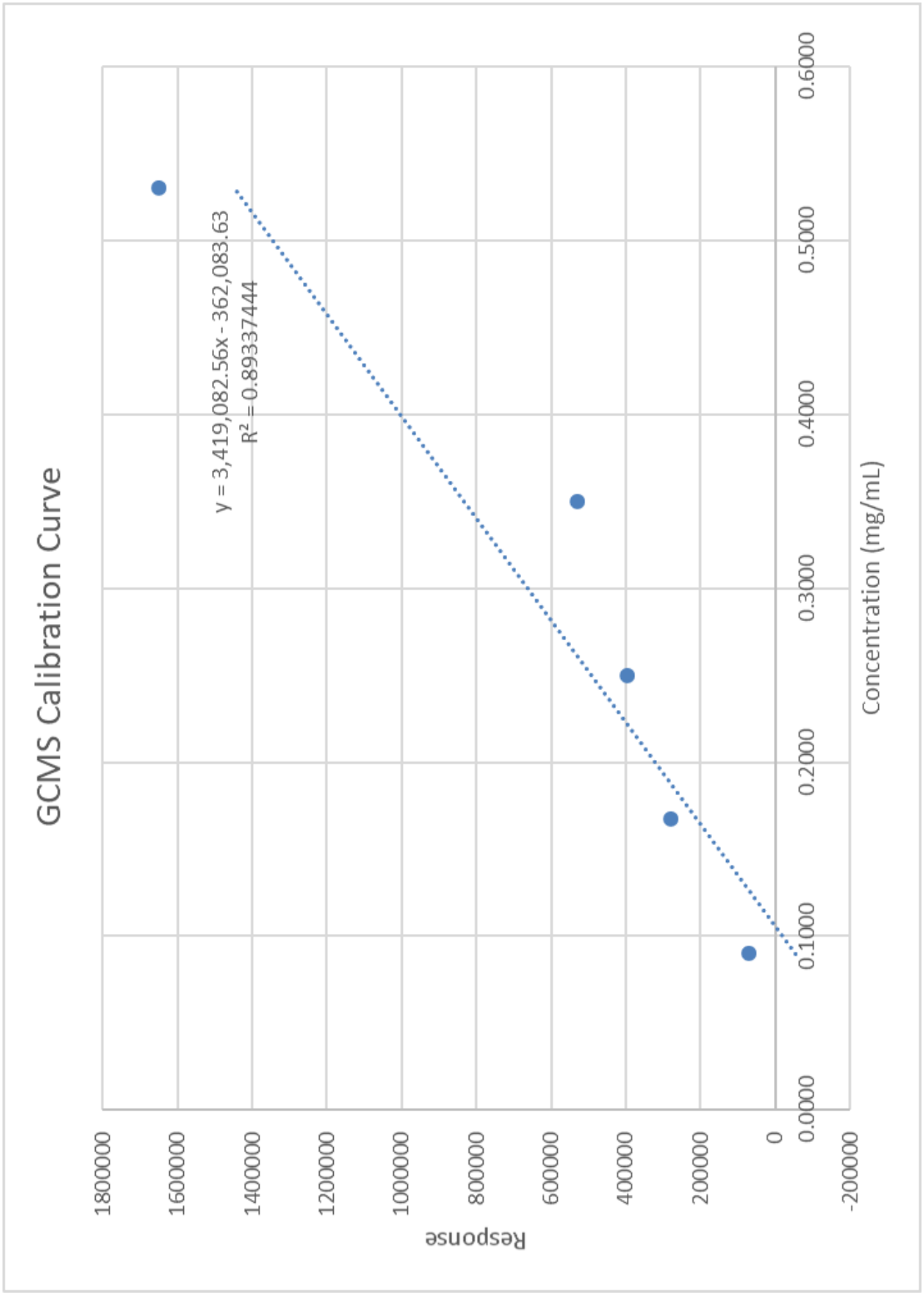


FIGURE 19. Curve of 100 Evidence Samples

FIGURE 20. Chromatogram CBD 1.54, CBN 1.96, THC 2.32

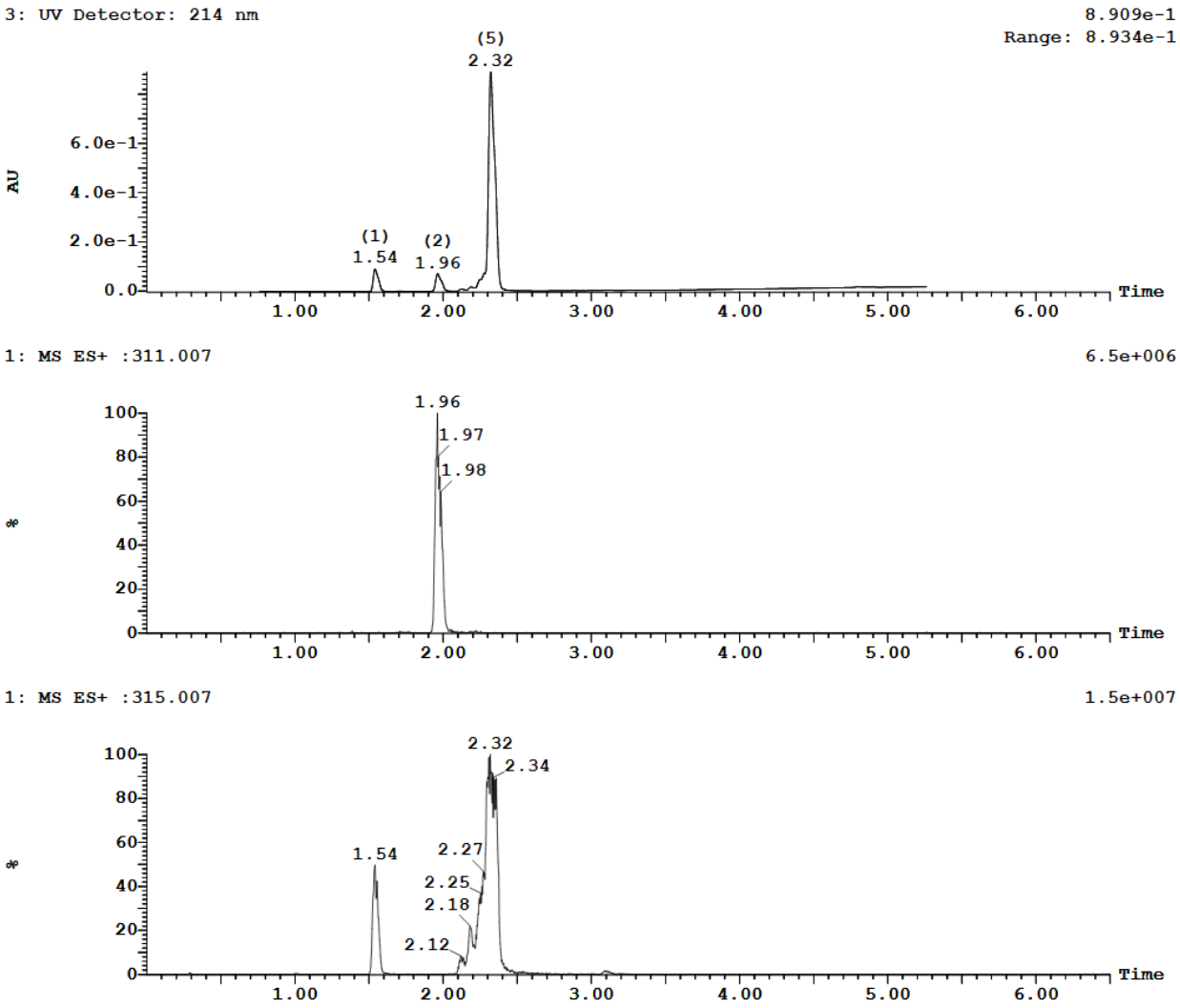
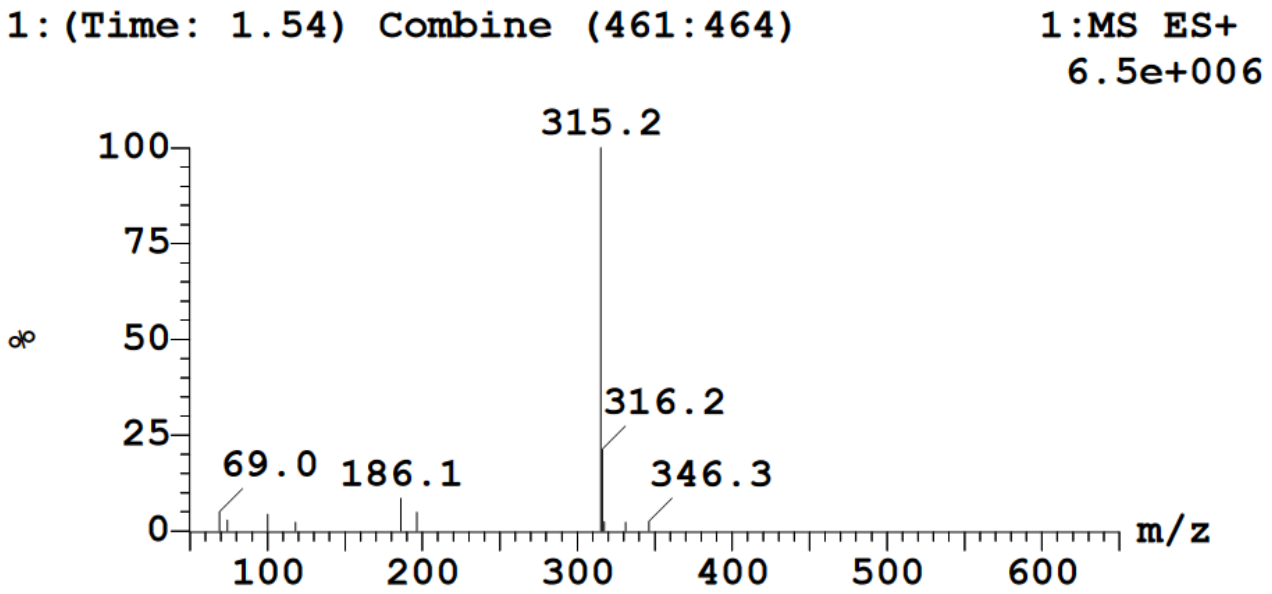


FIGURE 21. Low & High Cone Voltage Spectrum of CBD



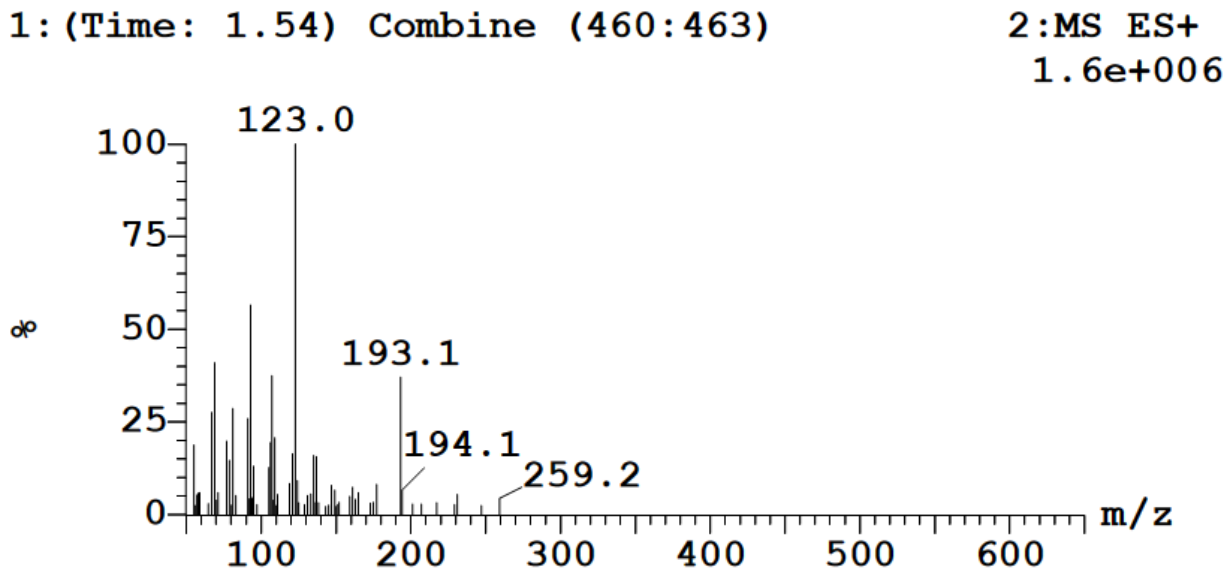


FIGURE 22. High and Low Cone Voltage Spectrum of CBN

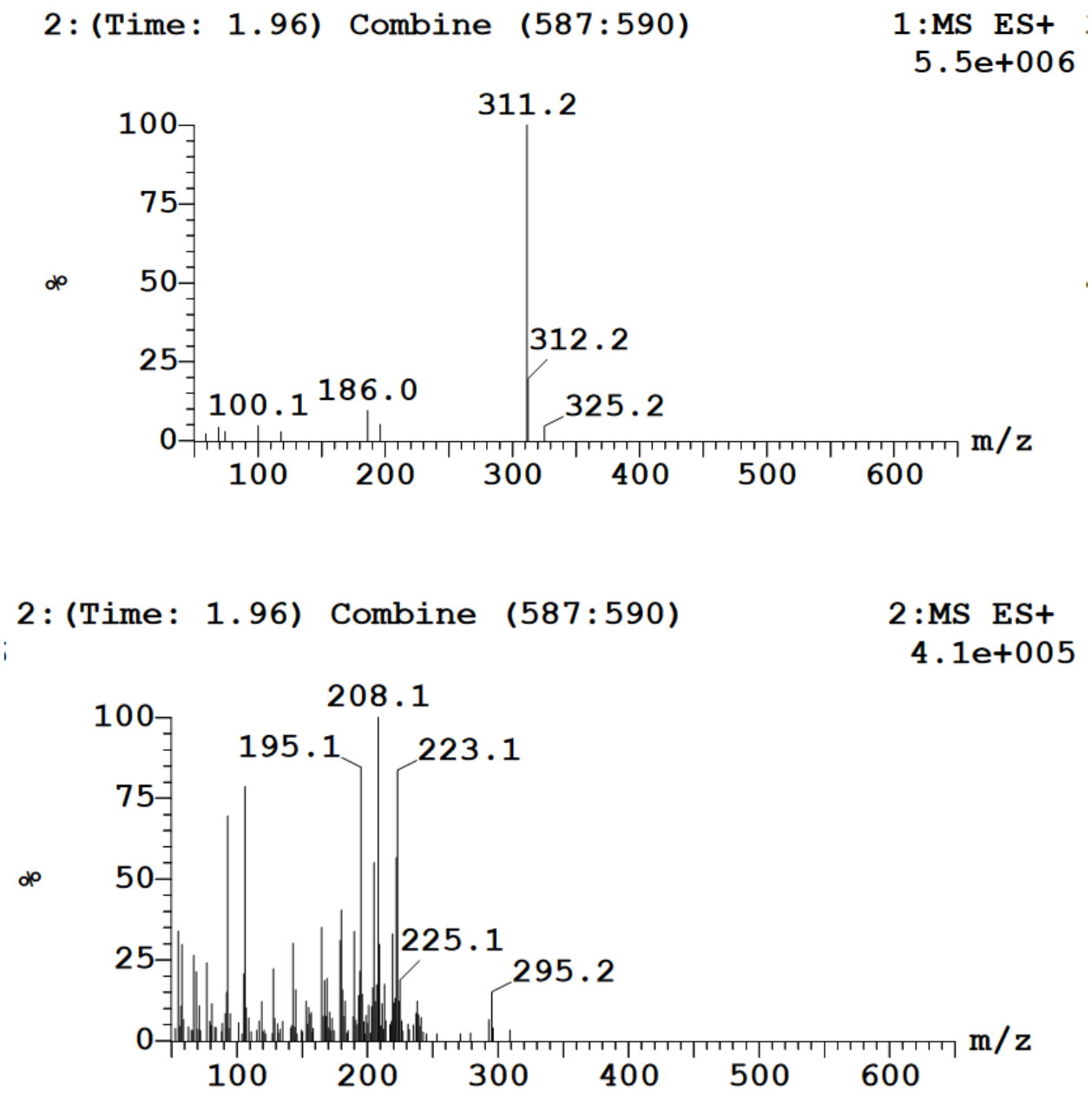
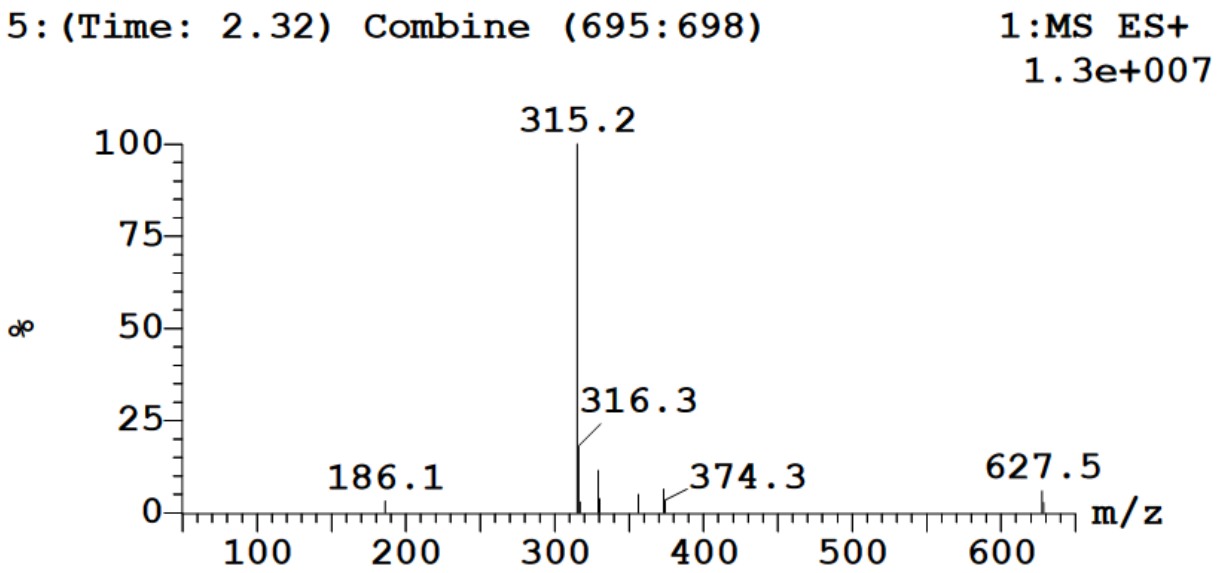
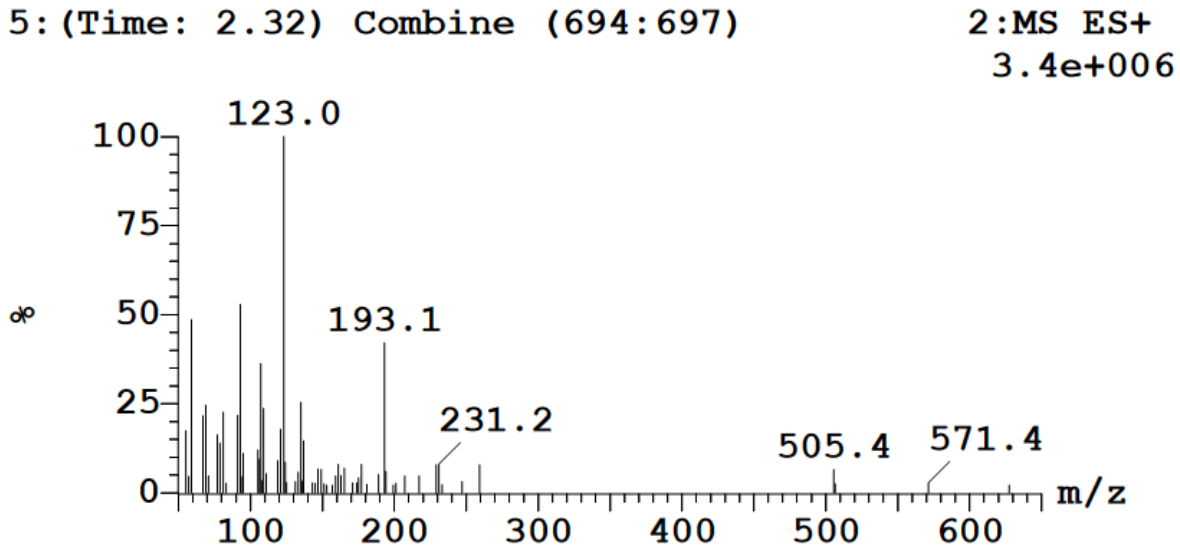


FIGURE 23. High and Low Cone Voltage Spectrum of THC





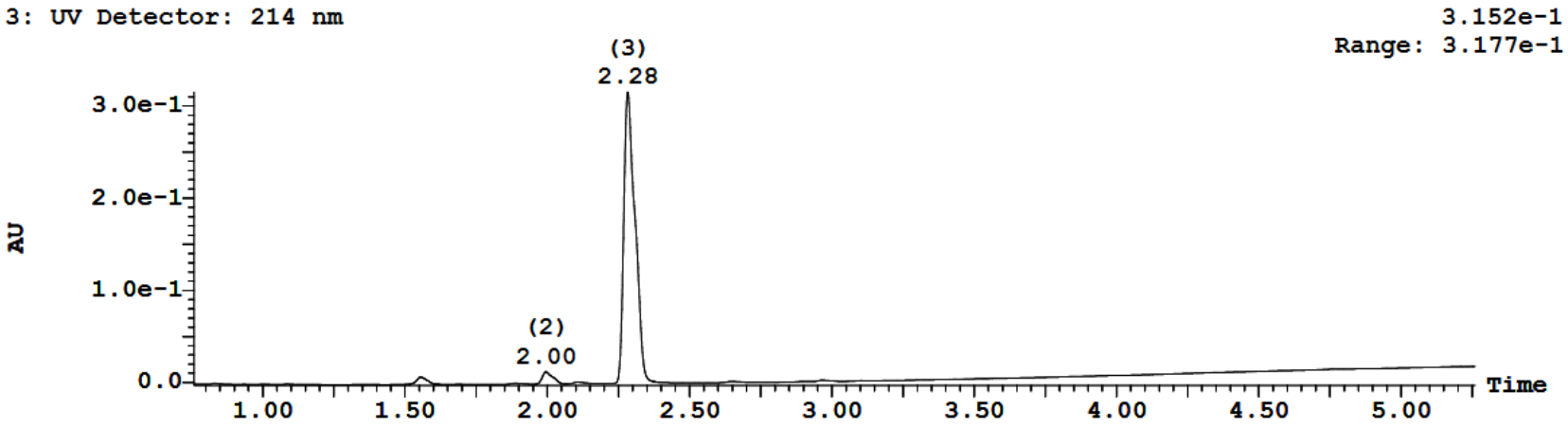


FIGURE 24. Evidence Sample Chromatogram

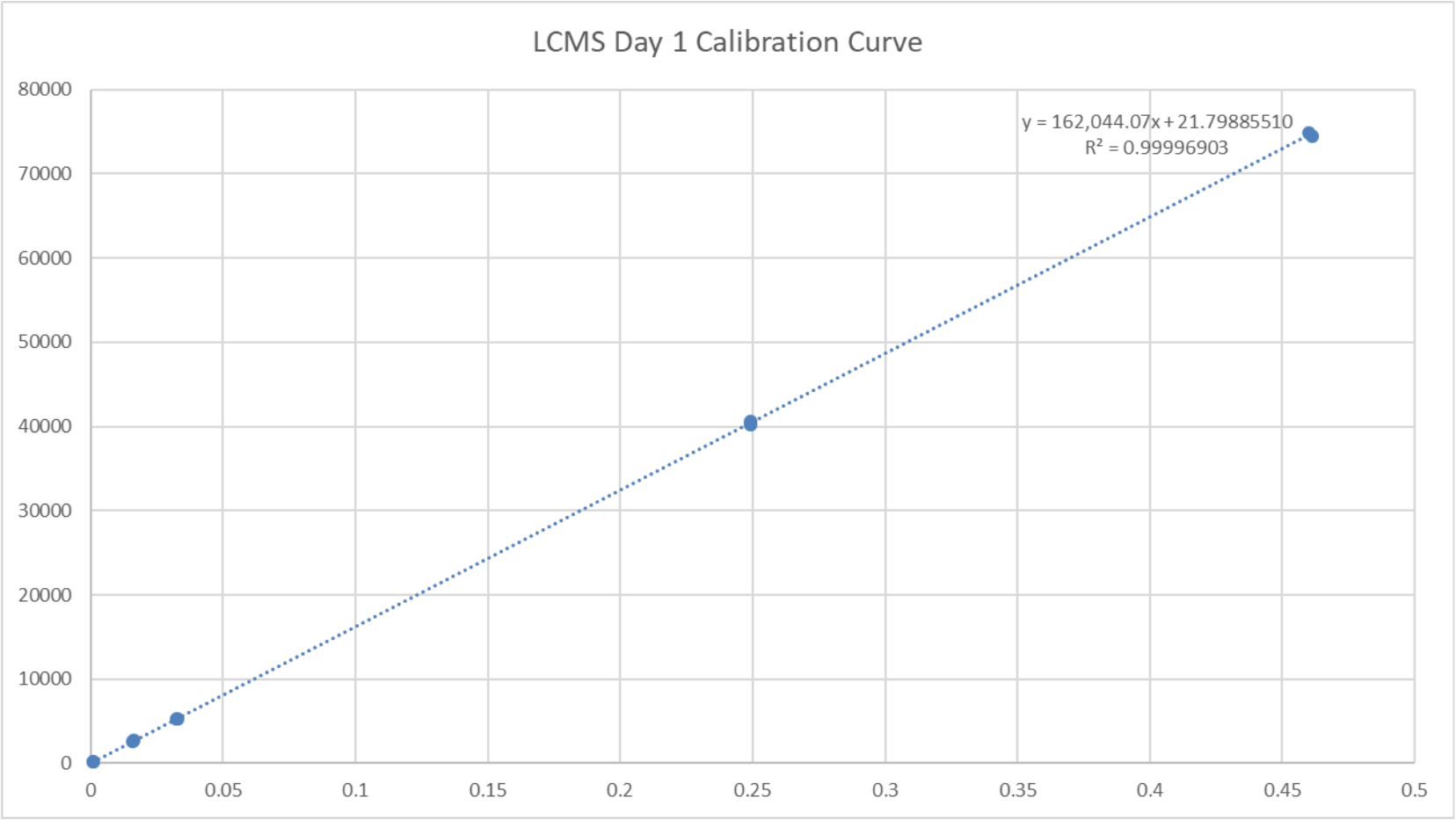


FIGURE 25. LCMS Day 1 Calibration Curve

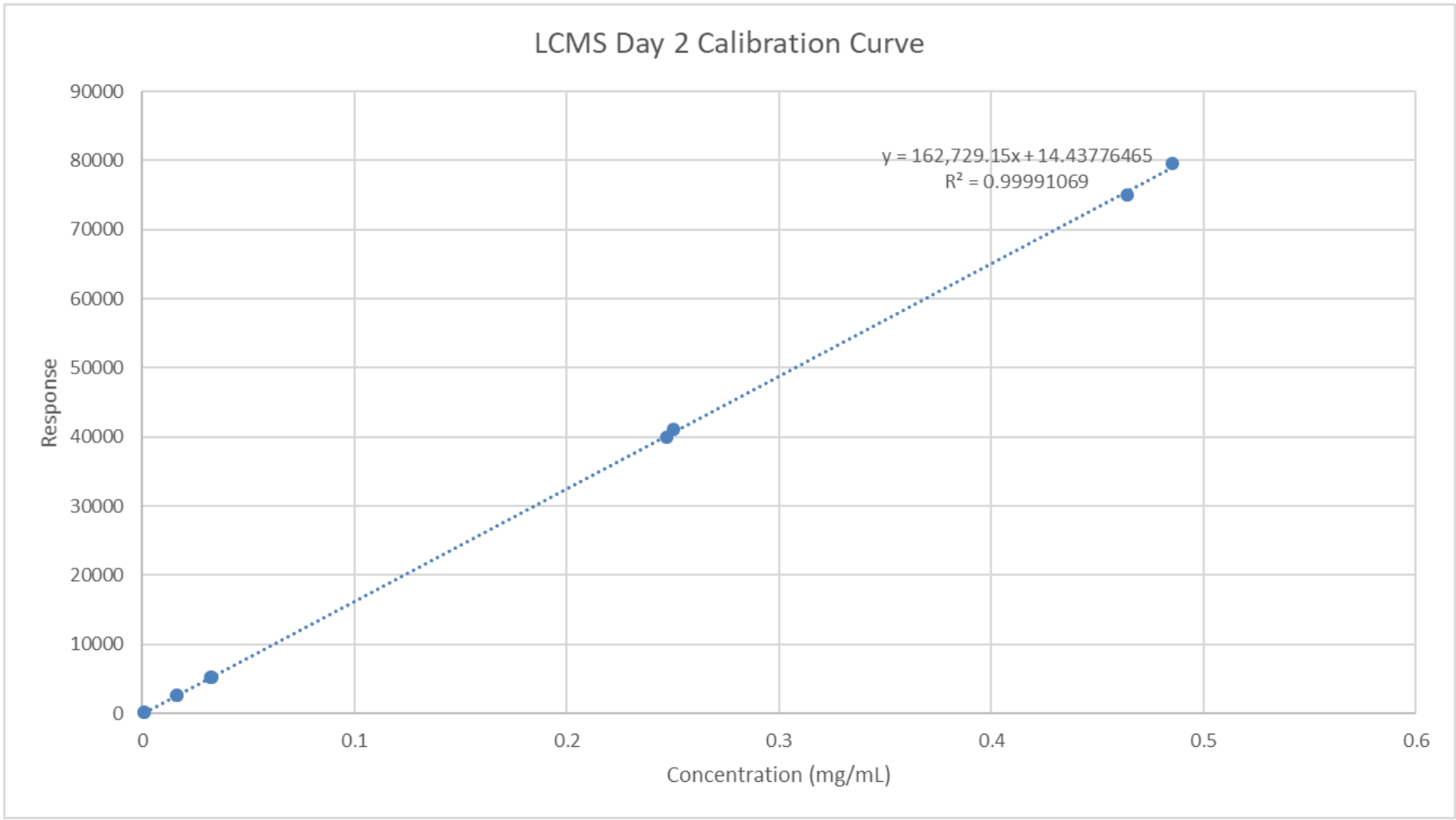


FIGURE 26. LCMS Day 2 Calibration Curve

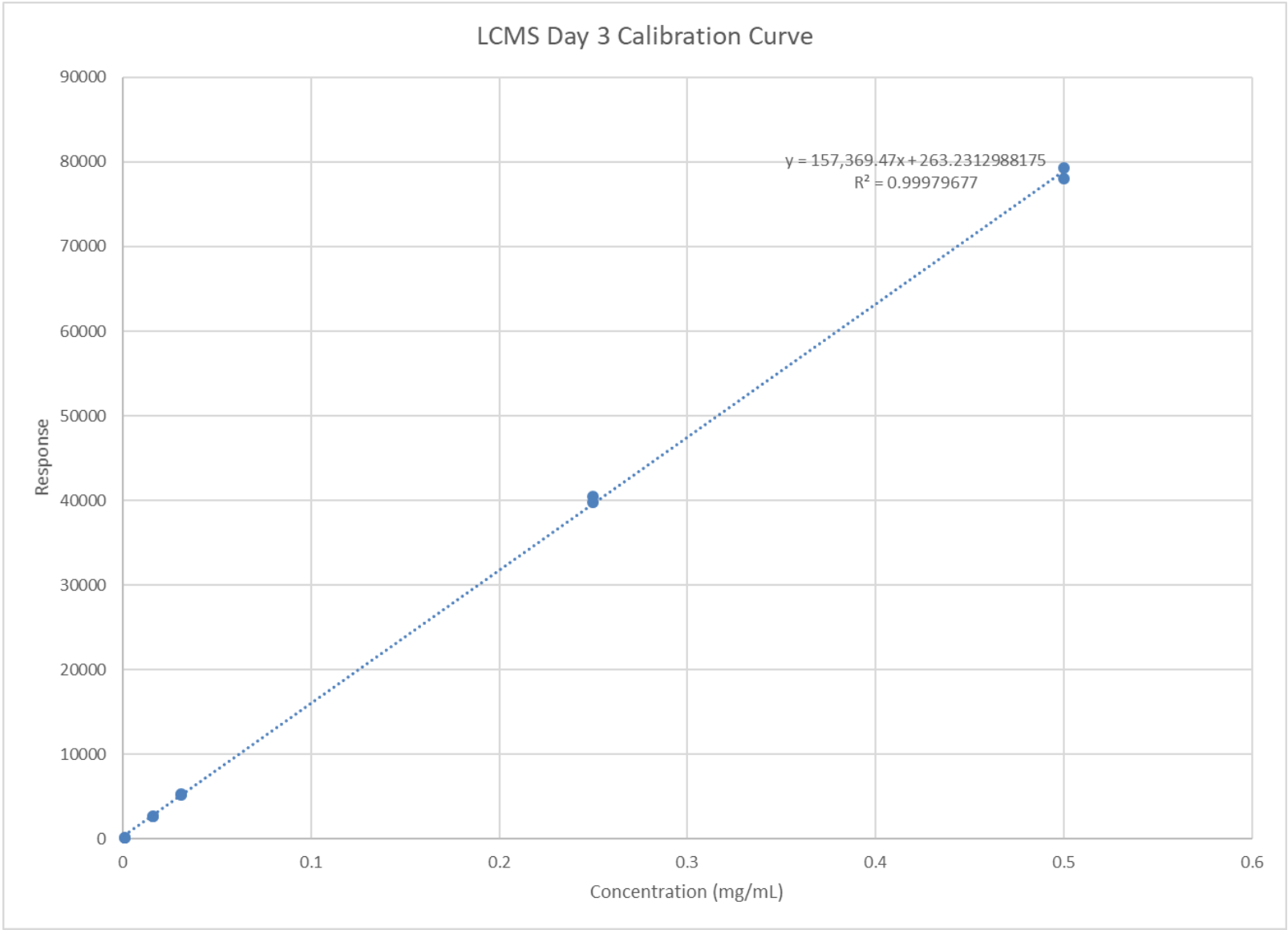


FIGURE 27. LCMS Day 3 Calibration Curve

FIGURE 28. Resin Left Behind in the Grinding Step