Evaluating Water Solubility Enhancement Methods to Increase the Bioavailability of Cannabidiol

By

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(Under the Direction of Dr. Michael Bartlett)

ABSTRACT

Cannabidiol (CBD), the major non-psychoactive component of cannabis sativa, is shown to have a number of therapeutic benefits including pain management, treatment of epilepsy and sleep disorders, and many others. CBD has low bioavailability due its poor water solubility. This study uses ultra-pure and highly stable crystalline CBD manufactured by Purisys Inc. and investigates five approaches to increase the water solubility: (1) addition of a surfactant, (2) particle size reduction, (3) creation of an amorphous solid dispersion, (4) spray drying, and (5) creation of cyclodextrin inclusion complexes. Of the five approaches, the cyclodextrin inclusion complexes had the most significant effect on the CBD water solubility, increasing the water solubility over 7 mg/mL, which is 9,000 times higher than unmodified crystalline CBD. The increase in water solubility allows for more efficacious CBD drug formulations that require less CBD to induce a therapeutic effect, while minimizing potential toxicity and adverse drug reactions. Further research should confirm the results of this study and investigate combining solubility methods like cyclodextrin inclusion complexes with spray drying.

INDEX WORDS: CBD, Solubility, BCS Class II, Amorphous solid dispersion, Cyclodextrin inclusion complex, Spray drying, Particle size reduction

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DEDICATION

This thesis is dedicated to my beautiful wife, Shellie. Without her support and love I would not have completed this research and thesis. She encouraged me to pursue this degree and supported me through its entirety. She motivated me to work when I was lacking the desire and encouraged me to rest when I needed it. She picked up the slack when I was too busy to help around the house. I am deeply thankful for the hours she spent proof reading and helping format this thesis. She had great patience and understanding with me as I had to take many Saturdays and weeknights away from fun activities to work on this project.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Cannabidiol (CBD), the major non-psychotomimetic component of cannabis, has numerous therapeutic benefits from pain management to PTSD treatment.[1,2] Interest in CBD has increased over the past few decades as it has gained popularity as a substitute for traditional opioid and chemical-based drugs. This increase, in part, is due to the lower potential for abuse compared to opioids, while maintaining similar therapeutic effects.

Due to the physiochemical properties of CBD, the compound has very poor bioavailability when taken orally.[3] The poor bioavailability of CBD leads to the majority of the drug administered never reaching the target site, and therefore reducing the potential effects of the drug. To overcome the poor oral bioavailability, formulations are created with higher concentrations of CBD to elicit the desired therapeutic response. This increased active pharmaceutical ingredient (API) concentration alters the risk for toxicity and severe adverse drug reactions.[4,5] Increasing the oral bioavailability of CBD would allow a CBD drug product to be created containing less API, decreasing the potential toxicity while maintaining the therapeutic efficacy.

The majority of previous studies use a hemp-derived or chemically extracted CBD, which has low purity and poor stability. This study utilizes crystalline CBD, synthetically manufactured by Noramco/Purisys, that is highly stable and ultra-pure, which is the optimal API for a CBD finished dosage.

Bioavailability is affected by many factors; this study evaluates those factors and investigates the best path to increase bioavailability. The Biopharmaceutics Classification System (BCS) is the well-known standard for evaluating the physical properties of orally administered drugs, specifically permeability and solubility with considerations of pH and dissolution. This data is used to inform early development of drug formulation and acts as a surrogate for in vivo studies, allowing APIs that meet certain criteria to waive extensive in vivo testing. The BCS classifies CBD as a Class II drug, meaning it has high intestinal permeability and low water solubility. The solubility is assessed over a biologically relevant pH range of 1.2 to 6.8 at 37 ^{O}C, using the highest dose strength of an immediate release product. Highly soluble is defined as the highest dose strength being fully soluble in 250 mL or less of aqueous media over the stated pH range and temperature (Figure 1). Ideally, permeability is informed via human pharmacokinetic studies, but can also be determined by in vitro methods using Caco-2 cells, human colorectal adenocarcinoma cells that model the intestinal epithelial barrier.[6] The efficacy of BCS Class II drugs is limited by the water solubility. There are three general categories of solubility enhancement methods used to address the issues of BCS Class II drugs: physical modifications, chemical modifications, and miscellaneous. Physical modifications include but are not limited to nano-suspensions, particle size reduction, altering polymorphic form, solid dispersions, creation of a co-crystal and emulsions. Chemical modifications include change of pH, salt formation, complexation, and use of buffers. Spray drying and addition of surfactants fall into the miscellaneous category. The goal of this research is to advance the properties of crystalline CBD, specifically water solubility, to make it a viable API for formulation in an oral solid dosage form (OSD) that overcomes CBD's poor oral bioavailability.

CHAPTER 2

BACKGROUND

There are over one hundred phytocannabinoid compounds found in the cannabis sativa plant. CBD and delta-9-tetrahydrocannabinol (D9-THC) are the two most abundant compounds. D9-THC is the primary psychoactive compound associated with the euphoric "high" feeling attributed to cannabis use. CBD, the primary non-psychoactive compound, produces the relaxed, calming sensation associated with cannabis use.[7] Like many of its derivatives and related compounds, CBD has numerous biological effects found to be therapeutic and medicinal. Scientific research is discovering that CBD can aid in treating inflammation, anxiety, sleep, pain, PTSD, epilepsy, and many other ailments.[1,2,8–10] CBD acts as a negative allosteric modulator of the cannabinoid receptor type 1 (CB1) and an inverse agonist of the cannabinoid receptor type 2 (CB2). Both CB1 and CB2 are G-coupled protein receptors (GCPR) that control the release of inhibitory and excitatory neurotransmitters, D9-THC acts as a partial agonist for the CB1 and CB2 receptors. The receptor interactions of CBD inhibit or minimize the effects of D9-THC, so when both are present the CBD competes for the receptor. This results in diminished effects of the D9-THC.[11,12]

The benefits to CBD over traditional opioid or chemical treatments are greatly debated. Many studies show increased therapeutic effects of CBD and other cannabinoids when compared to opioids.[13] It has also been shown that cannabis derived drug products tend to have a lower risk of addiction when compared to opioids.[14] This potential has generated a lot of interest in the current market as awareness of the abuse potential of opioids continues to grow.

CBD is found in many products on the market for non-medicinal designations such as beverages, supplements, ointments, and lotions. The majority of these nutraceuticals claim to exert the anti-inflammatory, anti-anxiety, and stress relieving properties that CBD possesses. There are also numerous ongoing clinical trials for drug products with CBD as the API.[15] However, due to the psychotropic effects of the closely related D9-THC and other phytocannabinoids, regulatory entities have concerns about cannabinoids as APIs. There is not the abundance of conclusive evidence in existence that is required for finished drug products to be FDA approved and allowed on the market. Also, the FDA approval process is long, expensive, and rigorous. Because of this, there is only one FDA approved drug on the market with CBD as the API, Epidiolex, a pediatric epilepsy drug. It is a solution taken orally at 2.5mg/kg twice a day for children 2 years and older.[16] There is a concern about giving young children such a high dose of API. If the bioavailability of CBD could be increased, then less drug would have to be administered, and could reduce the potential for future liver toxicity and other adverse drug reactions.

CBD exists as one of many compounds found naturally in the cannabis plant. The primary way of collecting isolated CBD is through biological or chemical extraction from the cannabis plant. Regardless of process, CBD extracted from the plant has significant variability in purity, low yields, and poor stability, making it expensive and time consuming to generate an isolate with the purity desired to create an FDA approved drug product. An additional obstacle arises from the allowable levels of D9-THC. According to the DEA, CBD must contain less than 0.3% D9-THC.[17] To overcome these issues, Purisys developed a synthetic route of creating crystalline CBD with high purity, over 99%, and a significantly longer shelf-life. The advantages of this crystalline CBD are numerous. The improved purity allows less of the material to be used

in formulations to still reach the desired loading. The stability allows the material to exist unchanged by impurity growth, even at accelerated conditions. The crystalline nature also makes the material very easy to handle and freely soluble in organic solvents, making analysis efficient and straightforward. However, the crystallinity also contributes to the poor solubility of CBD, so formulation solutions need to be applied to reach optimal drug efficacy.

The main obstacle facing a CBD oral dosage form is the low oral bioavailability. Bioavailability of a drug administered orally relies on several factors including drug permeability, dissolution rate, first-pass metabolism, pre-systemic metabolism, and susceptibility to effluent mechanisms.[18] Most of these factors affect the bioavailability of CBD to some degree. When CBD is taken orally it is highly absorbed in the stomach and metabolized by the liver. Many hepatic enzymes act on CBD including CYP1B1, CYP2C19, CYP2C9, and CYP3A4 from the CYP450 family.[12] Very little of the API makes it to the small intestine where it can be absorbed into the blood stream to be distributed to the target delivery site.

As previously stated, multiple factors contribute to poor bioavailability and thus many paths exist to address this issue. This study focuses on increasing the water solubility of CBD as a component of the poor bioavailability. Solubility of a drug is a crucial component in achieving the proper pharmacological effect, which is the result of having the desired concentration of drug in the systemic circulation. Only the drug particles that are fully dissolved will be able to be absorbed and reach the target site.[18] Based on the solubility study reported in the Purisys Pre-formulation Technical Package, CBD is “Practically Insoluble” in water. The United States Pharmacopeia (USP) and British Pharmacopeia (BP) classify “Practically Insoluble” as 10,000 and over parts of solvent required to dissolve 1 part of solute or less than 0.1 mg/mL (Figure 1).

Solubility is based on a solvents ability to interact and act on a solute. The higher the frequency that the solvent interacts with the solute the quicker and more completely it will dissolve.

There are many ways to address water solubility issues. Of the potential pathways, determining which ones to pursue presented a challenge. Evaluating the efficacy of methods in the literature and access to materials and availability of equipment were considered when determining the methods to be investigated for this study. The five methods selected for lab experimentation were (1) the addition of a surfactant to the diluent, (2) particle size reduction, (3) creating of an amorphous solid dispersion, (4) spray drying, and (5) cyclodextrin inclusion complexes. Each of these techniques have shown great success in increasing the water solubility of BCS Class II drugs.

Surface action agents, more commonly known as surfactants, are amphiphilic molecules which means they consist of a hydrophilic head and a hydrophobic tail. Surfactants are classified by the polarity of the hydrophilic head. Neutral heads are non-ionic, positively charged are cationic, and negatively charged heads are anionic. Zwitterionic surfactants also exist with both a positive and negative component. Surfactants break the surface tension of water by aligning themselves with their hydrophilic head in the water and the hydrophobic tail in the non-aqueous environment, whether that is air, oil, or drug. The surface tension of water is very high due to the strong cohesive forces between the water molecules. Surfactant molecules break up these strong forces by creating weaker molecular interactions between themselves and the water molecules. These weaker forces lower the surface tension and stabilize the lower energy interface, and this improves the dissolution of BCS Class II drugs like CBD. When a substantial amount of surfactant is added to the drug and water mixture, the critical micelle concentration (CMC) will be reached. For most surfactants that range is 0.05%-0.10%. Micelles are formed when the

hydrophobic API is surrounded by the surfactant molecules with the hydrophobic heads encapsulating the drug and the tails extending into the aqueous environment.[18,19] This method has proven very successful for many lipophilic drugs like CBD. Surfactants are numerous and found in many household and industrial products like soaps and detergents. Two were selected for this study Polysorbate 80 and Sodium Lauryl Sulfate (SLS). Polysorbate 80, also known as Tween 80, is a non-ionic surfactant used as emulsifier in foods and solubilizer in cosmetics in addition to its surfactant role in pharmaceutical products. SLS is an anionic surfactant with many similar uses as Tween 80 including use in many cleaning and hygiene products like laundry detergent, hand soaps, degreasers and more. These two compounds were selected due to commonality, availability, and efficacy in the literature.[20,21]

There is a strong inverse correlation between the particle size of a material and its solubility. Generally, as particle size decreases, solubility increases. This is due to a couple of primary factors. First, as previously stated, the solubility of a material is based on the ability of the solvent to act on the solute. Decreasing particle size increases the number of particles in the solvent, thus, increasing the frequency of interactions between solvent and solute. Smaller particle size also increases surface area allowing for more area for the solvent to act on the material.[22] There are many ways to reduce the particle size of an API. The most common is manual milling by mortar and pestle. More advanced techniques utilize grinding mills, such as jet mills, Fitz mills, or hydraulic mills like a colloid mill.

Solubility of a material is also greatly affected by its crystal structure. Crystalline material is comprised of neatly ordered molecules formed by a lattice structure. The molecules are held together by strong intermolecular forces that keep the molecule in a low energy state. For a crystalline material to dissolve, the crystal lattice must be broken, which takes a substantial

amount of energy. The energy required to break that crystal lattice is what causes the poor solubility of crystalline materials, because it is more energy efficient to stay in the crystalline form (Figure 2B). It has been shown that disrupting this rigid crystalline structure by forming an amorphous solid can significantly increase solubility[23]. Amorphous materials are a glassy-like solid with no order in the arrangement of the molecules. The amorphous nature of the material requires significantly less energy to dissolve the molecules since there is no crystal lattice to protect it. The glassy or amorphous state of a material is achieved by forcing the disruption of the intermolecular bonds and keeping the material from recrystallizing to the more stable crystalline state. This can be achieved in multiple ways. The most straight forward path is by heating the material to a molten high state then rapidly cooling the drug past the glass transition state. This inhibits the drug from cooling slowly and naturally recrystallizing (Figure 2A). The low energy barrier between the amorphous and crystalline form of the API results in very poor stability of the amorphous form because the drug prefers to be in the crystalline form.[24] Amorphous drugs require a stabilizing agent to increase the energy barrier between the amorphous state and the crystalline state. Polymers are used in addition to the API to create an amorphous solid dispersion (ASD). The presence of the polymer increases the glass phase transition energy thus stabilizing the ASD. There are many ways to create an ASD. The two ways investigated in this study are by the melt-quench method and spray drying. The melt-quench method uses the basic process described previously. A mixture of API and polymer is heated until the API is fully melted. Next, the molten drug-polymer mixture is supercooled past the glass transition point, creating an ASD. [25]

Spray drying involves a different chemical process to create an ASD. At its most basic form, spray drying uses an API-polymer mixture dissolved in a solvent or mixture of solvents,

known as a feed solution. That feed solution is then sprayed as a fine mist into a chamber filled with heated gas. As the hot gas evaporates the solvents in the mist, the resulting solid amorphous particles fall to the bottom of the chamber. Those particles are filtered based on particle size and collected. There are numerous specialized parts and settings for each component of the process as well as apparatuses with varying features.[26] Spray drying apparatuses vary in size from bench-top models to multi-story industrial models. All the parameters of the spray drying process can be customized based on the properties of the drug formulation and desired product characteristics, pending instrument capabilities. Compared to the other solubility enhancement methods investigated in this study, spray drying has the largest cost in regard to energy required and overall price of operation. Spray drying has been very successful in overcoming the solubility issues for BCS Class II drugs.[27–29] This technique was selected for this study due to proven success as well as access to a bench top spray dyer at the University of Georgia.

Another proven method in successfully increasing the water solubility of BCS Class II drugs is the creation of cyclodextrin inclusion complexes. Cyclodextrins (CD) are cyclic oligosaccharides made up of D-glucopyranose units, that exits in six-, seven-, or eight-unit toroidal shaped structures referred to as α, β, and γ CDs, respectively (Figure 3). The units are linked by α-(1,4) bonds and can be easily modified by methylation or hydroxypropylation for increased specialization of the CD. The exterior of the cone is hydrophilic due to the free hydroxyl groups and the inside, comprised of oxygen atoms and hydrogen atoms, creates a hydrophobic pocket that allows lipophilic molecules like CBD to hide within the pocket of the CD protected from the aqueous environment. The hydrophilic outer shell of the CD increases the interaction of the water molecules with the CD inclusion complex, thus, increasing the solubility of the API contained inside the complex.[30] The methylated and hydroxypropylated beta and

gamma CD have shown increased solubility when compared to unmodified beta and gamma CDs.[30,31] Five CDs were selected to compare the effects of each on the water solubility of CBD: beta, gamma, hydroxypropyl-beta, hydroxypropyl-gamma, and methyl-beta. Due to the small cavity size of the alpha CD, it is often too small to hold larger drug molecules like CBD, so it was not investigated in this study.[31]

CHAPTER 3

MATERIALS AND METHODS

Ultra-pure crystalline CBD was manufactured under GMP regulations and provided by Noramco/Purisys in Athens, Georgia. Excipients were kindly donated by the University of Georgia College of Pharmacy, specifically the labs of Dr. Gurvindar Singh Rekhi and Dr. Michael Bartlett located in Athens, Georgia. The Tween 80 was manufactured by Sigma Aldrich, headquartered in St. Louis, Missouri. Sodium Lauryl Sulfate was manufactured by Fisher Chemical in Hampton, New Hampshire. Polyvinylpolypyrrolidone (PVPP) was manufactured by EMD Millipore headquarters located in Burlington, Massachusetts. The cyclodextrins were manufactured by Cerestar locate in Hammond, Indiana. All solvents used in formulations and HPLC mobile phase preparations were ultra-pure, HPLC grade from various manufacturers. Water was pulled fresh daily from a regularly calibrated Elga purification system. All instruments, solvents, glassware and apparatus were property of Purisys and utilized in Purisys labs. All analytical instruments were within qualification at the time of use. Qualifications were performed by a vendor representative annually or quarterly.

Ultraviolet and Visual Spectrometry (UV/Vis)

Solutions were placed into Standard Q 10mm Quartz glass cuvettes and analyzed at an absorbance range of 190 nm to 400 nm. This range was selected to determine the primary and secondary lambda max values to be utilized for analysis of the formulated solutions. An Agilent 8435 spectrophotometer was used with ChemStation analysis software. In preparation for

analysis of CBD formulations by UV/Vis, a linearity standard of CBD in methanol was prepared and analyzed under the same UV/Vis conditions. Crystalline CBD was weighed into a glass volumetric flask creating a stock solution for each concentration of the linear curve. The stocks were then diluted to working solution concentrations of 5 μg/mL, 10 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL, and 300 μg/mL. A pure methanol blank was used as a 0 μg/mL CBD solution. This linear curve was prepared and run in triplicate for accuracy.

Differential Scanning Calorimetry (DSC)

A TA Instruments Q100 DSC was used for analysis. The method consisted of a 5 degree Celsius per minute ramp from 40^{O}C to 200^{O}C to determine the melting point and analyze the crystalline properties of different formulations. DSC operated under the same method parameters was also utilized to determine the compatibility between CBD and the excipients used in the formulations.

X-Ray Powder Diffraction (XRPD)

A Bruker D92 phaser was used to determine polymorphic form, as well as, to determine if the formulations were amorphous or crystalline. The instrument was scanned from 0 to 40 degrees theta. Parameters were optimized as a part of a Purisys method.

High Pressure Liquid Chromatography (HPLC) with Variable Wavelength Detector

An Agilent HP 1100 HPLC with Variable Wavelength detector or an Agilent 1260 was used with a diode array detector (DAD). The instrument was operated using a method validated by Purisys for CBD analysis. The solvent system consists of a formic acid in water for the aqueous

phase and a methanol and acetonitrile mixture for the organic phase. The stationary phase consisted of a Waters XBridge C18 column with a length of 150 mm, inner diameter of 3.0 mm, a particle size of 3.5 μm and a pore size of 130 Å. The column was heated to 40^{O}C. The detector monitored analyte absorbance at a wavelength of 215 nm.

Ultra-High Pressure Liquid Chromatography (UPLC) with Quadrupole Dalton (QDa) Mass Detector

A Waters H Class UPLC with a DAD and QDa detector was used with the same solvent system and parameters as mentioned above. An Acquity UPLC BEH Shield RP18 column was used with a length of 100 mm, inner diameter of 2.1 mm, a particle size of 1.7 micrometers and a pore size of 130 Å.

Surfactant Addition

Aqueous diluents were prepared volumetrically with two different surfactants, Sodium Lauryl Sulfate (SLS) and Tween 80. Each surfactant was prepared at two different concentrations of 0.07% and 0.14% weight by volume. A calibration curve was prepared by dissolving CBD in methanol at concentrations of 5 μg/mL, 10 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL, and 300 μg/mL. This calibration curve was analyzed by UV/Vis spectrometry. The primary lambda max for CBD was determined to be approximately 208 nm and the secondary lambda was approximately 274 nm. Absorbance was collected at both the primary and secondary lambda max values for this calibration curve. The calibration standards were prepared and run in triplicate. Those numbers were averaged and used to generate a multi-point calibration curve for each lambda max. This calibration curve was used to quantify the concentration of the API in the CBD in water with surfactant formulations. The CBD in surfactant diluent solution stocks were

heated at 37 ^{O}C for 24 hours and analyzed via the parameters stated previously to evaluate the effects of heat on the solubility of CBD in the presence of surfactants.

Particle Size Reduction by Milling

A jet mill was used with settings determined by Purisys as a part of a CBD milling study. The material processed by the jet mill was selected out of the milling study because it resulted in the greatest reduction in particle size. For analysis, CDB was added to water to create a stock solution at a concentration of 1 mg/mL for both the milled CBD and unmilled CBD, for comparison. Additionally, a three-point calibration curve of the milled CBD in methanol was prepared at 10 μg/mL, 25 μg/mL and 100 μg/mL. The milled and unmilled CBD stocks were evaluated via UV/Vis to determine the level of solubility before further dilutions to working solution concentration levels were made. Due to very low absorbance of the CBD in the stock solutions, the stocks were analyzed on an UPLC with QDA according to the method parameters previously discussed. An aliquot of both stocks was heated at 37^{O}C overnight to analyze the effects of heat on the solubility. The solubility of both stock solutions and the milled CBD calibration curve were calculated from the CBD calibration curve used in the surfactant study.

Melt-Quench Amorphous Solid Dispersion

A mixture of 65:35 of CBD and PVPP was prepared by mixing the two components by mortar and pestle. The mixture was placed into a stainless-steel reactor and heated by a heating mantle to 150^{O}C. The temperature was held at 150^{O}C for 10 minutes allowing the CBD to completely melt. The vessel was rapidly cooled using an ice bath for 30 minutes forcing the CBD into an amorphous solid. The solid product along with the starting materials and pre-melted mixture

were evaluated by DSC and XRPD to determine the crystalline properties. The amorphous solid was prepared at approximately 1.0 mg/mL in water. This mixture was allowed to sonicate for 5 minutes, then stir for 1 hour to allow for dissolution. The water solubility of the solid product was originally analyzed using UV/Vis. No absorbance was detected at either lambda max (208nm or 274 nm). An aliquot of sample was taken and placed into storage at 5 ^{O}C. The bulk mixture was placed at 37^{O}C to stir overnight. Both mixtures were analyzed by HPLC due to the higher sensitivity of HPLC over UV/Vis spectrometry. The previously stated HPLC method and parameters were used for analysis. The solubility of the milled and unmilled stocks were calculated using the CBD in methanol calibration curve from the milling study.

Spray drying

Spray drying was evaluated with intended use on a Buchi B290 Mini Spray Dryer instrument, with use provided by the University of Georgia College of Pharmacy. The solubility of CBD was investigated in solvent mixtures of no more than 20% organic solvent, due to the restrictions of the instrument. First, 100 mg of CBD was dissolved in 20 mL of organic solvent then 80 mL of water was added. The organic solvents used were acetone, ethanol and methanol. Next CBD was mixed with one of three polymers commonly used to stabilize spray drying feed solutions, hydroxypropyl methylcellulose (HPMC), Kollidon 30, and Kollidon V64. The mixtures were then dissolved by acetone and water was added to reach a final ratio of 20:80. A final approach was tested by dissolving the polymer in water and CBD in acetone, then gradually adding the two mixtures together.

Cyclodextrin (CD) Complexes

Five different types of Cyclodextrins (Beta, Gamma, Hydroxypropyl-Beta, Hydroxypropyl-Gamma, and Methyl-Beta) were evaluated for CBD solubility enhancement. All CBD:Cyclodextrin complexes were prepared by mixing each of the cyclodextrins with water to create a 200mM solution. An excess of CBD (500 mg) was added to 5 mL of CD preparation and stirred for approximately 24 hours at 37^{O}C. The mixtures were centrifuged, and the supernatant was filtered through a 0.45um PTFE filter. The filtrate was placed in an amber HPLC vial and analyzed via the validated CBD HPLC method. A CBD standard was prepared at 0.3 mg/mL in methanol for calculation of the CBD solubility levels of the mixtures. The CBD peak for Hydroxypropyl-Beta (HP-Beta) CD:CBD sample and the Methyl-Beta CD:CBD sample overloaded the detector. The two samples were diluted by half and analyzed again via HPLC. The diluted Methyl-Beta CD:CBD was still too concentrated and overloaded the detector, so it was diluted again by half and analyzed via HPLC.

CHAPTER 4

RESULTS AND CONCLUSIONS

A baseline of CBD properties such as purity, solubility, melting point, particle size etc, was taken from the Noramco Cannabidiol Technical Package. Additional information not included in the tech package such as XRPD, DSC, and UV/Vis spectra of the raw API was collected as a part of this research. CBD concentration for analysis was calculated by using the desired drug loading of a CBD formulation and sensitivity of the UV/Vis instrumentation. The final concentration range was determined to be 5 micrograms per milliliter to 300 micrograms per milliliter. A research progression approach was implemented in this study evaluating one method to increase the water solubility of CBD then moving on to subsequent methods pending the success of the previous method.

The linear standard curve for UV/Vis analysis made from CBD in methanol was prepared and run in triplicate. The absorbance values produced from the three runs were averaged to create a curve used to calculate the concentration of CBD in trial formulations. Analysis of these CBD standard preparations on an absorbance range of 190 nm to 400 nm showed CBD had a primary lambda max around 208 nm and a secondary lambda max around 274 nm (Figure 4). At higher concentrations, 50 μg/mL and higher, the absorbance of CBD saturated the detector at the primary lambda max. At those higher concentrations the secondary lambda max, 274 nm, was used for calculations. The average absorbance of CBD was plotted against the corresponding concentrations to create an Absorbance vs Concentration curve to be used for quantifying the amount of CBD in formulations preparations. A graph was created for the primary lambda max,

excluding concentrations that overloaded the detector and for the secondary lambda max. A trend line was generated for each plot. The correlation coefficient for the line generated from the primary lambda max was 0.9669 (Figure 5). The correlation coefficient for the secondary lambda max was 0.9999 (Figure 6).

Addition of Surfactants

The first method evaluated was the addition of a surfactant to the dissolution media (water). The amphiphilic nature of the surfactants lowers the interfacial tension between the hydrophobic CBD and the water because the intermolecular forces between the surfactant and water molecules are much lower than between two water molecules. This reduction in the energy barrier allows for greater access of the diluent to the drug thus increasing wettability. SLS and Tween 80 were two commonly used surfactants to increase dissolution of BCS Class II drugs. A concentration of 0.07% and 0.14% w/v was found to be a common concentration of surfactant used in the literature to increase compound solubility. The solutions were monitored by UV/Vis at 225 nm. This wavelength was selected originally because it was the detection wavelength chosen in the validated CBD HPLC method and this analysis was conducted prior to determining the lambda maxes. Analysis of the CBD diluted in water with surfactant did not show a significant increase in CBD solubility for either surfactant at either concentration. The solutions prepared from the heated stock also did not show any significant increase in solubility. Due to negligible solubility increase with the addition of a surfactant to the dissolution media, alternative approaches were pursued.

Particle Size Reduction by Milling

The next approach investigated the effects of particle size on water solubility of CBD, more specifically if there was an inverse correlation between water solubility and particle size. It has been shown with specific compounds that a decrease in particle size increases the water solubility of that compound due to a few factors including increased surface area, thinner diffusion layer, and others. A milling study was previously conducted by Noramco scientists to determine the ideal mill type and parameters to efficiently reduce the particle size of CBD. The milling study used CBD material from the same GMP synthetic process that is used in this water solubility study. Multiple mills with various instrument parameters were evaluated in the study. The particle size from each trial was determined by a Malvern particle size analyzer using a validated method. For the purpose of this study, the material with the smallest particle size was analyzed for water solubility. A L1A Fitzmill/Jet mill with optimized parameters yielded the smallest particle size from the study. Rarely does a material exist in a completely uniform size, rather the particles exist in a distribution or range of sizes similar to a bell curve (Figure 7). Ideally that range would be very small, reflecting the most uniform size distribution possible. Particle size measurements are reported using the letter D and a percentage, normally D10, D50, and D90. These numbers indicate what percentage of the material is less than or equal to a specific particle size. For example, a D90 of 100 μm means that 90% of the material analyzed is 100 μm or less in diameter. When compared to the particle size of the unmilled CBD, the milled CBD had a five times reduction in D90, four times reduction in D50 and three times reduction in D10. The 1 mg/mL stock solution of the milled CBD was analyzed via UV/Vis and compared to an unmilled mixture of CBD in water at the same concentration. There was no clear absorbance at either the established primary or secondary lambda max values, 208 nm and 274 nm

respectively (Figure 8). A three-point calibration curve of milled CBD in methanol (10 μg/mL, 25 μg/mL, and 100 μg/mL) was also evaluated via UV/Vis to determine the effects of milling on CBD solubility in an organic solvent. (Table 1). The 1 mg/mL stocks were placed in a 37^{O}C oven overnight to investigate the effects of heat on the solubility of the milled CBD.

The samples were then run on an Acquity H-Class LC with QDa due to the higher sensitivity of the LC instrument and method. A QDa detector was used to positively identify the CBD peak in water in case of any analyte-solvent interactions that may have altered the retention time of the CBD peak. It was determined there was no shift in CBD retention time due to the aqueous solvent when compared to the CBD in methanol standard injections (Figure 9). The increased sensitivity of LC did show absorbances of both the milled and unmilled CBD in water. For the purpose of quantitation, a calibration curve at concentrations of 5 μg/mL, 10 μg/mL, 25 μg/mL, 50 μg/mL, and 100 μg/mL was prepared using the unmilled CBD in methanol and analyzed via LC (Figure 10). First, there was no significant increase in solubility of the milled CBD in methanol when compared to the unmilled CBD in methanol (Table 2). Solubility for the four sample preparations; milled RT, milled 37^{O}C, unmilled RT and unmilled 37^{O}C; was calculated based on the above stated calibration curve (Table 3). There was no significant increase in solubility of the unmilled 37^{O}C when compared to the RT. There was also no significant increase in solubility of the milled CBD at RT when compared to the unmilled CBD at RT. However, there was a two-fold increase in solubility of the milled CBD at 37^{O}C when compared to the other preparations. Even though there was an increase in solubility, the milled CBD at 37^{O}C was still considered insoluble in water by USP solubility standards previously shown in Figure 1.

Melt-Quench Amorphous Solid Dispersion

Altering the physical structure of CBD from a crystalline compound to an amorphous compound was the next method evaluated. PVPP was chosen as the stabilizing polymer in the CBD ASD. The melt-quench processes yielded a solid with a balm-like consistency. The CBD:PVPP ASD was analyzed via DSC to ensure it was amorphous. The individual components (CDB and PVPP) along with the pre-melted CBD:PVPP mixture were also analyzed via DSC and XRPD for comparison (Figure 11, 12). The DSC thermogram and XRPD diffractogram both confirm that CBD was crystalline, PVPP was amorphous, and the mixture had crystalline CBD still present. The thermogram of the ASD showed that the material was a majority amorphous, yet a very small endothermic event was present around the CBD melting point. This could be the result of some crystalline CBD that did not fully melt during the process or that recrystallized during the quench. The ASD was not analyzed via XRPD due to the balm-like consistency of the material as well as the limited amount of ASD produced. Once the amorphous nature was confirmed the ASD was analyzed for water solubility. 100 mg of the ASD was weighed into a volumetric flask with approximately 80 mL of water added. This preparation was placed in a sonicator for 5 mins to aid in dissolution. The preparation then stirred for 1 hour after which water was added to volume to create a 1mg/mL solution. The sample was analyzed via UV/Vis however no peaks were observed at either of the CBD lambda maxes (208 nm and 274 nm). (Figure 13) An aliquot was taken and stored at ambient temperature while the stock was placed in an oven at 37°C overnight. Both the ambient temperature (RT) and the 37°C samples were analyzed by HPLC (Figure 14). The concentrations of both samples were calculated using the peak areas gathered from the HPLC data and the standard curve equation from the milled CBD HPLC analysis. The solubility of CBD in the ASD showed an increase when compared to the unmilled and milled

CBD solubility. The RT ASD sample showed a six-fold and a five-fold increase when compared to the unmilled CBD and milled CBD, respectively, at the same conditions. The 37°C ASD showed an eight-fold and three-fold increase in solubility when compared to unmilled and milled CBD, respectively, at the same conditions. (Table 4) In spite of the large increase in solubility relative to the previous methods, a solubility of less than 3 μg/mL for a stock prepared at 1 mg/mL is still considered practically insoluble by USP criteria.

Spray Drying

The first approach to create a viable CBD spray drying feed solution proved unsuccessful as the CBD precipitated out of solution as the water was added. The CBD:polymer mixtures also did not fully dissolve in the 20% organic solvent mixture. Similarly the third approach of added the polymer dissolved in water to the CBD dissolved in acetone resulted in incomplete dissolution. It was determined that the water solubility of CBD was insufficient for creating a feed solution of only 20% organic solvent. The addition of an inert loop, Buchi B290 additional modification that allows the use of higher organic solvent in the feed solution, was evaluated. The cost of the inert loop was out of the scope of this project. Due to the restrictions of the instrument available, spraying drying could not be fully evaluated as a method for increasing the water solubility of CBD.

Cyclodextrin (CD) Complexes

The final approach evaluated was the creation of cyclodextrin inclusion complexes with CBD. Cyclodextrins are cyclic oligosaccharides that form a toroid configuration that allows hydrophobic compounds to be encapsulated within the cyclodextrin cavity. CDs are also

hydrophilic which causes an increase in the water solubility of the hydrophilic compound inside of the CD. After preparation and analysis of the five CBD:CD complexes via HPLC, it was found that the modified CDs; HP Beta CD, HP Gamma CD, and CH3 Beta; led to a significant increase in water solubility when compared to the unmodified CBD in water as well as the other solubility enhancement methods (Table 5). The CBD:HP Beta CD increased the water solubility of CBD the most with a solubility of over 7 mg/mL, more than nine thousand times higher than the milled CBD at 37^{O}C (0.76 μg/mL) and almost three thousands times higher than the ASD at 37^{O}C (2.44 μg/mL). The CBD:HP Gamma CD had a CBD solubility of 673 μg/mL and the CBD:CH3 Beta CD had a solubility of over 4 mg/mL.

Based on these results the CBD:HP Beta CD showed a significant increase in solubility, bringing CBD solubility from practically insoluble to slightly soluble. Despite the solubility range still being less than optimal for CBD, somewhere in the soluble to freely soluble range, increasing the solubility in such a drastic way was meaningful progress in the goal of increasing the bioavailability of CBD. This progress shows the possibility to increase the solubility of CBD to a level that decreases the potential toxicity from having to administer such a highly concentrated dose to achieve therapeutic effect. In short, we could be able to administer a drug product with less CBD while still eliciting a therapeutic effect. Despite this progress there is still substantial amount of work to reach the goal of highly soluble CBD.

CHAPTER 5

FUTURE DIRECTIONS

This study demonstrated an increase of the water solubility of CBD; however, more research is needed on how to further increase the solubility of CBD. The next step in this research would be to evaluate precision and increase robustness of the CD inclusion complex data. Performing multiple preparations and analyses of the previously conducted experiments would be the first step in addressing robustness. This would confirm the data generated and demonstrate precision of the preparation technique and analytical method. Increasing the preparation replicates would also highlight any variability in the interaction between the CBD and the CDs. The primary reported solubility data must be confirmed before moving forward. Researching other CDs is another future direction that would be beneficial in selecting a specific cyclodextrin that results in the highest CBD loading, thus, increasing water solubility to the maximum level possible. Once the optimal CD is selected, the formulation and process should be optimized. Experimenting with various ratios of CBD to CD in the formulations, different temperatures and reaction times would be used to optimize the CBD loading of the CD inclusion complex. Another area worth investigating is the combination of reduced particle size with the CD inclusion complexes. The increased solubility from smaller particles could have a combined increase when used with the CD complexes. Alternatively, the size of the CD cavity may be the limiting factor in CBD solubility thus the particle size of the API would not have an effect on solubility. This would be done by comparing the solubility of milled CBD:CD inclusion complexes to unmilled CBD:CD at various CBD loading concentrations.

Pursuing spray drying more in-depth would be another possible future direction. As stated previously, spray drying shows significant improvements in the water solubility of BCS Class II drugs. It would be beneficial to experiment with a spray drying instrument equipped with the inert loop attachment which allows above 20% organic solvent in the feed solution. As previously demonstrated, CBD requires higher organic solvent present in the diluent to be fully soluble. Also, combining cyclodextrin inclusion complexes with SDD could help to overcome the initial feed solution solubility issue that was discovered in the initial SDD lab work. It is possible that the increased solubility of CBD from the CD inclusion complexes would lend to sufficient CBD solubility in an 80:20 aqueous to organic solvent solution that is required for a spray drying instrument without the inert loop attachment.

As literature shows, there are numerous ways that the water solubility of BCS Class II drugs has been increased. This study assessed four of them, with promising results using the cyclodextrin approach. Any of the previously stated solubility enhancement methods, such as creation of a co-crystal, a nano-suspension, or prodrug, altering polymorphic form, could be pursued as future directions for this research.

CHAPTER 6

LIMITATIONS

Many of the limitations were discussed along with the future directions. The primary limitation is the lack of robustness. The majority of solubility enhancement methods were only performed once or at one concentration or using a single set of parameters. Each technique should have been thoroughly investigated to determine its effects on the water solubility of CBD, whether in a positive or negative way. With access to more time and resources, each component within each method of the study would have been performed in triplicate and the results averaged to ensure accuracy. Increasing testing replicates would also allow for statistical analysis to be performed on the data to evaluate data precision by producing standard deviation between the replicates. For the surfactant study specifically, increasing robustness would have consisted of investigating more surfactants at various concentrations based on literature research. Another improvement for the surfactant study would be analyzing how the addition of an excess amount of CBD in the stock preparations affected the overall solubility. As this would not be a long-term solubility solution due to very poor yield, it could give more insight on how drug solubility with surfactants is affected by the amount of API present. Failing to further investigate the formation of micelles was an additional limitation of this study.

Robustness was also the major limitation for the particle size study. Preparing the stock solutions in triplicate is needed to ensure accuracy and precision. Also, evaluating CBD material with several different particle sizes would allow a correlation to be made between particle size and solubility.

There were many areas for improvement and robustness in the ASD formulation. Multiple polymers and multiple formulation compositions should have been evaluated. Also, the temperature conditions for the melt and the quench could have been optimized. This could be done by designing a set of experiments that use various melting temperatures, hold times, quenching temperatures, and quench times to determine the optimal parameters to create an ASD. Additionally, using an apparatus where the state of the CBD mixture could be visually confirmed would have ensured complete uniform melting of the API before the vessel was quenched. As stated previously, all aspects of the study should have been performed in triplicate at a minimum to ensure accuracy and precision.

The majority of the limitations of the CD component of this research are mentioned as future directions, those being robustness, evaluation of drug loading, optimization of reactions times and temperatures, and investigation of various types of CDs.

FIGURES AND TABLES

Figure 1. Biopharmaceutics Classification System (BCS) solubility definitions[32]

Descriptive Term (Solubility Definition)	Parts of Solvent Required for One Part of Solute	Solubility Range (mg/mL)	Solubility Assigned (mg/mL)
Very soluble (vs)	<1	>1000	1000
Freely soluble (fs)	from 1 to 10	100–1000	100
Soluble (s)	from 10 to 30	33–100	33
Sparingly soluble (sps)	from 30 to 100	10–33	10
Slightly soluble (ss)	from 100 to 1000	1–10	1
Very slightly soluble (vss)	from 1000 to 10000	0.1–1	0.1
Practically insoluble (pi)	>10000	<0.1	0.01

Figure 2.(A)-Thermodynamic relationship of crystalline and amorphous state (Glass) as a function of temperature. (B)- Different energy states of crystalline drug, amorphous drug, and amorphous solid dispersion. (C)-Amorphous solid dispersion produces a stable supersaturation state under non-sink conditions of micro-dissolution study, while more energetic amorphous form of drug dissolves rapidly however is not stable and gradually crystalizes toward the most stable crystalline state (Lu et al., 2016).[33]

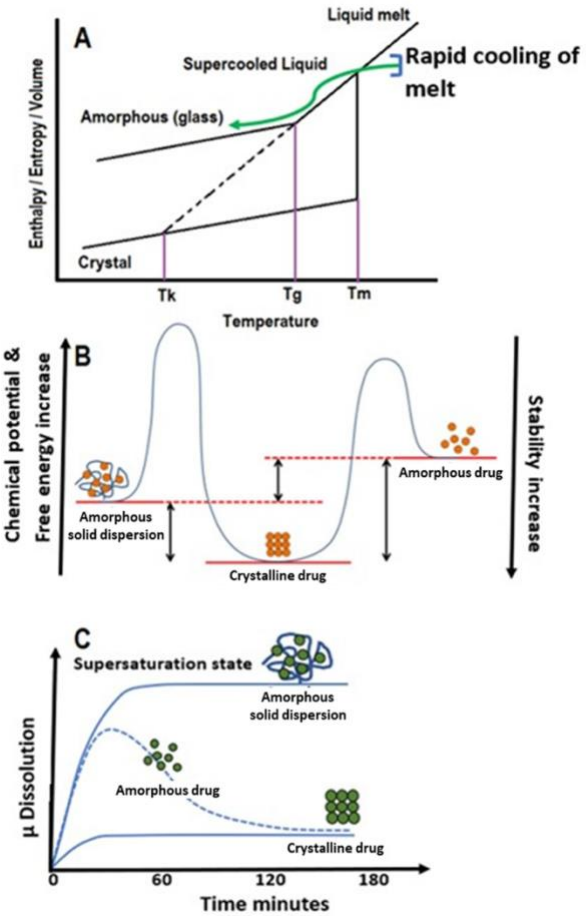


Figure 3. Cyclodextrin (CD) structures of (a) the monomer unit, (b) the overall shape, and (c) the structure of alpha, beta, and gamma CD.[34]

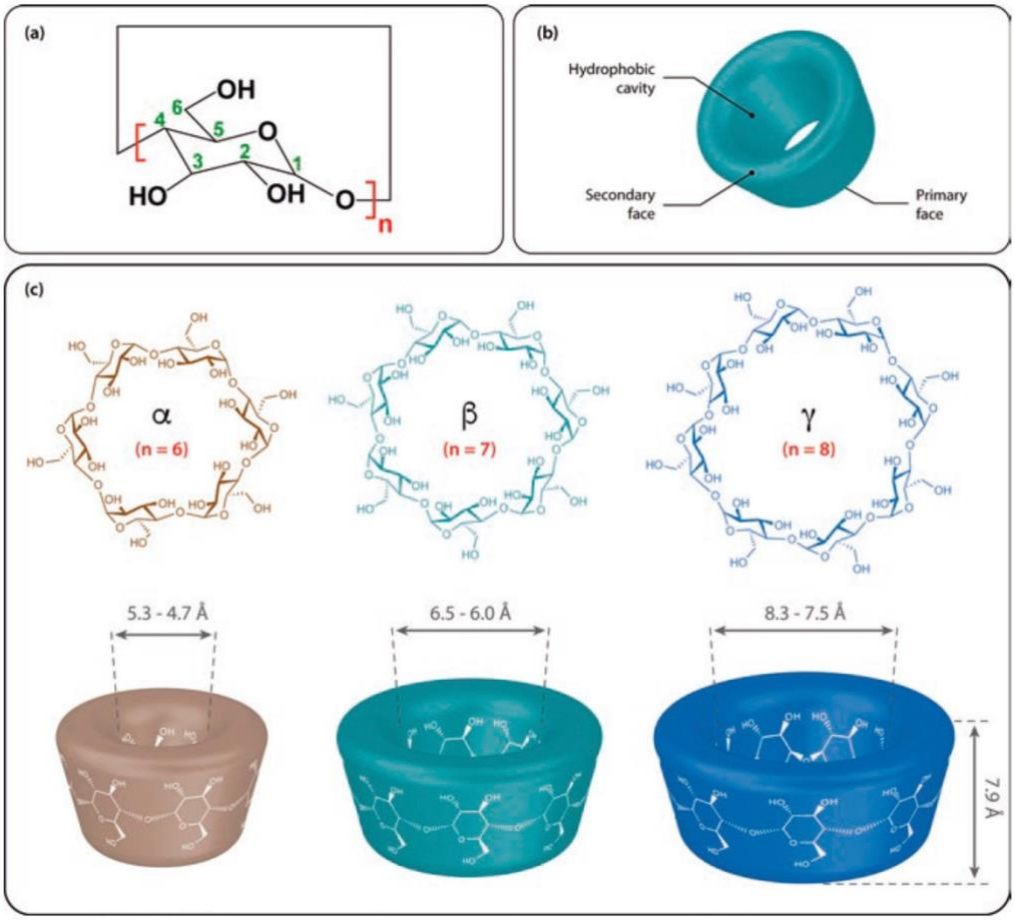


Figure 4. UV/Vis spectrum of CBD dissolved in Methanol standard curve. Concentrations 0 µg/mL to 300 µg/mL.

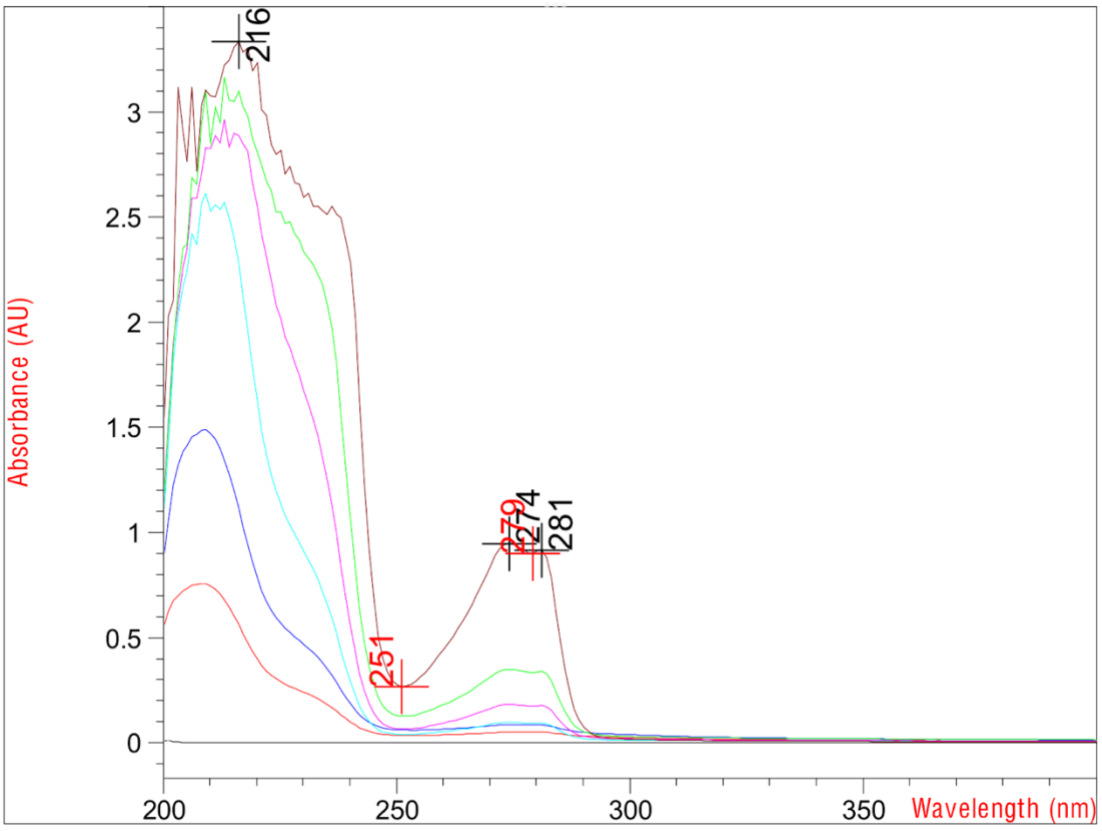


Figure 5. Linearity of CBD via UV/Vis at primary lambda max of 208 nm

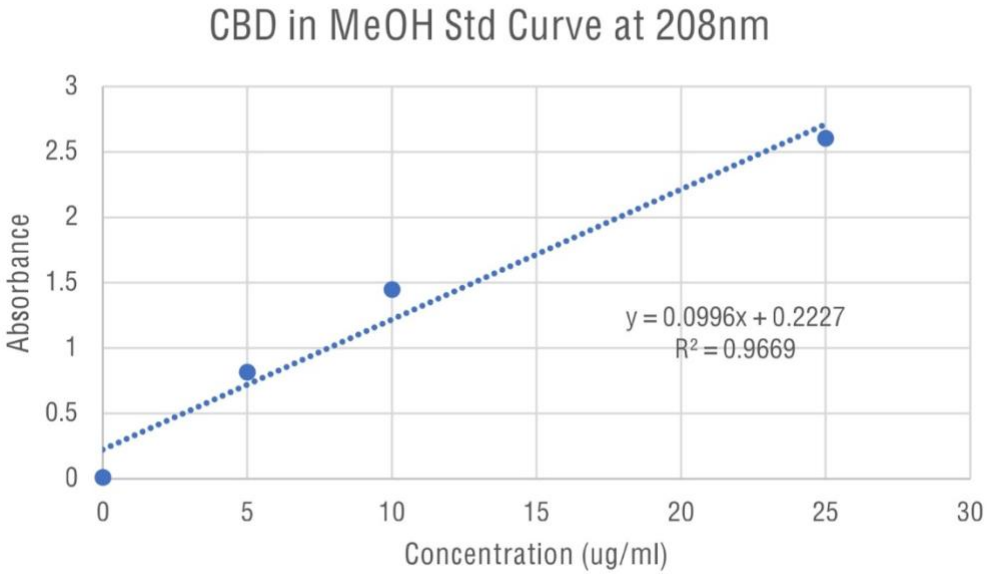


Figure 6. Linearity of CBD via UV/Vis at secondary lambda max of 274 nm

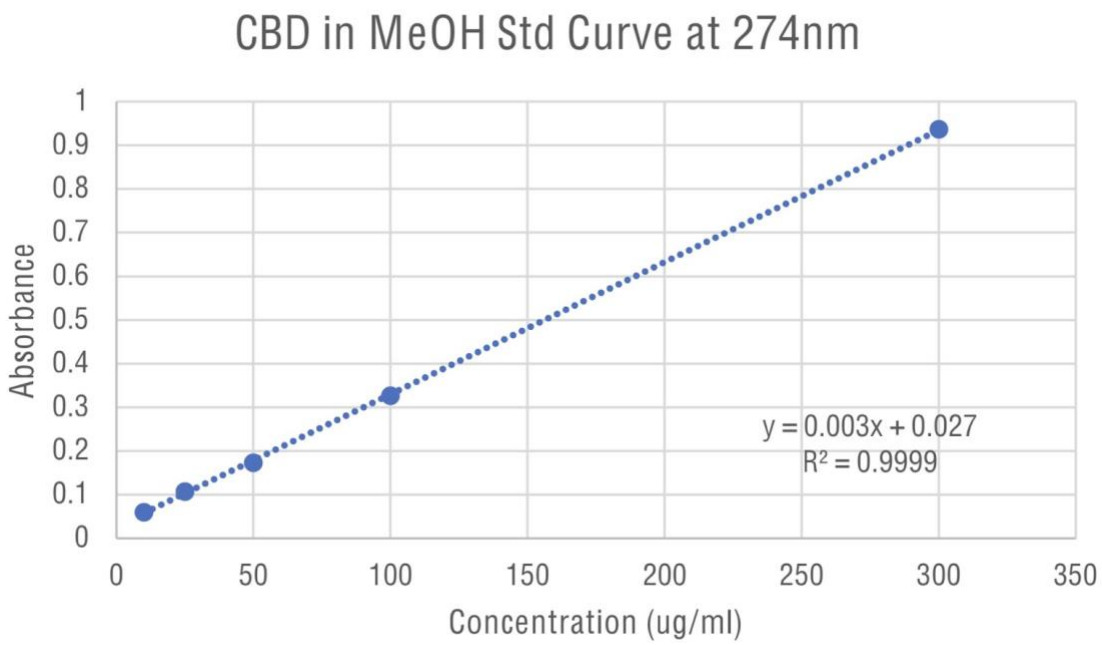


Figure 7. Particle Size Distribution example, showing D10 (Dv0.1), D50 (DV0.5), and D90 (Dv0.9). [35]

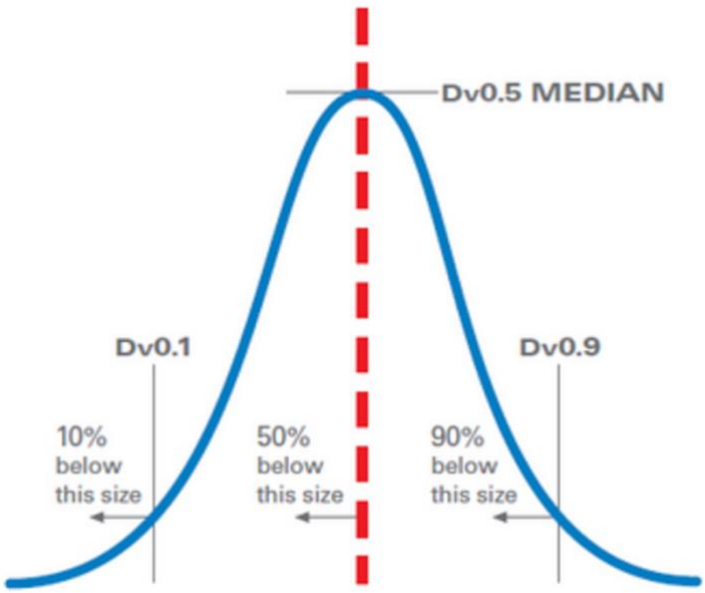


Figure 8. UV/Vis Spectrum of milled (blue) and unmilled (red) CBD at 1.0 mg/mL.

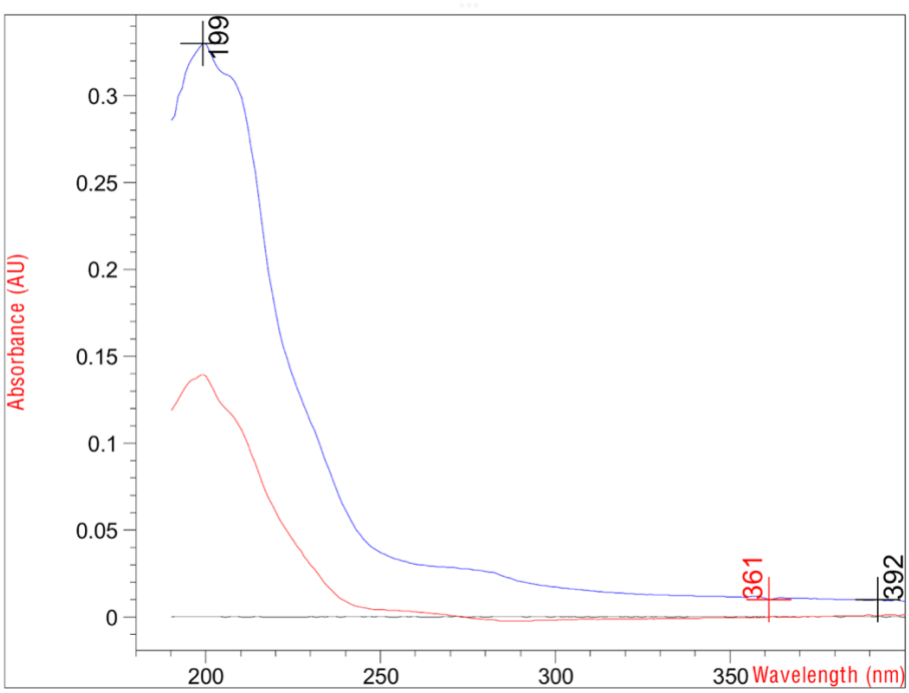


Table 1. Solubility of milled versus unmilled CBD via UV/Vis when dissolved in methanol. This is further confirmed by HPLC data. Negative values were the result of an inaccurate UV/Vis reading potentially connected to dirty curette. The analysis on UV/Vis was not repeated due to confirmation from HPLC.

	Unmilled		Milled	
CBD Conc (mg/mL)	208 nm	274 nm	208 nm	274 nm
10	1.3079	0.026455	1.2607	-0.064006
25	2.5502	0.08625	2.4224	-0.008485
100	2.9897	0.30225	2.9793	0.30349

Figure 9. LC Chromatogram of Unmilled CBD in water at 1 mg/mL shows the same retention time as CBD in methanol of 6.9 minutes.

Peak Results

	SampleName	Name	RT	Area (µV*sec)	% Area	Cal Curve Id	Result Id
1	C16224 CBD in H2O	Cannabidiol (CBD)	6.90	1788	1.632	13465	13484
Mean							

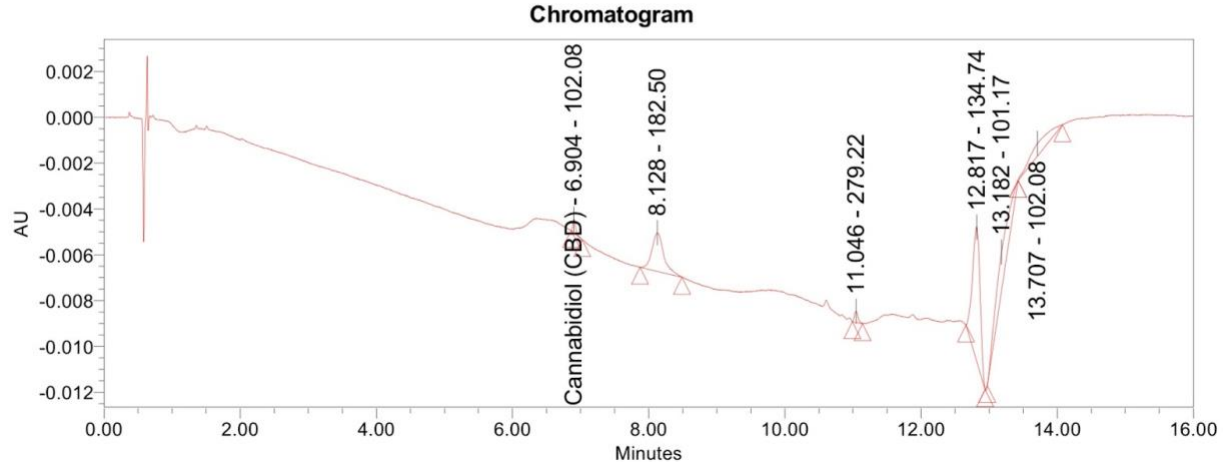


Figure 10. Linearity Curve of CBD Standards via HPLC

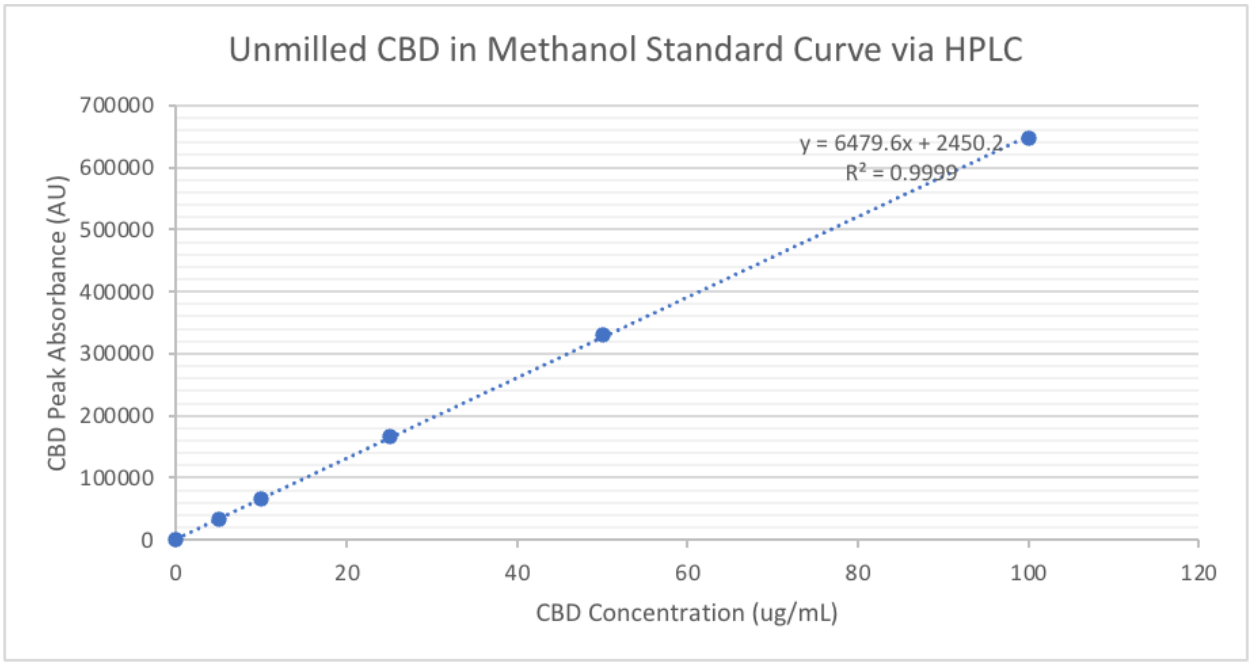


Table 2. Comparison of milled CBD and unmilled CBD peak areas both dissolved in methanol via HPLC confirms the UV/Vis data that milling does not have an effect on CBD solubility in methanol.

	Peak Area (AU)	
CBD Conc (mg/mL)	Unmilled	Milled
10	66362	66273
25	167136	163053
100	647778	652354

Table 3. Comparison of milled CBD and unmilled CBD water solubility by HPLC analysis of 1 mg/mL stock solutions. Solubility was calculated using the standard curve in Figure 6.

	Solubility (μg/mL)	
Condition	Unmilled	Milled
RT	0.28	0.36
37°C	0.30	0.76

Figure 11. XRPD Diffractogram of CBD, PVPP and pre-ASD mixture showing CBD is crystalline, PVPP is amorphous and the mixture still contains crystalline CBD.

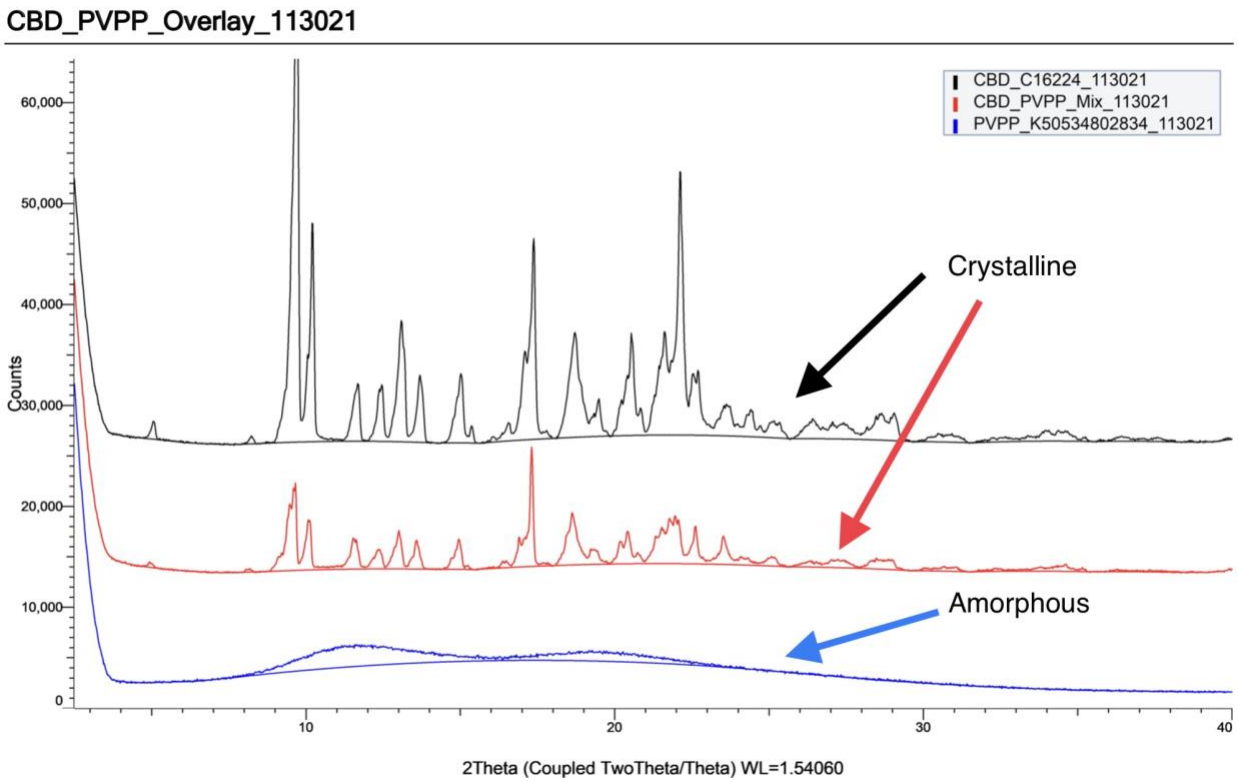


Figure 12. DSC Thermogram of CBD, PVPP, pre-ASD mixture and CBD:PVPP ASD shows CBD is crystalline, PVPP is amorphous, pre-ASD mixture contains crystalline CBD and ASD is amorphous.

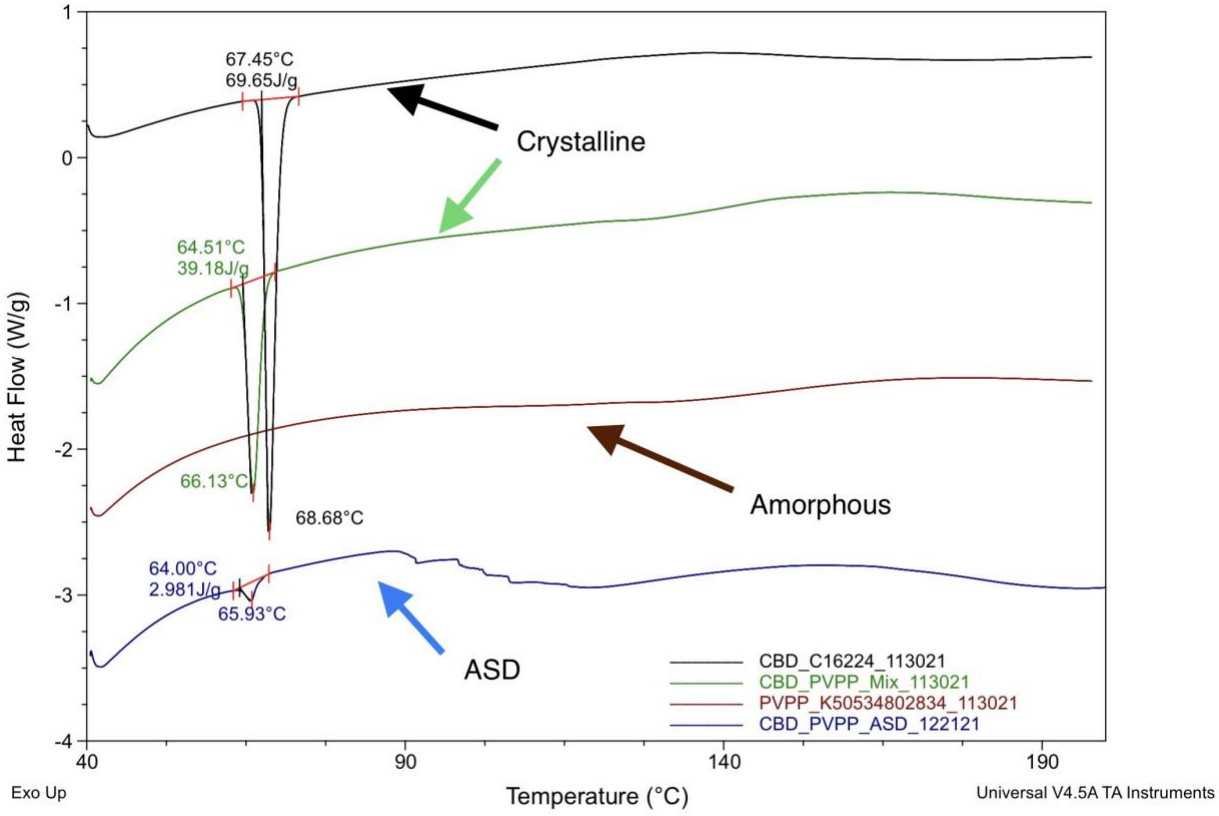


Figure 13. Analysis of CBD:PVPP ASD via UV/Vis shows no absorbance at either CBD lambda maxes, 208 nm or 274 nm, due to low sensitivity of the instrument.

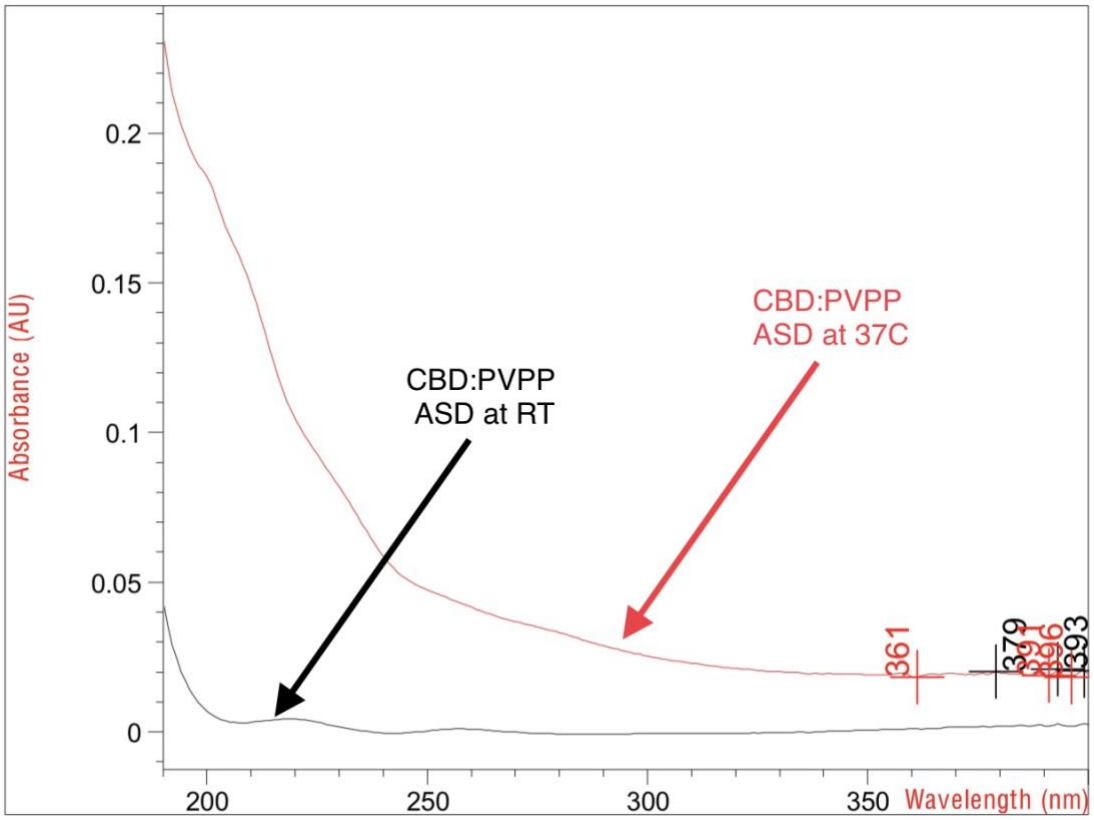
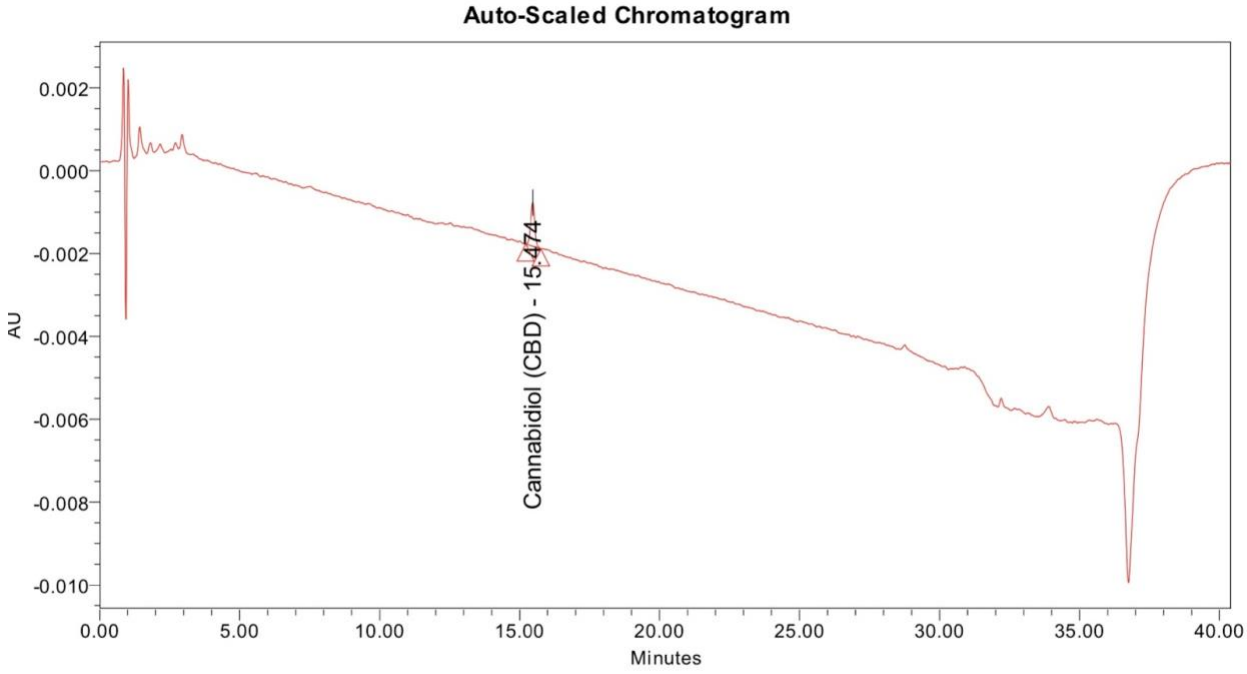

Absorbance (AU)
0.2
0.15
0.1
0.05
0
200
250
300
350
Wavelength (nm)
CBD:PVPP
ASD at 37C
CBD:PVPP
ASD at RT
361
379
391
396
393

Figure 14. HPLC Chromatogram of CBD:PVPP ASD. Higher sensitivity shows presence of CBD in the 1 mg/mL ASD stock. Retention time matches that of the CBD standard injected in the same run.



Peak Results

	SampleName	Name	RT	Area	Height	Amount	Assay_Corr
1	CBD ASD RT	Cannabidiol (CBD)	15.474	11011	1056	0.00036	0.04

Table 4. CBD solubility of the ASD for material at RT and 37C shows the sixfold increase in solubility at RT and the eightfold increase at 37 ^{O}C

	Solubility (µg/mL)	
Condition	CBD Control	ASD
RT	0.28	1.79
37^{o}C	0.3	2.44

Table 5. CBD Solubility from Cyclodextrin Complexes

Preparation Name	CBD Concentration (μg/mL)
Beta CD:CBD	4
Gamma CD:CBD	2
HP Beta CD:CBD	6066
HP Gamma CD:CBD	637
CH3 Beta CD:CBD	8970
HP Beta CD:CBD Diluted 1*	7124
CH3 Beta CD:CBD Diluted 1	1170
CH3 Beta CD:CBD Diluted 2**	4116

*Dilution used for HP Beta CD:CBD solubility concentration. Other preparation saturated the detector.
**Dilution used for CH3:CBD solubility concentration. Other preparations saturated the detector.

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