

HPLC Analysis of Cannabinoid Content in Commercial Cannabidiol Oils

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ABSTRACT

Cannabidiol oil (CBD oil) is a widely popularized herbal remedy used in anxiety treatments and FDA approved as an epilepsy medication for rare conditions. Tetrahydrocannabinol (THC), a cannabinoid producing intoxicating effects, is often present in these CBD oils as it is hard to fully extract. Federal regulations restrict THC content to below 0.3%

(-)- Δ^9 -tetrahydrocannabinol (Δ^9 -THC) by weight. However there are currently no restrictions on (-)- Δ^8 -tetrahydrocannabinol (Δ^8 -THC) content and CBD oil company labels can be deceiving as to the exact THC content in their oils. Thus, High Performance Liquid Chromatography (HPLC) is used to find the concentration of components in CBD oils. HPLC is a laboratory test that identifies the presence of a component in a mixture and calculates the quantity of that component in the compound. The objective of this experiment is to adapt HPLC methods to identify and quantify the concentration of cannabidiol, cannabinol, (-)- Δ^9 -tetrahydrocannabinol, and (-)- Δ^8 -tetrahydrocannabinol in 4 commercial CBD oils: Hillside Botanical CBD Oil, Ropana 500mg Nectar, VT. Hemp Co. CBD Isolate, and Z-Botanicals CBD Tincture. Results show three of the CBD oils had no THC content; Hillside had a concentration of 3.65 $\mu\text{g/mL}$ THC. All CBD oils, expectedly, had moderate CBD concentrations, the highest being VT Hemp CBD Isolate which had a CBD concentration of 91.78 $\mu\text{g/mL}$. It is notable that all of the CBD oils had labels indicating 1.36 $\mu\text{g/mL}$ concentrations of CBN even though there was no detectable CBN. In conclusion, the three cannabinoids explored in this experiment can be identified and quantified using the given method.

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) is a method of identifying and quantifying components in a mixture. It does this by the presence of its main compartment, the column. A sample enters the HPLC system and exits the column in the form of compound bands; these bands are picked up by the UV VIS detector. The detector measures absorption of UV wavelengths when passed through a sample. The HPLC system then sends the information it collects to a computer; this information is in the form of the absorption times and will be shown in a chromatograph.

In the experiment, the HPLC system was used to analyze cannabinoid content in CBD oil. Cannabidiol oil, commonly abbreviated to CBD oil, is a commercial oil applied topically or taken orally. Generally, CBD oil is composed of the cannabinoid cannabidiol along with medium-chain triglyceride oil (MCT), a type of coconut oil. The CBD oil market is widespread and in high demand, however, there is little validation of labeling on CBD oil. It is currently only FDA approved in treating two seizure disorders Dravet and Lennox-Gastaut syndromes, both severe forms of childhood epilepsy ([FDA 2018](#)). The expectation is, with more research, CBD

will become a trusted drug and avidly used in the medical world. There has been much conflict surrounding the federal legality of CBD oil. The federal law has stated that CBD oil is legal for public purchase as long as the Δ^9 THC concentration is below 0.3% by weight ([Akpan et al. 2019](#)). Many CBD companies mark the exact amount of THC content, however, not reporting the exact amount of THC content is not uncommon.

Cannabidiol, one of the main components in CBD oil, comes from the plant *Cannabis sativa* ([Malone et al. 2021](#)). *Cannabis sativa* contains cannabinoids, around 120, that serve as the psychoactive components of the plant ([Widelski et al. 2016](#)). These chemicals can be extracted using supercritical carbon dioxide. Four different cannabinoid standards were used in the experiment. CBD, generally anticipated to be the most prominent cannabinoid present in CBD oil, has been known to play a role in mood stabilization, along with anxiety prevention ([Ashton 2021](#)). The second and third cannabinoids are variations of tetrahydrocannabinol, also known as THC. THC is a psychoactive agent and a stimulant also found in *Cannabis sativa* ([Brust 2009](#)). The two types of THC used in this experiment were Δ^8 -THC and Δ^9 -THC. Both Δ^8 -THC and Δ^9 -THC have intoxicating effects, however Δ^9 -THC has stronger effects. Δ^8 -THC, expressed in lower quantities in the cannabis plant, may produce psychoactive effects ([McKeil 2021](#)). The final cannabinoid standard used in this experiment was CBN. This chemical comes as a result of the breakdown of THC ([Yetman 2021](#)). Like CBD, there is little research to validate its many herbal remedy claims of curing cancer, treating depression, and improving arthritis side effects ([Johnson 2020](#)).

The objective of this experiment was to adapt HPLC methods to discover whether the amount of cannabidiol, cannabinol, (-)- Δ^9 -tetrahydrocannabinol, and (-)- Δ^8 -tetrahydrocannabinol in Hillside Botanical CBD Oil, Ropana 500mg Nectar, VT. Hemp Co. CBD Isolate, and Z-Botanicals CBD Tincture.

MATERIALS AND METHODS

The experiment's methods were adapted from the paper *Leaner and greener analysis of cannabinoids* (Mudge et al. 2017). The HPLC used in this experiment was an Agilent 1100 with a C18 column. The column maintained a temperature of 4 degrees Celsius (Mudge et al. 2017). The mobile phase was composed of Solvent A, 10mM Ammonium Formate (pH of 3.52), and Solvent B, Acetonitrile. The gradient flow was set to a rate of 0.6 mL/min and the gradient itself was 0-8 mins; 52-66% Solvent B, 8-8.5 mins; 66-70% Solvent B, 8.5-13 mins; 70-80% Solvent B, 13-15 mins; 80% Solvent B (Mudge et al. 2017). The injection volume was set to 5 μ l and the detection set to 220 nm. Additionally, after every run, the system ran for 7 minutes to restore the column back to equilibrium.

Six different concentrations of standards were used to create standard curves. These concentrations were created using an equal amount of cannabidiol (CBD), cannabinol (CBN), (-)- Δ^8 -tetrahydrocannabinol (Δ^8 -THC), and (-)- Δ^9 -tetrahydrocannabinol (Δ^9 -THC), diluted in methanol. The concentrations were 3.2 μ l/mL, 32 μ l/mL, 64 μ l/mL, 80 μ l/mL, 100 μ l/mL, and 500 μ l/mL. The retention times and area of the peak created on the chromatogram were recorded to create a standard curve. These six concentrations were each run through the HPLC system three times.

Four different commercial cannabidiol oils were tested in this experiment; Hillside Botanical CBD Oil, Ropana 500mg Nectar, VT. Hemp Co. CBD Isolate, and Z-Botanicals CBD Tincture. 25 µl of the Z-Botanical Tincture oil, the VT Hemp Co. Isolate, and the Ropana 500mg Nectar was diluted with methanol in a 10 mL volumetric flask. Then, the oils were transferred to larger vials where they were sonicated for 15 minutes, vortexing every 5 minutes for 30 seconds each. The CBD oil mixtures then sat in the freezer at -20 degrees Celsius overnight and the same sonication and vortexing process was repeated the following day. The three CBD oil mixtures were then transferred into HPLC vials and ran through the system. 12.5 µl of the Hillside Botanical Oil was diluted with methanol in a 10 mL volumetric flask; this oil was prepared differently as it had a difficult time dissolving. The concentration was sonicated for 15 minutes, vortexing for 30 seconds every 5 minutes. The Hillside Botanical mixture was then transferred to an HPLC vial and ran through the HPLC system. The CBD oils were run through the HPLC machine two times.

For each time a sample ran through the HPLC system, a chromatogram was provided. In addition to each chromatogram, a table listing retention times, heights of the curves, and areas underneath the curves was also provided. For each standard, the six concentrations of each cannabinoid in relation to their attributing areas of the peaks they created were graphed. From this graphed data, a linear regression model could be created and the amount of variance in the data could be calculated by looking at the R^2 value.

RESULTS

CBD Standard Curve

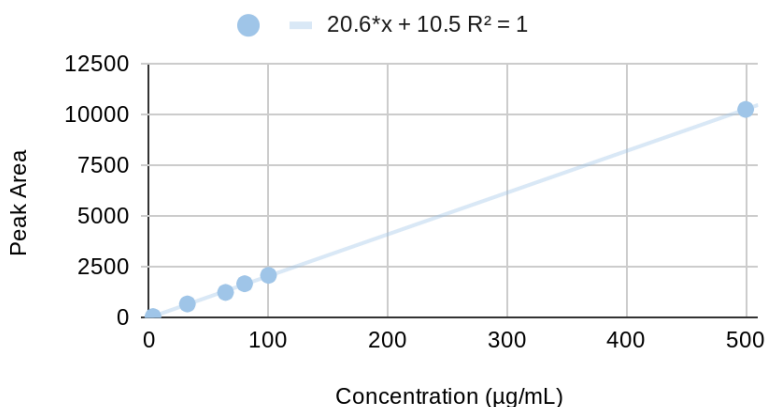


Fig.1 Standard Curve Created by Three Repeats of Six CBD Concentrations. This graph depicts the linear regression line from the CBD standard concentrations. The x-axis represents the concentration in ug/mL and the y-axis represents the peak area of each concentration. The CBD standard concentrations have an R^2 value of 1. Directly left of the R^2 value is the linear equation of the standard curve ($y=20.6x+10.5$). This equation allows for the determination of the concentration of CBD present in the CBD oils.

CBN Standard Curve

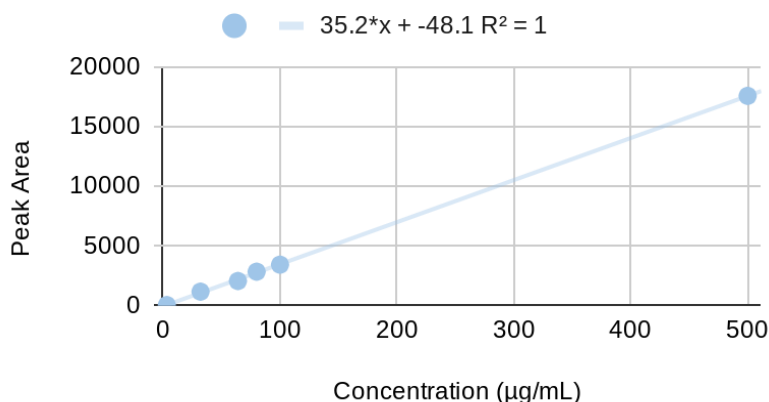


Fig.2 Standard Curve Created by Three Repeats of Six CBN Concentrations. This graph depicts the linear regression line from the CBN standard concentrations. The x-axis represents the concentration in ug/mL and the y-axis represents the peak area of each concentration. The CBN standard concentrations have an R^2 value of 1. Directly left of the R^2 value is the linear equation of the standard curve ($y=35.2x-48.1$). This equation allows for the determination of the concentration of CBN present in the CBD oils.

THC Standard Curve

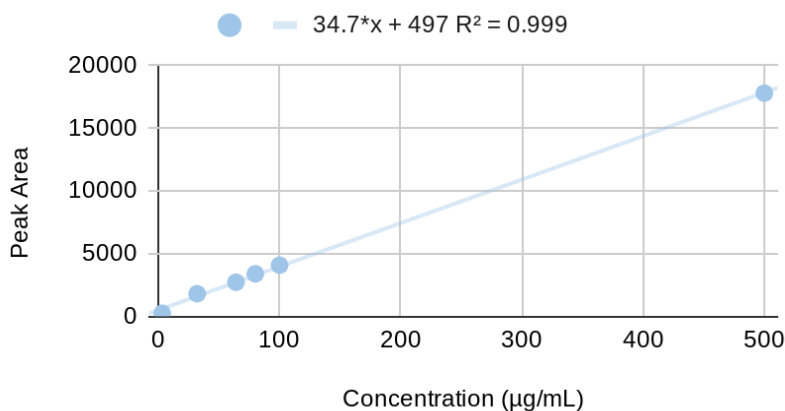


Fig.3 Standard Curve Created by Three Repeats of Six THC Concentrations. This graph depicts the linear regression line from the THC standard concentrations. The x-axis represents the concentration in ug/mL and the y-axis represents the peak area of each concentration. The THC standard concentrations have an R^2 value of 1. Directly left of the R^2 value is the linear equation of the standard curve ($y=34.7x+497$). This equation allows for the determination of the concentration of THC present in the CBD oils.

<u>CBD Oil</u>	<u>Conc. CBD (µg/mL)</u>	<u>Conc. CBN (µg/mL)</u>	<u>Conc. THC (µg/mL)</u>
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Ropana 500mg Nectar	62.09 ± 0.93	1.36 ± 1.39	-2.72 ± -3.00
VT Hemp Co. CBD Isolate	91.78 ± 0.98	1.36 ± 1.39	-0.56 ± -3.00
Hillside Botanical CBD Oil	37.6 ± 0.91	1.36 ± 1.39	3.65 ± 3.00
Z-Botanicals CBD Tincture	75.74 ± 0.95	1.36 ± 1.39	-0.29 ± -3.00

Fig.4 Table Exhibiting Concentrations of Cannabinoids in Commercial CBD Oils. This table shows the concentrations (in $\mu\text{g/mL}$) of three cannabinoids examined in the four CBD oils. The first columns show the CBD oils and the top rows indicate the corresponding cannabinoid concentrations. The measured concentration, followed by the uncertainty is given in the center cells. The uncertainty was calculated through error propagation. In the final column, the concentration of THC, three out of the four THC concentrations are negative. This indicates the actual THC concentration is zero.

DISCUSSION

This experiment concludes that, using the given method, the chosen cannabinoids can numerically be detected in the given CBD oils. The VT Hemp Co. CBD Isolate had the greatest amount of CBD and the Hillside Botanical CBD Oil had the least amount of CBD. Although slightly higher, the number of mg in 1 fl oz. of the Ropana 500mg CBD Oil stayed in the low 500s. There is an elevated level of error associated with the CBN and THC test, presumably due to the fact that these peaks could not be accurately represented in the chromatograms. There appears to be variation present among data, due to repeating the CBD oils only twice, further tests must be run to ensure the data is concrete.

Taking into consideration error, possible sources include the fact that many of the peak areas were integrated manually and bring in the concern of human error; the HPLC system failed to identify them or identified them incorrectly, the fact that this experiment was mostly machine focused decreases yet does not expel the potential of bias, and the negative concentrations of the THC concentrations raise some concern in respects to calculating errors.

Further steps for experimental improvement include using the fraction collector to gather specific cannabinoids to test them individually on the model organisms *Caenorhabditis elegans*, using different CBD oils in expectation of seeing different results, exploring the identification of different cannabinoids, and repeating the experiment in hopes of solidifying the dependency of data.

RESOURCES

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