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Title: Separating and Quantifying THC and THCA Using High Performance Liquid Chromatography

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Abstract approved:_____

A method was developed for a High Performance Liquid Chromatography (HPLC) instrument coupled with a UV-Vis detector to separate and quantify Δ^9-tetrahydrocannabinol (THC) and Δ^9-tetrahydrocannabinolic acid (THCA). Initial conditions for method parameters were chosen from literature, and then an experimental approach was employed to optimize every parameter that had a significant impact on the method. The final method has a gradient mobile phase at flow rate 0.8 mL/min and an oven temperature of 40 °C with an Alltima HP C18 AQ column. Mobile phase A is 0.1% formic acid in water and mobile phase B is 0.1% formic acid in acetonitrile. Mobile phase B is pumped from 65% to 80% following a linear 8-min time gradient, followed by a linear 1-min step gradient back down to 65%, with total collection ending after 11 minutes. This method produces a peak for THC around 3.60 minutes and a peak for THCA around 4.67 minutes. Calibration curves were made from standards, where the Limit of Detection (LOD) and Limit of Quantification (LOQ) is calculated for THC as 30 ppm and 100 ppm, respectively, and calculated for THCA as 9.1 ppm and 30 ppm, respectively. A hemp oil sample was acquired from Amazon and has an undetectable amount of THC and a calculated THCA concentration of 47.2 ppm. The hemp oil sample was spiked with 10 µL of THC and 10 µL of THCA and has an undetectable amount of THC and a calculated THCA concentration of 56 ppm. Future work includes obtaining higher concentrations of THC and THCA to test using this method and identifying other peaks found in the hemp oil chromatograms.

SEPARATING AND QUANTIFYING THC AND THCA USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Approved by the Department Chair

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Dean of the Graduate School and Distance Education

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Table of Contents

List of Tables

List of Figures

Introduction

Marijuana refers to the dried leaves, flowers, stems, and seeds from the *Cannabis sativa* or *Cannabis indica* plant.[1] The plant is grown for medicinal, recreational, and spiritual purposes. The main active ingredient in cannabis is called Δ^9-tetrahydrocannabinol (THC), shown in Figure 1A. THC is found in the resin secreted by glands of the plant and is the chemical responsible for most of marijuana's psychological effects.[2] Δ^9-tetrahydrocannabinolic acid (THCA) also occurs naturally in the plant but is non-psychoactive and not defined as an illegal drug federally (Figure 1B).[3] THCA is the precursor to THC, and the major structural difference between them is that THCA contains a carboxyl group on carbon 2. Upon smoking, cooking, or vaping the marijuana plant, THCA decarboxylates to become THC.[4] It takes three hours to completely decarboxylate THCA into THC at 100 °C and four hours at 98 °C.[5] Full decarboxylation will occur in 10 minutes at 160 °C and seconds above 220 °C.

Because marijuana contains THC, the plant has been classified as a Schedule I substance since Congress passed the Controlled Substances Act as Title II of the Comprehensive Drug Abuse Prevention and Control Act of 1970.[6] A Schedule I substance is defined as a drug with no currently accepted medical use and a high potential for abuse.[6] Although certain states have legalized marijuana, the drug is federally prohibited. The federal government has the legal authority to arrest anyone possessing marijuana.

In the state of Kansas, there are no laws that allow the use of recreational marijuana or medical cannabis. The first possession of marijuana charge is treated as a misdemeanor. The next is treated as a felony, with the severity level depending on how

much is in possession.[7] Although marijuana is illegal in Kansas, there are types of cannabis that are not. The Kansas Senate Bill 263 (SB 263), known as the Alternative Crop Research Act, was passed in April 2018 and allows the Kansas Department of Agriculture, either alone or in coordination with a state institution of higher education, to grow and cultivate industrial hemp and promote the research and development of industrial hemp.[8] Industrial hemp is defined by SB 263 as all parts and varieties of the plant *Cannabis sativa L* that contain a Δ^9-THC concentration of no more than 0.3 percent on a dry weight basis.[8]

Once marijuana is seized by police, crime labs confirm the plant's identity. Gas chromatography mass spectrometry (GC-MS) is commonly used in crime laboratories to analyze cannabis samples, but this technique is problematic when trying to determine the level of THC. The oven temperatures used in GC methods are usually between 250 - 275 °C, depending on the column used.[9] The temperature of the GC column would instantly decarboxylate the THCA in any sample, converting all THCA into THC.[10] Since this technique does not differentiate between the two substances, the reported amount of THC would be the sum of the original THC and the decarboxylated THCA. Because of this, labs using GC report the results as "THC Total." To accurately report the results of psychoactive THC in a sample, unmodified THC and THCA must be separated.

These results of THC and THCA determination are valuable, especially if the state or federal government changes the laws concerning marijuana and how much a person can possess. The THC content varies in a bottle of hemp oil, a blunt, and a brownie. Because of this, if federal laws change to allow a certain quantity of THC to be on someone's person, each individual sample seized and brought to the lab would have to

be tested. As such, these calculations should not include THCA as it is not psychoactive and the current methods that use GC-MS should not be. If someone smokes a blunt, all of the THCA would indeed decarboxylate into THC; however, the amount of THCA should not be included in the total if the blunt isn't smoked. This would be similar to arresting someone for drunk driving if they were just drinking outside near their car. A defense attorney would argue that the amount of THC is too high because of the included THCA. To avoid this, lab technicians would need to be able to separate THC from THCA to accurately quantify the amount of THC in the sample.

A method using high-performance liquid chromatography (HPLC) and an ultra-violet (UV) detector was created to accurately separate and quantify the amount of THC and THCA. An experimental approach was used to optimize every parameter variable that had a significant impact on the method, including column length, flow rate, oven temperature, UV wavelength, mobile phase composition, and time gradient. This was achieved by using THC and THCA standards to test each modification of the experimental method. Once a final method was developed, the method was confirmed with a sample of hemp oil containing unknown amounts of THC and THCA.

The HPLC-UV instrument consists of six major parts--solvent, pump, injection, column, detector, and waste, as shown in Figure 2. First is the HPLC solvent. Commonly, there are two solvents used in HPLC methods to help with the separation of samples through a difference in pH. The solvents are called the mobile phase because they carry the sample through the system. Next is the HPLC pump. The high-pressure pump meters a specified flow of the mobile phases. Once the instrument has solvent running through

the system, the injector introduces the sample into the stream. The model used in this research is a manual-injection HPLC.

After the sample enters at the injection port, it is carried to the HPLC column. The column contains chromatographic packing material that elutes components of the sample at different rates due to the components' varying degrees of interaction with the packing materials. A C18 reverse-phase column was used in this study, as this type of column is most commonly used in literature pertaining to cannabinoids.[11,12,13] The C18 means that an 18-carbon chain is covalently attached to the packing particles. A reverse phase column contains hydrophobic packing particles, in this case the C18, so that hydrophilic molecules will elute quickly out of the column, whereas hydrophobic molecules will stay in the column longer before ultimately being eluted out. After leaving the column, the separated components of the sample pass through the UV detector. The mobile phase then exits as waste.

HPLC-UV was chosen for this method because it is what was available in the lab. However, HPLC-UV also has many advantages over other instruments. As previously discussed, GC-MS will not accurately detect the amount of psychoactive THC in the original sample. It should also be noted that although GC-MS is quick and reliable, it is expensive; HPLC is not as expensive because it does not utilize a mass spectrometer.[14] Diode-Array Detection (DAD) detectors are popular in HPLC methods, but UV/Vis detectors are the most common in the industry, have lower noise, and are inexpensive.[15,16]

Methods have been developed for the separation and quantification of many cannabinoid components using instrumentation besides HPLC-UV. Coulter et al. (2011)[12] used LC-MS-MS, Hazekamp et al. (2004)[17] used HPLC-UV/FLD, and Lehmann et al.

(1995)[13] used HPLC-DAD. There have been some methods created specifically for HPLC-UV, such as by Swift et al. (2013)[11], Zivovinovic et al. (2018)[18], and Mandrioli et al. (2019)[19] but these methods did not use a column with the same dimensions of those obtainable for this research. Also, in both of these methods, THC eluted after 8 minutes--the method developed in this research aims to have both THC and THCA elute before 5 minutes.

The goal of this research is to create a method using the Agilent HPLC-UV to accurately separate and quantify THC and THCA from an oil sample. This method can be employed in the future by state, federal, and private labs in Kansas or within the United States as a whole to allow certain quantities of the drug.

Materials and Methods

Samples. Hemp Oil 300 mg Hemp Extract, by Absolute Nutrition (Stillwater, OK, USA), was obtained from Amazon. The sample was stored at room temperature.

Chemicals. Acetonitrile, formic acid, methanol, and chloroform were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Δ^9-tetrahydrocannabinol and Δ^9-tetrahydrocannabinolic acid standards were purchased as 1 mg/mL solutions in methanol and acetonitrile, respectively, from Cerilliant (Round Rock, TX, USA). All sample dilutions for the Δ^9-tetrahydrocannabinol calibration curves were prepared in methanol and all sample dilutions for the Δ^9-tetrahydrocannabinolic acid calibration curves were prepared in acetonitrile. Standards were stored at -80 °C and kept on ice with salt when in use.

Standard Solutions (HPLC Calibration). Calibration standards for both Δ^9-tetrahydrocannabinol and Δ^9-tetrahydrocannabinolic acid in the range 5−100 ppm were prepared by dilution from the respective 1 mg /mL standards. Calibration standards were stored at -80 °C and kept in ice with salt when in use in the lab.

Apparatus and Instrumentation. The HPLC system was a Prominence LC-20AB Liquid Chromatograph and SPD-20AV UV/Vis Detector from Shimadzu (Columbia, MD). The column was an Alltima HP C18 AQ, 150 mm x 2.1 mm, 3-µm particle size packing from Alltech (Columbia, MD).

Final Method used in HPLC Analysis. A gradient mobile phase at flow rate 0.8 mL/min and oven temperature 40 °C was used with an Alltima HP C18 AQ column for the final method. Mobile phase A was 0.1% formic acid in water. Mobile phase B was 0.1%

formic acid in acetonitrile. The concentration of mobile phase B was set at 65% to avoid a peak shift at the beginning of collection. Mobile phase B was pumped from 65% to 80% in a linear 8-min time gradient, followed by a linear 1-min step gradient returning to 65%, with total collection ending after 11 min. This was followed by 2 min of column conditioning with acetonitrile. Injection volume was 10 µL. The UV detector monitored 230 nm and 270 nm for Δ^9-tetrahydrocannabinol and Δ^9-tetrahydrocannabinolic acid. At the end of each sample set, the column was washed with 10 uL of acetonitrile, and an injection of 10 µL of acetonitrile was made to flush the manual-sampler needle. LabSolutions (Shimadzu) was used to calibrate and quantitate Δ^9-tetrahydrocannabinol and Δ^9-tetrahydrocannabinolic acid levels in ppm. Microsoft Excel spreadsheet software and Google Sheets was used to analyze the chromatograms.

Method Validation Experiments. Hemp oil samples were prepared for HPLC analysis by diluting 5 mg oil with 900 µL methanol and 100 µL chloroform.[13] Samples were then spiked with 10 µL of 100 ppm Δ^9-tetrahydrocannabinol standard, 10 µL of 100 ppm Δ^9-tetrahydrocannabinolic acid standard, both, or neither. All samples were sonicated for 15 min.

Results and Discussion

Methods from literature that were successful in separating compounds found in cannabinoids were chosen as a starting point to adapt and evolve for the HPLC instrument and column being used in this research.[11,12] To test these methods, standards of THC in methanol and THCA in acetonitrile were analyzed. These resulting sample concentrations were approximately 100 ppm--the concentrations needed to be strong enough so a peak was clearly visible on the resulting chromatogram. To test the successfulness of a method, the THC sample was run separately from the THCA sample to avoid split peaks. If both samples produced a peak that was narrow and even, the two chromatograms were overlaid to see if the two compound peaks were adequately separated, with peaks at least a minute apart.

There were six HPLC-UV parameters--column length, flow rate, temperature, wavelength, mobile phase composition, and time gradient, shown in Tables 1-6, respectively. Parameters were adjusted to increase separation, reduce overall runtime, and produce optimally shaped peaks.

The length of the column partially determines the runtime of the method and the degree of separation of the sample compounds--the longer the column, the longer the runtime and the more separation of the sample compounds. The starting column was a C18 reverse-phase column with the dimensions 250 mm x 4.6 mm, 5 µm. This column produced usable peaks; however, this column did not fit well into the HPLC oven, resulting in poor connection of the column. Leaks occurred often because of this, which is not ideal for a method, especially one that is used in a professional setting. The other problem associated with the column's long length was the runtime. The runtime was

usually longer than fifteen minutes, with one run giving a peak after 32 minutes. A successful method would ideally be under fifteen minutes, so ultimately this column was exchanged with a shorter C18 reverse-phase column with the dimensions 150 mm x 2.1 mm, 3 µm.

Figure 3 shows two chromatograms created with different column dimensions. The first chromatogram had the column with the dimensions 250 mm x 4.6 mm, 5 µm, and the second chromatogram had the column with the dimension 150 mm x 2.1 mm, 3 µm. The THCA peak eluted much faster using the shorter column compared to the longer column, about 4.21 minutes faster. A repeated method needs to have a short runtime to accommodate the high load of samples that are run in a day. Figure 3 demonstrates that, although a peak is present, ultimately the runtime was too long with the longer column for the method to be successful.

The flow rate also determines the runtime of the method. The higher the flow rate, the quicker a sample compound moves through the HPLC system. The flow rate needs to be high enough to minimize the runtime but also low enough to keep the instrument's pressure under 4000 psi, the instrument's limit. The two largest flow rates, 1.2 mL/min and 1.5 mL/min, were associated with the longer column. When the column was changed, the flow rate was also changed to go with the shorter column. The flow rate of 1.0 mL/min was too high, causing the pressure to exceed its limit and ultimately shutting down the run. The flow rate of 0.50 mL/min produced peaks but lengthened the runtime up to 22 minutes. The flow rate of 0.75 mL/min shortened the runtime and was well-below the pressure limit, so a final flow rate of 0.80 mL/min was chosen as it raised the

pressure but kept it just below its limit, as well as keeping the runtime down to 11 minutes.

Oven temperature is another parameter that affects the runtime--the higher the temperature, the shorter the runtime and the quicker the samples elute. A temperature of 37 °C was chosen as it was the midway point between oven temperatures found in literature.[11,13] The temperature was changed to 40 °C to lower the runtime. As seen in Figure 4, it is predicted from literature[5] that it would take 71.3 hours for THCA to decarboxylate to THC at 40 °C, assuming no change in decarboxylation mechanism. Only 0.23% of the THCA would be decarboxylated at 40 °C.

Another parameter was the choice of two detector wavelengths. From various literature sources, four wavelengths were used in different combinations--220 nm, 230 nm, 254 nm, and 270 nm.[11,18,20] The wavelengths 230 nm and 270 nm were chosen for the final method as, at these two wavelengths, the peaks produced from the chromatograms were the largest and easiest to see.

The mobile phases are used to change the degree of separation between two analytes. HPLC runs with the first mobile phase A and mobile phase B chosen from literature, 25 mM ammonium formate in a 50:50 mixture of acetonitrile and water and neat acetonitrile, did not produce any peaks. Mobile phase A was changed to 0.01% formic acid, a 50:50 mixture of acetonitrile and water, to see if the acid of the ammonium formate would help with the separation and production of peaks, as acid in mobile phases helps with shaping peaks by adding protons into the solution. The amount of formic acid was increased in mobile phase A and then added to mobile phase B. This combination of mobile phases resulted in split peaks, most likely because both mobile phases were too

similar to achieve complete separation. To combat this, the 50:50 mixture of acetonitrile and water was changed to just 0.1% formic acid in water to achieve the best separation of the two samples, resulting in the final choices for mobile phases.

Sometimes it is difficult to see which parameter to change to fix a problem presented in a chromatogram, so multiple parameters can be changed at once. Figure 5 shows two chromatograms created with different oven temperatures, wavelengths, and mobile phases. The first chromatogram produced a split peak for THC, and both THC and THCA had peaks that were too small to be valuable. To fix the split peak, mobile phase A was changed and the oven temperature was increased. To combat the smallness of the peaks, the wavelength of 220 nm was increased to 230 nm, resulting in higher and sharper peaks.

The last parameter was the time gradient. The time gradient is a program that changes the percentage of mobile phase B to mobile phase A that is being introduced to the flow of the system at any given time. For example, the beginning time gradient of 65-95 over 16 minutes means that at 0 minutes, the flow of the mobile phases into the system is 65% mobile phase B and 35% mobile phase A. Over time, mobile phase B will increase at the percentage that mobile phase A will decrease, so that at 16 minutes, mobile phase B is at 95% and mobile phase A is at 5%. The time gradient helps determine when the test samples will elute from the column. Many time gradients were tried to find a program that would successfully separate THC and THCA, at least a minute apart from each other. The time gradient of 65-100-65 over 0-10-15 minutes with collection ending at 22 minutes was successful, and so the program was tweaked multiple times to shorten the runtime while also keeping the adequate separation of the two peaks,

as shown in Figure 6. Ultimately, a successful time gradient of 65-80-65 over 0-8-9 minutes, with collection ending at 11 minutes, was chosen. Before each run, the concentration of mobile phase B was set at 65% instead of at the instrument's default of 100% so that there would not be a shift at the beginning of the collection.

This final method produced a peak for THC at 3.60 minutes on the chromatogram (Fig. 7). The peak has noticeable fronting. Fronting is commonly caused by two things--overloading the instrument with the sample or a degrading column. A smaller amount, 5 uL, was run to see if this fixed the fronting, but it did not. Thus, fronting on the chromatograms was due to the slight degradation of the older column. There is a small peak at 4.70 minutes, caused from a small amount of THCA still in the column from a previous run. This did not affect the THC peak in its height or location on the chromatogram--it only causes an extra peak to be seen. This method produced a peak for THCA at 4.67 minutes, seen in Figure 8. This peak also had some fronting, again from the slight degradation of the column. This peak was noticeably taller than the THC peak, having an intensity of roughly 6000 mV more comparatively, even though the concentrations were similar. The calculated retention times for THC and THCA were 2.93 minutes and 4.02 minutes, respectively, given by $t_r' = t_r - t_m$, where t_r is the retention time of the sample and t_m is the time when the mobile phases reach the detector.

Both chromatograms were overlaid to see if the peaks were separated (Fig. 9). Even with the fronting, the THC and THCA peaks do not overlap. The peaks have a calculated resolution time of around 2.71 minutes, given by $R = \Delta t_r / w_{av}$, where Δt_r is the difference between the two retention times of the sample peaks and w_{av} is the average width of the two sample peaks. Having a resolution time of 1 means that the peaks are

separated, so having a resolution time equaling 1 or higher is ideal. An acetonitrile blank (Fig. 10) was utilized to baseline the chromatograms.

Because the method was successful in separating the two peaks in a reasonable amount of time, the next step was quantifying the analytes. To do this, calibration curves, one for THC and one for THCA, were made for this method, shown respectively in Figure 11 and Figure 12. This was accomplished by diluting both of the original standards, THC in methanol and THCA in acetonitrile, to the appropriate concentrations. These new standards were made at concentrations of 5, 10, 20, 50, and 100 ppm. A chromatogram was made for each standard and the peak area concentration was reported by the HPLC-UV program. The calibration curves were made with the concentration in ppm on the x-axis and the peak area concentration on the y-axis.

The calibration curve equations are given for each curve, as well as the standard error of the slope and intercept and the R^2 value. The error of the slope and intercept describes the standard deviation of these values from the sample population. The lower the error, the lower the standard deviation and the better the fit of the equation. R^2 values tell how good data fits a best fit line. The higher the R^2 value, the better the correlation of the data. These values for the THC and THCA calibration curves are given in Table 7. The THC slope has an error of 0.011 and the THC intercept has an error of 0.5. The THC calibration curve has an R^2 value of 0.954, an indicator of good correlation.

The 20-ppm standard of the THCA calibration curve was over-concentrated due to human error when making it, so this data point was scratched. Using four data points. the THCA slope error is 0.068 and the THCA intercept error is 3.8, which is higher than expected. The 5-ppm standard value seemed below the calibration curve line

considerably, so the F-test was used to see if the data point was an outlier. The F-observed calculated value of 53.3 was well above the F-table value of 19.2, so the 5-ppm point was removed. The final THCA calibration curve has an R^2 value of 0.998, demonstrating very good correlation between the data points. The new slope has an error of 0.016 and the new intercept has an error of 1.0, much lower values compared to the values with the outlier.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated from both calibration curves, shown in Table 8. The LOD is approximately three times the minimum amount of the sample detected, given by $3 \times \sigma / s$, and the LOQ is approximately ten times the minimum amount of the sample detected, given by $10 \times \sigma / s$, where σ is the standard deviation of the response from the calibration curve line and s is the slope of the calibration curve line.

The next and final step of research was to test the method using a commercially available product. A THCA tincture from New Mexico was going to be purchased to test, but it required a cannabis card to obtain. Instead, hemp oil from Amazon was purchased. Neat oil will clog the column, so the oil was diluted and made ready for the HPLC by using a method from literature.[13] A sample of the oil dilution was tested as well as a spiked sample with an added 10 µL THC and 10 µL THCA.

The chromatogram of the oil sample is found in Figure 13. The THC peak came out at 3.52 minutes, and the THCA peak came out at 4.72 minutes. From the calibration curves, the concentration of THC was under the limit of detection and the concentration of THCA was calculated as 47 ppm. The chromatogram of the spiked oil sample is found in Figure 14. The THC peak came out at 3.72 minutes and the THCA peak came out at

4.96 minutes. From the calibration curves, the concentration of THC was under the limit of detection and the concentration of THCA was calculated as 56 ppm. The calculated retention times for THC and THCA in the unspiked sample were 2.76 minutes and 3.96 minutes, respectively. The calculated retention times for THC and THCA in the spiked sample were 2.94 minutes and 4.19 minutes, respectively. The unspiked chromatogram most likely had a delayed start to the runtime, explaining the earlier times of the peaks. The resolution of the unspiked sample was about 1.64 and the resolution of the spiked sample was about 1.96. Both chromatograms included other unknown peaks of compounds that were also in the hemp oil.

The chromatograms of THC and THCA were overlaid and compared. The unspiked sample elutes slightly quicker than the spiked sample, possibly caused by a delay of detection from when the sample was originally added into the system. Both values of THC and THCA were higher in the spiked samples than in the unspiked, which was expected. There were no known interferences with the hemp oil peaks as none of the peaks that eluted overlapped.

Compared with some other cannabinoid methods, the method developed in this research has the fastest elution time for THC and THCA and one of the shortest runtimes (Table 9). In the research done by Coulter et al.[12], both THC and THCA eluted after 5 minutes. Mandrioli et al.[19] had a method that eluted THC at 6.55 minutes and that eluted THCA at 7.65 minutes. The method created by Swift et al.[11] had THC eluting around 8.3 minutes. Zivovinovic et al.[18] had THC eluting around 8.90 minutes and THCA around 9.60 minutes. In the method developed by Hazekamp et al.[17], THC eluted around 11.51 minutes and THCA eluted around 15.39 minutes. The research by Lehmann et al.[13] had

THC eluting around 40 minutes and THCA around 47 minutes. Having a shorter runtime enables labs to run more samples per day, raising lab efficiency.

Future Work

Overall, the HPLC-UV method was successful in separating and quantifying THC from THCA. To continue on with this research, concentrated THC and THCA will need to be obtained with the federal government's permission and tested using this study's method. The federal amount of THC is currently set at 0.3%, which is equivalent to 3000 ppm. The LOD of THC from this study's method is 30 ppm. Although the method created in this research produces separated peaks that are quantifiable, it will not be relevant for a crime lab setting until higher concentrations of the compounds are tested and correlating calibration curves are made.

Another point of study will be to look into the other peaks found in the hemp oil chromatograms. Standards of other compounds commonly found in cannabis, such as CBD and CBDA, will be obtained and tested using this method to identify the unknown compounds. Knowing what compounds cause these mystery peaks will be useful for lab technicians and give them a more complete picture of the contents of a sample.

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Tables

Table 1. The HPLC column dimensions. Values are given by length x width, packing particle diameter.

Initial Column	Final Column
250 mm x 4.6 mm, 5 µm	150 mm x 2.1 mm, 3 µm

Table 2. The HPLC flow rates.

Parameter Variable	Longer Column Trials	Shorter Column Trials	Final Method
Flow Rate	0.50 mL/min 0.75 mL/min 1.00 mL/min	1.20 mL/min 1.50 mL/min	0.80 mL/min

Table 3. The HPLC oven temperatures.

Initial Temperature	Final Temperature
37 ℃	40 ℃

Table 4. The UV wavelengths.

Initial Wavelengths	Final Wavelengths
220 nm, 254 nm	230 nm, 270 nm

Table 5. The mobile phase compositions used by the HPLC.

Trials	Final Method
Phase A: 25 mM ammonium formate in acetonitrile/water 50:50 mixture Phase B: Acetonitrile Phase A: 0.01% formic acid in acetonitrile/water 50:50 mixture Phase B: Acetonitrile Phase A: 0.1% formic acid in acetonitrile/water 50:50 mixture Phase B: Acetonitrile Phase A: 0.1% formic acid in acetonitrile/water 50:50 mixture Phase B: 0.1% formic acid in acetonitrile	Phase A: 0.1% formic acid in water Phase B: 0.1% formic acid in acetonitrile

Table 6. The HPLC time gradients.

Parameter Variable	Trials	Final Method
Time Gradient	65-95 over 16 min 20-90-20 over 0-6-8 min 20-70-20 over 0-5.5-8 min 20-90-20 over 0-7.5-9.5 min, with collection ending at 11 min 20-30-90-20 over 0-2-6-8 min, with collection ending at 10 min 20-90-20 over 0-10-12 min, with collection ending at 14 min 30-90-30 over 0-6-8 min, with collection ending at 10 min 30-70-85-95-95-70 over 0-3-7-7.01-8-8.01 min, with collection ending at 10 min 60:100 over 0-24-30 min, ending at 30 min 60:100 over 0-16-30 min, ending at 30 min 60-100 over 0-8-16 min, ending at 20 min 65-100-65 over 0-10-15 min, ending collection at 22 min 65-90-65 over 0-10-14 min, ending collection at 15 min 65-80-65 over 0-6-8 min, with collection ending at 10 min	65-80-65 over 0-8-9 min, ending collection at 11 min

Table 7. The slope, intercept, associated error, and R^2 values of the THC and THCA calibration curves in ppm.

	THC Calibration Curve in ppm	THCA Calibration Curve in ppm
slope	0.085	0.33
standard error of slope	0.011	0.016
intercept	19.4	18.8
standard error of intercept	0.5	1.0
R^2 value	0.954	0.998

Table 8. The Limit of Detection and Limit of Quantification of the THC and THCA calibration curves in ppm.

	THC Calibration Curve in ppm	THCA Calibration Curve in ppm
LOD	30	9.1
LOQ	100	30

Table 9. The results of this research compared with other cannabinoid methods.

Research	THC elution time in min	THCA elution time in min	Column Dimensions	Runtime in min
This research	3.60	4.67	150 mm x 2.1 mm, 3 µm	11
Coulter et al.	5.00	5.00	50 mm x 2.1 mm, 1.8 µm	> 9
Mandrioli et al.	6.55	7.65	150 mm x 4.6 mm, 2.7 µm	8
Swift et al.	8.30	4.10	150 mm x 4.6 mm, 5 µm	11.2
Zivovinovic et al.	8.90	9.60	150 mm x 4.6 mm, 2.6 µm	15
Hazekamp et al.	11.5	15.4	250 mm x 4.6 mm, 5 µm	25
Lehmann et al.	40.0	47.0	200 mm x 2.0 mm	60

Figures

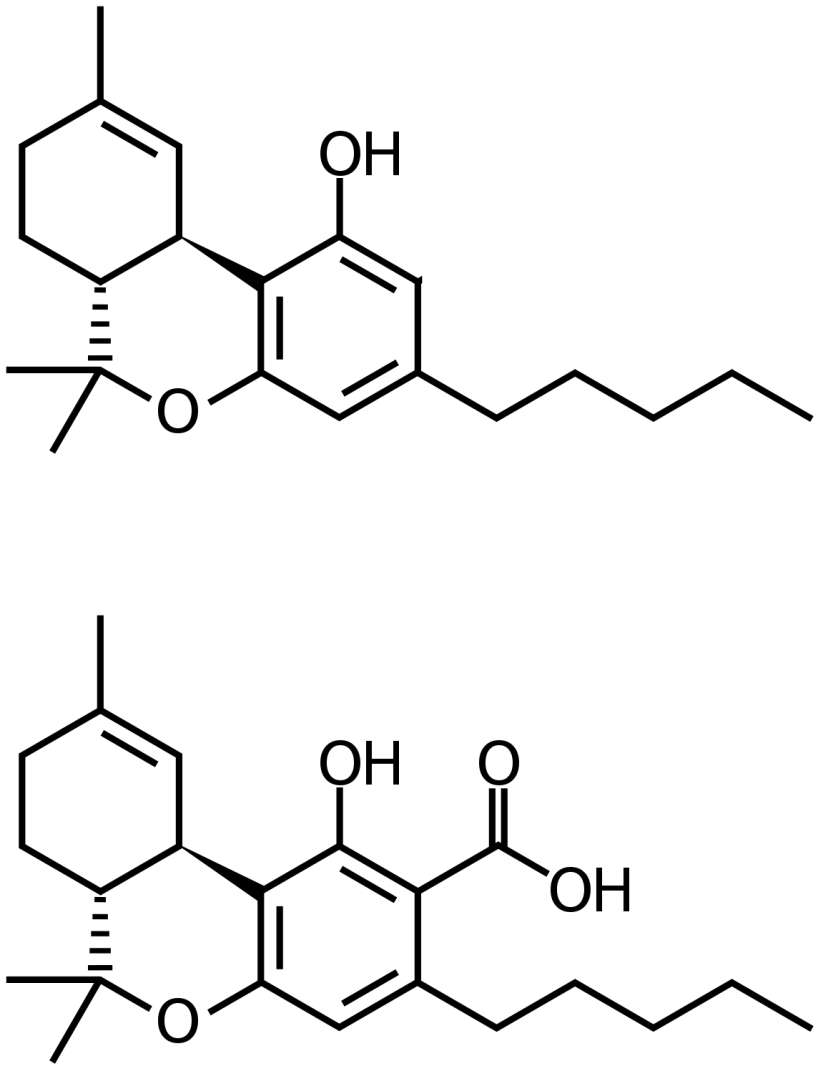

Figure 1: The structure of Δ^9-tetrahydrocannabinol (THC)(upper structure) and Δ^9-tetrahydrocannabinolic acid (THCA)(lower structure). Wikipedia, *Tetrahydrocannabinolic acid.* THCA has an added carboxy group on carbon 2 compared to THC.

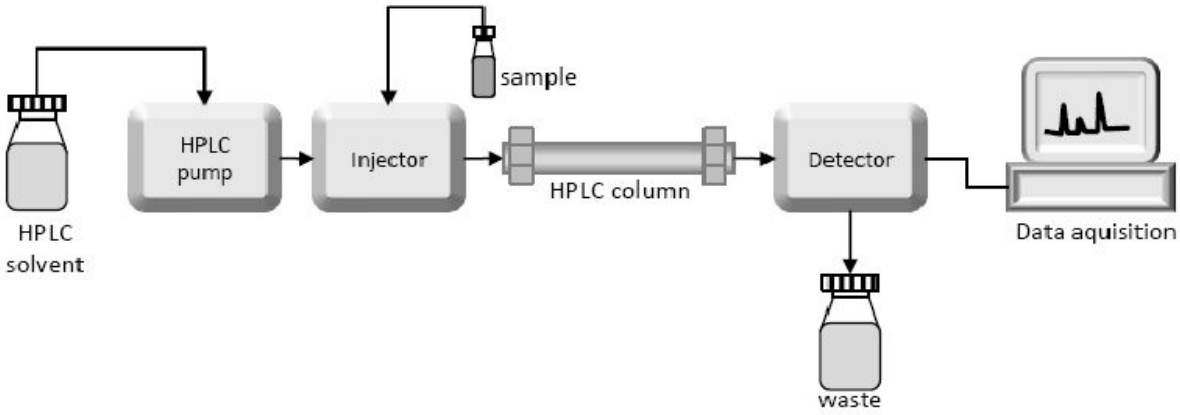

Figure 2: A diagram of a High Performance Liquid Chromatography (HPLC) System. Laboratory Info, *Instrumentation of HPLC.* The HPLC solvent is pumped through the system. Once loaded at the injector, the sample is carried by the mobile phase into the column. Here the sample components are separated and passed by the detector. The detector creates a chromatogram and the mobile phase and sample moves to waste.

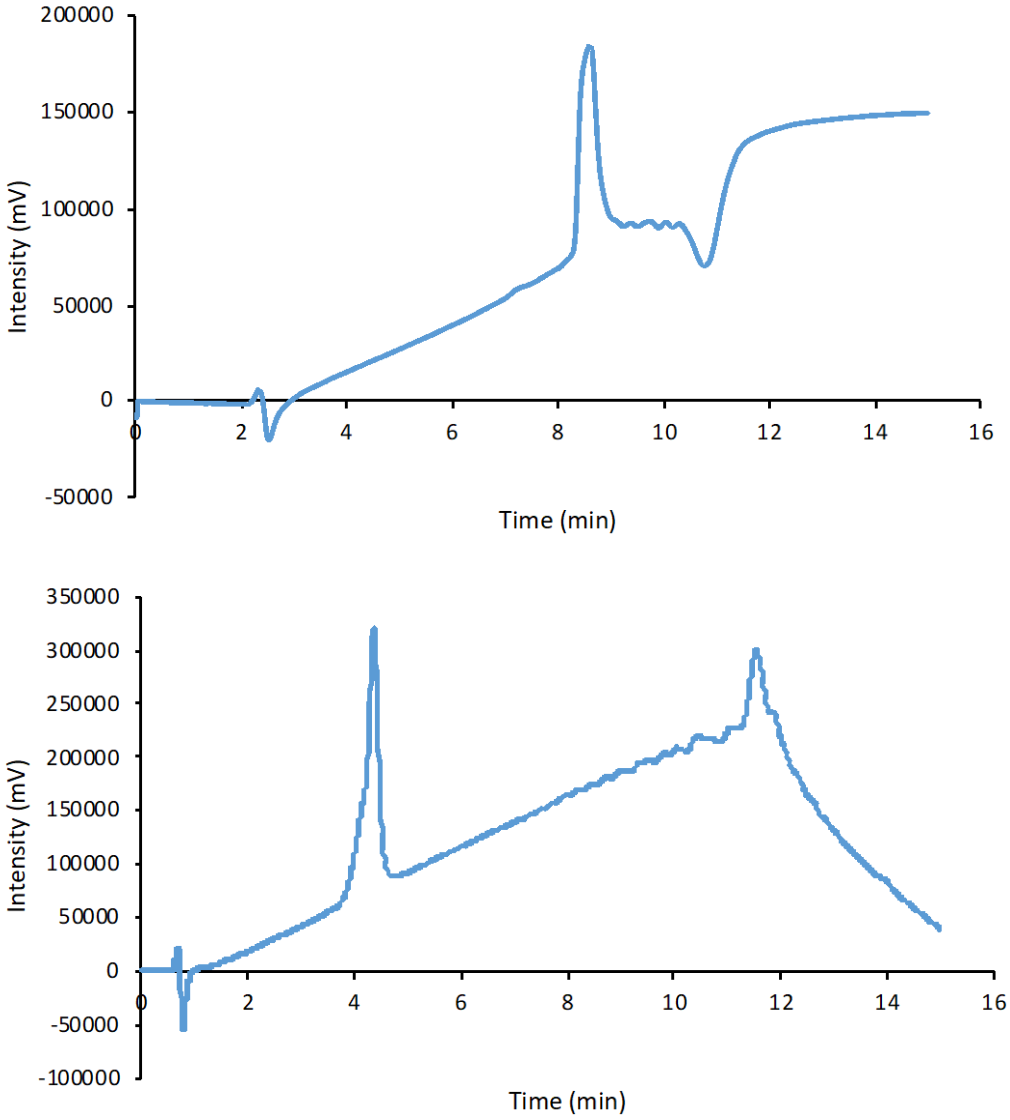

Figure 3. Chromatograms of THCA, not baseline adjusted, with differing column dimensions. The column dimensions of the first chromatogram (upper) are 250 mm x 4.6 mm, 5 µm, and the column dimensions of the second chromatogram (lower) are 150 mm x 2.1 mm, 3 µm. The THCA peak eluted much faster using the shorter column compared to the longer column, about 4.21 minutes faster. The mobile phases for both are 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The oven temperature for both is 40 °C. The flow rate for the first chromatogram is 1.00 mL/min and the flow rate for the second chromatogram is 0.75 mL/min. The time gradients are similar but not the same, with the first chromatogram having a time gradient of 60-100 over 0-8-12 min, ending at 15 minutes, and the second chromatogram having a time gradient of 65-100-65 over 0-10-15 min, ending at 22 minutes.

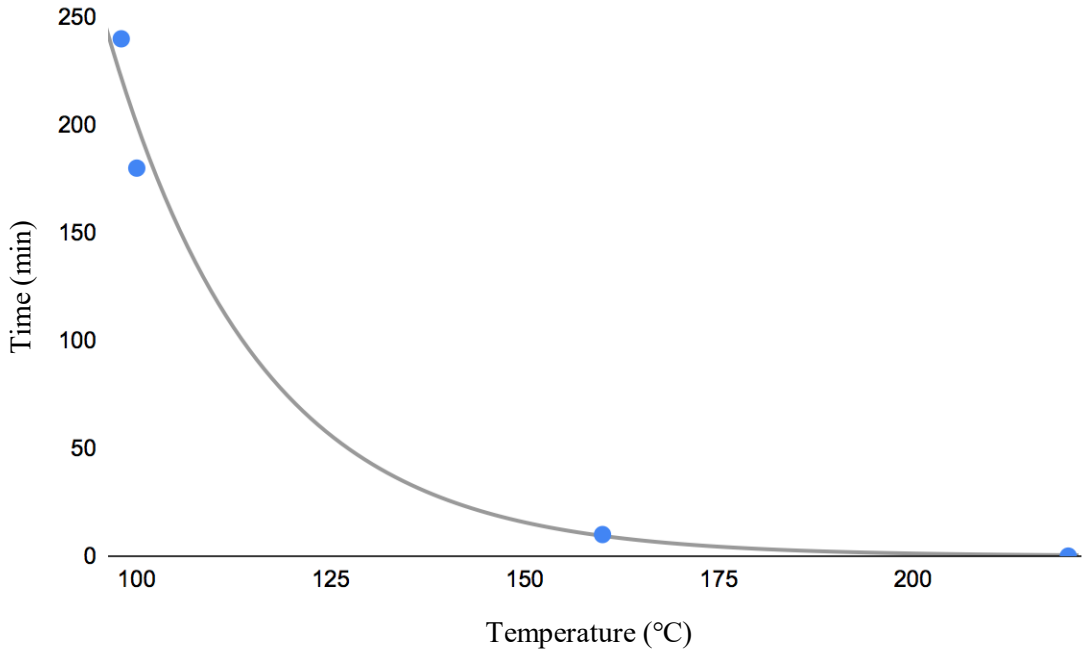

Figure 4. The time required to decarboxylate THCA into THC. The equation of the curve is $y = 32901*e^{-0.051x}$. The predicted time required to decarboxylate at 40 °C is 71.3 hours.

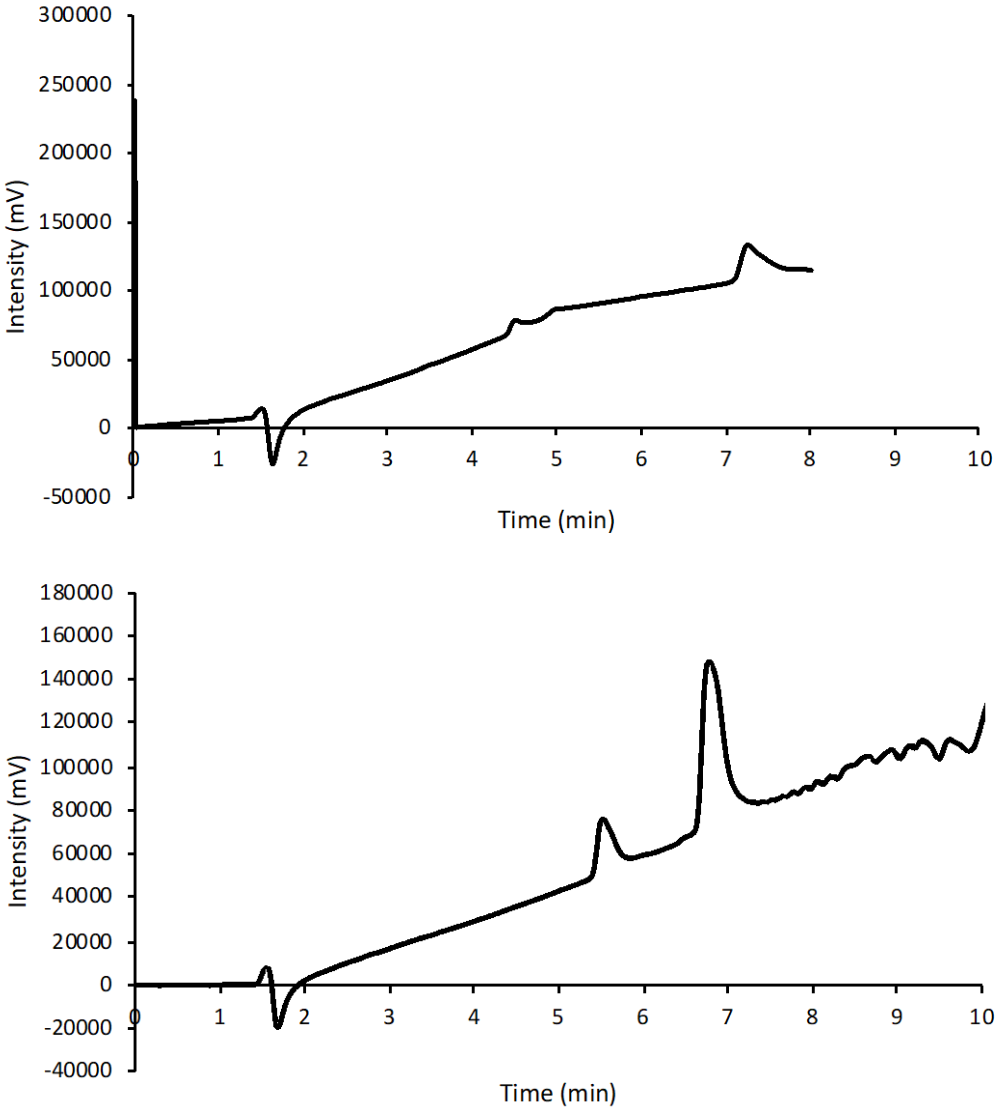

Figure 5. Chromatograms of THC and THCA, not baseline adjusted, with differing oven temperatures, wavelengths, and mobile phases. The oven temperature of the first chromatogram (upper) is 37 °C and the oven temperature of the second chromatogram (lower) is 40 °C. The mobile phases for the first chromatogram are 0.1% formic acid in a 50/50 mixture of acetonitrile/water and 0.1% formic acid in acetonitrile, and the mobile phases for the second chromatogram are 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The wavelength of the first chromatogram is 220 nm and the wavelength of the second chromatogram is 230 nm. The second chromatogram produced more prominent, as well as sharper, peaks, seen by the split THC peak in the first chromatogram. The column dimensions of both chromatograms are 250 mm x 4.6 mm, 5 µm. The flow rate for both chromatograms is 1.50 mL/min.

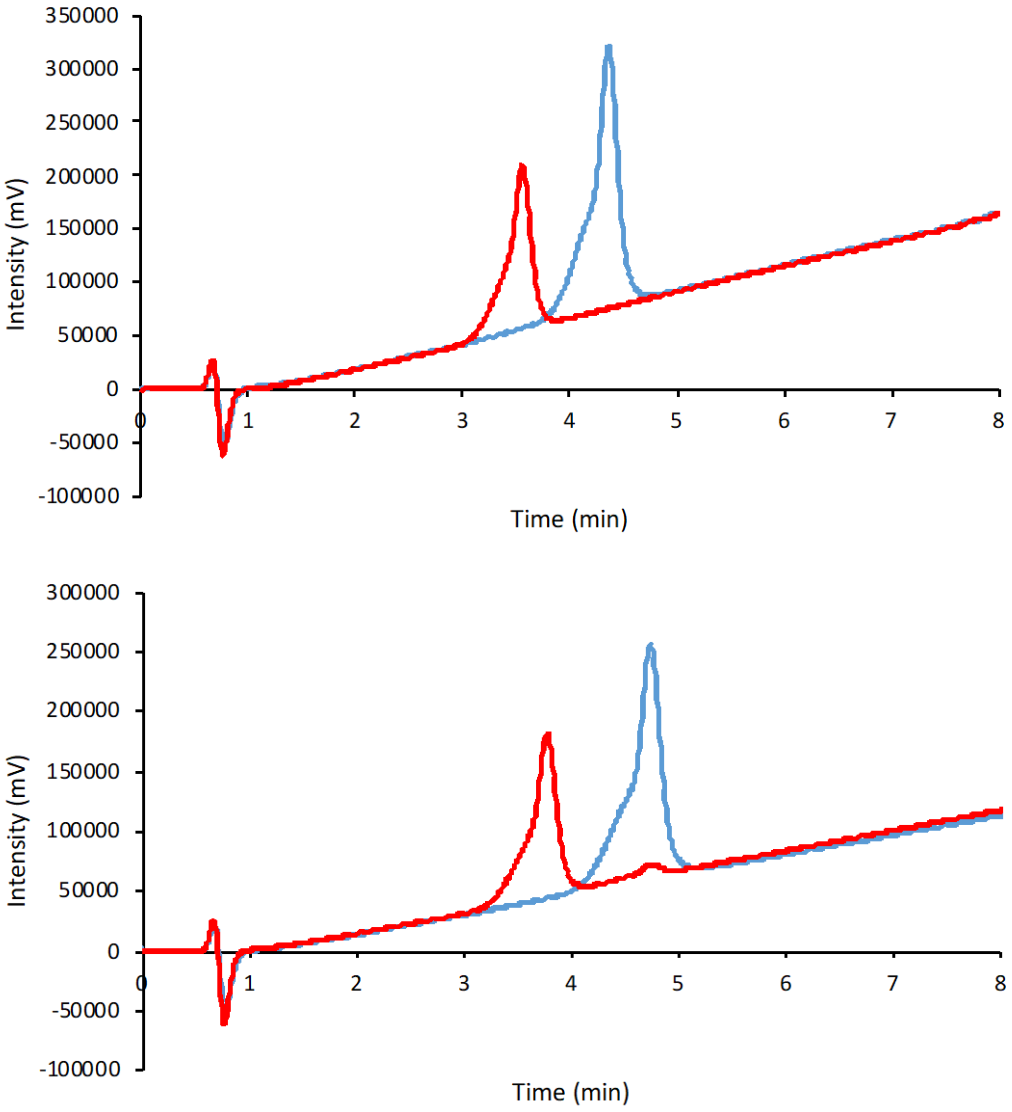

Figure 6. Chromatograms of THC and THCA overlaid, not baseline adjusted, with differing time gradients. The time gradient for the first chromatogram (upper) was 65-100-65 over 0-10-15 min, ending at 22 minutes, and the second chromatogram (lower) has a time gradient of 65-90-65 over 0-10-14 min, ending at 15 min. The column dimensions of both chromatograms are 150 mm x 2.1 mm, 3 µm. The mobile phases for both are 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The oven temperature for both chromatograms is 40 °C. The flow rate for both chromatograms is 0.80 mL/min. Even though THC eluted faster in the first chromatogram compared to the second, THC and THCA are better separated in the second chromatogram compared to the first, with a resolution of 3.81 compared with the resolution of the first chromatogram, 2.75.

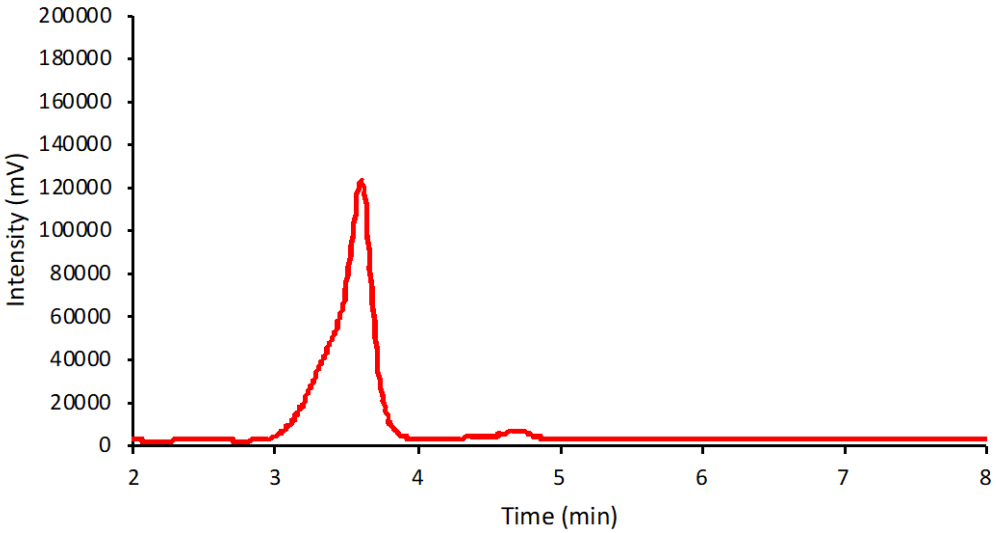

Figure 7. The chromatogram of the THC standard, shown in red, corrected with an acetonitrile blank. The THC standard peaked at 3.60 minutes. The small peak around 4.70 min is due to residual THCA from a previous run.

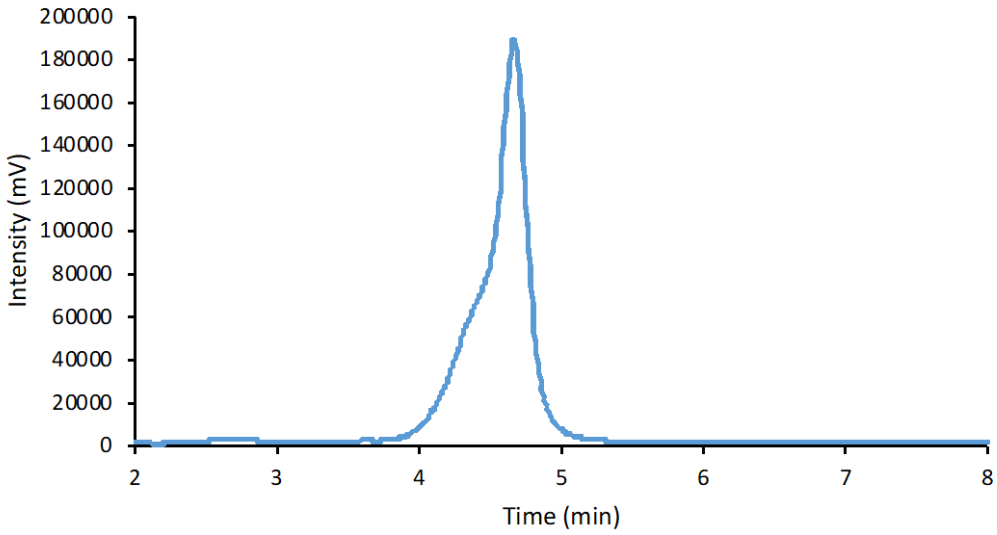

Figure 8. The chromatogram of the THCA standard, shown in blue, corrected with an acetonitrile blank. The THCA standard peaked at 4.67 minutes.

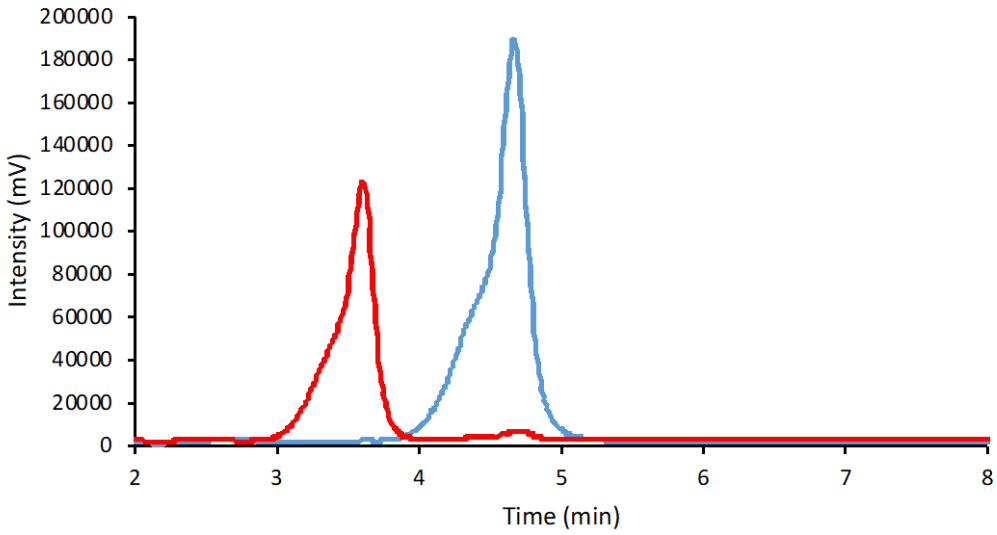

Figure 9. The THC (red) and THCA (blue) chromatograms overlaid. The resolution of the peaks was around 2.71, demonstrating good separation.

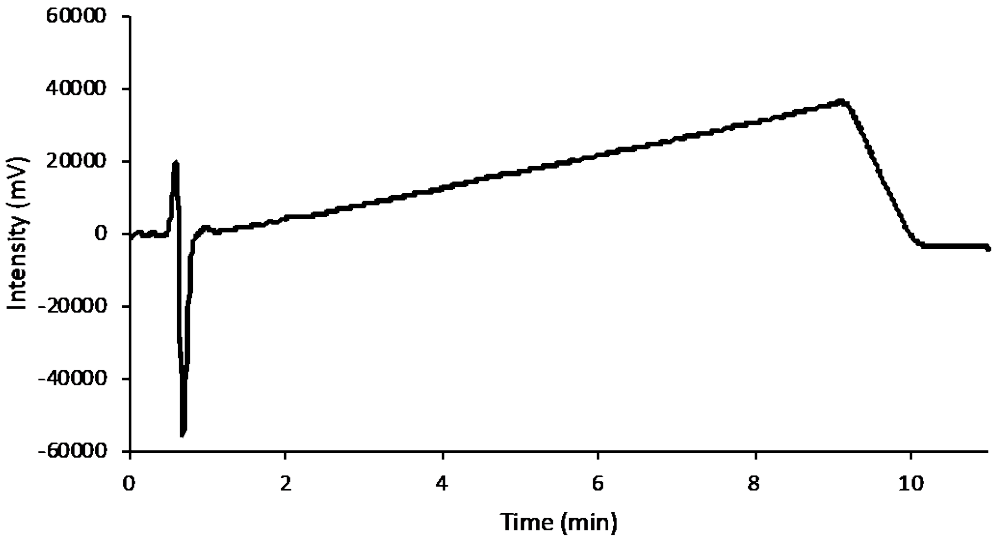

Figure 10. An acetonitrile blank. This blank was used to baseline all of the samples run.

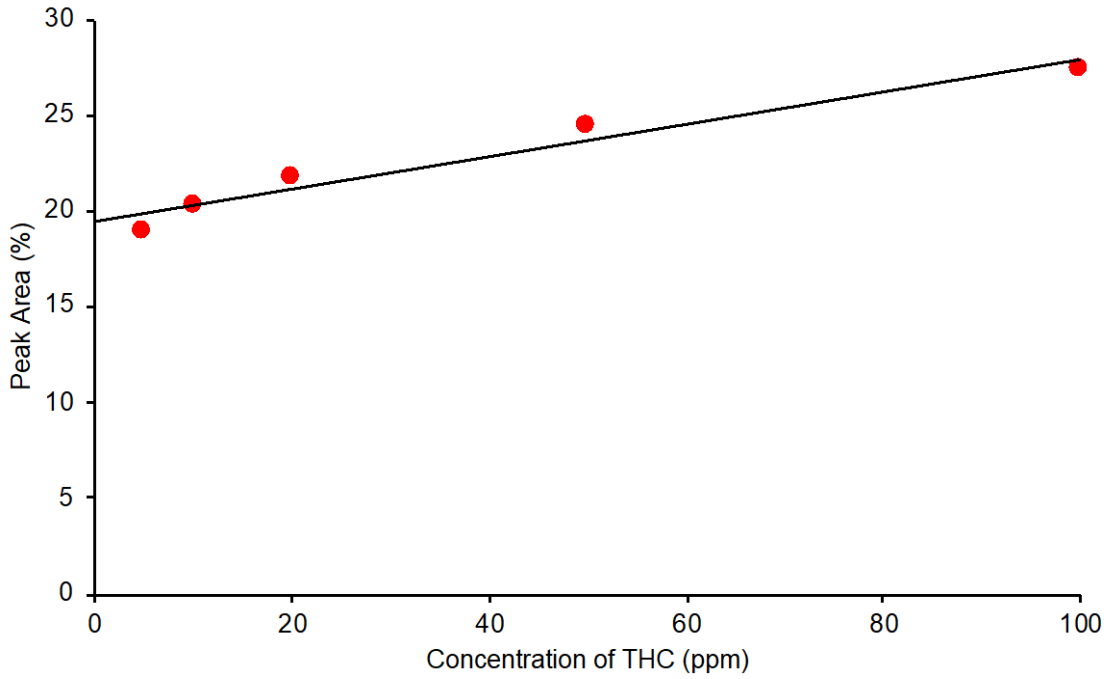

Figure 11. The THC calibration curve. The equation for the curve is $y = 0.085x + 19.4$. The standard error of the slope is 0.011 and the standard error of intercept is 0.5. The R^2 value is 0.954. The Limit of Detection is 30.0 ppm and the Limit of Quantification is 100. ppm.

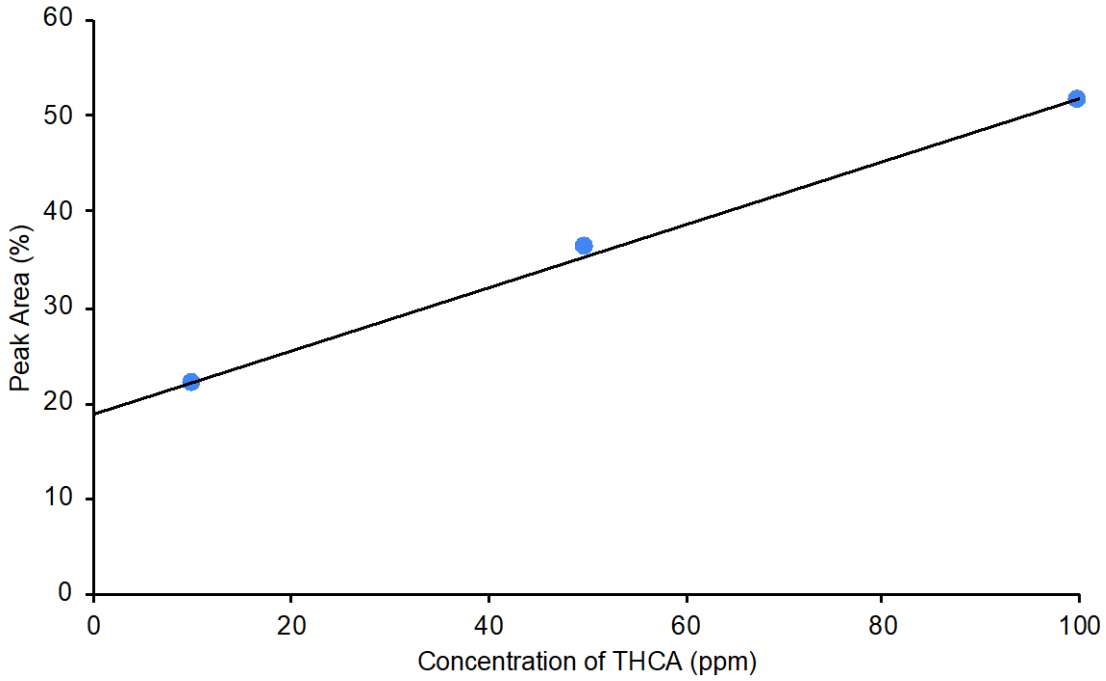

Figure 12. The THCA calibration curve. The equation for the curve is $y = 0.329x + 18.8$. The standard error of the slope is 0.016 and the standard error of the intercept is 1.0. The R^2 value is 0.998. The Limit of Detection is 9.1 ppm and the Limit of Quantification is 30 ppm.

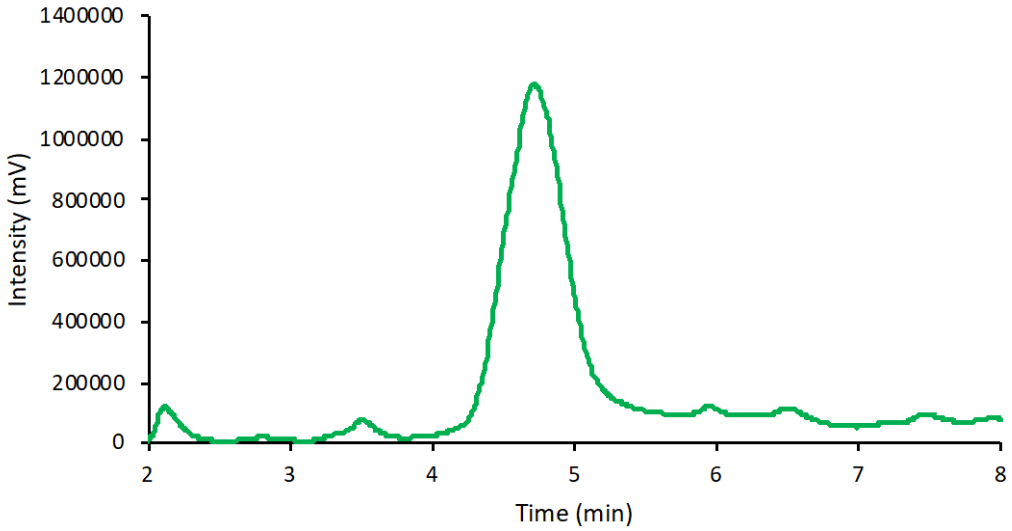

Figure 13. The chromatogram of a sample prepared from unspiked hemp oil solution, shown in green. THC peaked around 3.52 minutes and THCA peaked around 4.72 minutes. The THC concentration was below the LOQ (100 ppm). The THCA had a calculated concentration of 47 ppm. The calculated retention times for THC and THCA in the unspiked sample were 2.76 minutes and 3.96 minutes, respectively. The resolution of the unspiked sample was about 1.64.

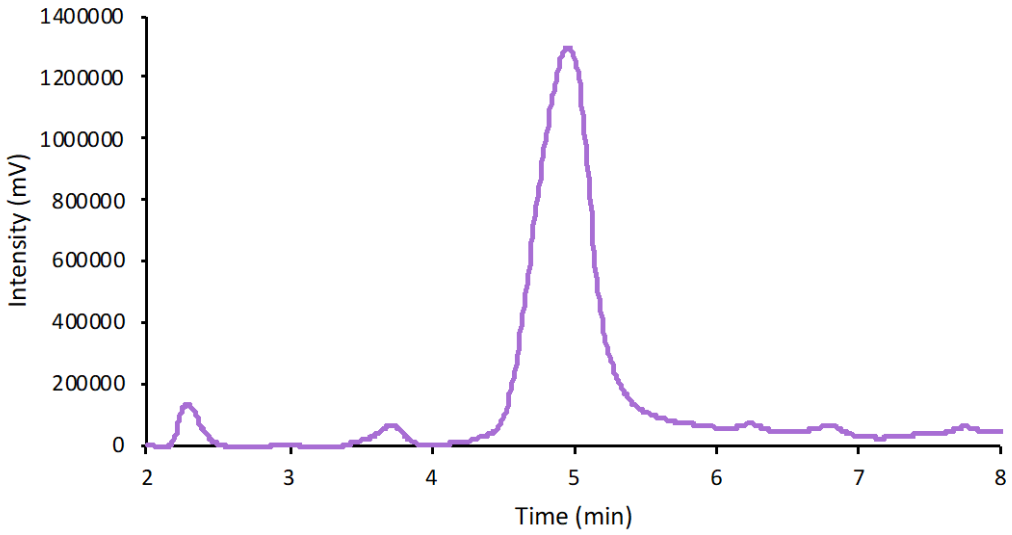

Figure 14. The chromatogram of a sample prepared from hemp oil solution spiked with THC and THCA, shown in purple. THC peaked around 3.72 minutes and THCA peaked around 4.96 minutes. The THC peak concentration was below the LOQ (100 ppm). The THCA peak had a calculated concentration of 56 ppm. The calculated retention times for THC and THCA in the spiked sample were 2.94 minutes and 4.19 minutes, respectively. The resolution of the spiked sample was about 1.96.

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