Cannabidiol and Tetrahydrocannabinol Determination Using High Performance Liquid Chromatography

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ABSTRACT

The purpose of this research was to create a fast method for cannabidiol (CBD), Δ9 and Δ8 tetrahydrocannabinol (THC) quantitation using reverse-phase high performance chromatography (RP-HPLC). Low UV detection sensitivity prevented creating a Δ9 and Δ8 THC calibration graph for quantitation. Elution mixtures of 85:15, 87:13, and 90:10 (v/v) percent methanol-water were tested for the mobile phase optimization. These compositions were tested for separation of a standard mixture of CBD, Δ9 and Δ8 THC. The 87:13 (v/v) methanol-water composition was optimal for this testing, producing analysis times under 5 minutes and resolutions of at least 1.7 or higher. Qualitative testing on hemp oil, containing added Δ8 and Δ9 THC, generated retention times (t_R), of 2.117, 3.919, and 4.314 minutes for CBD, Δ9, and Δ8 THC, respectively. HPLC detection comparisons showed a much greater UV sensitivity, by CBD, at 214 nm than at 254 nm.

The 87:13 (v/v) of methanol-water mobile phase was used for qualitative separation of CBD and the two THC isomers, and CBD quantitation. Two brands of hemp oil, with different CBD strengths, were used for CBD quantitation. Qualitative testing trials generated retention times (t_R) of 2.117, 3.919, and 4.314 minutes for CBD, Δ9-THC, and Δ8-THC, respectively. Following that a calibration curve was generated using CBD stock standard of 2.1 mg/mL, which was diluted to create the five standards of from 21.$_0$, and 105.$_0$ μg/mL. Three trials were run, detecting at 254 and 214 nm. Additionally, an external standard (that involves a single point calibration) was used at both 254 and 214 nm, due to poor dynamic range. The peak areas of each standard were used to create the two standards' plots and derive two calibration equations using the lower and upper three of the five concentrations points. The lower calibration equation was used to calculate the CBD concentrations in two hemp oil samples from their peak areas. Two average standard peak areas were used for the external standards calculations.

The average CBD in the hemp oil #1, with a declared value of 3500 mg/ 1 oz bottle (118 mg/mL single dose) was calculated at 3580 mg/1 oz bottle (121 mg/mL single dose) with a relative error of 2% of the declared concentration via the calibration plot method. However, the single point external standard method determined 43 and 50 mg/mL of CBD per single dose, with a relative error of -63% and -58%, using the 21.0 μg/mL and 52.$_5$ μg/mL reference standards, respectively at 254 nm. The hemp oil #2 with a declared value of 30,000 mg/ 1 oz bottle (1014 mg/mL single dose) sample produced CBD peak areas that were unreliable (RSD=75%), at 254 nm, but single point calibration method at 214 nm with reference standards, determined 489 and 748 mg/mL using the 21.0 μg/mL and 52.$_5$ μg/mL reference standards, at 214 nm, with a relative error of -40% and 26%, respectively.

1.0 INTRODUCTION

Cannabinoids are produced by a flowering plant found in eastern Asia, of the Cannabis genus, typically Cannabis sativa subsp. sativa and subsp. Indica[1]. Cannabis has been utilized for its fiber and for its therapeutic properties for thousands of years. It was introduced to western medical practice in the 1800s and was used until the laws of the 1930s prohibited its sale or use[2].

The most well-known of the cannabinoids, for therapeutic use, are cannabidiol (CBD) and tetrahydrocannabinol (THC) which are decarboxylation products of their precursors, cannabidiol acid (CBDA) and tetrahydrocannabinolic acid (THCA). Decarboxylation of these compounds occurs from aging or at temperatures above 110° C[4,5]. While CBD is legal in most places in the United States and elsewhere, THC remains illegal in many locales. The increased interest in these compounds has led to an increased need to quantify them in products being sold.

Cannabinoids are commonly assayed by gas chromatography (GC) or reverse phase high performance liquid chromatography (RP-HPLC). However, unlike GC, the use of HPLC requires no derivatization of the acidic cannabinoids. In addition, GC also requires higher column temperatures, due to the high boiling points of many of the non-derivatized cannabinoids[3,4,5].

The technique of HPLC provides an opportunity for both identifying and quantifying cannabinoids dissolved in a binary mixture of an organic solvent and water. By carefully choosing a binary mobile phase (M.P.) and a stationary phase (S.P.), cannabinoids of different polarity will travel at different rates through a column, separating into bands, represented as peaks in the recording software. The polarity of a substance will constantly be working toward equilibrium between dissolving in the M.P. and being adsorbed to the non-polar S.P. (the column). The intermolecular forces at work, in the reverse-phase HPLC columns are hydrogen bonding, dipole-dipole interactions, and Van der Wall forces[i]. The more polar compounds prefer the more polar phase, so tending to elute faster if the M.P. is more polar than the column, or eluting more slowly if the SP is more polar than the M.P. The time it takes to elute is called retention time, and under similar conditions will be approximately the same for the same compound, providing for tentative identification. The area of the peak created yields a measure of concentration. A sample with a known concentration will produce a peak area that can be used to determine concentration of the same compound, of unknown

concentration, by the ratio of areas. This technique is called an external standard technique. A more involved method creates a linear graph from a set of standards, run separately and plotted.

Creating a method for a particular type of elution, like cannabinoids with differing polarity, involves tailoring and testing MP solutions to make sure the elution finishes in a timely manner and that the compounds resolve, or separate, sufficiently. The purpose of this laboratory exercise was to develop an optimum M.P. composition for the fast separation and baseline resolution of CBD, Δ9-THC, and Δ8-THC. Using the optimum conditions, to determine CBD content in two over-the-counter CBD hemp-oil products advertised with a low CBD concentration (3,500 mg/30 mL bottle) and a high CBD concentration (30,000 mg/30 mL bottle).

2.0 EXPERIMENTAL

2.1 Chemicals:

Chemicals used included: methanol, HPLC Grade, Fisher Scientific, Waltham Massachusetts, CAS 67-56-1; water, HPLC Grade, Honeywell Research Chemicals, Morris Plains, NJ, CAS 365-4, acetonitrile, HPLC grade, Fisher Chemical, CAS-75-05-8. The organic hemp oil (HO #1) 3,500mg/30 mL bottle, Natgaenics (advertised with zero THC), amd Premium hemp oil (HO #2) 30,000 mg/30 mL bottle, were purchased from Amazon.com Online. Technical grade acetone was used for cleaning the glassware and HPLC syringe.

2.2 Equipment:

The equipment used included: Jasco HPLC #2 HPLC [PU-4180(pump), a rheodyne model six-port injector, UV-4075 (detector), LC-NETII/ADC, and an Eclipse XB reversed phase (C18, 150 mmx 4.6 mm i.d. x 5 μm dp) , Jasco Inc (Mary's Court MD), a 50-microliter syringe from Hamilton Company (Reno, NV), and a 100-1000 μL micropipette, VWRBrand International (Radnor, PA);

2.3 HPLC Conditions:

The detector wavelengths were 254 nm and 214 nm, the pump pressure was 1500 psi, the mobile phase was tested at an isotropic mixture of methanol and water at ratios of 85:15, 87:13, and 80:10, the flow rate was 1.5 mL/min, the sample size was 20 μL, and the temperature was room temperature (~15 °C).

2.4 Procedures

2.4.1 Preparation of Standards

Stand solution for qualitative and quantitative analysis. The CBD stock standard (2.1 mg/mL) was diluted x10, x8, x6, x4, and x2, with volumes of 200 µL of stock solution into, 1800, 1400, 1000, 600, and 200 µL of ACN. This yielded 210, 262.5, 350, 525 and 1050 µg/mL concentrations. Each of the previously mentioned five standards was further diluted x10, with volumes of 200 µL into 1800 µL of ACN. This yielded 21.0, 26.3, 52.5, and 105.0 µg/mL of calibrated standards. After each dilution, the diluted standards were placed in 4 mL vials, hand-mixed and placed in an ultrasonic bath for 2 min for complete dissolution. and placed in an ultrasonic bath for 2 minutes.

2.4.2 Hemp oil preparation

Hemp Oil 3,500 (3,500 mg/30 mL bottle) was one of the samples investigated was extracted by Dr. Andrea Mezencevova then mixed with acetonitrile (ACN) using a dilution factor of 81-fold. It will be referred to as HO#1 for the rest of the paper. Hemp Oil 30,000 (30,000 mg/30 mL bottle) was the second sample investigated and will be referred to as HO2 for the rest of the paper. It was also diluted in acetonitrile by Dr. Mezencevova by a factor of 81-fold. Both these dilutions, for HO#1 and HO#2, were diluted by a factor 100 and 1000 in ACN. For example, 100 µL and 10 µL aliquots of HO#1 HO#2 were further diluted to a volume of 10 mL in two separate volumetric flasks with ACN.

2.4.3 HPLC trials

To run an HPLC trial, first the HPLC pump, detector, and ADC were turned on. Then the controlling software was launched, a method was constructed, the flow and pump pressure were set, the detector wavelengths captured, the channels to be used captured, the output types, formats and methods saved. The pumps were turned on and the HPLC was allowed to equilibrate (until a stable baseline is obtained). The port is flushed a few times with methanol, to assure all previous samples were rinsed out prior to any injection.

Once the flat baseline was obtained, the HPLC was ready for injections to be executed. For each trial, 50 to 100 mL injected into the injection leaving the syringe in the port, while the valve is set in the load position, and the software set to the run/ready state. As soon as the

valve is switched to the inject (run) position, the trial is started. This injects 20 μL of standard or sample, while the remaining is flushed out of the loop. Approximately, two to three min after the start, the value is set to the load position, and the syringe removed. It can be loaded for the next run, at this point, so long as it remains in the load position. After the peaks have eluted, the software is instructed to stop the collection of data. Any external data can then be copied to an external drive and preparations for the next trial are made.

3.0 RESULTS AND DISCUSSION

3.1 Effect of methanol-water composition

Calibration and testing of CBD and THC required the development of an optimized mobile phase that gives a relatively good resolution and minimized analysis time. In the literature, a reverse-phase HPLC with isotropic mixture ratio common isotropic mixture ratio of 75:25 ACN/water is reported for eluting CDB and THC in 14.5 min e[2]. Due to the higher cost of ACN, the use of methanol and water was explored in this research. Because ACN is a stronger eluting agent than methanol, higher percents of methanol were substituted. Thus, the volume ratios of methanol:water at 85:15, 87:13, and 90:10 were tested.

Figure 1a-c shows the effect of methanol-water composition on the separation of CBD, Δ9-THC and Δ8-THC. Several trends are evident. Increasing the methanol content from 85 to 90% (v/v) in the M.P. decreases the total analysis time from 5.5 min to 3 min. However, resolution between CBD/Δ9-THC and Δ9/Δ8-THC peak pairs also change from 8.3, 5.6 to 2.2, 1.7 respectively.

The ADC failed to produce data on some of the trials, so chromatograph plot data was manually processed in a spreadsheet and the data produced for retention times, peak area, and efficiency data. The retention times were the same, to the level of precision measured for all cannabinoids measured. The peak areas for CBD were 4.8% less for manually calculated area values, using the corrected average peak areas. For the Δ9-THC peak areas, the corrected peak areas were the same. For the Δ8-THC peak areas, there was a 19% less difference. The difference, when it existed, was always less for the manual data. The reason for this is unknown, but the manual integration method will have some stepping differences from the ADC method with the ADC being able to integrate the peaks more continuously. This would lose some peak area.

The trends in NTP show a drop of about 25% from 85:15 to 90:10 in methanol-water composition for CBD. For Δ9-THC the NTP drop in that change was about 35%. For Δ8-THC the NTP drop was about 40%. The theoretical plate height, having an inverted relationship to NTP for a constant column length shows a similar type percent drop. The reasons for this are that as the strength of the eluent increases, with the higher methanol content, the retention time drops and the efficiency of the column drops, be that as a decrease in NTP or an increase in HETP. An anomaly with 87-13% methanol water M.P. testing showed a slight increase in efficiency over the 85-10% methanol water composition. The reason for this is unknown.

Retention time repeatability showed a high degree of repeatability for CBD, using both ADC and manual calculation methods, with an RSD of 0.27% at 85% methanol, below significant digit measures for 87% methanol, and below significant digit measures for 90% methanol M.P. content.

Trends for the effects of M.P. composition on the measured values match within value type whether ADC or manual calculation.

3.1.1 Choice of Detection Wavelength

The Jasco HPLC instrument offers dual UV wavelength channels. Several standard mixtures of CBD/THC specimens were run at 254 and 214 nm. The CBD peak was off scale at 214 nm, with poor response for THC, so 254 nm was chosen to generate chromatograms for mobile phase optimization. Generating a calibration curve at 214 nm [see Figure S2] with CBD, produced the lower chromatogram shown in Figure 3. The cutoff of the CBD peak brings the data produced into question.

Resolution at this concentration was 8.6 and 2.4, between the CBD/D9-THC and Δ9-THC/Δ8-THC, adjacent peaks*, changing to 8.8 and 1.6, at 87% (v/v) and and 5.6 and 1.7 at 90% (v/v) of methanol in water (Table 2).

*As calculated manually, no data was provided by ADC analyzer.

Pertinent data for the retention time, peak area, NTP, and HETP are found in table S1a-c in the supplemental information section.

Two hemp oil samples were run at a 90:10 methanol/water M.P. ratio.

In examining the chromatograms, a clear progression, from longer to shorter retention times is seen as the concentration of methanol increases in the mobile phase, as can be seen in Figures 1a-c.

Comparing the retention times and elution ratios of the three elutent mixtures demonstrates the 87:13 ratio to be a good compromise.

At the 87:13 ratio, the resolutions between CBD-Δ9-THC and Δ9-Δ8-THC are 5.6 and 1.7, respectively, with a total run time of less than 5 min. Therefore, 87% (v/v) methanol and 13% (v/v) water was chosen as the optimum mobile phase for isocratic elution, measured a wavelength of 254 nm, as a good tradeoff between total elution time and resolution. All calibrations and quantitation were performed using this mobile phase composition.

3.2 Qualitative Analysis of CBD and THC

Comparing the chromatograms shown in Figure S3, of hemp oil sample #1 (HO1), and hemp oil sample 2 (HO2) in Figure S3 and S4, HO1 has a clear peak at about 2.1 min. and HO2 has a much less pronounced peak at the same retention time. HO1 shows a peak for Δ9-THC at about 3.7 mins, with HO2 showing a similar peak at that retention time. The peak height for HO2 CBD is a little less than the peak for Δ9-THC. No Δ8-THC peak was observed on any of the hemp oil trials.

The first run was deemed non-repeatable and discarded. The rest gave an RSD of less than 0.01% for the t_R and 1.08% for the peak area. The RSDs for retention time and peak area values were under 2% and 20%, respectively, so were considered repeatable.

3.3 CBD Calibration

Poor detection of THC by HPLC-UV limited the quantitation portion of this research to CBD only.

3.3.1 Calibration trials at 254 nm:

Using a stock solution of 2.1 mg/mL, dilutions of x2, x4, x6, x8, and x10 gave concentrations that were then each diluted by a factor of 10 with ACN yielding concentrations of 105.0, 52.5, 35.0, 26.25, and 21.0 μg/mL, respectively. The specific process was mixing 200 μL of the stock solution to each of 1800, 1400, 1000, 600, and 200 μL ACN, then 200 μL of each

were added to 800 μL ACN to give the above dilutions. This can be found in Table S6, in the supplemental information section.

The retention times showed relative standard deviations (RSDs) of well below 2.0%, considered the cutoff for repeatable retention times. The RSDs for the peak areas were significantly below 20%, considered repeatable for peak area measurements. This data is in table S3, found in the supplemental information section.

The CBD calibration plots, in Figure S1, includes all 5 calibration points, measured at 254 nm. Visually the plot is less the linear and it shows a low R^2 value of 0.937. When it was broken into two plots, the first with the first three data points, and the second with the last three data point with the 0.035 mg/mL is common to both. These plots are in Figure 2a and 4b. The R^2 values were higher, as would be expected, and the plots were visually more linear. These were chosen for producing the calibration equations that follow.

For peak areas at 254 nm the two linear regression equations (equation 1 and 2) were for a three-point curve

$$\mathbf{y = 3923.6x - 44400} \qquad \mathbf{(1)}$$

$$\mathbf{y = 1214.3x + 52700} \qquad \mathbf{(2)}$$

These above two regression-equations allowed determining the unknown concentration values (mg/mL) in hemp oil as follows:

$$\mathbf{x = (y + 44400) / 3923.6 = \mu g/mL}$$

$$\mathbf{x = (y - 52700) / 1214.3 = \mu g/mL}$$

An example calculation using 14100 μV.s as the peak area:

$$\mathbf{(13700 + 44400) / 3923.6 = 14.8\ \mu g/mL}$$

They both equations suffer from a poor y-intercept, meaning a peak absorbance value of zero would yield 11.3 and -43.2 μg/mL of CBD. That said, the equations work well when the

absorbance being used for calculation is in the regions between 41000 – 94700 μV.s, and 94700 – 180000 μV.s.

An external method was also used. Concentrations were calculated using R_f values derived from the 52.5 μg/mL and 21 μg/mL standards. This was done for both hemp oil CBD peak areas, at 254 and 214 nm, for data that was repeatable.

254 nm example:

Using 21.0 μg/mL standard

R_{f254} = PeakArea/$Conc_{ExtStd}$ = 117000/52.5 = 2230

$Conc_{unk}$ = PeakArea $_{unk}$/ R_{f254} = 14100/2230 = 6.3 μg/mL

Or 52.5 μg/mL standard

R_{f254} = PeakArea/$Conc_{ExtStd}$ = 53600/21.0 = 2550

$Conc_{unk}$ = $PeakArea_{unk}$/ R_{f254} = 14100/2552 = 5.5 μg/mL

3.3.2 Trials at 214 nm:

In examining the data at 214 nm, the results were not considered acceptable. The R^2 value was approximately 0.71 and the CBD peak maxed out, making the area measurements unreliable.

The calibration plot from the absorbances at 214 nm is in Figure S2, the data for which is found in Table S4.

The retention times showed relative standard deviations (RSDs) of well below the 2.0% currently considered the cutoff for repeatable retention times. The RSDs for the peak areas were significantly below the 20% considered repeatable for peak area measurements.

In Figure S2, the area versus concentration regression doesn't show a linear fit. Because of the poor fit, the above data was not considered in deriving CDB values of unknowns.

A second area versus concentration regression was created from the upper two calibration standards (0.0525 and 0.105 mg/mL) and is shown in Figure S6.

That said, the absorption vs concentration data was collected and is found in Table 3. While the full calibration plot was useless, a second plot was used to create a calibration curve.

y = 22181x + 9000000

This regression produced concentration values (μg/mL) as follows:

x = (y - 9000000) / 22181 = μg/mL

Unfortunately, all hemp oil specimens measured absorbances of less than 9,000,000 μV.s which yield nonsensical values.

Also, the 0.0525 and 0.021 mg/mL standards were used as external reference and the following is an example of using an external reference to compute the concentration. The peak area data for HO1, at 214 nm, was deemed unrepeatable, with an RSD of 138%, exceeding the 20% exclusion criteria. Calibration data for 214 nm is in table S4, HO2 in table S8.

For instance,

For the 52.5 μg/mL standard,

R_{f214} = PeakArea/$Conc_{ExtStd}$ = 9900000/52.5 = 189000

$Conc_{unk}$ = $PeakArea_{unk}$/ R_{f214} = 1744000/189000 = 9.2 μg/mL

Or the 21.0 μg/mL standard,

R_{f214} = PeakArea/$Conc_{ExtStd}$ = 6060000/21.0 = 289000

$Conc_{unk}$ = $PeakArea_{unk}$/ R_{f214} = 1744000/289000= 6.0 μg/mL

3.4 CBD Quantitation

Three trials of samples in were tested, HO1 and HO2, diluted to the close to the same concentration (assuming the declared concentration was accurate), and mixed CBD, Δ8, and Δ9-THC. Due to poor detectability of THC, no quantitation was done on any of the hemp oil samples.

3.4.1 Hemp Oil 3500/30000 mg samples (HO1/HO2)

These samples had a base dilution of 81 fold during extraction procedure performed by the laboratory instructor was provided for analysis. The extracted hemp oil was then diluted 100 fold for the HO1, and 1000 fold for the HO2 samples. These were declared as 3500 mg/fluid ounce, or 118 mg/mL

and 30000 mg/fluid ounce, or 1014 mg/mL respectively. This resulted in dilutions of 8100 for the 3500 and 81000 for the 30000 samples.

At a λ of 254 nm, the HO1 dilutions gave an average area of 14100 μV.s. The standard deviation was acceptable, and the relative standard deviation was 1.2%, below the 20% cutoff. The full data can be found in Table 4.

Computing the value of the hemp oil yields 14.9 mg/mL, or 3.48 g/oz, which is 104% of the declared amount of 118 mg/mL or 3500 mg/oz.

Using the plot equation, the average computed concentration was 14.9 μg/mL, with a standard deviation of 0.27 μg/mL. Using the external standard R_f method, for the standard with the closest peak absorbance to the unknown yields 5.4 μg/mL. Using the higher standard external standard R_f gives the value of 6.1.

At 254 nm, the CBD peak was clear, and always with a t_R between 2.105 and 2.120 minutes for all the calibration trials. The peak absorbance data is found in Table 4. Comparison with declared values in Table 6.

Figure S3 is a sample chromatogram of HO1.

At a λ of 254 nm, the HO2 sample dilutions no ADC data was produced. Manual data processing produced an average area of 78.6 μV.s. The relative standard deviation was 76%, above the 20% cutoff. The data wasn't repeatable, therefore considered unacceptable. The full data can be found in table S3, in the supplemental information section.

Computing the concentration from the plot calibration equation was performed due to the unreliable data. At 214 nm, the calibration plot was non-linear, so was not used. The external R_f values were used to compute the concentration, yielding 6.0 μg/mL with the equation derived from the lower, or closest standard to the absorbance, and 9.2 μg/mL for the upper standard derived equation. These measurements had an accuracy of -52% and -26%, respectively, compared to the declared concentration of 1014 mg/mL. Comparison with measured vs declared values in table 6.

The peak absorbance data is found in Table 4. Figure S4 is a sample chromatogram of the HO2.

4.0 CONCLUSIONS

In determining an optimal mobile phase composition, elution mixtures of methanol and water of 85:15, 87:13, and 90:10 ratios were tested against a mixture of CBD, Δ9 and Δ8 THC. The 87% methanol mixture produced good retention times and component resolutions. This method was used for a qualitative testing of a hemp oil specimen containing added Δ8 and Δ9 THC. These runs generated retention times, t_R, of 2.117, 3.919, and 4.314 minutes for CBD, Δ9, and Δ8 THC, respectively.

A CBD stock standard of 2.1 mg/mL was employed to create working standards of 21, 26.$_3$, 35, 52.$_5$. and 105 μg/mL. The chromatograms measured at 214 nm produced non-linear calibration plots, so only data from 254 nm was used for calibration equations, though the 214 data was used for external standard determinations. These peak areas and their concentrations enabled the creation of two standards' plots and calibration equations. Breaking the dataset into two groups, lower and upper, produced more linear plots. The R^2 value jumped from below .94 to above 0.98 & 0.99. This resulted in two calibration equations, one linear from peak areas of 94700 to 180,000 μV.s, and another from peak areas of 41,000 to 94,700 μV.s. For the external standard, the 0.02625 and 0.0525 mg/mL values were used to produce the R_f values of 2550, and 2230 for the 254 nm peak areas, as well as 289000 and 189000 for the 214 nm peak areas.

The calibration equations were used to calculate the CBD concentrations in two hemp oil samples from their average corrected peak areas. The areas were obtained from x8100 and x81000 dilutions of HO1 and HO2, respectively. The average CBD in the Hemp Oil 3500 was calculated at 3460 mg/oz, with a relative error of -1% of the declared content, via the calibration plot/equation, and 1610 and 1470 mg/oz via the two external standards used, with a relative error of -62% and -57%, respectively. The Hemp Oil 30000 sample produced areas that were unreliable (RSD=75%), at 254 nm, but when calculated via external standard method, at 214 nm, the two results were 18.1 and 22.1 g/oz, with a relative error of -53% and 26%, respectively.

5.0 REFERENCES

(1) De Backer, B.; Debrus, B.; Lebrun, P.; Theunis, L.; Dubois, N.; Decock, L.; Verstraete, A.; Hubert, P.; Charlier, C. Innovative Development and Validation of an HPLC/DAD Method for the Qualitative and Quantitative Determination of Major Cannabinoids in Cannabis Plant Material. *Journal of Chromatography B* **2009**, *877* (32), 4115–4124. https://doi.org/10.1016/j.jchromb.2009.11.004.

(2) Aizpurua-Olaizola, O.; Omar, J.; Navarro, P.; Olivares, M.; Etxebarria, N.; Usobiaga, A. Identification and Quantification of Cannabinoids in Cannabis Sativa L. Plants by High Performance Liquid Chromatography-Mass Spectrometry. *Analytical and Bioanalytical Chemistry* **2014**, *406* (29), 7549–7560. https://doi.org/10.1007/s00216-014-8177-x.

(3) Analakkattillam, S.; Langsi, V. K.; Hanrahan, J. P.; Moore, E. Analytical Method Validation for Assay Determination of Cannabidiol and Tetrahydrocannabinol in Hemp Oil Infused Products by RP-HPLC. *Scientific Reports* **2022**, *12* (1). https://doi.org/10.1038/s41598-022-13737-6.

(4) Aizpurua-Olaizola, O.; Soydaner, U.; Öztürk, E.; Schibano, D.; Simsir, Y.; Navarro, P.; Etxebarria, N.; Usobiaga, A. Evolution of the Cannabinoid and Terpene Content during the Growth of Cannabis Sativa Plants from Different Chemotypes. *Journal of Natural Products* **2016**, *79* (2), 324–331. https://doi.org/10.1021/acs.jnatprod.5b00949.

(5) Wang, M.; Wang, Y.-H.; Avula, B.; Radwan, M. M.; Wanas, A. S.; van Antwerp, J.; Parcher, J. F.; ElSohly, M. A.; Khan, I. A. Decarboxylation Study of Acidic Cannabinoids: A Novel Approach Using Ultra-High-Performance Supercritical Fluid Chromatography/Photodiode Array-Mass Spectrometry. *Cannabis and Cannabinoid Research* **2016**, *1* (1), 262–271. https://doi.org/10.1089/can.2016.0020.

(6) Vitha, M. F. *Chromatography*; John Wiley & Sons, 2016., pg 147-149

(7) Analakkattillam, S.; Langsi, V. K.; Hanrahan, J. P.; Moore, E. Analytical Method Validation for Assay Determination of Cannabidiol and Tetrahydrocannabinol in Hemp Oil Infused Products by RP-HPLC. *Scientific Reports* **2022**, *12* (1). https://doi.org/10.1038/s41598-022-13737-6.

(8) Pourseyed Lazarjani, M.; Torres, S.; Hooker, T.; Fowlie, C.; Young, O.; Seyfoddin, A. Methods for Quantification of Cannabinoids: A Narrative Review. *Journal of Cannabis Research* **2020**, *2* (1). https://doi.org/10.1186/s42238-020-00040-2.

Figure Captions

Figure 1: Overlaid chromatograms showing the effects of methanol-water percent composition on the separation of cannabinoids (CBD, Δ9-THC, and Δ8-THC). Top chromatogram: 85% (/vv) methanol-15% (v/v) water, middle chromatogram: 87% (/vv) methanol-13% (v/v) water, bottom chromatogram: 90% (/vv) methanol-10% (v/v) water, and eluted using a reversed phase C-18 column (15 cmx 254 nm, with respective, labeled peaks at 2.602, 4.620, and 5.280 minutes, with an elution mixture of 85% methanol and 15% water. The resolution between these peaks was 8.3 and 2.2, 8.8 and 1.6, 5.6 and 1.7 respectively from the top to the bottom chromatograms.

Figure 2: Calibration plots showing (a) the lower three standard CBD concentrations (21, 26.3, and 35 μg/mL), and (b) upper three concentrations (35, 52.5, and 105 μg/mL) versus their corrected average peak areas, including the equation for linear regression and R^2 value. The inset equations in each plot is a linear regression equation that is used to determine the concentration of CBD in two commercial brands of hemp oil..

Figure 3: Overlay of CDB, Δ9-THC, Δ8-THC chromatogram at 254 and 214 nm.

Figure 1

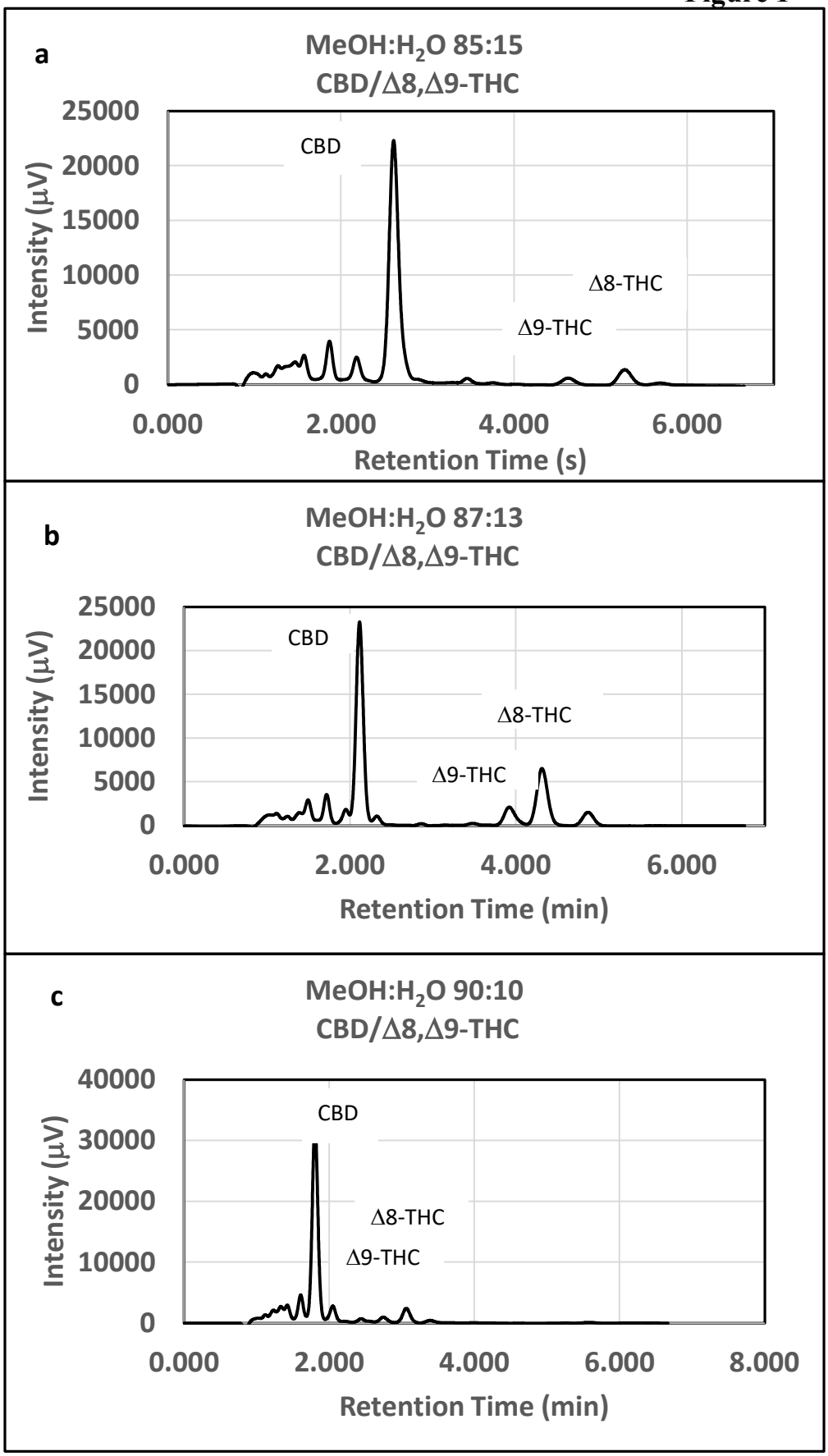

a
MeOH:H2O 85:15
CBD/Δ8,Δ9-THC
Intensity (μV)
25000
20000
15000
10000
5000
0
CBD
Δ8-THC
Δ9-THC
0.000
2.000
4.000
6.000
Retention Time (s)
b
MeOH:H2O 87:13
CBD/Δ8,Δ9-THC
Intensity (μV)
25000
20000
15000
10000
5000
0
CBD
Δ8-THC
Δ9-THC
0.000
2.000
4.000
6.000
Retention Time (min)
c
MeOH:H2O 90:10
CBD/Δ8,Δ9-THC
Intensity (μV)
40000
30000
20000
10000
0
CBD
Δ8-THC
Δ9-THC
0.000
2.000
4.000
6.000
8.000
Retention Time (min)

Figure 2

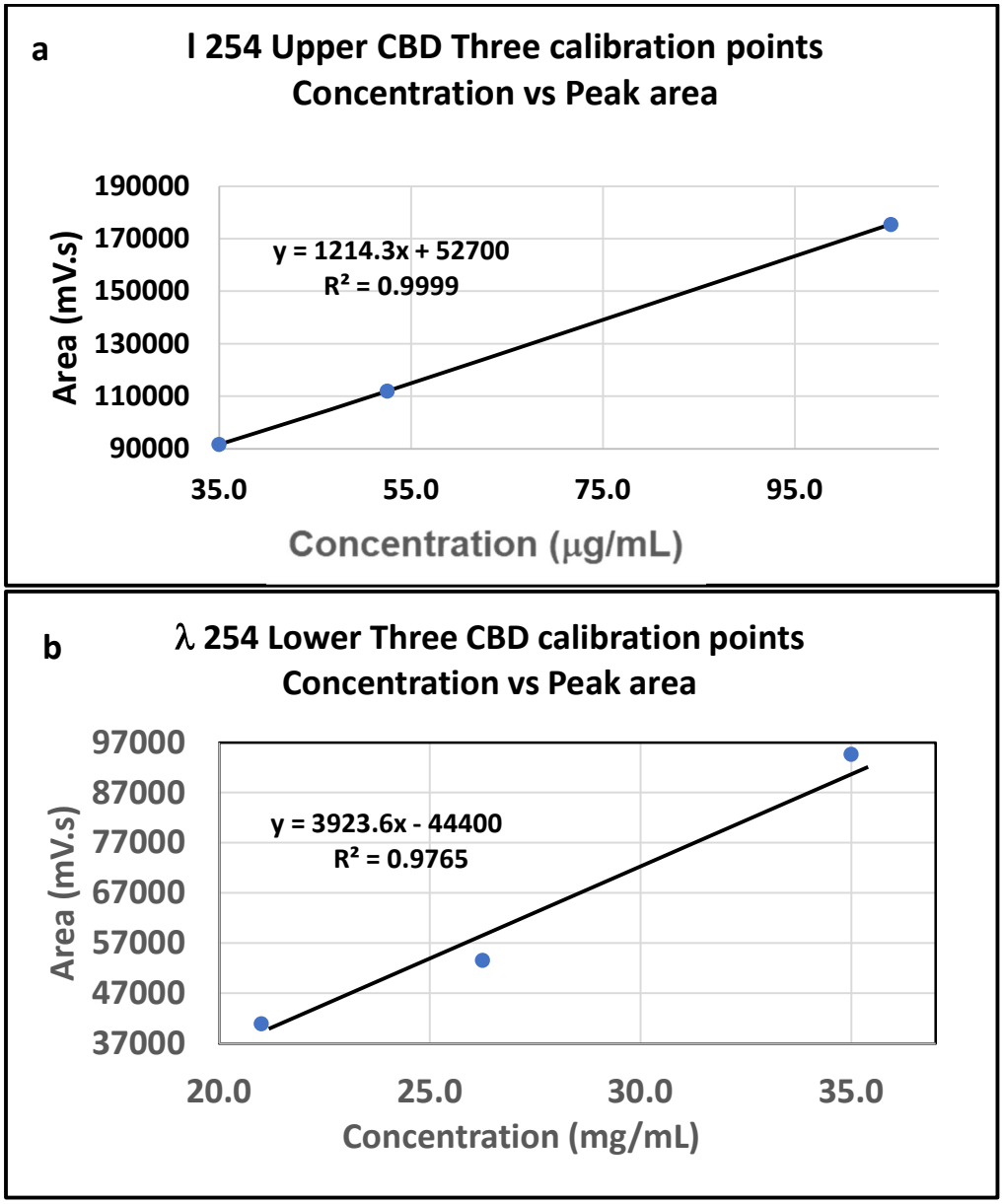

a
l 254 Upper CBD Three calibration points
Concentration vs Peak area
y = 1214.3x + 52700
R² = 0.9999
Area (mV.s)
190000
170000
150000
130000
110000
90000
35.0
55.0
75.0
95.0
Concentration (µg/mL)
b
λ 254 Lower Three CBD calibration points
Concentration vs Peak area
y = 3923.6x - 44400
R² = 0.9765
Area (mV.s)
97000
87000
77000
67000
57000
47000
37000
20.0
25.0
30.0
35.0
Concentration (mg/mL)

Figure 3

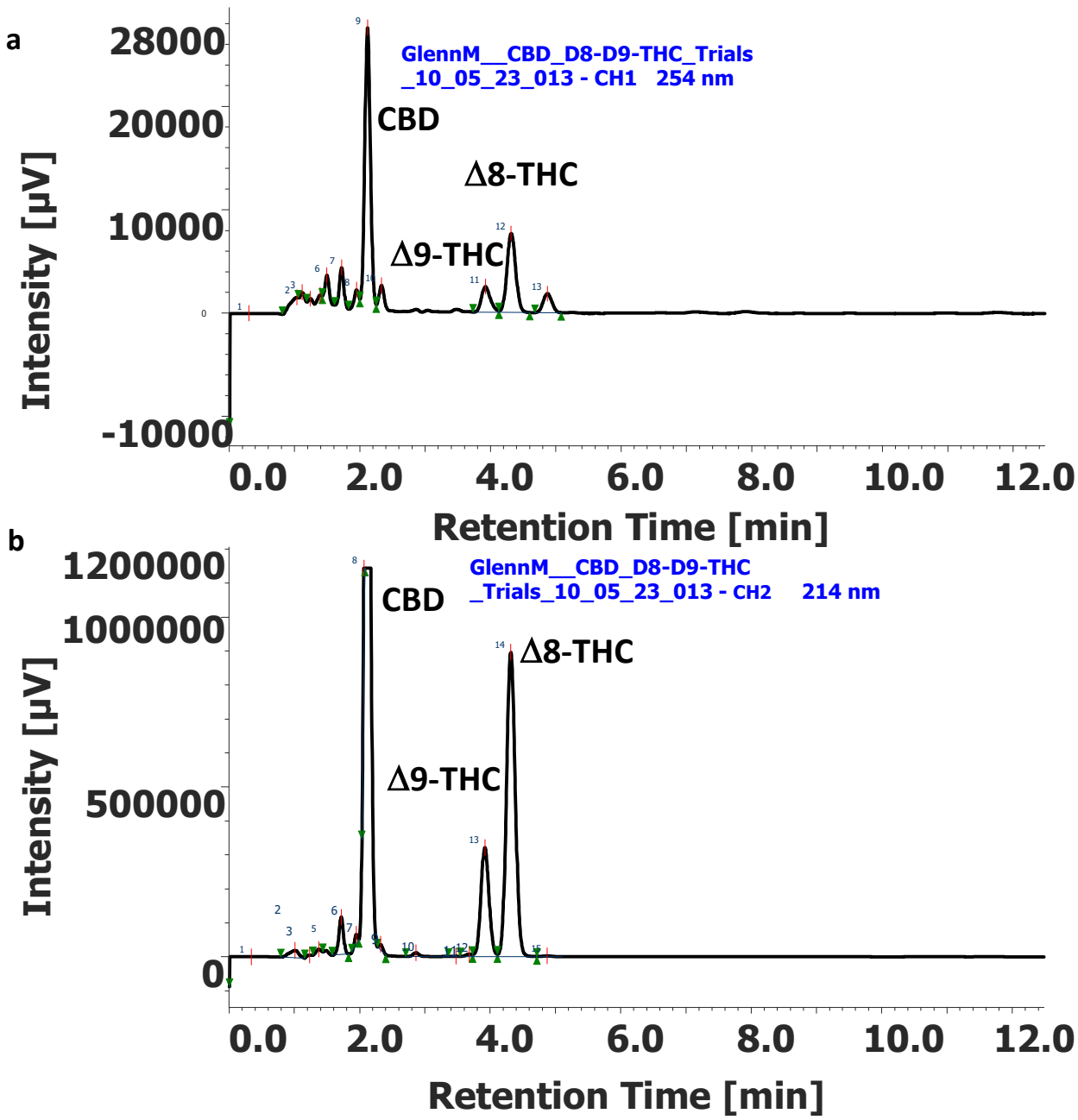

a
28000
20000
10000
0
-10000
Intensity [µV]
GlennM__CBD_D8-D9-THC_Trials
_10_05_23_013 - CH1 254 nm
CBD
Δ8-THC
Δ9-THC
0.0
2.0
4.0
6.0
8.0
10.0
12.0
Retention Time [min]
b
1200000
1000000
500000
0
Intensity [µV]
GlennM__CBD_D8-D9-THC
_Trials_10_05_23_013 - CH2 214 nm
CBD
Δ8-THC
Δ9-THC
0.0
2.0
4.0
6.0
8.0
10.0
12.0
Retention Time [min]

Table 1. CBD, Δ9-THC, Δ 8-THC peak areas, NTP, and HETP values, manual and ADC calculated

CBD	ADC Calculated				Calculated from Raw Data			
	Peak t_R (mins)	Area (μV.s)	N	HETP	Peak t_R (mins)	Area (μV.s)	N	HETP
Trial 1	2.117	143259	2666	0.00563	2.117	137405	2849	0.00526
Trial 2	2.117	145959	8753	0.00171	2.117	138782	2849	0.00526
Trial 3	2.117	145966	2651	0.00566	2.117	138782	2849	0.00526
Avg	2.117	145061	4132	0.00478	2.117	138323	2849	0.00526
Corr Avg	2.117	145000	4000	0.00400	2.117	138000	2849	0.00526
StdDev	-	1561	3082	0.00205	-	795	-	-
RSD	-	1.1	75	43	-	0.57	-	-
Δ9–THC								
Trial 1	3.917	19804	4811	0.0031	3.917	20195	3951	0.00380
Trial 2	3.920	20175	3984	0.0038	3.920	19397	5017	0.00299
Trial 3	3.920	20074	3980	0.0038	3.920	19397	4144	0.00362
Avg	3.919	20018	4199	0.0036	3.919	19663	3990	0.00392
Corr Avg	3.919	20000	4200	0.0036	3.919	20000	4000	0.00390
StdDev	-	191.8	408.4	0.0003	-	460.5	890	0.00096
RSD	0.04	0.96	9.7	8.9	-	2.3	22	25
Δ8-THC								
Trial 1	4.313	59545	6417	0.00234	4.313	59234	5017	0.00299
Trial 2	4.317	60984	6509	0.00230	4.313	58402	5259	0.00285
Trial 3	4.313	61107	6418	0.00234	4.313	58402	5259	0.00285
Avg	4.314	60545	6744	0.00224	4.313	58818	5138	0.00292
Corr Avg	4.314	60500	6700	0.00220	4.313	58800	5100	0.00292
StdDev	-	869	594	0.00018	-	480	139	0.00008
RSD	0.05	1.4	8.8	8.1	0.00	0.82	2.7	2.7

Conditions: Room temperature (~20°C), isocratic elution, detector wavelength of 254nm, pump pressure of 1500 psi, a sample size of 20 μL, and flow rate of 1.5 mL/minute.; ADC data taken from Automated Ada Collection unit, Data manually computed from raw data noted. Statistics derived may contain an extra significant figure if used for other calculations. Corr Avg above is the average corrected to standard deviation.

Table 2. Effect of composition on retention, resolution, NTP, and HETP values, at 85:15, 87:13, and 90:10 methanol/water (v/v) for CBD, Δ9-THC, and Δ8-THC.

Methanol:Water	85:15			87:13			90:10		
Analyte	t_R (min)	R_S	HETP (cm)	t_R (min)	R_S	HETP (cm)	t_R (min)	R_S	HETP (cm)
CBD	2.602		0.0050	2.117		0.0061	1.807		0.0048
%RSD	0.3		39.1	-		52.3	0.3		9.6
Δ9 THC	4.620	8.8	0.0034	3.919	8.8	0.0082	2.747	5.6	0.0039
%RSD	0.01		17.6	0.04		10.5	0.2		5.3
Δ8 THC	5.280	2.3	0.0029	4.314	1.6	0.0054	3.064	1.7	0.0022
%RSD	-		15.6	0.05		0.82	0.2		

Conditions: Room temperature (~15°C), isocratic elution, detector wavelength of 254nm, pump pressure of 1500 psi, a sample size of 20 μL, and flow rate of 1.5 mL/minute.

Table 3. Peak Area data for calibration standards measured at 254 and 214 nm

Concentration Standards (μg/mL)	Peak Area μV.s at 254 nm	Peak Area μV.s at 214 nm
21.0	41000	4600000
26.3	53600	6060000
35.0	94700	8670000
52.5	117000	9900000
105.0	180000	11100000

Conditions: Isocratic elution using 87% (v/v) methanol-13% (v/v) water, 254nm, pump pressure of 1500 psi, a sample size of 20 μL, and flow rate of 1.5 mL/min.

Table 4. Retention time and peak area data for HO1 and HO2 measured at 254 and 214 nm

Sample		**HO1**	**HO2**	**HO2**
Trial	Dilution	8100	81000*	81000
	Measure	254nm	254nm	214nm
1	t_R(s)	2.123	2.123	2.120
2	t_R(s)	2.123	2.123	2.120
3	t_R(s)	2.123	2.123	2.120
	Avg(s)	2.123	2.123	2.120
	Corr Avg	2.123	2.123	2.120
	SD(s)	-	-	-
	RSD(%)	-	-	-
1	A(μV.s)	14432	26	1742970
2	A(μV.s)	13969	143	1743229
3	A(μV.s)	14009	67	1745846
	Avg(μV.s)	14137	78.6	1744015
	Corr Avg	14100	80	1744000
	SD(μV.s)	256.5	60	1591
	RSD(%)	1.8	76	0.1

Conditions: Isocratic elution, detector wavelength of 254nm, pump pressure of 1500 psi, a sample size of 20 μL, and flow rate of 1.5 mL/minute.

* Peak data considered unrepeatable, based on high RSD value.

Table 3. Retention times and peak areas for CBD standards measured at 254 and 214 nm

Trial	Dilution x Conc (mg/mL)	ADC 10	8	6	4	2
		0.021	**0.02625**	**0.035**	**0.0525**	**0.105**
1	t_R(s)	2.123	2.123		2.120	2.123
2	t_R(s)	2.123	2.123	2.117	2.123	2.120
3	t_R(s)	2.127	2.120	2.123	2.117	2.117
	Avg(s)	2.1243	2.1220	2.1200	2.1200	2.1200
	SD(s)	0.0023	0.0017	0.0042	0.0030	0.0030
	RSD(%)	0.109	0.082	0.200	0.142	0.142
		λ = 254				
1	Area (μV.s)		53134		115562	179233
2	Area (μV.s)	40385	53775	91308	117232	181542
3	Area (μV.s)	41625	53772	92028	117441	179705
	Avg(μV.s)	41005	53560	91668	116745	180160
	corr avg	41000	53600	94700	117000	180000
	SD(μV.s)	877	369	509	1030	1220
	RSD(%)	2.14	0.69	0.56	0.88	0.68
		λ = 214				
1	Area (μV.s)	4520950	6016759		9826467	11000643
2	Area (μV.s)	4574080	6094606	8658062	9984053	11215529
3	Area (μV.s)	4759064	6056844	8676570	10013339	11101131
	Avg(μV.s)	4618031	6056070	8667316	9941286	11105768
	corr avg	4600000	6060000	8670000	9900000	11100000
	SD(μV.s)	124993	38929	13087	100509	107518
	RSD(%)	2.71	0.64	0.15	1.01	0.97

Conditions: isocratic elution, detector wavelength of 254nm, pump pressure of 1500 psi, a sample size of 20 μL, and flow rate of 1.5 mL/minute.

Table 6. HO1 and HO2 concentration calculations for plot and two external standard methods

CBD	Declared		Experimental Concentrations			% Error		
Method	Concentration		Plot	R_{f1}	R_{f2}	Plot	[1]R_{f1}	ǂ R_{f2}
HO1	3500	mg/oz	3575	1322	1514	2%	-62%	-57%
	118	mg/mL	120.9	44.7	51.2			
HO2	30000	mg/oz	*	14195	22091	*	-53%	-26%
	1014	mg/mL		480	747			

* No Reliable peak or calibration data available due to RSD greater than 20%.

[1] % error calculated using R_{f1} indicates response factor used at 214 nm

ǂ % error calculated using R_{f2} indicates response factor calculated at 254 nm

6.0 SUPPLEMENTAL INFORMATION

6.1 Run Details

The equipment used included: Jasco HPLC #2 HPLC [PU-4180(pump), UV-4075 (detector), LC-NETII/ADC, and a 150 mm ZORBAX Eclipse XB-C-18 Reverse phase column], Jasco Inc, Mary's Court Md; a 50-microliter syringe; and a 100-1000 μL micropipette, VWRBrand;

6.2 Calculations of CBD content in two brands of hemp oil using various methods.

Plot method for HO1 and HO2 at 254 nm

Calibration formula

Linear for peak areas of 41,000 to 94,700 μV.s

x = (y + 44400) / 3923.6

or linear for peak areas of 94,700 to 180,000 μV.s

x = (y - 52700) / 1214.3

An example calculation using 13700 μV.s as the peak area in Hemp oil #1:

(13700 + 44400) / 3923.6 = 14.8 μg/mL

$Conc_{undiluted}$ = measured concentration * dilution factor = 14.8 μg/mL x 8100 / 1000 = 120 mg/mL

External standard method

A 254 nm example for determining concentration of CBD in Hemp oil #1

Using 21.0 μg/mL standard

R_{f254} = Absorbance/$Conc_{ExtStd}$ = 117000/52.$_5$ = 2230

$Conc_{HO1}$ = $Absorbance_{\_HO1}$/ R_{f254} = 13700/2230 = 6.1 μg/mL

$Conc_{undiluted}$ = measured conc * dilution factor = 6.1 μg/mL x 8100 /1000 = 49 mg/mL

Or 52.$_5$ μg/mL standard

R_{f254} = Absorbance/$Conc_{ExtStd}$ = 53600/21.$_0$ = 2550

$Conc_{unk}$ = $Absorbance_{\_HO1}$/ R_{f254} = 13700/2550 = 5.4 μg/mL

$Conc_{undiluted}$ = measured conc * dilution factor = 5.4 μg/mL x 8100/1000 = 44 mg/mL

An example calculation using 80 μV.s as the peak area in Hemp oil 2:

(80 + 44400) / 3923.6 = 11.3 μg/mL

$Conc_{undiluted}$ = measured conc * dilution factor = 81000/1000 x 11.3 μg/mL = 915 mg/mL

External standard method

A 254 nm example for determining concentration of

Using 21.0 μg/mL standard

R_{f254} = Peak area/$Conc_{ExtStd}$ = 117000/52.5 = 2230 μV.s.mL/μg

$Conc_{unk}$ = Peak $area_{\_HO2}$/ R_{f254} = 80/2230 = 0.03 μg/mL

$Conc_{undiluted}$ = measured conc * dilution factor = 81000 / 1000 x 0.03 μg/mL = 0.07 mg/mL

Or 52.5 μg/mL standard

R_{f254} = Peak area/$Conc_{ExtStd}$ = 53600/21.0 = 2550 μV.s.mL/μg

$Conc_{unk}$ = Peak $area_{\_HO2}$/ R_{f254} = 80/2550 = 0.03 μg/mL

$Conc_{undiluted}$ = measured conc * dilution factor = 81000 / 1000 x 0.3 μg/mL = 0.073 mg/mL

A 214 nm examples for determining concentration of Hemp oil #2:

Using the plot method for returns negative concentrations:

$y = 22181x + 9000000$

This regression produced concentration values (μg/mL) as follows:

$x = (y - 9000000) / 22181 = \mu g/mL$

x = (1744000-9000000) / 22181 = -327 μg/mL

being negative concentrations are not physically possible, the equation is not viable.

Using the 52.5 μg/mL standard,

R_{f214} = Peak area/$Conc_{ExtStd}$ = 9900000/52.$_5$ = 189000 μV.s.mL/μg

$Conc_{unk}$ = Peak area$_{HO2}$/ R_{f214} = 1744000/189000 = 9.2 μg/mL

$Conc_{undiluted}$ = measured conc * dilution factor = 81000/1000 x 9.2 μg/mL = 750 mg/mL

Or the 21.0 μg/mL standard,

R_{f214} = Peak area/$Conc_{ExtStd}$ = 6060000/21.$_0$ = 289000 μV.s.mL/μg

$Conc_{unk}$ = Peak area$_{HO2}$/ R_{f214} = 1744000/289000= 6.0 μg/mL

$Conc_{undiluted}$ = measured conc * dilution factor = 81000/1000 x 6.0 μg/mL = 490 mg/mL

6.3 Significant Figures

Significant figures used were the significant figures of the raw data plus one, for all calculation results.

6.4 Raw Data and Supporting Documents

File and DB name info/locations for CBD HPLC Trials

HPLC2: ADC SN A247461868
PC (Full Device Name CHEMNSC246D3XFD.dmd.gsuad.gsu.edu)

Excel Files used in computation and plotting

- MobilePhaseSetup-CBD-THC.xlsm — Mobile Phase concentration trials and data processing
- CalibrationAnalyser.xlsm — CBD Calibration runs/raw data, dil calc, calibration plots
- HempOilCBDCalculations.xlsm — Hemp Oil Conc calc and raw data for peak processing
- MixedCBD_D9D8THCAnalyzer.alsm — Qualitative Run data and analysis

PowerPoint files with chromatogram figures

- CBD-D8-D9THC_Chromatogram.ppt — Chromatogram for the CBD, Δ8-THC, and Δ9-THC Qual Trial
- HO2_Chromatograms_1and2.ppt — Chromatograms for the HO1 and HO2 trials

PDF Reports and Raw Data

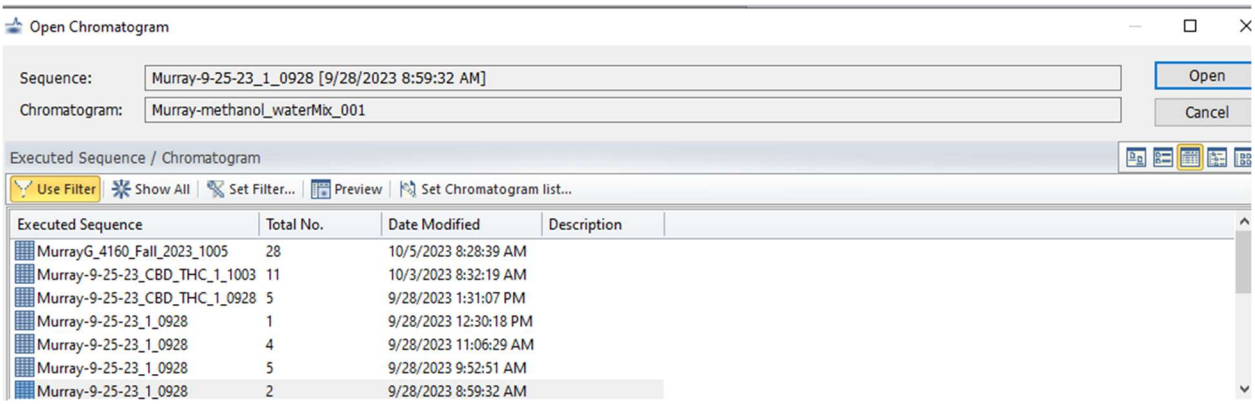
- Folder Desktop/Glenn TEST
- PDF Reports for 9_25_23 – 10_05_23
- Raw data for 10_05_23 & 9_28_only

Database

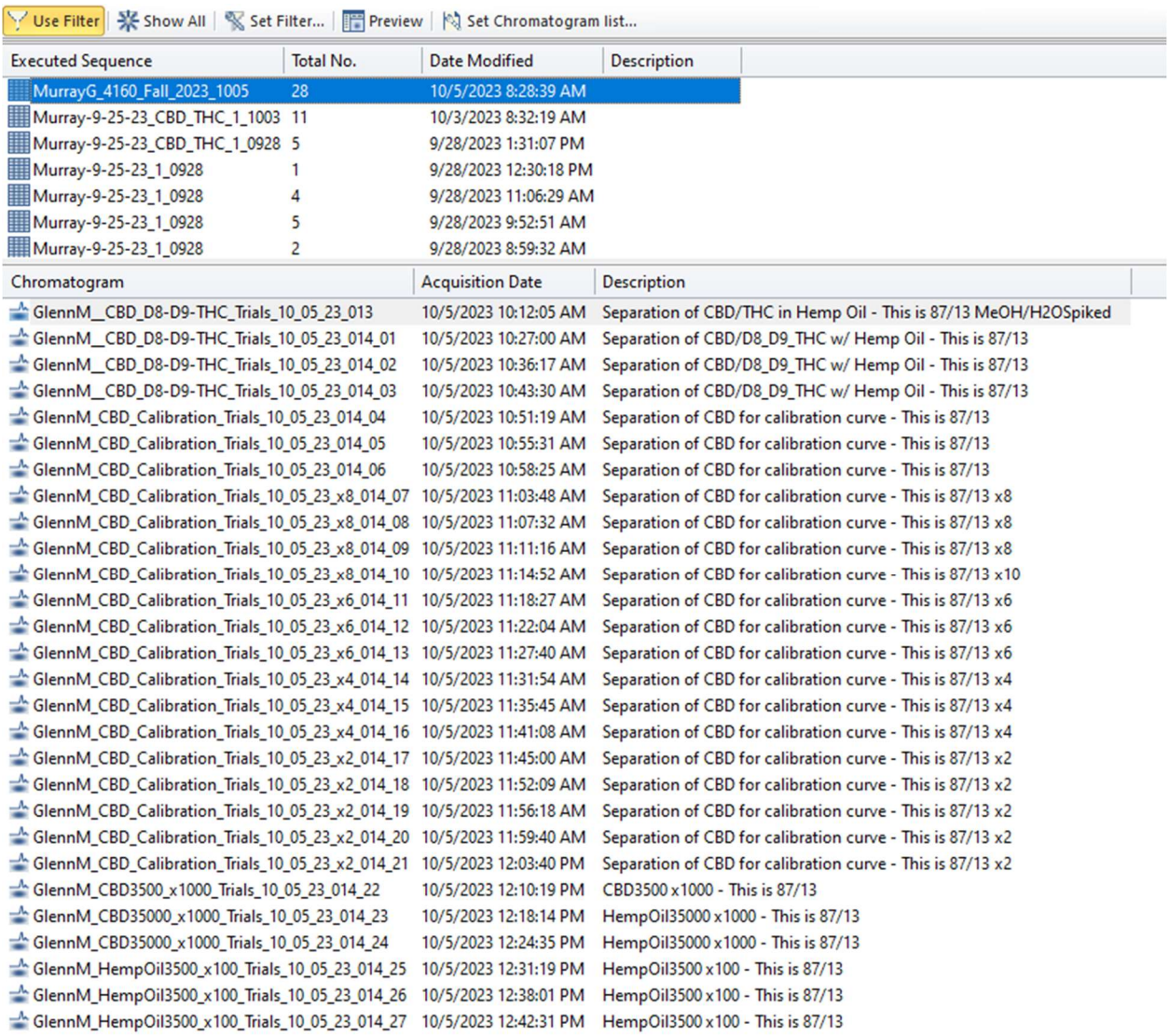
In Reverse Temporal Order

Sequence Runs

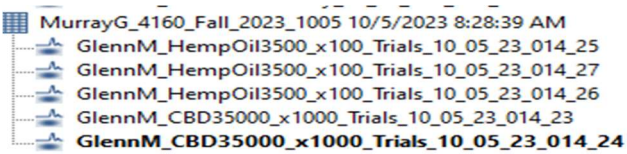
The database is organized into sequences, which contain chromatograms. Below are the table of sequences, and in the following pages, the chromatograms within those sequences. These can be access through the HPLC software under Project 1.



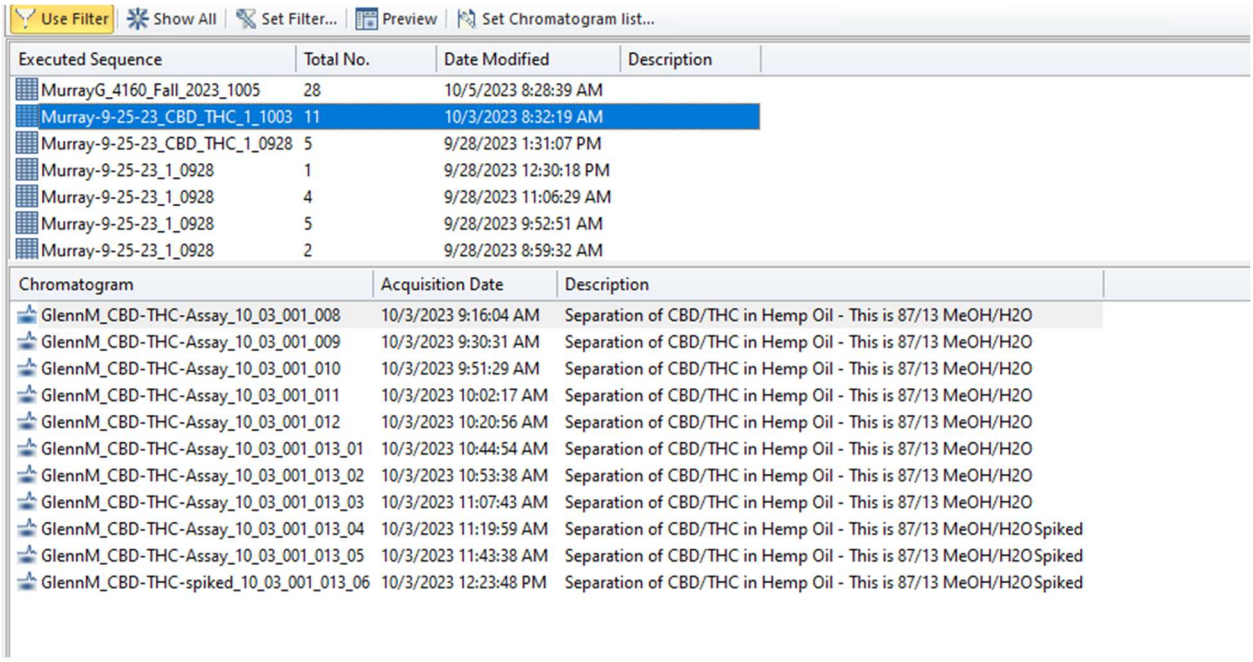
Sequence MurrayG_4160_Fall_2023_1005



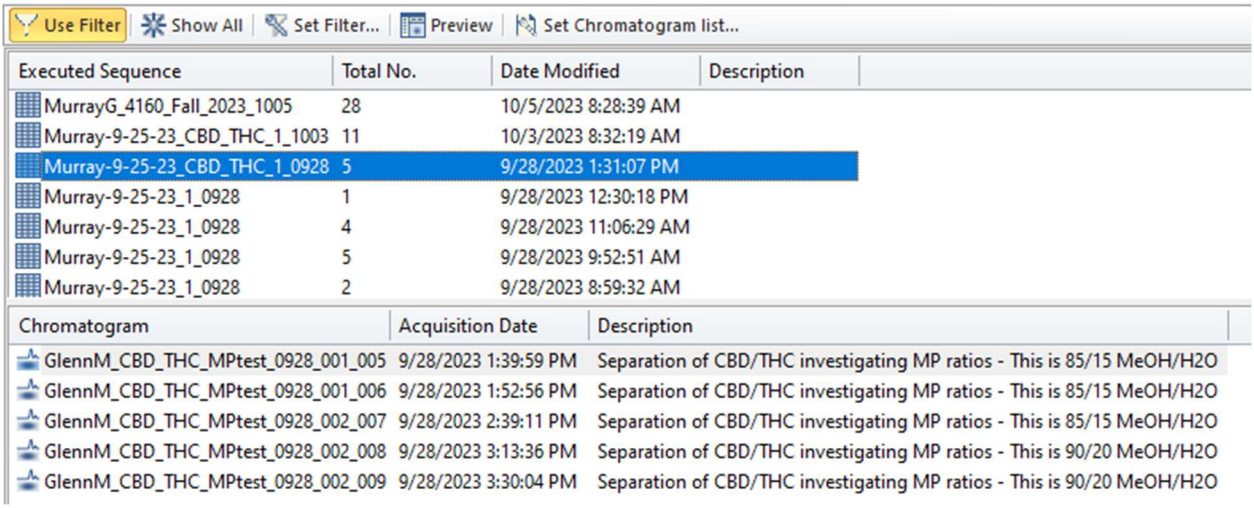
Hemp Oil Trials



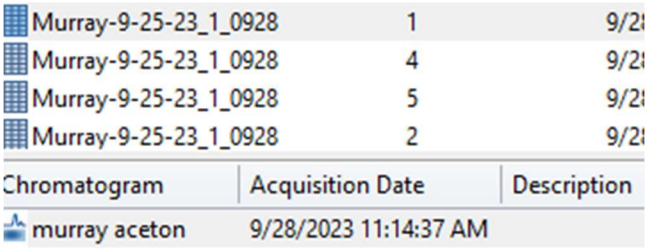
Sequence Murray-9-25-23_CBD_THC_1_1003 11 chromatograms



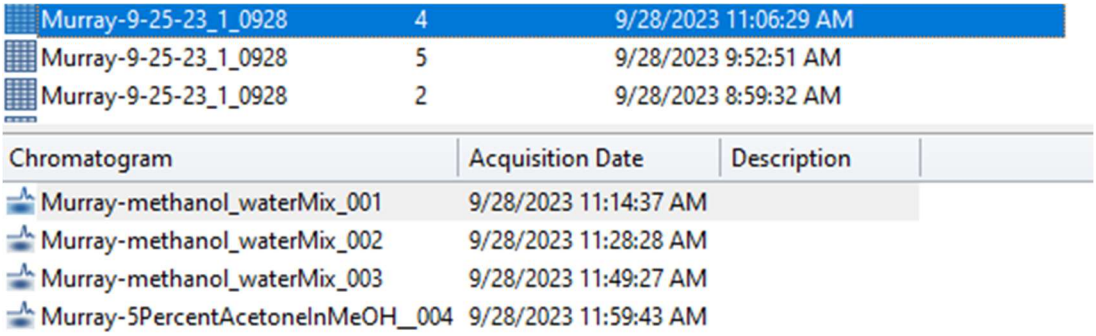
Sequence Murray-9-25-23_CBD_THC_1_0928 5 chromatograms



Sequence Murray-9-25-23_1_0928 1 chromatogram



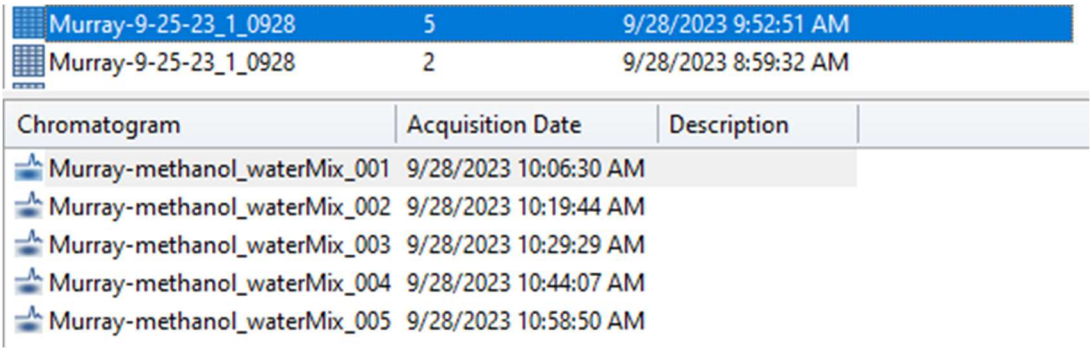
Sequence Murray-9-25-23_1_0928 4 chromatograms



Murray-9-25-23_1_0928	4	9/28/2023 11:06:29 AM
Murray-9-25-23_1_0928	5	9/28/2023 9:52:51 AM
Murray-9-25-23_1_0928	2	9/28/2023 8:59:32 AM

Chromatogram	Acquisition Date	Description
Murray-methanol_waterMix_001	9/28/2023 11:14:37 AM	
Murray-methanol_waterMix_002	9/28/2023 11:28:28 AM	
Murray-methanol_waterMix_003	9/28/2023 11:49:27 AM	
Murray-5PercentAcetoneInMeOH_004	9/28/2023 11:59:43 AM	

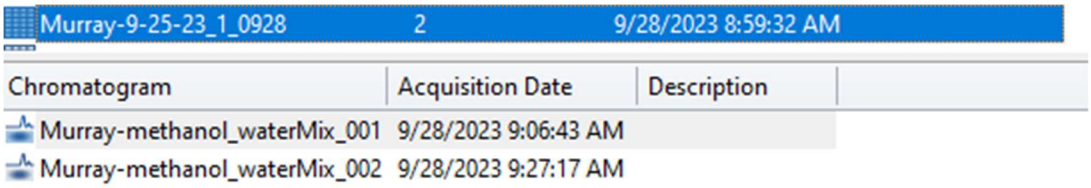
Sequence Murray-9-25-23_1_0928 5 chromatograms



Murray-9-25-23_1_0928	5	9/28/2023 9:52:51 AM
Murray-9-25-23_1_0928	2	9/28/2023 8:59:32 AM

Chromatogram	Acquisition Date	Description
Murray-methanol_waterMix_001	9/28/2023 10:06:30 AM	
Murray-methanol_waterMix_002	9/28/2023 10:19:44 AM	
Murray-methanol_waterMix_003	9/28/2023 10:29:29 AM	
Murray-methanol_waterMix_004	9/28/2023 10:44:07 AM	
Murray-methanol_waterMix_005	9/28/2023 10:58:50 AM	

Sequence Murray-9-25-23_1_0928 2 chromatograms



Murray-9-25-23_1_0928	2	9/28/2023 8:59:32 AM

Chromatogram	Acquisition Date	Description
Murray-methanol_waterMix_001	9/28/2023 9:06:43 AM	
Murray-methanol_waterMix_002	9/28/2023 9:27:17 AM	

6.5 Tables and Data

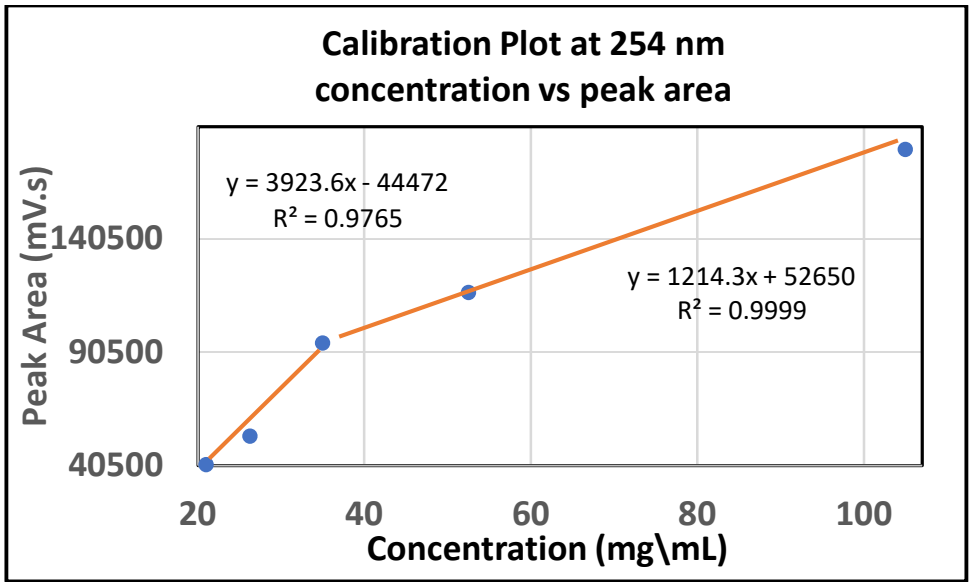


Figure S1 Calibration Plot at 254 nm, 0.21 – 0.105 mg/mL, broken into two approximate linear regions

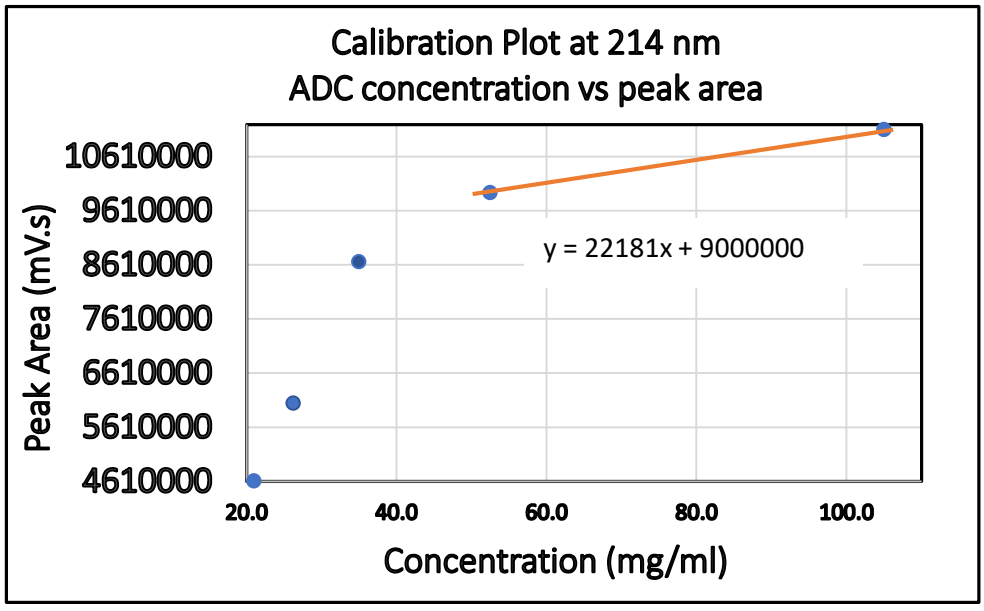


Figure S2 Calibration Plot at 214 nm

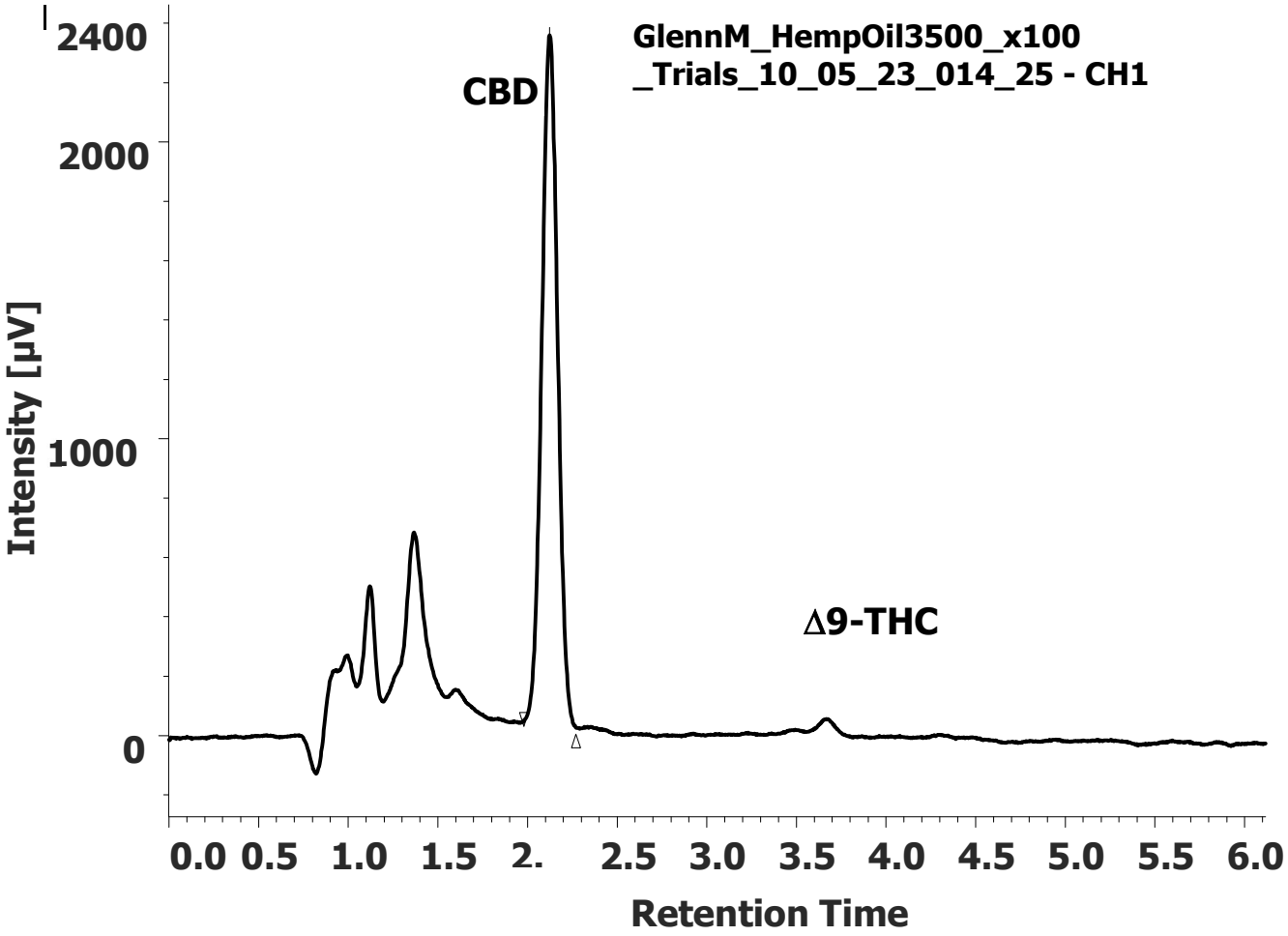


Figure S3 Example Chromatogram of HO#1 trial (dil = x8100) at 254 nm

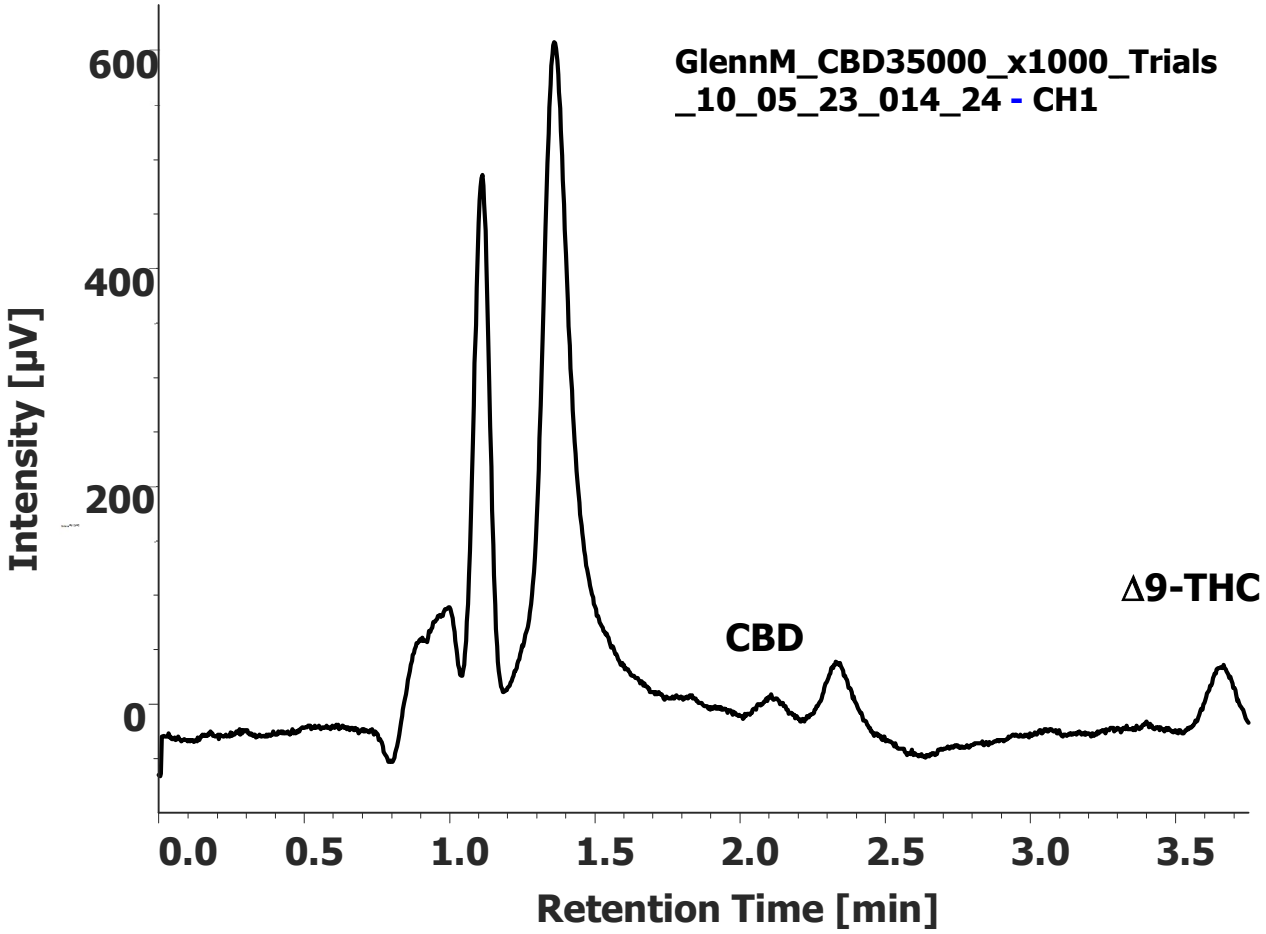


Figure S4 Example chromatogram of HO#2 trial (dil=x81000) at 254 nm

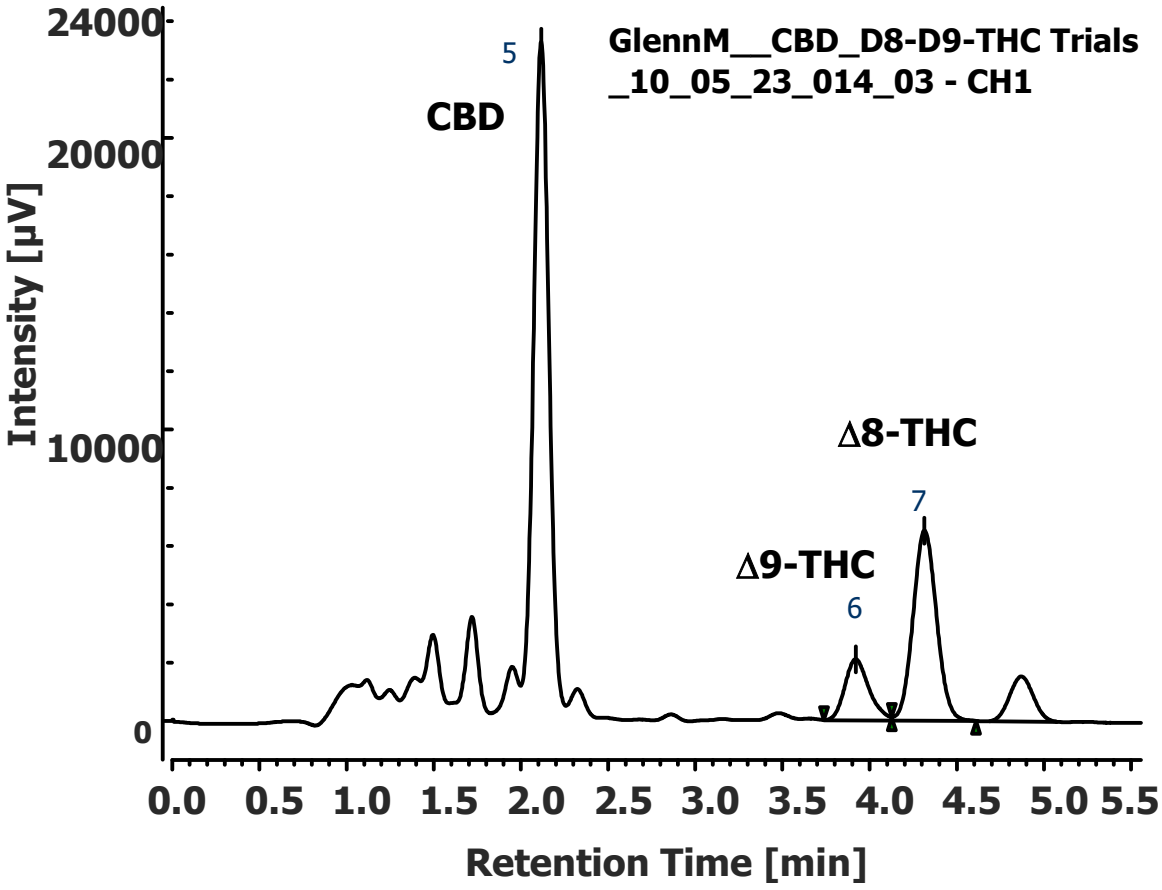


Figure S5 A trial chromatogram with CBD, Δ8, and Δ9 THC, using qualitative sample at 254 nm

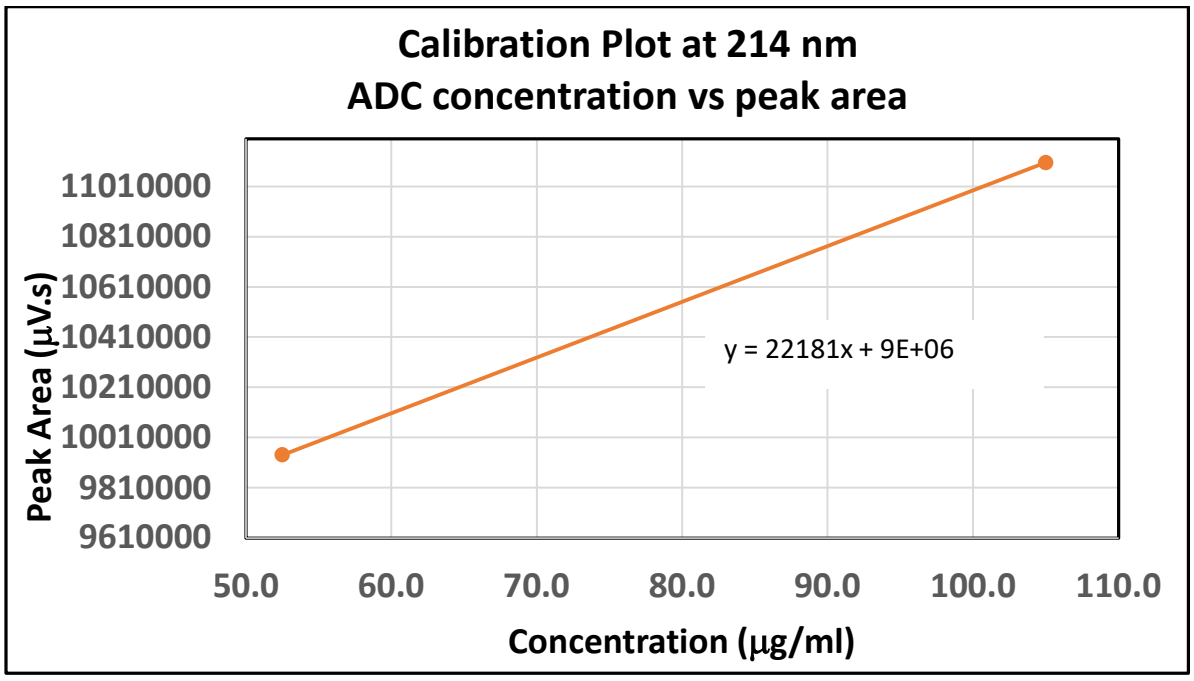


Figure S6 A calibration plot from the upper two calibration points measured at 214 nm, using standards of 0.0525 and 0.105 mg/mL

Table S1a CBD/THC 85:15 Retention times, peak area, and efficiency, ADC and manual calculation methods

CBD	ADC Calculated				Calculated from Raw Data			
	Peak t_R	Area	N	HETP	Peak t_R	Area	N	HETP
Trial 1	2.613	174415	2264	0.00663	2.613	166116	2780	0.00540
Trial 2	2.597	179512	2644	0.00567	2.597	175561	2456	0.00611
Trial 3	2.607	179646	5296	0.00283	2.607	175765	2475	0.00606
avg	2.602	177858	3401	0.00504	2.602	172481	2570.056	0.0058552
corr avg	2.602	178000	3000	0.00504	2.602	172000	2600	0.0059
StdDev	0.01	2982	1652	0.001973	0.01	5513	181.8476	0.00040
RSD	0.27	1.68	48.56	39.13	0.27	3.20	7.08	6.80
THC Δ9	NO ADT							
Trial 1					4.640	8714	3681	0.00407
Trial 2					4.617	6003	5022	0.00299
Trial 3					4.623	5903	4825	0.00311
avg					4.620	6873	4509.385	0.0033901
corr avg					4.620	7000	4500	0.0034
StdDev					-	1595	724	0.00060
RSD					0.01	23.20	16.05	17.58
THC Δ8								
Trial 1	5.300	16120	5164	0.00299	5.270	21360	4163	0.00360
Trial 2	5.280	15394	5251	0.00286	5.300	15019	5539	0.00271
Trial 3	5.280	15096	5296	0.00283	5.280	21360	4163	0.00360
avg	5.280	15537	5237	0.00289	5.290	19246	4621.942	0.0033046
corr avg	5.280	15600	5240	0.00289	5.290	19000	46200	0.003
StdDev	-	526.7	67.1044	8.3E-05	0.01	3661	794	0.00052
RSD	-	3.39	1.28	2.87	0.27	19.02	17.18	15.63

Table S1b CBD/THC 87:13 Retention times, peak area, and efficiency, ADC and manual calculation methods

CBD		ADC Calculated			Calculated from Raw Data			
	Peak t_R	Area	N	HETP	Peak t_R	Area	N	HETP
Trial 1	2.117	143259	2666	0.00563	2.12	137405	2849	0.00526
Trial 2	2.117	145959	8753	0.00171	2.12	138782	2849	0.00526
Trial 3	2.117	145966	2651	0.00566	2.12	138782	2849	0.00526
avg	2.117	145061	4690	0.00433	2.12	138323	2849	0.00526
corr avg	2.117	145000	5000	0.004	2.12	138300	2849	0.00526
StdDev	-	1561	3519	0.00227	-	795	-	-
RSD	-	1.08	75.02	52.35	-	0.57	-	-
THC Δ9	NO ADT							
Trial 1	3.917	19804	4811	0.0031	3.92	20195	3951	0.00380
Trial 2	3.920	20175	3984	0.0038	3.92	19397	5017	0.00299
Trial 3	3.920	20074	3980	0.0038	3.92	19397	4144	0.00362
avg	3.919	20018	4258	0.0036	3.92	19663	4370	0.00347
corr avg	3.919	20000	4300	0.0036	3.92	19700	4400.0	0.0035
StdDev	-	192	478.6	0.0004	-	461	568.1	0.00042
RSD	0.04	0.96	11.24	10.55	0.05	2.34	13.00	12.23
THC Δ8								
Trial 1	4.313	59545	6417	0.00234	4.31	59234	5017	0.00299
Trial 2	4.317	60984	6509	0.00230	4.31	58402	5259	0.00285
Trial 3	4.313	61107	6418	0.00234	4.31	58402	5259	0.00285
avg	4.314	60545	6448	0.00233	4.31	58680	5178	0.00290
corr avg	4.314	60500	6440	0.00233	4.31	59000	5200	0.00290
StdDev	-	868	53	1.9E-05	-	480	139.6	0.00008
RSD	0.05	1.43	0.82	0.82	-	0.82	2.70	2.74

Table S1c CBD/THC 90:10 Retention times, peak area, and efficiency, by ADC and manual calculation methods

CBD	ADC Calculated				Calculated from Raw Data			
	Peak tR	Area	N	HETP	Peak tR	Area	N	HETP
trial 1	1.810	192699	2313	0.006485	1.810	184198	2241	0.006694
trial 2	1.803	196489	2651	0.005658	1.803	188228	2224	0.006744
trial 3[1]								
Avg	1.807	194594	2482	0.006072	1.807	186213	2232	0.006719
corr avg	1.807	195000	2500	0.006072	1.807	186000	2230	0.006719
StdDev	0.005	2680	239	0.00	0.005	2849	12	0.00
RSD	0.27	1.38	9.63	9.63	0.26	1.53	1	0.52
Δ9-THC	NO ADT[2]							
trial 1	2.743	12416	1765	0.008499	2.750	4297	4484	0.003346
trial 2	2.750	11494	1903	0.007882	2.743	7472	2895	0.005181
trial 3[1]								
avg	2.747	11955	1834	0.00819	2.747	5884	3689	0.004263
corr avg	2.747	20000	1800	0.00819	2.747	19700	4400	0.0035
StdDev	0.005	652	98	0.00	0.00	2245	1123	0.00
RSD	0.18	5.45	5.32	5.32	0.17	38.16	30	30.44
Δ8-THC								
trial 1	3.07	21304	3555	0.004219	3.067	14803	2780	0.005396
trial 2	3.06	21442	3556	0.004218	3.060	17516	2780	0.005396
trial 3[1]								
Avg	3.06	21373	3556	0.004219	3.063	16159	2780	0.005396
corr avg	3.06	21400	3556	0.004219	3.063	16000	2780	0.005396
StdDev	0.00	98	1	0.00	0.00	1918	0	0.00
RSD	0.16	0.46	0.02	0.02	0.15	11.87	0	0.00

[1] The ADC failed to return data on trial 3 and no raw data was produced.

[2] The ADC failed to return data on Δ9-THC peaks, so raw data was used to calculate all table data.

Table S3 CBD Calibration Trials, ADC Calculated Peak Retention times and Areas at 254 nm

Trial	Dilution x Measurement\ Conc(mg/mL)	10 0.021	8 0.026_{25}	6 0.035	4 0.052_{5}	2 0.105
1	t_R(s)	2.123	2.123		2.120	2.123
2	t_R(s)	2.123	2.123	2.117	2.123	2.120
3	t_R(s)	2.127	2.120	2.123	2.117	2.117
	Avg(s)	2.124	2.122	2.120	2.120	2.120
	corr avg	2.124	2.122	2.120	2.120	2.120
	SD(s)	0.002	0.0017	0.0042	0.0030	0.0030
	RSD(%)	0.109	0.082	0.200	0.142	0.142
1	Area (μV.s)		53134		115562	179233
2	Area (μV.s)	40385	53775	91308	117232	181542
3	Area (μV.s)	41625	53772	92028	117441	179705
	Avg (μV.s)	41005	53560	91668	116745	180160
	corr avg	41000	53600	94700	117000	180000
	SD (μV.s)	877	369	509	1030	1220
	RSD (%)	2.14	0.69	0.56	0.88	0.68

Table S4 CBD Calibration Trials, Peak Retention times and Areas at 214 nm

Trial	Dilution x Measurement\ Conc(mg/mL)	10 0.021	8 0.02625	6 0.035	4 0.0525	2 0.105
1	t_R(s)	2.123	2.120		2.163	2.070
2	t_R(s)	2.120	2.123	2.093	2.090	2.067
3	t_R(s)	2.127	2.120	2.110	2.113	2.087
	Avg(s)	2.1233	2.1210	2.1015	2.1220	2.0747
	SD(s)	0.0035	0.0017	0.0120	0.0373	0.0108
	RSD(%)	0.165	0.082	0.572	1.759	0.520
1	A(μV.s)	4520950	6016759		9826467	11000643
2	A(μV.s)	4574080	6094606	8658062	9984053	11215529
3	A(μV.s)	4759064	6056844	8676570	10013339	11101131
	Avg(μV.s)	4618031.3	6056069.7	8667316.0	9941286.3	11105767.7
	SD(μV.s)	124993.4	38929.3	13087.1	100508.8	107518.0
	RSD(%)	2.71	0.64	0.15	1.01	0.97

Table S5 Mixed Hemp oil, Δ8 and Δ9 THC trials, Retention times and areas at 254 nm

Trial	Retention Time	
2	t_R(s)	2.117
3	t_R(s)	2.117
4	t_R(s)	2.117
	Avg(s)	2.1170
	SD(s)	0.0000
	RSD(%)	0.000
	Area	
2	A(μV.s)	143259
3	A(μV.s)	145959
4	A(μV.s)	145966
	Avg(μV.s)	145061.3
	SD(μV.s)	1560.9
	RSD(%)	1.08
	Conc (μg/mL)	76.8

Table S6 Dilutions of 2.1 mg/mL stock standard; working standards concentrations from Stock 2.1 mg/mL Stock Standard solution.

Stock std	ACN		Standards Concentration		Calculations x10 dilution
μL	μL	Dilution factor	mg/mL	Conc (μg/mL)	Conc (μg/mL)
200	1800	10	0.21	210	21
200	1400	8	0.2625	262.5	26.25
200	1000	6	0.35	350	35
200	600	4	0.525	525	52.5
200	200	2	1.05	1050	105

The stock solution was added in 200 ml amounts to give various dilutions, shown above, then each was diluted by 10 to give each of the final concentrations.

Table S7 Retention times/absorbance for qualitative mix

CBD	ADC Calculated			
	Peak t_R	Area	N	HETP
Trial 1	2.117	143259	2666	0.00563
Trial 2	2.117	145959	8753	0.00171
Trial 3	2.117	145966	2651	0.00566
avg	2.117	145966	4690	0.00478
corr avg	2.117	146000	5000	0.00227
StdDev	-	1561	3518	0.00500
RSD	0.00	1.07	75	52

THC Δ9	NO ADT			
	Peak t_R	Area	N	HETP
Trial 1	3.917	19804	4811	0.0031
Trial 2	3.920	20175	3984	0.0038
Trial 3	3.920	20074	3980	0.0038
avg	3.919	20018	4258	0.0036
corr avg	3.919	20000	4200	0. 0036
StdDev	-	192	478	0.0004
RSD	0.04	0.9	11.2	11.3

THC Δ8				
	Peak t_R	Area	N	HETP
Trial 1	4.313	59545	6417	0.00234
Trial 2	4.317	60984	6509	0.00230
Trial 3	4.313	61107	6418	0.00234
avg	4.314	61107	6448	0.00232
corr avg	4.314	61100	6450	0.00232
StdDev	0.00	868	53	-
RSD	0.00	1.4	0.82	1.0

Table S8 HO2 Retention time, Peak Area, and efficiency

214 nm				
HO2	t_R(mins)	Area (μV.s)	N	HETP (cms)
Trial 1	2.120	1742970	2565	0.00585
Trial 2	2.120	1743229	2581	0.00581
Trial 3	2.120	1745846	2585	0.00580
average	2.12	1744015	2577	0.00582
corr area	2.12	1744000	2580	0.00582
Stdev	-	1591	10.6	-
RSD	0.0	0.1	0.4	0.4

Table S9 Calculation of CBD in two brands of hemp oils (HO1 and HO2) at 254 and 214 nm

Contrasted Calculation Methods	254	nm				
	HO1			HO2		
Calculation Method	$R_{f\_21\mu g}$	$R_{f\_52.5\mu g}$	Plot	$R_{f\_21\mu g}$	$R_{f\_52.5\mu g}$	Plot
Peak Area Corrected Average	14100	14100	14100	80[1]	80[1]	80[1]
External Standard R_f value	2550	2230		2550	2230	
Concentration (μg/mL)	5.5	6.3	14.9	0.031	0.036	11.4
Dilution factor	8100	8100	8100	81000	81000	81000
Undiluted Concentration (μg/mL)	44700	51200	120900	2500	2900	920320
Undiluted Concentration (mg/mL)	44.7	51.2	120.9	2.5	2.9	920.3
Undiluted Concentration (mg/oz)	1322	1514	3575	74	86	27217
Declared Concentration (mg/oz)	3500	3500	3500	30000	30000	30000
% error	-62%	-57%	2%	-100%	-100%	-9%
	214	nm				
	HO1[2]			HO2		
Calculation Method	$R_{f\_21\mu g}$	$R_{f\_52.5\mu g}$	Plot	$R_{f\_21\mu g}$	$R_{f\_52.5\mu g}$	Plot[3]
Peak Area Corrected Average				1744000	1744000	1744000
External Standard R_f value				289000	189000	
Concentration (μg/mL)				6.0	9.2	
Dilution factor				81000	81000	
Undiluted Concentration (μg/mL)				480000	747000	
Undiluted Concentration (mg/mL)				480	747	
Undiluted Concentration (mg/oz)				14195	22091	
Declared Concentration (mg/oz)				30000	30000	
% error				-53%	-26%	
Plot function	**λ= 254**	**= (y+44500)/3923.6**		Below	94,700	μV.s
	λ= 254	**= (y-52600)/1214.3**		Above	94,700	μV.s
	λ= 214	**= (y-9000000)/22181**				

[1] **Peak data considered unrepeatable, based on high RSD value.**

[2] **No data reported at 214 nm by ADC.**

[3] **No valid plot method for HO2 at 214 nm, returns negative concentrations.**