Project: Determination of Cannabidiol in Four Cannabis Flower Bud Samples by Liquid Chromatography – Mass Spectrometry

Nicole Hanna & Jenna Middleton

CHEM 3170 Laboratory

Date(s) of Experiment: November 2nd & November 16th, 2022

Date of Submission: November 27th, 2022

Number of Pages: 21

Attached: 8

Abstract

This experiment was performed in order to determine the amount of cannabidiol present in four cannabis flower bud samples: Organic Sour Cookies, Kush Cookies, Lilac Diesel and Death Bubba. This was determined using liquid chromatography - mass spectrometry. LC-MS is an analytical technique that combines liquid chromatography which allows for separation based on polarity of an individual species, and mass spectrometry's ability to identify and quantify specific species. Ions were analyzed by a Q-TOF mass analyzer and detected by a sensitive electron multiplier detector. Samples were pushed through a 30°C C18 column, with an internal diameter of 1.8 µm and column width/length of 2.1 x 100 mm at a flow rate of 0.5 mL/min. Signals were recorded and produced analyte peaks as a function of concentration graphs. Five CBD stock solutions were prepared and analyzed to obtain a calibration curve which allowed for determination of CBD present in cannabis samples. The equation of least squares was determined to be $y = 1x10^6 x - 4 x10^6$ with a coefficient of correlation of 0.9561. The concentration of CBD present for Organic Sour Cookies was 36.95 ± 2.88 ppm with an RSD of 7.69%; Kush Cookies was 56.17 ± 4.51 ppm, with an RSD of 7.92%; Lilac Diesel was 59.88 ± 4.89 ppm, with an RSD of 8.07%; Death Bubba was 48.47 ± 3.76 ppm, with an RSD of 7.65%. The experimental procedure followed papers by McRae & Melanson (2020) and Romano & Hazekamp (2013).

Introduction

Liquid chromatography – mass spectrometry (LC-MS) is an analytical method used to separate and identify analytes. By combining the effects of liquid chromatography and mass spectrometry, this tool is beneficial for separating isomers that could not be differentiated using other instruments. Liquid chromatography separates individual analytes based on their polarity, by analyzing the stationary and mobile phases. As well as it monitors the compound separation relating to each analyte's affinities for the mobile phase. Each analyte is then passed through an ionization source in their gas phase after eluting through the column and is then passed to the mass spectrometer. Electrospray ionization (ESI) is used to ionize the sample, by charging the liquid eluent from the LC-MS and dispersing it as a fine spray, leaving molecular ions after evaporation, which are pushed to the mass analyzer. MS has the capability to analyze based on mass to charge ratios and uses a sensitive detector for specific ion determination. The mass analyzer is a quadruple time-of-flight (Q-TOF), which allows ion molecules to be analyzed after separation based on the time it takes to reach the detector; larger ions move slower and smaller ions move faster. This provides sensitive and accurate detection.

Cannabis, which was recently legalized in Canada and is widely used across the country, is a product commonly used as a psychoactive. Cannabis comes in many forms, and can be consumed via pills, edible substances, or smoking. Cannabis contains many antioxidant and anti-inflammatory properties; however, it can also cause health issues, like addiction, altered brain development and respiratory issues (Blessing et al., 2015; Volkow et al., 2014). Cannabinoid products typically contain two main active ingredients: cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (Δ^9-THC), the latter being the main psychoactive component. Δ^9-THC can cause dope-dependence, which increases intoxication, causes anxiety, impairment, and

psychotic-like symptoms. CBD is typically more calming, and acts as an entourage compound to reduce psychoactive effects (Catenza & Donkor, 2022). Due to this, it is important that CBD and THC content levels are accurate to ensure correct dosing and consumption, and to prevent adverse effects; thus, this experiment is warranted to ensure accurate labeling.

Experimental

Sample and Standard Preparation – Procedure

Stock solutions for both THC and CBD were prepared in the lab. The stock solution for CBD was prepared by diluting 10 mg of CBD powder, weighed using weighing paper, in 100 mL methanol, to obtain a 100 ppm stock solution. From this, five standards were prepared by diluting further with methanol to obtain concentrations of 5, 10, 15, 20 and 25 ppm. The THC stock solution was prepared by diluting 0.25 mL of THC liquid in 10 mL methanol, to obtain a 25 ppm stock solution. Five standards were prepared with concentrations of 5, 10, 15, 20 and 25 ppm. The volumes and concentrations for each standard can be seen in Tables 2 and 3.

The 1.0 g samples were prepared by first grinding the flower bud into smaller samples using a grinder obtained from the BC Cannabis Store. Samples were weighed to ensure 1.0 g was accurate. Grinded samples were then prepared by diluting them in 25 mL methanol in a 25 mL volumetric flask. All samples and standards were vortexed for approximately 5 minutes each. Samples were filtered through a 0.45 µm syringe filter into a test tube. The solvent in the samples was then evaporated over a boiling water bath, under a constant nitrogen stream. The leftover sample was reconstituted with 2.0 mL methanol. All samples and standards were transferred to LC-MS sample vials and ran using the parameters in Table 1. Samples were run in triplicate.

Chemicals and solvents

100 ppm CBD Stock, 100 ppm THC Stock, Methanol, Nitrogen, 99.7% Acetonitrile, 0.3% Formic Acid, 74.7% 18 MΩ Water, 25% Methanol, 0.3% Formic Acid

Sample Information

Organic Sour Cookies cannabis flower, 1.000 g; Kush Cookies cannabis flower, 1.000 g; Lilac Diesel cannabis flower, 1.000 g; Death Bubba cannabis flower, 1.000 g.

Instrument Information

Agilent Technologies G530 Accurate-Mass Q-TOF LC/S 1200 series, internal diameter of 1.8 µm and column width/length of 2.1 x 100 mm. Ionization Source: ESI+.

Table 1. Instrumental parameters of the Agilent Technologies Accurate-Mass Q-TOF LC/MS 1200 series

VCap:	3000 V	**Flow Rate:**	0.5 mL/min
Fragementor:	60.0 V	**Injection Size:**	5.0 µL
Gas Temperature:	350 ℃	**Column Temperature:**	30℃
Drying Gas:	8.0 L/min	**Solvent A:**	74.7% 18 MΩ water, 25% Methanol, 0.3% Formic Acid
Nebulizer:	15 psig	**Solvent B:**	99.7% Acetonitrile, 0.3% Formic Acid
Sheath Gas Temp:	325 ℃	**Elution Gradient:**	100% A to 100% B in 4 minutes, hold at 100% B for 8 minutes. After 30 s, 100% A, hold for 3 minutes.
Sheath Gas Flow:	8.0 L/min	**Stop time:**	5 mins
Acquisition:	100 - 500 m/z		

Table 2. Concentrations and volumes used for the CBD standard solutions

Standard	CBD concentration (ppm)	Volume of CBD standard solution (mL)	Methanol added (mL)	Final volume (mL)
1	5	0.075	1.425	1.5
2	10	0.150	1.35	1.5
3	15	0.225	1.275	1.5
4	20	0.300	1.200	1.5
5	25	0.375	1.125	1.5

Table 3. Concentrations and Volumes used for the THC standard solutions

Standard	THC concentration (ppm)	Volume of THC standard solution (mL)	Methanol added (mL)	Final Volume (mL)
1	5	0.3	1.2	1.5
2	10	0.6	0.9	1.5
3	15	0.9	0.6	1.5
4	20	1.2	0.3	1.5
5	25	0	1.5	1.5

Table 4. Cannabis samples used in the determination of the concentration of CBD in cannabis flower buds

Cannabis sample	Name of Cannabis Sample	Labeled THC concentration (mg/g)	Labeled CBD concentration (mg/g)	Company
W1	Organic Sour Cookies	7.0	<5.000	1964
W2	Kush Cookies	3.0	<0.300	3 Saints
W3	Lilac Diesel	11.0	<0.100	Redecan
W4	Death Bubba	7.0	<1.000	Namaste

Data and results

Table 5. Results of the peak area obtained for the five CBD standard solutions on the Agilent Technologies Accurate-mass Q-TOF LC/MS 1200 Series instrument at a column temperature of 30℃.

Standard	Concentration of CBD (ppm)	Peak Area
1	5	964969
2	10	7249784
3	15	17939941
4	20	21850933
5	25	25190968

Table 6. Calibration curve data and uncertainties obtained from the calibration curve of CBD (Figure 1) and the uncertainty tables in the Appendix

Cannabis Sample	W1	W2	W3	W4
Slope (m)	1261062.94	1261062.94	1261062.94	1261062.94
Uncertainty in slope (S_m)	156042.3851	156042.3851	156042.3851	156042.3851
y-intercept	-4276625.1	-4276625.1	-4276625.1	-4276625.1
Uncertainty in y-intercept (S_b)	2587670.214	2587670.214	2587670.214	2587670.214
Derived x	20.7815	31.5945	33.6755	26.2615
Uncertainty in unknown CBD concentration in cannabis bud (S_x)	1.5979	2.5020	2.7170	2.0840
R^2	0.9561	0.9561	0.9561	0.9561
Equation of the Line	$y= 1x10^6 x- 4 x10^6$	$y= 1x10^6 x- 4 x10^6$	$y= 1x10^6 x- 4 x10^6$	$y= 1x10^6 x- 4 x10^6$
% RSD	7.69	7.92	8.07	7.65
Propagation of Uncertainty (S_c)	36.95 ± 2.88 ppm	56.17 ± 4.51 ppm	59.88 ± 4.89 ppm	48.47 ± 3.76 ppm

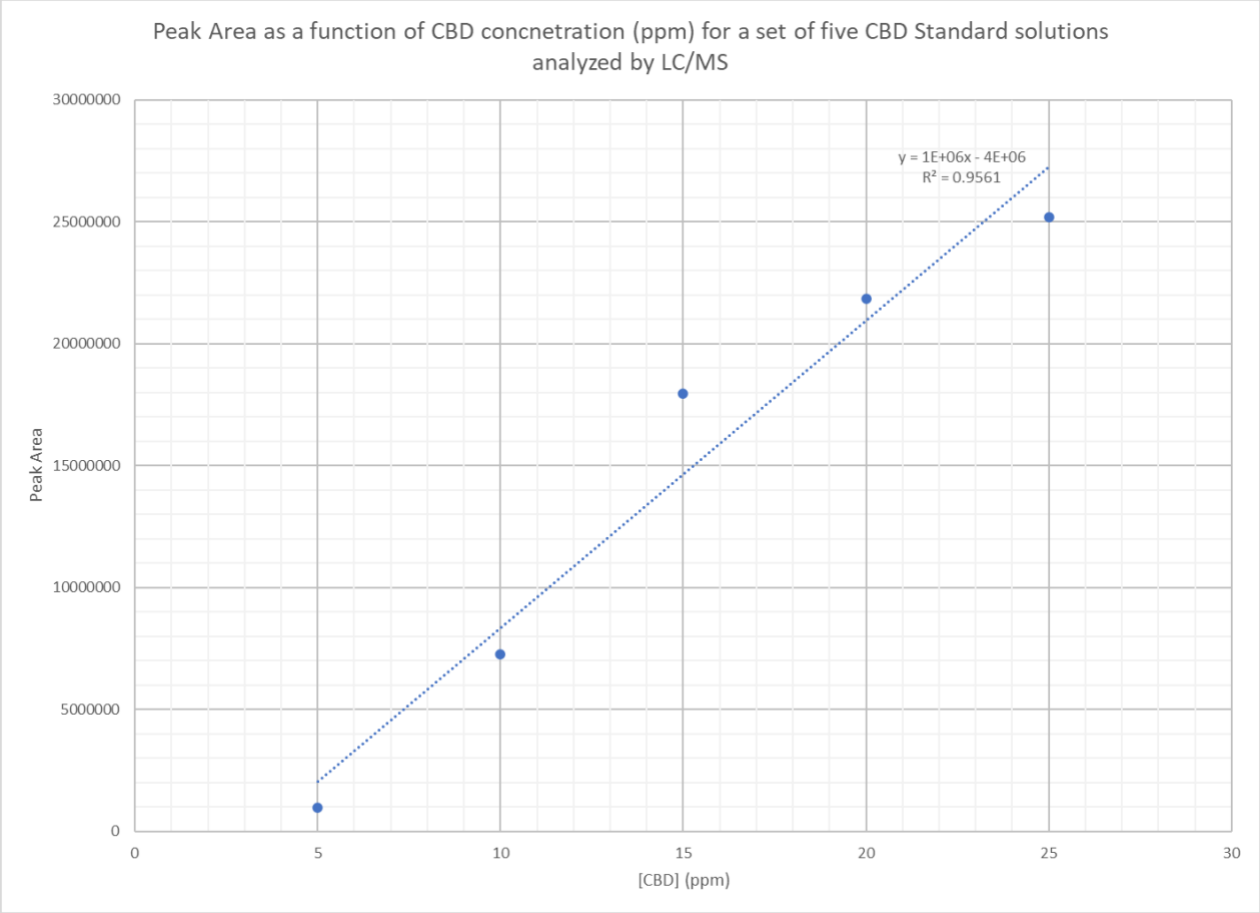


Figure 1. Calibration curve for the analysis of five CBD standards on the Agilent Technologies Accurate-mass Q-TOF LC/MS 1200 Series instrument at 30°C (n=4).

Table 7. Concentration of CBD present in weed samples

Samples	CBD concentration in samples (ppm)	Concentration in (mg/g)	Labeled Amount (mg/g)
W1 - Organic Sour Cookies	20.7815	0.03695	< 5.000
W2- Kush Cookies	31.5945	0.05617	< 0.300
W3 - Lilac Diesel	33.6755	0.05988	< 0.100
W4 - Death Bubba	27.2615	0.04847	< 1.000

Calculations → Sample calculations using Cannabis Sample 3

Uncertainty in the slope (S_m)

$$S_m = \sqrt{\frac{S_y^2 n}{D}}$$

$$S_m = \sqrt{\frac{(2467246.743)^2(5)}{1250}}$$

$$S_m = 156042.385$$

Uncertainty in the y-intercept (S_b)

$$S_b = \sqrt{\frac{S_y^2 \Sigma x_i^2}{D}}$$

$$S_b = \sqrt{\frac{(2467246.743)^2(1375)}{1250}}$$

$$S_b = 2587670.214$$

Uncertainty in unknown concentration of CBD in cannabis flower bud samples (S_x)

$$S_x = \frac{S_y}{|m|}\sqrt{\frac{1}{K} + \frac{1}{n} + \frac{(y-\bar{y})^2}{m^2 \Sigma (x_i - \bar{x})^2}}$$

$$S_x = \frac{2467246.743}{|1261062.94|}\sqrt{\frac{1}{3} + \frac{1}{5} + \frac{(38190320 - (73196595/5)^2}{(1261062.94)^2(250)}}$$

$$S_x = 2.7169$$

Concentration of CBD in standard solution → Standard solution 3

$C_1V_1 = C_2V_2$ $\quad C_1 = 100\text{ppm}$ $\quad C_2 = ?$

$V_1 = 0.225\text{mL}$ $\quad V_2 = 1.5\text{mL}$

$$C_2 = \frac{C_1V_1}{V_2}$$

$$C_2 = \frac{(100\text{ppm})(0.225\text{mL})}{1.5\text{mL}} \rightarrow C_2 = 15\text{ppm}$$

Concentration of CBD in cannabis flower buds → cannabis sample 2

$y = 38190320$ $\quad y = 1261062.94x - 4276625.1$

$$x = \frac{38190320 + 4276625.1}{1261062.94}$$

$$x = 33.68\text{ppm}$$

Calculation of %RSD

$$\%RSD = \frac{Sx}{\text{mean } x} \times 100\%$$

$$\%RSD = \frac{2.7169}{33.6755} \times 100\%$$

$$\%RSD = 8.1\%$$

Mass of CBD recovered

$$= 33.68 \frac{\mu g}{mL} \times 2.00\,mL$$

$$= 67.36\,\mu g$$

[CBD] in 1.5 mL aliquot

$$[CBD] = \frac{67.36\,\mu g}{1.5\,mL}$$

$$[CBD] = 44.91\,ppm$$

[CBD] in sample

$$[CBD] = 44.906 \times \frac{2.0\,mL}{1.5\,mL}$$

$$[CBD] = 59.875\,ppm$$

Propagation of Uncertainty for THC/CBD in cannabis sample

$$\left(\frac{Sc_2}{C_2}\right)^2 = \left(\frac{Svol1}{Vol1}\right)^2 + \left(\frac{Svol2}{Vol2}\right)^2 + \left(\frac{Sc_1}{C_1}\right)^2$$

$$\frac{Sc_2}{59.875\,ppm} = \sqrt{\left(\frac{0.08}{100\,mL}\right)^2 + \left(\frac{0.02}{1.5\,mL}\right)^2 + \left(\frac{2.7169}{33.68}\right)^2}$$

$$\frac{Sc_2}{59.875\,ppm} = 0.08172$$

$$Sc_2 = 4.89 \longrightarrow 59.875 \pm 4.89\,ppm$$

Discussion

Liquid chromatography - mass spectrometry (LC-MS) was used in this experiment to determine the concentration of cannabidiol (CBD) present in cannabis flower bud samples. The results of the analysis showed successful separation, and the concentration of CBD in each sample was determined to be: Organic Sour Cookies: 36.75 ppm ± 2.88 ppm; Kush Cookies: 56.17 ppm ± 4.51 ppm; Lilac Diesel: 59.88 ± 4.89 ppm and Death Bubba: 48.47 ± 3.76 ppm. Standards and cannabis solutions were run on the LC-MS, with samples being run in triplicate, by both the instructor and experimenters. The data was collected and analyzed using an Excel spreadsheet. Attached chromatograms represent all standards and cannabis flower sample peaks, which were characterized by their retention time and peak area. As shown in Table 5, peak area increased with the increase of concentration in the standard solutions, as expected. When analyzing the cannabis samples, it was hard to distinguish between both CBD and THC; therefore, CBD concentration was analyzed due to their co-elution.

The experimental procedure was derived using multiple papers. Background information was taken from *Quantitative Determination and Validation of 17 Cannabinoids in Cannabis and Hemp using Liquid Chromatography-Tandem Mass Spectrometry* by McRae & Melanson (2020). Extra information for ethanol extraction was followed from *Cannabis Oil: Chemical Evaluation of an Upcoming Cannabis-Based Medicine* by Romano & Hazekamp (2013). The original procedure suggests the use of ethanol; however, in order to be consistent with the stock standard solutions, methanol was chosen as a solvent. There is no difference between ethanol and methanol for extraction, only that ethanol shows a higher yield (Lazarjani et al., 2021). LC-MS was chosen as it was determined to be the ideal method for quantification methods related to cannabis, based on findings in 2020 conducted by Lazarjani et al.

As seen in Figure 1, a calibration curve was generated using the obtained peak areas as a function of the standard solution concentration. Five standards were created using varying concentrations of stock CBD, and varying volumes of methanol to obtain a final volume of 1.5 mL (Table 2). The calibration curve was generated after running the standard solutions on the LC-MS, which was further used to determine the concentration of CBD present in the flower bud samples. The flower bud samples were ground and diluted in 25 mL methanol, filtered through a 0.45 μm syringe filter, and a nitrogen blow down was performed to evaporate the solvent. This increased the surface area and decreased vapor pressure, while maintaining the integrity of the samples (LabMate, 2021). Samples were run on the LC-MS to obtain peak areas, which was used to determine concentration and associated uncertainty. THC standards were also run on the LC-MS, seen in Table 3, but as mentioned above, CBD and THC co-elute thus THC concentrations were not considered as there was no way to accurately separate the two, and CBD can represent both.

The first sample of cannabis tested was Organic Sour Cookies (Weed 1), which was determined to have a CBD concentration of 36.95 ppm, or 0.03695 mg/g. This was calculated from the equation of the line, which was found to be $y= 1x10^6x - 4 x10^6$ using excel. The uncertainty in the slope and the y-intercept was calculated to be 156042.3851 mg/L and 2587670.214 mg/L, respectively. The percent RSD was 7.69%, which would indicate reasonable precision. The associated R^2 value was 0.9561, indicating 95% of the data fell within the linear range. The propagation of uncertainty was calculated to be 36.95 ± 2.88 ppm.

The second sample of cannabis tested was Kush Cookies (Weed 2). It was determined to have a CBD concentration of 56.17 ppm, or 0.05617 mg/g. This was calculated from the equation of the line, which was found to be $y= 1x10^6x - 4 x10^6$ using excel. The uncertainty in

the slope and the y-intercept was calculated to be 156042.3851 mg/L and 2587670.214 mg/L, respectively. The percent RSD was 7.92%, which would indicate reasonable precision. The associated R^2 value was 0.9561, indicating 95% of the data fell within the linear range. The propagation of uncertainty was calculated to be 56.17 ± 4.51 ppm.

The third sample of cannabis tested was Lilac Diesel (Weed 3), which was determined to have a CBD concentration of 59.88 ppm, or 0.05988 mg/g. This was calculated from the equation of the line, which was found to be $y= 1x10^6x - 4 x10^6$ using excel. The uncertainty in the slope and the y-intercept was calculated to be 156042.3851 mg/L and 2587670.214 mg/L, respectively. The percent RSD was 8.07%, which would indicate reasonable precision. The associated R^2 value was 0.9561, indicating 95% of the data fell within the linear range. The propagation of uncertainty was calculated to be 59.88 ± 4.89 ppm.

The fourth and final sample of cannabis tested was Death Bubba (Weed 4). It was determined to have a CBD concentration of 48.47 ppm, or 0.04847 mg/g. This was calculated from the equation of the line, which was found to be $y= 1x10^6x - 4 x10^6$ using excel. The uncertainty in the slope and the y-intercept was calculated to be 156042.3851 mg/L and 2587670.214 mg/L, respectively. The percent RSD was 7.65%, which would indicate reasonable precision. The associated R^2 value was 0.9561, indicating 95% of the data fell within the linear range. The propagation of uncertainty was calculated to be 48.47 ± 3.76 ppm.

All samples showed low concentrations of CBD when compared to the labeled amount, as seen in Table 7. Organic Sour Cookies had a labeled amount of <5.000 mg/g, with an obtained concentration of 0.03695 mg/g. Kush Cookies had a labeled amount of <0.300 mg/g, with an obtained amount of 0.05617 mg/g. Lilac Diesel had a labeled amount of <0.100 mg/g, with an obtained amount of 0.05988 mg/g. Finally, Death Bubba had a labeled amount of <1.000 mg/g,

with an obtained amount of 0.04847 mg/g. Low calculated concentrations could be due to experimental errors or could be due to inaccurate labeling and co-elution problems. Technically, all calculated values are within the acceptable labeled amount; however, as they are estimates of the total amount present, the amount of CBD present cannot be accurately accepted.

According to a study in 2022 conducted by Spindle et al., researchers found that for a sample size of 105 topical products, 18% were over labeled, having less than 10% CBD, and 58% were under labeled, having more than 10% CBD. Additionally, another study conducted in 2017, by Bonn-Miller et al., found that 26% of tested products contained less CBD than what was labeled, stating that this could negate clinical responses when used for medicinal purposes. Researchers advised that manufacturing and testing standards must be employed. Based on this, it could be incurred that future work is needed to accurately determine the amount of CBD present in samples. Future work should also be done to counteract the effect or separate THC and CBD, as these cannabinoids co-elute, causing issues when analyzing data. This could be done by optimizing conditions to lengthen the elution region by including phospholipids, as suggested by Jamwal et al., in 2017. Furthermore, the concentration of CBD standards could be expanded to include higher concentrations. As it is noted in Table 7, most of the samples had higher concentrations than that of the standards. By expanding this, concentration values present in the samples could be more accurately determined.

Possible sources of error in this experiment include pipetting of the samples into the LC-MS vials, the use of syringe filters, the nitrogen blow down and the samples themselves. When the samples were pipetted into the LC-MS vials, it was noticed that some had different levels of liquid than others. While this did not affect the runs on the LC-MS, it could have affected accuracy of the values obtained. The syringe filters, when filtering the solvent after being

dissolved, became easily clogged with flower bud flakes. The filters needed to be changed multiple times to effectively filter the entire solution, which could have led to a loss of product. In the future, gravity or vacuum filtration may give better results. The nitrogen blow down may have caused issues as the needle dispensing the gas was quite small and had issues remaining in place for long periods of time. As it was being constantly adjusted, this could have caused issues with the evaporation of the solvent. Additionally, the water bath used during the nitrogen blow down evaporated due to the amount of time it took for the nitrogen to evaporate all of the solvent. In the future, it would be easier to clamp a part of the needle and separate the cords to ensure it can be easily, and quickly adjusted. Lastly, the samples could have caused sources of error, due to the inconsistency between them. Lilac Diesel and Death Bubba were very sticky after being ground up, while Organic Sour Cookies and Kush Cookies were quite dry. The stickiness of the former could have caused issues when diluting with methanol. The stickiness of the Lilac Diesel sample could have caused the higher percent RSD value, unless impurities were introduced between the grinding of the second and third samples.

Conclusion

This experiment was conducted to determine the concentration of cannabidiol in various cannabis flower bud samples, using liquid chromatography - mass spectrometry. The determined concentration of cannabidiol in the Organic Sour Cookies sample was 36.95 ± 2.88 ppm, with an RSD 7.69%; in the Kush Cookies sample was 56.17 ± 4.51 ppm, with an RSD of 7.92%; in the Lilac Diesel sample was 59.88 ± 4.89 ppm, with an RSD of 8.07%; in the Death Bubba sample was 48.47 ± 3.76 ppm, with an RSD of 7.65%.

References

1. Benefits of Ethanol Extraction Systems for Extracting Cannabis https://www.cbgbiotech.com/blog/benefits-of-ethanol-extraction-systems
2. Blessing, E.M., Steenkamp, M.M., Manzanares, J. *et al.* Cannabidiol as a Potential Treatment for Anxiety Disorders. *Neurotherapeutics* 12, 825–836 (2015). https://doi.org/10.1007/s13311-015-0387-1
3. Bonn-Miller MO, Loflin MJE, Thomas BF, Marcu JP, Hyke T, Vandrey R. Labeling Accuracy of Cannabidiol Extracts Sold Online. JAMA. 2017 Nov 7;318(17):1708-1709. doi: 10.1001/jama.2017.11909. PMID: 29114823; PMCID: PMC5818782.
4. Catenza, K.F., Donkor, K.K. Determination of Heavy Metals in Cannabinoid-Based Food Products Using Microwave-Assisted Digestion and ICP-MS. Food Anal. Methods 15, 2537–2546 (2022). https://doi.org/10.1007/s12161-022-02315-1
5. Ferber SG, Namdar D, Hen-Shoval D, Eger G, Koltai H, Shoval G, Shbiro L, Weller A. The "Entourage Effect": Terpenes Coupled with Cannabinoids for the Treatment of Mood Disorders and Anxiety Disorders. Curr Neuropharmacol. 2020;18(2):87-96. doi: 10.2174/1570159X17666190903103923. PMID: 31481004; PMCID: PMC7324885.
6. Jamwal R, Topletz AR, Ramratnam B, Akhlaghi F. Ultra-high performance liquid chromatography tandem mass-spectrometry for simple and simultaneous quantification of cannabinoids. J Chromatogr B Analyt Technol Biomed Life Sci. 2017 Mar 24;1048:10-18. doi: 10.1016/j.jchromb.2017.02.007. Epub 2017 Feb 6. PMID: 28192758; PMCID: PMC5697720.
7. Lazarjani, M.P, Torres, S., Hooker, T. *et al.* Methods for quantification of cannabinoids: a narrative review. *J Cannabis Res* 2, 35 (2020). https://doi.org/10.1186/s42238-020-00040-2
8. Lazarjani, M.P., Young, O., Kebede, L. *et al.* Processing and extraction methods of medicinal cannabis: a narrative review. *J Cannabis Res* 3, 32 (2021). https://doi.org/10.1186/s42238-021-00087-9
9. McRae, G., Melanson, J.E. Quantitative determination and validation of 17 cannabinoids in cannabis and hemp using liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem* 412, 7381–7393 (2020). https://doi.org/10.1007/s00216-020-02862-8
10. Meng Q, Buchanan B, Zuccolo J, Poulin MM, Gabriele J, et al. (2018) A reliable and validated LC-MS/MS method for the simultaneous quantification of 4 cannabinoids in 40 consumer products. PLOS ONE 13(5): e0196396. https://doi.org/10.1371/journal.pone.0196396
11. Morehouse, Z.P., Ryan, G.L., Proctor, C.M. *et al.* A proposed method of sample preparation and homogenization of hemp for the molecular analysis of cannabinoids. *SN Appl. Sci.* 3, 762 (2021). https://doi.org/10.1007/s42452-021-04747-6
12. Romano LL, Hazekamp A. Cannabis oil: chemical evaluation of an upcoming cannabis-based medicine. Cannabinoids. 2013;1(1):1–11.
13. Spindle TR, Sholler DJ, Cone EJ, et al. Cannabinoid Content and Label Accuracy of Hemp-Derived Topical Products Available Online and at National Retail Stores. *JAMA Netw Open.* 2022;5(7):e2223019. doi:10.1001/jamanetworkopen.2022.23019
14. Valizadehderakhshan, M.; Shahbazi, A.; Kazem-Rostami, M.; Todd, M.S.; Bhowmik, A.; Wang, L. Extraction of Cannabinoids from *Cannabis sativa* L. (Hemp)—Review. *Agriculture* **2021**, *11*, 384. https://doi.org/10.3390/agriculture11050384

15. Volkow ND, Baler RD, Compton WM, Weiss SR. Adverse health effects of marijuana use. N Engl J Med. 2014 Jun 5;370(23):2219-27. doi: 10.1056/NEJMra1402309. PMID: 24897085; PMCID: PMC4827335.

Appendix

CHEM 3170 Weed 1 Uncertainty Table							
number of points		**[CBD] ppm**	**Peak Area**				
n=	**5**	**x_i**	**y_i**	**x_iy_i**	**x_i^2**	**d_i**	**d_i^2**
		5	964969	4824845	25	-1063720.6	1.1315E+12
		10	7249784	72497840	100	-1084220.3	1.17553E+12
		15	17939941	269099115	225	3300622	1.08941E+13
		20	21850933	437018660	400	906299.3	8.21378E+11
		25	25190968	629774200	625	-2058980.4	4.2394E+12
		Σx_i	Σy_i	Σx_iy_i	Σx_i^2	Σd_i	Σd_i^2
	Sums:	75	73196595	1413214660	1375	9.31323E-09	1.82619E+13
		D=	**1250**		S_y	**2467246.743**	
Method of least squares							
		slope=	1261062.94		intercept=	-4276625.1	
		R^2=	0.9561		S_b=	**2587670.214**	
		S_m=	**156042.3851**		y_-	14639319	
		x_-	15				
measured y value			number of replicates of unknown (k)				
	37753014		3				
	3065434		S_x=	**1.597905989**			
	24972183						
y=	21930210.33						
derived x=	**20.78154436**						

Figure 2. Uncertainty table for cannabis sample 1(W1), Organic Sour Cookies, for the concentration of CBD determined in flower buds from the calibration curve (n = 5, k = 3).

CHEM 3170 Weed 2 Uncertainty Table							
number of points		[CBD] ppm	Peak Area				
n=	5	x_i	y_i	x_iy_i	x_i^2	d_i	d_i^2
		5	964969	4824845	25	-1063720.6	1.1315E+12
		10	7249784	72497840	100	-1084220.3	1.17553E+12
		15	17939941	269099115	225	3300622	1.08941E+13
		20	21850933	437018660	400	906299.3	8.21378E+11
		25	25190968	629774200	625	-2058980.4	4.2394E+12
		Σx_i	Σy_i	Σx_iy_i	Σx_i^2	Σd_i	Σd_i^2
	Sums:	75	73196595	1413214660	1375	9.31323E-09	1.82619E+13
		D=	1250		S_y	2467246.743	
Method of least squares							
		slope=	1261062.94		intercept=	-4276625.1	
		R^2=	0.9543		S_b=	2587670.214	
		S_m=	156042.3851		y_-	14639319	
		x_-	15				
measured y value			number of replicates of unknown (k)				
	43362677		3				
	39986787		S_x=	2.501577583			
	23348646						
y=	35566036.67						
derived x=	31.59450691						

Figure 3. Uncertainty table for cannabis sample 2 (W2), Kush Cookies, for the concentration of CBD determined in flower buds from the calibration curve (n = 5, k = 3).

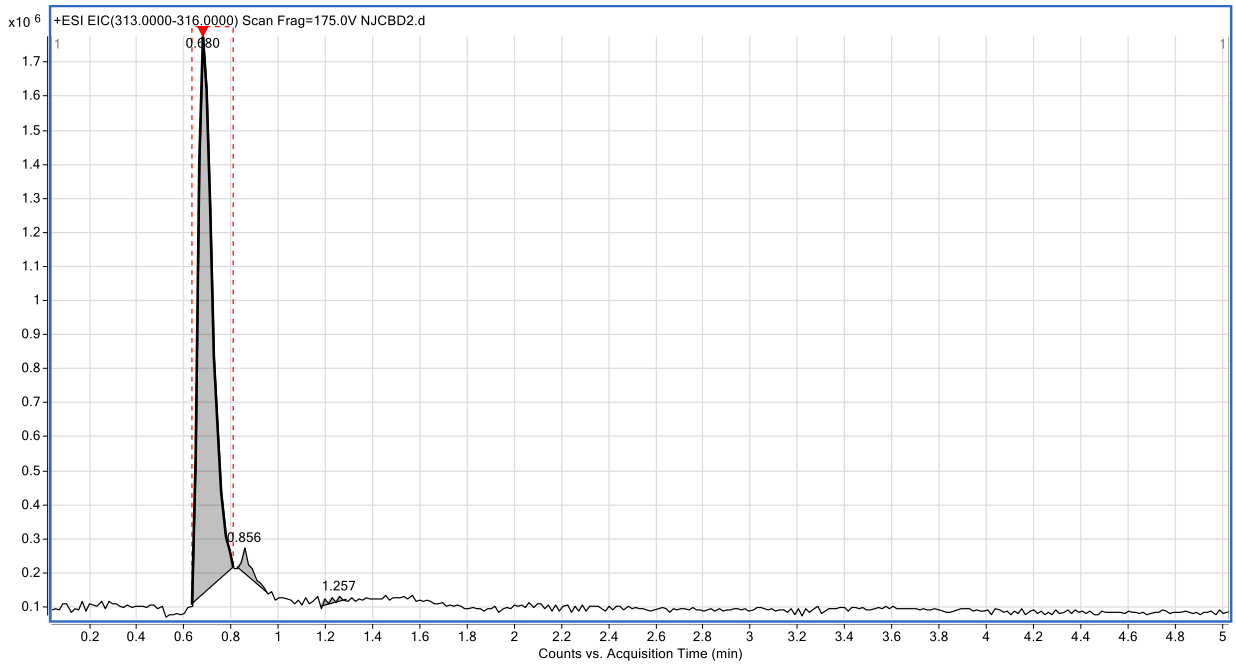
CHEM 3170 Weed 3 Uncertainty Table							
number of points		[CBD] ppm	Peak Area				
n=	5	x_i	y_i	x_iy_i	x_i^2	d_i	d_i^2
		5	964969	4824845	25	-1063720.6	1.1315E+12
		10	7249784	72497840	100	-1084220.3	1.17553E+12
		15	17939941	269099115	225	3300622	1.08941E+13
		20	21850933	437018660	400	906299.3	8.21378E+11
		25	25190968	629774200	625	-2058980.4	4.2394E+12
		Σx_i	Σy_i	Σx_iy_i	Σx_i^2	Σd_i	Σd_i^2
	Sums:	75	73196595	1413214660	1375	9.31323E-09	1.82619E+13
		D=	**1250**		S_y	**2467246.743**	
Method of least squares							
		slope=	1261062.94		intercept=	-4276625.1	
		R^2=	0.9543		S_b=	**2587670.214**	
		S_m=	**156042.3851**		y_-	14639319	
		x_-	15				
measured y value			number of replicates of unknown (k)				
	58906021		3				
	50794135		S_x=	**2.716927745**			
	4870804						
y=	38190320						
derived x=	**33.67551591**						

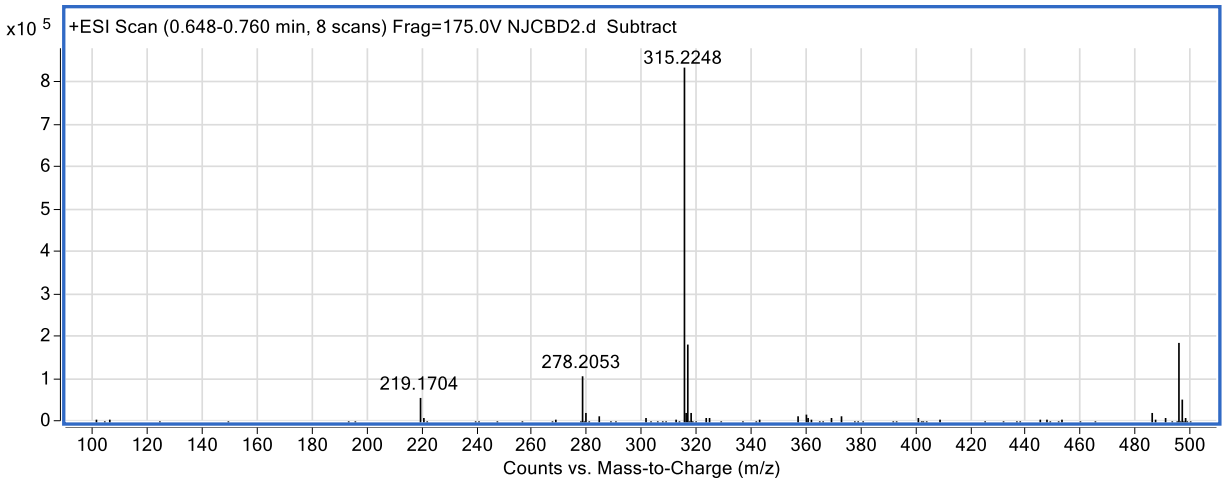
Figure 4. Uncertainty table for cannabis sample 3 (W3), Lilac Diesel, for the concentration of CBD determined in flower buds from the calibration curve (n = 5, k = 3).

CHEM 3170 Weed 4 Uncertainty Table							
number of points		[CBD] ppm	Peak Area				
n=	5	x_i	y_i	x_iy_i	x_i^2	d_i	d_i^2
		5	964969	4824845	25	-1063720.6	1.1315E+12
		10	7249784	72497840	100	-1084220.3	1.17553E+12
		15	17939941	269099115	225	3300622	1.08941E+13
		20	21850933	437018660	400	906299.3	8.21378E+11
		25	25190968	629774200	625	-2058980.4	4.2394E+12
		Σx_i	Σy_i	Σx_iy_i	Σx_i^2	Σd_i	Σd_i^2
	Sums:	75	73196595	1413214660	1375	9.31323E-09	1.82619E+13
		D=	1250		S_y	2467246.743	
Method of least squares							
		slope=	1261062.94		intercept=	-4276625.1	
		R^2=	0.9543		S_b=	2587670.214	
		S_m=	156042.3851		y_-	14639319	
		x_-	15				
measured y value			number of replicates of unknown (k)				
	41891955		3				
	36084559		S_x=	2.084107998			
	12329374						
y=	30101962.67						
derived x=	27.2615955						

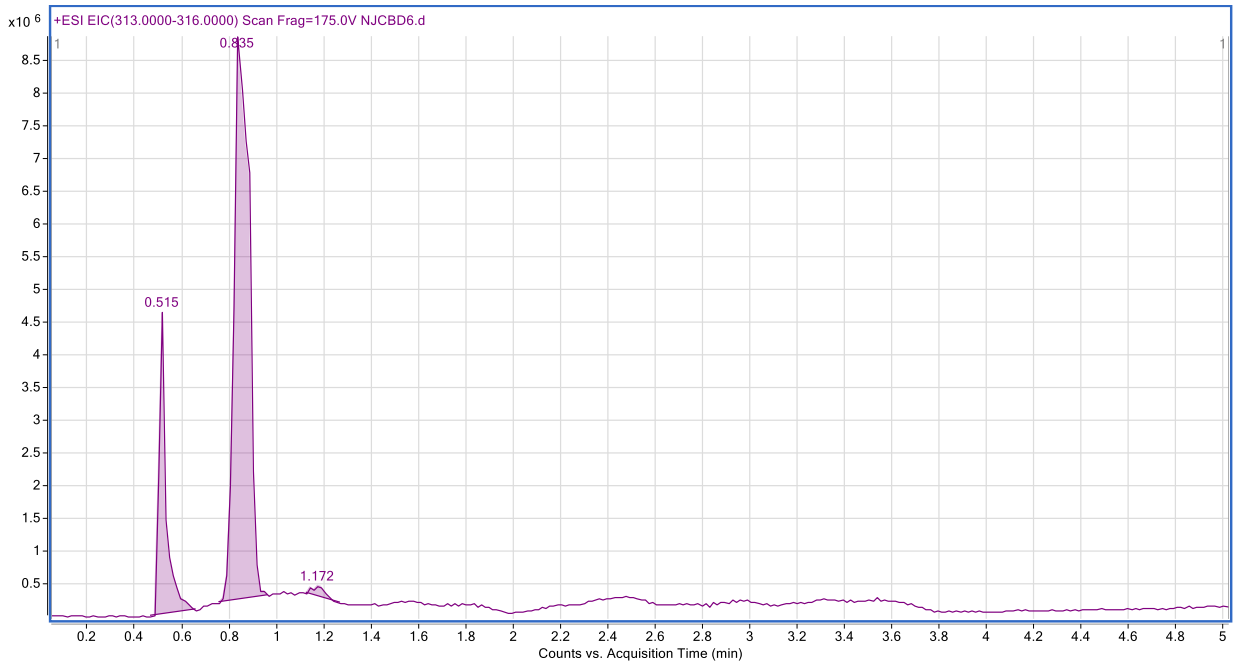
Figure 5. Uncertainty table for cannabis sample 4 (W4), Death Bubba, for the concentration of CBD determined in flower buds from the calibration curve (n = 5, k = 3).

CBD - Std 2

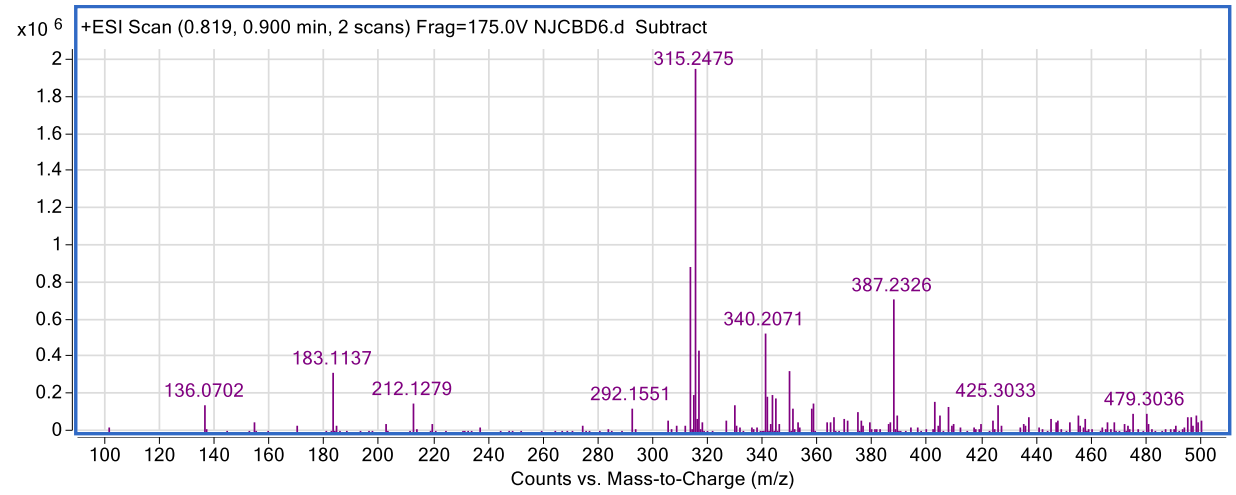

x10 6
+ESI EIC(313.0000-316.0000) Scan Frag=175.0V NJCBD2.d
0.680
0.856
1.257
Counts vs. Acquisition Time (min)


x10 5
+ESI Scan (0.648-0.760 min, 8 scans) Frag=175.0V NJCBD2.d Subtract
315.2248
278.2053
219.1704
Counts vs. Mass-to-Charge (m/z)

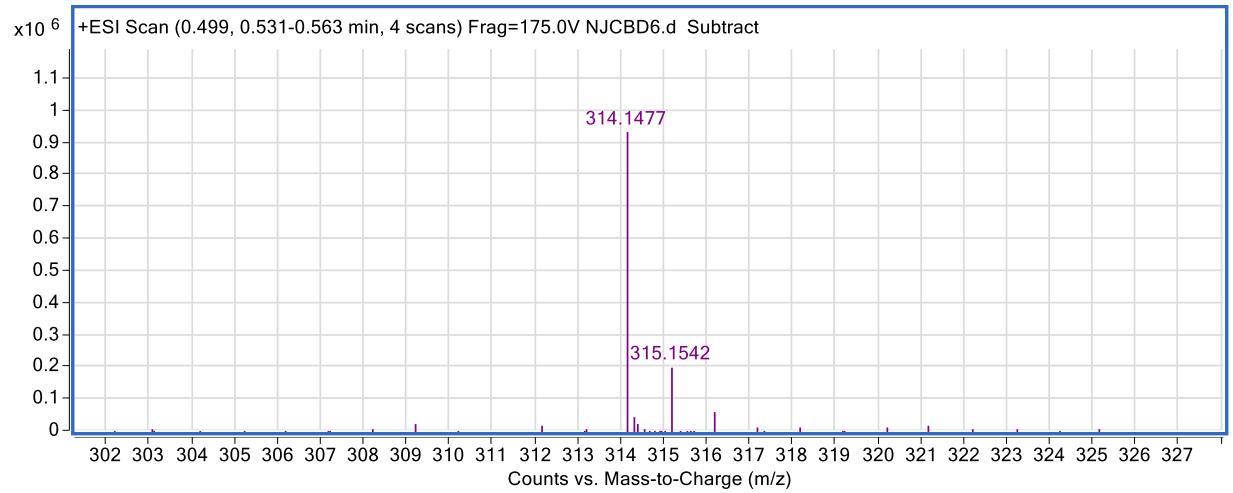
Weed 1



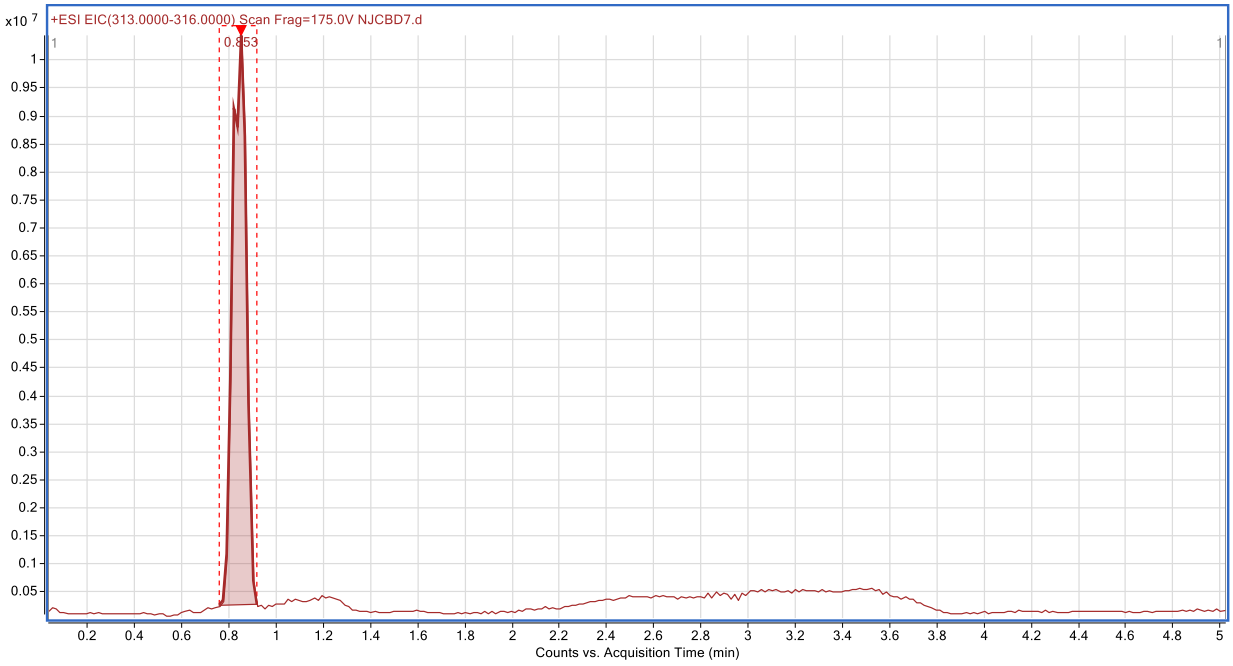
Big peak



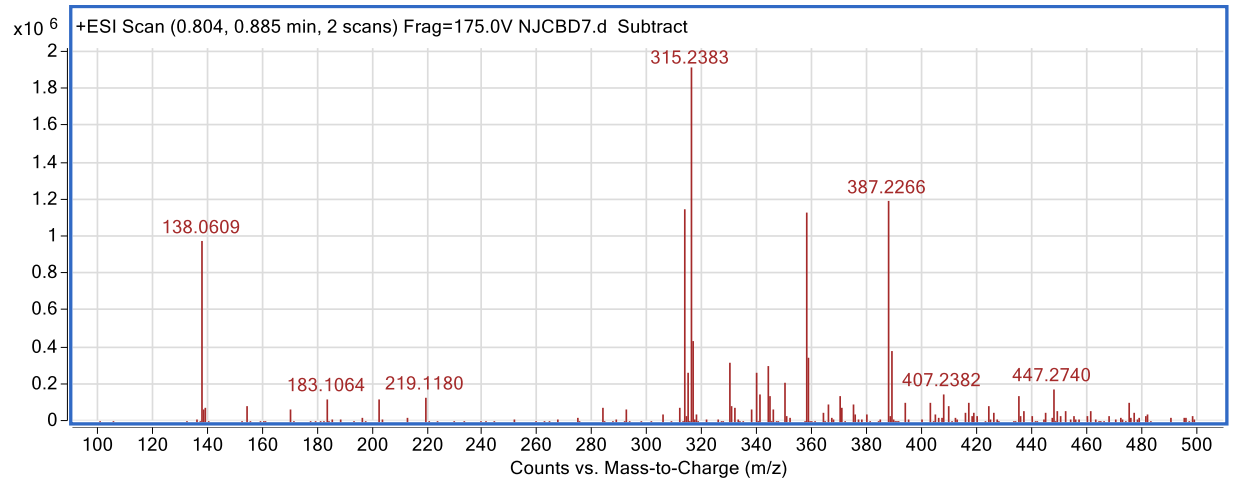
Small peak



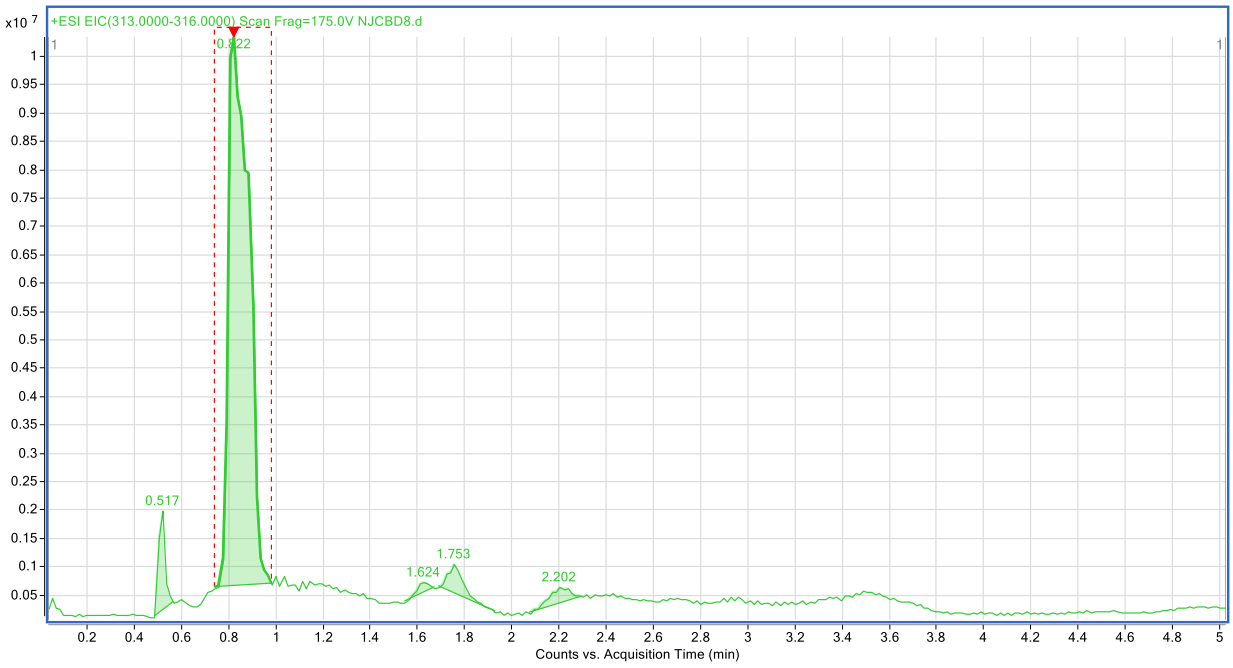
Weed 2



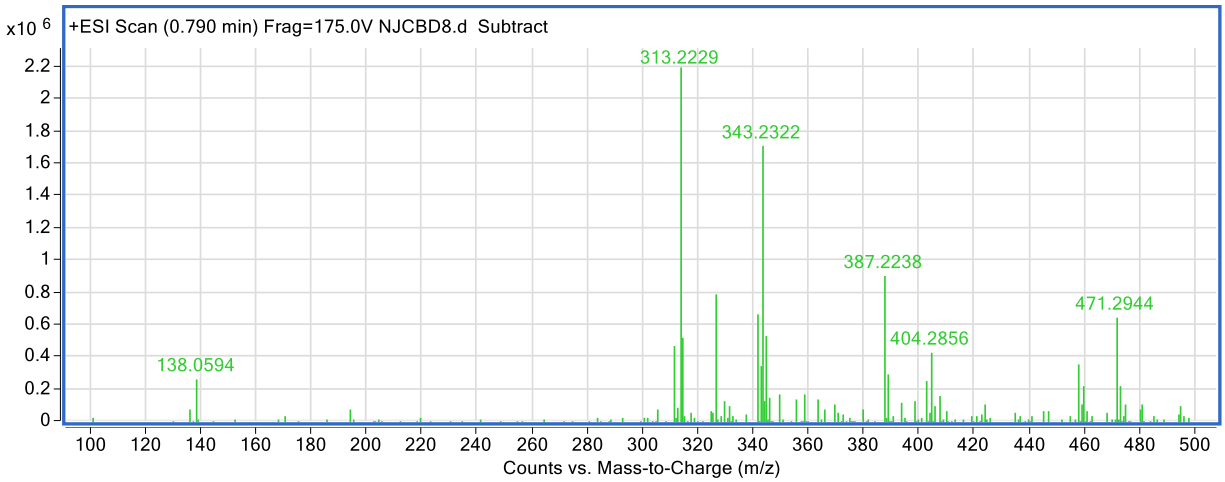
Only big peak



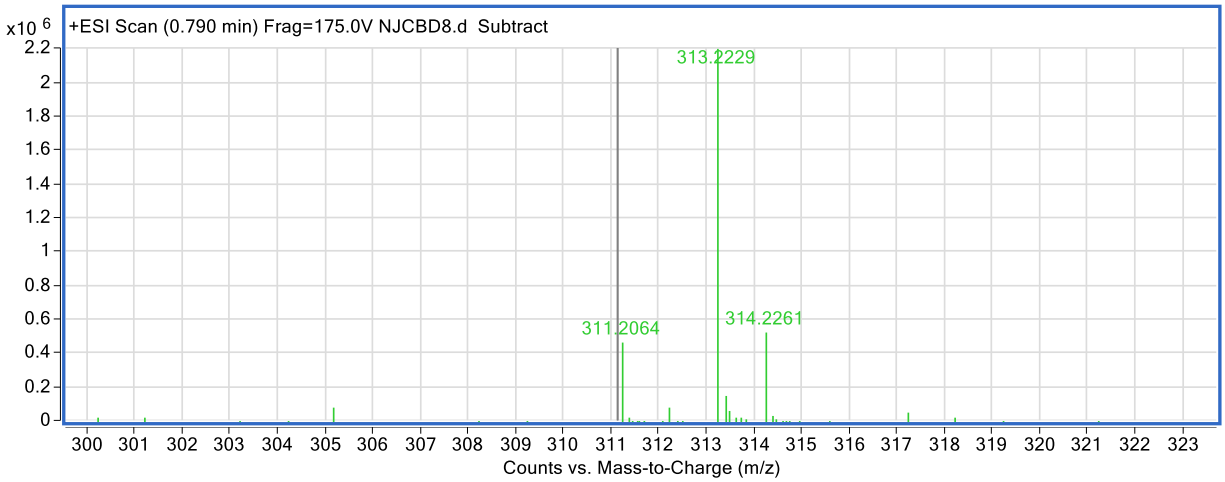
Weed 3



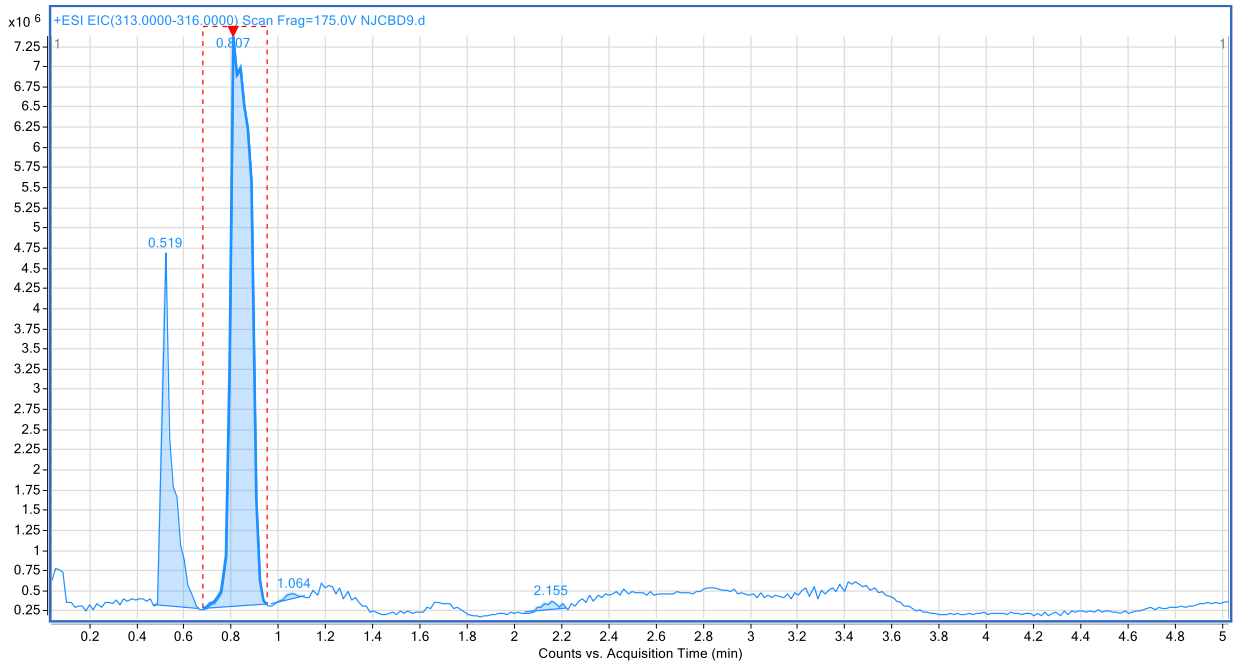
Big peak



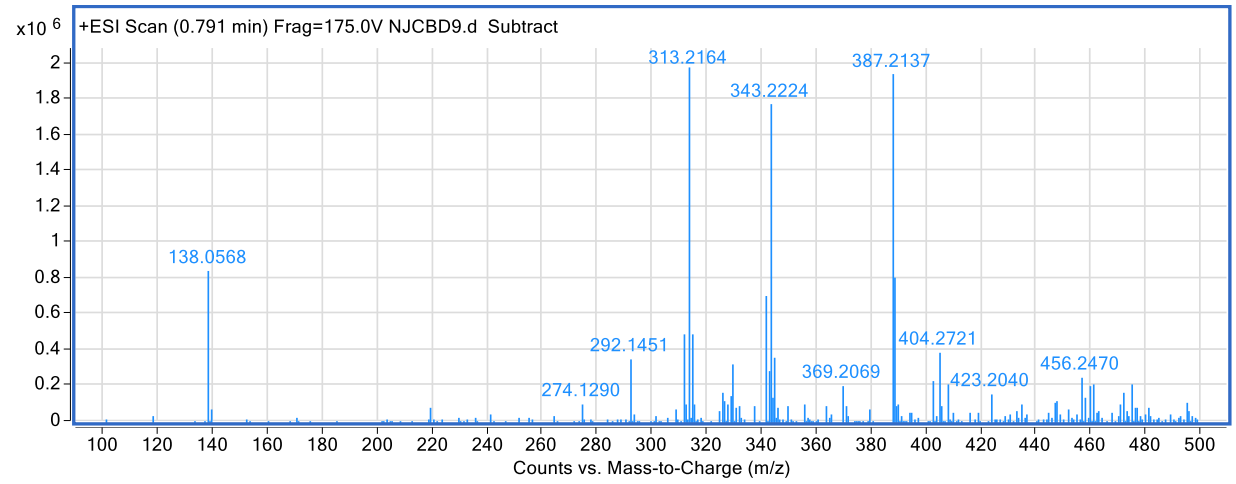
Small peak



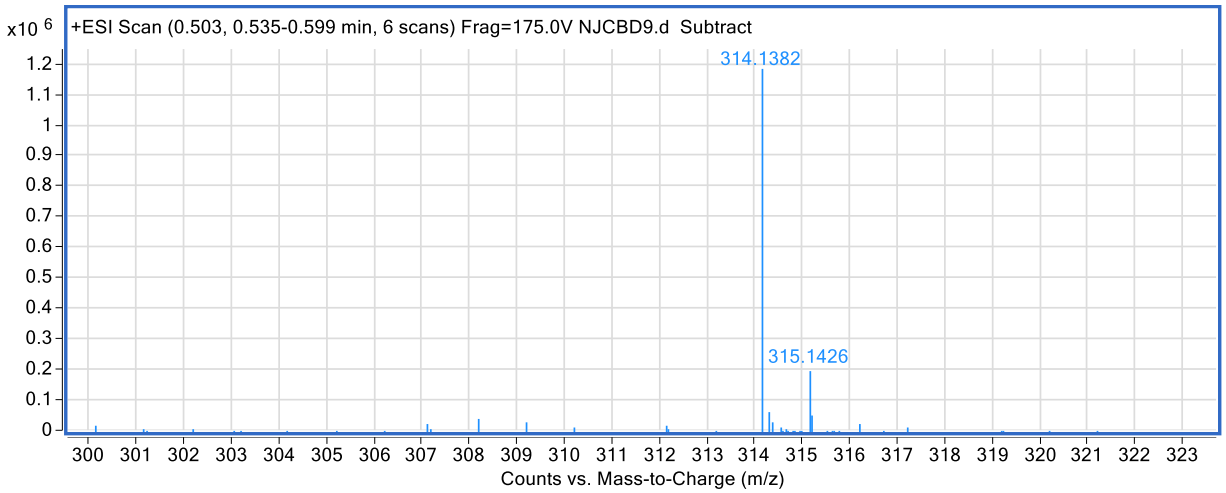
Weed 4



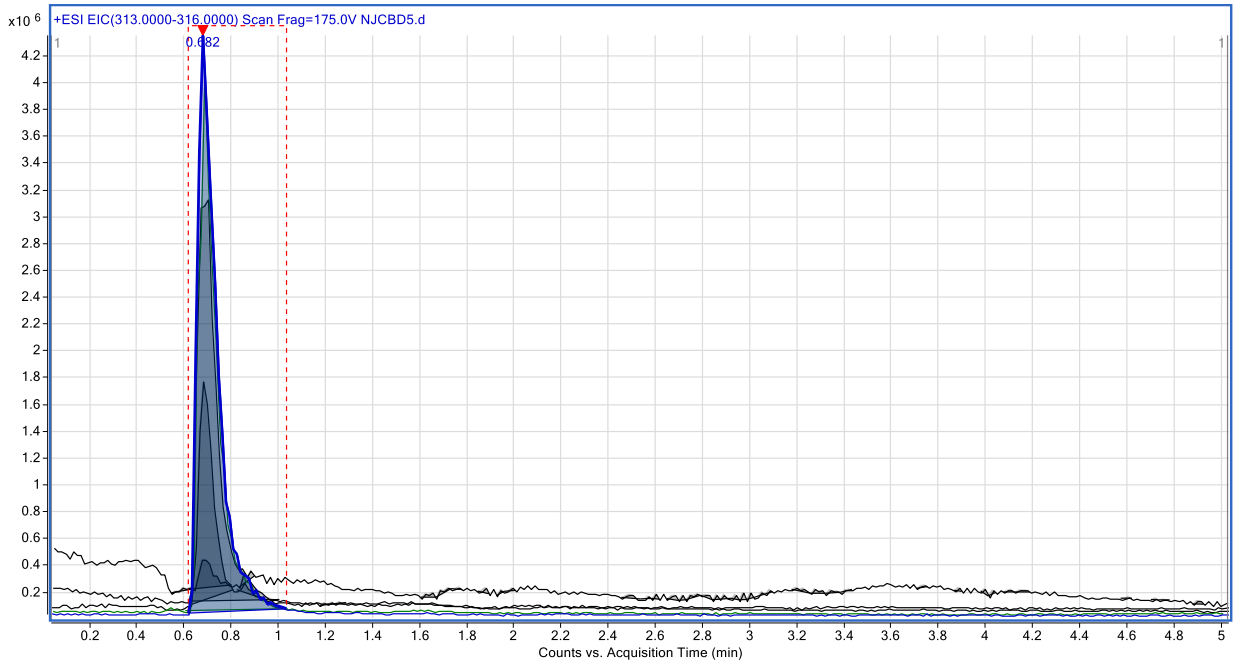
Big peak



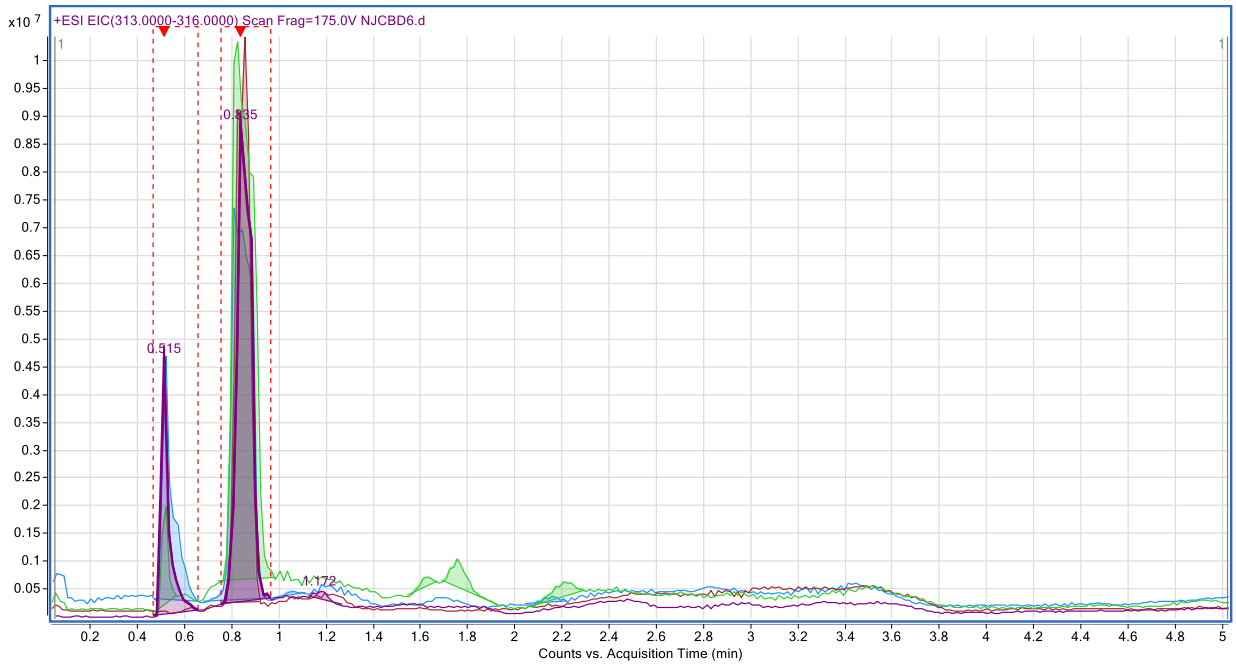
Small peak



Overlaid CBD Standards



Overlaid Cannabis Samples



CBD and THC Co-elution