EXTRACTION AND DETECTION OF DELTA 9- TETRAHYDROCANNABINOL FROM CANNABIDIOL OILS USING GC/MS

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CAPSTONE ABSTRACT

Extraction and Detection of Delta 9-Tetrahydrocannabinol from Cannabidiol Oils using GC/MS

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The process of detecting delta 9- tetrahydrocannabinol (THC) from CBD oil involves many different factors. Recently, a lot of research has been done in detecting THC in different cannabis products, however very little has been done to detect and even quantify THC from CBD oils. This study set out to validate an already established method and use this method to detect THC from hemp oils that claim not to contain THC on their labels. In order to do this the samples were extracted using a liquid-liquid extraction technique. The method validation consisted of determining the limit of detection (LOD) of CBD and THC and also the precision. Five calibrators were made at different concentrations ranging from 0.1-5 µg/ml. Positive and negative controls were also made to ensure that the experiment was working properly.

Three different blank oils consisting of olive oil, almond oil and coconut oil were used to conduct the validation study. These samples were done in duplicates as well and the

instrument used to conduct this study was the gas chromatograph-mass spectrometer (GC-MS). The LOD study was not able to be completed due to time constraints. The lowest concentration of CBD and THC that was analyzed was at 0.1 µg/ml. The retention times of CBD and THC were found to be 12.856 and 13.654 minutes respectively. The percentage coefficient of variation (%CV) was calculated for each oil at their respective concentrations using the peak areas of THC and CBD in order to find precision. It was noted that the majority of the samples had a %CV within 20%, which indicates decent precision; however, some samples were beyond that percentage due to complications in the extraction process creating fluctuations in the peak areas at each concentration analyzed.

After the LOD study was completed 6 different hemp oil samples were extracted and run as well using the same method. Results from these runs found that neither CBD nor THC was not detected in these oil samples. As a result, further studies that need to be done to enhance the extraction procedure and method to make it fully efficient.

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1.Introduction

1.1 Cannabis

This project highlights an area of concern in the cannabis industry by examining different cannabis products with improper labelling being sold on the retail market. Cannabis, also known as “marijuana” is the Genus from the family Cannabaceae. The number of species of marijuana is unknown due to the fact that there are many different strains being hybridized and grown daily. There is, however one generally accepted species that is broken down into three main subspecies.[1] The first subspecies is Cannabis sativa, which is a tall plant with a pale color and thin leaves. The second subspecies is Cannabis indica, which is a much shorter plant with dark green leaves compared to the sativa plant. The third subspecies is Cannabis ruderalis, which is the shortest of the three and is known as the parental strain due to its ability to be grown in any environment.

Figure 1: Diagram of Cannabis Species[22]

The main two species in circulation commercially are C. sativa and C. indica, as they are mostly used in the medicinal, recreational and illegal growing industry. The cannabis plant consists of over 400 chemical entities and more than 60 of them are known as

cannabinoid compounds.[1,2] Cannabinoids are chemicals that bind to the cannabinoid receptors in the body. The main cannabinoids that will be discussed are Cannabidiol (CBD) and delta 9-Tetrahydrocannabinol (i.e which will be henceforth written as "THC" for the purposes of this paper). CBD and THC are similar in structure as they possess a bi and tri-cyclic ring structure respectively. They have the same molecular formula and therefore follow the same pathway in the body.

Figure 2: Chemical structures of CBD and THC[24]

The rest of the cannabis plant contains other chemicals such as terpenes and flavonoids, which are responsible for the aroma and pigmentation of the plant respectively.

C. sativa is a strain that contains a higher THC content compared to C. indica.[2] THC is the principal psychoactive component in cannabis and can be found mainly in the bract and flower buds of the plant.

Figure 3: Diagram of the parts of the Cannabis plant[23]

THC is also very volatile and viscous in nature. It is recognized as a weak acid and a central nervous system (CNS) stimulant.[2] C. indica, on the other hand, has a higher CBD content as CBD occupies 40% of the plant.[2] CBD is the main non-psychoactive component of cannabis and has similar characteristics to THC; however, it is a CNS depressant.

1.2 Pharmacokinetics

Cannabis can be delivered into the body, through many different routes. Some common forms of cannabis administration are inhalation, oral administration, intravenous injection (IV) and transdermal.[3] The main forms in which it is administered is through inhalation via smoking and vaporization and through oral administration in the form of pills, edibles and sublinguals (tinctures).[3]

Inhalation and IV are the most efficient ways to receive cannabis products, as these methods deliver the chemical directly into the blood stream which causes quicker effects.[3] However, smoking and oral ingestion are reported to be the most common forms of administration. The bioavailability of cannabis after inhalation is affected due to the uncertainty in dose delivery. Oral administration has a lower bioavailability percentage which makes it a potentially less harmful route. This is due to the fact that delta 9-THC is subjected to first pass metabolism, which reduces its bioavailability.[2,3] It is metabolized by the liver before reaching systemic circulation, which decreases its concentration in the body making it less potent. THC is a hydrophobic/lipophilic molecule and can cross the blood brain barrier rather quickly.[8] This means that it can induce its effects on the body very quickly. It has a high partition coefficient, which means that it also has a large distribution volume. Rapid distribution in tissues and other organs decreases plasma concentrations. Due to the high lipophilicity, distribution occurs in highly perfused organs and tissues.[3]

1.2.1 Metabolism

When THC undergoes metabolism it produces psychoactive and non-psychoactive metabolites in the body through the liver by what is known as phase 1 and phase 2 metabolism.[3] The major metabolites produced are 11-hydroxy tetrahydrocannabinol (hydroxy THC) and 11 nor 9-carboxy tetrahydrocannabinol (carboxy THC) respectively.[3] Hydroxy THC is the psychoactive metabolite that is produced first through phase 1 metabolism, and then it is consequently metabolized to produce carboxy THC to facilitate elimination.[3]

Figure 4: Flow diagram of metabolism of THC[6]

1.2.2 Elimination

Approximately 80-90% of THC is excreted from the body as its metabolites through 65% feces and 20% urine.[3] THC undergoes slow elimination from the body which allows it to still be detected in urine while low or absent in blood. THC has a half-life of approximately 10-20 hours. Total elimination typically takes days to weeks and is all dependent on whether the dose taken was singular or chronic. An individual that takes a

chronic dosage would have a much longer elimination time compared to a singular dosage because the concentration of THC in the body would be higher.

1.3 Pharmacodynamics

In the body there exists a system known as the endocannabinoid system, which contains cannabinoids that are hydrophobic retrograde neurotransmitters and cannabinoid receptors.[4] Cannabinoid receptors are known as G protein coupled receptors that are targeted by endocannabinoids, phytocannabinoids and synthetic cannabinoids.[4] This facilitates the signaling of neurotransmitters to the brain. There are two types of cannabinoid receptors known as Cannabinoid Receptor 1 (CB1) and Cannabinoid Receptor 2 **(**CB2).[4] CB1 is the most abundant receptor and is expressed in the central nervous system, primarily in the brain. This explains how THC is able to bind to this receptor due to its ability to cross the blood brain barrier. CB2 is mostly associated with cells governing immune function, even though it is also expressed in the CNS. Both THC and CBD serve as ligands to different extents on different protein receptors (eg. GPR18, GPR55, GPR119).[4]

1.4 Effects of THC & CBD

The psychoactive effects of THC are facilitated by the activation of these CB1 receptors in the CNS. THC acts as a partial agonist on the CB1 and CB2 receptors.[6] This allows it to induce its psychoactive effects which includes euphoria and cognitive impairments at high doses.[6,7] THC has a similar chemical structure to that of an endogenous compound known as anandamide.

Figure 5: Diagram of Anandamide and THC structure[21]

Anandamide is a brain lipid that is responsible for brain function, as it attaches to neurotransmitters regulating mood or behavior. It also has the ability to bind to CB1 and CB2 receptors with high affinity and mimicking the psychoactive effects of plant derived cannabinoids[25]. However, once THC is present, it displaces the anandamide from the receptors to alter brain function.

CBD on the other hand is the non-psychoactive component and acts as an antagonist of the CB1 receptor. It plays the role of a negative allosteric modulator as it inhibits the

effects of THC and is presumed to have anxiolytic, anti-inflammatory and antipsychotic properties.[6,7]

1.5 Legal Status of Cannabis

Cannabis is listed as a Schedule I drug according to the Controlled Substances Act of 1970.[10] This makes the use of cannabis illegal on the federal level. Certain states, however, have enacted laws to legalize the use of cannabis products for both recreational and medical use. It should be noted that even though certain states may have laws stating that cannabis is legal, individuals can still be charged at the federal level with possession. This is combatted through certain states' medical laws which provide protection against arrest for possession of cannabis. Due to the proposed therapeutic value that cannabis presents, numerous efforts have been made to legalize its use on a federal level. In a New York Times report, it stated that in December 2020, the House of Representatives passed a landmark bill decriminalizing marijuana and making it legal on the federal level[27]. However, the passage of this bill was entirely symbolic as there was no chance it would be taken up by the Senate. Still, its passage signifies an evolving attitude towards marijuana. According to another news article written by Dezenski (2020) on the CNN website, five different states, including New Jersey voted for the legalization of recreational marijuana in the recent ballots. Another measure was also passed recently in Oregon known as the Drug Decriminalization and Addiction Treatment Initiative or Measure 110. Measure 110 decriminalizes the personal possession of small amounts of illicit drugs and reduces the penalty for possessing larger amounts[28]. With these new measures in place, usage rates may increase; therefore, it is very important to be able to have reliable and accurate testing for cannabis products.

1.6 CBD

Of the cannabis products being used in the majority of states, CBD products have become the most popular. According to a report written by Grand View Research, the CBD market is reportedly worth over 4.6 billion dollars and is expected to grow in the next 5 years[10]. Currently, there are a lot of CBD products being sold online, in convenience stores, dispensaries and even on the black market. The type of products that are seen include: E-cigarettes (vape pens), edibles (gummies, coffee, brownies etc), CBD shampoo, CBD skin creams and CBD/Hemp oils which are the most popular and the main focus of this research. The reported uses of CBD products are for pain relief medication, sleep improvement, perseverance and maintenance of brain health as well as for recreational activities.

To produce CBD/Hemp oil, the cannabis plant undergoes numerous methods of extraction. The two most popular methods are carbon dioxide (CO_2) extraction and solvent extraction.[4,10] CO_2 extraction involves using pressurized CO_2 in a chamber to extract cannabinoids from the cannabis plant turning the gas into a liquid which houses the extracts. Solvent extraction on the other hand involves heating the cannabis plant material in an organic solvent to extract the cannabinoids from the plant. The solvent is evaporated leaving the extracts behind. Numerous blogs and articles have stated that CO_2 extraction is the most recommended method as it yields more CBD than solvent extraction and it also has a higher purity factor reducing the residual contaminants left over[29,30]. However, this method is very expensive, and most companies cannot afford to do this type of extraction; therefore, they resort to solvent extraction.[1]

Figure 6: Extraction methods of CBD oil from the cannabis plant[10].

1.7 CBD Hemp Oil

Hemp is defined by the CBD Awareness Project as any cannabis crop that contains 0.3% THC or less in dry weight.[10] This is the main source of CBD oil. In 2018 the U.S Farm Bill legalized production and sale of hemp products, which are now currently being used in the medicinal and wellness market.[10] The FDA has also approved the only CBD medication (i.e. EPIDIOLEX <0.1% THC) for legal use. Based on the Farm Bill, any CBD/Hemp product that contains more than 0.3% THC can be deemed illegal on the federal level. This exposes an underlying problem with CBD/Hemp products: even though they have been legalized, there is still uncertainty regarding the concentration of THC in the majority of CBD products. This is due to the products having little to no regulation. Hemp oil is sold commercially as an herbal supplement at dispensaries, drug stores and even online. Depending where individuals purchase their hemp oils, it could result in repercussions for the user if illegal concentrations of THC are present. According to an article published by Johns Hopkins Medicine, six participants were given cannabis products to vape and it was reported that two out of the six participants tested positive for THC. After testing, the concentration of THC in urine was reported to be 0.39%.[12] This is a huge problem as this was just based on a single use of the product.

1.8 Significance of Research/Gap Analysis

Legal CBD/hemp products are used very often and for some individuals more than once a day, especially for medical reasons. This may lead to subsequent accumulation of THC in the body, as stated by the literature resulting in more THC-positive tests. A study done by Vandrey and his collaborators at the University of Pennsylvania in the Journal of American Medical Association (JAMA) found that roughly 21% of CBD/Hemp products sold on the internet contained THC, which was not listed on the labels.[12] This is a major concern and could affect the lives of many individuals taking these products without prior knowledge of the contents. Therefore, it is necessary to establish a reliable, reproducible method for the detection of THC in CBD products easily accessible for purchase.

Consequences of testing positive for THC include: loss of employment and future job opportunities, violation of parole and arrests at the federal level, especially if it was not prescribed. Many studies have been done on the concentrations of THC in the body after taking CBD products; however, not much research has been done on studying accurate methods to detect THC and also the concentration of THC in the products themselves before consumption, especially those sold online which do not include THC on their labels. Due to the lack of regulation it is likely that the THC concentration may vary across products being sold online.

2. Aims/Objectives

The aim of this research was to validate a pre-existing method to extract and detect delta 9-THC from CBD/Hemp Oil products. CBD/Hemp oil products available on the retail market, especially online that do not have delta 9-THC listed on the label or the label says 0% THC was the focus of this study. To achieve this six of the most popular CBD/Hemp oil products were purchased from six different manufacturers on Amazon.com. The method validation was done in duplicates for each blank oil sample to determine if the results were precise and reproducible. This validation was done using the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines in forensic science. SWGTOX was an organization that developed and disseminated standards to the forensic community to ensure proper protocols are observed[26]. The group disbanded in 2014, however its practices were transferred to the Organization of Scientific Area Committees (OSAC) and are still followed by many labs in order to maintain uniformity. Validation for this study entails limit of detection, limit of quantitation and linearity. If time allowed, then an inter and intra assay precision would also have been done. Analysis of six test oils was the next step to determine the presence of THC. If THC was detected in the oil samples and time allowed, quantitation would have been done to determine the concentration of THC in the oils and if they were above the legal threshold of 0.3%.

3. Materials and Methods

3.1 Materials and Instrumentation

Stock standard solutions of CBD (1mg/ml) and THC (1mg/ml), as well as internal standard solutions of CBD-d3 (100 µg/ml) and THC-d3 (100 µg/ml) were purchased from Certilliant. Methanol was used as the organic solvent and according to Jang E. et al (2020), it was recognized to be the best solvent to generate results for CBD and THC. To conduct method validation studies three different blank oils were purchased from Wegmans. These blank oils were almond oil, olive oil and coconut oil. Six different test hemp oils were purchased from different online sellers on Amazon. They were Nature's Beneficials hemp oil, Enhanced hemp oil, Newage Premium hemp oil, Healpark hemp oil, Cannamong premium hemp oil and Wonder Earth hemp oil. Each hemp oil purchased had the same reported concentration and they were selected because each of them had "0% THC" or "No THC" written on their labels. Validation studies using the blank oils were done first and then afterwards the same method was applied to the unknown hemp oil samples. The instrument used to conduct this experiment was the gas chromatograph (Agilent 6890N)/ mass spectrometer (Agilent 5973) using the Mass Hunter Software. This instrument was chosen as it works well with volatile analytes and it has high sensitivity in separating and identifying compounds.

3.2 Sample Preparation

The stock standards of CBD and THC were combined to make CBD/THC working solutions at 10 µg/ml and 1 µg/ml respectively. This was done by pipetting 1ml CBD and THC from their stock ampules into a volumetric flask and diluting it with methanol up to the mark on the flask. The internal standard solutions were also used to make a working solution of 10 µg/ml. The blank oil and test oil samples were extracted using a liquid-liquid extraction technique. For the validation study, aliquots (200 µl) of each blank oil were pipetted into different test tubes. Each tube was then spiked with the THC/CBD working solutions at decreasing concentrations. 50 µl of 10 µg/ml of the internal standard working solution was also added to each tube. After the samples for the validation study were prepared and run, the test hemp oil samples were prepared. Two hundred microliters (200 µl) of each test hemp oil sample was also pipetted into different test tubes and spiked with the same volume of internal standard as the blank oils. Through the extraction process the samples were then suspended in 3ml of methanol and then vortexed for approximately 1 hour. After the samples were vortexed, they were centrifuged at 2500 rpm for 10 minutes. The organic phase (top layer) of each sample was collected and transferred into a new test tube. The samples were then evaporated to dryness and then reconstituted with 200 ul of methanol. The extracts were transferred in GC/MS injection vials and 2 µl of the extract was injected into the GC/MS for detection. The same sample extraction procedure used by Jang E. et al (2020) was followed. An alteration was made to the procedure allowing the samples to evaporate to dryness instead of drying under nitrogen. This was due to budget constraints and not being able to get the nitrogen tank in time.

3.3 Method Validation

The method used in this experiment was an already established method, which was also developed and used by Jang. E et al (2020). The authors did many test trials using different conditions to optimize instrumental parameters, while also using past studies. These parameters were used in this experiment and were followed completely. A limit of detection (LOD) study was done using the SWGTOX validation guidelines to ensure that the method is reliable with the instrument and samples used in this experiment. A limit of detection (LOD) study was conducted using 3 different blank oils in duplicates and ran over a period of 3 consecutive days. The limit of detection is defined as the lowest concentration that an analyte can be reliably detected. This means that the instrument should yield a reproducible response greater than or equal to three times the noise level of the background signal from the negative samples and achieve acceptable predefined detection and identification criteria[26].

The calibrators were made at concentrations of 5 µg/ml, 3.5 µg/ml, 1.5 µg/ml, 0.5µg/ml and 0.1µg/ml respectively. These concentrations were chosen during method optimization, where the spiked oils provided detectable results at this range. A positive and negative control were also made for quality control measures and to ensure that the experiment was working properly and that no cross contamination occurred. The concentration of the positive control was 1.5 µg/ml. Olive, almond and coconut oil were used as sample oils and they were each made at decreasing concentrations from 5 µg/ml, 3.5 µg/ml, 1.5 µg/ml, 0.5 µg/ml and 0.1 µg/ml respectively in 30 different test tubes. The blanks oils followed the same extraction procedure mentioned and they were run over

three days. After the runs the retention times of the peaks identified were labeled for each sample. The peak areas were also calculated for each sample and used in the LOD study to help determine precision.

3.4 Hemp Oil Analysis

After the LOD and precision studies were completed, the method was then applied to the hemp oil samples to determine if THC was present. The test hemp oils followed the same extraction procedure mentioned above, however the CBD/THC standard working solutions were not spiked into the tubes. 50 µl of 10 µg/ml internal standard working solution were spiked into each test tube. After the tubes were run on the GC/MS, the results were analyzed to determine the presence of THC.

3.5 GC/MS Conditions

The column used was a 5% phenyl methyl siloxane capillary (HP-5MS, 30m x 250 um x 0.25 um). During the run the oven temperature for the GC was maintained at 80 degrees for 1 minute, increased to 240 degrees at 20 degrees/min, increased to 260 degrees at 5 degrees/min and then increased to 300 degrees at 20 degrees/min and maintained for 10 minutes. The total run time for each sample was 25 minutes. The carrier gas was helium and it was at a constant flow rate of 1ml/min at 9.4 psi. The injection volume for each sample was set to 2µl and the analysis was performed in selected ion monitoring (SIM) mode. The ions that were monitored were at m/z 299 and 314 for THC, m/z 231 and 246 for CBD, m/z 302 and 317 for THC-d3 and m/z 234 and 249 for CBD-d3.

Injection volume	2 µl
Injection Port Temperature	250 °C
Column	HP-5MS L = 30 m ID = 0.25 mm DF = 0.25 µm
Pressure	9.4 psi
Gas Flow	Helium: 1.0 ml/min
Temperature Programming	Start: 80 °C, hold 1 minute Ramp: 20 °C at 240 °C, Ramp: 5 °C at 260 °C, Ramp: 20 °C at 300 °C, Hold for 10 minutes Total Run Time: 25 mins
Transfer Line Temperature	250 °C
Mass Spectrometer	Selected ion monitoring (SIM) mode Electron impact (EI) ionization
Ions Monitored	THC: 299 and 314 m/z CBD: 231 and 246 m/z THC-d3: 302 and 317 m/z CBD-d3: 234 and 249 m/z

Table 1: GC/MS parameters used to detect presence of THC

4. Results

After the GC/MS runs were completed, the peak areas of the THC and CBD peaks from the calibrators were calculated at each concentration and used to plot calibration curves for each analyte, which can be seen in the figures below.

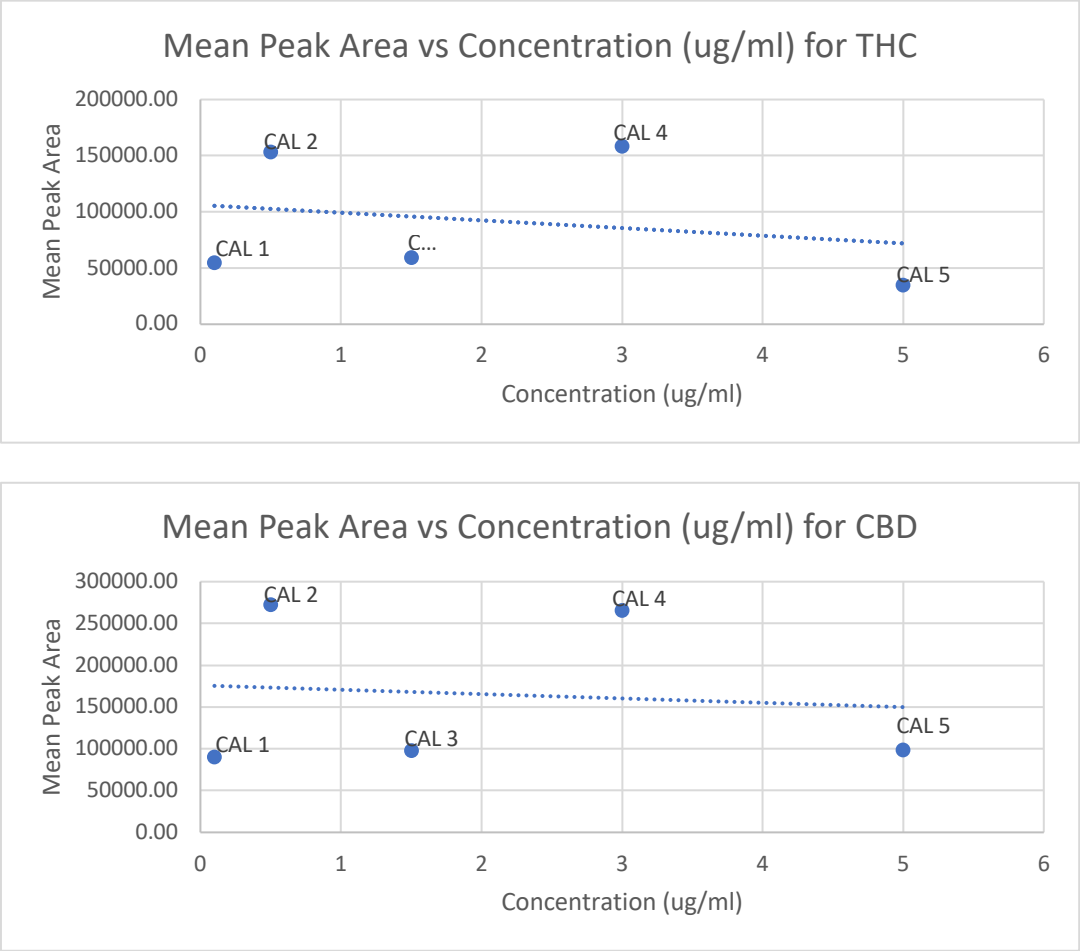


Figure 7: Calibration Curves of THC and CBD

Chromatograms of the three blank oils were also analyzed at each concentration. CBD and THC peaks were identified at 12.856 and 13.654 minutes respectively. Internal standard peaks were not clearly visible and therefore could not be identified.

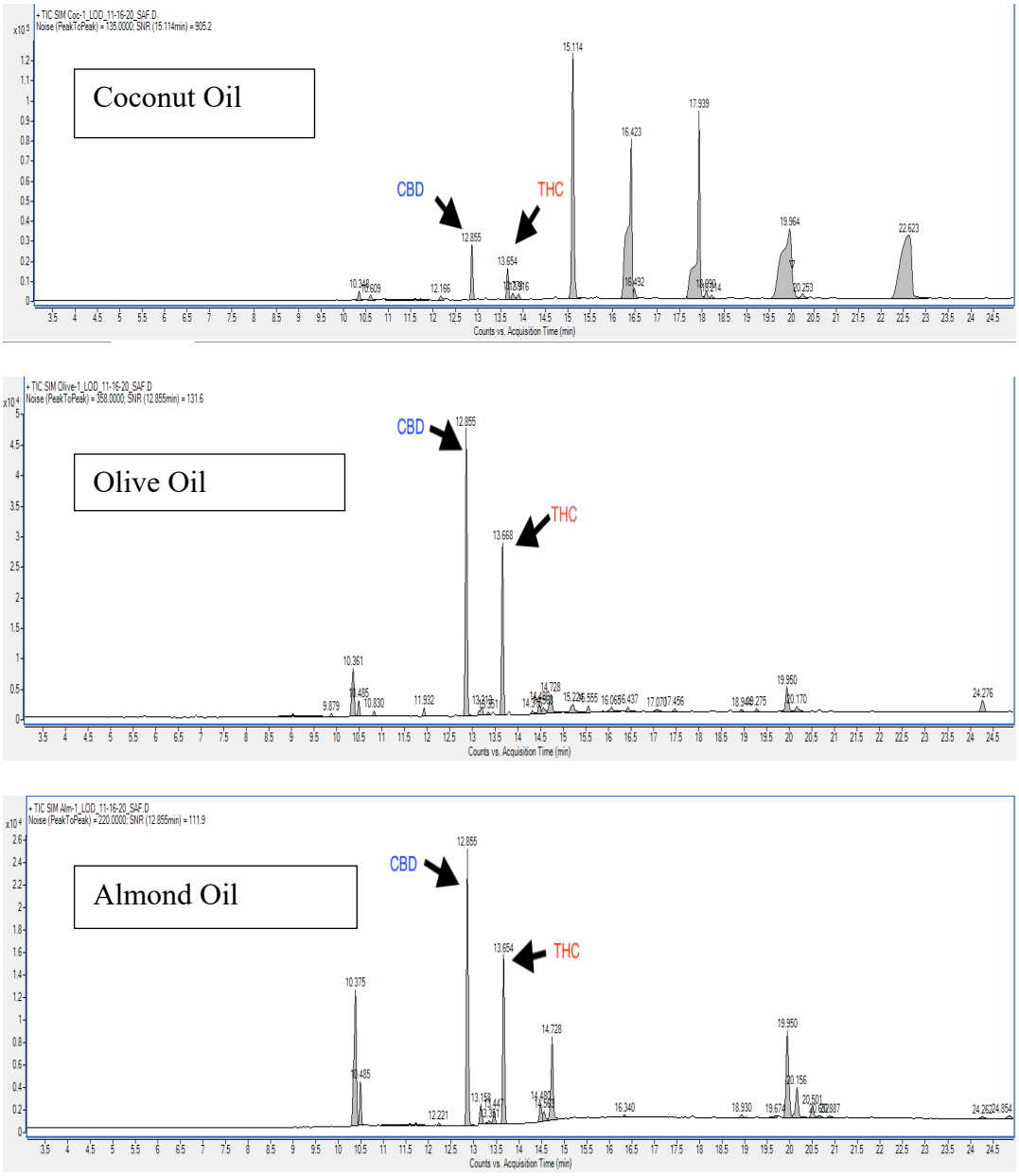


Figure 8: Chromatograms of Coconut oil, Olive oil and Almond oil illustrating CBD and THC peaks at 5ug/ml

The peak areas were gathered and used to calculate the precision of each type of oil at their respective concentrations. Precision refers to the degree of reproducibility or agreement

between repeated measurements. It is often expressed as the coefficient of variation (%CV). The mean and standard deviation of the peak areas for each sample at each concentration was calculated. The formula used to find %CV was:

CV(%) = (Standard Deviation/Mean) x 100

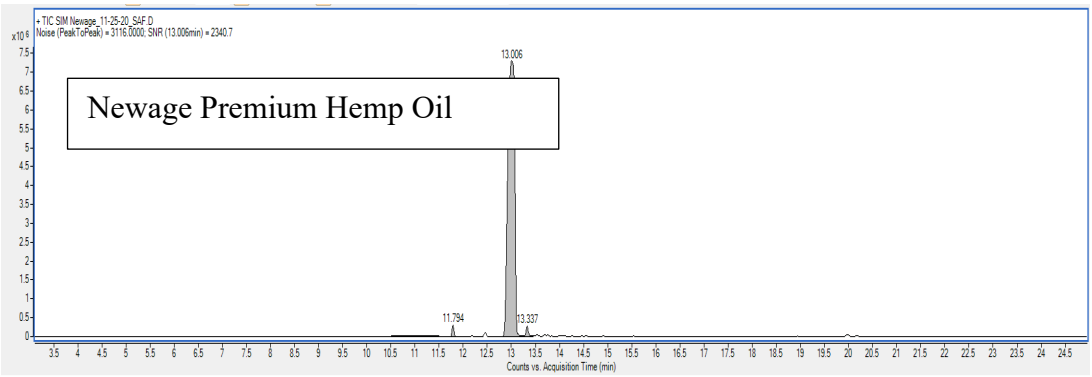
For readings to be considered precise the %CV must not exceed 20%. It can be seen in the table below some samples were above 20% (highlighted yellow), while the rest fell within the range.

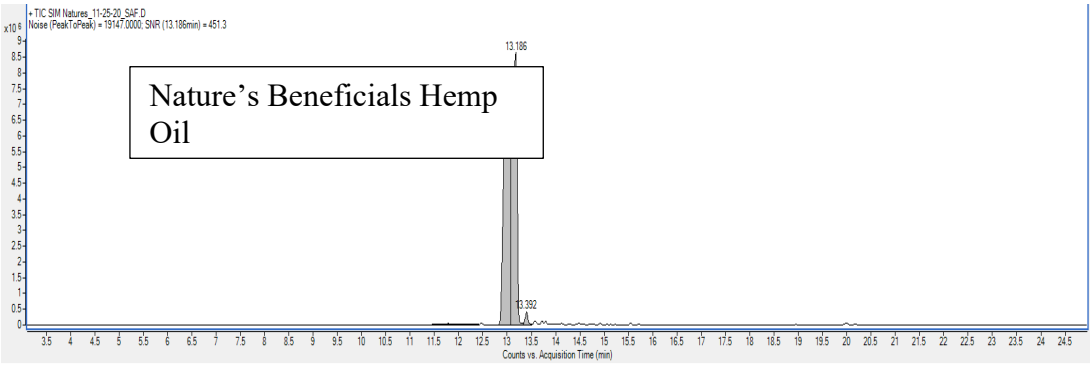
Precision Study of delta 9-THC										
Samples	Concentration (ug/ml)	Peak Area (Run 1)	Peak Area (Duplicate Run 1)	Peak Area (Run 2)	Peak Area (Duplicate Run 2)	Peak Area (Run 3)	Peak Area (Duplicate Run 3)	Mean	Standard Deviation (s)	%CV
Almond Oil	5	44562.79	46515.81	44734.64	44734.64	44058.21	48559.47	45527.59	1703.90	3.74
Almond Oil	3.5	37093.08	47749.12	39675.48	39675.48	38563.04	51881.97	42439.70	5937.05	13.99
Almond Oil	1.5	40638.4	29957.83	41924.25	41924.25	43821.96	33618.41	38647.52	5532.00	14.31
Almond Oil	0.5	40523.08	40697.54	45098.02	45098.02	44105.22	50244.17	44294.34	3574.56	8.07
Almond Oil	0.1	41724.33	36249.76	46345.73	46345.73	52310.82	39712.89	43781.54	5716.86	13.06
Olive Oil	5	80349.95	94961.35	100443.66	115489.32	155804.4	157764.26	117468.82	32470.02	27.64
Olive Oil	3.5	51112.03	59510.76	546496.57	74945.05	73704.62	100736.53	151084.26	194448.31	128.70
Olive Oil	1.5	53814.4	69503.86	59698.87	86640.03	66121.9	103474.78	73208.97	18542.06	25.33
Olive Oil	0.5	57501.02	72170.3	72185.66	79522.62	95335.31	87675.78	77398.45	13283.68	17.16
Olive Oil	0.1	48493.54	69542.73	59002.83	80075.87	75979.52	77830.7	68487.53	12397.96	18.10
Coconut Oil	5	47109.54	43179.57	49912.27	43883.81	48890.15	44174.07	46191.57	2843.83	6.16
Coconut Oil	3.5	34929.25	41827.25	39029.92	45744.82	41207.9	48271.61	41835.13	4746.95	11.35
Coconut Oil	1.5	40649.12	41515.24	46968.14	43966.88	39534.87	43568.31	42700.43	2690.87	6.30
Coconut Oil	0.5	37822.13	35618.36	45281.74	42235.38	39988.48	42506.32	40575.40	3496.59	8.62
Coconut Oil	0.1	29920.42	35341.38	33729.25	35258.39	38272.79	51031.44	37258.95	7276.01	19.53

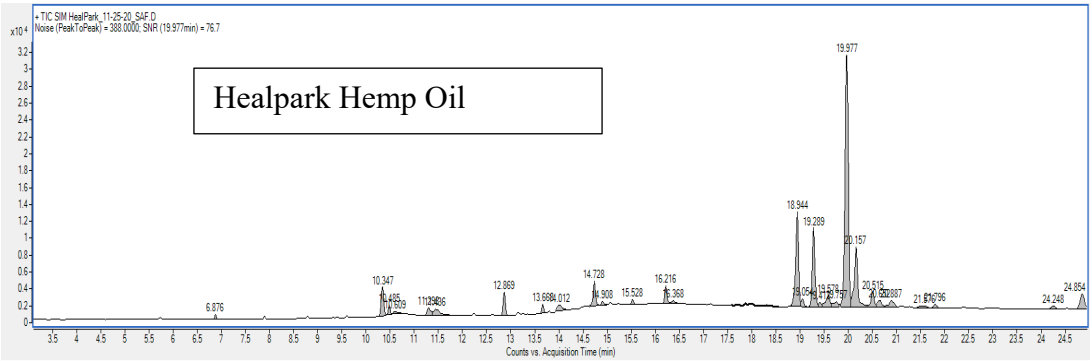
Precision Study of CBD										
Samples	Concentration (ug/ml)	Peak Area (Run 1)	Peak Area (Duplicate Run 1)	Peak Area (Run 2)	Peak Area (Duplicate Run 2)	Peak Area (Run 3)	Peak Area (Duplicate Run 3)	Mean	Standard Deviation (s)	%CV
Almond Oil	5	69967.85	71038.23	72594.85	72954.85	73603.5	81776.2	73655.91	4195.55	5.70
Almond Oil	3.5	59739.79	79192.97	64278.15	64278.15	63961.55	89104.39	70092.50	11459.94	16.35
Almond Oil	1.5	69504.1	50611.84	72360.07	72360.07	77317.73	58533.1	66781.15	10101.08	15.13
Almond Oil	0.5	68588.3	690473.23	75766.43	75766.43	79365.03	88808.25	179794.61	250266.99	139.20
Almond Oil	0.1	71756.78	62704.4	83511.19	83511.19	94782.77	72977.38	78207.29	11316.45	14.47
Olive Oil	5	131966.04	150931.26	174026.34	195612.16	280594.92	281967.28	202516.33	64662.86	31.93
Olive Oil	3.5	82721.81	94403.89	90767.14	126470.8	93137.92	177682.59	110864.03	36025.86	32.50
Olive Oil	1.5	88167.19	112927.98	102257.37	150270.36	114667.57	185796.03	125681.08	35939.79	28.60
Olive Oil	0.5	92099.99	117435.75	122935.86	136410.67	164287.07	153413.28	131097.10	26083.84	19.90
Olive Oil	0.1	77083.5	114642.43	101811.95	139502.3	131933.25	137634.06	117101.25	24432.03	20.86
Coconut Oil	5	82599.71	72385.07	86638.32	79051.34	85892.33	79791.66	81059.74	5248.57	6.47
Coconut Oil	3.5	62024.78	76878.54	68640.05	81384.34	83641.14	84457.99	76171.14	9044.20	11.87
Coconut Oil	1.5	75298.16	79887.43	83388.85	85367.52	81570.27	89383.25	82482.58	4815.53	5.84
Coconut Oil	0.5	72761.43	72874.25	81081.09	80159.64	76397.18	85378.56	78108.69	4996.29	6.40
Coconut Oil	0.1	55694.05	65847.03	63093.72	72418.15	78294	98846.92	72365.65	15118.26	20.89

Table 2: Peak Areas of THC and CBD at different concentrations used to calculate %CV for precision

After the LOD study was completed, the six different hemp oil samples were run and analyzed. No CBD or THC peaks were identified in any of the oils using this method. Chromatograms of each hemp oil can be seen in the figures below.







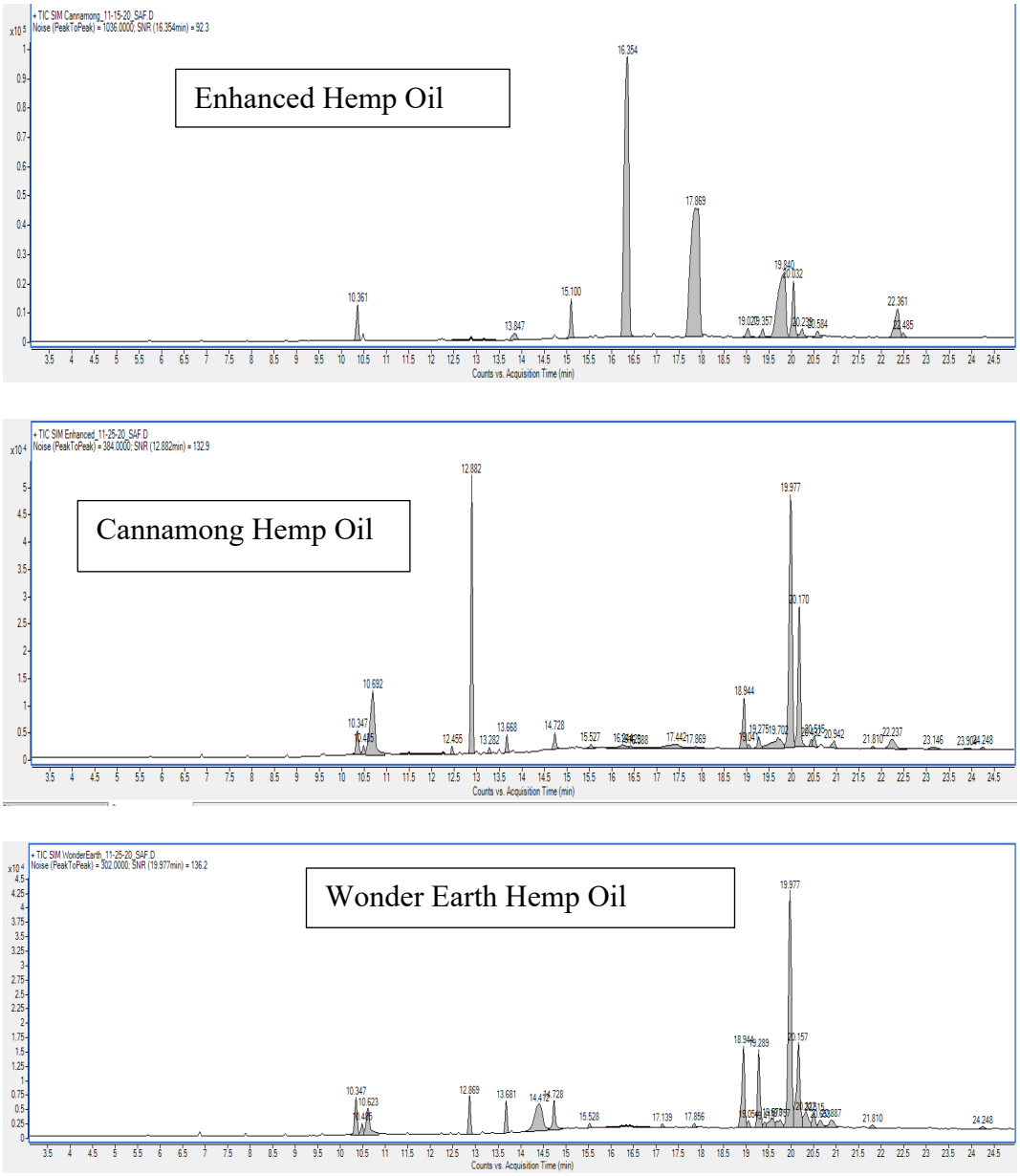


Figure 8: Chromatograms of 6 different hemp oils

5. Discussion

As mentioned before in the results, CBD and THC peaks were identified in each blank oil sample. The retention time for CBD was identified to be 12.856 minutes, while the retention time for THC was 13.654 minutes for the calibrators. The mean of the peak areas of both CBD and THC were calculated at each concentration over the three runs. This was used to plot a calibration curve for both CBD and THC (Figure 7). As CBD and THC concentration increases, the peak areas of each peak should also increase. However, from the results of the calibration curve there are fluctuations in the data points which affected the linearity of the curve. This might be as a result of matrix affects. Since the matrix in this experiment was oil, the viscosity of the oils made it difficult for the analytes to be extracted. It could also be a result of the GC/MS column being overloaded with sample. Cannabinoids have a tendency to stick on the columns or injection ports[20]. This may result in rising baselines or carryover into the other samples, which may explain why there were fluctuations in the peak areas. This proved to be a major issue for the internal standards as the internal standard peaks were not clearly visible and therefore could not be identified. This has an impact on the experiment, as the internal standard is used as a tool to ensure that the experiment is working properly. It is chemically similar to the analyte, however, has a different retention time and is used in quantitation studies to find the concentration of the analyte. Since the focus of the study was on detection of THC and not quantitation, the issues were minimal. In actual case work this would be a major issue as the concentration is normally required to determine whether or not THC is at the legal level.

During the LOD study using the three blank oils, it was also noticed that the viscosity of the oils proved to make the matrix very complex, hence not a lot of the analyte was extracted into the organic phase; therefore, resulting in peak are fluctuations for both CBD and THC. THC and CBD peaks were displayed on the chromatograms (Figure 8), however the internal standards that were spiked did not display any visible peaks on the chromatograms just like the calibrators. This is as a result of the same issues discussed regarding the calibrators. THC and CBD peaks were observed at the same retention times as that of the calibrators and the peak areas were also calculated for each. At each concentration for each blank oil THC and CBD peaks could be identified clearly and they had relatively high peak values. The peaks identified in coconut oil; however, have lower peak heights than the olive and almond oil. This may be a result of coconut oil being more viscous than the other two oils, therefore not as much analyte was extracted. The lowest concentration analyzed was 0.1 µg/ml for each oil. THC and CBD peaks were still visible at this concentration however, due to time constraints the samples were not analyzed at lower concentrations to determine a more accurate LOD in each oil. Even though an accurate LOD could not be established, the experiment was allowed to continue the since the reported concentration of hemp in the oils were 1.11 mg/ml which is higher than the concentrations analyzed for the LOD. The peak areas were used to calculate the %CV at each concentration for each blank oil sample. The majority of the samples had a %CV below 20%, which indicates that the method had decent precision even though here were fluctuations in the peak values. The samples highlighted in yellow had a %CV that exceeded 20% and were primarily from olive oil. This could be a result of the olive oil being more viscous than the other blank oils, which affected the extraction

of the analyte even more creating imprecise values. Carryover from the other runs could also explain these imprecise values. This can be corrected by increasing the temperature of the oven to burn the excess cannabinoids off the column so that it does not affect the other runs.

This means that if a very viscous sample is encountered during casework, then inaccurate readings might be reported which will affect not only the case, but the validity of the method used.

After the LOD and precision studies were completed, analysis of the test hemp oil samples were done. On the chromatograms in Figure 9 there were no CBD or THC peaks identified in the oils using this method. The peaks that were present consisted of the other components present in the oil, such as fatty acid residues and other flavoring components in the oils. As mentioned before, hemp oil is produced from the extraction of the cannabis plant and the plant consists of numerous components other from cannabinoids which explains why these other peaks were present. Newage Premium Oil and Natures Beneficial oil did not produce good chromatograms compared to the other oils because these two oils were more viscous, which made it more difficult to extract the analytes. CBD and THC could still be present in these samples, however due to the underlying factors that affected the extraction and method, it was not identified. Although the analyte of interest was not identified in these particular samples, there is a possibility of detecting THC if the extraction procedure is optimized. Also, this method has been used to identify all the analytes of interest in another study done by Jang E. et al (2020). The analysis of those samples along with the positive and negative controls were able to detect THC and

demonstrating the validity of this method. The method was decent for the narrow scope of this study, however based on the study this method would never be valid for casework samples.

6. Future Work

Future work using the results of this study can answer many questions that were raised during this project. First, a more detailed method validation could be done to really determine how reliable this method is. This study would include: calibration models, inter and intra assay precision, limit of quantitation, linearity carryover and interference studies. As stated before, only an LOD study was done along with calculating the %CV to find precision. As it relates to the LOD, a more comprehensive study can be done by analyzing lower concentrations to accurately determine the LOD for each analyte in different blank oils. In the experiment the LOD was cut off at 0.1 ug/ml, however lower concentrations could be analyzed to further determine a more accurate LOD value for the blank oils.

As it relates to the extraction procedure, more studies can be conducted on how to maximize the extraction of the analyte of interest. Due to the viscosity of the oil matrix, there were many complications in the results; therefore, different ways to enhance the extraction procedure so that the analyte can be efficiently extracted out of solution should be examined. Dilution of samples could be done so that the column is not overloaded and to also lessen the viscosity of the oil so that the analytes can be extracted efficiently and also not stick to the column or injection port. In a full validation, carryover studies would be done to analyze this issue. In order to remedy rising baselines and carryover it would be beneficial to inject a silyating agent vial between samples. The silyating agent derivatizes any components that may get stuck on the column. Raising the oven temperature can also aid in burning the component off the column. This may help to

explain or correct the fluctuation in peak areas. Different instrumentation can also be used to get better results. In another study done by Meng, Q. et al (2018) Liquid Chromatography- Tandem Mass Spectrometry (LC/MS-MS) was used. The extraction procedure used in this experiment involved acidifying the cannabinoids in order to extract them out of the organic solvent more efficiently. This could be beneficial as different instrumentation require different extraction procedures and this can help determine a more reliable and accurate method of examining the oils. Other articles where LC-MS/MS was used also illustrate different extraction procedures, which proved to be very effective in identifying different cannabinoids[31,32].

Other brands of CBD oils could also be examined at different concentrations for the presence of THC, as well as different CBD products that are currently on the market. CBD products are continuously evolving and different methods to analyze them need to be developed.

7. Conclusion

The instrumental method that was used for this study could not adequately be validated to determine reliability, however based on the results it was considered unreliable due to extraction inefficiencies. The weaknesses of this study were primarily due to the extraction procedures. The analyte could not be efficiently extracted due to the oil being too viscous and needing to be diluted more before being injected into the GC/MS. The method was still able to detect the THC and CBD peaks from the LOD study, which means that the instrument conditions were suitable for detection. Based on previous studies done by Jang E et al (2020), it was shown to be a reliable method to detect CBD and THC and to also quantify it. This method has the potential to be used in forensic labs to determine the presence of THC in complex oil samples. Further studies need to be carried out in order to enhance this method so that it can be more reliable. With this current method it would prove difficult to analyze oils, especially oils that may have little to no THC in them. Since some of the difficulty is primarily due to the extraction procedure, different alterations such as acidifying the sample and even adding a derivatization step can improve the extraction and identification of the analyte. These alterations were used in a previous study done by Meng, Q et al (2018). While this method was unsuccessful in providing adequate information as it relates to the validation of analytes of interest, there are many opportunities to improve this procedure to solve the problems encountered in this study**.**

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