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Synthetic Route Sourcing of Illicit at Home Cannabidiol (CBD) Isomerization to Psychoactive Cannabinoids Using Ion mobility-coupled-LC-MS/MS

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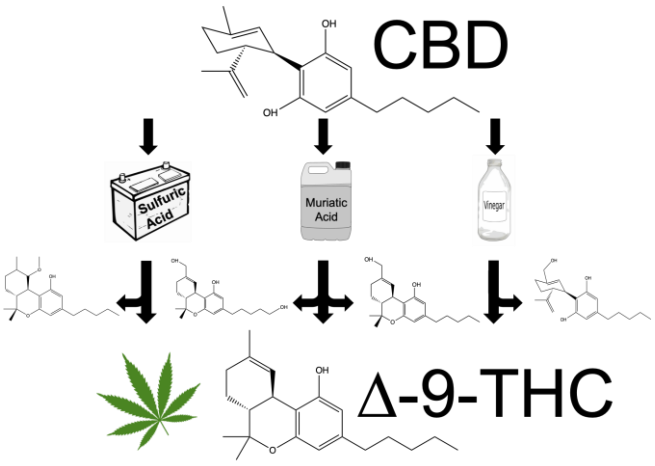
Graphical abstract



Highlights

- Rapid Isomerization of CBD to Δ-9-THC using chemicals that the general population can purchase
- Chemical route sourcing of cannabinoid isomerization reactions to monitor the synthetic route
- Isolation and analysis of cannabinoids using LC-MS and IM-MS

ABSTRACT
This study focuses on the chemical route sourcing of illicitly produced Δ9-Tetrahydrocannabinol (Δ9-THC) via the acid-catalyzed cannabidiol isomerization reaction. Each of the acid-catalyzed reactions used acids that are readily available for the general population such as battery acid, muriatic acid, and vinegar. After the acid-catalyzed isomerization was complete, an analysis using Liquid Chromatography-coupled-Mass Spectrometry (LC-MS)-coupled-ion mobility to confirm all synthetic impurities in the sample was conducted. The conducted chemical route sourcing allows law enforcement to be able to determine how CBD was converted to psychoactive cannabinoids. Specifically, 10-methoxy-THC, 11-hydroxy-THC, 11,5''-dihydroxy-Δ9-THC, and 5''-hydroxy-CBD were able to be used as indicators in the determination of the chemical route sourcing. Additionally, the ion mobility allowed for a rapid secondary separation of the psychoactive cannabinoids without the need for the long LC/MS analysis time.

KEYWORDS: Cannabidiol, Psychoactive Cannabinoids, Synthetic Route, Liquid Chromatography, Ion Mobility

Introduction

Marijuana legalization has created many judicial issues, raising concerns of safety for civilians as many companies are now extracting and selling Δ9-Tetrahydrocannabinol (Δ9-THC) as an illegal concentrate. Furthermore, daily cannabinoid users have increased from 9.8% of the population of the United States in 2007 to 13.39% in 2014, and now to 15.3% in 2017, according to the National Survey on Drug Use and Health[1, 2]. These users do not only smoke the leaf anymore, as more than 18% of cannabis users are now inserting these concentrated Δ9-THC products into their electronic cigarettes

and smoking the hash oil or wax[3, 4]. These oil extracts can be more than 80% Δ9-THC; however other cannabinoids, such as hexahydrocannabinols (HHCs), which have similar psychoactive properties, are being delivered in the remaining 20% of the sample[5, 6]. One study has found that out of 84 Cannabidiol (CBD) products, only 26 accurately labeled all of the cannabinoids in their product as other impurities existed[7]. As marijuana becomes legalized in more states, proper identification of all the cannabinoids in the sample is required to determine how the companies are extracting or converting the Δ9-THC, as impurities can be present in the samples after extraction or conversion[8, 9].

Converting CBD to Δ9-THC has been studied since the 1940's when Adams et al. determined that acid would catalyze the reaction of converting CBD to other psychoactive cannabinoids[10, 11]. However, no structures of the psychoactive cannabinoids were able to be determined until 1965 when Gaoni and Mechoulam were able to identify that using hydrochloric acid or p-toluenesulfonic acid in methanol results in tetrahydrocannabinols being formed[12]. Gradually, more studies were completed on the degradation pathway of CBD and its subsequent cannabinoids. It is well known that CBD will convert to other known cannabinoids such as Δ8-Tetrahydrocannabinol (Δ8-THC), Δ9-THC, and Cannabinol (CBN) when adding acid to the CBD[13]. This conversion of CBD to Δ9-THC using hydrochloric acid prompted a large field of research to determine if CBD could be converted to Δ9-THC during the digestion process due to a large amount of hydrochloric acid in the stomach[14-19]. Other research groups have found that Δ9-THC does get formed in the stomach as well as other cannabinoid metabolites such as 6ß-hydroxymethyl-d-9-tetrahydrocannabinol, which forms via an epoxide reaction[20]. This metabolite, 6ß-hydroxymethyl-d-9-tetrahydrocannabinol, has similar psychoactive properties as Δ9-THC. Although some researchers still argue the absence of CBD to Δ9-THC conversion in the human body, Δ9-THC metabolites have been detected up to 3 hours later when Δ9-THC is dosed orally[21-23]. Regardless of the conversion in the human body, numerous metabolites other than Δ9-THC are found during this isomerization Subsequent methods for the isomerization of CBD to Δ8-THC and Δ9-THC consisted of using sulfuric acid, BF_3Et_2O, or boron fluoride in methanol[24, 25]. The methods that have been found so far use chemicals that anyone can buy at low concentrations, which leads researchers to believe that these illicit drug dealers can start producing their own Δ9-THC since CBD is now legal[6]. However, each reaction has different yields of products, ranging from 40% to 80% isomerization of CBD to Δ9-THC. The remaining products are route-specific and can be monitored to determine the synthetic route that was used.

Currently, researchers have used analytical techniques that require long analysis times such as Liquid Chromatography coupled-Mass Spectrometry (LC-MS) and Gas Chromatography-coupled-Mass Spectrometry (GC-MS) to analyze and confirm the presence of both known and unknown cannabinoids that either are either synthetic or natural [26-29]. However, researchers were able to find that compounds such as cannabicyclohexanol, JWH-018, hexahydroxycannabinoids (HHCs), etc., which do have similar psychoactive properties as Δ9-THC, will form under experimental conditions with the proper chemical pathway[30, 31]. Additionally, some of these studies were limited due to the use of Single Ion Monitoring (SIM)[32], which prevents other impurities from being seen during the analysis. Little research has been conducted to determine the presence of impurities throughout these reactions. Currently, when performing illicit drug chemical profiling and source investigation procedures to confirm the presence of illicit drugs, the analysis must show chemical identifiers, such as the drug itself, reagents of the synthetic route, or the impurities known for the reaction. [33, 34]. For many years, impurities have been used to confirm the detection of a drug, such as methamphetamine, as well as to distinguish between the different synthesis routes, such as the Leuckart route and reductive amination route, based on the reagents used and impurities present[35-39]. This helps local law enforcement in finding the source of the chemicals. Unfortunately, studies of the impurities in the isomerization of CBD to Δ9-THC are largely underdeveloped for illicit drug reactions because there are so many different commercially available acids to the public.

This study monitors the different route-specific impurities that are generated in the isomerization reaction of CBD to subsequent psychoactive cannabinoids, such as Δ9-THC, using chemicals that can be purchased by the general population such as ethanol, battery acid (37% sulfuric acid), muriatic acid (30% hydrochloric acid), and vinegar (5.4% acetic acid) (Figure 1). After the synthesis, the analysis was conducted using LC-MS-coupled-ion mobility to confirm and identify all synthetic impurities in the sample, which can be used for synthetic route sourcing. The ion mobility allowed for a second degree of separation to confirm the presence of unknowns in the samples based on their collisional cross-section. This technique was rapid, requiring only 2 minutes to analyze all of the psychoactive cannabinoids and allowed for the determination of cannabinoids with more than 1 $\square^2$ difference. Among the three different routes, the battery acid method, the muriatic acid method, and the vinegar method produced 9, 12, and 7 different cannabinoids, respectively. Each of these methods allowed for sourcing of the synthetic route to be accomplished.

Methods

Reference Cannabinoids

A reference solution comprised of 7 cannabinoids at 1 mg/mL concentration in acetonitrile were purchased that included: Cannabidiolic Acid (CBDA), Cannaibgerol (CBG), Cannabinol (CBN), Δ8-THC, Δ9-THC, CBD, Cannabichromene (CBC), and Tetrahydrocannabinolic Acid (THCA) (Absolute Standards Inc., Hamden, CT, USA). These reference standards were further diluted from 1 mg/mL to 10 µg/mL concentration for analysis.

Battery Acid

1 gram of CBD (LaCore Enterprises, Melissa, TX, USA) was added to 35 mL of 95% v/v ethanol (Sigma Aldrich, St. Louis, MO, USA) in a 50 mL Round Bottom Flask (RBF) and placed in a hot water bath at 70 °C. Once the CBD was dissolved, 4 drops of 35% sulfuric acid (Sigma Aldrich, St. Louis, MO, USA) were added to acidify the solution, pH < 3. The mixture was allowed to reflux for 24 hours in the water bath of 70 °C. Once complete, 5 drops of 10 M NaOH (Fisher Scientific, Hampton, NH, USA) was used to basify the solution until the pH was greater than 10, which removes the sulfuric acid as sodium sulfate. The reaction was then filtered to remove the sodium sulfate, which allowed for just the cannabinoids to be in the remaining ethanol solution. The solution was sampled after 0, 1, 2, 4, 5, 6, and 24 hours to monitor the isomerization of the CBD. Each sample consisted of extracting 1 μL of the reaction solution at the specified time interval and diluting with 999 μL of HPLC grade acetonitrile (Fisher Scientific, Hampton, NH, USA) to generate a 1 mg/mL cannabinoid sample. This sample was then diluted to 10 μg/mL in HPLC grade acetonitrile for analysis.

Muriatic Acid

1 gram of CBD was added to 35 mL of 95% v/v ethanol in a 50 mL RBF and placed in a hot water bath at 70 °C. Once the CBD was dissolved, 47.3 μL of 37% hydrochloric acid (Fisher Scientific, Hampton, NH, USA) were added to the solution making it a final concentration of 0.05% HCl v/v with a pH of less than 5. The mixture was allowed to reflux for 24 hours in the water bath of 70 °C. The mixture was sampled after 0, 1, 2, 4, 5, 6, and 24 hours to monitor the isomerization of the CBD. Each sample consisted of extracting 1 μL of the reaction solution at the specified time interval and diluting with 999 μL of HPLC grade acetonitrile (Fisher Scientific, Hampton, NH, USA) to generate a 1 mg/mL cannabinoid sample. This sample was then diluted to 10 μg/mL in HPLC grade acetonitrile for analysis.

Vinegar

1 gram of CBD was added to 35 mL of 95% v/v ethanol in a 50 mL RBF and placed in a hot water bath at 70 °C. Once the CBD was dissolved, 1.909 mL of 99% glacial acetic acid (Sigma Aldrich, St. Louis, MO, USA) were added to the solution making it a final concentration of 5.2% acetic acid. The mixture was allowed to reflux for 24 hours in the water bath of 70 °C. The mixture was sampled after 0,

1, 2, 4, 5, 6, and 24 hours to monitor the isomerization of the CBD. Each sample consisted of extracting 1 μL of the reaction solution at the specified time interval and diluting with 999 μL of HPLC grade acetonitrile (Fisher Scientific, Hampton, NH, USA) to generate a 1 mg/mL cannabinoid sample. This sample was then diluted to 10 μg/mL in HPLC grade acetonitrile for analysis.

LC/MS Analysis

A Waters Acquity UPLC (Waters Corporation, Milford, MA, USA) and Waters Synapt G2-Si mass spectrometer (Waters Corporation, Milford, MA, USA) were used to analyze the samples using the MassLynx software (Waters Corporation, Milford, MA, USA). The experiment used a binary solvent method consisting of milliQ water containing 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B). The initial flow of the binary pump was set to 23% solvent A and 77% solvent B at a flow rate of 0.500 mL/min. The method then changed to 5% solvent A with 95% solvent B at a linear gradient over a period of 4 minutes and held for 2 minutes. The column was then reconditioned back to the original condition over a period of 5 minutes at a linear gradient. A 20 μL sample of 10 μg/mL isomerized CBD was injected to an Agilent proshell 120, C18, 2.7 μm, 4.8x50 mm column (Agilent Technologies, Santa Clara, Ca, USA) set at 40 °C. Masses within the m/z 300 to 360 range were analyzed by the mass spectrometer. The source temperature and desolvation gas set point was set to 80 °C and 500 L/h, respectively, with a capillary voltage set at 3 kV.

Battery Acid LC/MS/MS

After the initial LC/MS was completed for the battery acid method of conversion of CBD to Δ9-THC, MS/MS was used to confirm the other cannabinoids synthesized. For the battery acid conversion, the trap collision energy was set to 20 V and the mass spectrometer method was set to fragment *m/z* 315 at 0 – 1.05 min, 1.05 – 2.0 min was set to fragment *m/z* 347, 2.10 – 2.60 min was set to fragment *m/z* 333, 2.61 – 2.90 min was set to fragment *m/z* 329, 2.91 – 3.40 min was set to fragment *m/z* 333, 3.41 – 3.60 min was set to fragment *m/z* 347, 3.61 – 4.00 min was set to fragment *m/z* 315, 4.01 – 4.30 min was set to fragment *m/z* 315, 4.31 – 5.00 min was set to fragment *m/z* 347, 5.10 – 5.50 min was set to fragment *m/z* 315, and 5.51 – 11.00 min was set to fragment *m/z* 333.

Muriatic Acid LC/MS/MS

After the initial LC/MS was completed for the muriatic acid method of conversion of CBD to Δ9-THC, MS/MS was used to confirm the other cannabinoids synthesized. The LC-MS/MS method was set to fragment *m/z* 347 for 0 – 2.08 min at 20 V, 2.09 – 2.30 min was set to fragment *m/z* 333 at 20 V , 2.31 – 2.71 min was set to fragment *m/z* 347 at 20 V, 2.71 – 2.90 min was set to fragment *m/z* 315 at 23 V, 2.91 – 3.10 min was set to fragment *m/z* 329 at 15 V, 3.15 – 3.55 min was set to fragment *m/z* 333 at 20 V, 3.60 – 3.90 min was set to fragment *m/z* 315 at 23 V, 3.91 – 4.15 min was set to fragment *m/z* 315 at 23 V, 4.16 – 4.50 min was set to fragment *m/z* 315 at 23 V, 4.60 – 5.10 min was set to fragment *m/z* 347 at 20 V, 5.20 – 5.50 min was set to fragment *m/z* 333 at 20 V, 5.55 – 7.00 min was set to fragment *m/z* 315 at 23 V, and 7.10 – 11.00 min was set to fragment *m/z* 333 at 22 V.

Vinegar LC/MS/MS

After the initial LC/MS analysis was completed for the vinegar method of conversion MS/MS was used to confirm the other cannabinoids synthesized. The LC-MS/MS method was set to fragment *m/z* 347 for 0 – 2.10 min at 22 V, 2.11 – 2.60 min was set to fragment *m/z* 333 at 23 V , 2.61 – 3.20 min was set to fragment *m/z* 315 at 15 V, 3.21 – 3.65 min was set to fragment *m/z* 329 at 22 V, 366 – 3.90 min was set to fragment *m/z* 315 at 22 V, 3.91 – 4.18 min was set to fragment *m/z* 315 at 22 V, 4.18 – 5.30 min was set to fragment *m/z* 315 at 22 V, and 5.31 – 11.00 min was set to fragment *m/z* 315 at 22 V.

Ion Mobility Analysis

Ion mobility was conducted using ESI-MS-coupled-ion mobility using the traveling wave function on the Waters Synapt G2-Si mass spectrometer (Waters Corporation, Milford, MA, USA). The samples were injected at 20 μL/min and the mass range was *m/z* 300 to 360. The data was collected and averaged over 30 seconds for each *m/z* compound, resulting in a 2-minute analysis. The source temperature and desolvation gas set point was set to 80 °C and 500 L/hr, respectively, and the capillary was set at 3 kV. The traveling wave voltage and wave height were varied across the compounds being 20, 21, 1500, and 25 m/s and 7.2, 10.2, 32.7, and 14.2 V for *m/z* 315, 317, 311, and 359, respectively, in the reference method. The traveling wave voltage and wave height for the battery acid method were 29, 1500, 25, and 25 m/s and 15.8, 23, 15.8, and 14.8 V for *m/z* 315, 329, 333, and 347, respectively. The traveling wave voltage and wave height for the muriatic acid method were 29, 28, 25, and 14 m/s and 14.8, 15.2, 21.7, and 13.4 V for *m/z* 315, 329, 333, and 347, respectively. The traveling wave voltage and wave height for the vinegar method were 29 m/s and 13.4V for *m/z* 315 and 1500 m/s and 23 V for *m/z* 329, 333, and 347. The data analysis was completed using the software DriftScope (Waters Corporation, Milford, MA, USA). Collisional Cross-sections were calculated with the open source software IMPACT from the University of Oxford to determine the collisional cross-section sizes of each of the cannabinoids in the experiment using their xyz coordinates (Table 1)[40].

Results

Reference Cannabinoids

Reference cannabinoids were analyzed using the LC-MS method above. Peak times of the following standards CBDA, CBG, CBD, CBN, Δ9-THC, Δ8-THC, CBC, THCA, and using this LC-MS were 2.595, 2.79, 2.80, 3.636, 4.11, 4.20, 4.80, and 4.814 minutes, respectively (Figure 2A). The ion mobility of the reference standards resulted in *m/z* 311 with a drift time of 56 bins for CBN (Figure 2B), *m/z* 315 with a drift time of 27 bins, 48 bins, and 69 bins indicating three compounds with different collisional cross-sections, which are the CBD and either Δ9-THC or Δ8-THC and the CBC (Figure 2C), *m/z* 317 with a drift time of 59 bins for CBG (Figure 2D), and *m/z* 359 with a drift time of 64 bins for THCA and CBDA as there is less than 1 $\square^2$ difference in the collisional cross-sections. (Figure 2E).

Battery Acid Conversion

The 0-hour sample extraction resulted in only CBD being found at 2.80 minutes, which agrees with the reference samples of 2.80. After only 1 hour, the CBD peak was significantly reduced and large peaks at 2.54 and 3.25 min were found to be 8-OH-iso-HHC, 9α -OH-HHC, respectively. Additionally, a large doublet at 3.97 and 4.13 min developed after 24 hours, which is the Δ9-THC and Δ8-THC peak found in the reference. At 3 hours, the CBD peak was no longer visible and smaller peaks began to increase, such as the 1.96 (11-5''-dihydroxy-CBD), 2.87 (11-hydroxy-CBD), 3.56 (10-methoxy-THC), and 4.70 (9-methoxy-THC) peaks (Figure 3A). The ion mobility of the battery acid method resulted in *m/z* 315 with a drift time of 53 bins for Δ9-THC/Δ8-THC and the unknown cannabinoid eluting at 5.39 min (Figure 3B). The *m/z* 329 had a drift time of 74 bins for 11-hydroxy-CBD (Figure 3C), while the *m/z* 333 had a drift time of 59 and 71 bins for both the 8-OH-iso-HHC and 9α -OH-HHC (Figure 3D). The *m/z* 347 had drift times of 60 and 72 bins indicating the presence of 10-methoxy-THC/9-methoxy-THC and 11-5''-dihydroxy-CBD (Figure 3E).

The MS/MS fragmentation was completed for each of the compounds in the sample (S.1, S.4). The peak at 1.96 min (*m/z* 347.244) fragmented to *m/z* 329.203 (loss of hydroxyl group), *m/z* 311.197 (loss of two hydroxyl groups), *m/z* 287.215 (loss of C_3H_6O), *m/z* 271.167 (loss of $C_3H_7O_2$), *m/z* 231.137 (loss of $C_6H_{12}O_2$) *m/z* 217.118 (loss of $C_7H_{14}O_2$), *m/z* 193.116 (loss of $C_9H_{14}O_2$), and *m/z* 107.069 (loss of $C_{15}H_{24}O_2$). This fragmentation confirms the product 11-5''-dihydroxy-CBD (S.1, S.4). The peak at 2.54 min (*m/z* 333.232) fragmented to *m/z* 315.230 (Loss of hydroxyl), *m/z* 259.160 (loss of $C_4H_{10}O$), *m/z* 193.116 (loss of $C_9H_{17}O$), *m/z* 135.104 (loss of $C_{13}H_{26}O$), and *m/z* 93.0541 ($C_{14}H_{24}O_3$). This fragmentation confirms the product 8-OH-*iso*-HHC (S.1, S.4). The peak at 2.87 min (*m/z* 329.211) fragmented to

m/z 311.204 (loss of hydroxyl), *m/z* 287.201 (loss of C_3H_7), *m/z* 271.160 (loss of C_3H_8O), *m/z* 231.130 (loss of $C_6H_{12}O$), *m/z* 193.116 (loss of $C_9H_{14}O$), and *m/z* 107.074 (loss of $C_{15}H_{26}O$), which confirms the presence of 11-hydroxy-CBD (S.1, S.4). The peak at 3.25 min (*m/z* 333.232) fragmented to *m/z* 315.230 (loss of hydroxyl), *m/z* 259.160 ($C_4H_{10}O$), *m/z* 193.116 (loss of $C_9H_{14}O$), *m/z* 135.104 (loss of $C_{13}H_{26}O$), and *m/z* 93.0541 (loss of $C_{14}H_{24}O_3$). This fragmentation confirms the product of 9α -OH-HHC (S.1, S.4). The peak at 3.56 min (*m/z* 333.232) fragmented to *m/z* 315.230 (loss of hydroxyl), *m/z* 259.167 (loss of $C_4H_{10}O$), *m/z* 193.116 (loss of $C_9H_{14}O$), *m/z* 135.104 (loss of $C_{13}H_{26}O$), *m/z* 93.0541 (loss of $C_{14}H_{24}O_3$), which confirms the presence of 10-methoxy-THC (S.1, S.4). The peak at 3.97 min (*m/z* 315.230) fragmented as Δ9-THC, which was *m/z* 259.167 (loss of C_4H_9), *m/z* 193.116 (loss of C_9H_{15}), *m/z* 135.099 (loss of $C_{13}H_{24}$), *m/z* 123.024 (loss of $C_{12}H_5O_2$), *m/z* 107.069 (loss of $C_{13}H_{20}O_2$), and *m/z* 93.0499 (loss of $C_{14}H_{22}O_2$) (S.1, S.4). The second peak of this doublet occurred at 4.13 min and is known to be Δ8-THC (*m/z* 315.230), which fragmented as expected to at *m/z* 259.160 (loss of C_4H_9), *m/z* 193.116 (loss of C_9H_{15}), *m/z* 135.099 (loss of $C_{13}H_{24}$), *m/z* 123.024 (loss of $C_{12}H_5O_2$), and *m/z* 93.0499 (loss of $C_{14}H_{22}O_2$ (S.1, S.4). While no significant changes occurred in the fragmentation pattern, Δ8-THC is known to elute after Δ9-THC. The peak at 4.70 min (*m/z* 347.260) fragmented to *m/z* 315.222 (loss of methoxy), *m/z* 259.160 (loss of $C_5H_{12}O$), *m/z* 193.104 (loss of $C_9H_{14}O_2$), *m/z* 135.104 (loss of $C_{14}H_{28}O$), and *m/z* 93.0499 (loss of $C_{15}H_{26}O_3$) (S.1, S.4). This fragmentation is known to be 9-methoxy-THC. The peak at 5.39 min (*m/z* 315.222) fragmented to *m/z* 259.153 (C_4H_9), *m/z* 193.104 (loss of C_9H_{15}), *m/z* 135.099 (loss of $C_{13}H_{24}$), *m/z* 123.024 (loss of $C_{12}H_5O_2$), *m/z* 93.0499 (loss of $C_{14}H_{22}O_2$). This compound is assumed to be another tetrahydrocannabinol as it has the same fragmentation pattern as Δ9-THC (S.1, S.4).

Muriatic Acid Conversion

The 0-hour sample extraction resulted in only CBD being found at 2.80 min, which agrees with the reference samples of 2.80 min. After 1-hour the CBD peak was reduced, but still was the main peak present. However, other impurities began to increase after 24 hours such as at 2.18 min and 3.40 min, which were found to be 8-OH-iso-HHC and 9α -OH-HHC (Figure 4A). Additionally, the Δ9-THC and Δ8-THC doublet was found at 4.10 min and 4.22 min. Another peak at 3.77 min was found to be an isomerization peak of Δ9-THC and is thought to be Δ11-THC or Δ7-THC due to previous literature searches, however no standard was found to confirm this finding[41, 42]. Fragmentation was conducted and found that it has the same fragmentation and ratios as Δ9-THC and Δ8-THC, confirming that it is an isomer of Δ9-THC and Δ8-THC. This method did not isomerize all the CBD even at 24 hours, however the CBD peak was no longer the most prominent peak in the sample. The largest peak was found to be the 3.38 peak, which was 9α -OH-HHC. The ion mobility of the muriatic acid method resulted in *m/z* 315 with a drift time of 44 and 50 bins for CBD, Δ9-THC/Δ8-THC and the two unknown cannabinoids eluting at 3.77 and 5.60 min (Figure 4B). The *m/z* 329 had a drift time of 56 and 69 bins for 11-hydroxy-CBD and 11-hydroxy-THC (Figure 4C), while the *m/z* 333 had a drift time of 59 and 71 bins for both the 8-OH-iso-HHC and 9α -OH-HHC (Figure 4D). The *m/z* 347 had drift times of 46, 67, and 90 bins indicating the presence of 9-methoxy-THC, 11,5''-dihydroxy-Δ9-THC, and 11-5''-dihydroxy-CBD (Figure 4E).

The MS/MS fragmentation was completed for each of the compounds in the sample (S.2, S.4). The peak at 2.01 min (*m/z* 347.211) fragmented to *m/z* 329.195 (loss of hydroxyl group), *m/z* 311.189 (loss of two hydroxyl groups), *m/z* 287.178 (loss of C_3H_6O), *m/z* 271.153 (loss of $C_3H_7O_2$), *m/z* 231.117 (loss of $C_6H_{12}O_2$), *m/z* 107.060 (loss of $C_7H_{14}O_2$), which confirms the presence of 11-5''-dihydroxy-CBD (S.2, S.4). The peak at 2.18 min (*m/z* 333.224) fragmented to *m/z* 315.214 (Loss of hydroxyl), *m/z* 259.153 (loss of $C_4H_{10}O$), *m/z* 193.104 (loss of $C_9H_{17}O$), *m/z* 135.089 (loss of $C_{13}H_{26}O$), and *m/z* 93.0541 ($C_{14}H_{24}O_3$). This fragmentation confirms the presence of 8-OH-*iso*-HHC (S.2, S.4). The peak at 2.59 min (*m/z* 347.187) fragmented to *m/z* 329.219 (Loss of hydroxyl), *m/z* 311.181 (loss of two hydroxyl groups), *m/z* 271.146 (loss of $C_3H_7O_2$), *m/z* 205.098 (loss of $C_8H_{14}O_2$), and *m/z* 107.056 (loss of

$C_7H_{14}O_2$), which is a known fragmentation of 11,5''-dihydroxy-Δ9-THC (S.2, S.4). Residual CBD was left over after the synthesis, which is found at peak 2.82 min (*m/z* 315.214) and it fragmented to *m/z* 259.146 (loss of C_4H_9), *m/z* 193.104 (loss of C_9H_{15}), *m/z* 135.094 (loss of $C_{13}H_{24}$), *m/z* 123.019 (loss of $C_{12}H_5O_2$), and *m/z* 93.0457 (loss of $C_{14}H_{22}O_2$) (S.2, S.4). The peak at 2.97 (*m/z* 329.195) fragmented to *m/z* 311.173 (loss of hydroxyl), *m/z* 287.178 (loss of C_3H_7), *m/z* 271.146 (loss of C_3H_8O), *m/z* 231.117 (loss of $C_6H_{12}O$), *m/z* 193.089 (loss of $C_9H_{14}O$), and *m/z* 107.060 (loss of $C_{15}H_{26}O$), which is known to be 11-hydroxy-CBD (S.2). The peak at 3.38 (*m/z* 333.216) fragmented to *m/z* 315.214 (loss of hydroxyl), *m/z* 259.139 ($C_4H_{10}O$), *m/z* 193.092 (loss of $C_9H_{14}O$), and *m/z* 135.089 (loss of $C_{13}H_{26}O$). This peak confirms the presence of 9α -OH-HHC (S.2, S.4). The peak at 3.77 min (*m/z* 315.199) fragmented to *m/z* 259.146 (loss of C_4H_9), *m/z* 193.098 (loss of C_9H_{15}), *m/z* 135.094 (loss of $C_{13}H_{24}$), *m/z* 123.019 (loss of $C_{12}H_5O_2$), and *m/z* 93.0457 (loss of $C_{14}H_{22}O_2$), this fragmentation is similar to Δ9-THC and is believed to be Δ11-THC or Δ7-THC based on literature searches (S.2)[41, 42]. The peak at 4.10 min (*m/z* 315.214) fragmented as Δ9-THC is expected to at *m/z* 259.146 (loss of C_4H_9), *m/z* 193.1098 (loss of C_9H_{15}), *m/z* 135.089 (loss of $C_{13}H_{24}$), *m/z* 123.019 (loss of $C_{12}H_5O_2$), and *m/z* 93.0457 (loss of $C_{14}H_{22}O_2$) (S.2, S.4). The secondary peak of this doublet occurred at 4.22 minutes and is known to be Δ8-THC (*m/z* 315.207), which fragmented as expected to *m/z* 259.153 (loss of C_4H_9), *m/z* 193.098 (loss of C_9H_{15}), *m/z* 135.094 (loss of $C_{13}H_{24}$), *m/z* 123.019 (loss of $C_{12}H_5O_2$), and *m/z* 93.0415 (loss of $C_{14}H_{22}O_2$) (S.2, S.4). The peak at 4.88 min (*m/z* 347.244) fragmented to *m/z* 315.222 (loss of methoxy), *m/z* 259.153 (loss of $C_5H_{12}O$), *m/z* 193.098 (loss of $C_9H_{14}O_2$), and *m/z* 135.089 (loss of $C_{14}H_{28}O$), which confirms the presence of 9-methoxy-THC (S.2, S.4). The peak at 5.45 (*m/z* 329.187) fragmented to *m/z* 287.134 (loss of C_3H_7), *m/z* 259.153 (loss of C_5H_{11}), and *m/z* 229.057 (loss of $C_6H_{14}O$), which confirms the presence of 11-hydroxy-THC (S.2, S.4).. The peak at 5.60 min (*m/z* 315.207) fragmented similarly to Δ9-THC at *m/z* 259.153 (C_4H_9), *m/z* 193.098 (loss of C_9H_{15}), *m/z* 135.094 (loss of $C_{13}H_{24}$), *m/z* 123.015 (loss of $C_{12}H_5O_2$), and *m/z* 93.0457 (loss of $C_{14}H_{22}O_2$) (S.2).

Vinegar Conversion

The 0-hour sample extraction resulted in only CBD being found at 2.80 min, which agrees with the reference samples of 2.80. After 1-hour changes of CBD isomerization were only found as an increase in the peak at 3.77, which is thought to be Δ11-THC or Δ7-THC. After 4 hours, a noticeable peak of increasing Δ9-THC was found to increase. At 24 hours, the reaction was analyzed and found that 11-5''-dihydroxy-CBD, 8-OH-iso-HHC, CBD, 10-methoxy-THC, and Δ9-THC were found at 1.96 min, 2.22 min, 2.80 min, 3.50 min, and 4.11 min, respectively (Figure 5A). The compound at 3.77 min was found in this spectra and was the most abundant after 24 hours. The ion mobility of the battery acid method resulted in *m/z* 315 with a drift time of 31 and 42 bins for CBD, Δ9-THC/Δ8-THC and the two unknown cannabinoids eluting at 3.77 and 5.63 min (Figure 5B). The *m/z* 329 had a drift time of 60 bins for 11-hydroxy-CBD (Figure 5C), while the *m/z* 333 had a drift time of 54 for the 8-OH-iso-HHC (Figure 5D). The *m/z* 347 had a drift time of 62 bins, indicating the presence of 11-5''-dihydroxy-CBD (Figure 5E).

The MS/MS fragmentation was completed for each of the compounds in the sample (S.3, S.4). The peak at 1.96 min (*m/z* 347.211) fragmented to 315.214 (loss of methoxy), *m/z* 193.104 (loss of $C_9H_{14}O_2$), *m/z* 135.012 (loss of $C_{14}H_{28}O$), *m/z* 108.070 (loss of $C_{16}H_{31}O$), and *m/z* 93.0457 (loss of $C_{15}H_{26}O_3$), which confirms the presence of 11-5''-dihydroxy-CBD (S.3, S.4). The peak at 2.22 min (*m/z* 333.152) fragmented to *m/z* 315.207 (loss of hydroxyl), *m/z* 259.146 (loss of $C_4H_{10}O$), *m/z* 193.098 (loss of $C_9H_{17}O$), *m/z* 135.089 (loss of $C_{13}H_{26}O$), and *m/z* 93.0415 ($C_{14}H_{24}O_3$). This fragmentation confirms the presence of 8-OH-iso-HHC (S.3, S.4). Residual CBD was left over after the synthesis, which is found at peak 2.82 min (*m/z* 315.207) and it fragmented to *m/z* 259.146 (loss of C_4H_9), *m/z* 193.098 (loss of C_9H_{15}), *m/z* 135.089 (loss of $C_{13}H_{24}$), *m/z* 123.0195 (loss of $C_{12}H_5O_2$), and *m/z* 93.0415 (loss of $C_{14}H_{22}O_2$) (S.3, S.4). The peak at 3.50 min (*m/z* 329.179) fragmented to *m/z* 133.076 ($C_{12}H_{21}O_2$) and *m/z* 105.045 (loss of $C_{14}H_{25}O_2$), which confirms the presence of 5''-Hydroxy-CBD (S.3, S.4). The peak at 3.77 (*m/z* 315.207) fragmented to *m/z* 259.146 (loss of C_4H_9), *m/z* 193.098 (loss of C_9H_{15}), *m/z*

135.089 (loss of $C_{13}H_{24}$), *m/z* 123.015 (loss of $C_{12}H_5O_2$), and *m/z* 93.0415 (loss of $C_{14}H_{22}O_2$), this compound was also found in the muriatic acid conversion method and is believed to be Δ11-THC or Δ7-THC (Figure 6). The peak at 4.11 min (*m/z* 315.207) fragmented as Δ9-THC is expected to *m/z* 259.146 (loss of C_4H_9), *m/z* 193.098 (loss of C_9H_{15}), *m/z* 135.089 (loss of $C_{13}H_{24}$), *m/z* 123.015 (loss of $C_{12}H_5O_2$), and *m/z* 93.0415 (loss of $C_{14}H_{22}O_2$) (S.3, S.4). The peak at 5.63 min (*m/z* 315.207) is also found in the vinegar conversion method as it fragmented to *m/z* 259.153 (C_4H_9), *m/z* 193.092 (loss of C_9H_{15}), and *m/z* 135.089 (loss of $C_{13}H_{24}$) (S.3).

Discussion

The LC/MS analysis was able to separate all of the psychoactive cannabinoids, but required 11 minutes to complete the analysis, while the ion mobility separation using the traveling wave was able to separate the psychoactive cannabinoids that had a collisional cross-section greater than 1 □^{2} in less than 2 minutes, which agrees with current ion mobility techniques to separate Δ9-THC and CBD[43]. Due to the small collisional cross-section differences among the Δ7-THC, Δ8-THC, Δ9-THC, and Δ11-THC cannabinoids, separation using ion mobility was not completed as the collisional cross-sections were 131.355, 130.9739, 131.5067, and 131.3203 □^{2}, respectively. However, the reduction of time in the ion mobility analysis for the discovery of route-specific impurities was significantly decreased in the determination of the 10-methoxy-THC, 137.8878 □^{2}, and 11-5”-dihydroxy-CBD, 141.4486 □^{2}, as no LC/MS was required due to the large differences in these collisional cross-sections.

The isomerization of CBD to other known and unknown cannabinoids using sulfuric acid and muriatic acid occurred as expected from previous literature [12]. These methods of conversion have reported similar structures such as 9-methoxy-THC, 8-OH-iso -HHC, and Δ9-THC[12, 24]. However, this study utilizes the weak acidity of vinegar to complete the isomerization, while previous studies have used *p*-toluenesulfonic acid, sulfonic acid, and even BF_3 as the acid catalyst[24]. This study was able to utilize a weaker acid, such as vinegar (5.4% acetic acid) to isomerize CBD to 11,5’-dihydroxy-CBD, 8-OH-*iso*-HHC, 11-hydroxy-CBD, 9*α* -OH-HHC, 5’-hydroxy-CBD, Δ9-THC, Δ8-THC, and 9-methoxy-THC.

One significant impurity, found at 3.77 minutes, occurred in the muriatic acid method and the vinegar method. This compound is a cannabinoid peak as it fragments the exact same as both Δ9-THC, Δ9-THC. Upon further research, this peak may be either Δ11-THC or Δ7-THC. These compounds have a longer retention time than CBD, but a shorter retention period than Δ9-THC and Δ8-THC [41, 42].

Another significant impurity was the compound that eluted around 5.6 min. This compound was found in all the isomerization reactions and was not found in the reference LC/MS analysis. This compound is believed to be a tetrahydrocannabinol due to similar fragmentation patterns. This compound eluted later than Δ9-THC, eliminating the thought that it may be either Δ11-THC or Δ7-THC. One possible explanation could be that this compound is Δ10-THC, although current research is limited and currently no standard exists to confirm this theory[44].

Conclusion

The battery acid method was the only method to produce 10-methoxy-THC, which eluted off at 3.56 minutes. The battery acid method also produced a large peak at 5.39 minutes, while the other methods did not result in this cannabinoid being synthesized.

The muriatic acid method produced the greatest number of impurities in the sample. Specifically, this method produced both 11-hydroxy-THC and 11,5”-dihydroxy-Δ9-THC, which were not found in the other methods. This is interesting because this is the procedure that could occur in the human body. The isomerization of CBD using 0.05% HCl may occur in the human body generating these unknown cannabinoids, which warrants further research to be conducted on these isomers for toxicity and pharmacokinetics. This study allows researchers to begin isolating these impurities to study toxicity on the human body.

The vinegar method was the only method to produce 5''-hydroxy-CBD, which eluted off at 3.50 minutes. The battery acid and muriatic acid methods produced 11-hydroxy-CBD.

Additional studies should also be conducted to determine if any other conventional acids can convert CBD to psychoactive cannabinoids. Acids such as boric acid and phosphoric acid can be readily purchased over the counter and should be tested to determine the capability of CBD isomerization. Subsequent studies should also focus on establishing a resolution capable of separating compounds with less than 1 $\square^2$ collisional cross-section difference.

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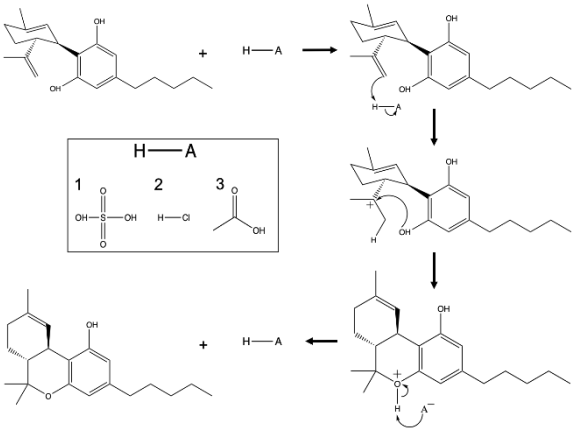

Figure 1 Proposed isomerization reaction of CBD to Δ9-THC with the addition of the three acids used, (1) sulfuric acid, (2) hydrochloric acid, (3) acetic acid.

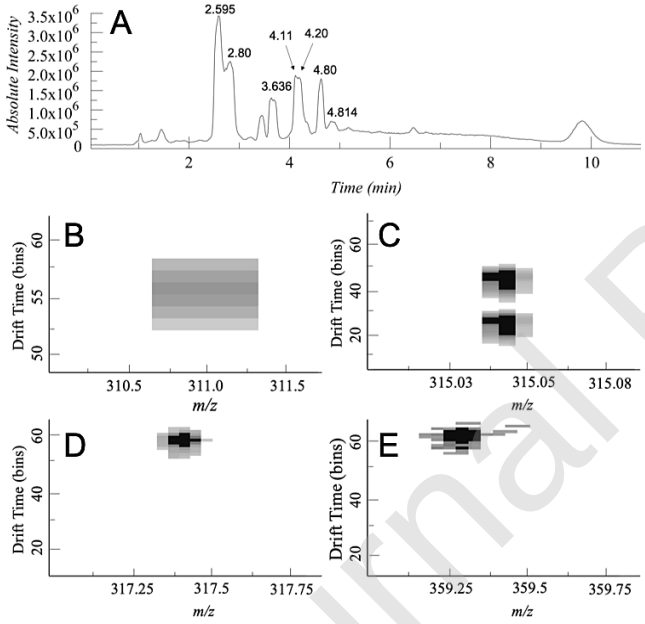

Figure 2 Reference cannabinoids analysis LC/MS (A), ion mobility of CBN (B), ion mobility of CBD, Δ9-THC/Δ8-THC, and CBC (C), ion mobility of CBG (D), ion mobility of THCA and CBDA (E).

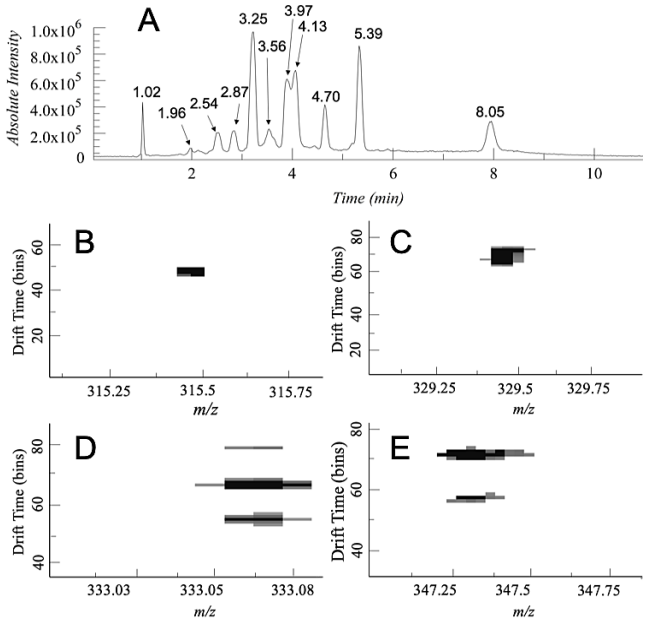

Figure 3 Battery acid method of converting CBD to psychoactive cannabinoids LC/MS (A), ion mobility of Δ9-THC/Δ8-THC/Δ11-THC/Δ7-THC (B), ion mobility of 11-hydroxy-CBD (C), ion mobility of 8-OH-iso-HHC and 9α-OH-HHC (D), ion mobility of 10-methoxy-THC/9-methoxy-THC and 11-5''-dihydroxy-CBD (E).

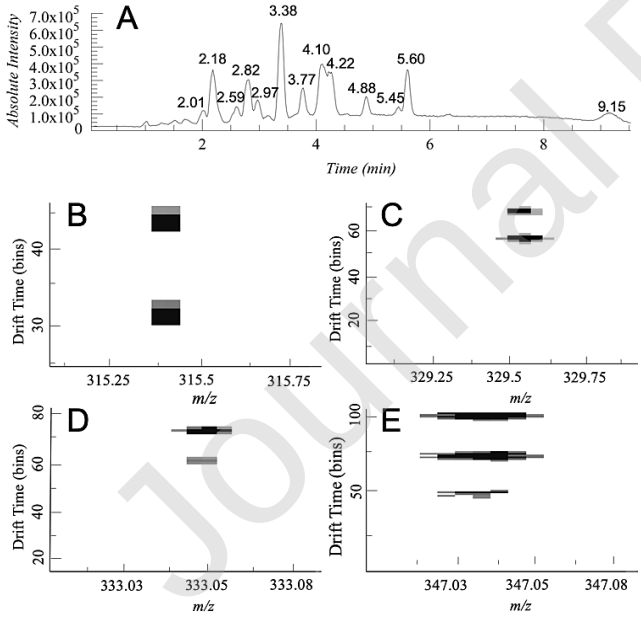

Figure 4 Muriatic acid method of converting CBD to psychoactive cannabinoids LC/MS (A), ion mobility of CBD, Δ9-THC/Δ8-THC/Δ11-THC/Δ7-THC (B), ion mobility 11-hydroxy-CBD and 11-hydroxy-THC (C), ion mobility of 8-OH-iso-HHC and 9α-OH-HHC (D). ion mobility of 9-methoxy-THC, 11,5''-dihydroxy-Δ9-THC, and 11-5''-dihydroxy-CBD (E).

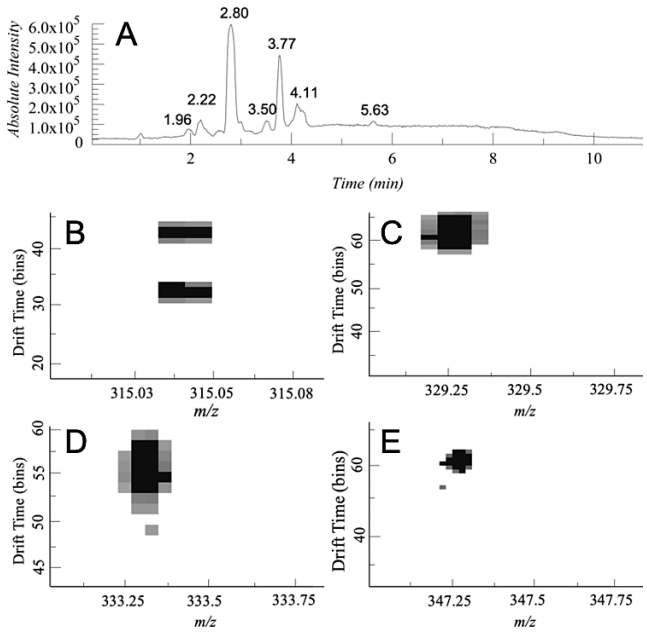

Figure 5 Vinegar method of converting CBD to psychoactive cannabinoids LC/MS (A), ion mobility of CBD, Δ9-THC/Δ8-THC/Δ11-THC/Δ7-THC (B), ion mobility of 11-hydroxy-CBD (C), ion mobility of 8-OH-iso-HHC (D), ion mobility of 11-5''-dihydroxy-CBD (E).

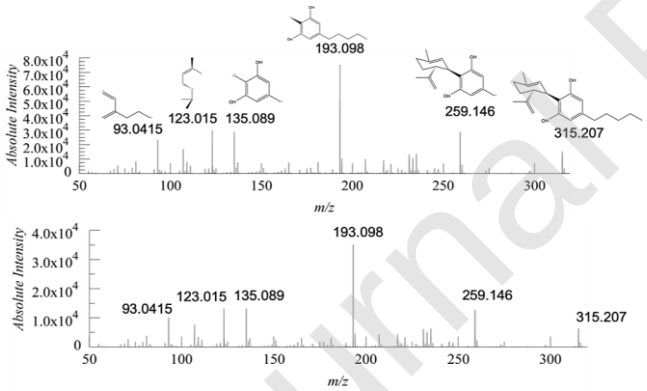

Figure 6 MS/MS fragmentation of Δ9-THC (Top) compared to Unknown Cannabinoid at 3.77 min (Bottom)

Table 1 Reference cannabinoids and psychoactive cannabinoids identified using the Battery Acid (BA), Muriatic Acid (MA), and Vinegar (V) methods. LC/MS average Retention Times (R.T.) across the samples are listed along with the corresponding *m/z* values and Collisional Cross-Section (CCS) of the molecule.

Compounds	R.T. (min)	Method	CCS (□^{2})	m/z
11,5”-dihydroxy-CBD	1.98	BA, MA, V	141.4486	347
8-OH-*iso*-HHC	2.38	BA, MA, V	129.9111	333
11,5”-dihydroxy-Δ9-THC	2.59	MA	135.0111	347
CBDA	2.595	Ref	141.1308	359
CBG	2.79	Ref	131.9371	317
CBD	2.80	MA, V	126.8266	315
11-hydroxy-CBD	2.92	BA, MA, V	136.9755	329
9α -OH-HHC	3.31	BA, MA, V	135.4929	333
5”-hydroxy-CBD	3.5	V	137.8392	329
10-methoxy-THC	3.56	BA	137.8878	347
CBN	3.64	Ref	130.0004	311
Δ11-THC	3.77	MA, V	131.3203	315
Δ7-THC	3.77	MA, V	131.3550	315
Δ9-THC	4.06	BA, MA, V	131.3928	315
Δ8-THC	4.18	BA, MA, V	130.9739	315
9-methoxy-THC	4.79	BA, MA, V	137.0959	347
CBC	4.80	Ref	138.8955	315
THCA	5.81	Ref	140.9308	359
11-hydroxy-THC	5.45	MA	131.5067	329