CHALLENGES IN DETERMINING DELTA-8-THC IN CANNABIDIOL CONVERSION PRODUCTS: WHY LABS MAY REPORT ERRONEOUS RESULTS

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HIGHLIGHTS

- Most commercially available Δ^8-THC is synthesized by conversion of cannabidiol (CBD) under acidic conditions that produce variable amounts of intermediate substances including Δ^9-THC and side-products such as Δ^8-*iso*-THC, $\Delta^{4(8)}$-*iso*-THC, and other substances.
- Δ^8-THC obtained by conversion of CBD and substances made from it require analysis to assess the purity of the products, to identify impurities, and to determine compliance with the regulatory limit for Δ^9-THC.
- Many hemp laboratories analyze the Δ^8-THC obtained from conversion of CBD using reversed-phase high performance liquid chromatographic (RP-HPLC) methods that were developed and validated for analysis of phytocannabinoids in plant materials. These methods are fit-for-purpose for analysis of phytocannabinoids including Δ^8-THC extracted from plant materials, but they are not suitable for determining Δ^8-THC produced by conversion of CBD because some of the side-products from the conversion reaction can interfere with the determinations of Δ^8-THC and Δ^9-THC thereby invalidating the quantitative estimates for these two substances.
- Therefore, the suitability of methods for analysis of any materials containing Δ^8-THC obtained by conversion of CBD must be verified by a method validation study that includes an assessment of the effects of various side products on determinations of Δ^8-THC and Δ^9-THC.
- However, method validation studies for assessing the suitability of new and existing methods have been hampered because reference standards of Δ^8-*iso*-THC, $\Delta^{4(8)}$-*iso*-THC, and other impurities have not been available.
- This impediment to method validation studies has been addressed by Cayman Laboratories which recently announced the commercial availability of a reference standard for Δ^8-*iso*-THC. The availability of this standard has enabled laboratories to validate analytical methods to determine Δ^8-THC contaminated with Δ^8-*iso*-THC produced from the conversion of CBD.
- KCA Laboratories in Nicholasville, Kentucky has used this reference standard to document the co-elution of Δ^8-THC and Δ^8-*iso*-THC under RP-HPLC conditions and has validated a sensitive and specific gas chromatography with mass spectrometric (GC-MS) detection method for determining Δ^8-THC, Δ^9-THC, and Δ^8-*iso*-THC in CBD conversion products.

PROBLEM STATEMENT

Recent consumer interest in the use and consumption of delta-8-THC (Δ^8-THC) has led to the need to analyze synthetic Δ^8-THC for purity and compliance with regulations regarding limits on Δ^9-THC. Furthermore, several states now require testing of Δ^8-THC because they are treating it similarly or identically to Δ^9-THC.[1] For example, Ohio now requires determination of all tetrahydrocannabinols to verify that the regulatory cap on total THC in medicinal products is not exceeded.[2] Furthermore, Δ^9-THC is being synthesized by acid-catalyzed isomerization of cannabidiol (CBD) isolated from hemp and offering it for sale throughout the US.[3]

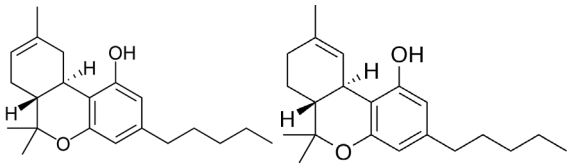
Consequently, laboratories have had to develop and validate methods that are fit-for-purpose for measuring Δ^8-THC in the presence of Δ^9-THC and other reaction side-products. In developing these methods, laboratory personnel have experienced challenges in determining Δ^8-THC due to interference from substances that are not present in plant materials but that are produced during the acid-catalyzed intramolecular cyclization of CBD that is typically used to produce commercial quantities of Δ^8-THC. Furthermore, they have encountered challenges in determining Δ^9-THC accurately at the 0.3% limit particularly when the sample contains reaction side products that interfere with or mask its determination. Recently, the USP Cannabis Panel reviewed the production of commercial quantities of Δ^8-THC from the acid-catalyzed intramolecular ring closure of CBD and identified the importance of testing synthetic Δ^8-THC using scientifically valid methods.[4] Furthermore, the USP Cannabis Panel expressed a need for systematic clinical investigations using test articles containing purified and well-characterized Δ^8-THC. Therefore, the purpose of this communication is to identify and discuss some of these challenges in determining Δ^8-THC and how to overcome them in the cannabis laboratory to meet the quality standards that have been identified by the USP Cannabis Panel and others.

[1] https://www.reuters.com/legal/litigation/controlling-cannabis-classification-delta-8-thc-2021-09-22/
[2] https://www.cincinnati.com/story/news/2021/06/21/ohio-regulates-delta-8-thc-medical-marijuana-products/7578880002/
[3] https://cbdoracle.com/news/hemp-delta-9-thc-products-lab-study-consumer-safety-and-legality/
[4] https://www.usp.org/sites/default/files/usp/document/our-science/usp-delta-8-final-12-2-21.pdf

Phytocannabinoids are secondary plant metabolites produced by *Cannabis sativa* Linnaeus, of which the most well-known is the psychoactive compound (-) Δ^9-*trans*-tetrahydrocannabinol (THC or Δ^9-THC) [1-5]. The cannabis plant produces both (-) Δ^9-*trans*-tetrahydrocannabinolic acid (tetrahydrocannabinolic acid, THCA) and cannabidiolic acid (cannabidiol acid, CBDA) from cannabigerolic acid but decarboxylation of each in the presence of light, air or (especially) heat produces THC and CBD, respectively [6-11]. Decarboxylation may occur to some extent in the plant before harvesting and is accelerated by heating and exposure to air [9].

Δ^8-THC is (-)-*trans*-Δ^8-tetrahydocannabinol and, as its name would suggest, is closely related chemically and pharmacologically to Δ^9-THC. They differ in the position of the double bond in the cyclohexenyl ring (see Figure 1) which affects the shape of the ring. The close structural similarity between Δ^8-THC and Δ^9-THC has two important consequences. The first is that both substances bind to cannabinoid receptors to produce similar pharmacologic effects including psychoactivity, but they differ in relative potency with Δ^9-THC being somewhat more potent [12; 13]. The second is that they exhibit similar physicochemical properties that affect the analyst's choice of analytical approaches particularly when measuring small amounts of Δ^9-THC in the presence of large amounts of Δ^8-THC to determine compliance with regulatory limits for Δ^9-THC.

Figure 1. (-)-trans-Δ^8-THC (left) and (-)-trans-Δ^9-THC (right)



Synthesis of Δ^8-THC by treating CBD with various acids has been known from the early 1940s. Roger Adams and his research group at the University of Illinois in Champaign reported in the early 1940s that they had synthesized two different tetrahydrocannabinols from CBD using different acids and reaction conditions [14-20]. They recognized that they had made two different psychoactive substances that were closely related isomers, but they were unable to determine their chemical structures and assign their stereochemistry. These two psychoactive substances were later identified as Δ^8-THC and Δ^9-THC in 1964 by Mechoulam's group in Israel [21-25] using instrumental methods of analysis that were not available to Adams and his group in Illinois more than twenty-five years earlier.

Mechoulam's group synthesized both Δ^8-THC and Δ^9-THC and assigned the correct stereochemistry to them [21; 22; 26; 27]. Furthermore, they identified Δ^9-THC as the main psychoactive substance in various cultivars of *C. sativa* L [23]. Due to the differences in the conformation of the cyclohexenyl ring, Δ^8-THC and Δ^9-THC are shaped somewhat differently, and consequently torsion in the cyclohexenyl ring in Δ^9-THC is greater than that in Δ^8-THC due to intramolecular repulsion between the phenolic group and the hydrogen atom on the carbon atom at C-10 [28]. One of the consequences of this intramolecular interaction is that Δ^9-THC isomerizes to Δ^8-THC particularly in the presence of acids thereby reducing this interaction [24; 28; 29]. Therefore, small quantities of Δ^8-THC are typically found in *Cannabis* plant material due to isomerization of Δ^9-THC. However, the amount of Δ^8-THC in *Cannabis* plant material is low and insufficient for commercial production.

Since the amount of Δ^8-THC in hemp is too low for it to serve as an economical source, the recent demand for Δ^8-THC in commerce has been met by synthesis from CBD using acidic reaction conditions first used by the Adams group at the University of Illinois with some modifications to improve yield [24; 25; 30; 31]. The reaction conditions have been modified and common reagents substituted for less common ones so that the synthesis can be performed in facilities that are not as well equipped as academic and commercial pharmaceutical synthesis laboratories. An important consequence of these changes is that several reaction side-products are produced under reaction conditions that are commonly used [32].

The side products are challenging to analyze because many have the same molecular formula and the same lipophilicity as Δ^8-THC and Δ^9-THC. Consequently, they are difficult to separate from Δ^8-THC and Δ^9-THC by methods that rely on differences in lipophilicity such as reversed-phase high performance liquid chromatography (RP-HPLC). Analysts have been unable to assess whether their RP-HPLC methods for determination of phytocannabinoids in plant materials can differentiate them from as Δ^8-THC and Δ^9-THC because reference standards of the side-products have been not available until recently. The recent availability of one of these (*i.e.*, Δ^8-*iso*-THC) provides a means by which analysts can test their RP-HPLC cannabinoid methods to determine whether Δ^8-*iso*-THC is resolved from Δ^8-THC. This is an important question because Δ^8-*iso*-THC is almost always present in synthetic Δ^8-THC [33].

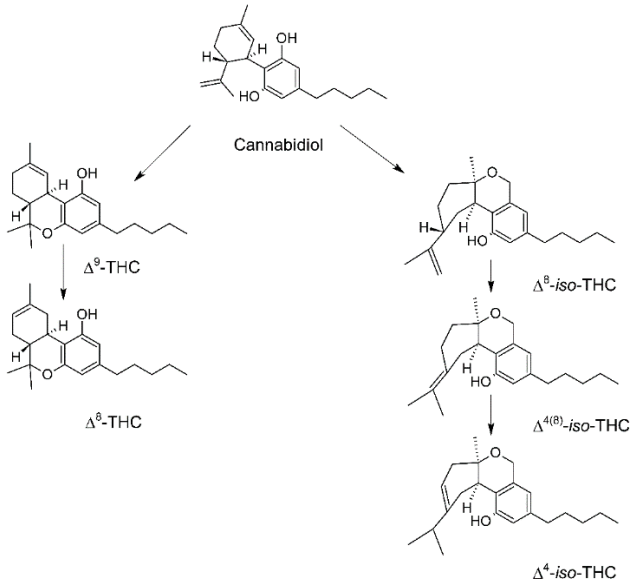
SYNTHESIS OF Δ^8-THC AND Δ^9-THC FROM CANNABIDIOL

Both Δ^8-THC and Δ^9-THC have been synthesized from CBD under differing conditions including the type and the amount of acid catalyst, the reaction temperature and the reaction time, the solvent used to dissolve the reactants, and whether

water or atmospheric oxygen is present or excluded [31]. Varying the conditions affects the relative amounts of Δ^8-THC and Δ^9-THC as well as the type and amounts of the side products [31]. The reaction scheme for synthesizing both Δ^8-THC and Δ^9-THC from CBD is shown in Figure 2.

In addition to establishing the structures of Δ^8-THC and Δ^9-THC, Mechoulam's group investigated the acid-catalyzed intramolecular cyclization reaction mechanism by preparing Δ^8-THC from CBD in refluxing benzene using *p*-toluenesulphonic acid as the acid catalyst. They also prepared Δ^9-THC from CBD in very dilute ethanolic hydrochloric acid. When they then treated the Δ^9-THC (obtained from CBD in the second synthesis) in refluxing benzene with *p*-toluenesulphonic acid, they obtained Δ^8-THC indicating that Δ^9-THC is an intermediate in the synthesis of Δ^8-THC when stronger acids, higher temperatures, or water or alcohols are present [30]. Therefore, findings for Δ^9-THC as a side-product in the synthesis of Δ^8-THC from CBD are expected [31]. Furthermore, multiple side-products, depending on reaction conditions, have been reported [31; 32]. These side-products include Δ^7-THC, Δ^{10}-THC, Δ^{11}-THC, 11-hydroxy-CBD, 11-hydroxy-THC, 5'-hydroxy-CBD, 11,5'-dihydroxy-CBD, 11,5'-dihydroxy-THC, Δ^8-*iso*-THC, $\Delta^{4(8)}$-*iso*-THC [32]. Various substituted hexahydrocannabinols (HHC) such as 9α-hydroxy-HHC and 8-hydroxy-*iso*-HHC have also been reported [32]. When the synthesis is carried out in methanol or ethanol, the methoxy or ethoxy derivatives 9-methoxy-HHC and 10-methoxy-HHC or 9-ethoxy-HHC and 10-ethoxy-HHC are formed [32]. The largest number of products is observed when HCl is used as the acid catalyst, and the only acid reported to catalyze the formation of 11,5′-dihydroxy-Δ^9-THC [32] is HCl [32]. On the other hand, 10-methoxy-THC is produced only with sulfuric acid in methanol and 5′-hydroxy-CBD is produced only by acetic acid [32]. Based on these findings, identification of certain reaction side-products could reveal information about the reactants and reaction conditions used to synthesize the Δ^8-THC or Δ^9-THC but reference standards for most of these substances are unavailable.

Figure 2. Acid-catalyzed cyclization of CBD to the THC series (left) and iso-THC series (right) in dry toluene under nitrogen at 0°C using BF_3-dietherate as the acid catalyst.



Additional reaction conditions to synthesize Δ^8-THC and Δ^9-THC from CBD using different acid catalysts and reaction temperatures and solvents have been identified [31]. A mechanism for the synthesis of Δ^8-THC and Δ^9-THC from CBD in dry toluene under an inert atmosphere using boron trifluoride (BF_3)-dietherate as an acid catalyst at -10 - 0°C has been proposed [31]. The reaction proceeds along two parallel paths (see Figure 2) in which the phenol adds across the terpenyl double bond to form members of the *iso*-THC series or the endo double bond to form members of the THC series [31]. The initial products formed under these acidic conditions are Δ^8-*iso*-THC and Δ^9-THC, respectively [31]. Both Δ^8-*iso*-THC and Δ^9-THC are isomerized under the reaction conditions to the more thermodynamically stable $\Delta^{4(8)}$-*iso*-THC and Δ^8-THC, respectively so that the end products are predominately $\Delta^{4(8)}$-*iso*-THC and Δ^8-THC mixed with Δ^8-*iso*-THC and Δ^9-THC and possibly Δ^4-*iso*-THC [31].

Those products formed under acid-catalyzed reaction conditions in the presence of water and various alcohols result from the addition of phenol, water, methanol, or ethanol to one or both double bonds in CBD. Therefore, the abundances and identities of side-products formed in the synthesis of Δ^8-THC from CBD depend on multiple factors including reaction conditions that may be difficult to reproduce and control in laboratories operated by persons with limited training or experience with laboratory procedures. Consequently, synthetic Δ^8-THC may contain reaction side-products that are not phytocannabinoids except for Δ^9-THC. Therefore, the analysis of synthetic Δ^8-THC

requires a different approach from the analysis of the small quantities of Δ^8-THC typically found in hemp because the matrices are completely different owing to the presence of various side products that are not phytocannabinoids found naturally in hemp. The following section will address specific factors involved in the analysis of Δ^8-THC in hemp and synthetic Δ^8-THC from acid-catalyzed isomerization of CBD.

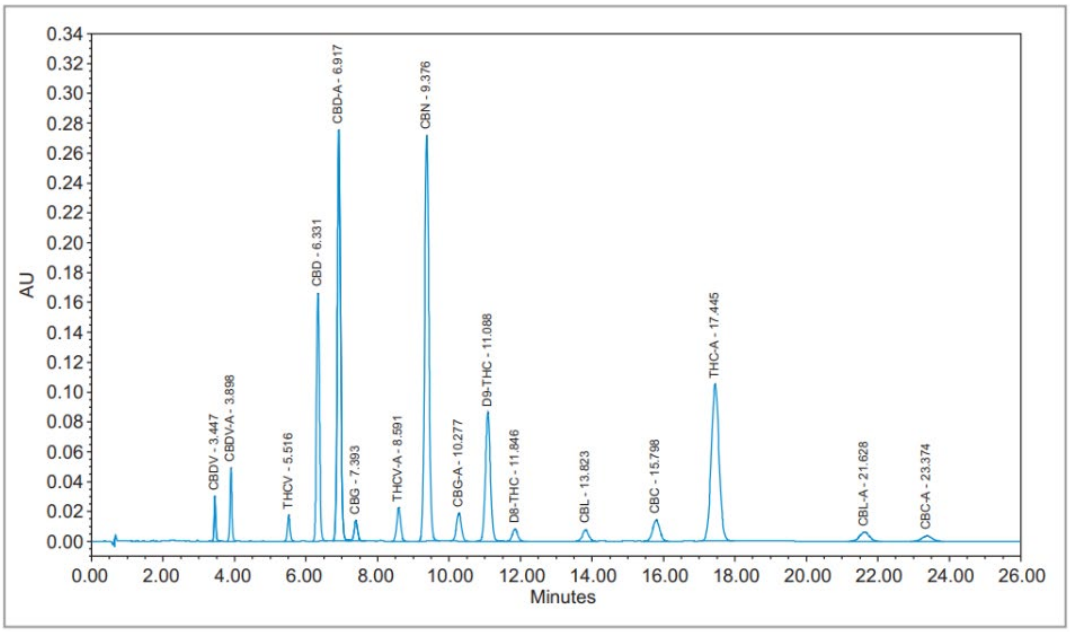
Differing synthetic approaches and different isolation and purification procedures are being used to obtain Δ^8-THC and Δ^9-THC from CBD (Sams, personal communication). Consequently, some of the commercially available Δ^8-THC contains substantial amounts of Δ^9-THC and as much as 25-35 % (w/w) as unidentified substances that include the reaction side-products listed earlier and perhaps other substances (Sams, personal communication). Therefore, there is a need for determining the purity and amount of Δ^8-THC or Δ^9-THC in the presence of reaction side-products such as those described above but RP-HPLC methods developed to determine Δ^9-THC in *Cannabis* plant material have not been validated for determining synthetic Δ^8-THC or Δ^9-THC from conversion of CBD because reference standards of key side-products have not been available until recently (*i.e.*, March 2022).

ANALYSIS OF Δ^8-THC AND Δ^9-THC FROM THE ACID-CATALYZED CONVERSION OF CBD

The analysis of plant material from *C. sativa* L for neutral and acidic phytocannabinoids is usually performed by RP-HPLC-PDA analysis of extracts of the dried plant material. Analytical separations are performed on C18 or similar reversed-phase columns using a mobile phase consisting of a mixture of methanol or acetonitrile and water containing a pH modifier to control the ionization and therefore the elution of the acidic cannabinoids. Since the separation by RP-HPLC depends primarily on the lipophilicities of the analytes, those substances with similar lipophilicities elute at similar retention times and those that are more lipophilic elute later than those that are less lipophilic. The cannabinoids with two phenolic groups (*e.g.*, CBD and cannabigerol (CBG)) are more polar than those with one phenolic group (*e.g.*, various THC isomers) and therefore elute considerably earlier. Those cannabinoids with shortened alkyl side chains such as (-)-Δ^9-tetrahydrocannabivarin (Δ^9-THCV) and cannabidivarin (CBDV), the propyl homologues of Δ^9-THC and CBD, respectively, have shorter retention times compared to the normal (*i.e.*, pentyl-substituted) phytocannabinoids because those cannabinoids with shorter chain lengths are less lipophilic [34]. RP-HPLC methods are capable of separating mixtures of 16-24 or more phytocannabinoids from plant sources in 10-20 minutes (Sams, personal observation).

Since Δ^8-THC and Δ^9-THC are structural isomers differing only in the location of a double bond in the cyclohexenyl ring (Figure 1), they have nearly identical lipophilicities and consequently elute in a narrow retention time window under reversed-phase HPLC conditions. Baseline separation of Δ^8-THC from Δ^9-THC is one of the more challenging separations in mixtures of phytocannabinoids. The chromatogram in Figure 3 illustrates the separation of a standard mixture of phytocannabinoids in a method validated for the analysis of *Cannabis* plant material. Note that Δ^8-THC and Δ^9-THC elute in a narrow retention time window but that they are completely resolved from each other with Δ^9-THC eluting just before Δ^8-THC (see Figure 3).

Figure 3. RP-HPLC Chromatogram of Acidic and Neutral Cannabinoid Standards[5]



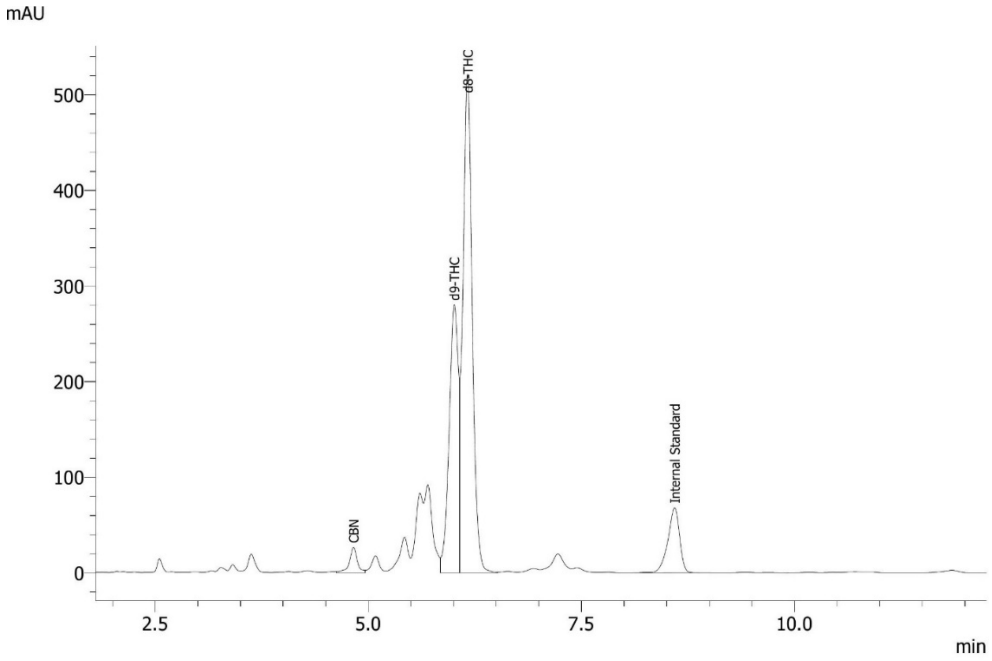
The PDA detector acquires ultraviolet-visible (UV-VIS) absorption data throughout each HPLC analysis, so the spectra acquired during sample analyses can be compared with those of reference standards to assess and verify identities of detected substances. However, many cannabinoids and side-products are so chemically similar that their UV-VIS spectra are nearly identical. Therefore, the PDA detector doesn't have sufficient specificity to differentiate many of these substances from others. Therefore, identifications of cannabinoids by RP-HPLC-PDA in extracts of plant materials are based largely on comparisons of retention times. When properly validated, RP-HPLC-PDA methods can be demonstrated to be fit-for-purpose for determining all major phytocannabinoids as well as many minor phytocannabinoids in *Cannabis* plant materials [35-43].

Some laboratories have also reported results of analysis of synthetic Δ^8-THC and Δ^9-THC using RP-HPLC-PDA, apparently based on the assumption that the only difference between it and plant extracts is the abundance of Δ^8-THC relative to that of Δ^9-THC. However, the validity of the results cannot be assured without verifying that reaction side-products are adequately resolved from the target analytes (specifically Δ^8-THC and Δ^9-THC) in Δ^8-or Δ^9-THC THC obtained by the conversion of CBD.

The inadequacies in using RP-HPLC to analyze a Δ^8-THC isolate obtained from the conversion of CBD is shown in Figure 4. Examination of the chromatographic data revealed a peak identified as cannabinol (CBN) and a peak attributed to Δ^9-THC eluting immediately before and incompletely resolved from the large peak tentatively identified as Δ^8-THC. Furthermore, this large peak is skewed to the right suggesting co-elution with another substance (Sams, personal observation). The substances eluting between the peak attributed to CBN and that attributed to Δ^9-THC are all unidentified substances although they are commonly observed in RP-HPLC analyses of conversion materials. Due to the lack of specificity of the PDA detector, all identifications must be considered tentative until confirmed by analysis using a method that has been validated for determination of Δ^8-THC or Δ^9-THC from conversion of CBD.

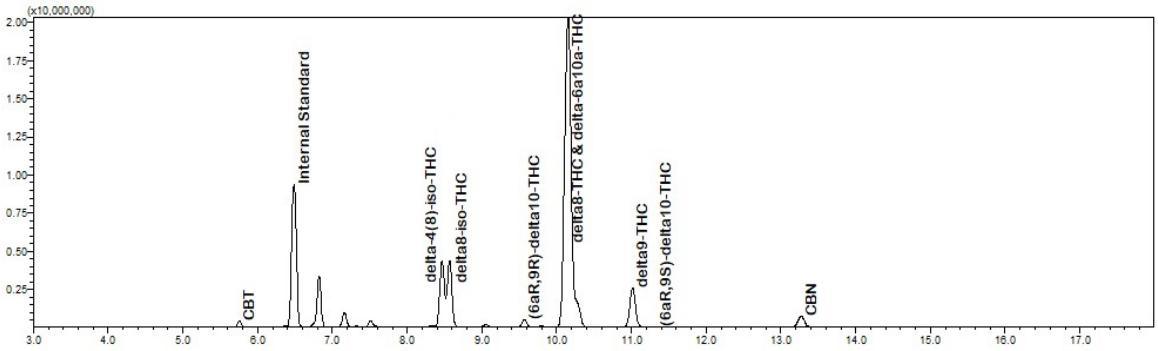
[5] https://www.waters.com/webassets/cms/library/docs/720006426en.pdf

Figure 4. RP-HPLC-PDA Analysis of a Δ^8-THC Isolated from Conversion of CBD



These results indicated the need for re-analysis of the sample using a method with selectivity based on different principles of separation and improved specificity based on mass spectral detection to assist in identification of unidentified substances in the RP-HPLC-PDA chromatograms. Since liquid chromatography with electrospray detection is typically based on reversed-phase separations, we opted to re-analyze the sample by gas chromatography-mass spectrometry (GC-MS) to achieve separation by a different separation principle (*i.e.*, volatility compared to lipophilicity). Using a GC-MS method with a selective stationary phase, we obtained the results for this sample as shown in Figure 5.

Figure 5. GC-MS Analysis of Δ^8-THC Isolated from Conversion of CBD



The results of the re-analysis of this Δ^8-THC sample by GC-MS provided information that was not obtainable by RP-HPLC-PDA analysis. The presence of Δ^9-THC was evident in the GC-MS data by the well-resolved peak at approximately 11 minutes. The amount of Δ^9-THC determined by GC-MS represented about 5 % of the total weight of the sample. Although this concentration was substantially greater than the regulatory limit of 0.3%, it was substantially less than the estimate obtained by RP-HPLC analysis. The two peaks between 8 and 9 minutes were identified as $\Delta^{4(8)}$-*iso*-THC and Δ^8-*iso*-THC, respectively (see Figure 2). These substances co-elute with Δ^9-THC and Δ^8-THC, respectively, during RP-HPLC analysis and interfere with their determinations. Therefore, the reported concentrations of Δ^9-THC and Δ^8-THC in this sample are lower when determined by a validated GC-MS method compared to a RP-HPLC-PDA method validated for phytocannabinoids. The concentration of Δ^8-*iso*-THC in the sample was determined by the GC-MS method developed by us using the reference standard which is now available.[6] GC-MS analysis also confirmed the presence of (6a*R*,9*R*)-Δ^{10}-THC, (6a*R*,9*S*)-Δ^{10}-THC, and $\Delta^{6a,10a}$-THC. These substances elute after Δ^9-THC under RP-HPLC conditions but were not identified (see Figure 4). Although Δ^8-THC and $\Delta^{6a,10a}$-THC are incompletely resolved chromatographically under GC-MS conditions (see Figure 5), they are readily differentiated by different fragmentation patterns and ion abundances (Sams, personal communication).

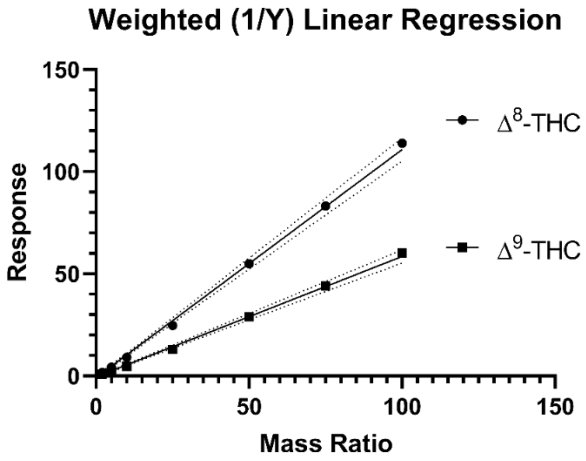
SOLUTION

Based on the RP-HPLC results that we obtained early in the expansion of the commercial market for Δ^8-THC prepared by conversion of CBD, KCA Labs evaluated GC-MS as an alternate method of analysis of these materials because we had determined that reaction side-products were interfering with the determinations of both Δ^8-THC and Δ^9-THC by RP-HPLC methods. We had observed distorted peaks for both Δ^8-THC and Δ^9-THC in chromatograms reported by us and others. To obtain insight into the nature of these side products, we synthesized Δ^8-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC by acid-catalyzed intramolecular ring closure of CBD using a published method [31]. We demonstrated that these side-products interfered with the RP-HPLC-PDA determination of Δ^8-THC and Δ^9-THC, respectively. The co-elution of these isomers was previously reported by others [31]. Furthermore, we determined that our GC-MS method provides complete separation of Δ^8-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC from Δ^8-THC and Δ^9-THC and that GC analysis provides separation of Δ^8-THC from Δ^9-THC. In addition, mass spectral detection provides much greater specificity of identification of these and other substances in mixtures of CBD conversion materials than is available by PDA detection. Based on these observations, we concluded that we would not use RP-HPLC-PDA for the determinations of Δ^9-THC and Δ^8-THC in these materials due to the interference from $\Delta^{4(8)}$-*iso*-THC and Δ^8-*iso*-THC; and that we would use GC-MS for the analysis of CBD-conversion products instead of RP-HPLC-PDA.

We quickly determined that Δ^8-THC and other neutral cannabinoids are amenable to analysis by GC-MS without derivatization. Although the acidic forms of Δ^8-THC and Δ^9-THC undergo decarboxylation (at least partially) under the elevated temperatures required for GC-MS analysis [44-46], they are not present in the Δ^8-THC and Δ^9-THC materials made from conversion of CBD. The neutral forms (*e.g.*, CBD, CBN, Δ^8-THC, and Δ^9-THC) are stable, exhibit excellent peak shape, and can be quantified (see Figure 6) with excellent accuracy and precision under the GC-MS conditions that we had optimized (Sams, personal observation). The quantification of Δ^9-THC and other neutral cannabinoids by GC-MS was found to be a fit-for-purpose means of determining Δ^9-THC in CBD conversion products due to the superior resolution of it from $\Delta^{4(8)}$-*iso*-THC and Δ^8-THC thereby assuring its detection in the presence of substances that interfere with its detection by RP-HPLC-PDA.

6 https://www.caymanchem.com/product/33864/%CE%B48-iso-thc

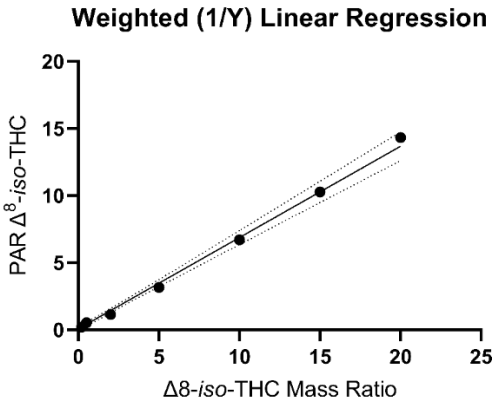
Figure 6. GC-MS Calibration Curves for Determination of Δ^8-THC and Δ^9-THC



The recent commercial availability of a reference standard of Δ^8-*iso*-THC[7] enables every laboratory to determine whether its RP-HPLC method resolves Δ^8-*iso*-THC from Δ^8-THC. If the peaks are not resolved, estimates of the purity of Δ^8-THC determined by RP-HPLC-PDA will be higher than actual due to the contribution from Δ^8-*iso*-THC. Most samples of Δ^8-THC submitted for analysis to KCA Laboratories contain detectable amounts of Δ^8-*iso*-THC and some samples contain appreciable amounts of it (Sams, personal observation). Therefore, estimates of Δ^8-THC determined by RP-HPLC methods are frequently higher than estimates obtained by GC-MS methods due to the co-elution of Δ^8-*iso*-THC and Δ^8-THC in RP-HPLC methods.

Using the GC-MS/MS method described above for determining Δ^8-THC and Δ^9-THC, we have demonstrated the suitability of this method for determining Δ^8-*iso*-THC in CBD conversion mixtures. The calibration line prepared using the internal standard method and with weighting by 1/Y where Y is the peak area ratio (PAR) is linear over the range of 0.2 to 20. A typical calibration line for Δ^8-*iso*-THC is shown in Figure 7.

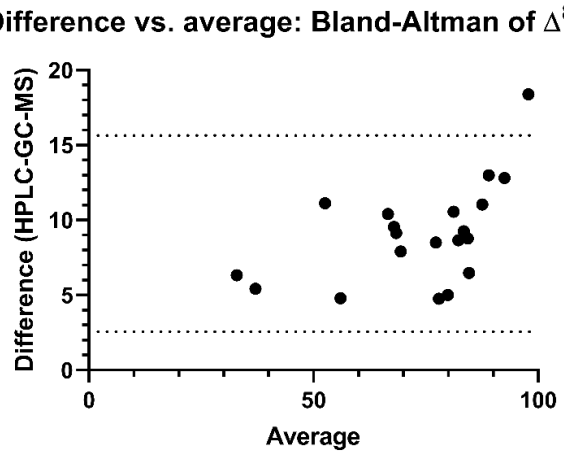
Figure 7. GC-MS Calibration Curve for Determination of Δ^8-iso-THC



7 https://www.caymanchem.com/product/33864/%CE%B48-iso-thc

In our experience, the contribution of Δ^8-*iso*-THC to the apparent purity of Δ^8-THC can be substantial (see Figure 8) because Δ^8-*iso*-THC is present in many Δ^8-THC samples. For example, the Bland-Altman plot obtained from the estimates of the purity of Δ^8-THC from RP-HPLC-PDA and GC-MS analysis in Figure 8 illustrates the contributions of Δ^8-*iso*-THC to the estimate of Δ^8-THC. In every case, the estimate from HPLC was greater than that from GC-MS and a peak attributable to Δ^8-*iso*-THC was observed in the GC-MS analysis of each of these samples. Therefore, the difference between the two methods is due to the co-elution of Δ^8-*iso*-THC with Δ^8-THC when determined by RP-HPLC methods due to the lack of specificity of these methods.

Figure 8. Bland-Altman Plot of Δ^8-THC Determined by HPLC and GC-MS Methods



GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS OF CBD CONVERSION PRODUCTS FOR Δ^8-THC

The neutral phytocannabinoids from cannabis have been analyzed by GC-MS and GC-FID for decades due to the excellent resolving power that can be obtained using capillary columns with bonded stationary phases [36; 41; 47; 48]. On non-polar polydimethylsiloxane stationary phases, the elution order typically follows the boiling points of the solutes, with lower boiling solutes eluting earlier.[8] Accordingly, analysis of cannabinoids on polydimethylsiloxane stationary phases results in Δ^9-THC eluting before Δ^8-THC because the boiling point of Δ^9-THC is lower than that of Δ^8-THC. However, CBD and cannabichromene (CBC) are not adequately resolved on polydimethylsiloxane stationary phases so other phases with different selectivity are necessary to resolve these two cannabinoids in mixtures particularly when non-selective detectors such as the FID detector are used. These phases have been identified and are being used to determine cannabinoids by GC-FID and GC-MS methods.

Bonded stationary phases with extensive substitution of phenyl groups for the methyl groups of the polydimethylsiloxane stationary phases have found application in the GC separation of cannabinoids. The phenyl groups in mixed dimethylpolysiloxane-dimethyl-diphenyl bonded stationary phases account for the primary (*i.e.*, p-p) interaction with the phenyl groups in cannabinoids and determine the order of elution. Stationary phase columns containing 35% phenyl 65% dimethylpolysiloxane improve the resolution between CBD and CBC and afford complete resolution of Δ^8-THC and Δ^9-THC.[9] Furthermore, the elution order of Δ^9-THC and Δ^8-THC is reversed compared to that on non-polar phases as well as the order based on boiling points (Sams, personal observation).

We have analyzed numerous CBD reaction mixtures by GC-MS using a 30 m x 0.25 mm x 1.50 mm Rxi 35 Sil MS (Restek) capillary column[10] containing a 35% phenyl 65% dimethylpolysiloxane stationary phase. The column was operated isothermally at 240 °C for 15 min and then ramped at 25 °C per minute to 315 °C and held for one min before returning to starting conditions. Under these conditions, CBC, CBD, CBN, Δ^8-THC, Δ^9-THC, Δ^8-*iso*-THC, and Δ$^{4(8)}$-*iso*-THC were adequately resolved and the order of elution of Δ^8-THC and Δ^9-THC was reversed compared to that on

[8] https://www.restek.com/en/chromablography/chromablography/dont-overestimate-cannabidiol-during-medical-cannabis-potency-determinations-with-gas-chromatography.--use-stationary-phase-selectivity-for-accuracy-and-hydrogen-for-fast-analysis/
[9] https://lab-comp.hu/wp-content/uploads/2015/06/Restek_GC_Fused_Silica_Capillary_Columns.pdf
[10] https://www.restek.com/en/products/columns/gc-columns/Fused-Silica-Capillary-Columns/8496/

non-polar stationary phases (Sams, personal communication)

SUMMARY

Reversed-phase liquid chromatographic methods have been demonstrated to be suitable for determining phytocannabinoids in plant materials and isolates of plant materials, but they may not be suitable for determining Δ^8-THC and Δ^9-THC in synthetic mixtures from the CBD. Specifically, side-products from the *iso*-THC series and possibly other substances are inadequately resolved from Δ^8-THC and Δ^9-THC by RP-HPLC methods. Consequently, quantitative estimates for both Δ^8-THC and Δ^9-THC determined by RP-HPLC separations of synthetic mixtures are likely to be inaccurate. On the other hand, both Δ^9-THC and Δ^8-THC are resolved from each other and from reaction side-products from the *iso*-THC series and positional isomers of THC by GC-MS methods on capillary columns containing 35% phenyl 65% dimethylpolysiloxane stationary phases.

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