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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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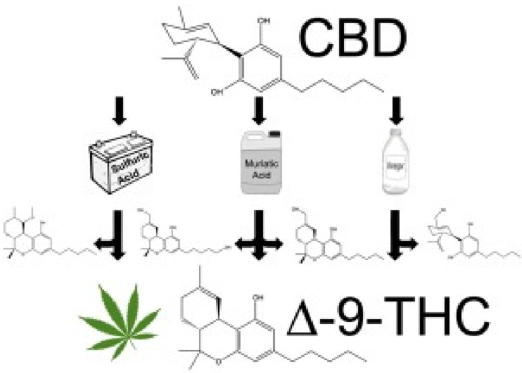
Abstract

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.

Graphical abstract



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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], [15], [16], [17], [18], [19]]. Other research groups have found that Δ9-THC does get formed in the stomach as well as other cannabinoid metabolites such as 6β-hydroxymethyl-Δ9-tetrahydrocannabinol, which forms via an epoxide reaction [20]. This metabolite, 6β-hydroxymethyl-Δ9-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 h later when Δ9-THC is dosed orally [[21], [22], [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], [27], [28], [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], [36], [37], [38], [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) (Fig. 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 min to analyze all of the psychoactive cannabinoids and allowed for the determination of cannabinoids with more than 1 $Å^2$ 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.

Section snippets

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 g of CBD (LaCore Enterprises, Melissa, TX, USA) was added to 35 mL of 95% v/v ethanol…

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 min, respectively (Fig. 2A). The ion mobility of the reference standards resulted in *m/z* 311 with a drift time of 56 bins for CBN (Fig. 2B), *m/z* 315 with a drift time of 27 bins, 48 bins, and 69 bins indicating three compounds with different collisional…

Discussion

The LC/MS analysis was able to separate all of the psychoactive cannabinoids, but required 11 min 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 $Å^2$ in less than 2 min, 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…

Conclusion

The battery acid method was the only method to produce 10-methoxy-THC, which eluted off at 3.56 min. The battery acid method also produced a large peak at 5.39 min, 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…

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Author contributions

The manuscript was written through contributions of all authors.…

CRediT authorship contribution statement

Thomas D. Kiselak: Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Rachel Koerber:** Data curation, Methodology, Writing - review & editing. **Guido F. Verbeck:** Conceptualization, Methodology, Visualization, Supervision, Writing - review & editing.…

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2022, Results in Chemistry

Citation Excerpt :

…As concerning the thermal and acidic conversion of CBD, several data are available in literature. In most cases, the compound reacted in acidic conditions [15] (including in artificial gastric juice) [16] and/or at high temperature [17] to form different THC regioisomers and isoTHC. The kinetic parameters for the degradation of different cannabinoids were examined in dried cannabis resins at different pH values, and the activation energy for the first order degradation of CBD in aqueous solution at pH = 2 was measured in 131.21 kJ mol−1 [18].…

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> *Citation Excerpt :*
>
> …HS-MCC-GC-IMS has similarly found application in the analysis of VOCs in goat cheese [182]. A number of recent papers dealt with cannabis analysis by IMS [42,236–239], where a particular focus has been on the use of IMS as a fast screening method for tetrahydrocannabinol (THC) and cannabidiol (CBD) [236,237]. Terpenoids are another important class of plant natural products which have benefited from the application of IMS, for example the analysis of lanostane-type triterpene acids by UHPLC-IMS-MSE using an in-house constructed MS and IMS database [240], and the analysis of terpenes in essential oils using HS-GC-IMS [232].…

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