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Does cannabidiol (CBD) oil convert to psychotropic cannabinoids including tetrahydrocannabinol?

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Abstract

Cannabidiol (CBD) is a non-psychotropic cannabinoid that belongs to the *Cannabis Sativa* L plant. This naturally occurring cannabinoid has gained huge interest in recent years because of the potential therapeutic and pharmacological effects it has upon ingestion and inhalation. Cannabidiol has not only been regarded as a medicinal product but is widely sold over the counter (OTC) in the form of concentrated oils, food supplements, cosmetics, and electronic cigarettes. Regulation of OTC products has proven difficult because of the narcotic nature of the cannabis plant and it has been advised that CBD might be degraded into psychotropic cannabinoids, namely tetrahydrocannabinol (THC).

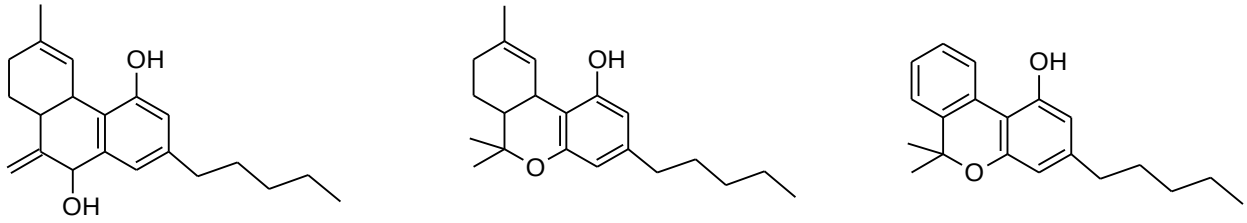
In current literature, orally administered CBD tablets have shown high incidence of drowsiness through ingestion. This is particularly of interest because in a previous study comparing the effects of Δ^9-THC and CBD in a sleep-wake cycle, it was found that THC promoted sleep whereas CBD caused wake-inducing effects perhaps suggesting evidence of THC in OTC products. This project aims to carefully review studies that have investigated degradants and metabolites of CBD and summarise findings of CBD degradation processes, specifically the results of *in-vivo* and *in-vitro* studies. Additionally, method development and instrumentation efficiency will be explored for the separation and quantification of CBD and THC to investigate if this can affect the degradation kinetics. To achieve these aims various literature searches were performed using key words to access relevant peer reviewed research. A thorough analysis of each paper was performed to provide an in-depth understanding of the potential degradation of CBD oil and other details such as method development, extraction efficacy, product recovery and a general picture of CBD in the medicinal and consumer industries.

In vitro and *in vivo* findings were presented that showcase a huge amount of controversy and highlight weakness in the argument that supports the conversion of CBD to THC. Many of the *in-vitro* investigations did not report changes to the psychological and motor functioning of participants and in those that did, the data was limited due to the small number of participants, no comparison to control groups and potentially inaccurate feedback that should be interpreted with caution. Although controversial, the evidence supporting acidic degradation is still viable, therefore product contamination and mislabeling has also been considered as well as variation and inconsistencies in production methods and biochemical influences such as 'The Entourage Effect'.

Keywords: Cannabidiol, Tetrahydrocannabinol, Entourage effect, Biochemistry, Pharmacokinetics, Cyclisation, Acid-degradation.

Introduction

Cannabis Sativa is a herbaceous species that originates from Central Asia[1], with the earliest uses dating back as far as 2700 B.C[2]. The interest in Cannabis was renewed in the 1990's with the discovery of cannabinoid receptors in the human body and the recognition of an endogenous cannabinoid system in the brain[3], a pivotal point for CBD research. The psychoactive and pharmacotherapeutic effects are attributed to a small number of cannabinoids (~60) from the nearly 500 compounds that belong to the plant[4]. Of this small subset, the major phytocannabinoids that exhibit psychotropic effects include Δ^8-THC, Δ^9-THC and the non-psychotropic include CBD, and cannabinol (CBN)[5]. The molecular structures of CBD, CBN and THC are shown in Figure 1, which are main compounds of interest in this study as well as some primary active metabolites of THC.



Cannbidiol (CBD) *Tetrahydrocannibinol (THC)* *Cannabinol (CBN)*

Figure 1: Structures of Cannabidiol (CBD), Tetrahydrocannabinol (THC) and Cannabinol (CBN)[1]

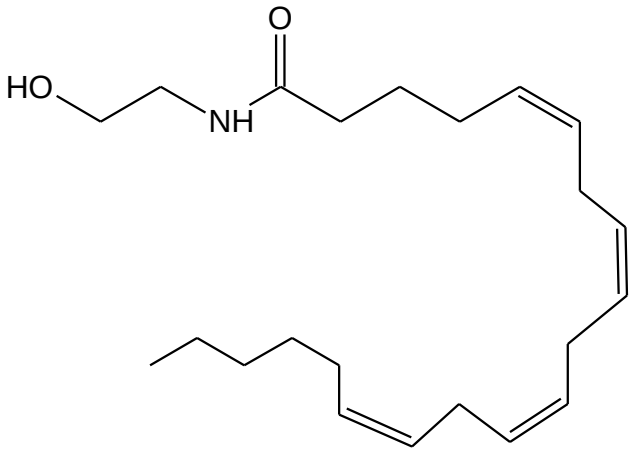
As shown in Figure 1, CBD and THC share some similarities and differences in their molecular structure which ultimately affect the way they react. Both CBD and THC have the same number of carbon, hydrogen, and oxygen atoms (21, 30 and 2 respectively) however the difference in molecular structure dramatically effects the reactivity and characteristics of these molecules[6]. The three rings (phenol, pyran and cyclohexane) that constitute the structure of THC, provide rigidity and are the main noticeable differences in comparison to the CBD molecule which exhibits a central 2-Methylidenecyclohexan-1-ol group. Geometrically, CBD is not a planar molecule due to the strong repulsion between both hydroxyl groups and the limonene ring[6].

Bioavailabilty and Pharmacokinetics of CBD

Cannabinoid receptors can be found throughout mammalian species; these receptors are part of the endocannabinoid signaling system that includes enzymes that are responsible for the synthesis and degradation of endocannabinoids. Endogenous CB_1 and CB_2 cannabinoid receptors can be found in various regions of the body; CB_1 receptors are associated with the brain, central nervous system and in tissue, whereas CB_2 receptors are found mostly in adipose tissue, the reproductive and immune system, the gut and other peripheral organs such as the eyes and muscles[7]. Cannabidiol acts as a molecular target to receptors that are found in mammalian tissue where it can act as an anagonist[4]. Biological agonists are chemicals that bind to receptors to activate them and generate a biological response. Not to be confused with an antagonist, a molecule that blocks action against the activity of an agonist. Exogenous CB_1 and CB_2 cannabinoid receptor ligands (CB_1 and CB_2 molecules originating outside of the body) are agonist molecules that are coupled through guanine nucleotide binding proteins. The source of these agonist molecules is likely from consumption or ingestion of a cannabinoid containing

substance such as CBD oil. The activation of these receptors has been demonstrated to reduce neuropathic and inflammatory pain and pain associated with multiple sclerosis[7,8,9]. The CB_1 receptors involved in this have been reported to specifically help manage muscle spasms, spasticity, and tremors due to their presence on nerve terminals which can mediate transmitter release when activated by agonists[8]. Cannabidiol also has an agonist effect on one of the serotonin 1A receptors (5-HT1A) found in the central peripheral nervous system, where it supports anxiolytic and antidepressant properties[10]. However, this data presents a modest affinity to the receptor[10], therefore additional work is required to compare the potential of CBD at other serotonin receptors to gain a broader understanding of how CBD can support antidepressant properties. Cannabidiol also acts as an agonist of the vanilloid receptors TRPV1 that are mostly expressed in the sensory nerve fibers and smooth muscle cells. The TRPV1 receptor does not present as great importance in comparison to the effects mentioned above and is only responsible for causing a hot, pungent sensation in the oral cavity[10], which is likely caused by the presence of the CB_1 receptors found within the nerve terminals and the molecules ability to manage the biological response.

Cannabidiol is directly involved in increasing anandamide signaling of endogenous cannabinoid receptors. Anandamide molecules, shown in Figure 2, are lipid mediators that act as ligands of CB_1 receptors. They are also primary molecular targets that are responsible for the pharmacological effects of Δ9-THC[9]. The molecular mechanism for the signaling events of cannabinoid receptors has not been fully established, therefore to fully understand the extent of these biological responses within the body further research into these mechanisms is required.



Anandamide

Figure 2: Anandamide molecule[9]

A host of other studies have clarified the effectiveness and therapeutic potential of CBD in the treatment of various diseases, predominately mental health disorders to include anxiety, depression, severe paranoia, and schizophrenia. In a crossover study comparing CBD to nitrazepam (a drug used to treat anxiety and insomnia), it was found that a high dose of CBD (160 mg), increased the patient's overall duration of sleep[11]. In six other clinical trail's, oral capsules containing CBD in a range of 600-

1000 mg were administered to patients suffering from mental health disorders (schizophrenia and anxiety disorder). Of these trials, five out of the six observed entirely positive effects which included improved recognition to emotional facial expression, reduced anxiety, and cognitive impairment[12,13,14,15,16,17]. Administration of CBD is not limited to treatment in humans, it has also shown anti-inflammatory, antipsychotic and anticonvulsant properties in animals[5].

Pharmacokinetics in the human body

The pharmacokinetic profile of CBD has been determined in the recent literature concerning bioavailability in the human body; this includes the evaluation of maximum serum concentration (C_{max}) and time to the maximum measured plasma concentration (T_{max}). Oromucosal spray and oral tablets are methods that are commonly used to uptake CBD into the human body. Evaluation of these methods and understanding the bioavailability and half-life of various consumer products gives insight into their ability to provide therapeutic effect and to monitor periods of maximum uptake to assess potentially undesired effect at its maximum.

Oralmucosal spray

In a systematic review of 24 eligible records, it was found that serum concentration was at a maximum between 1-4 hours after use, with C_{max} values occurring faster after inhalation when compared to oral ingestion[18]. The authors of this review evaluated various research papers that explore the efficiency of oromucosal sprays containing CBD with singular doses between 10-20 mg. One study that investigated the bioavailability of oromucosal spray, found that C_{max} was between 2.5 and 3.3 ng/mL and the T_{max} was between 1.64 and 4.2 hours[18]. It was also determined that C_{max} is dose-dependent; dosing of 20 mg/day resulted in 1.5 ng/mL C_{max} , while 60 mg/day saw an increase to 4.8 ng/mL C_{max}[18]. This has been supported by other research groups; one study evaluated the effect of gradually increasing single dosages on C_{max} values, which resulted in an exponentially increasing C_{max} value from 0.4 to 1.2 and 2.2 ng/mL following a 5, 10 and 20 mg/day dosing regimen, respectively[19]. Increases in CBD bioavailability have also been observed under fed and fasted conditions; in one study 12 men were given a single 10 mg dose/day of CBD oromucosal spray. Those in a fed state resulted in a C_{max} value of 3.7 ng/mL and those in a fasted state had a C_{max} value of 1.2 ng/mL, a 3-fold higher bioavailability when used alongside food[18].

Oral capsules

C_{max} following oral administration is also a dose dependent value. One research group found that a dose of 10mg CBD resulted in a C_{max} of 2.47 ng/mL at 1.27 hours and a dose of 400 or 800 mg resulted in a value of 181 ng/mL at 3.0 hours and 114 ng/mL at 1.5 hours (400 mg) and 221 ng/mL at 3.0 hours and 157 ng/mL at 4.0 hours (800 mg)[18]. However, the higher doses were co-administered with intravenous fentanyl (a potent opioid) which may have had an influence on the overall bioavailability and the results could be considered skewed. Another study that involved eight male and female smokers found that a dose of 800 mg oral CBD resulted in a C_{max} of 77.9 ng/mL and a T_{max} of 3.0 hours, this is much lower than the previous study which suggests that co-administration may increase bioavailability resulting in an unrealistic representation. An increase of dosing does suggest an effect on the overall bioavailability however the higher doses do not present a great difference, suggesting a saturation effect. Similarly to oromucosal spray, fed and fasted states have an effect on bioavailability of CBD in the form of oral capsules. In

one study 12 male and female participants in a fasted state were administered oral capsules containing 5.4 mg of CBD which resulted in a mean C_{max} of 0.93 ng/mL. This was followed by the same subset consuming a breakfast meal 1 hour after the capsules which saw an increased mean to 1.13 ng/mL. Another group that consisted of the administration of oral CBD tablets and an oromucosal spray to nine men found that those that had taken the oral capsules had a 4-fold increase in C_{max} when compared to spray (2.1 ng/mL vs 0.5 ng/mL).

Half-life

The half-life of CBD across various modes of administration was also evaluated which found that after taking oral capsules (20 mg) the half-life was 1.09 to 1.97 hours and for oromucosal spray (5-20 mg) the half-life was between 1.44 and 10.86 hours. However, it was found by a different research group that after chronic oral administration the half-life lasted between 2-5 days. Chronic administration refers to the slow development or long-lasting effect after administration. When comparing the pharmacokinetic differences between oral capsules and oromucosal spray it can be suggested that oral formulation is rather advantageous regarding higher bioavailability and higher dosing amounts (400-800 mg), whereas oromucosal spray is generally administered at a maximum of 20 mg/dose with subsequently lower C_{max} values. In some cases, the C_{max} for CBD capsules was significantly higher, therefore for consumers looking for greater uptake of CBD with higher dosing, oral forms may be the preferred method. It is noted however, that bioavailability of CBD in oromucosal spray form is much longer than in oral capsules and may be desired over capsules. It was discovered that after oral administration bioavailability of CBD spanned for much longer periods of time, in one study significantly longer periods between two to five days were reported.

From the research summated it can be suggested that the bioavailability of CBD and maximum serum concentration is dose dependent in both forms (oral spray and capsule form), where increased dosing resulted in an greater C_{max} values for both methods. For oromucosal spray, doses of 20 mg/day resulted in a C_{max} value of 1.5 ng/mL and 60 mg/day saw an increase to 4.8 ng/mL. Where oral capsules were used higher doses of 800 mg vs lower doses of 10 mg were evaluated, which resulted in a huge difference of 77.9 ng/mL and 2.47 ng/mL, respectively. Fed and fasted states were evaluated which resulted in higher bioavailability for those that had consumed a meal prior to being administered CBD. In experiments that utilised a fed state prior to CBD administration in both forms (oral mucosal and capsule) higher plasma levels were observed. Oral mucosal spray contributed a three-fold higher bioavailability when used during a fed state vs a fasted state (1.2 vs 3.7 ng/mL) and four-fold increase for oral capsules (0.5 vs 2.1 ng/mL).

Cannabidiol is a well-accepted non-psychotropic cannabinoid in the medical and consumer market, with well evidenced therapeutic effects demonstrated from controlled studies. It has been demonstrated that CBD has a positive safety profile, as recorded in the positive results in treatment. However, CBD is not without scrutiny and debate; many researchers have observed psychotropic side effects in humans and other mammalian species that are typically associated with THC, raising concerns regarding the stability of CBD once investigated. Various types of analyses including *in-vitro*, whilst *in-vivo* studies have been conducted to evaluate the potential formation of psychotropic cannabinoids. *In-vivo* are concerned with the simulation of gastric and physiological conditions through forced acidic degradation,

in-vitro studies focus on the effects reported by volunteers and patients that have been administered CBD in various different forms, their blood and urine samples and the reporting of any undesired or unexpected effect.

Transformation of CBD to THC is likely to cause effect

Transformation of CBD to THC is likely to cause consumer and medicinal products to suffer changes that are likely to be caused when under acidic conditions. It has been found that CBD transforms into Δ^9-THC via an acid-catalyzed cyclisation, and in oxygen is oxidized to dimeric and monomeric hydroxyquinones[19]. A proposed reaction mechanism is given in Figure 3 showing the acidic cyclisation route, giving THC as the resultant product with regeneration of the acidic species[20]. In the first step an acid is added to a solution containing CBD which involves the pi bond of the methylene group attacking the proton of the acid resulting in the generation of a tertiary carbocation. The second step shows the oxygen of the hydroxyl group attacking the positively charged carbocation resulting in a cyclic rearrangement. The final step involves the proton from the hydroxyl group being lost which regenerates the acidic species and the product THC is formed.

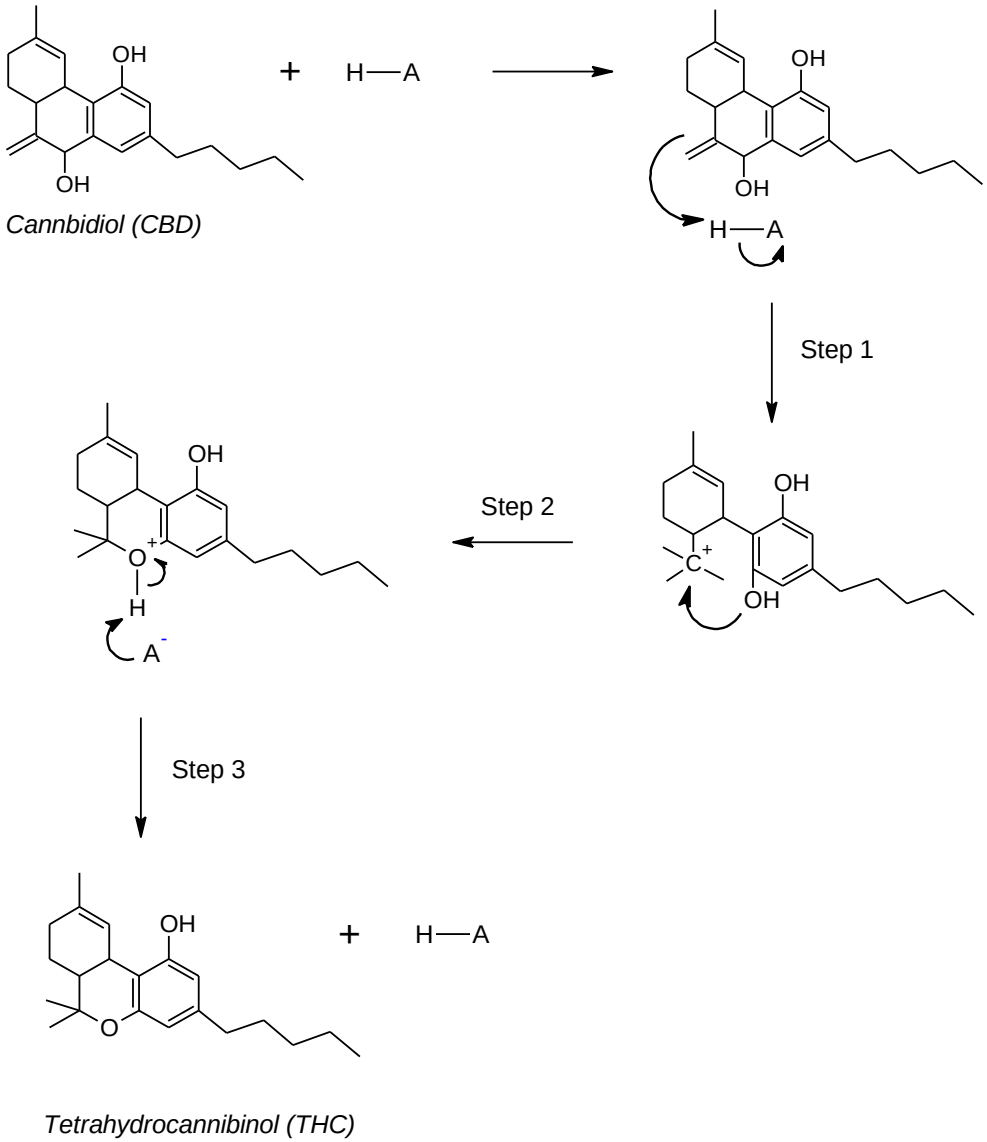


Figure 3: Acid catalyzed cyclisation reaction of CBD to THC[20]

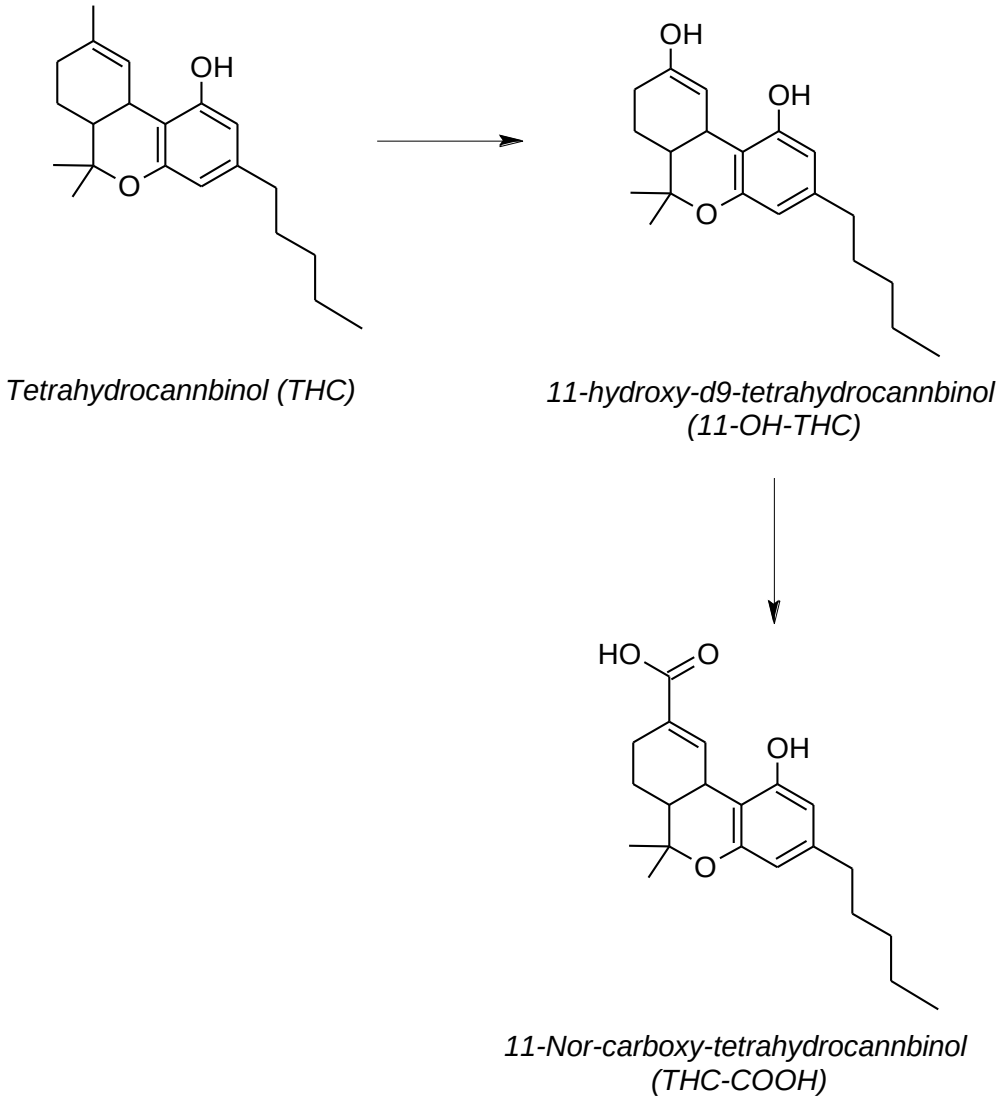
The mechanism proposed in Figure 3 is insightful and helps to understand the potential steps associated with acid cyclisation, however more research is required to evidence this mechanism. Supporting evidence in a recent epilepsy paper found that pediatric patients showed high incidence of drowsiness (21%) and fatigue (17%) after taking orally administered CBD[5]. These symptoms were also observed in five other studies[21,22], coupled with poor cognitive and motor function[22,23] and one study finding strong sedative effects[25]. These effects are typically associated with THC not CBD and may suggest the degradation as shown in Figure 2. Other research groups have found that Δ^9-THC can be formed in the acidic conditions of the stomach, as well as other metabolites including 6β-hydroxymethyl-Δ9-tetrahydrocannabinol, that have psychotropic properties similar to THC[26] raising further questions regarding the use of CBD products. In mammalian research it was demonstrated that CBD conversion produced THC-like effects, including hypothermia and catalepsy in mice[27]. Studies such as these support the claim that CBD does convert to THC, however for the three trials that mentioned fatigue, sleepiness, poor cognitive and motor function, the number of participants was low with the highest number of participants reaching only 225[21] and the lowest with only 10 participants[23]. This is not enough to conclusively confirm degradation, as typically thousands of participants are required to obtain a more accurate picture.

Effects of high doses of cbd in human trials

The effect of high dose CBD in human trials has been explored in various research papers[21,22,23]. The findings from an epilepsy patient study sparked huge interest in this field and has been pursued in various review papers which disagree completely with the sedative and fatigue like effects demonstrated in previously mentioned studies[28,29]. In one investigation, a research group conducted two separate studies to evaluate the possibility of interconversion of CBD with the participation of healthy volunteers[28]. The first study had a participation of 60 mixed sex volunteers between the ages of 18 and 50. The administration of 600 mg of oral CBD took place after a standardized meal and blood samples were taken every 30 minutes for six hours in total. The second study had the same number of participants, but the administration of CBD was during a fasted period (eight hours). Blood samples were taken from both groups and analysed using an ultra-performance liquid chromatography (UPLC) instrument coupled with a mass spectrometer.

The results of both studies found no signs of Δ^8THC or Δ^9THC in whole blood after oral administration. In a different study, a sample of eight men and women suffering from Huntington's disease (a neurodegenerative disease) were administered 10 mg/kg/day of CBD oil[30]. The blood samples obtained after 15 weeks were tested for parent CBD and THC, not the subsequent metabolites. The authors found detectable levels of CBD however THC was not detected in the plasma. In a third study 16 healthy volunteers received 600 mg of oral CBD in a double-blind experiment[31]. The authors reported no elevation of THC; however, levels of 11-hydroxy-D9-tetrahydrocannabinol (11-OH-THC) and 11-Nor-9-carboxy-THC (THC-COOH) were not only detected but a steady increase was observed in the 3 hours after acute administration was observed[31]. These psychoactive metabolites of THC can exhibit commensurable and sometimes greater effects than THC[31,32]. Various metabolic routes for the conversion of THC to THC-COOH have been proposed in the literature; however, the main route is shown in Figure 4 where THC metabolises into

THC-COOH *in-vivo*[32]*.* It has been suggested that THC interacts with specific cannabinoid receptors in the body which include the opioid and benzodiazepine receptors resulting in the primary active metabolite (11-OH-THC) and the primary inactive metabolite (THC-COOH)[32]. A mechanism of action has not yet been established however it has been suggested that the 11-OH-THC metabolite is a product of hydroxylation of the methyl side-chain, which is enzymatically catalyzed by CYP2C9, an enzyme responsible for metabolizing non-steroidal drugs[29].



Tetrahydrocannbinol (THC)

11-hydroxy-d9-tetrahydrocannbinol (11-OH-THC)

11-Nor-carboxy-tetrahydrocannbinol (THC-COOH)

Figure 4: Metabolic route of THC to THC-COOH proposed to take place within the human body[32]

The results of the high dose CBD studies allude to two major suggestions; the first is that the cyclisation of CBD to THC may occur, but that the THC molecule does not exist long enough in the acidic conditions of the gastric region before being converted into the primary active metabolites (Figure 4). The second suggestion is that CBD may degrade or cyclize to these metabolites rather than THC itself. The metabolic route that has been proposed (Figure 4) has been confirmed by the detection of the primary psychotropic metabolite's (THC-COOH and 11-OH-THC)[31].

The other investigations cited gave no mention of evaluating metabolites but rather focused on the concentration of THC after oral CBD dosing[28,30]. Further investigation should be carried out to ascertain whether or not the proposed mechanism is realistic and to assess a wider range of molecules to include THC and all the primary active metabolites.

Methods for quantification of cannabinoids and acid degradation

Two common approaches when investigating the forced acid degradation of CBD include *in-vivo* and *in-vitro* studies*. In-vivo* approaches tend to utilize human participants or animal testing to identify how CBD is affected in biological systems. Fed and fasted states and periods of time where alcohol and caffeine are in constraint are thematic. The subsequent blood, urine or plasma samples are then evaluated. *In-vitro* studies often recreate the conditions associated with the gastrointestinal region by using various acids, namely hydrochloric acid (HCl), (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer and simulated gastric fluid (SGF), which is typically prepared using (0.1 M, 7 mL) HCl buffer solution in water (1 L pH of 1.2), 3.2 g of pepsin and 2.0 g of NaCl and is maintained at a temperature of 37°C[5]. Incubation periods are commonly used in the preparation of CBD samples with a variety of temperatures and periods observed. Incubation periods are used to mimic *in-vivo* conditions to nominally represent the maximal time of exposure of the substrate to the environment. Incubation periods also highlight rate kinetics and relationships between concentration of CBD used and CBD degradation/THC formation. The most commonly observed kinetics are first-order[1]. Once these experiments have been completed a variety of analytical techniques that have been developed for the qualification and quantification of cannabinoids can be applied. These include ultra-performance or high-performance liquid chromatography (UPLC or HPLC respectively) with UV detection, tandem mass spectrometry (MS) and gas chromatography that can be coupled with a flame ionisation detector (GC-FID) or a mass detector (GC-MS). Other commonly used detectors coupled with HPLC include MS/MS with UV detection.

Review aims

This review considers the safety, stability, and efficacy of CBD products by analyzing the findings from multiple studies that focus on the potential acid degradation of such products to produce THC. It is important to monitor stability, especially from a manufacturers point of view because CBD treated patients and consumers in general may be exposed to levels of THC and other psychotropic cannabinoids that may exceed the threshold for a physiological response. As CBD products become increasingly popular so does the importance to quantify and characterise cannabinoid profiles to ensure uniformity and quality of the preparations. A range of quantitative and qualitative techniques have been renewed in the literature, alongside various advances and developments, all of which will be critically evaluated in this review.

Method

Relevant papers were critically evaluated for factors such as participant numbers, methods used, results and findings and for evidence supporting the acid degradation of CBD to THC. A combination of keywords such as ‘cannabidiol’, ‘cannabinoids’, ‘acid degradation’, ‘quantification’ and ‘analytical’ were used and research papers from Google Scholar, Cochrane Library (Wiley), PubMed, ACS Publications and

Plymouth University Primo databases have been searched in English. In total the number of papers considered was 77. The methods were used for *in-vitro* and *in-vivo* type analyses were reviewed in order to compare the effect of CBD, reported THC like effects and potential contamination, mislabeling and the stability of consumer products. This section will critically evaluate the results of various methods to highlight trends or conflicting results.

In-vitro studies

Cannabinoid samples and their origin

To assess stability of CBD, forced degradation experiments have been conducted and documented in the literature to highlight the potential production of psychotropic products such as Δ^8-THC and Δ^9-THC, using an *in-vitro* approach. Each investigation used various cannabinoid samples (Table 1), which indicates trends in manufacturer concentration and purity, and highlights variance in the final concentration of the stock solution used in analysis. Of the seven publications reviewed here, six purchased reference CBD/cannabinoid samples that ranged in concentration (1-3 mg/mL). From the seven different science and technology companies there was no preference towards one particular source. The final study prepared a sample of in-house CBD that was extracted from plant material by maceration in methylene chloride for 24 h[33]. The concentration of extracted sample was 1 mg/mL, which was then diluted to 10 µg/mL. A trend was identified for the manufacturer's concentration, which showed that most research groups purchased cannabinoid standards at 1mg/mL. The concentration of the purchased stock solutions after dilution varied greatly, with a range of 10 ng/mL to 40 mg/mL depending on what analytical instrument was used.

Table 1: Cannabinoid samples (manufacturer concentration, stock concentration and purchase manufacturer)

MATERIALS	PURITY	MANUFACTURERS CONCENTRATION	CONCENTRATION STOCK SOLUTION	MANUFACTURER	REFERENCE
Synthetic CBD	99%	1 mg/mL	40 mg/mL	Zynerba Pharmaceuticals	[5]
	-		200-400 µg/mL	Supelco Cerilliant	[33]
	100%		2 µg/mL	Sigma Aldrich	[34]
	99.9%		10 ng/mL	LGC standards GmbH	[35]
	-		5-50 µg/mL	Restek Corporation	[36]
Reference solution containing 7 cannabinoids including CBD	-		10 µg/mL	Absolute standards INC.	[37]
In-house isolated CBD prepared from cannabis plant material	-	3 mg/mL	1 mg/mL	The National Center for Natural Products Research, University of Mississippi	[38]

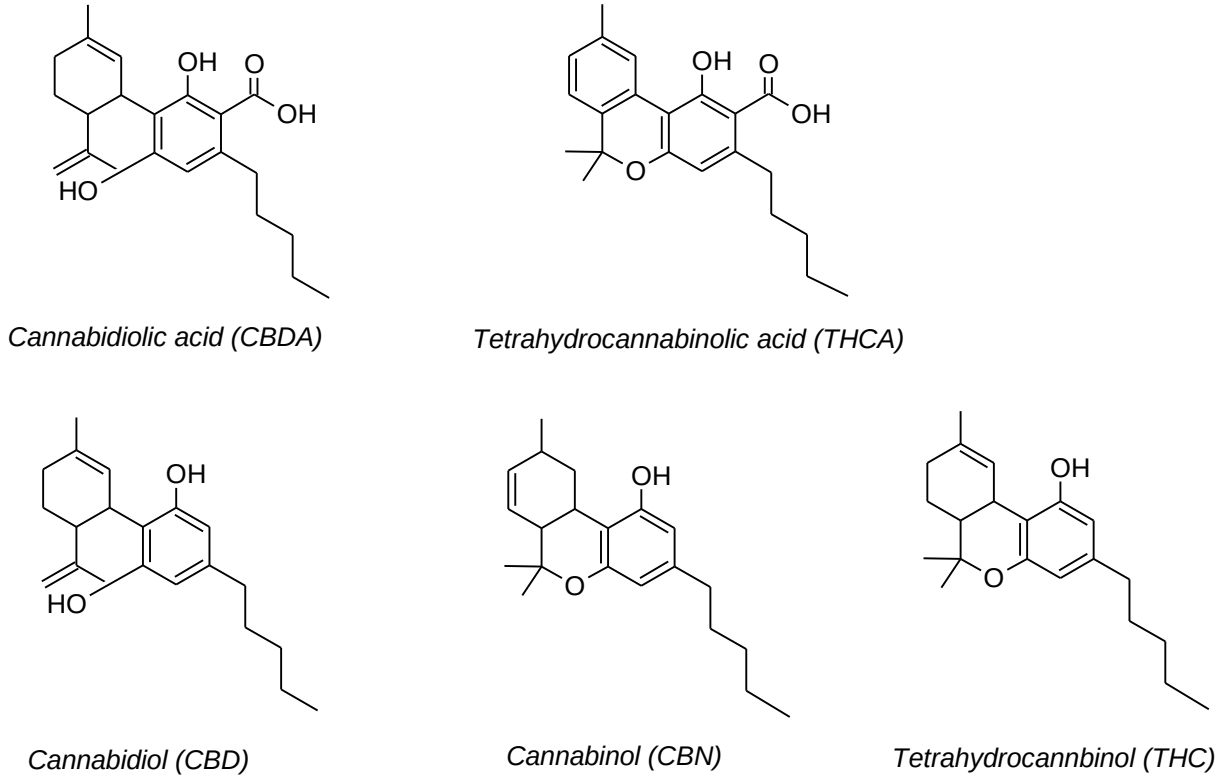
Commonly used instrumental techniques

Multiple papers have reported the successful and rapid separation of cannabinoids using various instrumental analytical methods. These include HPLC, UPLC and GC with subsequent detectors that include flame ionisation detection (FID), mass spectrometry (MS), ultra-violet (UV) and photo diode array detection (PDA). Some methods employ two detectors, for example HPLC-UV-PDA or HPLC-MS/MS (two MS detectors). Other methods including ultra-high-performance supercritical fluid chromatography (UHPSFC) have also been investigated. This section aims to critically evaluate each method, highlighting advantages and disadvantages and drawing attention to similarities or differences across all methodologies. Extraction

and separation of cannabinoids from plant material and purchased standards will also be discussed including the subsequent method of separation and quantification.

Key properties of common HPLC methods

Instrumental parameters of various HPLC instruments are shown in Table 2, which have been derived from seven research papers[5,34,38,39,40,41]. Some of the major compounds analysed are shown in Figure 4 which include CBDA, THCA, CBD, CBN and THC.



Cannabidiolic acid (CBDA) *Tetrahydrocannabinolic acid (THCA)*

Cannabidiol (CBD) *Cannabinol (CBN)* *Tetrahydrocannbinol (THC)*

Figure 5: Cannabinoid compounds featured in analysis[1]

Some themes have been established; six of the seven studies used a gradient elution with isocratic elution typically disregarded. One study mentioned that isocratic elution resulted in a long analysis run time of 36 minutes, which suggests the method is unsuitable for routine determination where a larger volume of samples is considered[40]. Of these seven methods, two utilised acetonitrile as mobile phase B[34,39], the remaining methods varied.

Table 2: HPLC and UPLC instrumental parameters (mobile phase, operating mode, column, flow rates, temperatures and detectors used)

INSTRUMENTAL PARAMETERS							
ANALYSIS TYPE	**UPLC[5]**	**UPLC[39]**	**UP CONVERGENCE CHROMATOGRAPHY[39]**	**HPLC[40]**	**HPLC[34]**	**UHPSCF[38]**	**HPLC[41]**
Mobile Phase A	2 mM ammonium formate pH 4.8	Water	CO_2	Methanol: water	0.1% formic acid	CO_2	0.1% formic acid and 2 mM ammonium formate
Mobile Phase B	Methanol	Acetonitrile	200 proof ethanol		Acetonitrile	Isopropanol: water (80:20)	0.1% formic acid in methanol and acetonitrile (50:50)
Operating mode	Gradient	Gradient: Mobile phase B: Starting at 73% with linear increase to 90% over 6 minutes	Gradient: Mobile phase B: Starting at 3% with linear increase to 13% over 9 minutes	Isocratic	Gradient: 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 min and held for 2min	Gradient: 4.0% B to 9.0% B in 4.5 min, and then to 30.0% B in the next 2.5 min (hold 3 min)	Gradient: 83% mobile phase B to 98% mobile phase B in 6.5 min
Column properties	Waters HSS $C_{18,}$ 50 x 2.1 mm x 1.8 µm	ACQUITY UPLC CSH $C_{18,}$ 130 Å x 1.7 µm, 2.1 x 50 mm	Trefoil Cel1 C_{18}, 2.5 µm, 3.0 X 150 mm	Zorbax C_{18}, 3.5 µm, 100 x 4.6 mm	Agilent C_{18}, 2.7 µm, 4.8 x 50 mm	ACQUITY UPC $C_{18,}$ 1.7 µm, 3.0 X 150 mm	NexLeaf CBX II, 1.8 µm, 3.0 x 100 mm
Flow rate	0.5 mL/min	0.6 mL/min	2.0 mL/min	1.0 mL/min	0.5 mL/min	1.4 mL/min	0.5 mL/min
Oven temperature	50 °C	30 °C	50 °C	25 °C	-	30 °C	30 °C
Injection volume	10 µL	2 µL	2 µL	10 µL	20 µL	1 µL	5 µL
Detector	UV-MS	PDA-MS	PDA-MS	UV	MS/MS	PDA MS	MS/MS
Wavelength	222 nm	225 nm	225 nm	220 nm	-	220 nm	-
Mass range	315.2	-	-	-	300-360 m/z		-

Carbon dioxide appeared twice for the mobile phase A[38,39], two other studies used 2mM ammonium formate and formic acid[5,41] and another two used water (one in methanol)[39,40]. There is no obvious pattern for mobile phase A however the three methods mentioned indicate some similarities. The flow rates for each method ranged from 0.5 to 2.0 mL/min, however the most popular method was 0.5 mL/min used in three studies[5,34,41]. A theme was observed for the oven temperature where

30°C was utilised three times[38,39,41] and 50°C twice[5,39] with the least common temperature at 25°C employed only by one study. A range of 2 to 20 µL was used for injection volume, however the most common volumes were 2 and 10 µL. Detection methods included MS/MS, PDA-MS, and UV-MS, suggesting these techniques are generally preferred for the quantification of cannabinoids, with MS/MS occurring twice and PDA-MS three times. Where wavelength data was available the wavelengths ranged from 220-225 nm, however the most typical absorbance wavelengths were 220 nm and 225 nm, both of which appeared twice. In summary some themes have been identified, which suggest that the most popular parameters employed for the quantification of cannabinoids using HPLC methods include a flow rate of approximately 0.5 mL/min, the employment of MS/MS or PDA-MS detection, 220-225 nm wavelength set-point, and an injection volume between 2-10 µL.

Incubation periods, physiological buffers and their effect on cbd

Incubation periods and physiological buffers are often used in cannabinoid degradation experiments to assess the stability of oral capsule products. Acidic physiological buffers are used to recreate the conditions of the gastrointestinal region to investigate the effects of acidity on CBD containing products. Various incubation periods have been trailled to indicate the rate kinetics of any degradation that may occur. One study group used a sample of 1.0 mL synthetic CBD (40 mg/mL) in simulated gastric fluid (SGF) and another sample of the same concentration in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer for analysis to detect possible psychoactive products via acid catalyzed cycliziation[5]. Good linearity by HPLC-UV was observed for CBD (R^2 = 1.000), Δ^8-THC (R^2 = 1.000) and Δ^9-THC (R^2 = 0.999) and it was reported CBD degradation and THC product formation was rapid.

In SGF, CBD degraded 85% after 60 min incubation and 98% after 120 min incubation which demonstrated first-order rate kinetics of -0.0031 min^{-1}. The formation of THC isomers plateaued as CBD was consumed and the THC levels were impacted by secondary degradation to other unknown substances. In the HEPES buffer there was no evidence of CBD conversion to Δ^8-THC or Δ^9-THC. This research is crucial in understanding the rate of degradation which can used to estimate conversion of CBD in the body after oral dosing and to estimate at what concentration is CBD bioavailable for degradation in a gastric environment. In a second study 165 mg of crystallized CBD was dissolved in 10 mL of ethanol and prepared in 0.1 M hydrochloric acid (HCl)[39]. This sample was then incubated at 60°C for 24 H prior to analysis using reversed phase-UPLC.

A reference standard containing a matrix of various cannabinoids including CBD, Δ^8-THC and Δ^9-THC was also eluted as a reference. After exposure to acidic conditions significant degradation products were observed. Degradation products eluted at 2.9, 4.3 and 4.5 mins which indicated the presence of CBG, Δ^8-THC and Δ^9-THC, respectively. Peak identity was achieved using PDA and UV detection with retention time matching. Information regarding the stereochemistry was achieved by re-injecting the sample into a UPC system by convergence chromatography which indicated the main naturally occurring isomer (Δ^9-THC) was fully separated from the less common isomer Δ^8-THC. This is very important because Δ^9-THC has been documented to be 6-100 times more potent than Δ^8-THC[39]. Although two studies have provided strong evidence that CBD can degrade and subsequently produce psychoactive products this has been opposed strongly in the literature. In one review it was suggested that the *in-vitro* model used to study the conversion of CBD to Δ^8-

THC and Δ^9-THC did not accurately represent a gastrointestinal environment[29]. The SGF was considered highly artificial with no mention of including gastric enzymes or electrolytes in the fluid, therefore resulting in test conditions that deviate markedly from a natural gastric environment[29]. For SGF to better match physiological conditions it has been suggested the medium contains HCl, sodium chloride (NaCl), water and pepsin achieving a pH of approximately 1.2. This preferred method of preparing SGF however, still significantly deviates from natural conditions. Gastric pH is likely to lie in a pH range of 1.5-1.9 and can reach 3.5. Gastric fluid also contains many proteins and enzymes which are involved in digestion such as gastric amylase and gastric lipase, in addition to other inorganic analytes such as calcium, sodium, and potassium. Gastric transit time is variable, with an average order of time has been established to be between 2.5 to 3 hours.

In the second study featured in this section[39] where CBD was dissolved in 0.1 M HCl, it could be argued that the conditions do not truly represent acidity in the gastric region. Although significant degradation was observed these results cannot be used to provide evidence for physiological degradation, which is important for assessing stability of consumer products such as oral capsules. Acid degradation gives a good insight for product stability however is not useful for relating to human consumption. The structures of CBD and THC shown in Figure 5, differ slightly, and the hydroxylation of the methyl chain on CBD molecules is likely caused by the enzyme CYP2C19, which also contributes to the suggestion that recreation of gastrointestinal conditions is unattainable.

Method comparison of hplc and gc

Commonly used techniques in potency testing of cannabinoids include HPLC, UPLC and GC, all of which have been successful[41]. The major advantage of HPLC over GC is that the acid components, tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) can be characterised before conversion to their corresponding free forms (THC and CBD). This is a particularly useful method for OTC products, edible materials containing extracted tinctures and for original plant material potency testing. Gas chromatography does not detect the acid forms (THCA and CBDA) directly because the carboxylic acid groups found in the acid forms convert to free cannabinoids by in-situ decarboxylation in the intense heating of the GC injector port which is typically maintained around 200-300^{o}C.

GC mimics the conversion and decarboxylation process that takes place during smoking of plant material. Whilst GC is not appropriate to study the acid forms of cannabinoids, one study developed a method where silyated cannabinoids were used to avoid the decarboxylation process in the GC injection port[41]. Derivatisation can also be used prior to GC analysis, but may add extra time to the overall analysis. In another study GC vacuum ultra-violet (VUV) analysis was used, resulting in the rapid detection of cannabinoids[41]. This method had a very high limit of detection (LOD), which is rather disadvantageous in detecting analytes in biological matrices[41], although the specific LOD was not mentioned it is an important consideration, nonetheless.

Gas chromatography is faster than HPLC methods with an analysis time of approximately 20 minutes compared to an average run time of 36 minutes for HPLC, therefore is often preferred but is not advantageous for testing of acidic

cannabinoids[42,43]. One of the more widely practiced techniques in industry for the quantification of cannabinoids is HPLC coupled with UV and PDA detection due to its robust and inexpensive approach. Instruments with tandem use HPLC and ESI spray interfaces coupled with MS detection have demonstrated high efficacy and clear resulting chromatographs that identify acidic and non-acidic analogues of cannabinoids. One research group analysed a mixture of 16 cannabinoids with each analyte prepared at a concentration of 10 ppm in methanol. The major analytes of interest were CBD, CBG, CBN, Δ^8THC and Δ^9THC. Using this the identification of neutral compounds via positive ionisation (Δ^9THC and CBD) and their acidic analogues in negative ionisation mode was successful[42]. Coelution of CBD and CBG was reported during this separation however by implementing MS detection differences in molecular weight were identified and fragmentation patterns were obtained for both molecules, resulting in highly specific data where all compounds were distinguished[42].

Various disadvantages have been identified in the literature concerning the use of HPLC. An average run time of approximately 25-36 minutes has been reported which makes the method unsuitable for a large number of samples, especially for routine testing[40]. Secondly, in a previous report the peaks for CBD, cannabigerol (CBG) and THCA were reported inadequate for accurate determination and were non-distinguishable from the other compounds. In contrast to this, methods that utilised UPLC with tandem MS and UV have displayed excellent chromatographs with little to no interference in multiple publications[43,44]. In one report, authors commented on the high reliability of UPLC instruments used especially for orthogonal separation of naturally occurring cannabinoids (CBD, Δ^8THC and Δ^9THC) by reversed phase convergence chromatography[39]. It was highlighted, however, that the retention times of Δ^8THC and Δ^9THC are extremely close and therefore special diligence must be implemented to avoid misidentification[39].

Matrices that consist of multiple cannabinoids have been reported to co-elute, which may adversely affect the reproducibility of the analyte ionisation, especially for methods that rely on HPLC-MS electrospray ionisation[29]. To avoid co-elution, some method developments have been made. Firstly, samples containing CBD have been spiked with concentrations of THC to verify any possible matrix effect. Secondly, changes to selectivity of the chromatography and MS can be made to improve chromatograms visualization[29]. For example, in one study the mass transition m/z 313>245 for CBD was filtered, resulting in higher resolution[29]. Liquid chromatography often employs atmospheric-pressure chemical ionisation (APCI) and electrospray ionisation (ESI) sources. These methods usually generate a pronated molecule without effective fragmentation, allowing diagnostic information to be acquired[42]. Cannabinoids also have carboxylic and phenolic functional groups that are not ionized effectively using these ionisation techniques[41], and HPLC-MS generally offers a few advantages over other techniques such as GC-MS which include greater separation efficacy, higher sensitivity, and mass identification[42,43].

It is important to consider the impact detectors have on the overall efficacy of cannabinoid separation and characterisation. One of the more versatile hardware configurations that has been suggested is the use of a GC-MS channel with a second injector and a FID in a second channel[44]. GC-MS channels can be used with a small diameter capillary column for a reduced flow rate and subsequently higher resolution[45,46]. GC-FID has an edge over GC-MS because it makes use of cheap

authentic standards while MS usually requires equivalent deuterated standards which are much more expensive and often not available for all cannabinoids[46]. This is because FID consists of a more accurate quantitative response with respect to MS, therefore often corresponding deuterated standards are used to give a more accurate response, although these are often commercially unavailable for all minor cannabinoids[46]. It has also reported that GC-FID has a markedly lower sensitivity than GC-MS, where GC-FID only has a sensitivity of approximately 1 µg/mL, whereas GC-MS can reach values below 1 ng/mL[46]. Another important consideration when choosing the appropriate instrument for analysis is the cost including set-up, running and maintenance. These factors are very important in industry especially for routine analysis. It has been reported that the running of GC and HPLC instruments is rather cost effective, however the cost of equipment can be increased when coupled with more expensive detectors. For example, for HPLC, coupling of MS detectors will further the cost, which has been reported to be more expensive than GC-MS[41].

Key properties of common GC-MS and GC-FID methods

Some of the key properties from the GC-MS methods used across four different investigations has been summated in Table 3. GC is particularly ineffective for the quantification of cannabinoids in their acid forms due to high column temperatures where the acidic forms of the cannabinoids undergo decarboxylation. However, derivatisation prior to analysis can be performed to preserve the cannabinoid structure and also increase volatility which results in an improved peak shape[46]. One study used silylated cannabinoids to avoid in-situ decarboxylation and deemed this method pertinent for analysis[46], although this has been contradicted by other studies. One research group found the LOD when using a silica capillary column was high suggesting that detection of analytes in biological matrices is rather ineffective unless derivatization has been perfomed[43]. In all mentioned studies helium was the preferred carrier gas because it provides higher efficacy than other commonly used gases such as nitrogen and hydrogen[42]. Each study used different oven programmes, including the temperature ramp and holding times. Where available, a mass range of 40-500 m/z was observed which suggests the range is the most applicable for cannabinoid analysis. The mass range of the main cannabinoids has been reported between 316.5 and 310.4 which validates the mass range chosen in the methods shown in Table 3.

Some key properties of GC-FID methods are shown in Table 4. Nitrogen was featured twice as the carrier gas and helium only once. It has been reported in previous studies that nitrogen has greater efficacy and low cost, whereas helium is efficient but not as affordable[49]. Various capillary columns were used including the use of a silica column which may have been used as an attempt to reduce in-situ decarboxylation.

As demonstrated successful separation of cannabinoids can be achieved using GC-FID and GC-MS, with various different oven programmes whilst avoiding decarboxylation to produce non-psychotropic metabolites. One study reported a high LOD therefore suggested prior derivatization was required to prevent in-situ decarboxylation, however the LOD value was not included[46].

Table 3:. Key properties of common GC-MS methods (capillary column)

CAPILLARY COLUMN PROPERTIES	STARTING OVEN TEMPERATURE °C	OVEN PRCOCESS °C	CARRIER GAS	MASS RANGE M/Z	CANNABINOIDS ANALYSED	REFERENCE
Silica capillary DB1	10	10/min increase until 280. Hold for 30 min	Helium	-	CBD	45
Silica capillary DB1	100	10/min increase until 280. Hold for 30 min		-	Δ^9-THC, CBD, CBGA, CBDA, CBN, CBG, THCA	45
Phenylmethyl siloxane capillary column (5% cross linked)	50	6/min until 300. Hold for 4 min		40-500	CBG, CBD, CBGA, THC, CBC, THCA, CBDA, CBN	46
Poly-5% diphenyl-95% dimethyl polysiloxane capillary column (cross linked)	45	2/min increase until 100 reached. Then 5/min up to 250. Hold for 5 min.		40-500	CBD-CBDA	47
Phenylmethyl siloxane capillary column (5% cross linked)	50	6/min until 300. Hold for 4 min		40-500	THC, CBC, CBN, CBG, CBD	46

Table 4: Key properties of common GC-FID methods (capillary column)

CAPILLARY COLUMN PROPERTIES	STARTING OVEN TEMPERATURE °C	OVEN PRCOCESS °C	CARRIER GAS	CANNABINOIDS ANALYSED	REFERENCE
Capillary DB5	60	3/min increase until 240. Hold for 5 min	Nitrogen	CBD	50
Silica capillary DB1	100	10/min increase until 280. Hold for 30 min	Nitrogen	Δ^9-THC, CBD, CBGA, CBDA, CBN, CBG, THCA	46
Poly-5% diphenyl-95% dimethyl polysiloxane capillary column (cross linked)	45	2/min increase until 100 reached. Then 5/min up to 250. Hold for 5 min.	Helium	CBD-CBDA	49

Summary of *in-vitro* methods

It can be advised that HPLC coupled with MS detection or UV-PDA are the most effective methods for separation of cannabinoid matrices and for the identification and quantification of compounds. HPLC is a great method because changes can be made easily to improve the quality of results; for example when considering HPLC-MS, m/z filters can be altered resulting in higher resolution spectra and co-elution can also be avoided using this method by spiking samples with known concentrations of cannabinoids to indicate to avoid any potential matrix effect[29]. It has been reported in the literature that HPLC-MS is significantly more cost effective

than HPLC-UV and GC-FID/MS due to the greater confidence afforded in identifying compounds, therefore would be perform very well in routine analysis to reduce costs[41]. Methods that utilize HPLC rather than GC have shown significant advantages which include greater separation, higher sensitivity, and greater mass identification when coupled to an MS detector. Additionally, HPLC can identify acidic forms of cannabinoids whereas GC methods cannot perform this analysis with great confidence. The efficiency of decarboxylation conversion remains questionable, which provides researchers with no confidence and suggests that GC methods may not be suitable for this type of analysis[38]. It has been reported that in-situ decarboxylation can be avoided, however one to avoid decarboxylation results had a very high LOD[44]. Derivatization can be used to avoid decarboxylation; however this step requires more time and resources and can be avoided all-together when employing HPLC[29]. As shown in Table 2 the methods utilised by six research groups show only the employment of UV-PDA and MS detectors[5,34,40,41,42], UV-PDA was described as a robust and inexpensive method that was capable of producing clear chromatographs of acidic and non-acidic analogues[29]. Further research has found that certain HPLC methods have inadequate resolution for chromatographic separation when using complex matrices[38]. It was also discovered that HPLC is ineffective without tandem mass spectrophotometric techniques such as triple quadrupole MS and these instruments often require optima grade organic solvents, feature high maintenance costs, and require expensive instrumentation that is not appropriate for routine analysis[38].

In-vivo studies

Oral dosing of CBD in human trails

Various studies have been designed to investigate the potential for acid degradation of CBD to THC in the body due to the acidic conditions of the gastric region, typically ranging in pH between 1.5-1.9[29]. It has been reported in various papers that after oral CBD administration strong sedative effects, poor cognitive/motor function and high drowsiness was observed[5,23,24]. Unlike THC, CBD does not cause acute effects on cognitive and motor function, therefore reports of undesired and unexpected effects from oral CBD administration have sparked great interest in this field of research. In this section CBD dosage concentrations across various groups that include a range in age, gender and health status will be assessed to identify any changes to the patient's motor and cognitive functions and any psychological responses. The methods observed in each study will also be analysed to indicate trends in dosing, study type and length of time period the studies were conducted over.

The first study was conducted using a placebo controlled randomized double-blind study of oral CBD in patients with Dravet syndrome (a rare drug-resistant epilepsy that presents as prolonged seizures with fever that effects one side of the body)[51]. In total, 120 patients between the ages of 2.3 and 18.4 years were administered 20 mg/kg/day of oral CBD split into two dosages for a total of 14 weeks. It was reported that 20% of patients experienced fatigue and 13% experienced lethargy[51]. In another study, 72 patients suffering with sleep disorders were assessed by daily oral dosing of 25 mg/day CBD capsules for a total of 4 weeks[11]. Three of the patients reported mild sedative effects whilst two patients discontinued the trial due to complaints of fatigue. These two studies in particular raise suspicion due to the undesired side effects observed that are not typically associated with taking CBD, which supports

the claim that CBD may convert to THC in the body. In another randomized double-blind placebo-controlled study 16 healthy male subjects between the ages of 20-42 years were given 600 mg/day CBD in the form of oral capsules; no psychological difference between the placebo and CBD capsules was reported[52]. A further study conducted a small-scale investigation where six male and four female volunteers were given 200 mg/day oral CBD capsules for one week and advised to abstain from alcohol, then during the second week the volunteers were given 1g/kg of alcohol whilst continuing CBD dosing[23]. It was observed that alcohol and CBD alone did not caused significant impairment of psychomotor performance, however the combination of alcohol and CBD simultaneously resulted in significant decrease in accuracy for finger tapping responses (1 min extra response time) and cognitive function was also impaired. However, the researchers concluded that no evidence was provided to suggest that CBD inhibited any pharmacological effects[23]. Lastly, a double-blind study with patients suffering with heroin use disorder was conducted across a mixed gender group of 50 participants between the ages of 21 and 65 years[53]. Each day 400 and 800 mg tablets were administered randomly to patients to assess acute, short-term consequences of CBD administration to heroin cravings and cognitive function. There was no significant difference in cue-induced cravings which was assessed using the Heroin Craving Questionnaire Scores utilised by the researchers.

In summary, the data from the studies mentioned above indicate that any significant changes to the physiological state or motor and cognitive ability of the participants were not consistent or comparable to the other studies. It was found that patients in two different trials reported mild to severe psychological effects that even resulted in the discontinuation of their participation[11,51]. The remaining research groups collectively agreed no side-effects on the patient's psychological state, cognitive or motor function, which suggested that CBD is safe and well tolerated. It is important to mention that there are many limitations in clinical case studies that rely on the feedback from participants and results should be interpreted cautiously. In one of the mentioned studies[53], patients had previously been taking strong opioid drugs that have been known to change the physical structure and physiology of the brain which can result in long-term imbalances in the neuronal system[52].

It has also been suggested that deterioration of the brains white matter, which affects the ability to regulate behavior and respond to stressful situations, is a likely side effect of taking opioid drugs[52,53,54]. With that in mind it is possible that the results reported from patients in that particular study may have had a limited ability to make genuine observations and report accurate side effects during CBD treatment. There was also no comparison to a control group of healthy volunteers using CBD as treatment or in groups suffering with opioid withdrawal trialing a placebo, therefore the results are significantly biased and can be considered inaccurate, which are therefore rather fruitless when comparing to other studies. The hypothesis proposed by some researchers[5,23] about the conversion of CBD to THC under acidic conditions such as gastric fluid has raised some doubt. This proposal has not been confirmed by *in-vivo* studies where it is expected that some of the metabolites associated with THC such as 11-OH-THC or THC-COOH should be detectable in the blood or urine, however this has not been observed by multiple groups trialing oral CBD[31,23]. Nonetheless there is still clear evidence in the literature that acidic degradation of CBD to THC has taken place, therefore further investigation into the products themselves, concerning stability and contamination is recommended.

Results and discussion

Are THC-like effects caused by contamination of cbd products or mislabeling?
Intoxicating side-effects that are synonymous with THC have been reported in anecdotal clinical studies[11,51], whilst other studies have reported the formation of psychotropic compounds from CBD products during acid-degradation experiments[5,39]. Although this is a topical research area, there is still no definitive evidence on whether CBD undergoes acid-degradation cyclisation in the body resulting in psychotropic side effects typically associated with THC and related analogues. For this reason, this section aims to investigate whether product contamination and mislabeling of CBD containing products could be a cause of these reported side effects. Commercial OTC CBD products are typically comprised of extracts sourced from whole hemp plants and are extracted by supercritical CO_2 with the use of polar solvents such as isopropanol and ethanol[33]. Further purification is required to ensure a pure CBD product however this step is frequently disregarded due to the high cost. Therefore, CBD extracts are regularly sold as a cannabinoid mixture rather than pure CBD[33]. In the case of further purification, methods such as partial fractionation are employed using supercritical CO_2 and the extracts are then considered chemically pure[33].

Many researchers have commenced investigation to study the possible influence of THC contamination in commercial CBD products and mislabeling which results in incorrect dosage amounts of CBD and THC[33,57,58]. One study assessed potential contamination by sampling 67 CBD containing products that were registered as food supplements in Germany[33]. Samples were analysed using an HPLC-MS/MS method to assess THC content and were measured against the lowest observed adverse effect level (LOAEL) which has been determined by the European food safety authority as 2.5 mg/day of THC[55]. Out of the 67 samples, 17 samples (25%) had the potential to exceed the LOAEL and 29 samples (43%) exceeded the LOAEL and were classified unsuitable for human consumption. Overall, it was concluded that all samples were non-compliant with EU regulations.

The average dose of THC that leads to intoxication and adverse psychotropic effects is considered to be in a range of 10-20 mg/day for cannabis inhalation and resorption of orally ingested THC will vary depending on the individual, therefore side effects will vary[33]. A single oral dose of THC in the region of 20 mg results in what has been described as a “high sensation” otherwise referred to as dysphoria in adults that is typically maintained between 1-4 hours[33]. In some adult’s smaller doses of 5 mg have resulted in similar symptoms[33] therefore it can be suggested that THC dosages of 5 mg and above can put consumers at risk of intoxication. In one of the CBD supplements sampled in this particular study, a concentration of 30 mg THC in a bottle of 10 mL CBD extract was observed, which explains the adverse effects experienced by some consumers. Any CBD product with doses of approximately 1 mg of THC/serving provide the possibility to achieve intoxicating and psychotropic effects, therefore it has been suggested that these products are unsafe for human consumption. Routine testing and more time spent on purification must be performed to avoid putting consumers at risk[33].

In another study conducted by the FDA (Food and Drug Administration) in 2020, 102 ‘Black Market’ CBD products were tested to indicate THC contamination and

mislabeling[56]. It was discovered that 18% of products contained less than the specified amount of CBD, 47% contained approximately 20% of the total amount specified and 38% contained more than 120%. The most worrying statistic revealed that 49% of all products contained THC. The Journal of the American Medical Association published the results of an investigation assessing 84 samples of purchased CBD containing products[57]. Of the samples tested, 21% contained THC which claimed to be in relatively high concentrations that could be responsible for intoxication in children[57]. In another study published by the National Institute of Health, it was found that many products were mislabeled, with 26% of products containing less CBD than advertised and 46% containing more which indicates high variability within products and poor standardization[58]. This research group also indicated that oil-based products are more likely contain the accurate concentration of CBD when compared to extracted tinctures and vaporization liquids (45%, 25% and 12.5% respectively)[58]. These rather variable results may explain some of the side effects reported in the literature such as fatigue, drowsiness, and decreased appetite[5,22,23,24,25]. It has also been discovered that more than 40% of children with epilepsy who were given CBD orally exhibited THC like symptoms[59].

Quality traits of cannabidiol products and the influence of volatile terpene profiles 'The entourage effect'

Terpenes and cannabinoids share the same biosynthetic pathways due to the terpenophenolic profile of CBD[60]. In raw cannabis plant material, terpenes are in fact stored and secreted together amongst cannabinoids in glandular trichomes (specialized surface hairs found on cannabis plants that are sites for biosynthesis and storage for metabolites[60]). One study in particular found that terpene and cannabinoid compounds can be found in all trichrome types of cannabis plant material[61]. Terpenes found in cannabis flowers not only contribute to the aromatic profile of CBD extracts, but they also contribute to the therapeutic abilities of CBD oil by acting as co-activating agents that enhance the beneficial activity of phytocannabinoids in humans. This synergic action of cannabinoids and terpenes is known as "The Entourage Effect" which is the suggested contribution to the effect of cannabinoids from the addition of terpenes. This means that inactive terpenes can accompany primary endogenous cannabinoids to increase the overall activity. This concept has been described as a botanical synergy, where the dominant molecule gains the support of other plant derivatives such as terpenes and flavonoids to achieve the maximum pharmacological effect. The described synergy can also apply to THC and CBD, where both molecules co-activate each other, enhancing the beneficial effects of each compound. Around 200 different terpenoids have been isolated previously from Cannabis plants and can be found in concentrations of approximately 1%, with concentrations of 10% within trichomes[60].

It has been reported in a previous study that even low or negligible concentrations of terpenoids can increase or decrease activity levels in rodents and are productive in behavioral effects due to their high potency[61]. Classification of terpenoids has been assessed by the US FDA and federal emergency management agency (FEMA) who recently published a listing of 50 cannabis terpenes that are encountered routinely in commercial and illegal CBD containing products. Of these listed, eight predominate terpenes were classified as the most popular which include: limonene, linalool, α-pinene, terpinolene, ocimene, myrcene, β-caryophyllene and humulene. A schematic has been provided in Figure 6 which represents some of the commonly

found terpenes in cannabis plant material that facilitate the pharma-therapeutic effects of CBD. For example, myrcene (Figure 6), a terpene found in Cannabis strains is known to decrease anxiety and induce relaxation. In some Cannabis sativa strains that are rich in limonene enhancement of feelings of alertness and arousing behavior were observed which are likely attributed to the enhancement of some THC strains and CBD like affects[62]. Cannabis indica strains that are rich in myrcene are known to induce relaxation and decrease anxiety, therefore it is assumed that combining terpenes with cannabinoids enhances mood stabilizing and therapeutic effects.

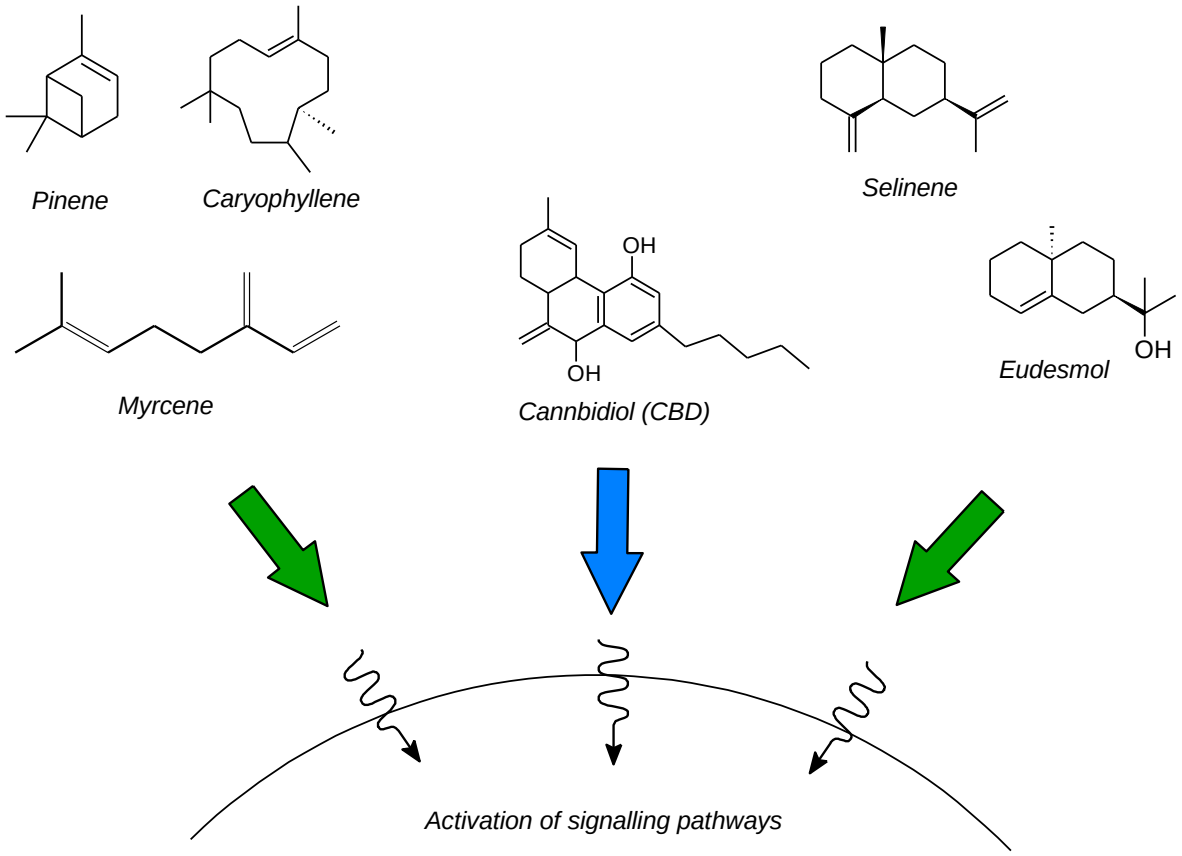


Figure 6: "The Entourage Effect" of terpenes and cannabinoids entering a cell[61]. Squiggly arrows represent the synergistic co-activating process that happen before pharmacotherapeutic effects are observed. Green arrows represent co-activating molecules, blue arrow representing CBD.

One study investigated the terpenoid contribution to the herbal synergy of Cannabis, where the nomenclature "chemovar" was used to describe the unique biochemical attributes of a particular cannabis plants that consist of predominantly of THC and CBD[62]. The researchers found that β-myrcene was the most common terpene in cannabis chemovars in the USA and in Europe, which is likely the cause of narcotic sedative effects, otherwise known as "couch-lock"[62]. In another study, the synergy between CBD and THC was evaluated, which concluded many positive benefits including anti-anxiety, antipsychotic effect, and the reduction of tachycardia[63]. It was also found that terpinolene predominant chemovars were found to be energizing in humans, however in animals produced sedative like effects[64]. Further double-blind

testing human and animal studies should be undertaken to understand this difference. The suitability of cannabis to the treatment of psychiatric conditions remains questionable, however the success of this may be improved with certain chemovars that contain generous CBD but minimal THC[65]. Chemovars with high linalool and limonene has been suggested for possible reduction in depression and anxiety and therefore may also be appropriate for such treatments[65].

The same research group that investigated terpenoid contribution also performed a patient survey that consisted of 30 participants conducted over a 7-week period[62]. The survey was designed to evaluate various side-effects reported by patients after smoking medical grade cannabis. The participants were split into 6 groups and were given cannabis twice a day via inhalation that consisted of varying cannabinoid ratios and terpene profiles to compare notable differences in side effects that may be attributed to certain terpenoids (Table 6). Some of the side effects that were investigated include sedation, anxiety, alertness, focus and energy. The researchers found that non-myrcene dominant terpene profiles provided an increase in energy and alertness, whilst in the myrcene dominant cannabis sample a decrease in both was reported. In the terpinolene dominant samples increases in energy were reported, whereas samples containing ocimene and no terpinolene produced a calming effect. The limonene and pinene dominant chemovars produced increased focus, particularly in the pinene groups. Limonene, linalool and ocimene dominant groups promoted positive and ‘inspired’ attitudes, as reported by participants in the mood metric section of the survey.

Table 4: Comparator terpene groups[62]

TERPENE CLASS	COMPARATOR TERPENES
A	Pinene, myrcene
B	Limonene, linalool, humulene, caryophyllene
C	Myrcene, ocimene
D	Terpinolene, ocimene
E	Myrcene, ocimene, linalool, caryophyllene, pinene
F	Linalool, myrcene, caryophyllene, limonene

With the understanding that the predominance of certain terpenoids such as myrcene in CBD containing products are likely the cause of sedative effects, this may begin to explain some of the undesirable side-effects that have been reported in previous studies such as drowsiness and fatigue[11,21,22,25,51]. Low to moderate doses of CBD have been proven to induce ‘alertness’ and the ability to counteract drowsiness which has been mentioned in other studies[61], therefore this contradiction may be explained by “The Entourage Effect” phenomena. Other researchers have turned to chemovar profiling and entourage effects to understand and predict the unique aromatic profile characteristics of many chemovars. One research group in particular created an intuitive report called ‘PhytoFacts’ which displays a complete chemical analysis of terpenoids and cannabinoids in Cannabis plant samples[66] and has been illustrated and featured in many publications[62,67]. The researchers utilise a

pie chart to display expected 'entourage effects' that are associated with specific terpenoids in a given chemovar. An algorithm was also designed by a collection of customer inputs who had used certain chemovars, which can then be applied to the biochemical effects. To reduce or eliminate some of the undesired effects in CBD products selective breeding of low myrcene chemovars may also be suitable. Manufacturers may want to evaluate the specificity of their chemovars and raw cannabis plant so that drowsiness and sedation are reduced significantly. Some researchers have reported that terpenoid production can be controlled with light exposure[67]. In general, greater light exposure increases terpenoid yield, however this decreases soil fertility, and it was also mentioned that to achieve controlled terpenoid yield and high crop quality, repeated selective breeding should be maintained[67].

An overwhelming amount of positive and relevant effects that are attributed to terpenoids found in CBD products have been reported in the literature, even at low concentrations of approximately 0.05%[62]. D-limonene is a ubiquitous molecule found with great abundance in nature that demonstrates powerful anxiolytic and antidepressant properties, which has been demonstrated in mice[68]. This research has been confirmed in a human clinical study where hospitalised patients suffering with depression were exposed to a limonene containing citrus fragrance, with subsequent normalization of 'Depression Scores'. Of the 12 patients assessed, nine successfully discontinued anti-depressant medication and serum evidence of immune stimulation was observed[69]. Limonene has also been successful in the production of apoptosis (a process of elimination to rid the body of damaged cells) in breast cancer treatment. High doses were given to patients in Phase II which resulted in apoptosis[70].

In a different study that compared pure CBD to high content CBD hemp oil in the treatment of breast cancer cells it was found that botanical drug preparations (those containing pure CBD) were more effective than the pure samples at reducing cell viability[70]. Conversely another research group examined if CBD oils isolated from hemp would be more potent in reducing cancer than pure CBD, thus supporting "The Entourage Effect" and found no increased efficacy for CBD oils. This suggests that in reducing cancer cells, there may not be any effect. The researchers also noted that contributions from the less abundant molecules such as phytochemicals and flavonoids are very minor[71]. Synergistic interactions between cannabinoids and terpenes (inter-entourage) and between different cannabinoids (intra-entourage) has been reported by many other researchers where the whole plant extract had superior effect to purified cannabinoids, which contribute lower concentrations of terpenoids and phytochemicals[72,73,74].

Other promising indications of terpenes used in medical and clinical scenarios have been reported in the literature. For example, α-pinene has been used in the treatment of agitation that is typically associated with dementia[75]. It was also reported that alongside treatments using THC, therapeutic effects were increased when α-pinene was used. Another example of chemovars that contribute synergistic effects are those containing linalool, caryophyllene and limonene, these specific chemovars have shown clinical efficacy in reducing burns and in epilepsy treatment[76]. The suitability of cannabinoids to treat certain psychiatric conditions remains questionable, however in the studies discussed here there is positive indication. Clinical success may also be improved by focusing on the use of chemovars that are generous in linalool, limonene, and CBD, with minimal THC and

myrcene to avoid sedative effects but to increase efficacy for the improvement of anxiety and depression. Further evidence of this synergy can be evaluated with randomized double-blind clinical trials or by evaluating psychometric differences in brain activity depending on the terpenoid contribution or profiling of a specific chemovar.

Is the stability of cbd containing products compromised by stressful environments?

In the scientific community, concerns have been raised on the quality of CBD containing products such as oils, vaping products, and edible tinctures. These issues mostly concern thermal and photo-stability and mislabeling which has been evaluated previously in this review. The thermal and photo-stability of commercial CBD products has emerged as a major issue, however, to date only one research group has evaluated the chemical stability upon storage where multiple commercial samples are considered[77]. Upon investigation of thermal stability, the researchers found that CBD samples stored at approximately 4 °C decreased in concentration by an average of 5%, samples stored at room temperature decreased in concentration between 7-11%, and samples stored at 37 °C decreased in concentration by approximately 10% on average. This suggests that to preserve CBD, samples should be stored at lower temperatures and should not reach close to room temperature. This may prove difficult especially during transit and when products are stored in a commercial building such as a shop. The same research group also investigated the photo-stability of CBD samples by storing solutions for 30 days at room temperature. Identical samples were placed alongside each other, one wrapped in aluminum foil (dark control) and one under natural sunlight (light control).

The results showed a detrimental effect on the chemical stability of CBD in natural light where an average decrease in concentration of 13% was observed, whereas dark control samples only demonstrated an average decrease of 4%. It is evident that thermal and photo-stability are important for the consideration of CBD products, especially in regard to the potential degradation that may cause the formation of psychoactive products such as THC. These alarming data sets discussed here, where the concentration of CBD samples decreased by a noticeable amount may be explained by incorrect manufacturing practices or by degradation of the phytocannabinoids due to incorrect storage conditions which can be reduced if further time is spent on planning the correct storage considering thermal and photo stability.

Conclusions

Does CBD oil convert to psychotropic cannabinoids including THC? The literature regarding this inquiry is rather disconcerting and will remain this way until further research into acid degradation and *in-vivo* testing is undertaken that evaluates not only the results of forced degradation in natural and realistic conditions but also the source of the sample. When evaluating the literature for *in-vivo* testing it was evident that sample testing was not employed by researchers prior to the participants being administered CBD. Many participants reported side effects such as fatigue and drowsiness[5,22,23] and some reported that these effects were so severe they discontinued the course of CBD[11,51]. When considering the effects of environmental stress on CBD samples, the composition of terpenoids found within CBD that may augment ‘The Entourage Effect’ and the ubiquitous mislabeling it is possible that the results of clinical studies could be inaccurate and therefore the reports from patients

not entirely accurate. The acid degradation of CBD products via metabolic routes may be more accurately assessed by considering to a greater degree the source of the CBD, and the stability of the given sample which may have been mislabeled or degraded in stressful environments prior to administration. Considering the terpenophenolic profile of CBD samples is also very important because of 'The Entourage Effect' that has been reported to co-activate the existing physiological and psychotropic effects of CBD and THC. Myrcene in particular has been proven to induce sedative effects and terpinolene is known to create an energizing effect[62,63], which may have influenced the effects or indeed co-activated CBD in human studies.

Many other important areas of the literature must be evaluated thoroughly to gain a full understanding of how CBD may be affected by external stresses, the biochemical composition of the CBD, and the source it originated from before any assumptions can be made about acidic degradation. Method development must also be considered as many inconsistencies were identified in this review which may affect the data and conclusions drawn. Conversion of CBD to psychotropic cannabinoids is contested with many research papers supportive of this statement and many others against it. Suggesting additional work is required to investigate this further as the topic appears confusing and misleading. CBD is often referred to as non-psychotropic when compared to THC, however given that CBD has shown pharmacological benefits for multiple mental health conditions including anxiety, depression, and schizophrenia it is more beneficial to consider CBD as non-intoxicating. The major suggestion, however, is that CBD could be converted to THC after exposure to gastric fluid that has been simulated. In the literature, three types of gastric fluid were featured that include SGF, HEPES and HCL.

After thorough investigation of these papers, it can be concluded that none of them accurately represent the true conditions of the gastric region, suggesting that the results of these experiments should not be directly compared to natural metabolic degradation. The researchers found that HEPES buffer did not contribute to any degradation, whereas SGF and HCL both did. Immediately HEPES type buffers can be disregarded in this instance due to no degradation taking place, however method development may be employed for SGF type fluids to ensure a more realistic version is created before performing CBD degradation experiments. Due to the complexity of the natural conditions in the gastric region that range in acidity and organic substances such as pepsin, mucus, and proteins creating a realistic SGF will be challenging.

Another consideration is that the acidity in the stomach changes frequently throughout eating cycles. The conditions in the experiment using HCl as the fluid for acid degradation were extreme and did not represent the gastric region palpably, although insightful the pH of the acid used in this investigation (pH approx. 1.5) was unrealistic in comparison to natural conditions. Various mechanisms that suggest the probable route of degradation of CBD to THC and some of the by-products associated with the routes have been suggested by researchers. Although the isomerisation reactions and routes of degradation that have been proposed (Figures 3 and 4) are rather insightful and can be considered realistic, they have not been confirmed fully and further research must be undertaken these mechanisms.

Of the papers reviewed that evaluated blood and urine samples after CBD administration, only one reported traces of THC-COOH and 11-OH-THC, which does

support the likelihood of the metabolic degradation of CBD to THC (Figure 4), however this is one study, and more research must be undertaken. Another budding issue to mention is the inconsistencies across human studies where CBD was administered for patient survey and psychological feedback questionnaires. The first of which was the very small number of participants featured in these investigations, which ultimately limited the integrity of the results. Clinical trials typically feature thousands of participants which confirms efficacy, evaluate its effectiveness and monitors side effects. For all the papers mentioned in this review however, the highest number of participants was 120 and the smallest group was 10, which dramatically reduces reliability of the results. Some of the participants recruited for CBD trials were also being treated for the side effects associated with addiction after consuming opioid type drugs. These types of drugs are known to cause damage to the brain, which could impact the integrity of the patient reports. Further investigation with larger participant sizes and with those who are fit and well could improve the results by giving a more realistic and genuine response.

CBD is an intriguing compound that has exceptional diversity with regard to the observed effects. It has been demonstrated that CBD does convert to THC in more extreme forced acidic conditions however there seems to be no compelling evidence that CBD undergoes bioconversion and cyclisation to THC during human metabolism, therefore consumers should not experience undesired side effects. Armed with the knowledge that some monoterpenoids display prominent narcotic like profiles that are seemingly responsible for sedative effects, with careful consideration of the source of the CBD product and selective breeding these compounding effects can be avoided. If CBD products are correctly labelled and controlled within the right environments to maintain the integrity of the product, consumers should be advised that CBD is a safe and therapeutic treatment that does not cause intoxicating effect.

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