Cannabinoid profile and regulatory compliance of non-scheduled cannabinoid-containing products in South Africa

By

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Declaration of originality

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Abstract

Cannabis contains numerous chemical compounds and has a multitude of therapeutic and pharmacological properties. The most-studied compounds in the cannabis plant are the cannabinoids, of which Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) are the most well-known. South African cannabis legislation has seen momentous changes in recent years, and recreational and medicinal cannabis use was legalised in 2018. There are a variety of cannabis products available for purchase in South Africa, many in the form of CBD oils. The aim of this study was to determine the cannabinoid profile in cannabinoid-containing products and to determine the compliance of these products to South African regulations.

Six CBD oils available in South Africa were selected for analysis and purchased online in both a 'summer' and 'winter' batch. A robust, validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed for the analysis of four cannabinoid compounds; THC, CBD, cannabinol (CBN) and tetrahydrocannabinolic acid-A (THCA). Cannabinoid content and batch-to-batch conformity was assessed in two batches of the six CBD oils. Targeted analysis was achieved with a C18 Phenomenex Gemini column (2 x 100 mm) using an isocratic gradient programme consisting of 10 mM ammonium formate in water: acetonitrile (0.1% formic acid) 32.5:67.5 at a flow rate of 0.4 mL/min for 13 minutes. Additionally, the immediate and outer container labels of all CBD oils were scrutinized to determine compliance to labelling regulations.

The optimised method was validated according to the International Conference on Harmonisation (ICH) guidelines, and the limit of detection (LOD) was determined using the calibration curve for each analyte. Regarding CBD concentration, the selected CBD oils failed to meet label claims as advertised CBD content was mislabelled in almost all CBD oils. Three CBD oils contained CBD concentrations between one quarter and one half higher than the advertised amount, while two contained double, and two contained triple the advertised CBD content. Conversely, the measured CBD concentrations in two CBD oils were between a quarter and a half less than the advertised CBD content. The disparity between advertised and measured CBD content may be ascribed to the differences in cannabinoid extraction

and detection methods employed, as well as the type of cannabis strain used. Concentrations of THC, CBN and THCA were not detected in significant amounts in five of the six CBD oils (<0 mg/mL, with detection limits of 293.2 ng/mL for THC, 30.9 ng/mL for THCA and 47.2 ng/mL for CBN). High concentrations of both THC and CBN were detected in one CBD oil; at 47.97 mg THC and 15.4 mg CBN in batch 1, and 131.5 mg THC and 26.3 mg CBN in batch 2, respectively.

A lack of batch-to-batch conformity regarding cannabinoid content was noted for all samples of both batches. This may be a consequence of seasonal variation in cannabis plants used for CBD oil production. An inspection of the immediate and outer container labels revealed that the selected CBD oils complied with most labelling requirements stipulated by The Medicines and Related Substances Act, (Act 101 of 1965); with the exception of one CBD oil, which contained no labels besides the proprietary name.

South Africa still has many hurdles to overcome in terms of effective cannabis regulation, and measures need to be implemented to ensure unscheduled cannabis products used for medicinal purposes adhere to local regulations. The importance of cannabis education cannot be underestimated; and both cannabis users and health care professionals should be well-informed on clinical and legislative aspects to encourage a sustainable and bright future for the South African cannabis industry.

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I hope my research, findings and efforts will be beneficial to the scientific community and future of the South African cannabis industry.

List of abbreviations

CB	Cannabinoid
CBC	Cannabichromene
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBG	Cannabigerol
CBGA	Cannabigerolic acid
CBN	Cannabinol
CDA	Central Drug Authority
CPC	Centrifugal partition chromatography
CYP	Cytochrome P450
EMA	European Medicines Agency
ESI	Electrospray ionisation
FDA	Food and Drug Administration
GABA	γ-aminobutyric acid
GMP	Good manufacturing practice
GPR55	G protein-coupled receptor 55
HCP	Health care professional
HIV	Human Immuno-deficiency virus
HPLC	High performance liquid chromatography
HPLC-PDA	High performance liquid chromatography-photo diode array
ICH	International Conference on Harmonisation
IS	Internal standard
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MCT	Medium chain triglyceride
MRM	Multiple reaction monitoring
MS	Mass spectrometry
PDA	Photo diode array
RPLC	Reversed phase liquid chromatography
SAHPRA	South African Health Products Regulatory Authority
SFE	Supercritical fluid extraction
S/N	Signal to noise
Δ^9-THC	Δ^9-Tetrahydrocannabinol

TRPV1	Transient receptor potential cation channel subfamily V member 1
THCA	Δ^9-Tetrahydrocannabinolic acid-A
UNODC	United Nations Office on Drugs and Crime
USA	United States of America

Glossary

Abuse of Dependence-producing substances and Rehabilitation Centres Act (Act no. 41 of 1971): Provides for the prohibition of various drug-related activities; including the detention and interrogation of certain persons and the establishment and registration of rehabilitation centres and hostels.

Central Drug Authority (CDA): An advisory body established in terms of the Prevention of and Treatment for Substance Abuse Act (Act no. 70 of 2008) and is mandated to assist in the fight against substance abuse in South Africa.

Decriminalisation: The act of relaxing penalties associated with cannabis to make cannabis use or possession a non-criminal offense (as opposed to legalisation).

Drugs and Drug Trafficking Act (Act No. 140 of 1992): An act passed in South Africa concerning various criminal activities relating to drugs, such as criminalising the possession, sale or manufacture of drugs and other matters concerned therewith, including an obligation to report information to the police.

Endocannabinoid: Endogenous ligands of cannabinoid receptors that are expressed throughout the central nervous system and periphery.

Legalisation: The act of abolishing a law previously deeming something as illegal/prohibited, and will no longer be subject to criminal penalties (as opposed to decriminalisation).

Medicines and Related Substances Act (Act 101 of 1965): A set of regulations published by the South African Health Products Regulatory Authority (SAHPRA).

Medical Innovation Bill: A bill proposed in February 2014 seeking to make provisions for innovation in medical treatment and legalise the use of cannabinoids for medical purposes and beneficial commercial and industrial uses.

South African Health Products Regulatory Authority (SAHPRA): A statutory regulatory authority concerned with governing the manufacture, distribution, sale and marketing of medicines, scheduled substances, medical devices and *in vitro* diagnostic devices (IVDs); including the regulation of clinical trials and related matters which are of public interest.

The Medical, Dental, and Pharmacy Act (Act No. 13 of 1928): An act passed in South Africa that prohibited the use, sale and production of "habit forming drugs"; of which restrictions on cannabis use are included.

The United Nations Single Convention on Narcotic Drugs (1961): An international treaty that aims to combat drug abuse and trafficking through coordinated international cooperation directed at limiting the possession, use, trade, distribution, import, export, manufacture and production of narcotic drugs exclusively.

United Nations Office on Drugs and Crime (UNODC): A United Nations (UN) office established in 1997 to assist the UN in addressing issues of illicit trafficking and abuse of drugs, as well as crime prevention and criminal justice.

World Health Organization (WHO): A specialized organization concerned with international public health and member of the United Nations Development Group.

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Chapter 1: Introduction

The cannabis plant has a complex biological profile, and contains over 400 chemical compounds, of which 60 are cannabinoids.[1,2] Δ^9-Tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) are among the most well-known and widely-researched cannabinoids found in cannabis products, including CBD oils.[3,4] Cannabis products are available in a variety of formulations, many of which are unscheduled and freely available in South Africa, following the recent change in cannabis legislation. Globally, cannabis legislation differs between countries and is subject to regulation by each country's own constitution and laws. Determination of cannabinoid content in commercially sold preparations is prudent in order to assess the quality and safety of unscheduled CBD oils.

1.1 Cannabis plant

The cannabis plant is commonly used for its fibres, seeds, seed-based oils and medicinal purposes.[5] Cannabis is not indigenous to South Africa and is thought to have been introduced to southern Africa as early as the 15th century by Arab or Indian traders where it was used as a herbal remedy by Khoisan and Bantu tribes.[6] The Khoisan tribes used the word 'dacha' to refer to cannabis which was then adopted into the word 'dagga', a commonly used colloquial term in South Africa used to refer to cannabis.[7] Resin secreted by the female cannabis plant can also be eaten, and is commonly referred to as 'hashish'.[8,9]

Cannabinoids are a unique class of low-molecular weight lipophilic compounds acting on cannabinoid (CB) receptors within the body.[1,2] Cannabinoids are located in the seeds, stalks, flowers and leaves of the cannabis plant. Cannabinoids include phytocannabinoids (cannabinoids derived from the cannabis plant), endocannabinoids (endogenous cannabinoid neurotransmitters that bind to cannabinoid receptors in the body) and synthetic cannabinoids.[10] Of the phytocannabinoid compounds, tetrahydrocannabinol is the most active isomer, with the delta-9 (Δ^9) form being the main psychoactive compound.[11] Cannabidiol is the main non-psychoactive component of cannabis, eliciting no biological interaction with endogenous cannabinoid receptors.[8,12] Although cannabinoids are among the most well-known and most researched constituents of the cannabis plant,[13] other cannabis constituents include flavonoids, alkaloids, terpenes, steroids, cardiac glycosides, resins, saponins and quinones.[14]

There are three strains of Cannabis that are most commonly cultivated; *Cannabis sativa*, *C. indica* and *C. ruderalis* (family Cannabaceae).[15,16] The chemical composition varies between species, and as such affects pharmacological activity. With regard to cannabinoid content; *C. sativa* has a higher THC content, which results in mood-lifting and intoxicating effects, and is thus preferred by users seeking 'a high'.[13,17] *C. indica* contains a higher CBD content and provides calming, sedative and relaxing effects.[18] Hemp is a species of the cannabis plant grown for industrial purposes due to its lower THC content.[19]

1.2 Medicinal and recreational use of cannabis

1.2.1 Cannabis formulations

Cannabis extracts are available in different formulations, and the THC and CBD content varies between formulations.[10,20] Cannabis products can be THC or CBD dominant, or a hybrid product containing high concentrations of both CBD and THC. Administration may be topical (lotions or balms), oral (tablets, oils, edibles or tinctures),[21] or inhaled via smoking.[22] Other formulations include capsules, suppositories, and vaporising oils.[15,23,24] Cannabis can also be dried, cured and processed into cigarettes and/or vapours, where it is commonly referred to as 'marijuana', 'weed' or 'pot'.[2,8,25]

Modern medicinal cannabis preparations may refer to pharmaceutical products containing purified phytocannabinoids or synthetic forms of cannabinoids approved by regulatory authorities.[26] Synthetic cannabis products that mimic the effects of specific cannabinoids are also available in the form of liquids, tablets and sprays.[3] Δ^9-Tetrahydrocannabinol is available in synthetic forms, known as Dronabinol (Marinol®, Syndros®) and Nabilone (Cesamet®).[27,28,29]

1.2.2 Use of cannabis for medicinal purposes

The complex pharmacology of cannabis allows for a variety of medicinal uses, and cannabis can be indicated for the treatment of depression, nausea, pain, glaucoma, insomnia, multiple sclerosis and inflammatory diseases.[2,3,29,30]

One of the most noteworthy indications for cannabis is its role in the treatment of chronic pain.[24,31,32] It has also been reported that cannabinoids demonstrate analgesic properties for the treatment of chronic pain associated with various

conditions which include: cancer[33], multiple sclerosis[29] and human immunodeficiency virus (HIV).[34] The analgesic activity is ascribed to anti-nociceptive effects in descending pain pathways[12] and modulation of rostral ventromedial medulla neuronal activity.[32]

Cannabis also provides anti-inflammatory properties[29] through inhibition of prostaglandin synthesis[2] and acts as an effective anti-emetic in the treatment of chemotherapy-induced nausea and vomiting.[33] Appetite stimulation in patients with HIV or acquired immunodeficiency syndrome, as well as cancer patients on chemotherapy treatment is also facilitated by cannabis use.[35] Improvement of patient-reported multiple sclerosis spasticity symptoms have also been reported,[24,35] where nabiximols (Sativex®) were the primary cannabis plant extracts studied in aiding symptoms.[29] Furthermore, cannabis may aid in improving short-term sleep outcomes in individuals with sleep disturbance associated with obstructive sleep apnoea syndrome, fibromyalgia and multiple sclerosis.[35]

Cannabidiol in particular, has been shown to have a variety of therapeutic indications and medicinal benefits; including anxiolytic, anti-epileptic and anti-inflammatory effects.[10] These include pain relief for neurogenic pain, as well as pain and swelling associated with inflammation in arthritic and rheumatic diseases.[11] Cannabidiol also exhibits anxiolytic effects in individuals with anxiety disorders,[11,13,36] and shows potential in improving self-injurious behaviour and anxiety in young patients with autism spectrum disorder.[37,38,39] Furthermore, hyperactivity and sleeping problems in children with autism spectrum disorder may be improved; although further research needs to be conducted to assess the safety and efficacy of CBD as either monotherapy or an adjunctive treatment of symptoms.[37,40]

Cannabidiol has also been found to be moderately effective in controlling dystonic movement disorders.[11] In June 2018, the Food and Drug Administration (FDA) approved Epidiolex®, a purified CBD formulation, for the treatment of two rare but severe forms of epilepsy, namely Lennox-Gastaut syndrome and Dravet syndrome.[41] The European Medicines Agency (EMA) followed suit in September 2019 and approved the marketing authorisation of Epidyolex® for adjunctive therapy of seizures.[42]

Currently, CBD is being investigated as a potential therapy for the treatment of acute myocarditis and heart complications which may develop as an acute inflammation of

the heart tissue triggered by viral infections, such as Influenza or COVID-19.[43] Acute myocarditis and cardiovascular complications are one of the main risk factors associated with COVID-19 deaths,[43,44] and phase II clinical trials to investigate CBD as a potential therapy for acute myocarditis have been granted FDA approval.[43] Combined phase II and III clinical trials to evaluate CBD as a cardio-protective treatment for COVID-19 patients with pre-existing cardiovascular conditions, are currently underway.[43]

1.2.3 Cannabis as a recreational drug

Cannabis is a popular recreational drug, and the world's most widely used illicit drug,[20] frequently sought after for its psychotropic properties.[45,46] Recreational cannabis use is becoming increasingly common in both developed and developing countries,[46] and it is estimated that between 147-162 million people worldwide use cannabis recreationally.[46,47] Users may experience a feeling of being calm and relaxed, or the feeling of being intoxicated as THC interacts with cannabinoid receptors; [8,17] many of which are found in the parts of the brain influencing pleasure, memory and thought.[17] It is due to these psychotropic properties that cannabis has become a prohibited substance, or substance/drug of abuse.

Cannabis is often considered a gateway drug, and chronic cannabis use may lead to the development of cannabis addiction and dependence.[8,17,47] Adolescents experimenting with recreational tobacco, cannabis and alcohol were found to be more likely to experiment with other illicit drugs.[48] Statistical data from Australia, Canada, the European Union and the United States of America (USA) reveal that in recent years the number of patients admitted to treatment centres for substance abuse due to cannabis addiction, has surpassed those admitted for alcohol addiction.[49] These statistics are alarming, since regular recreational cannabis use amongst adolescents and young adults may jeopardise normal psychosocial development, as adolescent brain development is affected.[38,50]

1.3 Pharmacology of cannabinoids

1.3.1 Cannabinoid compounds

Cannabinoids are classified as being either psychoactive or non-psychoactive. Of the cannabinoid compounds, seven are considered important for clinical or scientific

reasons (Table 1). These cannabinoids, among others, are thought to have synergistic, additive or antagonistic effects on THC and modulate its response and activity.[8,12]

Table 1: Clinical and/or research significance of the seven most abundant cannabinoid compounds found in cannabis plants.

Cannabinoid	Significance (clinical or research)
Δ^9-THC	• Abundant in cannabis products (cigarettes, tinctures, consumables)[21] • Main psychoactive compound producing psychoactive effects.[4] • Wide range of pharmacological activities (analgesic, appetite stimulating, anti-emetic and psychological effects).[4,8,10]
Δ^9-THCA	• Inactive acid precursor of Δ^9-THC, and may be converted to Δ^9-THC via decarboxylation reactions.[51] • Possible presence in cannabis products due to decarboxylation.[30]
CBD	• Found in large concentrations in cannabis products (CBD oils, consumables, CBD teas).[21,52] • Produces many pharmacological effects (analgesic, anti-emetic, anxiolytic, anti-epileptic, anti-inflammatory effects).[4,8,10,12]
CBDA	• Inactive precursor of cannabidiol, and may be converted to CBD via decarboxylation reactions.[51]
CBN	• One of the main psychoactive cannabinoid compounds.[8] • Possible presence in cannabis products.[53]
CBG	• Known to induce sedation.[10]
CBC	• Present in high concentrations in cannabis plant.[10] • Shows sedative effects and may enhance the effects of THC.[10]

Δ^9 -THC: Δ^9-Tetrahydrocannabinol, Δ^9-THCA: Δ^9-Tetrahydrocannabinolic acid-A, CBD: Cannabidiol, CBDA: Cannabidiolic acid, CBN: Cannabinol, CBG: Cannabigerol, CBC: Cannabichromene.

Psychoactive cannabinoids include Δ^9-THC and its isomer, Δ^8-tetrahydrocannabinol (Δ^8-THC); as well as cannabinol (CBN), a non-enzymatic oxidation breakdown product of THC.[54] The psychoactive effects of both CBN and Δ^8-THC are considered less powerful than Δ^9-THC,[8] and Δ^8-THC is present in much lower concentrations in the cannabis plant in comparison to Δ^9-THC. Thus, its psychoactive ability is reduced.[8,54]

Cannabinoid compounds without psychoactive properties include; CBD, Δ^9-tetrahydrocannabinolic acid A (THCA), cannabidiolic acid (CBDA), cannabigerol (CBG), and cannabichromene (CBC).[8,12,13] The cannabinoid acids CBDA and THCA are the inactive acid precursors of CBD and Δ^9-THC, respectively.[55] The biosynthetic pathway of all cannabinoids begins with cannabigerolic acid (CBGA), and all cannabinoids are synthesized *in vivo* in a carboxylated form.[56] A schematic representation of the formation of cannabinoid acids from CBGA is provided in Figure 1. Although classified as non-psychoactive, cannabinoids such as CBC may have sedative effects,[10] and CBC has been reported to enhance the psychoactive effects of THC.[8]

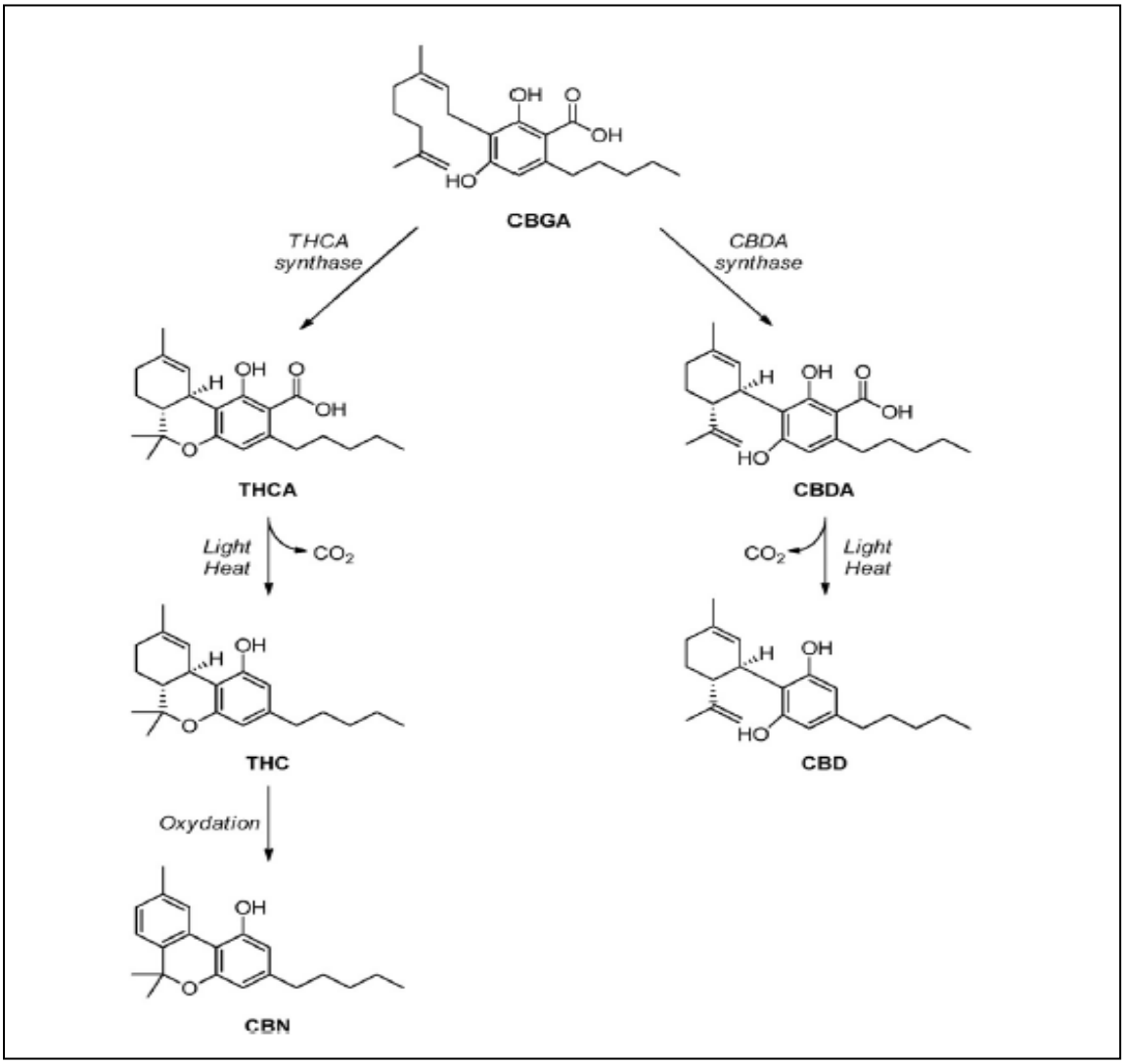


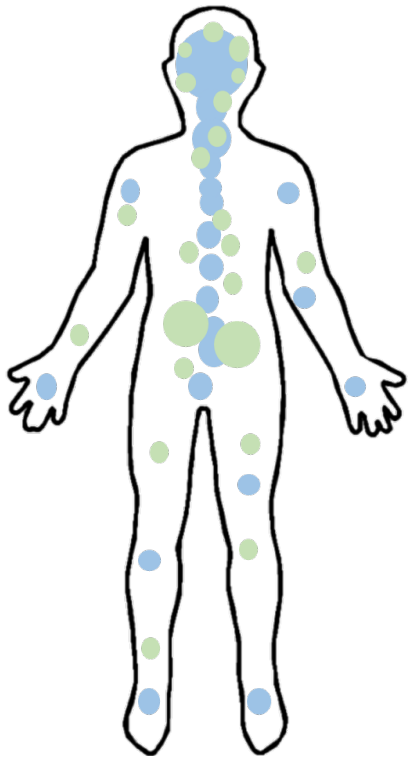
Figure 1: Schematic representation indicating the biosynthetic pathway of the cannabinoids Δ^9-tetrahydrocannabinol (Δ^9-THC), tetrahydrocannabinolic acid-A (THCA), cannabidiol (CBD), cannabinol (CBN) and cannabidiolic acid (CBDA) beginning at cannabigerolic acid. (Adapted from Citti *et al.*[55])

1.3.2 Pharmacology

1.3.2.1 Endocannabinoid system

The human endocannabinoid system consists of cannabinoid receptors, endocannabinoids and enzymes involved in their degradation and synthesis.[57] Although the physiological function of cannabinoid receptors is not completely understood, most cannabinoid reactions uniquely support homeostasis and are mediated by agonistic or antagonistic actions at specific receptors sites.[10,58] Pharmacological effects are exerted via CB_1 and CB_2 cannabinoid receptors present in the central and peripheral nervous system, respectively (Figure 2).[24]

CB_1 receptors were discovered in the late 1980s, and are located in the cerebral cortex, limbic areas (such as the hippocampus and amygdala), basal ganglia, brain stem, thalamus and cerebellum. The CB_2 receptors are expressed in immune cells found in the spleen, tonsils and bone marrow, amongst others.[1,8] Cannabinoid receptors are usually activated endogenously by derivatives of endocannabinoids,[33] and the first endocannabinoid to be identified was arachidonoyl ethanolamide (also known as anandamide) in 1992.[12] Anandamide and 2-arachidonoylglycerol are the most widely studied endocannabinoids.[8,12] Anandamide modulates nociceptive signals by acting on CB_1 receptors and is released in response to inflammation and nerve injury, whereas 2-arachidonoylglycerol is induced in response to nociceptive signals.[32] The discovery of the G protein-coupled receptor 55 (GPR55) and the transient receptor potential cation channel subfamily V member 1 (TRPV1) may be reclassified as CB_3 and CB_4 receptors, respectively. GPR55 is largely expressed in dorsal root ganglion neurons, and is activated by cannabinoids such as Δ^9-THC and methanandamide (anandamide analogue).[8,59]



Human endocannabinoid system

CB_1 receptors

Located in the central nervous system, including:

- Cerebral cortex
- Limbic areas (i.e. hippocampus and amygdala)
- Basal ganglia
- Brain stem
- Thalamus
- Cerebellum

CB_2 receptors

Located in the peripheral nervous system and immune cells, including:

- Spleen
- Tonsils
- Bone marrow

Figure 2: Diagram indicating the location of cannabinoid receptors (CB_1 and CB_2) found throughout the human body in the human endocannabinoid system[1,8,24] (image created by author) CB_1 receptor location shown in blue, and CB_2 receptor location shown in green.

Endocannabinoids modulate neural conduction of pain signals and are produced by cells in response to cell injury.[32] Endocannabinoids undergo *de novo* synthesis from post-synaptic membrane–lipid precursors and once released, are able to exert a wide range of physiological effects. These may affect the neural, metabolic, reproductive, vascular and immune systems.[13] CB_1 receptors function to maintain homeostasis by regulating synaptic neuro-transmission and influence the function and activity of other neurotransmitters such as γ-aminobutyric acid (GABA), monoamines and opioid systems.[8,12] The CB_2 receptors alter immune cells by modifying cytokine release, and are known to increase in response to peripheral nerve damage.[28,32] When activated, CB_2 receptors can affect inflammatory and immunosuppressive activity.[29]

1.3.2.2 Pharmacological effects

The pharmacodynamics of individual cannabinoids and the role of the endocannabinoid system is still not fully understood. From animal studies it is evident that the pharmacological and therapeutic effects of cannabis cannot be attributed to THC alone, nor to any other single cannabinoid.[11] Rather, cannabinoids interact synergistically to produce pharmacological effects.[60] Although THC and CBD are isomers of each other, their affinity and potency to CB receptors differ, and their pharmacological effects are markedly different.[26,61] Cannabinoids interact with a myriad of neurotransmitters and neuromodulators such as acetylcholine, dopamine, GABA, noradrenaline, serotonin, glutamate, prostaglandins, opioid peptides and histamine.[17]

The majority of THC's pharmacological effects are mediated by agonistic actions at CB_1 and CB_2 cannabinoid receptors. Activation of CB_1 receptors affects nervous system function by producing psychoactive effects, whereas activation of CB_2 receptors does not.[17] However, the anti-emetic effects of THC are thought to be mediated by both CB_1 receptors and non-CB mechanisms.[17] Cannabinol has centrally acting effects similar to THC. However, these effects are not as prominent due to additional carbon double bonds in their chemical structure resulting in decreased psychoactivity.[2]

On the other hand, CBD has a low binding affinity to endogenous cannabinoid receptors, and therefore does not produce psychoactive effects due to antagonistic activity at the CB_1 receptor. Instead, CBD interacts with GPR55 and TRPV1 receptors,[60] and is also known to modulate the effects of THC by inhibiting several THC-mediated CB_1 mechanisms.[13,17,62] Cannabidiol can also modulate THC levels when acting as an antagonist to the CB_1 receptor.[17,63] Thus, a higher ratio of CBD:THC ratio may not induce psychoactive effects, whereas a high THC:CBD ratio is likely to lead to psychoactivity. Consequently, CBD is able to provide medicinal benefits without psychoactive effects. Analgesic and anti-inflammatory effects of CBD are mediated by dual cyclooxygenase and lipoxygenase inhibition,[29] preventing the metabolism of arachidonic acid and subsequent release of prostaglandins and leukotrienes[27], respectively, thus, preventing an inflammatory response. A summary of cannabinoid affinity to CB1 and CB2 receptors is provided in Table 2.

Table 2: Affinity to cannabinoid receptors for major cannabinoid compounds.

Cannabinoid compound	Psychoactive effect	Interaction with CB_1 and CB_2 receptors
Δ^9-Tetrahydrocannabinol	Yes	Partial agonist of both.[10]
Cannabinol	Mildly psychoactive	Partial agonists of CB_1 and CB_2 (albeit with lower affinity than THC).[8,54]
Cannabidiol Cannabigerol Cannabichromene Cannabidiolic acid Δ^9-Tetrahydrocannabinolic acid-A	No	CB_1 and CB_2 antagonists.[2,4,54]

1.3.3 Adverse drug reactions

Cannabis is able to induce psychoactive effects affecting perception and mood; which may impair one's ability to perform normal daily activities.[47] Cannabis use may result in impaired concentration, judgement and motor coordination; as well as short-term memory loss and slower reaction times.[1,9] This is especially relevant when performing tasks such as driving or operating machinery.[8,24,64] There also appears to be an increased risk of short-term adverse effects associated with cannabinoid use, such as the development of asthenia, problems with balance, confusion, dizziness, disorientation and drowsiness.[65,66] Cannabis use may also lead to acute psychosis in adolescents, even in those without a history of mental illness.[20]

Long-term cannabis use also carries respiratory and cardiovascular health risks such as tachycardia and changes in blood pressure.[66,67] $^9\Delta$-Tetrahydrocannabinol may also increase oxygen demand and cardiac work, resulting in increased cardiac output.[10] Furthermore, carcinogens and mutagens found in tobacco smoke are also found in cannabis smoke, and smoking cannabis has been shown to produce airway inflammation and decrease pulmonary function.[8,17,66] Long term and frequent cannabis use may also cause dysphoria, paranoia and anxiety.[68] However, these reactions appear to be dose-related and are more prevalent in naïve users and those that are psychologically vulnerable.[8] Cases of the development of schizophrenia and psychosis have also been reported.[8,24,46,68]

Cannabis tolerance may develop in chronic users, thereby aggravating cannabis dependence and addiction if the dosage is escalated to achieve pharmacological effects.[8] Cannabis use disorder presents itself in the same way as any other substance abuse disorder and cannabis addictions are common, especially if there is a high THC:CBD ratio.[49]

1.4 Analysis of cannabinoids in different preparations

Many analytical methods developed to determine cannabinoid content are for the analysis of biological samples (blood and urine) for drug screening purposes,[69] rather than quality control or potency testing. The most widely used methods for the identification of cannabinoids in biological matrices are gas chromatography (GC)[56,70] and reversed phase high performance liquid chromatography (HPLC),[30,71] coupled with various detection techniques.[63,72] These include mass spectroscopy (LC-MS/MS), photo diode array (HPLC-PDA) and ultra-violet spectroscopy (LC-UV).[73,74]

There are a variety of medicinal cannabis products available for purchase in South Africa, many of which are in the form of CBD oils. During the production of CBD oils, various extraction procedures may be employed to extract cannabinoids from cannabis plant material and remove unwanted cannabis plant material in order to purify CBD oils. Common methods for extraction and purification of cannabinoids from various matrices include alcohol extraction,[75] centrifugal partition chromatography (CPC), supercritical fluid extraction (SFE) and supercritical carbon dioxide (CO_2).[76]

Methods employed for content or safety testing of cannabinoids in cannabis products may require techniques or procedures specific to the type of cannabis product. The chemical structures of THC and CBD are isomers[51], and it is important that the selected method is able to distinguish between isobaric cannabinoids with similar precursor masses.[30] The chemical structures of the analytes; CBD, THC, THCA and CBN are provided in Figure 3.

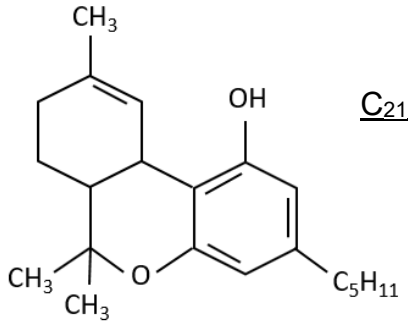
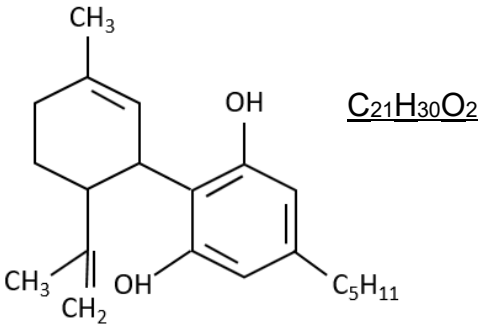
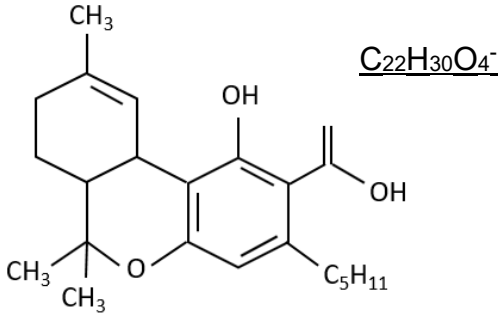
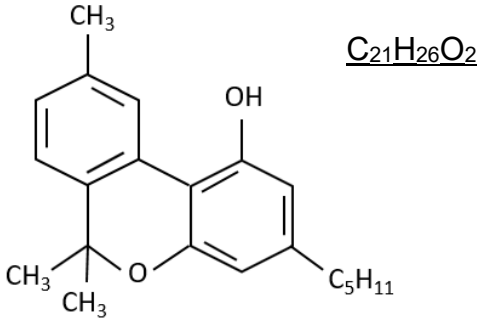
 $C_{21}H_{30}O_2$	 $C_{21}H_{30}O_2$
Δ^9-Tetrahydrocannabinol	**Cannabidiol**
 $C_{22}H_{30}O_4^-$	 $C_{21}H_{26}O_2$
Δ^9-Tetrahydrocannabinol acid-A	**Cannabinol**

Figure 3: Chemical formula and structure of cannabinoid compounds with a significant presence in cannabis products.

1.4.1 Methods of analysis

1.4.1.1 Gas chromatography

Gas chromatography methods provide a rapid analysis,[63] and are commonly used for the quantification and analysis of cannabinoids found in cannabis products.[70,77] However, the cannabinoid acids CBDA, CBGA and Δ^9-THCA may be difficult to determine with GC methods as they are decarboxylated into their neutral forms as heating occurs during analysis.[55,72] Thus, to quantify the neutral forms of CBDA, CBGA and Δ^9-THCA with GC methods, an extra derivatization step is mandatory.[30,63,76] This step is not necessary with HPLC methods, where both the neutral and acidic forms of these acids are able to be determined as the high temperatures used for GC analysis are not required.

1.4.1.2 Liquid chromatography ultra-violet spectroscopy

Analytes are determined primarily by their retention times with LC-UV methods,[69] which may be problematic for the analysis of cannabis products, as isomeric or co-eluting analytes such as CBD and THC may be misidentified.[76]. Thus, when compared to LC-MS/MS, LC-UV methods are less specific. Furthermore, detection of analytes are less selective at a lower range of wavelengths, as terpenes have been shown to absorb UV light at wavelengths where cannabinoids are usually detected.[74,78]

1.4.1.3 Liquid chromatography tandem mass spectrometry

LC-MS/MS allows for the specific detection of cannabis compounds and offers a wide dynamic range for the quantification of cannabinoids.[76,79] LC-MS methods use mass spectra of known cannabinoids for identification, and are capable of producing a targeted analysis of analytes with known mass to charge (*m/z)* ratios, corresponding to individual chromatographic peaks.[71] MS/MS detection measures the response of individual fragments of each compound,[80] and closely related compounds can be separated with LC-MS/MS analysis while ensuring the integrity of the original structure[79]. Thus, isobaric analytes and/or analytes with the same fragmentation pattern (such as THC and CBD) are able to be distinguished with LC-MS/MS analysis by their retention times and individual precursor and product ion masses. This allows for an extremely sensitive method for the detection of multiple analytes in a single analysis, producing excellent resolution and confirming purity of eluted peaks.[30,72]

HPLC methods are also more suitable for the analysis of liquids and polar edibles, whilst GC methods are ideal for volatile compounds extracted from flowers and concentrates.[63] LC-MS/MS facilitates the separation and quantification of compounds in a mixture, and relies on the separation of analytes based on the difference in solubility between the stationary and mobile phases.[81] It is therefore crucial that the analytes are soluble in the mobile phase.[81] CBD oils are solvent extracts from the cannabis plant dissolved in an edible oil.[30,61] Thus, sample preparation for LC-MS/MS analysis of CBD oils does not require extraction methods such as SFE and solid phase extraction, as cannabinoids are already in a solution suitable for analysis.

Furthermore, using reversed phase liquid chromatography (RPLC) is advantageous as non-polar compounds (such as cannabinoids) have an affinity for the non-polar stationary phase,[82] and results in shorter preparation time.[70] LC-MS/MS is also a more universally applicable technique as an additional hydrolysis or derivatization step is not required.[81] The quantification of unknown concentrations of selected analytes using LC-MS/MS methods is possible as known concentrations of analytes can be analysed at different concentration ranges to provide a calibration curve. A plot of analyte concentration relative to isotope-labelled internal standard (IS) concentration is plotted, from which the cannabinoid concentration can be derived via extrapolation.

1.4.2 Important cannabinoids

The four most important cannabinoids are CBD, THC, CBN and THCA based on their pharmacological effects, psychoactivity and concentration.[3,30,51] Each of these are described separately.

1.4.2.1 CBD

Cannabidiol is one of the main cannabinoids in the cannabis plant, and is well-known due to its pharmacological effects.[3,4] Cannabidiol is widely used as either a primary or concomitant treatment for a variety of medical conditions, where it provides analgesic, anti-emetic, anti-spasmodic and appetite-stimulating effects without causing psychological changes.[13,62] A survey of over 2000 participants in North America indicated that almost 60% of participants reported the use of CBD to treat a medical condition; the most common being chronic pain, followed by arthritis/join pain and anxiety.[43,83] The remaining participants indicated the use of CBD to promote general health and well-being.[83]

Apart from certain CBD-containing preparations excluded from schedules (schedule 0) according to the Medicines Act, CBD is still classified as a schedule 4 substance in South Africa (thus subjected to stricter regulations). Cannabidiol is the main constituent in CBD oils, thus developing a robust method for the determination of CBD in CBD oils would be beneficial.

1.4.2.2. THC, CBN and THCA

Although THCA has no psychoactive properties, it may be converted to THC via decarboxylation reactions triggered by stress conditions such as heat or UV light. Many regulatory authorities define the total THC content in cannabis products by the sum of THC and THCA multiplied by the molecular weight ratio of THC/THCA.[69]

Tetrahydrocannabinol and CBN are two of the main psychoactive cannabinoids in cannabis[70] and are synthesized by catalytic enzymatic reactions in the cannabis plant (refer to Figure 1).[17] Cannabinol may undergo oxidation to THC during plant aging and maturation, and may be indicative of poor storage conditions or improper extraction procedures.[4] Decarboxylation reactions may also occur during the extraction of cannabinoids from the cannabis plant[4] and during heating when smoking, vaping or baking cannabis for recreational use.[30,56,84] Thus, potential exposure to heat and UV light during production and/or storage of CBD oils may incur changes to cannabinoid composition and lead to the formation of THC. The possible presence of THC in CBD oils has significant consequences, as THC is classified as a schedule 6 substance according to South African medicine schedules (excluding processed products from cannabis material that do not exceed 0.001% THC). Although South African cannabis laws do not make mention of CBN or THCA content in CBD-containing products, both have the potential to enhance intoxicating activity or cause sedative-like effects.[4]

1.5 Cannabis regulation

1.5.1 Medicine schedules

The legality and scheduling of cannabis and cannabis-based products varies between countries and is subject to regulation by each country's own constitution and laws. A medicine schedule is a system of classification used to determine a medicine's benefit versus risk level by assessing its safety profile. A variety of factors establish a medicine's schedule: dosage form, strength, dose, route of administration, indication, duration of treatment or a combination of these factors.[85] Classifying medicines into schedules permits different levels of regulatory control over different types of pharmacological products (whether conventional or complementary).[85,86] Medicines with a low potential for harm and abuse/low incidence of side effects would be classified as 'low risk', while medicines with a high

probability of side effects and/or addiction are regarded as 'high' risk.[73] The scheduling of medicines determines whether these are available over-the-counter (OTC) or upon prescription from a pharmacist or medical doctor only.[73]

In South Africa, medicines fall into a schedule ranging from schedule 0 to schedule 8. Table 3 contains a summary of the medicine schedules in South Africa and a description of each schedule. Schedule 0 medicines are those with the least restrictions, and are defined as medicines which may be sold in an open shop (i.e. any setting, including general dealers), without prescription.[85] Schedule 1 medicines do not require a prescription, but may only be sold by a health care practitioner or wholesaler of pharmaceutical products (i.e. not sold in an open shop). Schedule 2 – 8 medicines may only be sold upon prescription from a registered health care practitioner and are more strictly regulated than schedule 0 and 1 medicines.[85,87] The most illicit substances which carry the strictest regulations are those with an extremely high potential for addiction or abuse, with little medicinal value.[88] These substances are classified as schedule 7 and 8 medicines, and are only made available with special permission for limited scientific or medical purposes.[85]

Table 3: Summary of the medicine scheduling system in South Africa.

Schedule	Description
0	Medicine is considered safe, and available for purchase in any store without prescription (E.g. Paracetamol).[87,89]
1 & 2	No prescription required, although medicine is only available over-the-counter after consulting a pharmacist (e.g. antihistamines, non-steroidal-anti-inflammatory drugs).[90,91]
3 & 4	Medicines are only available upon prescription from an authorised prescriber, and the indicated condition(s) requires a professional medical diagnosis (e.g. oral anticoagulants, antimalarials, oral antibiotics).[88]
5	Schedule 5 medicines are similar to those in schedule 4, but are potentially addictive. Thus, repeat prescriptions are limited to 6 months (e.g. sedatives and anti-depressants).[87]
6	Schedule 6 medicines are moderately to highly addictive, and patients must be closely monitored. Repeat prescriptions are limited to no more than 30 days (e.g. opioids).[90,92]
7 & 8	Substances require a permit and are highly addictive and strictly controlled with little medicinal value (e.g. heroin, methamphetamine).[88,90]

In addition to being classified under a schedule, medicines in South Africa are also classified into various categories. Category A and C medicines are those which are intended for only human and only veterinary use respectively, and are ready for administration without any further manipulation (i.e. only a vehicle is added to the medicine).[89] Category B medicines are intended only for human use, but cannot be administered without further manipulation. Complementary medicines intended for both human and veterinary use and which are ready for administration without further manipulation are classified as Category D medicines. [89]

1.5.1.1 South African cannabis schedules

Cannabis was officially proclaimed illegal in South Africa in 1928 after the introduction of The Medical, Dental and Pharmacy Act of 1928.[6,93] Since then, various other acts concerning the use of cannabis have been introduced in South Africa. The Abuse of Dependence-producing Substances and Rehabilitation Centres Act was introduced in 1971, listing cannabis as a “prohibited dependence-producing drug”. This act states that anyone found in possession of more than 115 grams of cannabis, or proven to be the owner or person in charge of cannabis plantations on cultivated land is presumed to be guilty of trading cannabis.[94] These laws are also reiterated in the Drugs and Drug Trafficking Act, enacted in 1992. The Act lists cannabis as an “undesirable dependence-producing substance” (with the exception of dronabinol, which is listed as a “dangerous dependence-producing substance”).[95]

In 2014, the Medical Innovation Bill was introduced in the South African National Assembly with the intention to make provisions for the legalisation of cannabinoids for medical purposes.[96] However, the Bill was later rejected in November 2017.[97] Subsequently, in 2016, the Central Drug Authority (CDA) recommended decriminalising the use of cannabis; although its use remained illegal.[98]

In March 2017, The South African Health Products Regulatory Authority (SAHPRA); (formerly known as the Medicines Control Council) issued guidelines for the cultivation and manufacture of cannabis and cannabis-related pharmaceutical products.[99] Thereafter, in November 2017, CBD was down-scheduled from a schedule 6 drug to a schedule 4 drug by SAHPRA for industrial purposes. This

included the manufacture of products with no pharmacological action or medicinal purpose, and those for analytical laboratory purposes. However, cannabis still remained a prohibited schedule 7 drug and strictly controlled substance only available under certain conditions.[91,100,101]

In September 2018, the Constitutional Court handed down a judgement declaring that it would no longer be a criminal offense for an adult to cultivate, possess or use cannabis for personal consumption (whether recreational or medicinal) in a private place.[102] However, at this time, cannabis-containing products (including extract cannabis oils) were not permitted to be cultivated in private and then sold to the public. In May 2019, the Minister of Health excluded certain CBD-containing preparations from being strictly regulated under the Medicines Act.[103] Thus, preparations with a maximum daily dose of 20 mg CBD without claiming to treat or cure any medical condition, as well as processed products not exceeding 0,001% THC and 0,0075% total CBD were exempted from being assigned a scheduling status for a period of one year.[103,104] Any CBD preparations that did not meet the above requirements remained classified as schedule 4. This change in legislation allowed for the sale of cannabis products (both registered or unregistered) provided that CBD and THC content do not exceed legal limits.

These regulations were updated in May 2020, and the personal consumption of the cannabis plant for private use currently remains legal in South Africa. Cannabis laws were also updated to include preparations of CBD not exceeding 600 mg per sales pack as a Complementary Medicine (Category D), as those excluded from being assigned a medicine schedule.[105] According to the latest regulations, THC was down-scheduled and is listed as a Schedule 6 drug except when processed products contain less than 0,2% THC and are intended for industrial purposes only. This is also applicable to processed products containing no more than 0,001% THC, and schedule 7 entries for cannabis, dronabinol and tetrahydrocannabinol were deleted.[105] However, medicines containing THC intended for medicinal purposes still need to be registered by SAHPRA and can only be bought at a pharmacy with a prescription from an authorised prescriber. A timeline describing the history of cannabis legislation in South Africa is provided in Figure 4.

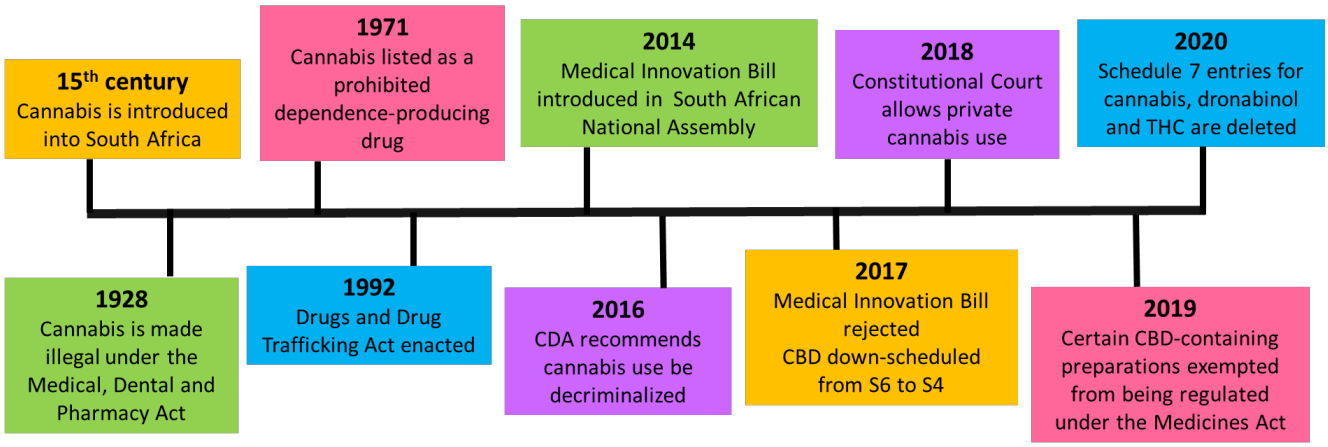


Figure 4: Timeline indicating the history of laws pertaining to cannabis in South Africa. (CDA: Central Drug Authority, CBD: cannabidiol)

1.5.1.2 International medicine schedules

Globally, each country has its own medicine schedule system based on regulations set by local regulatory authorities. Countries may assign different schedules for cannabis, or for individual cannabinoids (such as THC or CBD). Some countries allow the medicinal use of cannabis products containing high CBD content, with minimal to low THC content.[106] In many countries, CBD may be legal as a constituent of approved prescription medicines such as Sativex® and Epidiolex®, although it remains illegal in the form of non-approved cannabis extracts.[4]

Many countries have also adopted the policies of the United Nations Office on Drugs and Crime's (UNODC) Single Convention on Narcotics Drugs of 1961, wherein cannabis is classified as a schedule 4 drug.[107] The Single Convention provides an international framework recognizing the medicinal value of narcotic drugs (such as cannabis) and ensures the availability for medicinal and research purposes while impeding diversion into illicit channels and abuse.[99] According to the convention, drugs listed under schedule 4 are subject to strict controls, such as prohibiting the production, manufacture, trade, use or possession if deemed necessary.[107] Countries which participated in the Convention by virtue of ratification, accession or succession to the Protocol of 25 March 1972 include South Africa, USA, the United Kingdom and Australia, amongst others.[107] However; since then, cannabis legislation has changed in many countries, and in December 2020, UNODC's governing body voted to down-scale cannabis to a schedule 1 drug.[108]

1.5.2 Cannabis legislation

Cannabis use is classified as either legal, illegal or decriminalised. Decriminalisation is the removal of criminal sanctions for the possession or use of small amounts of cannabis.[66] Although this does not mean cannabis use is legal, those found to be in possession of cannabis would only face minor penalties or fines instead of prosecution or arrest. Defining cannabis as an illegal substance signifies that those found to be in possession of cannabis or cannabis related products would face prosecution and/or imprisonment.[109] In contrast, legalisation of cannabis implies that all penalties related to cannabis use are revoked.[66,110] A summary of the legal status of cannabis in 27 countries (as of August 2021) is provided in Table 4.

1.5.2.1 Cannabis use for medicinal purposes

Cannabis use for medicinal purposes is legal in many countries, some of which include: South Africa, Canada, Australia, New Zealand, Germany, Italy, the Netherlands, Switzerland, Jamaica, Argentina and Colombia.[111,112]

Since November 2018, the United Kingdom has legalised the use of cannabis-based products for medicinal use when prescribed by a medical practitioner under strictly controlled circumstances.[109] This includes the treatment of multiple sclerosis, cases of severe epilepsy, chemotherapy-induced nausea and vomiting, or when existing treatment options have proven to be ineffective.[113] In the USA, medicinal cannabis use is currently legal in 36 states, four US territories and the district of Columbia.[114] However, these laws differ between states in terms of how cannabis may be cultivated, distributed and consumed; as well as for the conditions it may be prescribed for.[115]

Medicinal cannabis use still remains illegal in most African and Asian countries, although countries such as Zimbabwe and Lesotho have taken steps towards legalising cannabis.[116,117] In 2017, Lesotho was the first African country to legalise the cultivation and export of cannabis for medicinal use although cannabis use remains illegal.[116] In April 2018, Zimbabwe legalised the use of cannabis for research and/or medicinal purposes.[117] Individuals and businesses in Zimbabwe are able to apply for licences to cultivate cannabis for medicinal or scientific use, which allows them to possess and sell cannabis, cannabis oil, and dried cannabis products.[118]

Table 4: Summary of the global cannabis legalisation status, applicable on August 2021.

		Legalisation status	
Continent/Region	**Country**	**Medicinal**	**Recreational**
Asia	China	Illegal since 1985.[119]	
	India	Illegal[111]	Illegal, exceptions made for the use of 'bhang'.[111]
	Indonesia	Illegal since 1927.[119]	
	Japan	Illegal since 1948.[119]	
	Saudi Arabia	Illegal[119]	
	Thailand	Legal since 2018.[120]	Illegal but often unenforced.[111]
	United Arab Emirates	Illegal[119]	
Africa	Lesotho	Illegal[118]	Illegal but often unenforced.[118]
	Nigeria	Illegal[119]	
	South Africa	Legal since 2018.[104]	Legal for personal use since 2018.[104]
	Zimbabwe	Legal since 2018.[112,118]	Illegal[118]
North America	Canada	Legal since 2001.[112]	Legal since 2018.[121]
	Jamaica	Legal[121]	Decriminalised since 2015 for possession of up to 56.6 g or 5 plants.[121]
			Legal for those of Rastafari faith.[111]
	Mexico	Legal since 2017 for cannabis products containing not more than 1% THC.[121]	Legal for private, recreational use since June 2021.[121]
	United States of America	Legal in 36 states, 4 territories and the district of Columbia.[3,122]	Legal in 18 states, 2 territories and the District of Columbia.[111]
			Decriminalised in 13 states and 1 territory.[111]

(Green: legal, red: illegal, yellow: decriminalised)

		Legalisation status	
Continent/Region	**Country**	**Medicinal**	**Recreational**
Europe	France	Cannabis-derivatives in medicinal products are legal since 2013 only under strict circumstances.[111]	Decriminalised since September 2020.[45]
	Germany	Legal at federal level since 2017.[112]	Illegal but often decriminalised.[45]
	Italy	Legal since 2013, with a prescription.[111]	Decriminalised for personal use.[121]
	The Netherlands	Legal since 2000.[112]	Decriminalised for possession up to 5g.[121]
	Switzerland	Legal since 2011 under special conditions.[112]	Decriminalised for possession of up to 10 g since 2012.[121]
	The United Kingdom	Legal since 2018 for cases of severe epilepsy, chemotherapy-induced nausea and vomiting, or multiple sclerosis when prescribed by a registered specialist.[113]	Illegal since 1928.[45]
Oceania	Australia	Legal at federal level in all states.[111]	Decriminalised in the Northern Territory and South Australia. Decriminalised for personal use in the Australian Capital territory.[121]
	Fiji	Illegal[119]	
	New Zealand	Legal since 2018.[111]	Illegal[111]
South America	Argentina	Legal since 2017.[121]	Decriminalised for personal use in small amounts in private locations since 2009.[121]
	Brazil	Legal in amounts exceeding 0.2% THC only under extenuating circumstances since 2015.[111] Less restrictions for amounts below 0.2% THC.[111]	Illegal, although punishment varies according to the amount found in possession.[111]
	Colombia	Legal since 2015.[121]	Legal for cultivation of up to 20 plants since 2015.[121] Decriminalised for possession of up to 20 g since 2012.[121]

(Green: legal, red: illegal, yellow: decriminalised)

Medicinal cannabis use has been legal in South Africa since 2018. Currently, there are no registered cannabis or cannabinoid-containing products for medicinal use in South Africa, although medicines approved/registered in other countries to which SAHPRA aligns itself with, may be used in South Africa. This includes Epidiolex® oral solution (registered by the FDA and EMA) and Sativex spray (registered in the United Kingdom and Canada).[88]

1.5.2.2 Cannabis use for recreational purposes

According to UNODC, as of 2013, an estimated 181.1 million people were users of cannabis for non-medical purposes globally.[123] Recreational cannabis use remains illegal in many countries, although some countries have decriminalised cannabis for personal use (Table 4).[46,109] A press release by the UNODC in 2020 stated that cannabis-related arrests accounted for approximately half of all drug-related offenses.[124]

Recreational cannabis use is legal in South Africa for personal consumption in a private place in accordance with the Constitutional Court judgement in September 2018.[104] However, cannabis use only remains legal if the above-mentioned conditions are met. Recreational cannabis products that are not intended for medicinal use do not require a license to be imported, manufactured or sold as 'recreational cannabis products' as long as they adhere to the exclusion notice declared by the Minister of Health. However, importers and manufacturers must be able to provide verifiable proof of the CBD and/or THC content of the product and comply with the provisions of other applicable legislations such as the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972).[88] Recreational cannabis products include a variety of hemp and CBD balms and creams, CBD edibles and mints, CBD vape juices and CBD edible capsules.[125] Some online stores also sell THC-based tinctures, capsules, suppositories and vaporising oils.[126]

1.6 Safety, quality and efficacy of cannabis products

Despite the potential benefits of legalising cannabis in South Africa, the decision to remove certain CBD-containing products from schedules to the Medicines Act may open a plethora of loopholes. Unregistered products remain the most frequent source of cannabis products, despite the development, registration and availability of registered medicinal cannabis products.[33] Possible reasons for this are that they are

cheaper, require no prescription (if unscheduled) and are thus more readily available compared to registered cannabis products. People may also resort to cannabis products as an alternative source of treatment if conventional, registered medicines do not provide relief of symptoms/illness. Cannabinoid and excipient content in registered cannabis medicines have been modified to ensure products have a high therapeutic value and satisfactory safety profile.[33]

According to South African law, applicants wishing to introduce cannabis-containing products onto the market must apply to register the product with SAHPRA, and any unregistered cannabis-containing products not approved by SAHPRA are considered illegal.[104] Compliance to good manufacturing practices (GMP) requirements; as well as the safety, quality and efficacy of prospective products need to be assessed prior to approval and registration. As of August 2021, SAHPRA had received 21 licence applications for the cultivation of cannabis for medicinal use, of which one was withdrawn. From the remaining applications, 16 were inspected and subsequently issued a cannabis cultivation license. However, no CBD- or THC-containing medicines have been registered by SAHPRA to date.[104,105] However, medical practitioners may apply for controlled access of unregistered cannabis products/specific cannabinoids for individual patients, if these preparations have been approved and/or registered by regulatory authorities that SAHPRA aligns itself with.[88]

1.6.1 Recommendations to ensure quality

Cannabis licenses must be applied for by anyone wishing to grow, cultivate, import, export or manufacture cannabis or cannabis resin.[99] Cannabis products may be produced by private individuals on a small-scale, or by large-scale manufacturers and independent companies,[51] and most unscheduled cannabis products in South Africa include a variety of CBD and hemp oils.[88] Cannabis laboratories may require large amounts of capital to start up, and are also subject to specific requirements and standards concerning equipment, hygiene, storage, personnel training and GMP standards.[127,128]

These products are often not subjected to the same stringent quality control tests and GMP requirements that registered pharmaceutical products have to undertake.[88] Quality control testing ensures that cannabinoid content and the quality of CBD oils

does not differ between batches. Testing also determines how shelf-life and stability of products are affected by external factors, and whether degradation of compounds occur during storage.[4] The recent changes to cannabis laws raises the question of how batch-to-batch consistency will be achieved with unscheduled products as it is unknown whether other quality control tests, such as stability testing, is performed on these products prior to sale and marketing.

1.6.2 Recommendations to ensure safety

The removal of CBD from Schedules to the Medicines and Related Substances Act (Act 101 of 1965), allows for the legal production and sale of CBD-dominant products without having to go through the entire drug development process. The average medicine may take a number of years to go from conception to market,[58] and seeing as though no THC or CBD-containing medicines have been registered in South Africa to date (August 2021),[105] those seeking cannabis medicines as a form of alternative or adjuvant treatments are now able to access cannabis-based products. Cannabis oils have become a popular choice amongst people seeking cannabis as a form of therapy for many reasons. A cross-sectional study of CBD users conducted by *Corroon* and *Phillips* indicated that the majority of users reported the use of CBD in sublingual sprays, drops and tinctures.[83] These formulations are easy to administer, have a low potential for intoxication, and eliminate the stigma often associated with smoking cannabis.[51]

Methods of extraction and purification of CBD oils may affect the composition of the final product.[76] Cannabidiol oil is a solvent extract from the cannabis plant which is then dissolved in an edible oil,[51] and specialised extraction procedures (e.g. SFE or CPC techniques) are usually employed to extract CBD and other cannabinoids from raw cannabis plant material.[4] Small-scale manufacturers may lack the equipment and facilities needed to correctly isolate CBD from other cannabinoids, while removing any impurities.[128] This makes it difficult to ensure the safety of CBD oils comprising legal amounts of cannabinoids, while providing therapeutic effects with minimal side effects. Furthermore, there are no mandatory quality and safety tests to assess whether there are any harmful or unknown substances in these products i.e. possible contamination. Investigating which cannabinoids are present in various CBD oils, as well as their quantities, assists the investigation into whether CBD oils

adhere to the current legislation in South Africa, and serves in the interest of public safety.

Additionally, unscheduled products are often not subject to other regulations as per the Medicines and Related Substances Act,[91] such as labelling and package insert requirements. All pharmaceutical products in South Africa intended for human administration must contain a label on the immediate container, including information in English and one other official South African language.[91] Important information such as the concentration of active ingredients (i.e. CBD and THC) can be relayed to consumers to ensure correct dosing. Labelling requirements pertaining to the immediate and outer container labels of medicines for human administration stipulated by SAHPRA are listed in Table 5. Unscheduled cannabis products such as CBD oils are classified as Category D (complementary medicines) in South Africa, for which additional labelling requirements are needed (Table 6).

Table 5: Immediate and outer container labelling requirements that pertain to cannabis products in South Africa according to Act 101 of 1965.

Labelling information for medicines intended for human administration		
Regulation	**Immediate container label**	**Outer label**
Bar code	Required	Not required
Batch number	Required	Required
Category/class of medicine	Required	Not required
Dosage instructions	(Recommended dosage) Required	Not required
Expiry date	Required	Required
Indications for use	Required	Not required
Manufacturing date	Required	Not required
Medicine dosage form	Required	Required
Medicine schedule	Required	Not required
Name of active ingredients and quantity	Required	Not required
Net quantity of contents (expressed in an appropriate unit/volume)	Required	Not required

Labelling information for medicines intended for human administration		
Regulation	**Immediate container label**	**Outer label**
Proprietary (trade) name	Required	Required
Registration/application number	Required	Required
Storage instructions	Required	Not required
The name of the holder of the certificate of registration/manufacturer	Required	Not required
The warning: “Keep out of reach of children”	Required	Not required

Table 6: Immediate and outer container labelling requirements for complementary medicines in South Africa.

In the case of complementary medicines (Category D)		
Regulation	**Immediate container label**	**Outer label**
The words “Complementary medicine”	Required	Required
The words “Health supplement”	Required	Not required
The words “This unregistered medicine has not been evaluated by the SAHPRA for its quality, safety or intended use.”	Required	Not required

1.6.3 Recommendations to ensure efficacy

Cannabis restrictions and laws imposed by both South African, and international Governments continue to hinder the manufacture, production and testing of cannabis and cannabis-containing products for medicinal use. A scarcity of clinical evidence and post-marketing surveillance means there is a lack of information available regarding drug-drug interactions and contra-indications associated with cannabis-derived pharmaceuticals and individual cannabinoids.[29] This information is usually revealed during clinical trials and post-marketing surveillance.[51]

Although clinical studies regarding the chronic use of cannabis are scarce, what is known at present from data collected from Epidiolex® is that CBD may interact with drugs metabolised by the same Cytochrome P450 (CYP) enzyme complexes.[10,22] *In vitro* studies indicate that CBD, THC and CBN may exhibit competitive inhibition on

various CYP enzymes; namely CYP2C9, CYP1A1/2 and CYP1B1.[129,130] Furthermore, cannabinoids are highly protein-bound in plasma, and may interact with other drugs with a high affinity to plasma proteins.[22] However, it is unclear at what concentration these effects are observed *in vivo*, especially since bioavailability depends on various factors, including route of administration, gastrointestinal absorption, hepatic metabolism and renal excretion.[22]

The possibility of unintended drug-drug interactions between CBD and THC-containing products and herbal or conventional medicines is highly likely. A study published in *Medical Cannabis and Cannabinoids* showed that some medications may not function as intended when used in conjunction with cannabis products.[29] The majority of medications listed have a narrow therapeutic index, varying from cardiac medications to antifungals and antibiotics.[22] Drug interactions between anti-seizure medications and CBD have been noted, and plasma concentrations of the benzodiazepine, Clobazam, have been shown to increase when administered with CBD.[26] Additionally, drug-drug interactions with other medications may affect cannabinoid concentrations through induction or inhibition of CYP 450 enzymes and/or phase II conjugation reactions.[26] However, the absence of clinical testing and post-marketing surveillance in unregistered cannabis products results in information regarding drug interactions associated with other medicines co-prescribed with cannabis, being largely unknown. These include antiretrovirals, anti-epileptics and chemotherapeutic agents.[129]

1.7 Aim and objectives

The aim of this study was to determine the cannabinoid profile in cannabinoid-containing products and to determine the compliance of these products to South African regulations.

The objectives of this study were to:

- optimise the analysis of the cannabinoid reference standards using the LC system,
- optimise the analysis of the cannabinoid reference standards using the LC-MS/MS system,

- quantify four selected cannabinoids in the purchased products using both methods,
- analyse and compare different lot numbers of purchased products to assess quality and consistency,
- propose recommendations for the regulation of cannabinoid containing products.

Chapter 2: Materials and methods

Approval to conduct this study was received from the Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria (Appendix I).

2.1 Chemicals, reagents and products

All solvents used during sample preparation and chromatography were of analytical grade. Reference standards of THCA (1000 µg/mL) (# A0155758) and a cannabinoid mix standard containing THC, CBD and CBN (1000 µg/mL) (# A0151144) were procured from LECO Africa (Johannesburg, South Africa). The internal standard (IS) CBD-D3 (100ug/mL) (# FE04021902) for analysis in positive mode was purchased from Cerilliant (Johannesburg, South Africa).

Acetone (Merck®, purity >99.5%) was purchased from Sigma-Aldrich (Pty) Ltd. (Johannesburg, South Africa). Hexane (Merck®, purity >99.9%) was procured from Merck (Darmstadt, Germany); and acetonitrile (ROMIL®, purity >99.9%) and methanol (ROMIL®, >99.9%) were purchased from Microsep (Pty) Ltd. (Johannesburg, South Africa).

Two batches of six commercially available CBD oils (which will remain anonymous) were purchased online from websites that advertise and sell CBD oils with the option of delivery within South Africa. These oils are referred to as CBD Oil ‘A’, ‘B’, ‘C’, ‘D’, ‘E’ and ‘F’. The number ‘1’ is used to denote the first batch of CBD oils purchased, whereas number ‘2’ refers to CBD oils from the second batch (but same supplier). The first batches of all CBD oils were purchased in January-February 2020 (‘Summer batch), and the second batches purchased between July-August 2020 (‘Winter batch). All CBD oils were stored at ambient room temperature and both were analysed prior to their expiry date. Cannabinoid content was determined in all selected CBD oils across both batches to assess adherence to SAHPRA regulations. Furthermore, batch to batch consistency and conformity was assessed by determination of cannabinoid content and an inspection of the physical characteristics (colour, consistency and transparency) in all CBD oils.

2.2 Preparation of analytes

2.2.1 Standards

An aliquot (100 µL) of the certified cannabinoid mix standard (CBD, THC, CBN; LECO Africa, South Africa) and 100 µL of the certified THCA reference standard (LECO Africa, South Africa) was added to 800 µL of methanol to form a stock solution of 1.0 mL. The stock solutions were aliquoted at 20 µL and stored at -18 ºC until analysis. Upon analysis, different concentrations of stock solutions (0.0, 5.0, 12.5, 25.0, 50.0, 75.0 and 100.0 µg/mL) were diluted by the addition of acetone, and 10 µL of the isotope-labelled CBD-D3 (internal standard, IS) to obtain a constant concentration of 40 µg/mL for all calibration standards.

All storage instructions and conditions for the cannabinoid reference standards and the isotope-labelled IS were met as per manufacturer's guidelines, and were used prior to their expiration dates.

2.2.2 Sample preparations

A dilution/extraction method was used to prepare the CBD oils investigated. An aliquot (50 µL) of each CBD oil was added to 10 mL of acetone in 20 mL clear glass vials (57 X 27.5 mm; Separations, SA). Each sample was vortex mixed on a VM-300 IKA vortex mixer for 30 seconds until the mixture was dissolved and no precipitation or oily droplets were observed. Thereafter, another 10 mL of acetone was added.

Tubes were left to stand for 5 min, after which an aliquot (20 µL) was pipetted into clear glass conical base inserts (6 x 31 mm). An aliquot (20 µL) of the isotope-labelled CBD-D3 IS was added to each vial and the content mixed by pipetting several times. Thereafter the inserts were placed inside 2 mL amber glass autosampler vials (12 x 32 mm) and subjected to analysis.

2.3 Instrumentation

2.3.1 HPLC analysis

The HPLC system used consisted of a SIL-20 CHT auto-sampler, an FCV-11AL solvent selector with model LC-20ADxR pumps, and a DGU-20A3 micro vacuum degasser (Shimadzu, Kyoto, Japan). Separation of the four cannabinoid reference standards was performed using a Phenomenex Gemini C18 column (2 X 100 mm)

with a particle size of 3 µm. The injection volume was 4 µL. Isocratic elution was used for the analysis with a buffer system consisting of 10 mM ammonium formate in water: acetonitrile (0.1% formic acid) 32.5:67.5 at a flow rate of 0.4 mL/min for 13 min. The system was equilibrated for 1 min prior to the run, and returned to starting conditions in the last 3 min to recondition the column. Data acquisition was by diode array detection at 215 nm with a 10 nm bandwidth. System control and data analysis was performed using LabSolutions software (Shimadzu, Kyoto, Japan).

2.3.2 LC-MS/MS

The Agilent HPLC 1100/1200 system used consists of a degasser, a binary LC pump, column oven and autosampler linked to a Sciex QTRAP triple quadrupole mass spectrometer equipped with a “turbo V” electrospray ionisation (ESI) source. For data comparison, separation was achieved using the Phenomenex Gemini C18 column (2 X 100 mm) and the column temperature was kept at ambient temperature (20°C ± 1°C). The conditions for the mobile phases for LC-MS/MS analysis were as follows: mobile phase A consisted of 10 mM ammonium formate in water and mobile phase B consisted of 0.1% formic acid in acetonitrile. The system was equilibrated for 1 min prior to the run, and returned to starting conditions in the last 3 min to recondition the column. Analyst™ 1.5.2 software was used for data acquisition and analysis.

2.3.3 UPLC-MS/MS

Untargeted analysis for method optimisation was performed on an Acquity UPLC system which comprised of a binary solvent manager, column compartment and a temperature-controlled sample manager followed by a Synapt G2 QTOF mass spectrometer (Waters, Milford, MA, USA). The binary solvent manager allows gradient elution of two different solvent mixtures. For analyte separation, the Phenomenex Gemini C18 column (2 X 100 mm) was used. The analytical column compartment was kept at ambient temperature (20°C ± 1°C) and the sample manger temperature was set to 8°C. Mobile phase A consisted of 10 mM ammonium formate in deionised water and mobile phase B consisted of 0.1% formic acid in acetonitrile. MassLynx 1.4 software (Waters, Milford, MA, USA) was used for data acquisition and quantitative data analysis.

2.3.4 Mass spectrometry

To determine the optimised mass spectrometric detection parameters for each reference standard, the four cannabinoid reference standards were tuned individually using the manual tuning function on the Analyst 1.5.2. These MS conditions were optimised in both the positive and the negative mode. After initial tuning of the mass spectrometer parameters, they were re-optimised at the elution conditions of different standards from the chromatographic system using targeted multiple reaction monitoring (MRM). Optimised source conditions for various mass spectrometric parameters were used for acquisition in both the positive and negative ESI mode (Table 7).

Table 7: Mass spectrometric conditions as determined during optimisation of the method.

Parameter	Setting
Curtain gas (CUR)	23.0
Declustering potential	90.0
Collision gas (CAD)	Medium
Ion spray voltage (IS)	5000 Volts
Temperature (TEM)	450.0
Ion source gas 1	28.0
Ion source gas 2	36.0

The collision energy (CE) varied for each analyte (Table 8). THC (315.4617→ 193.3) and CBD (315.232 → 193.3) were used as the quantifier ions while THC (315.4617→ 259.0) and CBD (315.232 → 280.7) were used as qualifier ions.

Table 8: Mass spectrometric parameters of the four cannabinoid compounds investigated.

Compound	Molecular mass (g/mol)	Precursor ion (m/z)	Product ion (m/z)	ESI Polarity	Collision Energy (Volts)
Δ^9-THC	314.4617	315.4617	193.0 259.0	+	30
Δ^9-THCA	358.471	359.471	341.2	+	32
CBD	314.4617	315.232	193.0 259.0	+	30
CBN	310.430	311.10	293.2	+	32

Δ^9-THCA: Δ^9-Tetrahydrocannabinolic acid-A, Δ^9 -THC: Δ^9-Tetrahydrocannabinol, CBD: Cannabidiol, CBN: Cannabinol.

2.4 Method validation

The validation process followed the guidelines as set out by FDA and the International conference on harmonisation (ICH) for analytical methods for the pharmaceutical analysis of drugs.[74,131] The analytical method for quantitation was validated for matrix effects, recovery, linearity, limit of detection (LOD), limit of quantification (LOQ), carry-over, inter and intraday precision and accuracy and analyte stability.

2.4.1 Linearity and linear range

Blank matrix was spiked with the certified reference standards to form a seven-point calibration curve. Linearity was established across seven different concentrations of stock solution (0.0, 5.0, 12.5, 25.0, 50.0, 75.0 and 100.0 µg/mL) and samples were injected for LC-MS/MS analysis in triplicate runs on three separate days. Linear equations (y= mx+c) and the coefficient of determination (r^2) for each analyte were determined mathematically using the Analyst ® 1.5.2 software.

2.4.2 Limit of detection and limit of quantification

Analyte peak area over internal standard peak area vs. analyte concentrations were used to construct linear calibration curves for CBD, THC, THCA and CBN over the range (0-100 µg/mL). The LOD was determined by the calibration curve for each analyte, as the lowest measured analyte concentration that can be distinguished

from the noise at the analyte retention time. The average signal to noise (S/N) ratios were determined at the lowest concentration levels for CBD, THC, THCA and CBN by Analyst software. The LOQ was defined as the lowest concentration from which it is possible to quantify the analyte with an acceptable level of accuracy and precision, and was estimated at 5 times the S/N ratio at the analyte retention time using both precursor and product ions during MRM fragmentation analysis.

2.4.3 Intra- and inter-day precision and accuracy

A seven-point calibration curve set was prepared at the following concentrations of stock solution: 0.0, 5.0, 12.5, 25.0, 50.0, 75.0 and 100.0 µg/mL. The stable isotope labelled standard was spiked into the calibration standards at a fixed concentration of 10 µg/mL, and the total volume was made up to 40 µg/mL with the addition of acetone.

The standards were analysed in triplicate on a single day to generate a set of calibration curves to assess intra- and inter-day accuracy and precision which was expressed as a ratio of analyte peak area over IS peak area. The same calibration set was analysed in triplicates on Day 7 to assess analyte stability, recovery and matrix effects.

2.4.4 Stability

Analyte stability in solvent was assessed at ambient temperature (±19 ºC) on Day 1 and again on Day 7. The ratio of the internal standard peak area over analyte peak are for each analyte was compared between freshly prepared samples prepared on Day 1, and those re-injected after storage at ambient temperature for 7 days.

2.4.5. Percentage recovery and matrix effects

The analytes of interest in this study are contained in a variety of pure medium chain triglyceride (MCT) oils, rather than aqueous based biological matrices. Recovery was tested by adding a known amount of the analytes to refined coconut oil and comparing this to the same number of analytes added to mobile phase B. Only the oil-based analytes were extracted the same way as the samples. Determination of percentage recovery and matrix effect was obtained by injecting and eluting a methanol blank using the optimised chromatographic programme while infusing a constant 10 µL/min flow of 50 µL of analyte stock solution in 350 µL of solvent

containing 0.1% formic acid in acetonitrile with 10% deionised water through a T junction just after the HPLC column. The eluent was monitored as if a sample had been injected and the change in signal intensity showed any matrix effects.

2.5 Statistics

For method validation, three triplicate seven-point calibration curves were analysed on Day 1 and Day 7; and all data was analysed using the built-in statistical functions of Analyst 1.5.2 which determines both accuracy and precision. It also performs the linear regression calculation for the best fit formulas for calibration curves, the correlation coefficient and coefficient of determination. The validation process was in accordance with the ICH guidelines for validation of analytical procedures.

2.6 Assessment of label compliance

Pharmaceutical products intended for human use are required to contain labelling information on both the immediate and outer container labels (Refer to Table 5). This information communicates significant information to the user; including the batch number, dosage instructions, name and quantity of active ingredients and storage instructions, amongst others. Unscheduled cannabis products classified as category D (complementary medicines) in South Africa are also subject to additional labelling requirements, such as those intended to inform users that the product is an unregistered health supplement or complementary medicine that has not been evaluated by SAHPRA (Table 6). The labels of all the purchased CBD oils were scrutinized to determine whether they complied to, or violated SAHPRA labelling regulations.

Chapter 3: Results

3.1 Method optimisation

The optimised method providing the best peak shape, sensitivity and mass spectrometric parameters was the final method used for analysis, following adjustments of mass spectrometric and chromatographic parameters. During this study, both targeted and untargeted analyses were performed on six commercially available CBD oils. Optimisation of each analyte was performed by direct infusion (untargeted), followed by manual tuning of mass spectrometer parameters on the Analyst system (targeted).

A sensitive and selective analysis of cannabinoids was achieved with a C18 Phenomenex Gemini column (2 x 100 mm) which showed complete baseline separation of THC and CBD despite identical MRM transitions. The gradient programme was optimised to the conditions listed in Table 9 and allowed sufficient time for column equilibration, and removal of lipophilic, oily residues from CBD oil extracts. Acetone was chosen as the primary solvent for analysis as it provided the best clean-up, recovery and separation of analytes from the oils in which they were formulated. Standard curves at concentrations ranging from 0.01 to 100 µg were developed using the optimised method to determine the concentration range for analysis.

Table 9: Gradient programme for sample analysis using LC-MS/MS.

Time (min)	% B
0.01	0.4
0.02	67.5
3.00	72
4.50	85
6.50	85
6.75	98
8.00	98
8.25	67.5
13.00	67.5

The flowrate was 0.4 mL/min.

The mass spectrometer used in this study had an ESI source capable of both ESI+ (positive) and ESI- (negative) ionisation. Precursor ion (Q1) scans were performed for all four analytes using the initial source conditions, however, Q1 scans of the analytes showed preferential ionisation and greater sensitivity in the ESI+ mode. The collision energy was optimised for each analyte, and lower collision energies (± 30 V) resulted in greater sensitivity when compared to higher collision energies between 80 – 120 V. The retention times for the analytes in positive acquisition mode are provided in Table 10. Representative extracted ion chromatograms (XICs) of the reference standards of THC, CBD, THCA and CBN and the isotope-labelled IS, CBD-D3, spiked into a blank matrix are shown in

Table 10: Average observed retention time for the analytes CBD, THC, CBN and THCA in ESI positive (+) acquisition mode.

Analyte	Retention time (min)
CBD-D3	2.9-3.1
CBD	3.0
THC	4.9-5.1
CBN	4.2-4.5
THCA	6.0-6.2

CBD-D3: Cannabidiol-D3 IS, CBD: Cannabidiol, THC: Tetrahydrocannabinol, CBN: Cannabinol, THCA: Tetrahydrocannabinolic acid-A

3.2 Method validation

The coefficient of determination (r^2) values were >0.97 for all standard analytes. Calibration curves for THC, CBD, THCA and CBN are provided in Figure 6. A summary of the quantitative data, LOQ and LOD for the four cannabinoid compounds is provided in Table 11. The fitting equation and correlation coefficient was determined using Analyst ® software. For all four standards, the LOQ was 5 µg/mL.

Table 11: Summary of the quantification range, correlation coefficient and LOQ and LOD of the four cannabinoid compounds.

Analyte	Quantification range (µg/mL)	Fitting equation	Correlation coefficient (r^2)	LOD (ng/mL)	LOQ (µg/mL)
CBD	0-100	y = 0.0315x	0.9937	149.3	5
THC	0-100	y = 0.0737x	0.9952	293.2	5
CBN	0-100	y = 0.0132x	0.9947	47.2	5
THCA	0–100	y = 0.0124x	0.9760	30.9	5

CBD: Cannabidiol, THC: Tetrahydrocannabinol, CBN: Cannabinol, THCA: Tetrahydrocannabinolic acid-A

The results of intra- and inter-day precision and accuracy measured on Day 1 and Day 7 for each analyte are provided in Table 12**.** Samples dried by evaporation were reconstituted in 30 µL methanol (analytical grade) and vortex mixed for approximately 30 seconds on Day 7. Intra- and inter-day accuracies ranged from 93.6% to 123.7% for CBD, 93.2% to 134.2% for THC and 91.7% to 110.3% for CBN. Precision values were below 9.6% for CBD, 9.0% for THC and 14.8% for CBN. For THCA, intra- and inter-day accuracies varied between varied greatly, and were between 65.0%-188.2%. Precision for THCA was reported as below 18.3%. An error on Day 7, in which double the amount of internal standard was added at the 25 µL sample, resulted in low inter-day accuracy for THC, explaining the outlying value. For the Day 7 repeat calibration, the 5 µg/mL sample of THCA had dried out completely and was reconstituted with methanol and vortex mixed as described above, however, inter-day accuracy remained poor as it is suspected that the analyte did not completely dissolve.

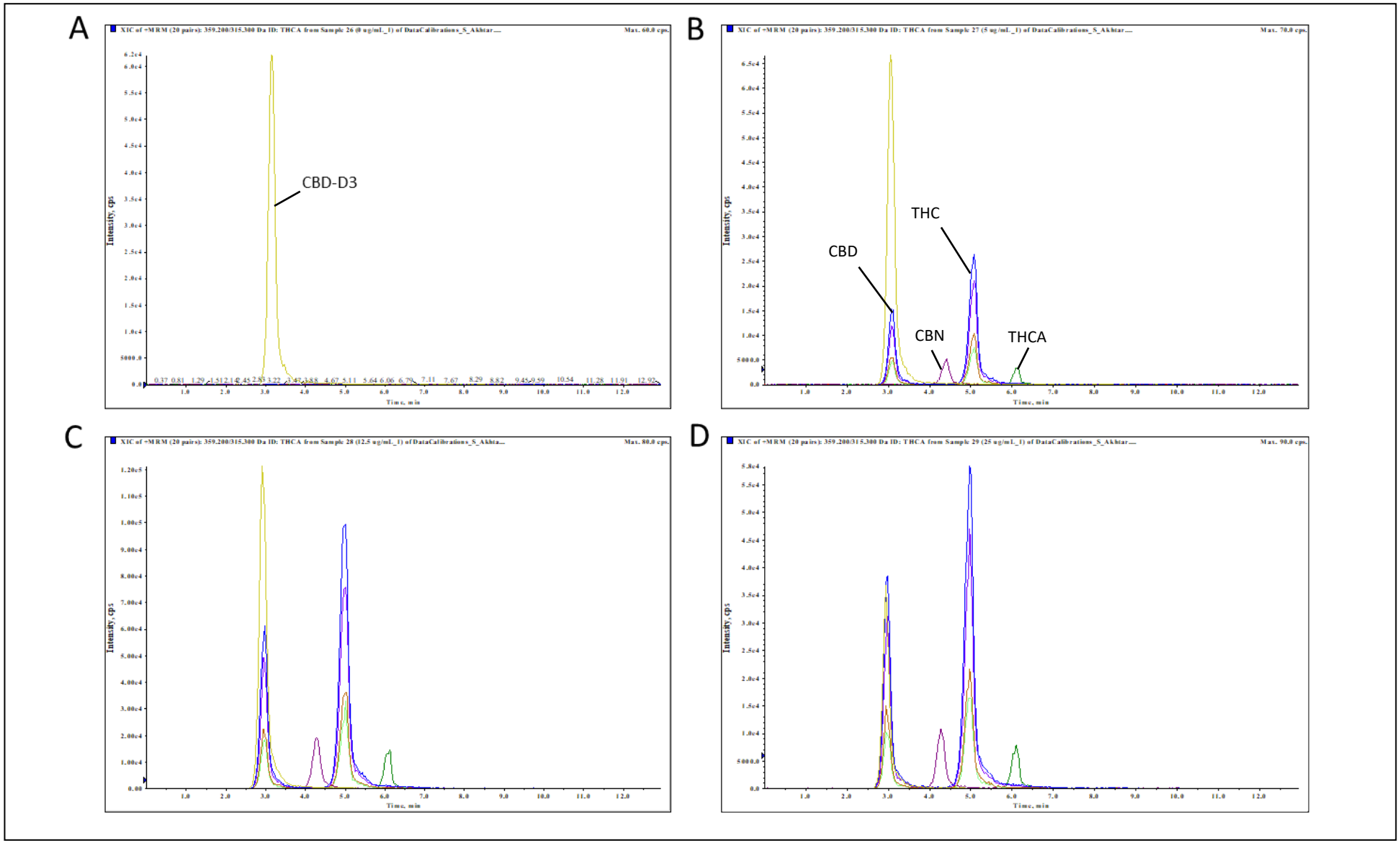


Figure 5: Extracted ion chromatograms of THC, CBD, CBN and THCA reference standards analysed in positive (+) ESI mode at A) 0 µg/mL, B) 5 µg/mL, C) 12.5 µg/mL and D) 25 µg/mL. The yellow line denotes CBD-D3 (IS).

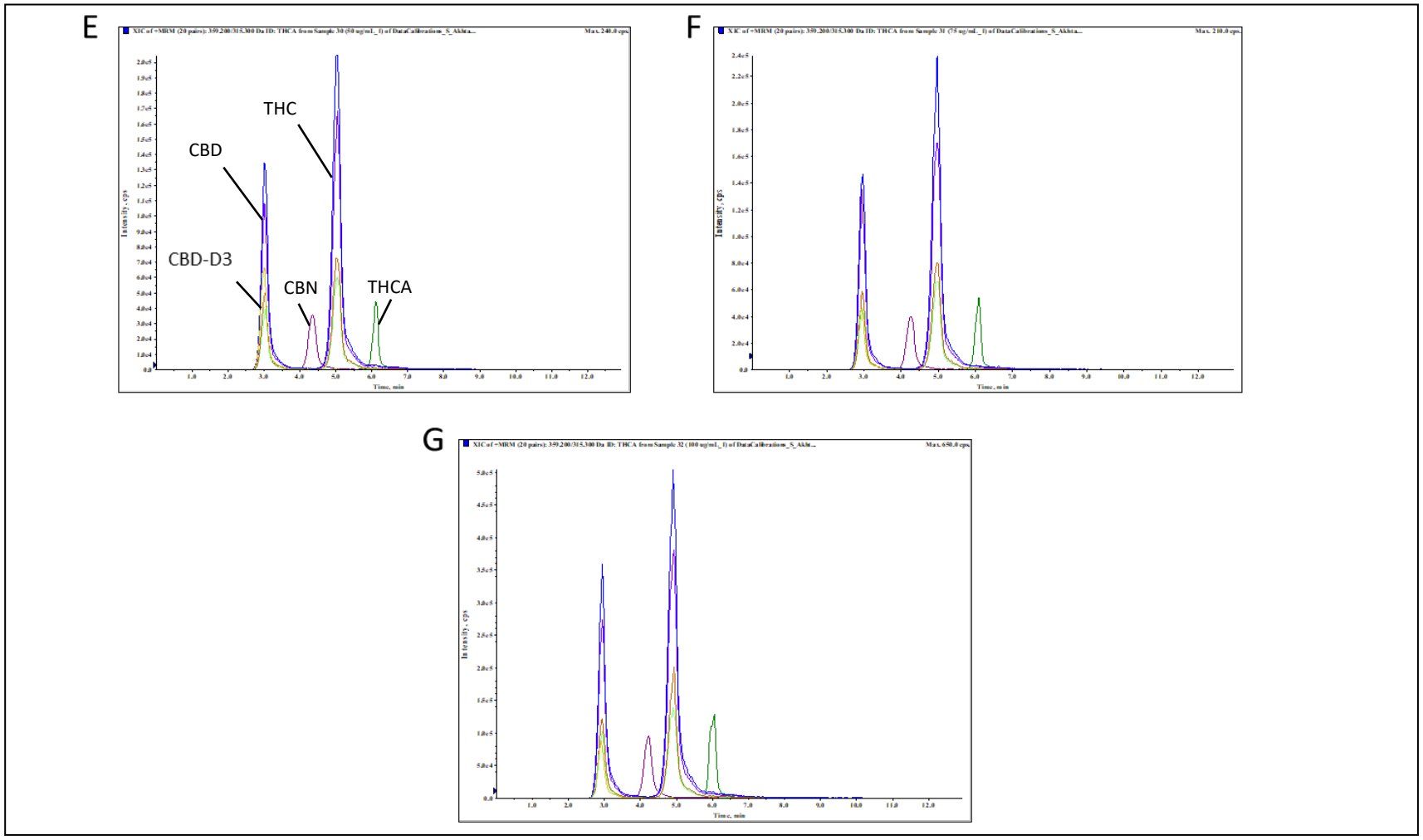


Figure 5: Extracted ion chromatograms of THC, CBD, CBN and THCA reference standards analysed in positive (+) ESI mode at E) 50 µg/mL, F) 75 µg/mL and G) 100 µg/mL. The yellow line denotes CBD-D3 (IS).

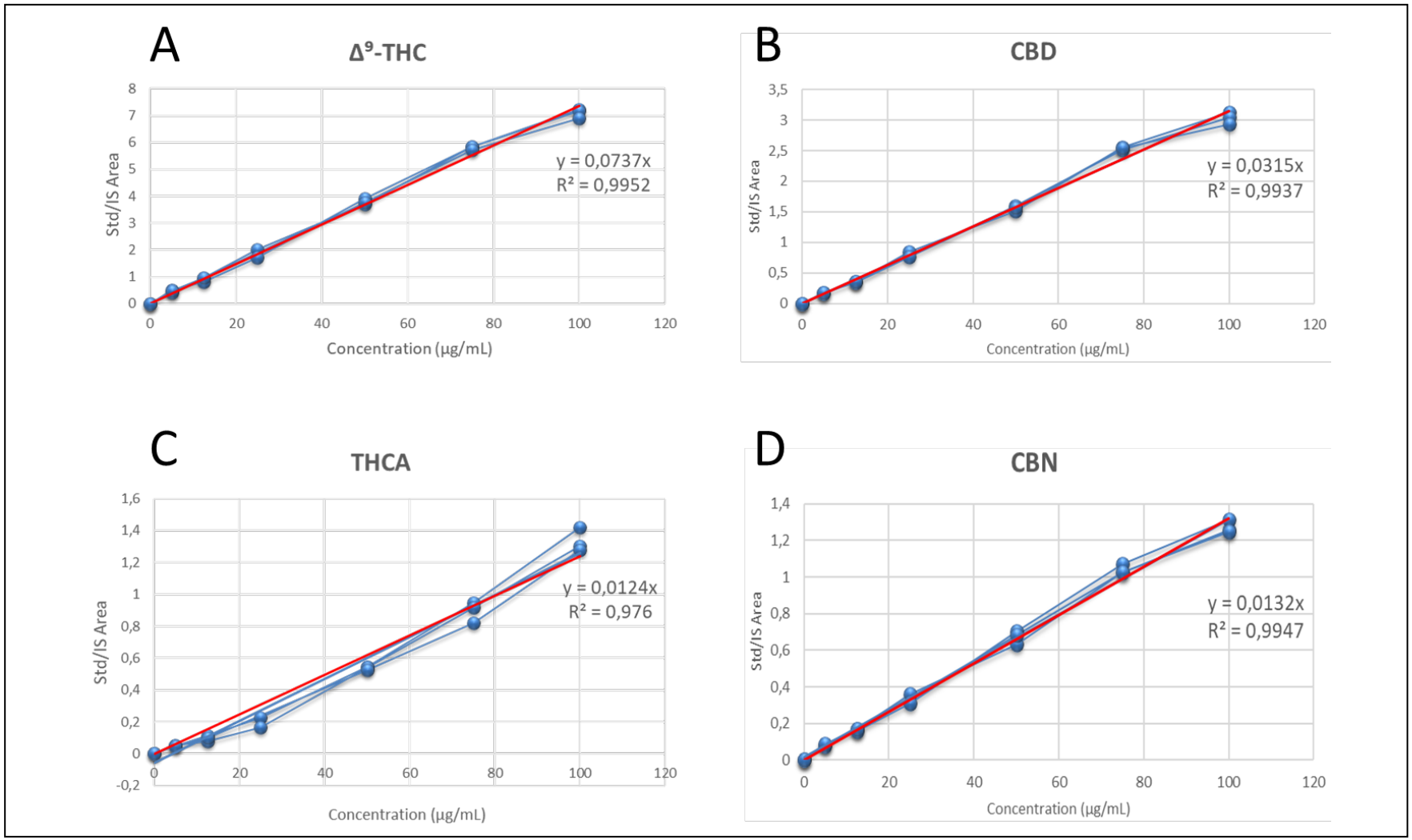


Figure 6: Calibration curves for the standard compounds A) THC, B) CBD, C) THCA and D) CBN with the average trend-line for triplicate runs indicated in red (n=3).

Table 12: Intra and inter-day precision and accuracy for THC, CBD, CBN and THCA.

Analyte	Nominal concentration (µg/mL)	Measured concentration (mean ± SEM) (µg/mL)	Accuracy (%)	Precision (%)
CBD				
Intra-day	0	0.0	N/A	
	5	5.8 ± 0.4	116	6.7
	12.5	11.7 ± 0.4	93.6	3.5
	25	26.8 ± 0.4	107.2	1.3
	50	49.8 ± 0.9	99.6	1.8
	75	81.1 ± 0.2	108.1	0.2
	100	95.3 ± 2.4	95.3	2.5
Inter-day	0	0.0	N/A	
	5	6.2 ± 0.1	123.7	0.9
	12.5	13 ± 0.3	103.6	2.0
	25	27.4 ± 2.6	109.4	9.6
	50	50.3 ± 1.2	100.7	2.5
	75	77.6 ± 5.5	103.4	7.1
	100	97.2 ± 2.6	97.2	2.6
Analyte	**Nominal concentration (µg/mL)**	**Measured concentration (mean ± SEM) (µg/mL)**	**Accuracy (%)**	**Precision (%)**
THC				
Intra-day	0	0.02	N/A	
	5	6.7 ± 0.6	134.2	8.2
	12.5	12.9 ± 0.5	103.3	3.9
	25	27.2 ± 2.0	108.7	7.5
	50	51.2 ± 1.4	102.4	2.7
	75	79.6 ± 2.1	106.2	2.6
	100	95.4 ± 4.2	95.4	4.4
Inter-day	0	0.02	N/A	
	5	6.6 ± 0.4	132.1	6.5
	12.5	15.5 ± 0.2	123.9	1.6
	25	12.5 ± 1.1	50.2	9.0
	50	46.6 ± 1.2	93.2	2.5
	75	78.5 ± 3.2	104.6	4.1
	100	101.8 ± 6.8	101.8	6.7

Analyte	Nominal concentration (µg/mL)	Measured concentration (mean ± SEM) (µg/mL)	Accuracy (%)	Precision (%)
THCA				
Intra-day	0	0.02	N/A	
	5	4.5 ± 0.5	89.8	11.2
	12.5	8.6 ± 1.3	68.4	15.3
	25	18.1 ± 3.2	72.4	17.7
	50	46.2 ± 1.2	92.5	2.6
	75	75.7 ± 5.3	100.9	7.0
	100	112.1 ± 6.1	112.1	5.5
Inter-day	0	6.7 ± 0.03	N/A	
	5	9.4 ± 0.3	188.2	3.3
	12.5	10.5 ± 0.1	83.7	0.9
	25	16.2 ± 1.3	65.0	8.2
	50	48.4 ± 2.6	96.8	5.4
	75	76.8 ± 3.4	102.4	4.4
	100	101.7 ± 18.6	101.7	18.3
Analyte	**Nominal concentration (µg/mL)**	**Measured concentration (mean ± SEM) (µg/mL)**	**Accuracy (%)**	**Precision (%)**
CBN				
Intra-day	0	0.0	N/A	
	5	5.4 ± 0.6	107.6	12.0
	12.5	11.5 ± 1.1	91.7	9.3
	25	25.6 ± 2.8	102.6	11.0
	50	51.2 ± 1.7	102.4	3.4
	75	79 ± 3.0	105.3	3.8
	100	96.4 ± 2.4	96.4	2.5
Inter-day	0	N/A	N/A	
	5	4.6 ± 0.7	92.2	14.8
	12.5	11.6 ± 0.4	92.5	3.0
	25	27.6 ± 1.1	110.3	4.0
	50	51.1 ± 1.6	102.2	3.0
	75	78.6 ± 5.5	104.8	7.0
	100	96.3 ± 2.4	96.3	2.5

Stability curves comparing the measured and expected concentrations (in µg/mL) on Day 1 and Day 7 for CBD, THC, CBN and THCA are provided in Figure 7. All four analytes showed good stability, which were indicative thereof that degradation of analytes did not occur between Day 1 and Day 7 when stored at ambient temperature.

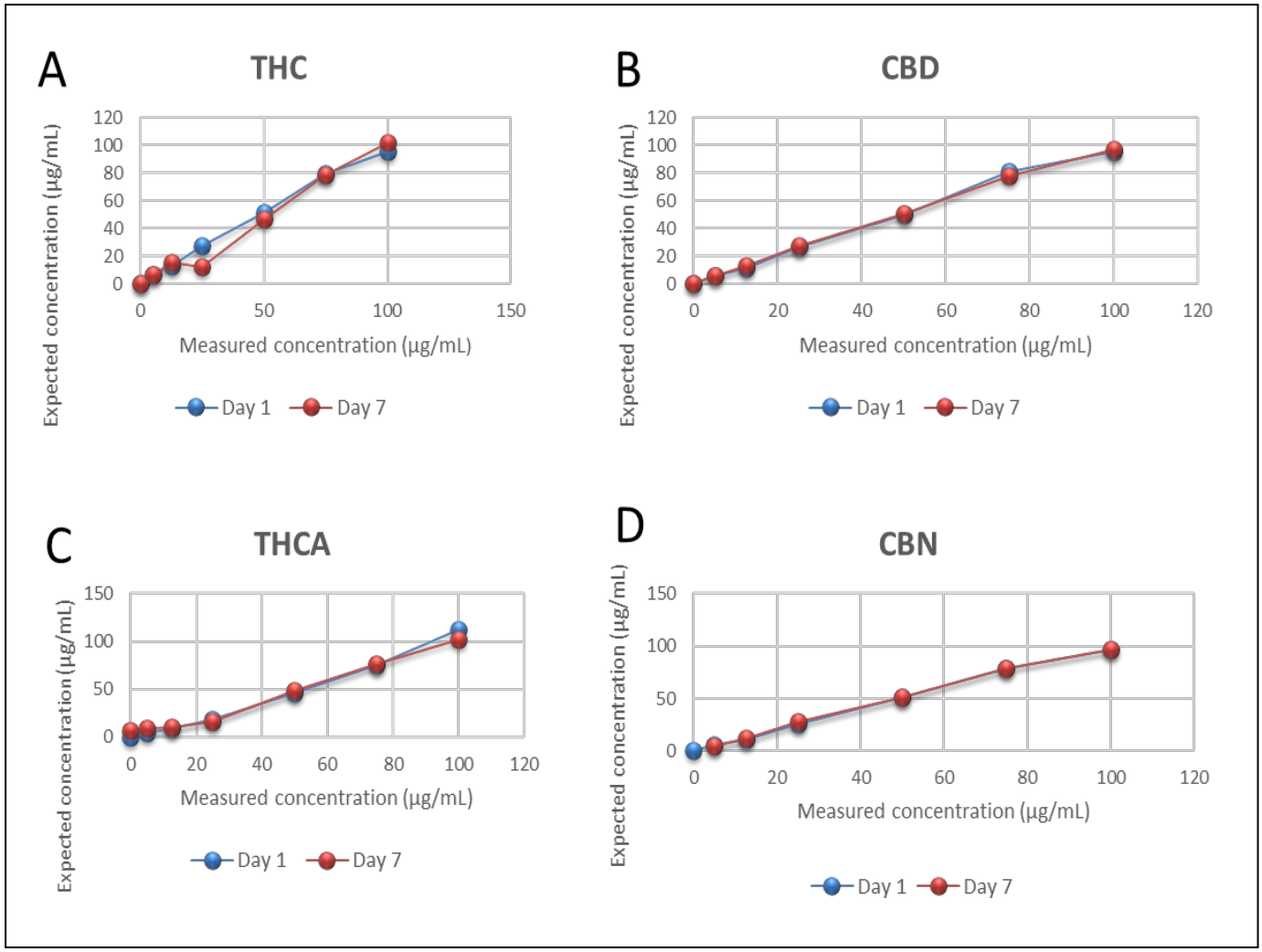


Figure 7: Stability curves showing the expected versus the measured concentration for A) THC, B) CBD, C) THCA and D) CBN on Day 1 (blue line) and Day 7 (red line).

The signal-to-noise ratio was >10 for all analytes, and the analytes showed good recovery following elution of a methanol blank and direct infusion of stock solution and solvents (Figure 8).

No matrix effects that could affect the quantitation of the analytes were observed, as suppression of the analytes at their respective retention times was not seen during the constant-rate infusion. The suppression of ionisation between 9-10 min of the run did not impact the optimised method, as elution and quantitation of analytes was completed by this time.

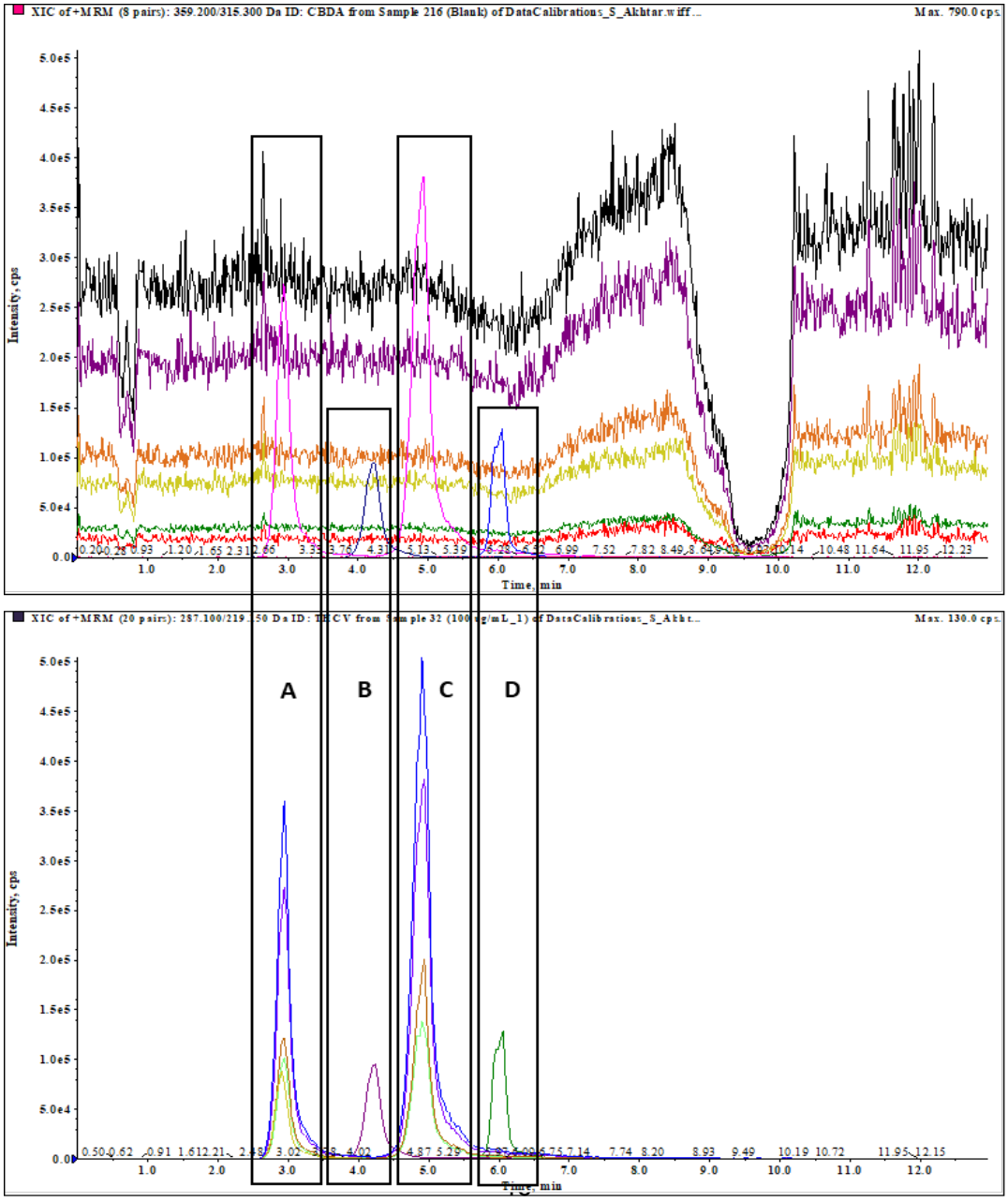


Figure 8: Evaluation of matrix effects observed for A) CBD, B) CBN, C) THC and D) THCA. An extracted ion chromatogram (top graph) of the constant-rate infusion of the four analytes is superimposed with analyte peaks of CBD, CBN, THC and THCA at their respective retention times (bottom graph)

3.3 Cannabinoid content and quality

To obtain approximate cannabinoid concentrations in mg/mL of the formulations, or per container; the analyte concentration values from the calibration curves were multiplied by the dilution factor used during sample preparation and the volume per container of each respective CBD oil. Only the measured concentrations of CBD could be used to compare and assess cannabinoid content and batch-to-batch conformity. The measured CBD concentration (in mg per bottle) was compared to the advertised CBD concentration contained on the outer labels of CBD oil A, B, C, D and E. Cannabidiol concentration was not specified on the immediate container of CBD oil F, therefore, only the measured concentration could be denoted. Results showing the advertised versus measured CBD concentrations are provided in Table 13**,** and represented graphically in Figure 9.

Table 13: A comparison between the reported (on label) and measured CBD concentrations per bottle in the oils analysed.

CBD oil	Batch number	Volume per bottle (mL)	Reported CBD concentration (mg)	Average measured CBD concentration (mg)
A	1	30	800	1080
	2			480
B	1	10	465	349
	2			848
C	1	20	468	595
	2		156	442
D	1	10	400	591
	2			474
E	1	30	300	815
	2			644
F	1	10	None listed	0
	2			5

Following analysis, CBD concentration was found to be underlabeled in eight, and overlabeled in two CBD oils; and measured CBD concentrations for both batches of the six CBD oils ranged from 0 – 1080 mg. The analysed CBD oils were not expected to contain noteworthy concentrations of other cannabinoids; and concentrations of THC, CBN and THCA were not specified on labels. However, the presence of THC and CBN were detectable in CBD oil F at noteworthy concentrations (Table 14). Measured CBN concentrations were also detected in the second batch of CBD oil B and C, at 3.5 mg and 2 mg, respectively. THCA was not detected in any CBD oils. However, this is not ascribed to the sensitivity of the assay as the method was sensitive enough to detect concentrations of THCA as low as 5 µg/mL, as evident from the calibration curves for THCA (Figure 6).

Table 14: Average measured cannabinoid content in CBD oil F determined from duplicate runs.

Batch number	Volume per bottle (mL)	Measured CBD concentration per bottle (mg)	Measured THC concentration per bottle (mg)	Measured CBN concentration per bottle (mg)
1	10	0	47.97	15.4
2		5	131.5	26.3

In addition to cannabinoid content, the consistency of each CBD oil was assessed to determine whether seasonal variation exists between different batches of the same CBD oil. Batch-to-batch consistency was compared in all CBD oils, except CBD oil C which was purchased in two batches containing different amounts of CBD (Figure 9).

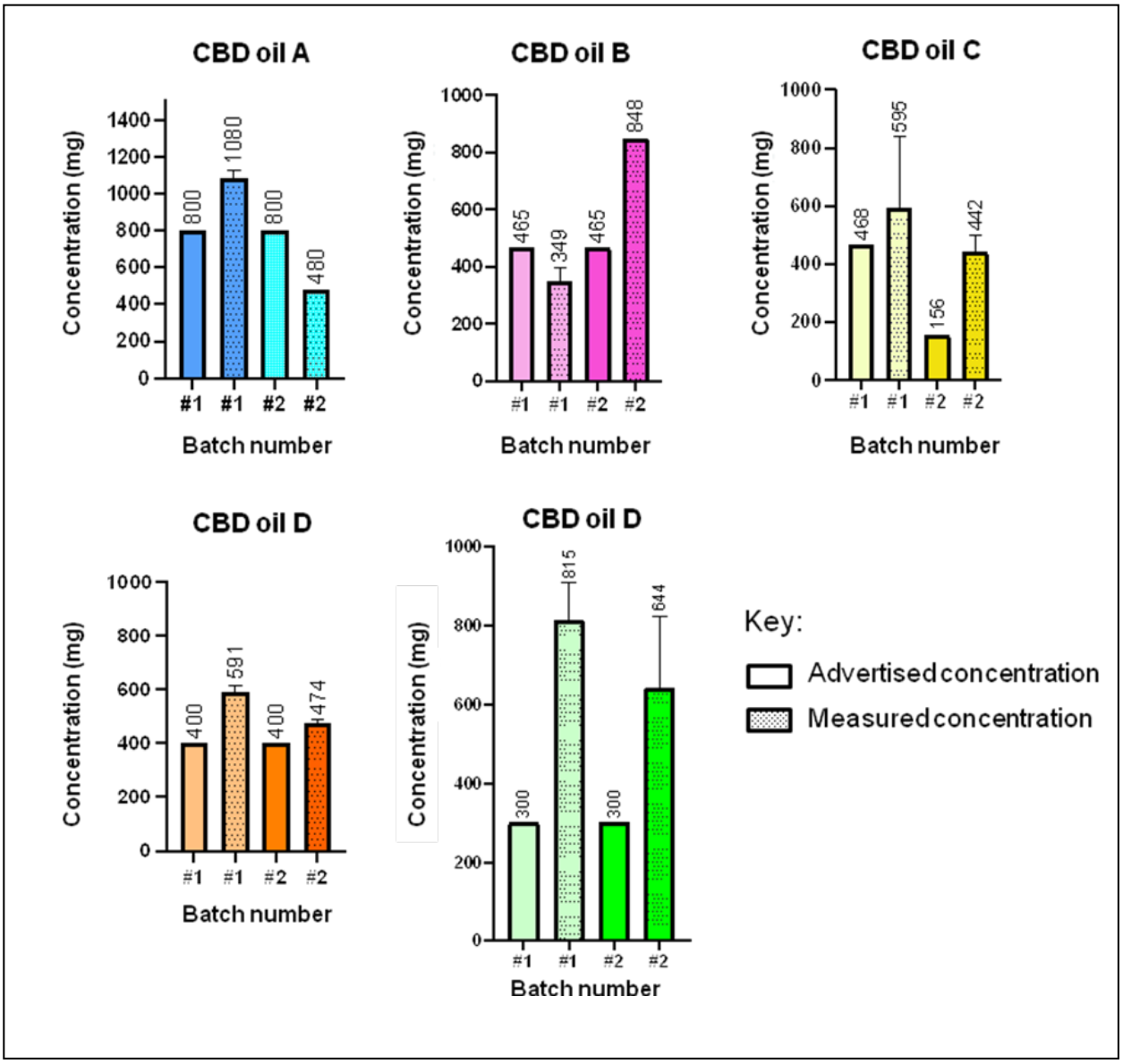


Figure 9: The difference between advertised and measured CBD concentration in the first and second batches of purchased CBD oils. (CBD oil F is excluded as the advertised CBD concentration was not listed on the outer container).

The physical appearance of all CBD oils was assessed between batches, and the results are tabulated (Table 15). The colour, consistency and transparency between 'summer' and 'winter' batches of CBD oils remained the same; with the exception of the first (summer) batch of CBD oil C, where precipitates were observed in the bottle. The second batch of the same oil did not contain any precipitates and appeared clear.

Table 15: Physical characteristics of analysed CBD oils.

	Colour			Consistency	Transparency	
CBD oil	**Gold/light yellow**	**Gold-light brown**	**Black/brown**	**Watery**	**No precipitates**	**Precipitates observed**
A1	√			√	√	
A2	√			√	√	
B1	√			√	√	
B2	√			√	√	
C1		√		√		√
C2		√		√	√	
D1	√			√	√	
D2	√			√	√	
E1		√		√	√	
E2		√		√	√	
F1			√	√	Unclear, sample was too dark to assess transparency	
F2			√	√		

Letters indicating CBD oil, numbers indicate batch 1 or 2

3.4 Analysis of CBD oil labels

Pharmaceutical products in South Africa are required to contain certain labelling information on the immediate and outer containers according to regulations of Act 101 of 1965. A comparison of information provided on the labels of all CBD oils is presented in Table 16.

Table 16: Comparison of information contained on the immediate and outer container labels on both batches of the 6 CBD oils analysed.

Label	A		B		C		D		E		F	
	1	2	1	2	1	2	1	2	1	2	1	2
Batch number	Y	Y	Y	Y	N	N	Y	Y	Y	Y	N	N
CBD content (mg)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Dosage instructions	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Excipient content	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Expiry date	Y	Y	Y	Y	N	N	Y	Y	Y	Y	N	N
Manufacturer details (phone number or address)	Y	Y	Y	Y	N	N	Y	Y	Y	Y	N	N
Statement that the product has undergone testing	N	N	N	N	N	N	Y	Y	N	N	N	N
Statement: “This product has not been evaluated by SAHPRA/FDA”	N	Y	N	N	N	N	N	N	Y	Y	N	N
Statement: “This product is a Health supplement”	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Storage instructions	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Volume (mL)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Warning against use during pregnancy/lactation	N	N	N	N	N	N	Y	Y	Y	Y	N	N
Warning to “Consult a doctor/physician before use”	Y	Y	N	N	Y	Y	Y	Y	Y	Y	N	N
Warning to discontinue use if adverse reactions appear	Y	Y	N	N	N	N	N	N	N	N	N	N

Label	A		B		C		D		E		F	
	1	2	1	2	1	2	1	2	1	2	1	2
Warning to "Keep out of reach of children"	Y	Y	Y	Y	Y	Y	N	N	Y	Y	N	N
Warning not to exceed recommended dose	Y	Y	Y	Y	N	N	N	N	N	N	N	N
Warning when driving/ operating heavy machinery	N	N	N	N	N	N	N	N	Y	Y	N	N

Numbers indicate batch, 'N' indicates No, 'Y' indicates Yes

The labels of all the purchased CBD oils were scrutinized to determine whether they complied with, or violated SAHPRA labelling regulations. A comparison of the presence or absence of labelling and safety information required for complementary medicines as per Act 101 is contained in Table 17. Both batches of oil F did not comply with any of the set regulations, except for the inclusion of the proprietary name. The following information was contained on either the immediate or outer container of all the remaining five CBD oils: proprietary name, dosage form, list of active ingredients and quantity, net quantity of contents, indications for use, storage instructions and class of medicine. Concerning CBD content, all CBD oils, with the exception of oil F, provided CBD content, volume and dosage instructions, on the label as required. Batch number, expiry date, the name of the holder of the certificate of registration/manufacturer (CBD oil A, B, D and E) and the warning "Keep out of reach of children" (CBD oil A, B, C and E) appeared on four CBD oils. However, non-adherence to regulations such as the provision of pregnancy warnings and omission of the statement that the product has not been evaluated by a regulatory body are evident. Additionally, labels containing the medicine schedule, registration number, manufacturing date and category of medicine were not present on any CBD oils.

Table 17: Labelling comparison of the immediate containers of both batches of the CBD oils investigated.

	Presence of labels required for Category D medicines, as per Act 101 of 1965											
	A		B		C		D		E		F	
	1	2	1	2	1	2	1	2	1	2	1	2
Bar code	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Batch number	Y	Y	Y	Y	N	N	Y	Y	Y	Y	N	N
Class of medicine (i.e. Health supplement)	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Dosage instructions	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Expiry date	Y	Y	Y	Y	N	N	Y	Y	Y	Y	N	N
Indications for use	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Manufacturing date	N	N	N	N	N	N	N	N	N	N	N	N
Medicine dosage form (i.e. Cannabis oil extract)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Medicine category (i.e. Category D: Complementary medicine)	N	N	N	N	N	N	N	N	N	N	N	N
Medicine schedule	N	N	N	N	N	N	N	N	N	N	N	N
Name of active ingredients and quantity	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Name of the holder of the certificate of registration/ manufacturer	Y	Y	Y	Y	N	N	Y	Y	Y	Y	N	N
Net quantity of contents	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Proprietary (trade) name	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Registration/application number	N	N	N	N	N	N	N	N	N	N	N	N
Storage instructions	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
The warning: "Keep out of reach of children"	Y	Y	Y	Y	Y	Y	N	N	Y	Y	N	N

Numbers indicate batch. 'N' indicates No. 'Y' indicates Yes

Chapter 4: Discussion

4.1 Optimisation of LC-MS/MS analysis

The development of a sensitive, robust method was achieved through optimisation of mass spectrometric parameters, column chemistry and solvent and mobile phase compositions. This demonstrated that LC-MS/MS analysis proves to be an effective method for the determination of cannabinoid content in CBD oils, and allows for the separation and detection of multiple analytes in a single analysis.

Many studies corroborate the use of C18 columns for reversed-phase liquid chromatography[30,55,76] and analysis of cannabinoids.[69] Methods employed by Lingyun *et al.* showed complete separation of cannabinoids from cannabis plant material using HPLC-PDA detection.[41] This method was adapted through the addition of volatile buffers and an appropriate LC-MS/MS column to befit MS detection. Initial analysis was performed on a C18 Waters BEH polymeric column (4.6 x 100 mm, 2.5 µm) and a Phenomenex Synergi column (2 x 75 mm, 4 µm). However, the best peak shape and compound separation, which added to the sensitivity and selectivity of the cannabinoid analysis was achieved with a C18 Phenomenex Gemini column (2 x 100 mm). The C18 column also showed optimal separation of isobaric analytes that could be clearly distinguished with no overlap, despite identical MRM transitions and similar precursor and product ion masses between THC and CBD. Furthermore, the 100 mm length of the Gemini column allowed sufficient separation of all four analytes (CBD, THC, CBN, THCA) with acceptable resolution.

Several mobile phases were tested to determine their effect on ionization and retention time of the analytes. A review of the different mobile phases selected for cannabinoid analyses using C18 columns revealed that the most common mobile phases are formic acid in water (A) and formic acid in acetonitrile (B).[72,76,132] In this study, the mobile phases found to provide the best chromatograms with smoother and narrower peaks were 10 mM ammonium formate in water (A) and 0.1% formic acid in acetonitrile (B).

In order to facilitate analysis, the separation of cannabinoids from oil components was crucial, as cannabis extracts in CBD oil are dissolved in various organic oils. Most CBD oils in this study contained MCT coconut oil. Through pilot studies it was discovered that the retention time of hydrophobic oily compounds (such as MCTs) were longer and had a later retention time compared to cannabinoids, due to a high affinity to the non-polar stationary phase. Various solvents were analysed in order to determine which solvent provided the best separation. These included acetone, methanol, hexane, methyl tert-butyl ether and isopropanol. It was revealed that acetone gave the best clean-up and recovery of analytes, especially CBD and THC. All cannabinoids were separated and identified from the chromatograms with minimal background noise using acetone. This is in contrast to pilot studies using hexane as a solvent, where low peak intensities of cannabinoids were observed, with a significant amount of background noise. Thereafter, a series of dilutions with 20 µL CBD oil in acetone were prepared (200, 400, 600, 800 µL, 1 mL, 2 mL, 3 mL, 4 mL and 5 mL). Narrower and smoother peaks were observed when a ratio of 20 µL CBD oil to 20 mL of acetone was used for preparation of the samples.

After initial optimisation, it was evident that the detection of all four cannabinoids was discernible in the ESI+ mode, showing greater sensitivity. The collision energy was optimised for each analyte, and lower collision energies (± 30 V) provided greater sensitivity. The optimised gradient programme, according to ICH guidelines, (Table 9) allowed for sufficient time for column equilibration and removal of lipophilic, oily residues from the CBD oil extracts.

4.2 Analysis of CBD oils

Mislabelling of CBD products is prevalent worldwide,[39] and various international studies have queried the quality of over-the-counter, unregulated CBD oils based on results which show they do not contain advertised or reported amounts of CBD and THC.[51,53] All cannabinoid concentrations in CBD oils should meet label claims, without exceeding legal limits as per SAHPRA regulations.[101] Although even high doses of pure CBD oil formulations are well tolerated and considered safe,[51] and the potential for developing an addiction is low; CBD content should still adhere to legal limits. For safety testing and potency analysis, cannabinoids of importance included CBD and the psychoactive cannabinoids THC, CBN and THCA. The potency of cannabis products is represented by the percentage of THC or CBD content contained within. Cannabidiol concentrations measured in this study indicated that the selected CBD oils were either over- or underlabeled (Table 13). In fact, the measured CBD concentration in four CBD oils exceeded 600 mg per sales pack, which is prohibited for schedule 0 medicines. Consequently, dosage instructions and serving sizes (per drop) calculated to ensure ingested concentrations of CBD oil do not exceed 20 mg per day would be either over- or underestimated.

Laboratory tests on 102 CBD oils conducted by the FDA indicated that almost 17% contained less than 80% of the advertised CBD content, while a further 37% contained 120% more CBD than advertised.[133] Only 45% were within a 20% range of advertised CBD content, while almost 50% of the 102 CBD oils contained THC and THCA content higher than the limit of quantitation.[133] Another study of 84 CBD products purchased from 31 companies in the United States indicated that almost 70% of products purchased were mislabelled, containing either a higher or lower CBD content than advertised.[52] Furthermore, THC was detected in 21% of these products, even though THC content was presumed to be low or absent.[52] In yet another study conducted on 14 CBD oils commercially available in European countries, it was determined that 9 contained CBD concentrations different to the advertised amount.[4]

Research conducted at Massachusetts General Hospital on 97 participants using products labelled as having a 'high CBD content' found that CBD was not detected in urine samples of roughly 30% of participants, while THC was detected in the urine of almost 80% of participant's.[134] Similar results were observed in this study (Table 14), and despite purporting to be a CBD oil, CBD oil F contained low CBD concentrations and THC concentrations in excess of that which would be considered legal in South Africa (THC concentrations should not exceed 0.001% of the total preparation in processed products).[103,105] Common adverse drug reactions associated with CBD oils include diarrhoea, dry mouth, loss of appetite and fatigue.[39,135] High THC concentrations pose a danger to consumers, and may cause additional adverse effects affecting mental capacity; including paranoia, hallucinations and loss of memory.[53] Furthermore, unregulated content of psychoactive cannabinoids like THC and CBN may lead to the development of dependence and/or addiction. Cannabidiol is known to antagonize the activity of THC at the CB_1 receptor,[8,10] and clinical studies indicate that conversion of CBD to Δ^9-THC, following oral administration does not occur, even at high doses.[135,136] Thus, hallucinogenic side effects observed with CBD oils are usually attributed to unregulated THC concentrations present in CBD oils.[135] Concentrations of 10-20 mg of THC are enough to cause intoxication when smoking cannabis,[135] and sedative effects of cannabinoids appear to be dose-related.[20] Low concentrations may cause short-term memory impairment and loss of temporal awareness, whereas high concentrations may lead to panic and toxic delirium.[20,70] However, it is difficult to predict what concentration of THC may cause intoxication of orally ingested cannabis due to factors such as bioavailability and first pass metabolism.[135]

The increased specificity of LC-MS/MS analysis for the detection of cannabinoids[76] as opposed to other methods (such as GC-MS and LC-UV) may explain why measured CBD content is significantly higher than reported values in most of the CBD oils analysed. Differences in measured and reported CBD concentrations may also be attributed to variability in extraction and quantification methods employed by cannabis laboratories and manufacturers.[51] Due to their lipophilic nature, cannabinoids extracted in oil matrices appear at higher concentrations post-

extraction compared to cannabinoids extracted in other matrices, such as water.[61] Temperature and atmospheric conditions of extraction methods, such as supercritical CO_2 extraction are known to yield higher concentrations of CBD compared to other methods.[4] Thus, the type of extraction method used may justify the occurrence of higher-than-advertised CBD content determined in CBD oils analysed in this study. The detection of concentrations of CBN in CBD oils (i.e. both batches of CBD oil F, and the second batch of CBD oil B and C) may also be indicative of improper extraction procedures and/or inadequate storage conditions, as the formation of CBN may be a result of THC oxidation during plant aging.[4]

The taste, colour and viscosity of CBD oils may be affected by the type of solvents used in the extraction of cannabinoids.[51] The colour of CBD oils may also be affected by the presence of terpenes in solution.[63] No significant differences in the physical appearance of CBD oils was observed between batches, except for CBD oil C. Precipitates observed in batch 1 but not in batch 2, may be explained by differences in the extraction procedures used during the manufacturing of the two different batches (Table 15). Improper extraction in the first batch of CBD oil C may have allowed impurities to appear in the final product, affecting the physical appearance; although a more likely interpretation for the disparity in appearance may be seasonal variation of cannabinoid content found in cannabis plants used for production.

The sale of unregulated CBD oils also leaves consumers with no legal guarantees regarding the composition and quality of CBD oils.[4] Batch to batch conformity (for CBD) could only be assessed in CBD oil A, B, D and E. Batch 1 and 2 of CBD oil C were purchased at different concentrations, and the advertised CBD content was not listed on the outer container of CBD oil F. However, measured CBD concentration between the two batches of CBD oil F were negligible, at 0 and 5 mg, respectively (Table 14). Measured THC and CBN content in CBD oil F indicated that no batch to batch conformity was observed. Concerning the remaining CBD oils, CBD content varied between the first and second batch of CBD oils A, B, D and E, even though the advertised CBD concentration for all four CBD oils was listed as identical between both batches (Figure 9). Stability curves measuring analyte concentrations

on Day 1 and Day 7 (Figure 7) indicate that degradation of analytes did not occur, and S/N ratios for CBD demonstrate acceptable recovery. Thus, inconsistencies observed between the same batches of CBD oils cannot be attributed to an error in methodology, but rather a number of external factors. These may include differences in cultivation, extraction and manufacturing conditions and/or procedures between different laboratories and manufacturers.

Without consistency between batches of the same CBD oils, therapeutic effects may either be unknowingly heightened or reduced between batches based on the quality of a particular batch. Dosing regimens would need to be adjusted according to advertised CBD concentrations per batch. The implementation of identical analytical procedures by each company for every batch of CBD oils produced, would increase the likelihood of conformity between batches. However, this is not always guaranteed, due to fluctuations of cannabinoid content in the cannabis plant between seasons.[137] Fluctuations in cannabinoid concentrations are related to stages of plant development, and cannabinoid content is highest in flowering plants, and lowest in seedlings.[137] Differences in cannabinoid content may also be attributed to the use of different strains of cannabis between batches, with *C. sativa* strains containing higher cannabinoid concentrations.[138]

4.3 Labelling

All pharmaceutical products available in South Africa are expected to adhere to SAHPRA regulations in terms of quality and content, regardless of medicine schedule. This also encompasses labelling and safety information, however, this is often not enforced as unscheduled pharmaceutical products are not checked and/or regulated. In South Africa, unscheduled medicines are freely available to the public without prescription in both pharmacies, hospitals and shops.[85] Although only CBD oil A, C, D and F were manufactured in South Africa, all CBD oils were easily available for purchase online in South Africa and are thus subject to labelling requirements stipulated by SAHPRA.

According to the Medicines and Related Substances Act, certain labels are required to be present on medicines intended for human use (refer to Table 5 and 6).[89] The following labelling information was present on both the immediate and outer containers of CBD oils A, B, C, D and E: the proprietary (trade) name, medicine dosage form, name and quantity of active ingredients, net quantity of contents, volume of solution, dosage and storage instructions. Despite containing no labelling information other than the proprietary (trade) name, CBD oil F is being sold and marketed as a CBD oil; leaving users unaware and oblivious to the illicit cannabinoid content contained within. The name(s) of all active ingredients were listed on CBD oils A-E, although only CBD oil A and B contained a warning to not exceed the recommended dose (i.e. the maximum daily dose of 20 mg CBD per day).

South African cannabis legislation states that cannabis products may only remain unscheduled if the THC and CBD content in processed products is below 0.001% and 0.0075% respectively, while making only a general health claim.[88,91] This is often in conjunction with warnings stipulating that the product has not been evaluated for use by a regulatory authority such as SAHPRA. Although this particular warning is not a requirement for Category D medicine labels, the second batch of CBD oil A, and both batches of CBD oil E, conveyed this warning to potential CBD oil users. All CBD oils, excluding batch 1 of CBD oil A and both batches of CBD oil D, indicated that CBD oils are health supplements not intended to treat any specific conditions(s) (i.e. indications for use). Another significant requirement is for labels to display the medicine schedule, which was absent from the inner and outer container all 6 CBD oils. However, this may be explained by the initial grace period of one year given for CBD-containing preparations. Additionally, the absence of the registration/application number from all CBD oils is accounted for by the fact that unscheduled medicines do not need to be registered in order to be marketed and sold in South Africa. However, this type of information will be required for registered cannabis-containing medicines that may become available in South Africa in the future.

The expiry date and batch number are required to be present on CBD oils, and are important for quality control and tracking purposes. These were listed on all CBD

oils, excluding both batches of CBD oil C and F. On the contrary, the manufacturing date and description of the category of medicine (i.e. Category D; Complementary medicines) were absent from the immediate and outer containers of all 6 CBD oils, despite being legally required to do so.

Despite the exclusion of certain CBD-containing preparations from schedules to the Medicines Act, and research showing that CBD has a good safety profile,[33] the individual schedules of CBD, THC and the cannabis plant indicate that they carry stricter regulations. Thus, they should be treated as potentially dangerous or addictive substances; and the importance of warnings on questionable cannabis products new to local markets cannot be underestimated. Both batches of all CBD oils, excluding CBD oil B and F contained a warning advising potential users to consult a physician or medical professional before use. Of the six analysed CBD oils, only CBD oil A contained a warning to discontinue use if adverse reactions are observed, and CBD oil E was the only oil to contain a warning stating that precautions should be taken when driving motor vehicles or operating heavy machinery. Areas of the brain controlling co-ordination, judgement and memory are affected by THC in particular, and it is apparent that THC reduces psychomotor and cognitive skills.[70,139] Thus, cannabis use is a major risk factor for vehicular accidents as it impacts driving performance.[67,70,139] The bioavailability of THC in the human body depends on the route of administration and frequency of use, and may be detectable from 1.5 to 4.5 h following oral administration (depending on the administered dose).[70,140] Furthermore, it has been reported that even after cessation of cannabis administration, cannabinoid concentrations may still be detected in biological samples indefinitely.[30,55,140] A study conducted by Goodwin *et al.* in users of hemp oils containing high THC concentrations demonstrated that the plasma levels of THC and its metabolites may be comparable to plasma levels of those using dronabinol. Therefore, very low THC concentrations present in CBD oils may accumulate in the body of regular CBD oil users, resulting in an accumulation of THC over time.[38,70] Due to their lipophilic nature, concentrations of THC may also gradually enter the blood stream following adsorption to adipose tissues and/or poorly vascularised tissues.[70,139] Consequently, frequent, long-term CBD oil users

may unknowingly experience delayed psychoactive effects as a negative consequence. This also has significant repercussions for cannabis testing in the workplace and instances of roadside testing.

It is of utmost importance that CBD oils contain labels warning against the dangers of cannabis use amongst the youth, whether intentional or accidental. Cases of accidental ingestion of cannabis edibles in children have been reported,[39] although studies elucidating the safety and efficacy of cannabis in children are rare.[141] Cannabis use in children (except for medicinal purposes) is usually prohibited and discouraged worldwide,[142] and Epidiolex is the only FDA –approved CBD-containing medicine for children.[38] Adolescent cannabis use may cause structural and functional changes to the developing brain and there are reports that CBD may lead to seizures in toddlers.[20,39] Only four CBD oils (CBD oil A, B, C and E) contained the warning "Keep out of reach of children", adhering to SAHPRA regulations for Category D medicines.

Clinical trials involving cannabis are scarce, and pregnant women are generally excluded from clinical trials to avoid possible unwanted effects.[143] However, some studies show that prenatal cannabis use affects neurodevelopment,[144] is associated with a 50% chance of low birth weight, and an increased risk of foetal impairment and preterm birth.[64,145] Clinical information on the presence and effects of cannabinoids in breast milk are unknown,[145] although it is known that cannabinoids are able to cross many types of cell barriers, including the placental barrier due to their lipophillic nature.[144] Thus, there is a possibility that cannabinoids may be present in the breast milk of lactating others using cannabis products. CBD oil D and E were the only ones to contain a warning discouraging use during pregnancy and/or lactation, and these warning were observed on both batches.

4.4 Recommendations

Currently, there is no single analytical method which is able to simultaneously detect the presence of contaminants, and both cannabinoid and non-cannabinoid cannabis components.[63] There is also no standard method for the extraction and detection of

compounds in cannabis products.[38,51] Nor is there a general consensus on any particular method for the determination of cannabinoid content in South African laboratories. Numerous chemical constituents in the cannabis plant, and the many types of cannabis formulations available make it difficult to form an agreement in terms of which analytical method is best for cannabinoid quantification.[51,63]

The development of distinct sensitive, robust and generally-accepted methods for the extraction and quantification of cannabinoids may encourage standardisation across all local laboratories and manufacturers producing CBD oil. However, this may be challenging as mass spectrometric and chromatographic techniques are capable of adjustment, and must be modified and optimised for each instrument. Furthermore, extraction and manufacturing procedures may also depend on, and be limited to the equipment and facilities available. Thus, ensuring safe cannabis use, especially with unregistered cannabis products will continue to be a challenge. However, implementing guidelines pertaining to the use of specific extraction techniques may mitigate the presence of unwanted contaminants in the final product, while taking into consideration the variance in extraction efficacy between methods. Manufacturers of CBD oils may be reluctant to declare possible THC content in their products due to legal repercussions, and small-scale manufacturers of CBD oils may not even have the equipment or facilities to correctly quantify CBD or THC content. In order to avoid high THC content, a possible recommendation would be that manufacturers of CBD oils be required to use *C. indica* strains when producing CBD oils as it may increase the probability that unregulated CBD oils contain higher levels of CBD compared to THC.

Concurrent with the above-mentioned recommendations, stricter enforcement of compliance to labelling requirements on CBD oils needs to be ensured. Additional warnings and safety information is recommended to be added, which may include written warnings with instructions to exercise caution when performing activities that require attentiveness and focus. These include activities such as driving and operating heavy equipment and machinery. This warning should also be extended to include any activity that requires co-ordination, judgement and concentration; since

unregulated CBD oils may contain questionable amounts of THC and other psychoactive cannabinoids. Additionally, it is recommended that warnings advising against the use of cannabis products during pre-and post-natal periods should be a requirement to encourage safety of both mother and child. The inclusion and enforcement of the above warnings, in combination with warnings advising users to strictly adhere to only the recommended daily/prescribed dose would be worthwhile.

In addition to improving safety information and ensuring compliance to SAHPRA regulations, patients and health care professionals (HCPs) should be educated on the clinical evidence, indications, adverse drug reactions and legislation surrounding cannabis. According to an informal survey of medical marijuana specialty physicians in California, most patients that sought medical advice and/or recommendations for cannabis products were those that self-medicated with marijuana prior to its legalisation in the United States.[146] Since the legalisation of recreational and medicinal cannabis in South Africa, the general public are able to self-medicate and have the option of choosing from a variety of medicinal cannabis preparations. Unscheduled cannabis products do not require consultations or prescriptions from health care professionals prior to purchase, leaving consumers with the task of doing their own research about cannabis products. More research needs to be done on a South African audience to assess the public's knowledge and understanding of cannabis use and safety. However, HCPs should still be knowledgeable on the uses and indications of cannabis, in order to advise patients seeking medical advice.

The reluctance of HCPs to suggest cannabis as treatment options for patients due to either a lack of knowledge on pharmacological aspects of cannabis or scepticism in the safety and quality of unregulated CBD oils cannot be dismissed. An analysis of 26 international studies across countries such as the USA, Canada, Ireland and Australia revealed that health care professionals generally held favourable opinions regarding the medicinal use of cannabis in clinical practice.[147] However, the studies also revealed that HCPs that they lacked knowledge across both the legislative and clinical aspects of cannabis use.[147] If cannabis use continues to remain legal in South Africa, especially for medicinal use, then incentives to educate HCPs on the

role of the human ECS are recommended to be implemented. To mitigate potential psychoactive effects, HCPs should be aware that some unregulated CBD oils may contain trace amounts of psychoactive cannabinoids, and they should advise patients accordingly.

Cannabis legislation has legal, financial and social implications, and has a considerable impact on the lifestyle and health of those using cannabis products, especially the youth.[20] Educational measures implemented to educate both health care professionals as well as the general public would prove to be effective in ensuring the safe use of CBD oils. This could be in the form of safety data sheets, package inserts/patient information leaflets or pamphlets provided by manufacturers.

Chapter 5: Conclusion

Globally, countries have begun to relax stringent, age-old laws surrounding cannabis use, and have started to facilitate the research and/or legalisation of medicinal cannabis. The demand for pure CBD oils is one that is likely to increase as both HCPs and patients seek alternative forms of medicine to treat conditions and illnesses that CBD is known to provide beneficial effects for.

The South African cannabis market includes a variety of medicinal and recreational products easily available following the legalisation of cannabis in 2018, and South African cannabis legislation has also made provisions for CBD-containing products in particular. Numerous medicinal cannabis preparations include CBD oils, many of which contain concentrations of THC and/or psychoactive cannabinoids affecting product safety and efficacy.[51] However, the exclusion of certain CBD preparations from schedules to the Medicines Act means that it is a responsibility of the manufacturers to meet the criteria of a safe, good quality product that adheres to legal requirements. These standards may be difficult for small scale-manufacturers to achieve without the correct facilities and equipment to extract and convert raw cannabis plant material into a purified product.

The development of a robust, validated LC-MS/MS method affirms the utilization of LC-MS/MS for the detection and quantification of cannabinoids. Targeted analysis of six commercially available CBD oils indicated that most were mislabelled and failed to meet label claims, with some containing CBD concentrations exceeding 600 mg CBD per sales pack. This may impact consumers purchasing CBD oils regularly to relieve symptoms, thus it is important for CBD content to remain at therapeutic levels to provide health benefits. Additionally, high concentrations of the psychoactive cannabinoids CBN and THC were detected and measured in one CBD oil. This breaches South African laws for CBD-containing cannabis products to be considered as schedule 0 medicines, thus justifying the need for stricter regulations and enforcement of existing regulations. An assessment of the quality and batch to batch consistency of CBD oils also revealed that in all purchased products, there were differences in cannabinoid content between batches of the same product.

Regulations and/or guidelines concerning the manufacturing of cannabis products should be instituted as soon as possible to allow GMP guidelines to be implemented prior to marketing and sale of products. Additionally, an inspection of the immediate and outer container labels revealed that many labelling requirements for pharmaceutical products in South Africa (stipulated by SAHPRA) were present on the majority of selected CBD oils. However, minimal warning and safety information on the selected CBD oils is indicative of the need to include more information in an effort to improve safety.

In future, any steps taken towards cannabis reform in South Africa should take the existing measures of the cannabis market into consideration. Sensitive, robust and generally accepted methods must be developed to accurately extract, purify and quantify cannabinoids. This may ensure the production of quality products with minimal differences between batches. South Africans are able to purchase CBD oils without prescription or advice from health care professionals, thus emphasizing the importance of safe, efficacious products with adequate labelling and safety information. Lastly, the importance of improving cannabis education must not be underestimated. Initiatives taken to educate both patients and HCPs on how to successfully implement cannabis into new or existing treatment protocols will ensure that the future of cannabis in South Africa is brighter than its past.

Chapter 6: Study limitations and recommendations

The selection of CBD oils available for purchase online from various websites in South Africa were limited, and the purchase of CBD oils for this study was dependant on those able to be delivered to the University of Pretoria, given strict purchasing protocol. Thus, the number of samples tested in this study is not representative of all CBD oils available in South Africa. University approval for the order of CBD oils and cannabinoid standards for this study was difficult to obtain initially, as procurement of cannabis products was requested only a few months after the legalisation of cannabis products in South Africa.

This study only focused on the detection of four cannabinoids, based on pharmacological action and a high likelihood and/or prevalence in cannabis products. However, future studies undertaken to determine the presence of other cannabinoids may be beneficial as many chemical compounds may be present in very low quantities in cannabis products, resulting in potentiating effects. Thus, future research conducted on cannabis products focusing on the targeted analysis of different cannabinoid combinations to determine possible potentiating or deleterious effects of different cannabinoids would also be worthwhile. The possible presence of other compounds found naturally in the cannabis plant such as terpenes, saponins, flavonoids and many others were not determined using a targeted analysis. Thus, the true composition of compounds and chemical purity of the CBD oils analysed in this study is not known. Future studies utilizing an untargeted analysis to assess the complete profile may provide a clearer picture as to the true chemical composition of cannabis products.

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Appendices

Appendix I: Research Ethics Committee approval letter

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- ICRG #: IORG0001762 OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

22 September 2020

Approval Certificate
Annual Renewal

Ethics Reference No.: 661/2019
Title: Cannabinoid profile and regulatory compliance of non-scheduled cannabinoid-containing products in South Africa

Dear Ms S Akhtar

The **Annual Renewal** as supported by documents received between 2020-08-25 and 2020-09-16 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2020-09-16 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2021-09-22.
- Please remember to use your protocol number (661/2019) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)