

**An investigation of tetrahydrocannabinol, cannabidiol and cannabinol  
content of cannabis confiscated by the South African Police Service's  
Forensic Laboratories from various regions of South Africa**



By

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## Abstract

**Aim:** The aim was to quantify the tetrahydrocannabinol ( $\Delta^9$  - THC), cannabidiol (CBD) and cannabinol (CBN) levels in cannabis from various towns and cities in South Africa from samples confiscated by the SAPS Forensic Laboratories in an effort to assess the quality with regard to potency of the cannabis in a localised South African market.

**Introduction:** Cannabis, most commonly used illicit recreational substance globally, is from a preparation of flowering plants that synthesize a unique class of compounds known as the cannabinoids. Pharmacologically, the principal psychoactive constituent is a cannabinoid called  $\Delta^9$  - THC. The amount of  $\Delta^9$  - THC in conjunction with selected additional cannabinoid compounds, determines the strength or potency of the cannabis product. Recently, reports have speculated over the change in the quality of cannabis products over the past few decades specifically with regards to the increase in cannabinoid content which subsequently affects the strength of the drug product. South Africa currently has no monitoring programs or studies investigating the cannabis circulating the market.

**Methodology:** Selected cannabinoids were extracted in a methanol: chloroform (9:1 v/v) solution. Isocratic chromatographic separation was achieved on a Gemini 3 $\mu$  C18 110A (50 X 2.00mm) column with methanol and a 10mM ammonium acetate solution (1:1 v/v) as the injection solvent and acetonitrile and 0.1% formic acid as the mobile phase. Spectrometric analysis was performed on an AB Sciex 2000 mass spectrometer with turbo spray ionisation in the positive mode. The transitions of the protonated parent ions  $m/z$  315,  $m/z$  315 and  $m/z$  311, to the product ions  $m/z$  193, 193 and 223 was monitored for  $\Delta^9$  - THC, CBD and CBN respectively. The calibration curves fit quadratic regressions and the lower limit of quantitation for all three analytes was set at 1.56 $\mu$ g/ml.

**Results:** No CBD was detected in the cannabis sample studied. CBN and  $\Delta^9$  - THC was detected in all samples. All samples contained high levels of the degradation product. Gugulethu had the highest concentration of CBN at 19.05%, whilst Swellendam had the highest concentration of  $\Delta^9$  - THC at 20.62%. The average CBN and  $\Delta^9$  - THC was 6.68% and 4.84% respectively. The mean CBN/ $\Delta^9$  -THC ratio of all the samples was 1.91. The sum of  $\Delta^9$  - THC and CBN showed no distinct pattern in cannabinoid content between districts in and surrounding Cape Town.

**Conclusion:** The lack of CBD in the samples is indicative of a *Cannabis sativa* cannabis strain. Results indicate the samples used in this study are relatively old, with degradation of the active component evident. The results of this study in comparison to previous literature support an increase in cannabinoid content during the past few decades.

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## Abbreviations

CBD/ $\Delta^2$ -CBD -	Cannabidiol
CBN -	Cannabinol
CE -	Collision Energy
CXP -	Collision Cell Exit Potential
DP -	Declustering Potential
EMCDDA -	European Monitoring Centre for Drugs and Drug Addiction
HPLC – MS	High Performance Liquid Chromatography
GC – MS -	Gas Chromatography – Mass Spectrometry
LC -	Liquid Chromatography
LLQ -	Lower Limit of Quantitation
MRM -	Multiple-reaction monitoring
MS -	Mass Spectrometry
NIDA -	National Institute on Drug Abuse
NMR -	Nuclear magnetic resonance spectroscopy
SA -	South Africa
SAPS -	South African Police Service
UK -	United Kingdom
UNODC -	United Nations Office on Drugs and Crime
US -	United States of America

$\Delta^9$ -THC / (-)-trans  $\Delta^9$ -THC - Delta - 9- tetrahydrocannabinol

$\Delta^9$ -THC acid B /4-COOH-THC) - Delta - 9 - tetrahydrocannabinol-4-oic acid

$\Delta^9$ -THC acid A/2-COOH-THC - Delta - 9 - tetrahydrocannabinol-2-oic acid

## Part A: Research Protocol

### 1. Introduction

Cannabis, most commonly referred to as marijuana is an illicit recreational drug produced from the preparation of the *Cannabis* plant. Cannabis has been utilised for thousands of years as both fibre and as a recreational and medicinal drug. The earliest reported use of cannabis was 2900 BC in Chinese traditional medicine and has since then significantly incorporated itself in society forming the ethos of the largest drug cultures today and is the most commonly used illicit drug today (1). *Cannabis* is a genus of flowering plants that synthesizes chemical compounds known as cannabinoids. Pharmacologically, the principal psychoactive constituent of cannabis is a cannabinoid called tetrahydrocannabinol (THC). According to the United Nations Office of Drugs and Crime, the amount of THC in conjunction with additional cannabinol compounds determines the strength or potency of the drug product (2).

### 2. Literature Review

#### 2.1 Cannabis: Cultivation and Preparation Methods

Cannabis is marginally the most widely produced; trafficked and consumed illicit substance globally (3, 4). In 2011 cannabis was the largest contributor to the illegal drug production market with production of cannabis taking place across all continents and in almost all countries. According to the United Nations Office on Drugs and Crime (UNODC), in 2009 between 2.8% and 4.5% of the global population aged between 15 -

64 years, an estimated 125 - 203 million individuals, reported cannabis use at least once during the previous year alone (3).

The rise in popularity of cannabis as a recreational substance began in the second half of the 20th century. This, as well as the improvement of breeding techniques and the advancement of technology particularly in the field of hybridization and crossbreeding means that cultivation of cannabis now occurs at a much larger scale and is much more specific with the intention of maximizing the psychoactive potential than in previous years (5). The *Cannabis* genus includes three distinct sub species namely *Cannabis Sativa*, *Cannabis Indica* and *Cannabis Ruderalis* (6, 7). *Cannabis Sativa* is the plant species most commonly utilized for the production of cannabis since it is characterised by a high THC to cannabidiol (CBD) ratio, the amount of THC in relation to the additional cannabinoid compounds in the *Cannabis* plant. *Cannabis Indica* however has the more favourable growing cycle reaching maturity much sooner than *Cannabis Sativa*, so hybrid varieties with varying ratio of *Cannabis Sativa* and *Cannabis Indica* are incredibly popular (6,8,9). Cannabis intended for recreational use is available in different forms. Marijuana is the most widely consumed form resulting from the preparation from the dried flowering tops and leaves of the cannabis plant. Other available products included hashish, a resin made from the flower secretions of the cannabis plant, and hash oil, the essential oil extracted from the cannabis plant which is also the strongest preparation of cannabis (9, 10).

## **2.2 Chemistry of Cannabis**

The *Cannabis* plant is one of the most widely studied plants but the chemistry of the plant is still complex. There are more than 525 identifiable chemical constituents found in marijuana, the most distinctive and unique to the *Cannabis* plant are the cannabinoids (11). Currently, there are more than 80 identified cannabinoids that can be categorized into 10 subclasses including tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBD) (11, 12). THC subclass includes nine different isomers but the isomer delta-9- tetrahydrocannabinol ( $\Delta^9$  - THC) is the most active form

producing the physiological effects associated with marijuana use. Although THC has long been known to be the primary psychoactive constituent, recent evidence suggests that the effects of marijuana are due to THC in conjunction with additional cannabinoids specifically CBD which has been found to induce some pharmacological effects. CBN is the oxidation artefact of the breakdown of THC. Very little, if any exists in freshly dried marijuana and the age of marijuana samples can be estimated from the THC and CBN content depending on the storage conditions. Therefore, for the determination of quality of cannabis product samples it is essential to analyse the concentrations of THC, CBD and CBN (11, 12).

### **2.3 Marijuana Culture: Impact in Society**

The cannabis trade has a significant burden on society. The production of cannabis, the most commonly consumed illicit drug, occurs in almost all countries and across all continents. Both the indoor and outdoor variety of cannabis is relatively easy to cultivate a factor that has contributed to the increase in cultivation and trade. Outdoor cannabis is most common and is widely available whereas trends for indoor cannabis indicated that production is concentrated mainly in developed countries such as North America, Europe and the Oceania. Cannabis trafficking trends indicate that cannabis transportation is predominately intra-regional and that most cannabis is locally produced and consumed. According to the UNODC in 2012, Morocco is the leading country in resin production, followed by Afghanistan and Lebanon. Although there isn't much comprehensive information relating to the cannabis trend in Africa; usage is perceived to be widespread and on the increase (13, 14).

In addition to the impact the marijuana phenomenon has on the macro-level of society through its inter-continental cultivation and trade, marijuana usage impacts society on multiple different sub-fractions, one major influence being on crime. A number of studies have provided evidence for the positive association between drug misuse and crime. A meta-analysis conducted by Bennet *et al* to determine the statistical

association between drug misuse and crime indicated that there is a definite correlation between marijuana usage and crime. The meta-analysis reported that the odds of marijuana users offending are approximately 1.5 times greater in comparison to non-marijuana offenders. In addition they reported that heavy adolescent marijuana use is most associated with drug-related and property crime and not violent crime (15). An additional study conducted by Green *et al* discovered that heavy adolescent marijuana users are significantly more likely in comparison to non-users to have interactions with the criminal justice system in adulthood. Moreover, the study also showed that heavy adolescent marijuana usage increases the risk of engaging in criminal activities, with approximately 58.9% of the reported heavy adolescent marijuana users having an arrest record compared to only 34.8% of light/non-users. Heavy adolescent users also had more arrests on average than light/non-users and were also more likely to be arrested at younger ages. The study also showed that the more extensive criminal history of heavy adolescent users increases their chances of future incarceration. Green *et al* further elaborated on the association between drug misuse and crime examining the link between certain kinds of offences and illicit substances (16).

## **2.4 Trends In South Africa**

In parallel to numerous countries globally; the possession, sale, transportation and cultivation of cannabis is illegal in most African countries including South Africa (SA), with exception to the countries that have no laws regarding cannabis. According to the UNODC 2011 World Drug Report, in Africa between 3.8% to 10.4% , an estimated 21.6 - 59.1 million individuals between the age of 15 -64 years reported the usage of cannabis at least once during the preceding year making the estimated annual prevalence percentage of cannabis usage in Africa the second highest in the world (3). In SA, cannabis is the most commonly used illicit substance consumed by people of all ethnic groups and is second to only alcohol as the most extensively used drug (17). A study conducted by Parry *et al* that encompassed the 3 major metro cities in SA reported that similarly to global trends, cannabis usage is associated with crime (17, 18). Among their study population of 1000 arrestees in Cape Town, Durban and Johannesburg, 39% of

the study population tested positive for cannabis. The highest rates were in Cape Town with 50.2% of all arrestees testing positive for cannabis followed by Durban at 42.6% and then Johannesburg at 24.2%. The study also found that the top criminal charge was property offence (including by not limited to offences such as shoplifting; housebreaking; motor vehicle theft) at 31.7% followed by violent offences against a person at 26.1% and drug related offences (drug supply, possession, production, importation, exportation and cultivation) and additional offences (30.6%). The percentage of individuals that tested positive for cannabis was higher than in individuals less than 20 years of age at 58.8%, followed by the 21 - 25 age groups at 40% (17,18). In accordance to global trends, cannabis usage in SA is particularly popular among the adolescent. Parry *et al* reported that cannabis was the most common primary substance of abuse in adolescence, less than 20 years of age, seeking treatment across Cape Town, Durban and Gauteng in the observed time period (1997 – 2001). Cannabis also presented in the highest proportion of arrestees aged 20 and under younger in all three different year phases (19).

## **2.5 Increase in Potency**

Cannabis has changed dramatically since its rise in popularity in the late 1960's into 1970's, and recently reports have suggested that the cannabis on the market is significantly stronger in comparison (5,9). Differences between the cannabis from modern markets and the cannabis consumed in previous decades can be attributed to the natural evolution and maturity of the users and market. One of the main factors that contributed to the cannabis composition difference is due to the increase in knowledge by the cannabis user with specific reference to the part of the plant that is consumed (5). The THC distribution on the plant is localised with the highest concentrations of THC being present on the flowering buds, followed by the leaves closest to the buds. Much lower concentrations of THC are found in the leaves furthest away from the buds, followed by the stalks and the seeds of the *Cannabis* plant which do not possess any THC. While cannabis users in the 1970's predominately smoked the leaves, modern cannabis users smoke the more potent flowering buds (5,9). Therefore, one of the

factors contributing to the differences noted regarding strength or potency is independent of the cannabis itself, but rather the utilization of the plant in a more effective manner.

The other fundamental factor contributing to the reports of the increase in cannabis strength relates directly to the cannabis plant and can be separated into determinants relating to the genetic composition of the plant and determinants relating to its growing condition, both of which are crucial in affecting the THC concentration. As previously mentioned, a natural by-product of the advancing times was the assimilation of knowledge which enabled breeders to exploit the pros and cons of the two different subspecies. Experiments crossing sativa and indica strains led to the development of hybrid strains possessing the advantageous characteristics of both strains. The development of a “skunk”, a hybrid strains composed of 75% sativa and 25% indica was among the first to have the high THC property of the sativa along with the favourable growing cycle and yield of the indica. Since that time, hybridisation has become immensely popular with different strain composing of varying rations of the different subspecies practically dominating today’s cannabis market. Methods of cultivation techniques have also been improved on. A previously underutilized cultivation technique results in a special treatment of the plant known as sinsemilla, meaning without seeds. Sinsemilla are the unfertilized flowering buds of the female cannabis plant which do not produce any seeds and have a particularly high concentration of THC. The production of sinsemilla requires the breeder to differentiate between male and female plants early enough to ensure that the females are not exposed to the male pollen (5, 9). In 2006 the UNODC reported that over the last decade, sinsemilla cannabis has doubled in potency in a number of key markets with most high grade cannabis on the market being sinsemilla (5). The implementation of greenhouse technology promoted the movement towards indoor cultivation of cannabis. Indoor cannabis a several advantages over the traditional outdoor cannabis, one of it being indoor has a greater annual yield. Outdoor cultivation is restricted by the dynamic seasons limiting growers to one or two harvest per year. Indoor cultivation is not limited by nature with the growing cycles being regulated by alternating periods of light and dark from artificial sources (5). In 2004, the European Monitoring Centre for Drugs and Drug

Addiction conducted a study and concluded that only a modest increase in cannabis potency occurred and further attributed it to the use of indoor cultivated cannabis. The United States of America has reported the THC levels have risen over the last 25 years with indications that the potency has risen for about 4% to 8% since 1983 (9,20). Conversely, the United Kingdom Home Office study in 2008 found little change in the cannabis potency (20).

### **3. Justification for Research**

Certain research supports the theory that the quality of the cannabis products available has improved and the concentration of the psychoactive constituents specifically THC has increased. Since the influence and integration of cannabis extends to number fractions within society including crime, a quantitative investigation into the cannabis products available in the South African market is a necessity.

### **4. Hypothesis and Objectives**

We hypothesize the following:

- 1) The strength/potency of the marijuana samples collected in South Africa would have increased in comparison to previous research conducted.

The aims of the study are as follows:

- 1) To quantify selected cannabinoids of confiscated cannabis samples received from the South Africa Police Service Forensic Science Laboratories from different regions in South Africa with specific reference to the following cannabinoids:

- I. Delta-9- tetrahydrocannabinol ( $\Delta^9$  – THC)
- II. Cannabidiol (CBD)
- III. Cannabinol (CBN)

## **5. Research Methodology**

Quantification of the cannabinoids shall be performed according to guidelines provide by the United Nations Recommendation Methods for the Identification and Analysis of Cannabis and Cannabis products. The UNODC recommendation is based on the detection of total THC. The concentration of  $\Delta^9$  – THC, the primary psychoactive constitute, in combination with other cannabinoids is accepted as measure of potency or strength of the cannabis product sample. CBD, an additional cannabinoid found in cannabis products has recently been indicated to also cause pharmacological effects. CBN is a degradation product of THC and can estimate the age of cannabis product samples. Therefore for the characterisation of the cannabis products and determination of its quality, the concentration of THC, CBN and CBD shall be measured. During smoking or heating, tetrahydrocannabinolic acid (THCA) is decarboxylated and converted to THC, so the method proposed in this study aims to quantify the total THC, THC plus its precursor THCA. Research indicates that most marijuana consumed locally is cultivated locally therefore, results for this proposed study with be compared against previous research conducted with marijuana cultivated in South Africa.

### **5.1 Research Requirements**

- Permits providing permission to use the confiscated samples for research are being applied for from the South African Department of Health.

## 5.2 Sample Acquisition

Confiscated marijuana samples from different regions of South Africa will be obtained from the South African Police Service Forensic Science Laboratories.

## 5.3 Cannabinoids Reference Material and Reagents

The cannabinoids reference material of  $\Delta^9$ - THC, CBN and CBD at 1.0mg/ml was ordered and purchased from Cerilliant Co. All reference material will be stored at -80°C for the duration of the study. Lot numbers and expire dates were noted as follows:

- $\Delta^9$ - THC: Lot number:FE121112-02; Expires: 12/2017
- CBN: Lot number: FE092711-04; expires: 09/2015
- CBD: Lot number:FE121211-01; expires 02/2015

## 5.4 Analytical Procedures

Cannabis samples will be subjected to analysis by the following analytical procedures:

1) High Pressure Liquid Chromatography –Mass Spectrometer (HPLC-MS):

- Plant Extraction procedure: The confiscated marijuana sample will undergo sample preparation but grinding into a fine powder. A determined amount of grinded sample will be added to a solution of methanol-chloroform mixture. To extract the analyses the sample will be shaken and the placed in an ultrasonic water bath at ambient temperature. After a set period an aliquot of the clarified extract will be inject into the LC-MS.
- Standard Solution Preparation: Stock solutions of the reference material will be prepared, and will serve as the base for the working solution at different concentrations for the calibration range.

## **6. Impact of Knowledge**

The results of the study will be used for the student, Miss Rolanda Londt, to be complete a Masters in Biomedical Forensic Science degree.

## **7. Budget and Funding**

The following funding has already been allocated:

- R10,000 awarded by the UCT Equity scholarship for Miss Londt
- R40,000 awarded by the NRF Freestanding Masters

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## Part B: Structure Literature Review

### 1. Introduction

Marijuana, also known by a plethora of additional names such as dagga, ganja, pot or weed, is an illicit recreational drug produced from the preparation of the cannabis plant. Cannabis has been utilised for thousands of years as both a fibre and recreational or medicinal drug. The first reported use of cannabis was as early as 2900BC in Chinese traditional medicine and has since then significantly incorporated itself in society forming the ethos of the one of the largest drug cultures and is the most commonly used illicit substance today<sup>1</sup>. Cannabis is a genus of flowering plants that synthesizes a unique class of chemical compounds known as the cannabinoids. Pharmacologically, the principal psychoactive constituent of cannabis is a cannabinoid called delta-9-tetrahydrocannabinol but research into additional cannabinoids are proving activity. According to the United Nations Office of Drugs and Crime the amount of delta-9-tetrahydrocannabinoid, in conjunction with selected additional major cannabinoids, determines the strength or potency of the drug<sup>2</sup>. In recent years, significant emphasis has speculated that modern cannabis is substantially different from what was circulating in previous decades specifically regarding the potency of cannabis preparations<sup>3</sup>. Due to the magnitude of the cannabis market and the implications thereof that extend into many facets of society, comprehensive inquiry is a necessity.

### 2. Cannabis Chemistry: The Cannabinoids

The chemistry of cannabis is complex and consists of a substantial number of chemical constituents. There are over 500 known constituents but unique to cannabis are a class

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<sup>1</sup> (ProConOrg n.d.)

<sup>2</sup> (United Nations Office on Drugs and Crime n.d.)

<sup>3</sup> (UnitedNationsOfficeonDrugsandCrime 2009; United Nations Office on Drugs and Crime 2006)

of chemical compounds termed the cannabinoids<sup>4</sup>. Before 1965, little was known about the pharmacological profile of cannabis, the major active component had not been isolated in a pure form and its structure had not yet been determined. Publications on the cannabis constituents began in the early 19<sup>th</sup> century with the earliest published report on the chemistry of the cannabis occurring in 1840. Nonetheless, due to the unique properties of the cannabis compounds and restrictions created by the lack scientific advancements and technology, the active principle component remained elusive for some time<sup>5</sup>.

Cannabinol (CBN) was the first natural cannabinoid to be isolated and purified. In 1896 Wood *et al*, a research group from Cambridge reported the isolation of a compound referred to as CBN from the fractional distillation of an ether extract from cannabis resin material. The fractional distillation produced four principle fractions, one of them an ambered coloured viscous oil known as ‘Red Oil’. It was assumed to be homogenous, but later experimentation conducted in 1899 by Wood *et al* as well as by Dunstan and Henry, discovered that the oil was not homogenous and isolated a compound which was transferred the name CBN<sup>6</sup>. CBN’s structure was revealed and confirmed by synthesis in 1940 by Adam *et al*. Adam *et al* conducted further research which resulted in the isolation of an additional major cannabinoid termed cannabidiol (CBD). The end of the 1940’s saw the conclusion in attempts to isolate cannabis components by distillation. The technique proved only slightly effective in separating the complex chemical nature of the cannabinoids. The cannabinoids are non-alkaloid in comparison to other naturally occurring intoxicating substances, the chemical group consists of a large number of closely related compounds that do not crystalize in an underivatized state, most boil within the same temperature range, and therefore separation was rendered difficult or nearly impossible with current techniques. Due to the limitations on analysis the mass of early reported and published scientific knowledge of cannabis specifically relating to the structure of the active species was inaccurate. Research investigating the cannabinoids was almost entirely neglected for the following 20

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<sup>4</sup> (Brenneisen n.d.)(Elsohly & Slade 2005)

<sup>5</sup> (Nahas 1984; Nahas 1973b; Nahas 1973a)

<sup>6</sup> (Nahas 1973b; PatentStorm n.d.)

decades. The structure of CBD wasn't elucidated until 1963 by Mechoulam and Shvo and the active component, delta-9- tetrahydrocannabinol ( $\Delta^9$ - THC), was only identified the preceding year. Numerous reports suggesting the isolation of  $\Delta^9$ - THC have been published, most notably Wollner *et al* (1942) and DeRopp (1960) but the first authenticated isolation of the active cannabis principle in pure form was reported by Mechoulam and Gaoni in 1964. Hexane extract of cannabis hashish resin was separated by repeated column chromatography on florisil and alumina. All compounds were isolated, fractions and pure compounds were tested on rhesus monkeys and it was shown that no other compound except  $\Delta^9$ - THC induced psychotomimetic symptoms<sup>7</sup>.

Since the identification of the  $\Delta^9$ - THC, a considerable amount of research aided by modern scientific techniques such as chromatography, mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR), has been employed to deciphering the complexity of cannabis chemistry<sup>8</sup>. Today, over 60 naturally occurring cannabinoids present in cannabis have been characterised, and all compounds; their carboxylic acid analogs and their transformation products have be characterised. All compounds have been isolated and methods for their detection and quantitation have been developed<sup>9</sup>. Phytocannabinoids, the term designated for the naturally synthesized cannabis constituents, are classified as C<sub>21</sub> terpenophenolic compounds. They are generally all hydrophilic, readily soluble in lipids and alcohol (large octanol/water partition coefficient). Herbal or natural cannabinoids are divided into 10 subclasses of which  $\Delta^9$ - THC, CBD and CBN are included<sup>10</sup>.

$\Delta^9$ -THC, classified by the position of its double bond, is a non-crystalline, highly hydrophilic compound with a large octanol: water partition coefficient of 6.99 (figure one). It is relatively unstable being easily degraded by heat, light, acids as well as atmospheric oxygen. There are seven double bond isomers but only two  $\Delta^9$ - THC and  $\Delta^8$ - THC occur naturally.  $\Delta^9$ - THC, (-)-trans- $\Delta^9$ -THC, is considered the main stereoisomer

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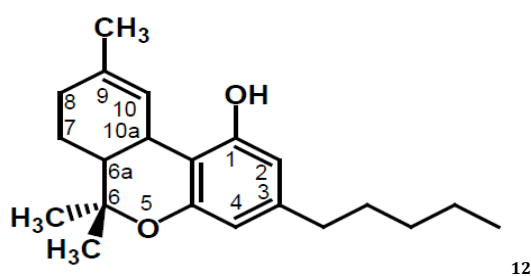
<sup>7</sup> (Nahas 1973b; Nahas 1984; PatentStorm n.d.)

<sup>8</sup> (Nahas 1984; Nahas 1973b)

<sup>9</sup> (Brenneisen n.d.; Stolker et al. 2004)

<sup>10</sup> (Brenneisen n.d.; Elsohly & Slade 2005)

and is the primary psychoactive constituent associated with cannabis. In addition, nine THC- type compounds with identified C<sub>1</sub> to C<sub>5</sub> side chains are known. Δ<sup>9</sup>-THC possesses an unsaturated bond located between C<sub>9</sub>-C<sub>10</sub> in the cyclohexane ring. Δ<sup>9</sup>- THC has four isomers centring around 2 chiral centres located at C<sub>4</sub> and C<sub>5</sub>. Only two isomers, (-)-cis-Δ<sup>9</sup>- THC and (-)-trans-Δ<sup>9</sup>- THC occur naturally with (-)-trans-Δ<sup>9</sup>-THC being the most abundant. In the cannabis plant, in addition to the Δ<sup>9</sup>-THC present, a major biogenic precursor, delta-9- tetrahydrocannabinol-2-oic acid (Δ<sup>9</sup>-THC acid A, 2-COOH-THC) and delta-9-tetrahydrocannabinol-4-oic acid (Δ<sup>9</sup>-THC acid B, 4-COOH-THC) are also present (figure two). Δ<sup>9</sup>-THC acid A is present to a much greater extent and over time or catalysed by heat like smoking, THCA is decarboxylated producing Δ<sup>9</sup>-THC (See figure three)<sup>11</sup>.



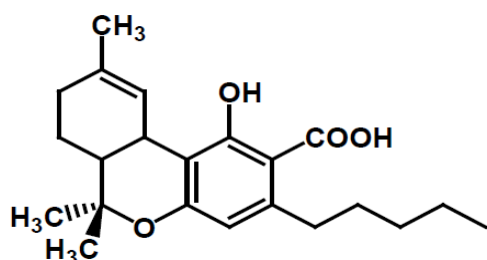
**IUPAC Name:** - (-)-(6aR,10aR)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol

**Molecular Formula:** C<sub>21</sub>H<sub>30</sub>O<sub>2</sub>

**Molecular Weight:** 314.4g/mol

**Log P:** 6.99 (octanol/water)

**Figure One:** The chemical structure and properties including the IUPAC name, molecular formulae and weight and Log P, of -Δ<sup>9</sup>-THC



**IUPAC Name:** (6aR,10aR)-1-hydroxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6h-benzo[c]chromene-2-carboxylic acid

**Molecular Formula:** C<sub>22</sub>H<sub>30</sub>O<sub>4</sub>

**Molecular Weight:** 358 g/mol

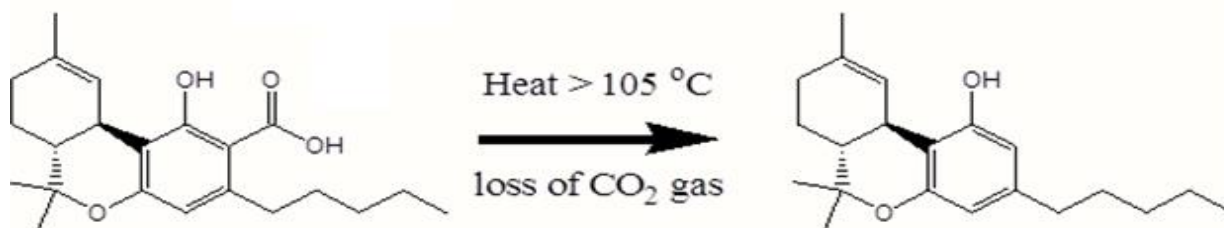
**Decarboxylation to THC:** Approx. 125-150°C

**Figure Two:** The chemical structure and properties including the IUPAC name, molecular formulae and weight of Δ<sup>9</sup>-THC Acid A

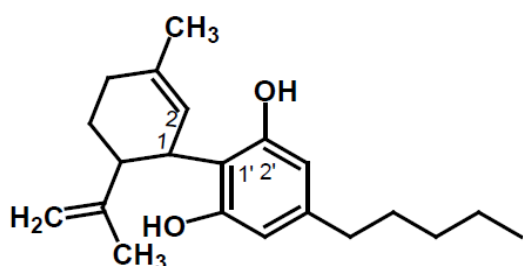
<sup>11</sup> (Brenneisen n.d.; Elsohly & Slade 2005; European Monitoring Center for Drugs and Drug Addiction. 2013; Leffingwell & Road 2003)

<sup>12</sup> (United Nations Office on Drugs and Crime n.d.)

<sup>13</sup> (United Nations Office on Drugs and Crime n.d.)



**Figure Three:** Diagrammatic representation of the decarboxylation of  $\Delta^9$ -THC Acid A to  $\Delta^9$ -THC



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**IUPAC Name:** 2-((1R,6R)-3-Methyl-6-(prop-1-en-2-yl)cyclohex-2-enyl)-5-pentylbenzene-1,3-diol

**Molecular Formula:**  $C_{21}H_{30}O_2$

**Molecular Weight:** 314.46 g/mol

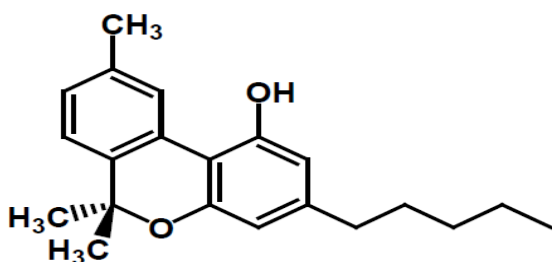
**Log P:** 5.79(Octanol/ water)

**Figure Four:** The chemical structure and properties including the IUPAC name, molecular formulae, weight and Log P, of CBD

$\Delta^2$ -CBD is a major constituent of cannabis which, along with  $\Delta^9$ -THC and CBN, is among the most abundant compound present. Similarly to  $\Delta^9$ -THC,  $\Delta^2$ -CBD is characterized as a hydrophilic compound with an octanol: water partition coefficient of 5.79 (figure four). It is photo-reactive, being degraded by light but at a different rate in comparison to  $\Delta^9$ -THC, and oxidizes in the presence of oxygen. According to formal numbering, the chemical nomenclature of  $\Delta^2$ -CBD differs from that of  $\Delta^9$ -THC in that its numbering is determined by a terpene ring rather than a pyran ring. There are seven double bond isomers of  $\Delta^2$ -CBD but only, the main isomer, 2-(6-isopropenyl-3-methyl-2-cyclohexen-1-yl)-5-pentyl-1,3-benzenediol occurs naturally that has chiral centres at positions  $C_1$  and  $C_6$ . Seven CBD-type compounds with  $C_1$  to  $C_5$  side chains have also been identified<sup>15</sup>.

<sup>14</sup> (United Nations Office on Drugs and Crime n.d.)

<sup>15</sup> (Mechoulam & Hanus 2002; Brenneisen n.d.; Elsohly & Slade 2005)



**IUPAC Name:** 6,6,9-trimethyl-3-pentyl-benzo[c]chromen-1-ol

**Molecular Formula:** C<sub>21</sub>H<sub>26</sub>O<sub>2</sub>

**Molecular Weight:** 310.43 g/mol

**Log P:** 6.23 (Octanol/ water)

**Figure Five:** The chemical structure and properties including the IUPAC name, Molecular formulae, weight and Log P, of CBN

An additional cannabinoid of analytical importance is cannabiol (CBN). CBN is not synthesized by the plant; rather it is considered a degradation artefact of  $\Delta^9$ -THC and is therefore not naturally present in cannabis. CBN is a fully aromatized derivative caused by the oxidation of  $\Delta^9$ -THC (figure five). Since CBN is not present in fresh and carefully dried cannabis, the presence of CBN is indicative of degradation. It has been reported that ratio of CBN to  $\Delta^9$  – THC can indicate age, with a ratio of less than 0.013 suggesting the sample is less than six months, and a ratio between 0.04 and 0.08 suggesting the sample is between one and two years old. Increases in CBN occur in conjunction with decreases in  $\Delta^9$ -THC, and levels of CBN are influenced by number variables, including the storage and experimental conditions that cannabis is subject to<sup>17 18</sup>.

### 3. Analytical Methods Utilized for the detection of Cannabinoids

A variety of analytical methods can be used for the detection of cannabinoids in cannabis samples. Analytical methods are influenced by whether analysis is qualitative, quantitative or both; on the availability or access to appropriate instrumentation; on the nature of the samples and with regards to forensic investigations the extent necessary to be consider legally acceptable. The United Nations Office on Drugs and Crime (UNODC) provides a comprehensive report detailing a variety of analytical methods

<sup>16</sup> (United Nations Office on Drugs and Crime n.d.)

<sup>17</sup> (United Nations Office on Drugs and Crime n.d.; Elsohly & Slade 2005; Brenneisen n.d.)

<sup>18</sup> (United Nations Office on Drugs and Crime n.d.)

used for the identification and analysis of cannabis and cannabis products. The report, which serves as a basis for this study, provides validated methods that serve as a guideline for numerous different techniques and procedures correlating to different analytical purposes and can subsequently be integrated into any laboratory setting. Gas chromatography (GC) and liquid chromatography (LC) tandem with mass spectrometry (MS) are the main instrumental techniques used for the determination of cannabinoids in forensic analysis<sup>19</sup>. The suitability of these techniques is left to the investigator or analyst's discretion to be determined by the purpose or specific aim of the analysis. Regarding the detection of cannabinoids, in particular  $\Delta^9$  - THC, due to its polarity the analyte requires derivitization prior to GC analysis to improve its volatility<sup>20</sup>. Derivitization renders sample preparation and analysis laborious and more expensive. LC analysis in contrast, does not require derivitization prior to analysis, rather exploits the solubility properties effectively making sample preparation simpler. This factor, in addition to apparatus availability was the reason why high performance liquid chromatography (HPLC) tandem MS was chosen for this study. No information regarding the analytical methodology utilised by the South African Police Service Forensic Laboratories in the analysis of cannabis products could be discerned.

#### **4. Factors Influencing the Strength of Cannabis**

According to the United Nations Office on Drugs and Crime (UNODC), the concentration of  $\Delta^9$ -THC in conjunction with additional cannabinol compounds, specifically CBD which has been shown to influence the effect of  $\Delta^9$ -THC, determines the strength or potency of the cannabis products<sup>21</sup>. The concentration of  $\Delta^9$ -THC in the cannabis plant and subsequent cannabis preparations are not constant and vary according to the genetic strain and cultivation techniques employed; the part of the plant used during the

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<sup>19</sup> (United Nations Office on Drugs and Crime n.d.; Vlase et al. 2010)

<sup>20</sup> (Vlase et al. 2010)

<sup>21</sup> (United Nations Office on Drugs and Crime n.d.)

production of the cannabis products as well as the specific preparation for administration<sup>22</sup>.

A key factor determining the concentration of  $\Delta^9$ -THC is the specific plant strain of the cannabis. The speciation of the genus cannabis is controversial and differs according to opinion but it is generally accepted to consist of one highly polymorphic species *C.sativa* L which is divided into several subspecies which include *C.sativa* subsp. *sativa*; *C.sativa* subsp. *indica*; *C.sativa* subsp. *ruderalis*<sup>23</sup>. Subspecies differ vastly in the phenotypic appearance as well as the chemical composition. Of particular relevance is the  $\Delta^9$ -THC: CBD ratio which greatly influences the subjective psychoactive and physiological effects of the drug. The sativa subspecies is known to have a higher  $\Delta^9$ -THC: CBD ratio in relation to the indica subspecies which conversely have a greater CBD:  $\Delta^9$ -THC ratio. Sativas are most commonly associated with the production of cannabis products because of their preferred subjective cerebral effects mediated by the high  $\Delta^9$ -THC content. Sativa induced effects are characterized as being more cerebral, energetic or euphoric whilst indica induced effects are known to be more physical, sedative, relaxing and less likely to induce anxiety<sup>24</sup>. In addition to pure strains, hybrid genetic strains from crossbreeding with varying ratios of *C.sativa* subsp. *sativa* and *C.sativa* subsp. *indica* are also incredibly popular. Improved breeding techniques aided in merging the beneficial and desired traits of the two subspecies yielding in product with the desired concentration of the major cannabinoids resulting in product with the desired taste; texture; physiological and psychoactive effect. Comparatively to sativa and indica, *C.sativa* subsp. *ruderalis* possesses a low  $\Delta^9$ -THC and CBD content thus serve little recreational use. The growth cycle, however, is not photoperiod dependant like the two other subspecies and begins flowering according to its life cycle, automatically changing into its flowering phase, reaching maturity sooner and can therefore be ready to harvest in less than 10 weeks from seed. This property of ruderalis is termed auto flowering and

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<sup>22</sup> (NationalCannabisPreventionandInformationCentre 2011; United Nations Office on Drugs and Crime 2006; Tsumura et al. 2012)

<sup>23</sup> (EncyclopaediaBritannicaOnlineAcademicAddition 2013; Hillig 2005; United Nations Office on Drugs and Crime n.d.)

<sup>24</sup> (ProConOrg 2013; Joy et al. n.d.; PatientsMarjuana 2011; PureAnalyticsCannabisPotencyandSafetlyScreening 2011; SenseiSeeds 2013)

the integration of this strain into hybrid strains is becoming increasingly popular since harvesting can occur more frequently than natural climate changes dictate<sup>25</sup>.

Advanced cultivation techniques have taken the science of cannabis growing to new heights by employing techniques with specific aim to maximise the psychoactive cannabinoid profile. The main production of cannabis globally is still outdoor cultivated cannabis. The cultivation of outdoor cannabis is however intricately dependant on climate and latitude. With the exception of ruderalis, the cannabis growth/flowering cycle is photo-dependant and only flower when the days grow shorter and exposure to light lessens. At higher climates this usually occurs before the plant has a chance to fully develop or is accompanied by frost. Indoor cannabis cultivation, however, is not limited by these restrictions. Indoor is mainly encountered in technologically advanced countries and has the advantage of occurring under artificial conditions where the photo period of the cannabis can be manipulated encouraging the flowering cycle and is therefore not limited to a few harvests per year rather can grow almost continually. The indoor movement has also seen the incorporation of hydro-culture into the agriculture of cannabis. Hydroponic agricultural techniques utilize a soil-less medium in which the roots of the plant are grown in a mineral rich nutrient solution only or in an inert medium such as gravel, mineral wool or pebbles. This technique is believed to provide a crop with greater cannabinoid concentrations in the cannabis product and with a shorter growing time<sup>26</sup>.

An additional cultivation technique, which can be utilized both indoor and outdoor, produces cannabis referred to as sinsemilla, meaning without seed. Sinsemilla is a special preparation which produces potent cannabis comprising exclusively of the unfertilized flowering buds of the female cannabis plant. Usually, the male cannabis plant fertilizes the female plant however, if grown in isolation; the flowering tops of the

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<sup>25</sup> (DutchSeeds n.d.; DutchPassionSeedCompany n.d.; BelowTheLion n.d.; United Nations Office on Drugs and Crime 2006; NationalCannabisPreventionandInformationCentre 2011)

<sup>26</sup> (United Nations Office on Drugs and Crime 2006; United Nations Office on Drugs and Crime 2012; United Nations Office on Drugs and Crime 2011; WikiHowtodoanything n.d.; NationalCannabisPreventionandInformationCentre 2011; UnitedNationsOfficeonDrugsandCrime 2009)

female plant remain unfertilized throughout maturity. These sinsemilla buds contain the highest concentration of  $\Delta^9$ -THC in comparison to any other plant part including the male flowering bud equivalent. The sinsemilla cultivation technique has been known since the 19th century, predominately in India, it was however only in the early to mid-1970s that this technique was incorporated into the cultivation of cannabis in the United States and in the 1980s in Europe. According to the UNODC 2006 world drug report, sinsemilla cannabis has doubled in potency in a number of key markets. All higher grade cannabis is usually sinsemilla, the potency of which is much higher than the seeded product. Although sinsemilla is the ideal preparation of the cannabis plant for the production of marijuana, sinsemilla comprised of only a small portion of the marijuana available in the illegal markets in comparison to seeded cannabis. In 2004, the potency of sinsemilla in the US averaged at approximately 10.5% compared to the 2.5% of lower grade cannabis. Similarly, in the Netherlands, the sinsemilla strength averaged close to 18% in comparison to 6% for imported cannabis for the same year<sup>27</sup>.

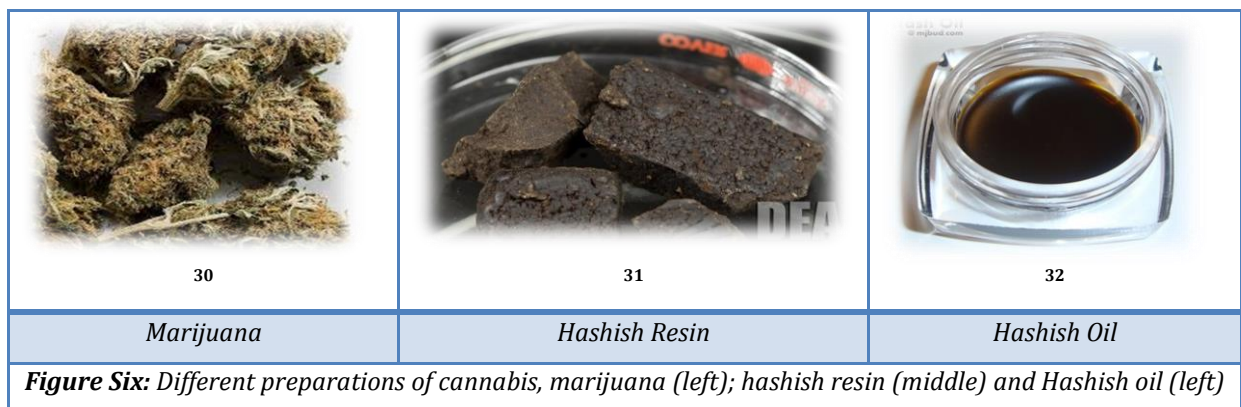
The distribution of  $\Delta^9$ -THC in the cannabis plant is not uniform and the part of the plant used influences the strength of the cannabis products. Approximately 10 - 12% of  $\Delta^9$ -THC present in the plant is found in the flowering buds of the female cannabis plant, the highest proportion in the entire plant. Approximately 1 - 2% can be found in the leaves and 0.1 - 0.3% in the stalks and less than 0.03% is present in the roots of the cannabis plant.

Cannabis intended for recreational use is available in different forms each with varying  $\Delta^9$ -THC concentration range (see figure six). The potency of the cannabis products are dependant on the specific preparation for administration. Marijuana is the most widely consumed form and is prepared from the dried flowering tops and leaves of the cannabis plant. Marijuana, or herbal cannabis product, results in the lowest  $\Delta^9$ -THC concentration and the estimated average of the products for  $\Delta^9$ -THC varies greatly. Almost all high-grade marijuana constitutes of sinsemilla with reported  $\Delta^9$ -THC

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<sup>27</sup> (United Nations Office on Drugs and Crime 2006; NationalCannabisPreventionandInformationCentre 2011; Tsumura et al. 2012; DutchPassionSeedCompany 2013)

concentrations reaching to as much as 17%. A stronger preparation of cannabis is hashish resin. Hashish is a resin made from the flower secretions of the cannabis plant. Traditionally hashish is produced by either rubbing the cannabis flowers until the resin sticks which is then removed and collected or by sifting the ground up, dried leaves which is subsequently compressed to make a resin brick. A more modern method of production is through alcohol extraction. Hashish is the second strongest preparation of cannabis, with concentrations ranging from an estimated 5% to 15%. The strongest preparation of cannabis is the essential oil referred to as hashish oil. The oil is highly potent and is produced by extracting the cannabinoids from the plant material with a solvent. The colour may range from clear to pale yellow through to brown or black. The concentration of  $\Delta^9$ -THC in hash oil can range from an estimated 15% to 50%<sup>28</sup>. One of the fundamental differences between modern marijuana consumption and previous decades is a perceived difference in strength. Previously, no discrimination was taken with regards to the part of the cannabis plant use, with the whole plant including the flowering buds, leaves and stalks being incorporated into the cannabis product. This is in stark contrast to modern usages which generally is focused in the highly concentrated flowers and leaves closest to the buds<sup>29</sup>.



<sup>28</sup> (NationalCannabisPreventionandInformationCentre 2011; UnitedNationsOfficeonDrugsandCrime 2009)

<sup>29</sup> (UnitedNationsOfficeonDrugsandCrime 2009; NationalCannabisPreventionandInformationCentre 2011; United Nations Office on Drugs and Crime n.d.)

<sup>30</sup> (UnitedStatesDrugEnforcement n.d.)

<sup>31</sup> (UnitedStatesDrugEnforcement n.d.)

<sup>32</sup> (MJBud n.d.)

## 5. Cannabinoid Trends: How Much Has Changed

The theory surrounding the change in quality of cannabis products over the past few decades is not novel and has been the subject of debate and speculation for some time. It was noted nearly 20 years ago that the potency of cannabis is much stronger in relation to previous generations. Since then reports with exaggerating figures, some claiming that modern cannabis is 20 – 30 times stronger, so much so it can be considered a completely different drug. The notion of a change in cannabis product is understandable considering the advancement in agricultural techniques employed with modern users being more knowledgeable about the product but the extent of which, supported by scientific information and relevant to the setting, is under scrutiny<sup>33</sup>.

Inquest into the changing cannabinoid profile has been conducted in many countries globally predominately in first world nations. A meta-analysis conducted in 2012 by Cascini *et al* concluded that there is a definite, recent and consistent increase in herbal cannabis potency globally<sup>34</sup>. This trend however isn't consistent for all cannabis products and countries. In the US, the National Institute on Drug Abuse (NIDA) Marijuana Potency Monitoring program (MPMP) conducts research into the potency of cannabis preparations seized domestically and includes domestic and internationally imported products. A study conducted between 1993 and 2008 determined that the change in strength of cannabis products during the past four decades was evident. The report showed a rise in mean  $\Delta^9$ -THC concentration from all confiscated cannabis preparations from 3.5% in 1993 more than doubling to 8.8% in 2008 with individual products showing a rise from 3.4% – 6.9% for marijuana, sinsemilla from 5.8% – 11.5% and hashish resin from 6.6% – 23.1%. The report stated that cannabis is more potent and the market for higher potency cannabis is growing, a trend that can be seen in the increase in sinsemilla seizures<sup>35</sup>. Studies conducted in the United Kingdom (UK), the

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<sup>33</sup> (National Cannabis Prevention and Information Centre 2011; United Nations Office on Drugs and Crime 2009; European Monitoring Center for Drugs and Drug Addiction. 2008; United Nations Office on Drugs and Crime 2006)

<sup>34</sup> (Cascini et al. 2012)

<sup>35</sup> (Mehmedic et al. 2010)

Netherlands and Italy reported similar findings. The 2008 Home Office Cannabis potency study from the UK reported that the proportion of herbal cannabis increased from an estimated 30% in 2002 to 55% by 2004/2005. Furthermore, 80.8% of the examined material was classified as herbal cannabis and of that 97% was classified as high grade sinsemilla. Moreover, the study determined the mean  $\Delta^9$ -THC of traditional imported herbal cannabis was 8.4% and the mean  $\Delta^9$ -THC of sinsemilla was twice that at 16.2%<sup>36</sup>. In 2004, the Netherlands, a country with liberal cannabis laws, reported that the average  $\Delta^9$ -THC of Dutch home-grown marijuana, known as Nederweit, was significantly higher at 20.4% than imported marijuana at an estimated 7.0%  $\Delta^9$ -THC. Similarly, Dutch hashish, known as Nederhasj, the  $\Delta^9$ -THC levels was determined to be 39.3% which is significantly more in comparison to 18.2%  $\Delta^9$ -THC from imported hashish. Moreover, the Netherlands reported in general that the average  $\Delta^9$ -THC of domestic Dutch marijuana and hashish as well as imported hashish was significantly higher than previous years, almost doubling over a period of five years<sup>37</sup>. Similarly, a study conducted in Italy found that during 1997 – 2000, marijuana and hashish increased moderately from 2.5% to 7% and 4.5% to 6% respectively, but over 2002 – 2004 a marked increase in potency of both was seen from 10.7% to 15% in marijuana and from 9.8% to 15.3% in hashish<sup>38</sup>. In accordance with the previous studies, similar findings were reported in a number of countries representing key markets including Australia, New Zealand, England and Japan<sup>39</sup>.

Even though, there is substantial evidence that supports the upward potency trends over the earlier four decades, there is evidence shows a stabilisation in the  $\Delta^9$ -THC in the UK and parts of Europe since the peaks in the late 1990s/ early 2000s. Furthermore some counties have not shown any significant increases in potency while other countries have no monitoring programs or studies. Even though selected individual countries reported upward trends, evidence from Europe as a continent does not support claims that the modern cannabis is 10 or more times more potent than 10 or 20 years ago as concluded by the European Monitoring Centre for Drugs and Drug

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<sup>36</sup> (Hardwick 2008)

<sup>37</sup> (Pijlman et al. 2005)

<sup>38</sup> (Licata et al. 2005)

<sup>39</sup> (Swift et al. 2013; Tsumura et al. 2012; Potter et al. 2008)

Addiction (EMCDDA). The EMCDDA reported mean potencies trends over a period of five years between 1997 – 2003 in seven European countries including the UK, Netherlands, Germany, Czech Republic, Portugal, Austria and France. During that period, no overall trend in mean potency was evident in the countries with the exception of the Netherlands. The EMCDDA however, determined that the rise seen after 1999 in the Netherlands was due to the domestically produced cannabis resin, nederhasj, which is not only uncommon in the Netherlands but elsewhere also. In addition, recent data collected in 2005 from the Netherlands showing further increase in  $\Delta^9$ -THC after the 2003 contains bias since the material sampled was purchased in Dutch coffee shops, establishments that are legally permitted to sell cannabis, the quality of which is generally superior and therefore may not be representative of all cannabis products consumed. The EMCDDA did however verify the emergence and rise of high grade herbal cannabis from intensive indoor cultivation methods or sinsemilla. The report did caution however that in some cases no distinction was made between sinsemilla and traditional herbal cannabis<sup>40</sup>.

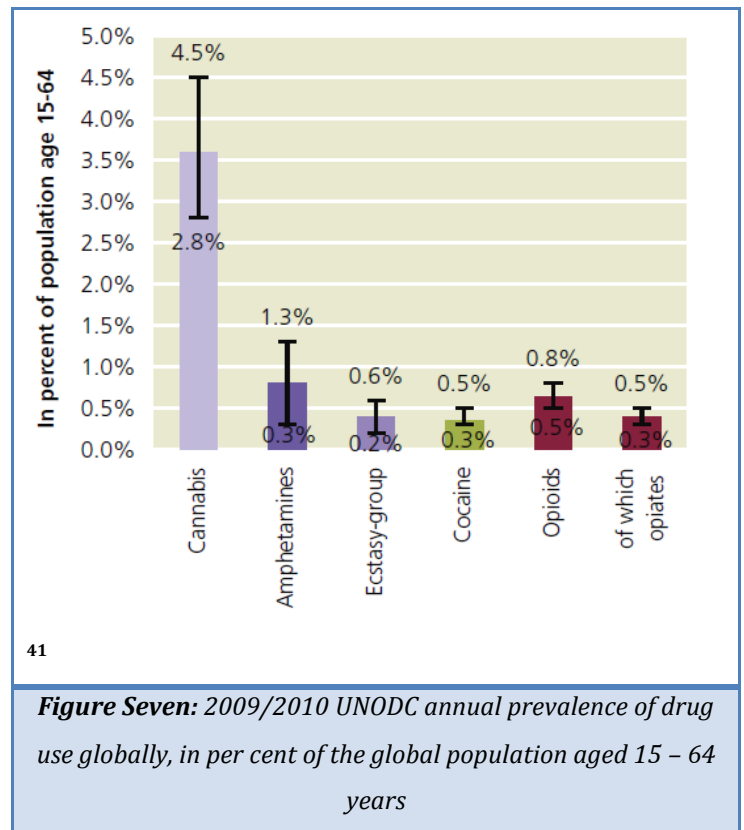
## **6. Cannabis: Forensic Implications and Impact in Society**

The legality of possession, cultivation and distribution of cannabis and products varies between country but generally possession, cultivation and trade of cannabis and cannabis products are illegal in most countries and have been since the beginning of cannabis prohibition in the late 1930's. Despite this, cannabis has consistently been the most marginally consumed illicit substance globally, with reports suggesting that there are currently between 119 million and 224 million cannabis users worldwide. In 2011 the UNODC world drug report stated that cannabis herb was globally the largest illicit drug product followed by cannabis resin (see figure seven). Indoor and outdoor cannabis is relatively easy to cultivate a factor that has contributed to the increase in cultivation and trade to such a degree that herbal cannabis production occurs across all

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<sup>40</sup> (European Monitoring Center for Drugs and Drug Addiction. 2008)

continents and in almost all countries. Trends for indoor cannabis suggest that production is concentrated mainly in developed countries such as North America, Europe and the Oceania. In contrast, cannabis resin production is more localised (figure eight). In the Near and Middle East, South-West Asia, North Africa cannabis resin is more prominent. Recent trends indicate that there is shift in resin production from Morocco, a key and leading North African resin supplier, decreasing while Afghanistan and India show an increase in production activity. Cannabis trafficking statistics indicate that

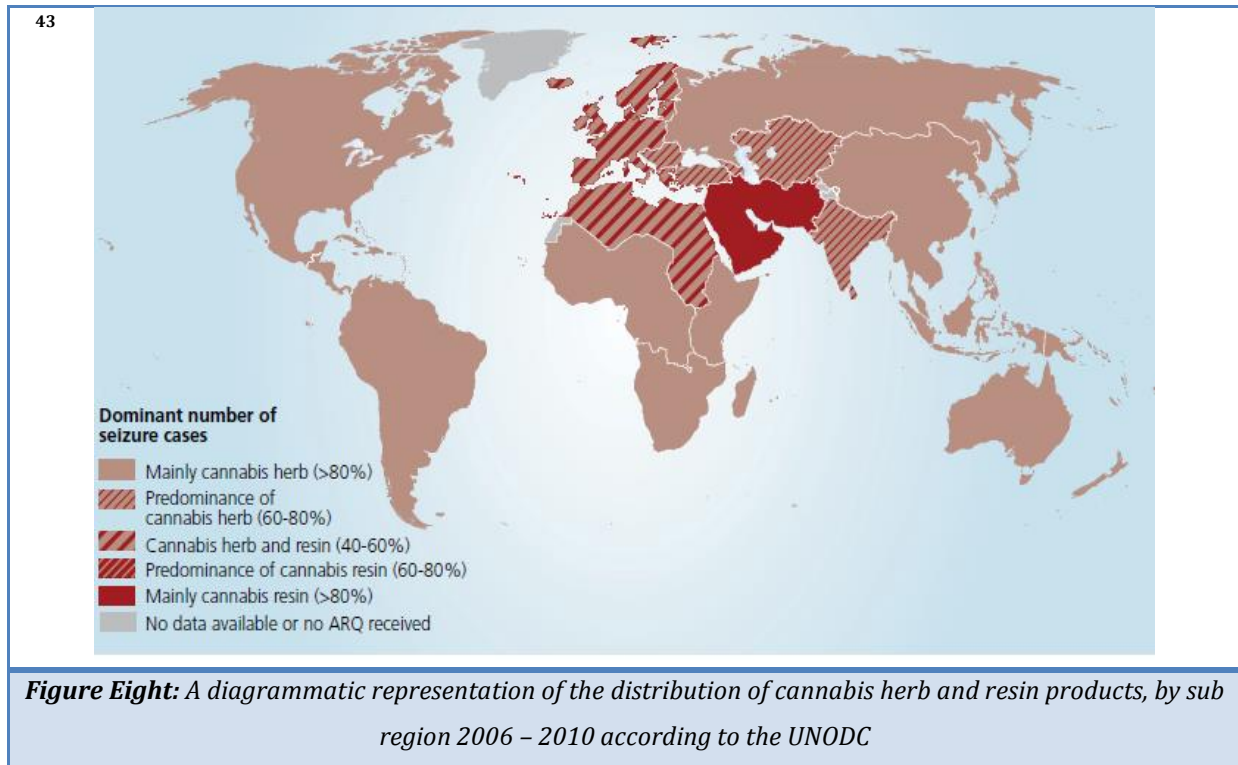


transportation is predominately intra-regional and that most cannabis is locally produced and consumed. Production of herbal cannabis is widespread therefore much of the demand for cannabis is covered by local production, a factor that is considered safer as it involves less trafficking and reduces the risk of seizures. Resin production however is more concentrated and trafficking occurs over larger distances. Afghanistan, Morocco and Lebanon are globally the leading resin production countries. Resin produced in Morocco is destined for consumption in West and Central Europe and North Africa, while Afghanistan generally serves as the source for neighbouring countries, but recent trends indicate a shift in resin supply favouring Afghanistan<sup>42</sup>. Due to the magnitude of the cannabis market and the legal implications thereof, the cannabis market places a considerable burden on governments and international agencies in terms of prosecution of criminal acts associated with cannabis, monitoring program if there are as wells as treatment resulting from negligent usage. With the avocation of the beneficial properties of cannabis use receiving much publicity, a transition is occurring with, in addition to the legalisation of cannabis for medical purposes, many countries are re-evaluating the legal status of the use of recreational cannabis. Many countries

<sup>41</sup> (United Nations Office on Drugs and Crime 2011)

<sup>42</sup> (United Nations Office on Drugs and Crime 2011; United Nations Office on Drugs and Crime 2012)

such as North America, South America and parts of Europe have decriminalised the possession of small quantities whilst in a few countries namely the Netherlands, North Korea and in the US states of Colorado cannabis is effectively legal.



In addition to the impact the marijuana phenomenon has on the macro-level of society through its cultivation and trade, marijuana usage extends to multiple different sub-fractions within society. A number of studies have provided evidence for the positive association between drug misuse and crime. A meta-analysis to determine the statistical association between drug misuse and crime indicated that there was a definitive correlation between marijuana usage and crime; with the odds of marijuana users offending being approximately 1.5 times higher in comparison to non-marijuana offenders. A study conducted by Green *et al* to determine whether heavy adolescent marijuana use can lead to criminal involvement in adulthood showed that heavy adolescent marijuana users are much more likely than non-users to have interactions with the criminal justice system. The study also showed that heavy adolescent

<sup>43</sup> (United Nations Office on Drugs and Crime 2012)

marijuana use increases the risk of engaging in criminal activities, with approximately 58.9% of the reported heavy adolescent marijuana users having an arrest record compared to only 34.8% of light/non-users. The heavy adolescent users also had more arrests on average than light/non-users and were also more likely to be arrested at younger ages. The study also showed that the more extensive criminal history of heavy adolescent users the greater their chances of additional or future incarceration. Studies further elaborated on the association between drug misuse and crime examining the link between certain kinds of offences and illicit substances. The meta-analyses found that heavy adolescent marijuana use is most associated with drug-related, property crime and not violent crime. This evidence is supported by additional studies that prove positive that marijuana associated crime may be based on an economically need<sup>44</sup>. The correlation between marijuana and crime might therefore be due to marijuana's association with other 'harder' recreational drug use since it is considered a gate way drug and a large proportion drugs usage occurs in conjunction with marijuana. In addition, individuals who are more likely to partake in the use of marijuana might also simply have more socially deviant behaviour tendencies.

## **7. Cannabis Culture in South Africa**

In parallel to numerous countries globally; the possession, sale, transportation and cultivation of cannabis is illegal in most African countries including South Africa (SA), with exception of countries that have no law regarding cannabis. According to the UNODC 2011 World Drug Report, in Africa between 3.8% to 10.4% , an estimated 21.6 - 59.1 million individuals, between the age of 15 -64 years reported the use of cannabis at least once during the preceding year making the estimated annual prevalence percentage of cannabis usage in Africa the second highest in the world<sup>45</sup>. In SA specifically, cannabis is the most commonly used illicit substance consumed by people

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<sup>44</sup> (Bennett et al. 2008; Green et al. 2010)

<sup>45</sup> (United Nations Office on Drugs and Crime 2011)

of all ethnic groups and is second to only alcohol as the most extensively used drug. In accordance to the global trend, cannabis is particularly popular among the youth<sup>46</sup>. A study conducted by Peltzer and Ramlagan to analyse the cannabis use trends in SA reported that cannabis circulating in SA is either cultivated in SA with major imports including neighbouring countries such as Swaziland, Lesotho, Mozambique and Zimbabwe. Similarly, many of the neighbouring countries also serves as the major export destinations for cannabis cultivated in SA but only small portions are exported to European markets in particularly the UK<sup>47</sup>.

A study conducted to examine the cannabis use trends in SA reported that similarly to global trends, cannabis usage is associated with crime. A study conducted by Parry and Plüddeman showed that among their study population of 1000 arrestees in Cape Town, Durban and Johannesburg, 39% of the study population tested positive for cannabis. The highest rates were in Cape Town with 50.2% of all arrestees testing positive for cannabis followed by Durban at 42.6% and then Johannesburg at 24.2%. The study also found that the top criminal charge was property offence (including by not limited to offences such as shoplifting; housebreaking; motor vehicle theft) at 31.7% followed by violent offences against a person at 26.1% and drug related offences (drug supply, possession, production, importation, exportation and cultivation) and additional offences (30.6%). The percentage of individuals that tested positive for cannabis was higher in individuals less than 20 years of age at 58.8%, which was followed by the 21 - 25 age groups at 40.1%<sup>48</sup>.

Cannabis research, with regards to potency studies or trends is very limited in Africa and SA. SA does not have any cannabis potency monitoring programs, nor has there ever been an investigation into the potential increase in potency in cannabis collected in SA over a period of time. In 1980 Field *et al* conducted a study to investigate the composition of cannabis with regards to its psychoactive principles from samples

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<sup>46</sup> (Charles D H Parry et al. 2004; Carney et al. 2013)

<sup>47</sup> (Peltzer 2007)

<sup>48</sup> (Charles D.H. Parry et al. 2004)

obtained from various geographical regions of SA. The samples were analysed using GC- MS and were obtained from three regions of SA namely Kokstad in Kwa-Zulu Natal (then Transkei); the Pongola district in Kwa- Zulu Natal and the Tzaneen district of Limpopo (then Transvaal). The samples were classified according to sex, part of the plant and age specifically into young plants less than 6 weeks old, medium aged plants 6 weeks to 3 months and old plants from between 3-6 months old. The concentration of  $\Delta^9$ -THC was recorded in % mass/mass and the results showed that the growing buds presented the highest concentration of  $\Delta^9$ -THC in all categories. The study found that in the samples collected in Kokstad, the growing tips of young plants has a  $\Delta^9$ -THC of 4.24%; male medium aged plants was 1.87% and 1.62% for female plants in the same category, and the old plants from 1.63 and 2.32 for male and female plants respectively. In Pongola  $\Delta^9$ -THC percentage for young plants was determined to at 2.15%; 1.41% and 2.51% for medium aged male and female plants. And lastly, in Tzaneen, the growing tip of the young plants was 1.35%; 4.77% and 3.83% for male and female medium plants respectively and 3.08% for old plants. Findings from this study support facts that is now common knowledge such as the cannabinoid content of the roots, stems and seeds contain trace quantities of cannabinoids and the highest concentration of cannabinoids are localised to the flowering buds. In addition, the samples, less than 6 months old contained only trace amounts of CBN, indicating very little if any degradation in fresh samples. Since no studies or reports exploring the cannabinoid content of cannabis from SA has emerge since that time, it serves a reasonable baseline for an indication of the quality of cannabis previously<sup>49</sup>.

## 8. The Purpose of this Study

*“the complex political, medical, cultural and socioeconomic issues associated with cannabis necessitates not only public and governmental scrutiny, but especially scientific inquire” - 50*

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<sup>49</sup> (Field, B.I; Arndt 1980)

<sup>50</sup> (Mehmedic et al. 2010)

Certain research support the theory that the quality of cannabis products has improved and the concentration of the psychoactive constitutes specifically  $\Delta^9$ -THC has increased. Since the influence and integration of cannabis extends to number fractions within society, quantitative investigation into the cannabis products available in the South African market is a necessity.

## 9. Aims

The aims of the study are as follows:

- 1) To quantify selected cannabinoids of confiscated cannabis samples received from the SAPS Forensic Science Laboratories through tandem HPLC – MS/MS analytical techniques with specific reference to:
  - IV. Delta-9- tetrahydrocannabinol ( $\Delta^9$  – THC)
  - V. Cannabidiol (CBD)
  - VI. Cannabinol (CBN)
- 2) To elucidate a trend in cannabinoids of the cannabis regionally

## PART C: Publication Ready Manuscript, with Instructions to Authors

### Forensic Science International

#### **An investigation of tetrahydrocannabinol, cannabidiol and cannabinol content of cannabis confiscated by the South African Police Service Forensic Laboratories from various regions of South Africa**

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Keywords:  $\Delta^9$ -Tetrahydrocannabinol; Cannabinol; Cannabidiol  
South Africa; Cannabis Potency; Forensic Science

## 1. Introduction

Cannabis, produced from the preparation of the cannabis plant is the most commonly abused illicit substance globally. The chemistry of cannabis is complex and consists of a substantial number of chemical constituents. There are over 500 known compounds but unique to cannabis are a class of chemical compounds termed the cannabinoids<sup>1</sup>. Today, over 60 naturally occurring cannabinoids are known of which  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), cannabidiol (CBD) and cannabinol (CBN) are included<sup>7</sup>.  $\Delta^9$ -THC is the main psychoactive component associated with cannabis use. Chemically, it is relatively unstable being easily degraded by heat, light, acids as well as atmospheric oxygen<sup>8</sup>. CBD is another major constituent of cannabis which, along with  $\Delta^9$ -THC, is among the most abundant present in cannabis. It is photo and thermo reactive and is readily oxidized by oxygen.  $\Delta^9$ -THC has long been known to be the principle psychoactive compound in cannabis, but recent reports have indicated that the physiological and psychological effects of cannabis are also influenced by CBD<sup>12</sup>. The degradation of  $\Delta^9$ -THC forms CBN an additional cannabinoid of analytical importance. CBN is not synthesized by the plant; rather it is considered an artefact and is therefore not naturally present in cannabis. Since CBN is not present in fresh and carefully dried marijuana, the presence of CBN is indicative of degradation. Furthermore, it has been reported that ratio of CBN to  $\Delta^9$  - THC can indicate age in a sample. Increases in CBN occur in conjunction with decreases in  $\Delta^9$ -THC, and levels of CBN are influenced by a number of variables, including the storage and experimental conditions that cannabis sample is subject to<sup>14 15</sup>. The concentration of  $\Delta^9$ -THC in conjunction with additional cannabinol compounds, specifically CBD, influences the strength or potency of the cannabis<sup>16</sup>. According to the UNODC 2011 World Drug Report, in Africa between 3.8% to 10.4% , an estimated 21.6 - 59.1 million individuals between the age of 15 -64 years reported the usage of cannabis at least once during the preceding year making the estimated annual prevalence percentage of cannabis usage in Africa the second highest in the world<sup>51</sup>. In SA specifically, cannabis is the most commonly used illicit substance consumed by people of all ethnic groups and is second to only alcohol as the most extensively used drug. Cannabis research, with regards to potency studies or trends is very limited in Africa

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<sup>51</sup> (United Nations Office on Drugs and Crime 2011)

and SA. SA does not have any cannabis potency monitoring programs, nor has there ever been an investigation into the potential increase in potency in cannabis collected in SA over a period of time. Due to the magnitude of the cannabis market and the implications thereof that extend into many facets of society, comprehensive inquiry is a necessity. Possession, cultivation and distribution of cannabis and cannabis products are illegal in most countries and have been since the beginning of cannabis prohibition in the late 1930's. In addition to the impact the marijuana phenomenon has on the macro-level of society through its cultivation and trade, marijuana usage extends to multiple different sub- fractions within society. A number of studies have provided evidence for the positive association between drug misuse and crime.

## **2. Material and Methods**

### **2.1 Reference Standards and Chemicals**

Reference standards of CBD, CBN and  $\Delta^9$  - THC were ordered from Cerilliant Corporation® at a concentration of 1mg/ml. All standard solutions were stored at -20°C for the duration of the study. Chloroform was ordered from Sigma-Aldrich (Schnelldorf, Germany). The methanol (univAR, Washington, United States) used for the extraction as well as the injection solvent for chromatographic analysis were of high pressure liquid chromatographic grade. The acetonitrile (Sigma Aldrich) and 0.1% formic acid (Sigma Aldrich) were of HPLC grade and the ammonium acetate (Sigma Aldrich) was of analytical grade.

## **2.2 Samples**

Sample material was obtained from the South African Police Service Forensic Laboratory with permission from the Department of Health South Africa and the South African Police Service. 25g of 50 individually sealed cannabis samples from various cities and towns in South Africa were received in a sealed police issue evidence collection bag. The individual sample bags were numbered corresponding to a specific town or city. All cannabis samples received were dried herbal variety.

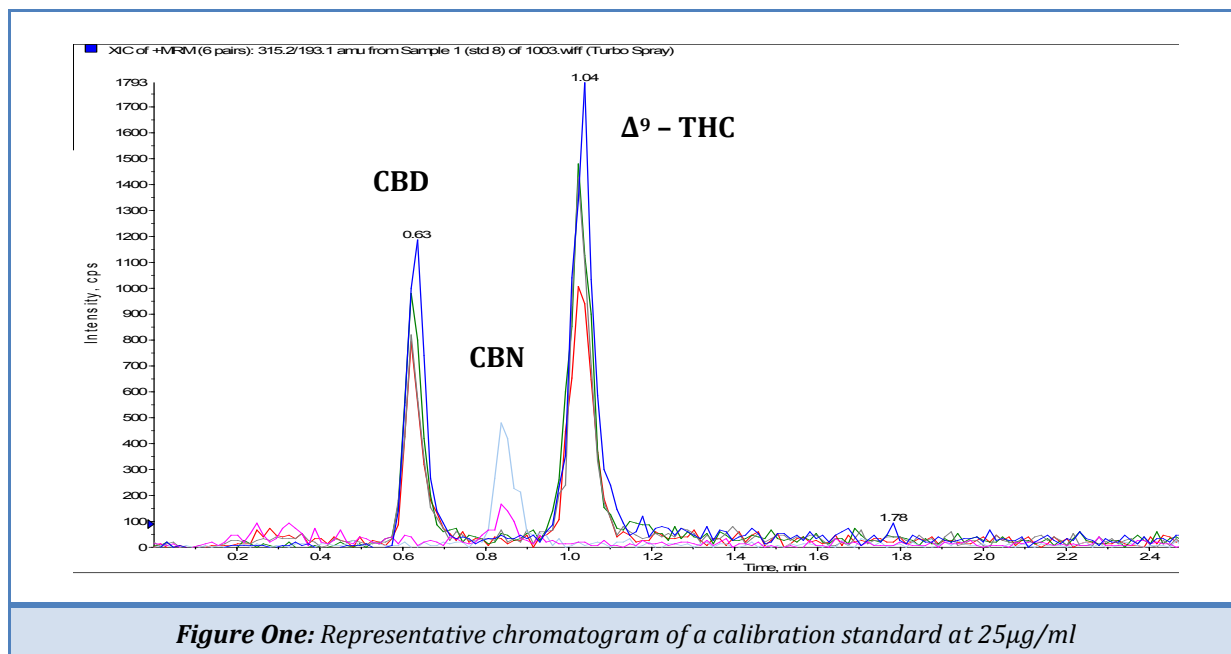
## **2.3 Extraction Procedure**

The cannabis material was prepared by removing the seeds and chopping/ grinding until fine. The samples were extracted in a solution of methanol-chloroform at 9:1 v/v and shaken overnight at 75 rpm. The samples were subsequently filtered with a 0.45 µm syringe filter and centrifuged for 5 minutes at 3500 rpm's, with a rotor radius of 13cm and RCF of 1500g set at 4 °C. A 200 µl aliquot of sample solvent was evaporated under nitrogen gas and redissolved in 200 µl of the methanol and 10mM ammonium acetate solution (1:1 v/v). The samples were briefly vortexed and transferred to HPLC autosampler vials. Ten microliters were injected onto the column.

## **2.4 Chromatographic Conditions**

Chromatography was performed on an Aligent 1100 series HPLC system. The samples were kept at 8°C. Isocratic separation was performed on a reverse phase Phenomenex Gemini 3µ C18 column 110A (50 X 2.00mm 3 micron) with a flow rate of 300 µl/min and injection volume of 10 µl. The mobile phase consisted of 0.1% formic acid and

acetonitrile (15:85 v/v). The injection solvent was a mixture of methanol and 10mM ammonium acetate solution (1:1 v/v). The elution orders of the cannabinoids were CBD, CBN and  $\Delta^9$  - THC with retention times of 0.6, 0.9 and 1.1 minutes, respectively. A representative chromatogram is presented in figure one.

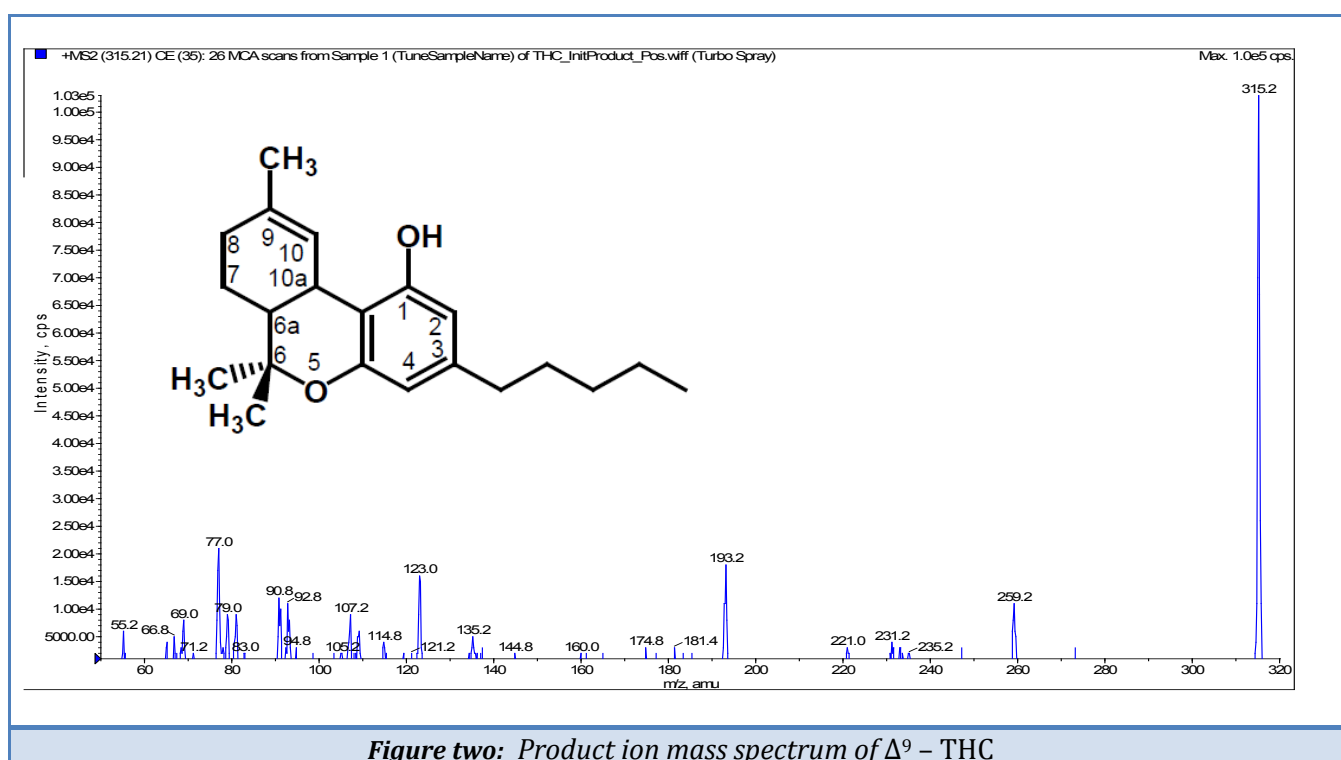


## 2.5 Mass Spectrometric Conditions

Analysis was performed on an AB Sciex 2000 triple quadrupole equipped with a turbo spray ionisation source in the positive ion mode and the ion spray voltage set at 5000v and source temperature at 400°C. The cannabinoids were detected using the multiple-reaction monitoring (MRM) mode. Data was captured and analysed in Analyst 1.4.2 software. The mass spectrometer parameters were optimised and are presented in table one. The same transitions of the protonated precursor ions  $m/z$  315.2 to the product ions  $m/z$  193.1 were monitored for  $\Delta^9$  - THC and CBD. The transitions of the protonated precursor ions  $m/z$  311.2 to the product ions  $m/z$  223.1 were monitored for CBN (table One). Product ion mass spectra of  $\Delta^9$  - THC, CBD and CBN are presented in Figures two, three and four respectively.

**Table One: Mass spectrometer parameters for  $\Delta^9$  - THC, CBD and CBN**

	Q1 Mass (amu)	Q3 Mass (amu)	Time (msec)	DP (volts)	CE (volts)	CXP (volts)
$\Delta^9$ - THC	315.21	193.10	150.00	31.00	31.00	6.00
CBD	315.17	193.10	150.00	46.00	27.00	10.00
CBN	311.15	223.10	150.00	51.00	27.00	12.00



**Figure two: Product ion mass spectrum of  $\Delta^9$  - THC**

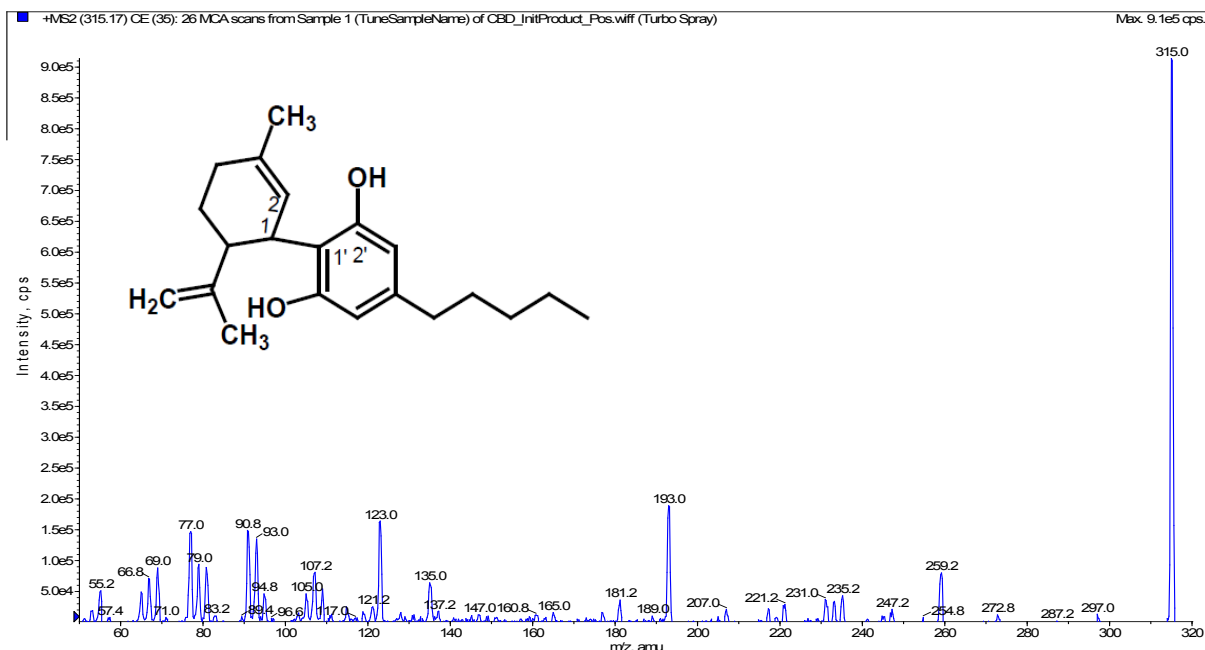


Figure three: Product ion mass spectrum of CBD

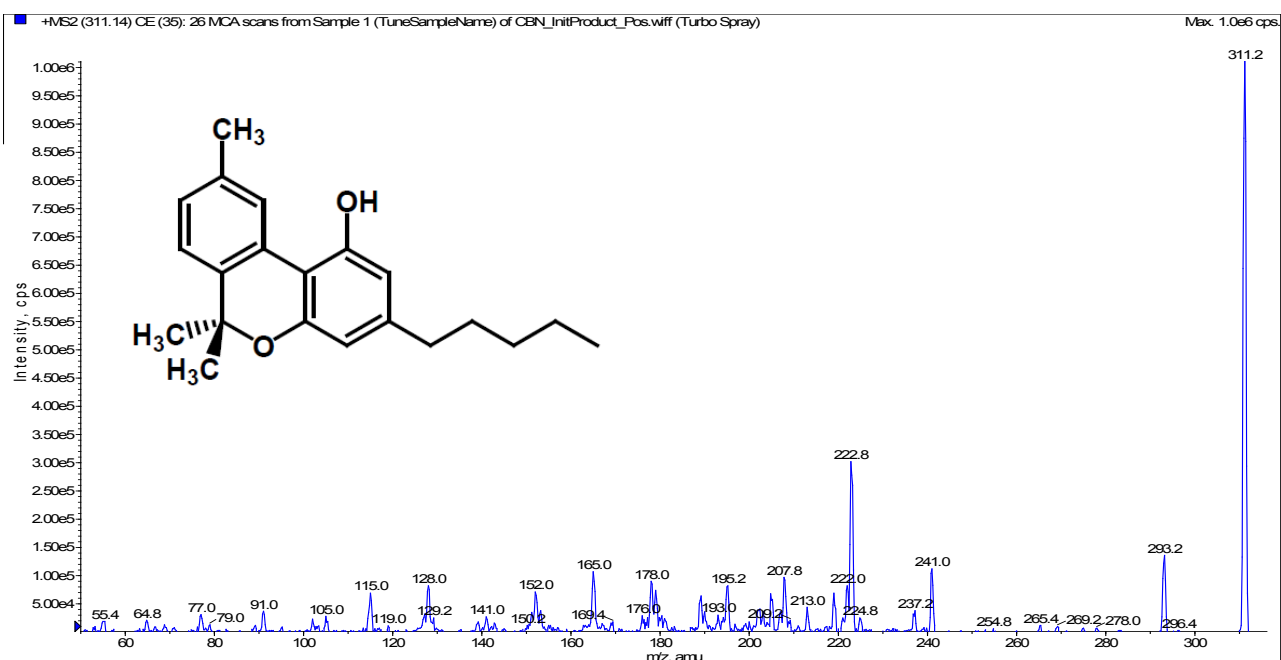


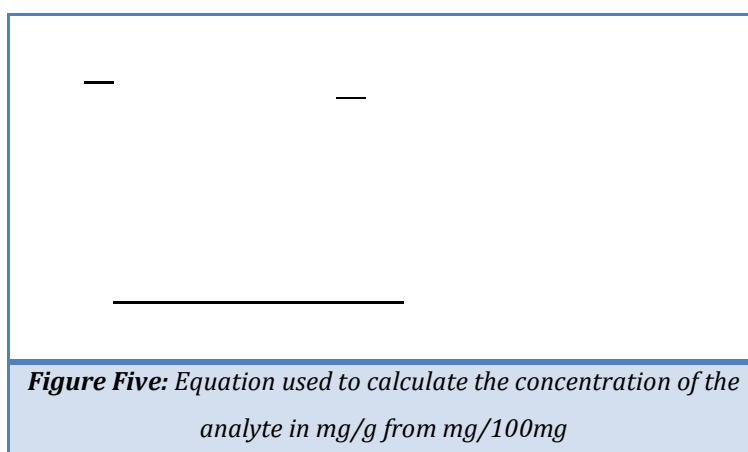
Figure four: Product ion mass spectrum of CBN

## 2.6 Calibration standard preparation and quantification

Stock solutions of  $\Delta^9$  - THC, CBD and CBN were prepared in a methanol and 10mM ammonium acetate solution at 1:1 v/v to a concentration of 1 mg/ml. Calibration standards of  $\Delta^9$  - THC, CBD and CBN were prepared in methanol and 10 mM ammonium acetate (1:1, v/v) to concentrations of 100, 50, 25, 12.5, 6.25, 3.13 and 1.56  $\mu\text{g/ml}$ .

Calibration curves were constructed. Quadratic regressions resulted in correlation coefficient of  $r=0.9978$ ,  $r=0.9999$  and  $r=0.9938$  for  $\Delta^9$  - THC, CBD and CBN respectively. The lower limit of quantification, determined from the calibration standards, for all 3 analytes was 1.56  $\mu\text{g/ml}$ .

The concentration of the analytes in mg/g was calculated as follows:



## 2.7 Confirmation

The identities of the cannabinoids detected in the cannabis samples were confirmed by applying the UNODC recommended methods for the identification and analysis of cannabis and cannabis productions. The elution order of CBD, CBN and  $\Delta^9$  - THC on a reverse phase HPLC served as confirmation. The elution order on a reverse phase column is influenced by the polarity of the analytes.  $\Delta^9$  - THC, being more hydrophobic

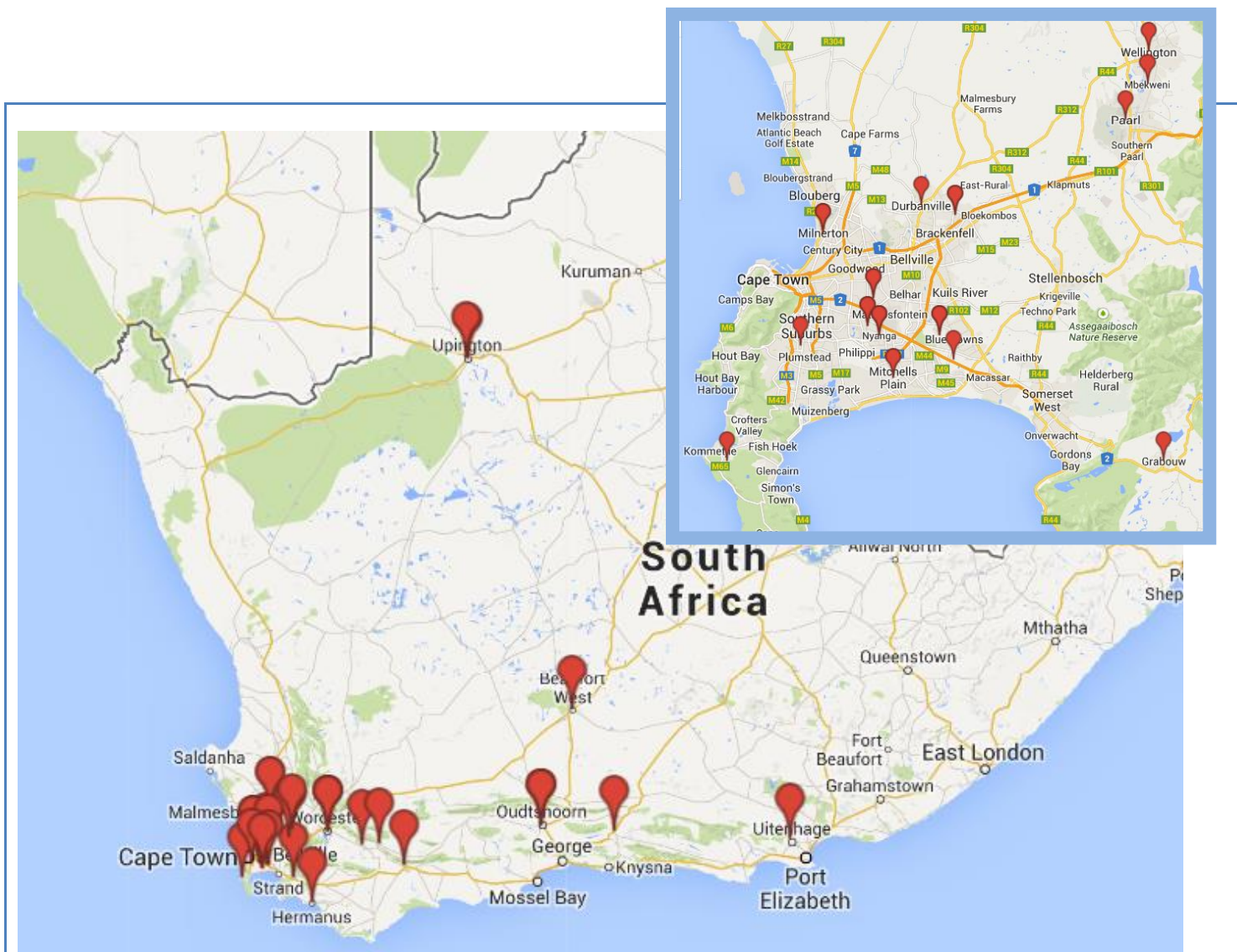
with a greater water partition co-efficient, is retained longer in the column in comparison to CBN and CBD, and thus will remain in the column longer and elute last. Furthermore, the relative LC retention times of the cannabinoids to that of the reference standards served as a distinguishing tool.  $\Delta^9$  - THC and CBD have the same Q1 and Q3 masses, therefore these standards were injected separately to confirm their identity.

## **2.8 Preliminary Study**

A preliminary study was conducted with the HPLC -MS assay via analysis of stored cannabis samples, provided by Prof Peter Smith from the Department of Pharmacology at the University of Cape Town, which served sample material in a previous research assignment. The samples were analysed with the HPLC -MS assay described earlier. The sample size consisted of 14 herbal cannabis samples from various parts of South Africa (figure ten). The samples were unfortunately old; with all samples showing discolour ranging from dark green to varying shades of brown. In addition the samples were very dry with whole flowering buds being indistinguishable from other plant material and therefore difficult to isolate. Results of the analysis are attached in addendum 1. There was no  $\Delta^9$  - THC present in any of the samples analyses, only significant amounts of the degradation product CBN was evident. Since the samples were bordering on the 15 year age, age determination as suggested in the UNODC recommendation methods would be impossible.

## **3. Results**

The sample distribution was predominately from the Western Cape, South Africa with most originating from sub districts with the Cape metropolitan and surrounding areas. Very few samples originate from areas outside the Western Cape (figure six)



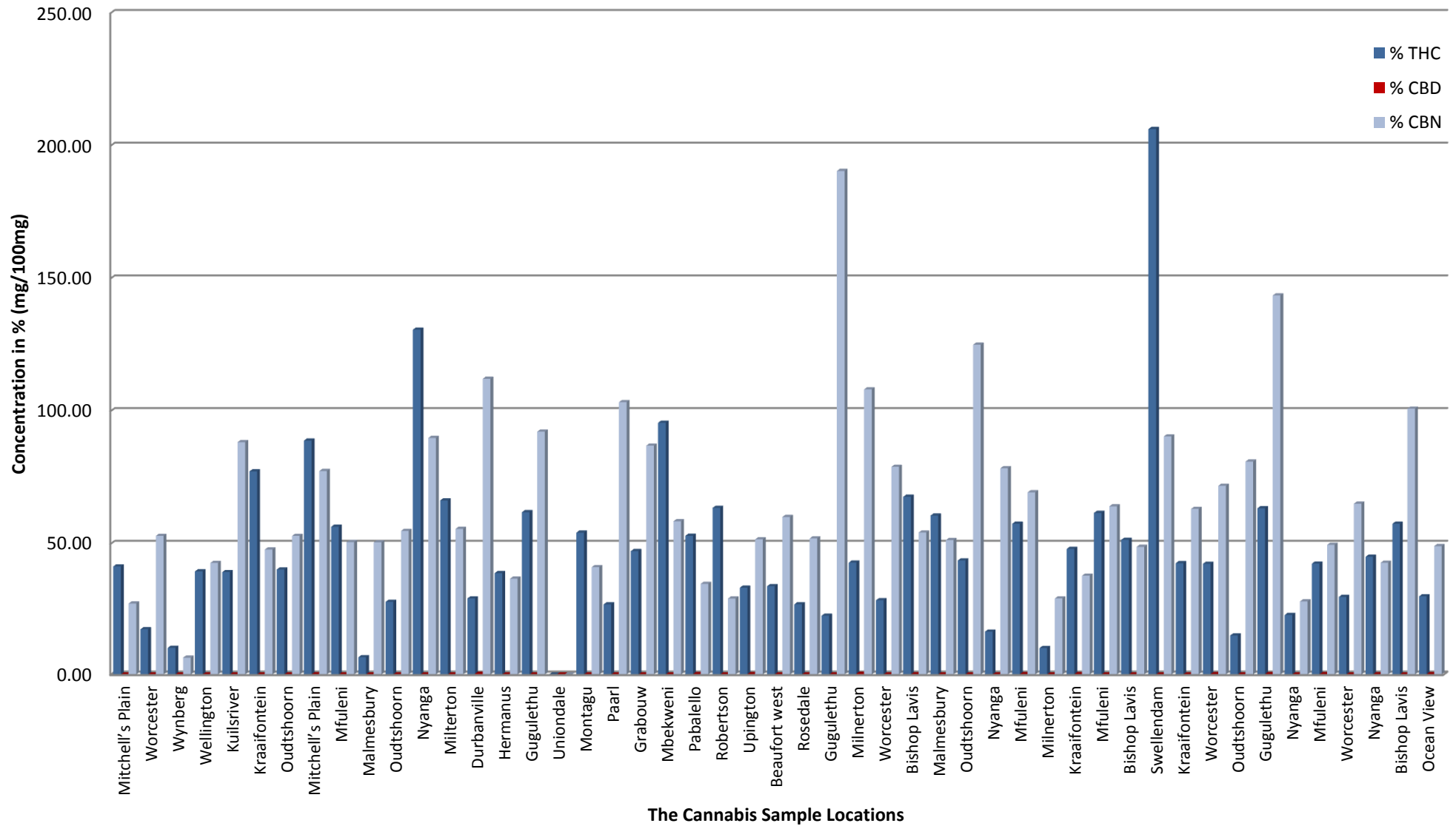
**Figure six** Geographical representation of locations that the cannabis samples originated from, with an enlargement of the Cape Metropolitan and surrounding areas

Results of the cannabis samples quantification included the number and corresponding station location, the retention time (Rt) in minutes, the calculated concentration in ug/ml and the converted concentration in % (mg/100mg) are presented in table two. The slight shift in retention times between analytes in the samples can be attributed to slight differences in chromatographic conditions on different days of analysis.

**Table Two: Results of quantification including the number and corresponding station location, the retention time (rt) in mins, the calculated concentration in ug/ml and the converted concentration in % (mg/100mg)**  
 BLQ = Below Limit of Quantification

Police Issue #	Station	Rt (min)			Calculated Con (ug/ml)			Concentration % (mg/100mg)			Police Issue #	Station	Rt (min)			Calculated Con (ug/ml)			Concentration % (mg/100mg)		
		THC	CBD	CBN	THC	CBD	CBN	THC	CBD	CBN			THC	CBD	CBN	THC	CBD	CBN	THC	CBD	CBN
1	Bishop Lavis	1.06	0.61	0.89	32.10	BLQ	33.11	62.47	BLQ	64.45	26	Beaufort West	1.00	0.64	0.83	17.08	BLQ	30.41	33.71	BLQ	60.02
2	Mitchell's Plain	1.31	0.85	1.15	21.20	BLQ	13.92	41.28	BLQ	27.10	27	Rosedale	1.30	0.00	1.14	13.72	BLQ	26.59	26.79	BLQ	51.92
3	Worcester	1.06	0.62	0.89	8.94	BLQ	27.37	17.28	BLQ	52.88	28	Gugulethu	1.30	0.86	1.14	11.41	BLQ	96.85	22.43	BLQ	190.39
4	Wynberg	1.01	0.00	0.85	5.23	BLQ	3.29	10.18	BLQ	6.41	29	Milnerton	0.94	0.58	0.78	21.66	BLQ	54.78	42.74	BLQ	108.11
5	Wellington	1.30	0.86	1.13	20.02	BLQ	21.65	39.39	BLQ	42.60	30	Worcester	1.02	0.59	0.86	14.59	BLQ	40.59	28.36	BLQ	78.90
6	Kuilsriver	0.98	0.62	0.81	19.85	BLQ	44.80	39.09	BLQ	88.20	31	Bishop Lavis	0.98	0.63	0.81	34.16	BLQ	27.35	67.63	BLQ	54.14
7	Kraaifontein	1.04	0.68	0.87	38.82	BLQ	24.00	77.20	BLQ	47.74	32	Mamesbury	1.28	0.00	1.12	31.13	BLQ	26.38	60.53	BLQ	51.30
8	Oudtshoorn	1.30	0.00	1.14	20.51	BLQ	27.05	40.09	BLQ	52.88	33	Oudtshoorn	1.29	0.71	1.14	22.02	BLQ	63.16	43.55	BLQ	124.92
9	Mitchell's Plain	1.01	0.56	0.85	44.71	BLQ	38.94	88.78	BLQ	77.32	34	Nyanga	1.29	0.00	1.13	8.24	BLQ	39.49	16.35	BLQ	78.36
10	Mfuleni	0.96	0.61	0.80	28.30	BLQ	25.40	56.33	BLQ	50.56	35	Mfuleni	1.07	0.72	0.90	29.62	BLQ	35.71	57.47	BLQ	69.29
11	Malmesbury	1.01	0.57	0.85	3.41	BLQ	26.16	6.58	BLQ	50.46	36	Milnerton	1.29	0.00	1.13	5.23	BLQ	15.06	10.08	BLQ	29.03
12	Oudtshoorn	1.04	0.59	0.88	14.27	BLQ	28.14	27.76	BLQ	54.76	37	Kraaifontein	1.06	0.71	0.89	24.35	BLQ	19.15	47.96	BLQ	37.73
13	Nyanda	1.30	0.86	1.13	66.83	BLQ	45.94	130.5 <sub>g</sub>	BLQ	89.79	38	Mfuleni	1.02	0.57	0.85	32.08	BLQ	33.38	61.54	BLQ	64.03
14	Milnerton	1.31	0.00	1.14	33.72	BLQ	28.27	66.24	BLQ	55.52	39	Bishop Lavis	1.30	0.00	1.14	25.89	BLQ	24.56	51.39	BLQ	48.75
15	Durbanville	0.99	0.66	0.83	15.03	BLQ	58.01	29.04	BLQ	112.07	40	Swellendam	1.30	0.00	1.14	21.24	BLQ	9.31	206.1 <sub>g</sub>	BLQ	90.35
16	Hermanus	1.29	0.00	1.13	20.15	BLQ	19.05	38.71	BLQ	36.60	41	Kraaifontein	1.28	0.00	1.12	21.82	BLQ	32.35	42.51	BLQ	63.04
17	Gugulethu	1.27	0.69	1.11	30.96	BLQ	46.18	61.80	BLQ	92.17	42	Worcester	0.95	0.61	0.79	21.68	BLQ	36.79	42.28	BLQ	71.75
18	Montagu	1.30	0.84	1.14	27.16	BLQ	20.59	0.00	BLQ	0.00	43	Oudtshoorn	1.25	0.84	1.10	7.59	BLQ	41.24	14.88	BLQ	80.87
19	Uniondale	1.04	0.68	0.87	< 0	BLQ	< 0	54.10	BLQ	41.00	44	Gugulethu	1.28	0.84	1.12	31.91	BLQ	72.35	63.29	BLQ	143.49
20	Paarl	1.04	0.62	0.88	13.69	BLQ	52.84	26.77	BLQ	103.28	45	Nyanga	1.05	0.60	0.89	23.07	BLQ	28.33	22.72	BLQ	27.91
21	Grabouw	0.93	0.69	0.77	23.95	BLQ	44.16	47.12	BLQ	86.87	46	Mfuleni	1.06	0.62	0.90	21.59	BLQ	25.25	42.33	BLQ	49.52
22	Mbekweni	1.03	0.66	0.87	48.90	BLQ	29.89	95.51	BLQ	58.38	47	Worcester	0.97	0.55	0.81	14.95	BLQ	32.83	29.62	BLQ	65.04
23	Pabalello	1.02	0.58	0.86	31.56	BLQ	20.65	52.91	BLQ	34.62	48	Nyanga	1.05	0.69	0.89	22.59	BLQ	21.44	44.99	BLQ	42.70
24	Robertson	1.29	0.00	1.13	32.20	BLQ	14.72	63.43	BLQ	29.00	49	Bishop Lavis	0.93	0.68	0.76	29.27	BLQ	51.36	57.44	BLQ	100.8
25	Upington	1.28	0.85	1.12	17.16	BLQ	26.71	33.15	BLQ	51.60	50	Ocean View	1.26	0.85	1.10	15.06	BLQ	24.73	29.85	BLQ	49.03

**Figure Seven: Cannabinoid Content of Cannabis Samples in percentage mg/100mg**



### **3.1 Classification of Cannabis**

Based on the  $\Delta^9$  – THC content, cannabis samples are divided into fibre type and drug type cannabis. Cannabis has for many centuries been used as a hemp, a practice that is still common today. Fibre type cannabis is legal to cultivate, and is characterised with low concentrations of  $\Delta^9$  – THC in comparison to the CBD. Since all the samples, excluding 19, have  $\Delta^9$  – THC in far greater quantities than CBD, all samples used can be classified as drug type cannabis.

### **3.2 Cannabinoids Levels in Cannabis Samples**

#### **3.2.1 CBD**

CBD was only detected in 13 of the 50 cannabis sample, but in all 13, the concentration was below the limit of quantitation and was thus considered as zero. Refer to figure seven, table one.

#### **3.2.2 % $\Delta^9$ – THC and %CBN**

$\Delta^9$  – THC was detected in all samples except sample 19, where no cannabinoids were detected. Swellendam presented with the highest concentration of  $\Delta^9$  – THC at 206.19%, whilst Malmesbury had the lowest concentration at 6.58%. The average  $\Delta^9$  – THC of all 50 samples was calculated to be 47.45%. Similarly, CBN was detected in all samples except sample 19. Gugulethu had the highest

concentration of CBN at 190.39%, whilst Wynberg presented with the lowest concentration of CBN at 6.41%. The average CBN percentage for the 50 samples was 64.75%. It was determined that 18 of the 50 cannabis samples had more CBN than  $\Delta^9$  – THC present. In addition, the average  $\Delta^9$  – THC at 47.45%, was lower than the average of CBN. Refer to figure seven.

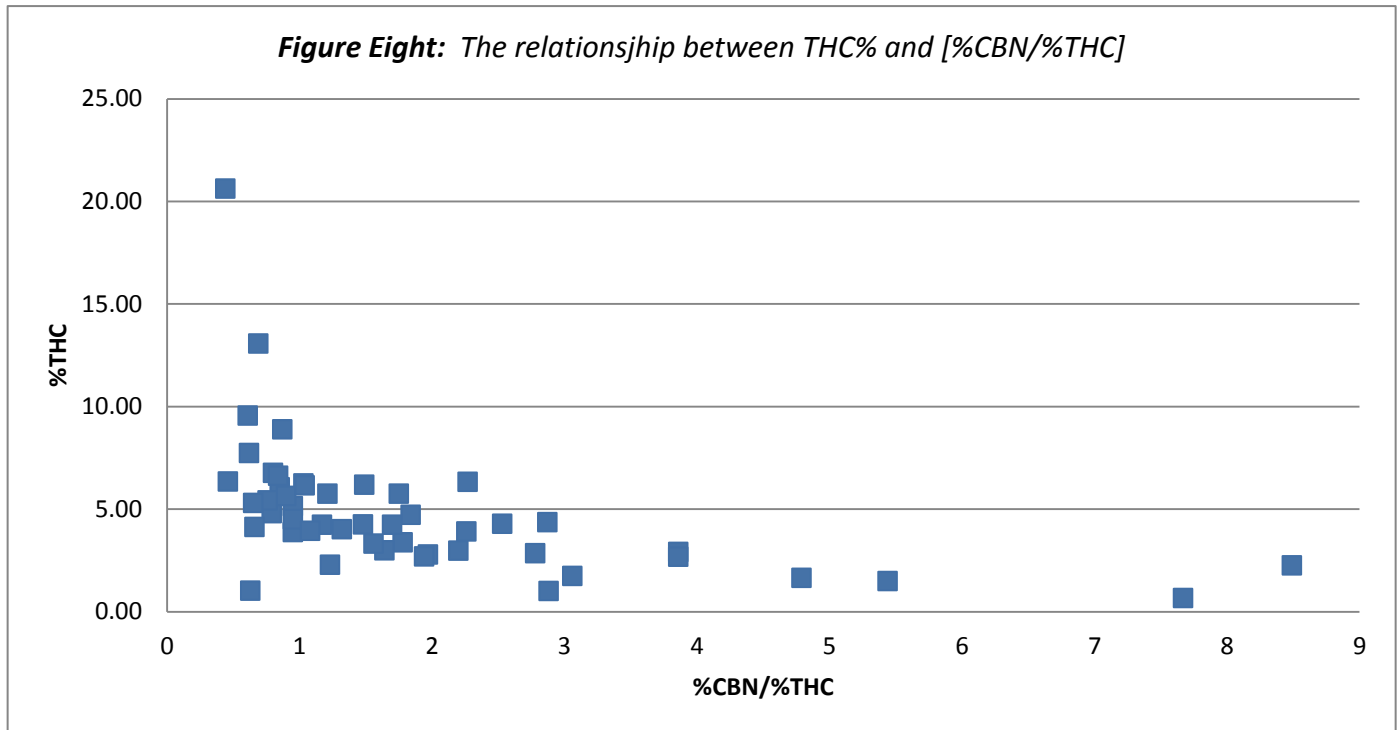
### **3.2.3 [%CBN/ %THC] Ratio as an Indicator of Sample Age**

The [%CBN/% $\Delta^9$  – THC] ratio provides an indication of the age of the samples. The sum of the cannabinoids were calculated for all samples and all values were considerably high (see table three). Gugulethu, with the greatest concentration of CBN had the largest [%CBN/%THC] ratio at 8.49. Similarly, Swellendam with the largest concentration of  $\Delta^9$  – THC, had the lowest [%CBN/%THC] ratio at 0.44. The average [%CBN/%THC] was 1.91, significantly lower than the maximum ratio presented in Gugulethu. Figure eight depicts the relationship between  $\Delta^9$  – THC and [%CBN/%THC] levels in the samples. The graph shows that as the concentration of  $\Delta^9$  – THC decreases the [%CBN/%THC] increases indicating long storage of samples. Most samples clustered between the 1- 3 value of [%CBN/%THC] and 0.00 – 15.00 of  $\Delta^9$  – THC%, but outliers at a  $\Delta^9$  – THC % greater than 20 and [%CBN/%THC] values greater than 4, indicate a trend.

### **3.2.4 [%THC+%CBN]**

CBN is the degradation product of  $\Delta^9$  – THC, therefore the sum of [%THC] and [%CBN] can provide an indication of the potential  $\Delta^9$  – THC levels of a sample and can therefore provide a means of comparison between aged samples. Swellendam, with the maximum CBN concentration had the maximum [%THC+%CBN] value at 296.54, whilst Wynberg has the minimum value of

[%THC+%CBN] at 16.58, and the average [%THC+%CBN] of the 50 samples was determined to be 112.20. Refer to table three.



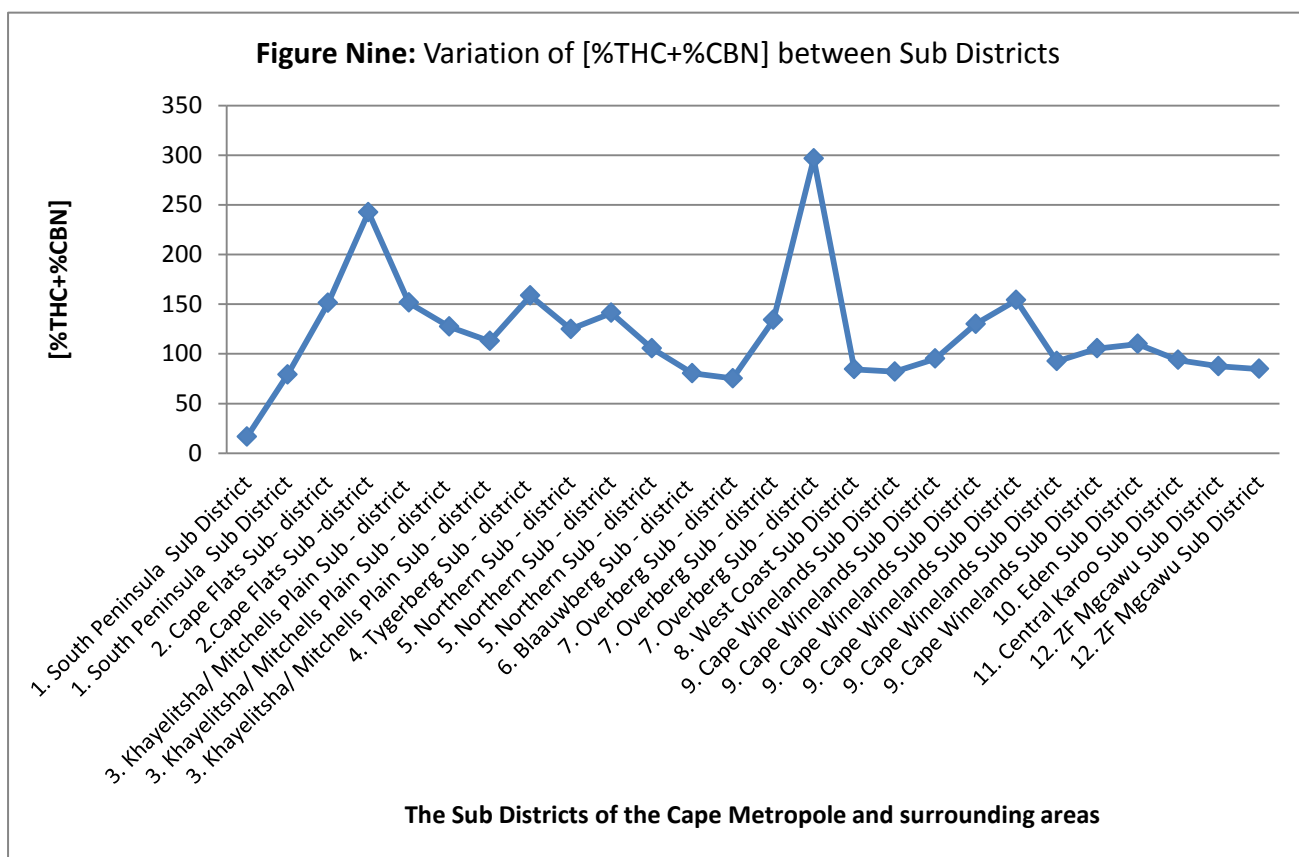
### 3.2.5 Sub District Characterisation

Variations between the [%THC+%CBN] of the sub districts of the Cape Metropolitan as well as surrounding areas were examined. The mean [%THC+%CBN] for towns that presented in more than one sample were calculated, and the areas were classified according to municipality designated sub districts. In instances where the district could not be determine such as Rosedale, numerous areas across South Africa with an area or town named Rosedale, the sample was excluded. The sub districts were arranged according their distance away from the city of Cape Town Metropolitan with the South Peninsula sub district as the orientation point. The sequential orders of the Sub – districts, as well as the towns that fall within a district, are as follows:

1. South Peninsula Sub-district: Ocean View; Wynberg

2. Cape Flats Sub-district: Nyanga; Wynberg
3. Mitchells Plain/Khayelitsha Sub-district: Mitchells Plain, Mfuleni;
4. Tygerberg Sub-district: Bishop Lavis
5. Northern Sub-district: Kraaifontein; Durbanville
6. Blaauwberg Sub-district: Milnerton; Table View
7. Overberg Sub-district: Swellendam; Grabouw
8. West Coast: Malmesbury
9. Cape Winelands: Mbekweni; Montagu; Robertson; Worcester; Wellington
10. Eden Sub-district: Outshornn; Uniondale
11. Central Karoo Sub-district: Beaufort West
12. ZF Mgcawi: Upinton; Palabello

A significant rise in the [%THC+%CBN] value can be seen arising from the Overberg sub-district as well as in the Cape Flats Sub-district. The South Peninsula sub-district presented with the lowest value at 16.58. No clear pattern is evident among the sub districts, although the majority of the values were high, falling between 50 and 300. See figure Nine.



**Table three: The [%CBN/%THC] ratio and [%THC+%CBN] value of cannabis samples**

	<b>Station</b>	<b>[%CBN/%THC]</b>	<b>[%THC+%CBN]</b>		<b>Station</b>	<b>[%CBN/%THC]</b>	<b>[%THC+%CBN]</b>
1	Bishop Lavis	1.03	126.92	26	Beaufort West	1.78	93.72
2	Mitchell's Plain	0.66	68.38	27	Rosedale	1.94	78.71
3	Worcester	3.06	70.16	28	Gugulethu	8.49	212.82
4	Wynberg	0.63	16.58	29	Milnerton	2.53	150.85
5	Wellington	1.08	81.98	30	Worcester	2.78	107.25
6	Kuilsriver	2.26	127.29	31	Bishop Lavis	0.8	121.77
7	Kraaifontein	0.62	124.94	32	Malmesbury	0.85	111.83
8	Oudtshoorn	1.32	92.96	33	Oudtshoorn	2.87	168.48
9	Mitchell's Plain	0.87	166.10	34	Nyanga	4.79	94.72
10	Mfuleni	0.9	106.89	35	Mfuleni	1.21	126.75
11	Malmesbury	7.67	57.05	36	Milnerton	2.88	39.11
12	Oudtshoorn	1.97	82.52	37	Kraaifontein	0.79	85.69
13	Nyanda	0.69	220.38	38	Mfuleni	1.04	125.57
14	Milnerton	0.84	121.76	39	Bishop Lavis	0.95	100.14
15	Durbanville	3.86	141.11	40	Swellendam	0.44	296.54
16	Hermanus	0.95	75.30	41	Kraaifontein	1.48	105.56
17	Gugulethu	1.49	153.97	42	Worcester	1.7	114.03
18	Montagu		0.00	43	Oudtshoorn	5.44	95.75
19	Uniondale	0.76	95.10	44	Gugulethu	2.27	206.78
20	Paarl	3.86	130.04	45	Nyanga	1.23	50.64
21	Grabouw	1.84	133.99	46	Mfuleni	1.17	91.85
22	Mbekweni	0.61	153.89	47	Worcester	2.2	94.65
23	Pabalello	0.65	87.53	48	Nyanga	0.95	87.68
24	Robertson	0.46	92.43	49	Bishop Lavis	1.75	158.24
25	Upington	1.56	84.75	50	Ocean View	1.64	78.88

#### 4. Discussion

Cannabis, as a recreational drug has an immense impact on society. It is, and has been for some time, been the most commonly abused illicit substance globally with a reported 224 million users. Production of cannabis extends across to almost all continents and occurs in almost all countries globally. The cultivation, possession and distribution of cannabis are illegal in most countries including SA. In SA, in parallel with global statistics, cannabis is the most widely used recreational substance, with usage transcending ethnic and cultural boundaries. Furthermore, cannabis often termed a “gate way drug” is therefore often associated with crime. The significance of cannabis in society demands knowledge and understanding of the drug. Currently, SA has no monitoring study aimed at examining the variations reported internationally regarding the changing cannabinoid content. This study aimed to investigate the cannabinoid content of cannabis sample’s confiscated by the SAPS forensic laboratories.  $\Delta^9$  – THC, the principle psychoactive constituent; its degradation product, CBN, and CBD, reported to influence the effect of  $\Delta^9$  – THC, was studied.

All samples received were classified as dry herbal cannabis preparations, regarded as the weakest preparation of the plant. The samples physical characteristics vary. Some samples consisted of large identifiable flowering buds, whilst others were already ground up or fine material. This provides some discrepancy in analysis since little is known about what part of the plant is included in the sample material, if any differentiation is made between the plant parts; if any flowering buds are included and the amount it constitutes in the sample provided. Differentiation between plant parts is imperative since the distribution of  $\Delta^9$  – THC varies, predominately localised in the flowering buds and leaves in the direct vicinity. For this reason, as much effort as possible was made to remove the seeds, large leaves and stalks during the extraction of the cannabinoids. Evidence of the discrepancy can be seen in the analysis of sample 19. This sample material was exclusively leaves. There was no identifiable flowering bud in the sample material provided by the SAPS only reasonably large leaves, and as a result no cannabinoids was detected in the sample.

The identification and quantification of  $\Delta^9$  – THC, CBD and CBN was performed with HPLC tandem MS. The MRM scans of the analytes reveal that the mass spectra of  $\Delta^9$  – THC and CBD are identical, with the same transitions from the protonated precursor ions ( $m/z$  315) to product ions ( $m/z$  193).  $\Delta^9$  – THC and CBD are chemically similar, only differing with an ester bond on the  $\Delta^9$  – THC and an alcohol on the corresponding position on CBD. Confirmation of analyte identity was achieved by the elution order. The cannabinoid profile of all the studied samples showed no presence of CBD, with detectable and varying quantities of  $\Delta^9$  – THC and CBN. The evident lack of CBD in all samples can suggest the genetic strain of cannabis plant. The two primary varieties of the genus *Cannabis* associated with the production of cannabis products are *Cannabis. L. Sativa* and *Cannabis. L. Indica*. The sativa strain is often characterised with high concentration of  $\Delta^9$  – THC and low quantities of CBD, whilst conversely high values of CBD with low concentrations of  $\Delta^9$  – THC are indicative of an indica strain cannabinoid profile. The significant lack of CBD in the SA confiscated samples is characteristic of almost pure sativa strains circulating the market.

A factor that greatly influences the quantification of cannabis samples is the age.  $\Delta^9$  – THC is a sensitive compound, being photo and thermo liable and degrading on exposure to air converting to its degradation artefact CBN. The analysis conducted in this study proved the samples were old, the degradation of the psychoactive component evident in the results with high levels of CBN. Gugulethu presented with the maximum CBN percentage at 190.29%, almost as high as the maximum  $\Delta^9$  – THC at Swellendam (206.19%), and exceeds the  $\Delta^9$  – THC percentages of most samples studied with the average CBN (64.75%) and being greater than the average of  $\Delta^9$  – THC of 47.75%. The cannabinoid profile for the study samples are depicted in figure seven and the amount of CBN is greater than the amount of  $\Delta^9$  – THC in most samples.  $\Delta^9$  – THC degrades at a faster rate in the first year of storage than for subsequent years, and a ratio of CBD to  $\Delta^9$  – THC of between 0.04 and 0.08 have been said to be indicative of a sample aged between one and two years old. The levels of CBN in the study samples greatly exceed the 0.08 value. Gugulethu had the maximum ratio at 8.49, suggesting the sample is the possibly the oldest and stored for the longest period. Swellendam presented with the

lowest CBN to  $\Delta^9$  - THC ratio at 0.44, suggesting the sample is comparatively the freshest but is still higher than 0.08 value marking two years. The mean ratio of the 50 study samples exceeded the 0.08 two year value mark at 1.91, with not one sample falling below a value of 0.5. The relationship between  $\Delta^9$  - THC and CBN, is graphically represented in figure eight, which shows that as  $\Delta^9$  - THC decreases, the [%CBN/% $\Delta^9$  - THC] ratio decreases.

Due to the relationship of  $\Delta^9$  - THC and CBN, with the CBN being effected by the concentration and degradation rate of  $\Delta^9$  - THC, and with samples that have such high levels of CBN, analysis of  $\Delta^9$  - THC and CBN in relation to one another is essential as it provides an valid manner of assessment between samples. The value encompasses both the amount of  $\Delta^9$  - THC present in the sample and the amount of CBN influenced by amount of  $\Delta^9$  - THC initially. And since the CBN to  $\Delta^9$  - THC of the samples used in this study indicated all samples could possibly be older than two year, the inconsistencies resulting from the differing degradation rates of  $\Delta^9$  - THC in successive years is reduced. Since, nationally, the variation in cannabinoid content between areas across SA cannot be studied due to limitations in the sample distribution; the cannabinoid profile of the sub districts of the City of Cape Town and districts of surrounding areas was examined. The average [%THC+%CBN] of towns and cities with more than one contribution to the sample population was calculated and if more than one sub district was present, it was included to possible elucidate a trend within the specific sub district. No clear distinct pattern was seen from [%THC+%CBN] values moving further away from the southern sub district of Cape Town. The Overberg district, which Swellendam falls under, shows a significant rise, almost doubling in comparison to its adjacent districts in the Overberg district and successive West Coast district. A peak is also evident in the Cape flats region, most liking as a result from collective effect of samples confiscated in the Nyanga area. In general all values fell between a 50 and 300 value. A slight increase in value can be seen from the West Coast sub district until the Cape Winelands, but in general regarding all sub-districts more samples will be needed to be examined to indicate a more definitive trend.

When considering previous literature regarding the cannabinoid profile of cannabis grown or confiscated in SA, of which there is very little, an article published by Field *et al* conducted in 1980, provides information relating the cannabinoid profile of that time. The study evaluated the cannabinoid compounds by GC-MS and classified plants according to age, sex and plant part. The study did, similar to the results of this study, showed that the cannabis in SA had very little CBD. The samples used by Field *et al* contained very little, trace quantities of CBN, indicating the samples were very fresh which is in contrast to the result of this study. The sum of [%THC+%CBN] for the Field *et al* study was calculated. The results indicate that, despite the difference in study methodology, a factor that is evident is the stark increase in the cannabinoid content in the past 30 years. The sum of [%THC+%CBN] was averaged for the plants (classified as young, medium or old) in the Field *et al* study, from the Kokstad (Transkei); Pongola (Natal) and Tzaneen districts, and the values were 1.76%; 2.50% and 2.17% respectively. The analysis of the Field *et al* was performed with GC-MS and method promotes the decarboxylation of  $\Delta^9$  - THC acid into  $\Delta^9$  - THC, which theoretically should increase the detection higher of the active component, but despite this the concentrations seen in this study's analysis is significantly higher. With the average [%THC+%CBN] of this study determined at 112.20% the results comparatively to the Field *et al* show a significant increase.

## 5. Study Limitations

A consistent problematic factor that affected this study was the lack of information about the samples provided. The sample material varied and no steps could be taken to provide consistency among the material that contributed to the cannabinoid profile. No information regarding age of the sample was received, or any storage conditions the material might have been subjected to. The analysis conducted in this study proved the samples were old, the degradation of the psychoactive component evident in the results. In addition, the stations indicated on the police information sheet are the station the samples were confiscated in. There is no information regarding where the sample originated from or what market regions it could have originated from.

Only one extraction per cannabis sample was performed during this study. To provide a more accurate assessment of the cannabinoids content, ideally more than one extraction per sample should be performed and the average calculated. Similarly, for a more detailed evaluation of the cannabis, additional cannabinoids should have been included in the study. A key cannabinoid that was excluded in this study that greatly affects the  $\Delta^9$  - THC is the  $\Delta^9$  - THC acid. Studies has showed that  $\Delta^9$  - THC acid is present in greater concentrations in comparison to  $\Delta^9$  - THC, and over time or as a result of heat, the acid decarboxylates into  $\Delta^9$  - THC. Therefore, for an accurate assessment of how strong the cannabis is, the acid should have been included in the cannabinoids assay, or the extraction method should include a step to promote decarboxylation. In addition, the analysis of the samples are only representative of one time frame, if the cannabinoid trend of samples is SA has to been assessed, the analysis of cannabis should occur over a longer period of time.

## 6. Conclusions

In conclusion, the cannabinoid content of cannabis is SA was investigated. All cannabis samples were classified as drug type, and were all of the herbal cannabis variety. The highest  $\Delta^9$  - THC concentration seen in the study was 20.62% but the average for all 50 samples studied was only 4.75%. Analysis showed the samples study were old, the degradation of the active component evident in the high values of CBN. The highest CBN concentration was 19.05% and the average for all 50 samples 6.48%. Results indicated that all the samples studied were older than two years, with Gugulethu showing the longest storage period. To provide a means of assessment between samples the [%THC+%CBN] for districts in and surround Cape Town was examined. No pattern was evident in cannabinoid content between districts. Furthermore, comparisons to previous research suggest the cannabinoid content in cannabis found in SA has increased significantly, but more research is needed for a more comprehensive view.

## PART D APPENDICES

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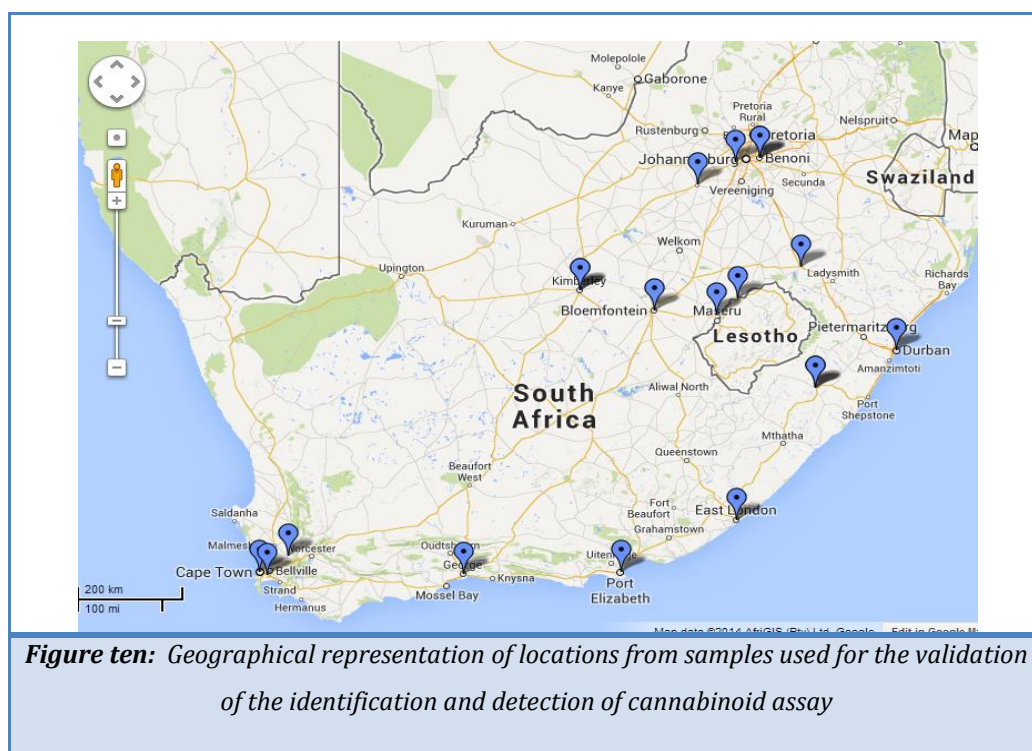
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### 3. Results from the Preliminary Study

#### 3.1 Samples

The sample size consisted of 14 herbal cannabis samples from various parts of South Africa, the locations of which are geographically depicted in figure 18. The samples were subjected to the previously described extraction method and HPLC- MS/MS conditions and parameters (see chapter 2 materials and methods.). The physical characteristics of the samples very old, with all samples showing discolour ranging from dark green to varying shades of brown. In addition the samples were very dry with whole flowering buds being indistinguishable from other plant material and therefore difficult to isolate.



### 3.2 Quantitation

Quantitation was achieved via a standard calibration curve with concentrations of 100; 50; 25; 12.5; 6.23; 3.13; 1.56; 0.780; 0.390; 0.195 $\mu\text{g/ml}$ . The calibration curves fits the quadratic regression with  $r=0.9957$ ;  $r= 0.9998$  and  $r=0.9938$  for  $\Delta^9$  – THC, CBD and CBN respectively. The lower limit of quantitation was established at 1.56 $\mu\text{g/ml}$ , 0.39 $\mu\text{g/ml}$  and 1.56 $\mu\text{g/ml}$  for  $\Delta^9$  – THC, CBD and CBN respectively. There was no  $\Delta^9$  – THC present in any of the samples analyses, and all detectable quantities of CBD fell below the limit of quantitation for the analyte. The analyte present in abundant quantities was the degradation product CBN.

**Table Four:** The quantification results of the preliminary study including Rt in minutes, calculated Concentration in ( $\mu\text{g/ml}$ ) and converted concentration in  $\text{mg/g}$

Police issue #	Station	Rt (min)			Calculated conc ( $\mu\text{g/mL}$ )			Concentration % ( $\text{mg/g}$ )		
		THC	CBD	CBN	THC	CBD	CBN	THC	CBD	CBN
1	George	N/A	N/A	1.2	BLQ	BLQ	24.5	0	0	4.63
2	Kimberly	N/A	N/A	1.198	BLQ	BLQ	16	0	0	2.99
3	Kokstad	N/A	N/A	1.205	BLQ	BLQ	36.7	0	0	7.22
4	Welkom	N/A	N/A	1.2	BLQ	BLQ	10.4	0	0	2.08
5	Potchestroom	N/A	N/A	0.974	BLQ	BLQ	23.6	0	0	4.68
6	Soweto	N/A	N/A	0.957	BLQ	BLQ	19.9	0	0	3.66
7	Benoni	N/A	N/A	1.199	BLQ	BLQ	27.5	0	0	5.40
8	Swaziland	N/A	N/A	0.878	BLQ	BLQ	17.2	0	0	3.44
9	Harrismith	N/A	N/A	0.939	BLQ	BLQ	25.7	0	0	5.14
10	Cape Town	N/A	N/A	0.977	BLQ	BLQ	19.6	0	0	3.92
11	Durban	N/A	N/A	0.854	BLQ	BLQ	33.9	0	0	6.78
12	Bloemfontein	N/A	N/A	0.945	BLQ	BLQ	16.6	0	0	3.32
13	Ladybrand	N/A	N/A	1.2	BLQ	BLQ	17.2	0	0	3.44
14	Fickburg	N/A	N/A	1.2						

### 3.3 Results

No  $\Delta^9$  – THC or CBD was detected in the sample used in the preliminary study. CBN was detected in all samples. Due to the lack of information as well as the lengthy storage period, no comparisons between samples could be made.