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COLLEGE OF BASIC AND APPLIED SCIENCES

DETERMINATION OF THE CONTENTS OF THREE MAJOR CANNABINOIDS IN CANNABIS SAMPLES FOUND IN GHANA

BY

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DECLARATION

I, Mercy Agyepong do hereby declare that the work contained in this thesis was undertaken solely by me under the supervision of Professor Vincent K. Nartey and Dr. Raphael K. Klake and that it has neither in part nor in whole been presented for another degree elsewhere.

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ABSTRACT

The possession, use, cultivation, sale and trafficking of any form of cannabis in Ghana without lawful authority is illegal. Incidentally the substance remains one of the most abused in the country. The aim of this research was to measure the content of three major cannabinoids - THC, CBN and CBD in cannabis samples cultivated and abused in Ghana. The research was encouraged to fill this gap of information against the backdrop of the current social outcry on the legalization of the substance in Ghana. A GC-MS method was utilized in the measurement of the content of THC, CBN and CBD as a percentage of the dry weight of thirty three illicit cannabis samples. Thirty samples were selected from exhibits available at the Forensic Science Laboratory of the Ghana Police Service, which originated from suspected plant materials seized by Police Officers across the regions of Ghana. The remaining three were uprooted from illegal cannabis farms in the Ashanti, Eastern and Volta regions. The cannabinoid contents measured for the seized samples analyzed were: THC{(0.93±0.07) - (15.14±1.22)} %; CBN {(0.67±0.05) - (3.23±0.26)} % and CBD {(0.88±0.07) - (1.19±0.09)} %. The THC content measured for the fresh samples were (6.88±0.55) %, (4.49±0.36) % and (5.46±0.44) % respectively for Ashanti, Eastern and Volta regions. The CBD content of the fresh farm samples were (1.33±0.10) %, (1.33±0.10) % and (1.18±0.10) % for Ashanti, Eastern and Volta regions respectively. The expanded uncertainties (k=2) in the measurement of the cannabinoids content were 8.06 %, 8.04 % and 7.44 % for THC, CBN and CBD respectively. The THC results of all the samples analyzed gave an indication of very high potent cannabis plant materials in Ghana. The THC content of the fresh samples collected from farms in the Ashanti, Eastern and Volta regions were higher and significantly different ($P<0.05$) from the seized samples from the three respective regions. The phenotype indices of the samples analyzed were greater than 1 and found in the range of 1.99 to 18.07; an indication that all the samples analyzed were drug type cannabis.

DEDICATION

I dedicate this work to my family for their support and prayers.

ACKNOWLEDGEMENT

I am grateful to the Lord God Almighty for his great mercy, grace and love upon my life.

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To my family, I say many thanks for your great support.

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LIST OF ABREVIATIONS

Δ^9 THC/THC	Delta-9-tetrahydrocannabinol
CBN	Cannabinol
CBD	Cannabidiol
GC-MS	Gas Chromatography- Mass Spectrometry
UNODC	United Nations Office on Drugs and Crime
FSL	Forensic Science Laboratory
Conc	Concentration
STDev	Standard Deviation
RSD	Relative Standard Deviation
TIC	Total Ion Chromatogram
µg/mL	microgram per milliliter
mg/mL	milligram per milliliter

CHAPTER ONE

INTRODUCTION

1.0 Background

Cannabis is reported as the most frequently trafficked drug worldwide and by far the most abused drug on the illicit drug market (United Nations Office on Drugs and Crime, 2009). Commonly known by names such as marijuana, marihuana, wee, weed and ganja, it is estimated, that between 2.8 % and 4.5 % of the world's population aged 15 to 64 corresponding to between 125-203 million people had used cannabis at least once in the reported year of 2010 (United Nations Office on Drugs and Crime, 2014).

In Africa, the 2014 World Drug Report indicates that, the most commonly abused drug is cannabis and that the annual prevalence of cannabis abuses in Africa, particularly West Africa, is much higher than the global average (United Nations Office on Drugs and Crime, 2014). Ghana, like other developing countries, has not been spared of the problems of drug abuse, which has become one of the many synonymous outcomes of rapid social change. Known locally in Ghana by names such as ganja, wee, ntampi, bongs, bonsam tawa and indian hemp, it is suggested that cannabis, alcohol and tobacco are the three most abused substances in the country (Akyeampong, 2005). In recent times, Ghana is recognized as a producer of cannabis for international drug trade with high consumption among the population (UNODC, 2000) and ranks second in West Africa in the illicit cultivation and exportation of cannabis (UNODC, 2007).

Cannabis is defined as a substance produced from cannabis sativa plant and used in three main forms namely; herbal cannabis, cannabis resin and cannabis oil (UNODC, 2012). The document further discussed that, the dried leaves and flower tops make up the herbal cannabis, the pressed secretions of the plant known as harshish or charash make up the cannabis resin and a mixture resulting from distillation or extraction of active ingredients of the plant make up the cannabis oil (UNODC, 2012). Cannabis contains a unique class of chemicals called Cannabinoids. About 120 of these compounds have been isolated from the plant (Morales, 2017) with Delta-9-tetrahydrocannabinol (Δ^9-THC), Cannabidiol (CBD) and Cannabinol (CBN) identified as the three major cannabinoids (Bruci et al., 2012).

The psychoactive ingredient in cannabis is THC and it is the cannabinoid responsible for psychotropic effects such as violence, aggression and psychosis, associated with users of the illicit drug (Di Forti et al., 2009; Sharma et al,. 2012) . On the other hand, studies have shown that cannabis has medicinal properties such as controlling non-cancer pain, controlling muscle spasm due to multiple sclerosis and treating wasting syndrome associated with AIDS (Clark et al., 2011). These mirroring effects of cannabis have earned it the description as the most controversial plant ever known worldwide (Bruci et al., 2012).

The psychotropic effects of cannabis which mostly lead to the commission of crime have informed the illegal status of the substance in most countries. However the status of cannabis as a controlled or narcotic substance varies globally from country to country and these variations are mostly based on the merits of the substance with respect to its medicinal and industrial benefits and its demerits as a psychotropic drug. Thus, whereas cannabis is totally an illegal substance in some countries, others have legalized the drug with varying degrees of limitations. For instance, the possession, use, cultivation, sale and trafficking of any type of cannabis in

Ghana without lawful authority is illegal (Narcotic Drugs Law, 1990), but most of the European Union, Canada and some states within the United States of America have recognized the value of hemp (fibre cannabis) and defined a legal limit of 0.3 % Δ^9-THC in the dry plant (UNODC, 2014).

Referred to as the potency, the THC levels in a cannabis sample primarily gives an indication of the strength of the substance. A research on cannabis samples from Albania reported mean THC levels ranging from 1.07 % to 12.13 % (Bruci et al., 2012). In a similar research, Tipparat et al., (2012) also looked into the characteristic cannabinoid content of cannabis plants cultivated in Northern Thailand and reported a mean THC content of 2.068 % dry weight.

Apart from the determination of the THC levels, characterization of cannabis has been done basically by using ratios of the percentage content of the three major cannabinoids. Notable in this classification system is the phenotype index defined by Fetterman (1971) which is the total THC content (% THC + % CBN) divided by the CBD content as detailed out in Table 1.1. Therefore cannabis can be divided into drug-type and fibre type and this has invariably influenced the legalization of the substance in certain countries where consideration has been given to economic and industrial value due to low levels of the psychotropic component - THC.

Table 1.1: Table of description of phenotype index

Phenotype	(%THC + %CBN)/%CBD	Class
Phenotype 1	Greater than 1	Drug type cannabis
Phenotype 2	Less than 1	Fibre type cannabis

A review of most literature revealed no information on the cannabinoids composition of cannabis in Ghana. However with a section of Ghanaians advocating for the legalization of substance, it has become highly imperative to investigate the phytoconstituents of cannabis cultivated and abused in Ghana to provide information on the character of the substance to serve as a good reference for any policy decision. This present study intends to give a good assessment of the character of cannabis samples found in Ghana to serve as a basis for a broader research into the field.

1.1 Problem statement

The major cannabinoids identified in cannabis are delta-9-THC, CBN and CBD. However the content of cannabinoids in cannabis are known to vary due to environmental conditions of farming, genetic makeup, mode of storage (heat, moisture and air), time of yield and synthetic preparations (Tipparat et al., 2012). The computational ratio of these cannabinoids composition may identify the cannabis plant (or product) as drug or fibre type. Countries as seen from the introduction to this study have researched and published findings on the cannabinoids content of cannabis grown or seized in these countries for reference and policy making. The problem identified by the researcher was that, even though there have been a number of studies on cannabis in Ghana, a review of most literature showed no information on the quantitative measure of the cannabinoids content. This study therefore seeks to fill this research gap.

1.2 Purpose of the study

The purpose of this study is to determine the percentage content of three major cannabinoids; THC, CBN and CBD in thirty illicit cannabis samples obtained from the Forensic Science Laboratory of the Ghana Police Service and three samples obtained from illegal farms in Ghana in order to determine the potencies and classify them into drug or fibre type with reference to the phenotype index. Instrumental analysis was done using Gas Chromatograph with Mass Spectrometer Detector (GC-MS).

1.3 Aim and objectives of the study

The aim of the study is to measure the percentage content of three major cannabinoids in thirty three cannabis samples found in Ghana in order to determine the potencies and classify as fibre or drug type cannabis.

1.3.1 Specific Objectives

- To determine the potencies of illicit cannabis samples found in Ghana, by the measurement of the percentage content of the three major cannabinoids.
- To classify the cannabis samples into drug or fibre type cannabis using the phenotype index.
- To determine the rate of decomposition of THC to CBN in the analytical samples.

CHAPTER 2

LITERATURE REVIEW

2.0 The cannabis plant

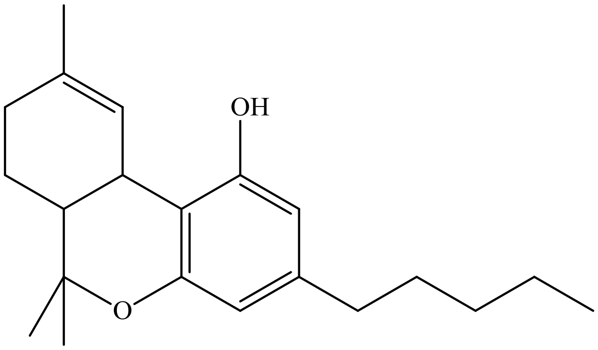
Cannabis is considered as of the genus Cannabis, family Cannabaceae and order Urticales (Kuddus et al., 2013). Different species of the plant have been identified, however, cannabis is the preferred designation for Cannabis sativa and Cannabis indica, and of minor significance, Cannabis ruderalis (Gloss, 2015). Figure 2.1 shows an image of Cannabis sativa, Cannabis indica and Cannabis ruderalis. Other researchers have suggested cannabis sativa as the unique species of the plant, with the genetic differences explained by variations at the subspecies-and-variety-levels or at a biotype-level of putative taxa (Small, 2015).

Cannabis is an annual crop plant propagated from seeds and grows vigorously when provided with a well drained soil, an open sunny location, ample nutrients and water (ElSohly et al., 2016). The plant is also known to be dioecious, thus having the male and female reproductive parts on separate plants (Clarke & Watson, 2007). Comparatively, plant species such as Hibiscus cannabinu, Acer palmatum and Dizygoteca elegantissima bear morphological features that show a degree of similarity to cannabis sativa. However the macroscopic and/or microscopic features eliminate this confusion. In addition, there exist other presumptive tests and analytical methods to distinguish cannabis from other plant materials (United Nations Office on Drugs, 2009).

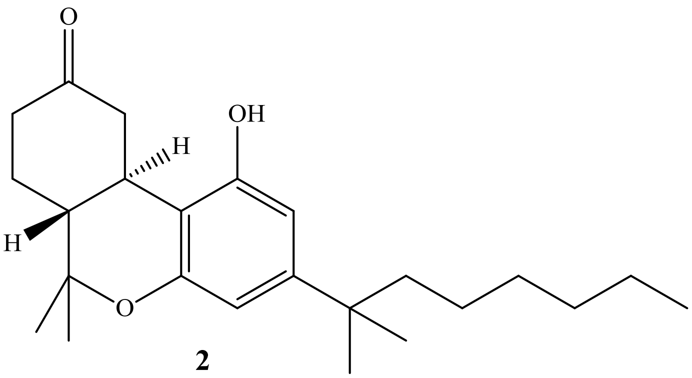
Figure 2.1: Leaves of Cannabis sativa (left), Cannabis indica (middle) and Cannabis ruderalis (right) (United Nations Office on Drugs and Crime, 2009)

2.1 Applications of cannabis

The history of the uses of cannabis is long and complex. It is an aged old medicinal plant described in almost every primeval hand book on plant medicine but its use has been banned in many countries due to its psychoactive effects. Records show that cannabis extracts were originally listed in the British and later the US pharmacopeia in the early nineteenth century (Madras, 2015). However for reasons of abuse and intoxication among others, the listings were removed in 1932 and 1941 respectively. Invariably the medicinal properties of the plant is still being explored till date. Ko et al., (2016) reported the medicinal use of cannabis in cancer patients to relieve symptoms including nausea and vomiting. The American Academy of Ophthalmology (2014) stated that cannabis is effective in the treatment of glaucoma, a vision impairment caused by optic nerve damage due to high intraocular pressure (IOP). According to the organization, cannabis is helpful in reducing intraocular pressure for short periods. Medical products that are currently approved for curative use include Dronabinol (1) and Nabilone (2) (Gloss, 2015).



1



2

Figure 2.2: Chemical structures of Dronabinol (1) and Nabilone (2)

Contrarily, other studies have raised concerns about the medicinal use of cannabis. Notable among these concerns are, the psychoactive nature of the substance, the difficulty in the determination of effective and safe dosage, the adverse effects of cannabis on the immune system of AIDS patients, risk of developing bipolar disorders and depression (Clark et al., 2011). Even though twenty six states in the United States have legalized the medical use of cannabis, the Drug Enforcement Agency (DEA) in agreement with the above concerns and more continue to classify it as an illegal schedule I drug. The agency asserts that, cannabis remains illegitimate with no scientific proof to show that it is better than approved drugs for any specific medical condition (Clark et al., 2011).

Cannabis is also known to be one of the oldest cultivated plants used for the production of oil from the seeds, and fibre from the stems for rope and fabrics. Its fibre (contains low THC of <0.3 %) is reported to have unique properties that are very useful in the automotive, bio-composite, medical and textile industries (Andre et al., 2016).

2.2 Chemistry of cannabis

The chemistry of Cannabis has been extensively studied with approximately over five hundred (500) compounds being identified (De Backer et al., 2009). Among these compounds is a unique class of chemicals called cannabinoids (Tipparat et al., 2012) with the identification of about 120 of these compounds (Morales, 2017). The compounds were identified as substances which are specifically recognized by the cannabinoid systems in the body (Sharma et al., 2012). Other classes of compounds found in cannabis plant apart from cannabinoids are Terpenoids and Flavanoids (Stott & Guy, 2004). Madras (2015) identified three sources of cannabinoids as;

- Phytocannabinoids: cannabinoid compounds produced by plants cannabis sativa or cannabis indica which is sometimes called herbal cannabinoids.
- Endocannabinoids: neurotransmitters produced in the brain or in peripheral tissues, and act on cannabinoid receptors of humans and animals.
- Synthetic cannabinoids: synthetic cannabis structurally analogous to phytocannabinoids or endocannabinoids and act by similar biological mechanisms.

The focus of this work would be on the phytocannabinoids or simply cannabinoids as they are the most active constituents in cannabis.

2.2.0 Cannabinoids

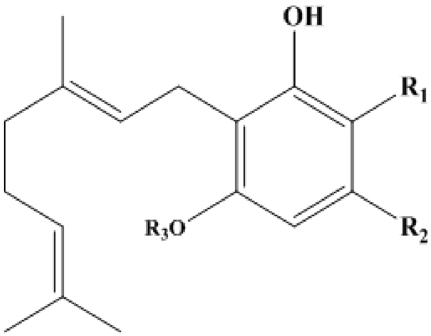
Cannabinoids are oxygen-containing C_{21} aromatic hydrocarbons found in cannabis (Morales, 2017). One hundred and twenty cannabinoids are estimated to have been isolated from cannabis (Morales, 2017). Delta-9-tetrahydrocannabinol (THC) is the main psychoactive component of cannabis whereas other major components but non psychoactive are cannabinol (CBN) and cannabidiol (CBD) (Bruci et al., 2012). Other known cannabinoids are: cannabichromene (CBC);

cannabidiolic acid (CBDA); cannabidivarin (CBD-V); cannabichromivarin (CBC-V); cannabielsoin (CBE) ; cannabigerol (CBG); cannabicyclol (CBL); cannabitriol (CBO); tetrahydrocannabinolic acid (THCA); tetrahydrocannabivarin (THC-V) (Stott & Guy, 2004). Much of our focus in the work would be centered on THC, CBN and CBD as their levels determine the character of cannabis.

2.2.1 Cannabigerol

The first cannabinoid isolated was cannabigerol (CBG) **(5)**. Its acid precursor cannabigerolic acid (CBGA) **(3)**, was reported as the first to be formed in the plant. Other cannabinoids in this group are on propyl side chain analogs and monomethyl ether derivatives (Shoyama et al., 1975). The derivatives were cannabigerolic acid monomethylether (CBGAM) **(4)**, cannabigerol monomethylether (CBGM) **(6)**, cannabigerovarinic acid (CBGVA) **(7)**, cannabigerovarin (CBGV) **(8)** (Gaoni & Mechoulan, 1964). The structures of these cannabinoids are in listed Table 2.1.

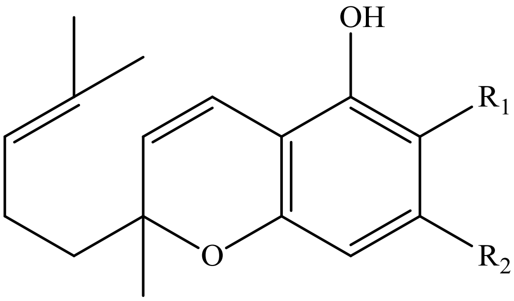
Table 2.1: Structures of some cannabigerol type cannabinoids with their pharmacological activity

Number	Structure	Main pharmaco-activity
3	 **R_1** **R_2** **R_3** COOH C_5H_{11} H	Antibiotics
4	**R_1** **R_2** **R_3** COOH C_5H_{11} CH_3	
5	**R_1** **R_2** **R_3** H C_5H_{11} CH_3	
6	**R_1** **R_2** **R_3** H C_5H_{11} H	Antibiotics Antifungal Anti-inflammatory Analgesic
7	**R_1** **R_2** **R_3** COOH C_3H_7 H	
8	**R_1** **R_2** **R_3** H C_3H_7 H	

2.2.2 Cannabichromene

Four cannabichromene (CBC) type cannabinoids have been identified. As shown in Table 2.2, cannabichromenic acid (CBGCA) **(9)**, cannabichromene (CBC) **(10)**, cannabichromevarinic acid (CBCVA) **(11)** and cannabichromevarin acid (CBCV) **(12)** are some known cannabichromene type cannabinoids (Gaoni & Mechoulan, 1964).

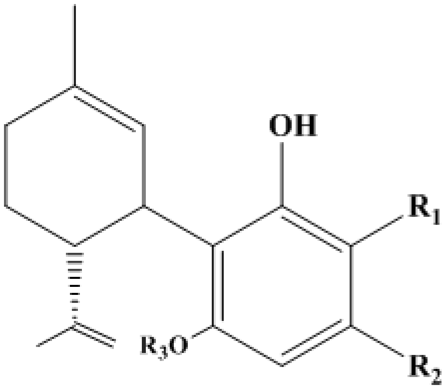
Table 2.2: Structures of some cannabichromene type cannabinoid with their pharmacological activity

Number	Structure	Main pharmaco-activity
9	 R_1 = COOH R_2 = C_5H_{11}	
10	R_1 = H R_2 = C_5H_{11}	Antibiotics Antifungal Anti-inflammatory Analgesic
11	R_1 = COOH R_2 = C_3H_7	
12	R_1 = H R_2 = C_3H_7	

2.2.3 Cannabidiol

The correct structure of cannabidiol (CBD) **(14)** was attributed to Mechoulam and Shvo (1963) after the first isolation in 1940 (Adams et al, 1940). Fiber-type cannabis (industrial hemp) is very rich in this cannabinoid and its corresponding acid precursor cannabidiolic acid (CBDA) **(13)**. Isolated in 1955 the first discovered cannabinoid acid was CBDA. Other examples are, cannabidiol monomethylether (CBDM) **(15)**, cannabidiol-C_4 (CBD-C_4) **(16)**, cannabidivarinic acid (CBDVA) **(17)**, cannabidivarin (CBDV) **(18)** and cannabidiorcol (CBD-C_1) **(19)** as shown in Table 2.3 (Gaoni & Mechoulan, 1964).

Table 2.3: Structures of some cannabidiol type cannabinoid with their pharmacological activity

Number	Structure	Main pharmaco-activity
13	 $R_1 = COOH$ $R_2 = C_5H_{11}$ $R_3 = H$	Antibiotic
14	$R_1 = H$ $R_2 = C_5H_{11}$ $R_3 = H$	Antibiotics Anti-inflammatory Analgesic Antioxidant

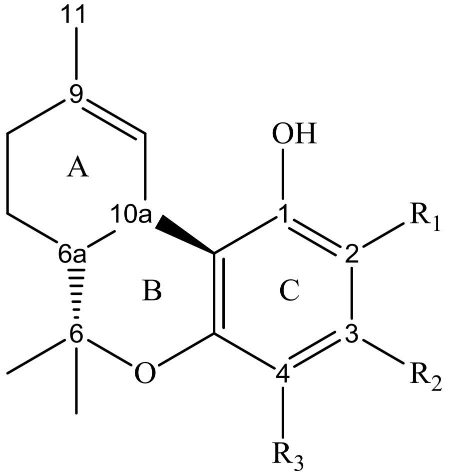
		Aniolytic Antipasmodic Antipsychotic
15	$R_1 = H$ $R_2 = C_5H_{11}$ $R_3 = CH_3$	
16	$R_1 = H$ $R_2 = C_4H_9$ $R_3 = H$	
17	$R_1 = COOH$ $R_2 = C_3H_7$ $R_3 = H$	
18	$R_1 = H$ $R_2 = C_3H_7$ $R_3 = H$	
19	$R_1 = H$ $R_2 = CH_3$ $R_3 = H$	

2.2.4 Δ^9-Tetrahydrocannabinol

Nine THC-type cannabinoids with C_1 to C_5 side chains are known. The major biogenic precursor is the Δ^9-tetrahydrocannabinolic acid A (THCA-A) **(20)**, whereas Δ^9-tetrahydrocannabinolic acid B (THCA-B) **(21)** is present to a much lesser extent. Δ^9-tetrahydrocannabinol (THC) **(22)** is the main psychotropic principle; the acids are not psychoactive. THC (6a,10a-trans-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo [b, d] pyran-1-ol) was first isolated in 1942 (Wollner et al., 1942), but Gaoni and Mechoulam assigned the correct structure in 1964 (Gaoni & Mechoulam, 1964). Other isolated THC type cannabinoids were; Δ^9-tetrahydrocannabinolic acid-C_4 (THCA-C_4) **(23)**, Δ^9-tetrahydrocannabinol-C_4 (THC-C_4) **(24)**, Δ^9-tetrahydrocannabivarinic acid (THCVA) **(25)**, Δ^9-tetrahydrocannabivarin (THCV) **(26)**, Δ^9-

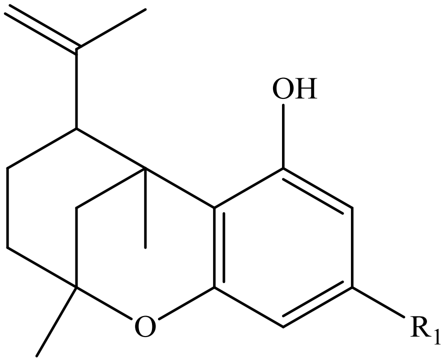
tetrahydrocannabiorcolic acid (THC-C_1) **(27)**, Δ^9-tetrahydrocannabiorcol (THC-C_1) **(28)** and Δ^7-cis-iso-tetrahydrocannabivarin **(29)** as listed in Table 2.4 (Gaoni & Mechoulan, 1964).

Table 2.4: Structures of some Δ^9-Tetrahydrocannabinol type cannabinoid with their pharmacological activity

Number	Structure	Main pharmaco-activity
20	 R_1 = COOH $R_2 = C_5H_{11}$ R_3 = H	
21	R_1 = H $R_2 = C_5H_{11}$ R_3 = COOH	
22	R_1 = H $R_2 = C_5H_{11}$ R_3 = H	Euphoriant Analgesic Anti-inflammatory Antioxidant Antiemetic
23	R_1 = COOH $R_2 = C_4H_9$ R_3 = H	

	or $R_1 = H \quad R_2 = C_4H_9 \quad R_3 = COOH$	
24	$R_1 = H \quad R_2 = C_4H_9 \quad R_3 = H$	
25	$R_1 = COOH \quad R_2 = C_3H_7 \quad R_3 = H$	
26	$R_1 = H \quad R_2 = C_3H_7 \quad R_3 = H$	Euphoriant Analgesic
27	$R_1 = COOH \quad R_2 = CH_3 \quad R_3 = H$ or $R_1 = H \quad R_2 = CH_3 \quad R_3 = COOH$	
28	$R_1 = H \quad R_2 = CH_3 \quad R_3 = H$	

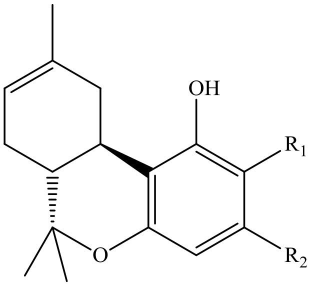
29



$R_1 = C_3H_7$

2.2.5 Δ8-Tetrahydrocannibinol

Δ^8-tetrahydrocannabinol (Δ^8-THC) **(31)** and its acid precursor tetrahydrocannabinolic acid (Δ^8-THCA) **(30)** are considered as THC and THC acid artifacts, respectively. The 8, 9 double-bond position is thermodynamically more stable than the 9, 10 position. Δ^8-THC is approximately 20 % less active than Δ^9-THC (Gaoni & Mechoulan, 1964; Wollner et al., 1942). Figure 2.3 show chemical structures of Δ^8-THC type cannabinoids.

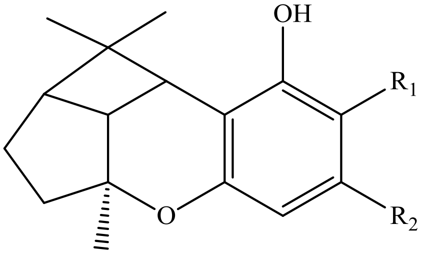


	R_1	**R_2**
(30)	COOH	C_5H_{11}
(31)	H	C_5H_{11}

Figure 2.3: Chemical structures of some Δ^8-THC type cannabinoids.

2.2.6 Cannabicyclol

Three cannabinoids characterized by a five-C-atom ring and C_1-bridge instead of the typical ring A are known: cannabicyclol (CBL) **(33)**, its acid precursor cannabicyclolic acid (CBLA) **(32)**, and the C_3 side-chain analog cannabicyclovarin (CBLV) **(34)**. Figure 2.4 show chemical structures of CBL. The cannabinoid is known to be a product of CBC when exposed to heat (Gaoni & Mechoulan, 1964; Wollner et al., 1942).

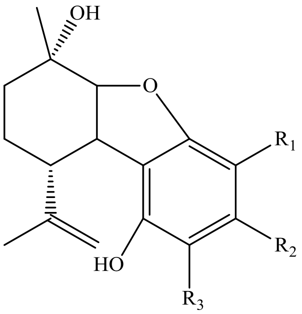


	R_1	**R_2**
(32)	COOH	C_5H_{11}
(33)	H	C_5H_{11}
(34)	H	C_3H_7

Figure 2.4: Chemical structures of some cannabicyclol type cannabinoids.

2.2.7 Cannabielsoin (CBE) type

Figure 2.5 shows the structures of three cannabielsoin (CBE) type cannabinoids namely; cannabielsoic acid A (CBEA-A) **(35),** and B (CBEA-B) **(36)** and cannabielsoin (CBE) **(37)** (Gaoni & Mechoulan, 1964; Wollner et al., 1942).

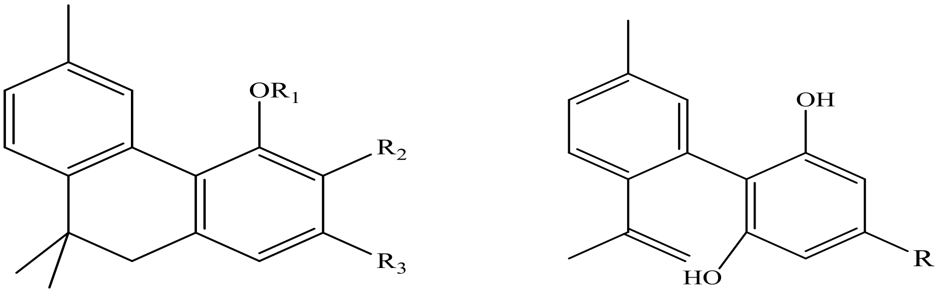


	R_1	**R_2**	**R_3**
(35)	COOH	C_5H_{11}	H
(36)	H	C_5H_{11}	COOH
(37)	H	C_5H_{11}	H

Figure 2.5: Chemical structures of some cannabielsoin type cannabinoids.

2.2.8 Cannabinol (CBN) and Cannabinodiol (CBND) types

Six cannabinol (CBN) and two cannabinodiol (CBND) type cannabinoids have been identified. The compounds are degradation products of THC. CBN was first mentioned in 1896 (Wood et al,. 1896) and its structure elucidated in 1940 (Adams et al., 1940). Examples are cannabinolic acid (CBNA) **(38)**, cannabinol acid (CBN) **(39)**, cannabinol monomethylether (CBNM) **(40)**, cannabivarin (CBN-C_4) **(41)**, cannabinol-C_2 (CBN-C_2) **(42)**, cannabiorcol (CBN-C_1) **(43)**, cannabinodiol (CBND) **(44)** and cannabinodivarin (CBVA) **(45)** (Gaoni & Mechoulan, 1964; Wollner et al., 1942)**.** Figure 2.6 shows the structures of this cannabinoid type.



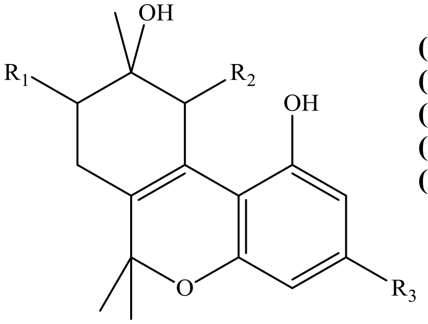
	R_1	R_2	R_3
(38)	H	COOH	C_5H_{11}
(39)	H	H	C_5H_{11}
(40)	CH_3	H	C_5H_{11}
(41)	H	H	C_4H_9
(42)	H	H	C_3H_7
(43)	H	H	CH_3

	R
(44)	C_5H_{11}
(45)	C_3H_7

Figure 2.6: Chemical structures of some cannabinol and cannabinodiol type cannabinoids

2.2.9 Cannabitriol (CBT) type

Nine cannabitriol (CBT) type cannabinoids have been identified, which are characterized by additional OH substitution. CBT itself exists in the form of both isomers and the racemate, whereas two isomers (9-a- and 9-b-hydroxy) of CBTV were identified. CBDA tetrahydrocannabitriol ester (ester at 9-hydroxy group) is the only reported ester of any naturally occurring cannabinoids. Cannabitriol (CBT) **(46)**, 10-ethoxy-9-hydroxy-delta-6a-tetrahydrocannabinol **(47)**, 8,9-Dihydroxy-delta-6a-tetrahydrocannadinol **(48)**, cannabitriolvarin (CBTV) **(49)** and ethoxy-cannabitriolvarin (CBTVE) **(50)** are some examples of cannabidiol type cannabinoids (Wood et al., 1896; Wollner et al.,1942). Figure 2.7 shows structures of cannabitriol type cannabinoids.



	R_1	**R_2**	**R_3**
(46)	H	OH	C_5H_{11}
(47)	H	OC_2H_5	C_5H_{11}
(48)	OH	H	C_5H_{11}
(49)	H	OH	C_3H_7
(50)	H	OC_2H_5	C_3H_7

Figure 2.7: Chemical structures of some cannabitriol type cannabinoids

2.3 THC content in cannabis

The potency of cannabis products is the level of the primary psychoactive constituent delta-9-THC (EMCDDA, 2010). In 2008, the reported mean THC content of cannabis resin ranged from 3 % to 16 % and the mean potency of herbal cannabis ranged from 1 % to 10 % for most European countries. The mean potency of sinsemilla was 12 % in Norway to over 16 % in the Netherlands and the United Kingdom (EMCDDA, 2010). A 2006 study showed that, two thirds of seizures of herbal cannabis in Switzerland ranged between 2-12 per cent THC (United Nations Office on Drugs and Crime, 2009). In Germany, the THC contents have been hovering around 8 %, showing no long-term change (EMCDDA, 2010). Also a study of cannabis potency in France showed 7 % to 13 % increase for herbal cannabis from 2009 to 2015 and 10 % to 23 % for the resin (Dujourdy & Besacier, 2017). Bruci et al., (2012) in a research on cannabis from different locations of Albania reported a range of 1.07 % to 12.13 %. In a similar research in Northern Thailand, THC levels were reported to range from 0.72 % to 1.480 % (Tipparat et al., 2012). The new trend to cultivate cannabis from seeds originating from high-THC cultivars and under indoor conditions has accounted for an increase in the potency of cannabis in recent times (Miller et al., 2003).

Apart from the determination of the THC levels, cannabis can be classified into fiber-type and drug-type basically by using defined ratios of the percentage content of the major cannabinoids of the substance. Two notable classifications are attributed to Fetterman et al., (1971) and Small et al. (1973). The phenotype index defined by Fetterman et al., (1971) was defined by the ratio: (% THC + % CBN)/% CBD and described in Table 2.5.

Table 2.5 Table of description of phenotype index

Phenotype	(%THC + %CBN)/%CBD	Class
Phenotype 1	Greater than 1	Drug type cannabis
Phenotype 2	Less than 1	Fibre type cannabis

Small et al., (1973) identified three chemotypes with the defined ratios as shown in Table 2.6.

Table 2.6: Table of description of Chemotypes

Chemotype	THC/CBD	Class
Chemotype 1	Greater than 1	Drug type plant
Chemotype 2	Close to 1	Intermediate drug plant
Chemotype 3	Less than 1	Fiber-type plant

These classifications have influenced the legalization of cannabis in certain countries where consideration has been given to economic and industrial use of the substance due to the low levels of the psychotropic ingredient.

A research on cannabis samples from Albania reported mean THC levels ranging from 1.07 % to 12.13 %. The phenotype ratios were found to range from 1.57 to 63.0 representing drug type cannabis (Bruci et al., 2012). In a similar research, Tipparat et al., (2012) also looked into the characteristic cannabinoids content of cannabis plants cultivated in Northern Thailand and reported phenotype ratios ranging from 12.6 and 84.09.

2.4 THC content in seeds

Cannabis seeds naturally contain no or very minimal THC. THC found in fibre cannabis or hemp product may be as a result of seed contact with the potent containing parts during processing (Elsholy, 2003; Ross et al., 2000; Leson et al, 2001). The seed, depending on the variety, may contain less than 0.5 Pg/g. However researches on hemp oil conducted in Switzerland, Germany and the United states have shown up to 3568 THC level (Gigliano, 2001). Notably cautious cleaning and drying of seed has been reported as a solution to this problem in the USA (Elsholy, 2003).

2.5 THC action in the body

The known Routes of Administration (ROAs) are active/passive smoking of cannabis (Sharma et al., 2012), vapouration of natural cannabis, vapouration of cannabis concentrates and ingestion of cannabis edibles (Russell et al., 2018). Other known ROAs are oromucosal/sublingual routes (eg tinctures, lozenges, sprays), transdermal (eg cannabis infused oils and creams), intravenous and rectal (Russell et al., 2018). The absorption, metabolism and effects of THC depends on the routes of administration (ROAs). Smoking has been found to be the most predominant ROA among all users and a very effective way of getting THC **(22)** into the brain (Russell et al., 2018).

After smoking THC is quickly absorbed into the blood flow and up to about 50 % is distributed into the body's circulatory system. The effects of THC are perceived within seconds or minutes reaching a maximum at 30 min right after smoking (Cone & Huestis, 1993). Lasting effects running for 2-3 hours have been reported (Coucke et al., 2016). The effects of oral consumption run from 30 min to two hours and has been reported to be more permanent lasting for up to 8 hours or more with heavy intake. Although oral route intake achieves limited concentrations in blood, psychotic signs may still be identified (Favrat et al., 2005).

THC acts by binding to the CB-1 and CB-2 receptors (Kalant, 2013). The natural (-)-trans isomer of THC is 6 to 100-fold more potent than the (+)-trans isomer. CBD is non psychoactive and it is reported to inhibit the potent action of THC by the enzyme cytochrome P450 3A11 which is known to transform THC to a more potent 11-hydroxy-THC. (Bornheim et al., 1995). The highly lipophilic nature of THC enables its distribution in the adipose tissue, liver, lung and spleen (Sharma et al., 2012). Continuous use of cannabis leads to accumulation in the fatty tissues. (Cone & Huestis, 1993). THC is bound to erythrocyte, lipoproteins and albumin in the plasma and an absorption of about 0.1 μmol/L THC in the plasma is enough for psychotropic effects (Cone & Huestis, 1993; Naef et al., 2004).

The liver is the key metabolizing organ for the biotransformation of Δ^9-THC to the non-psychotropic 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) **(52)** from the key metabolite-11-Hydroxy-Δ^9-THC (11-OH-THC) **(51)** (Figure 2.8). THC-COOH is excreted in urine mainly as a glucuronic acid conjugate -11-Nor- Δ^9-THC- Δ^9-carboxylic acid-glucuronide **(53)** and it is the compound of interest for most diagnostic and toxicological analysis of cannabis (Sharma et al., 2012). The half-life removal rate of up to 1200 mL/min from the plasma is reported to be 1-4 days. It is known that the total removal of a single dose of THC may take about five weeks and even far longer for habitual users. (Grotenhermen, 2003; Brenneisen, 2012; Iversen, 2003).

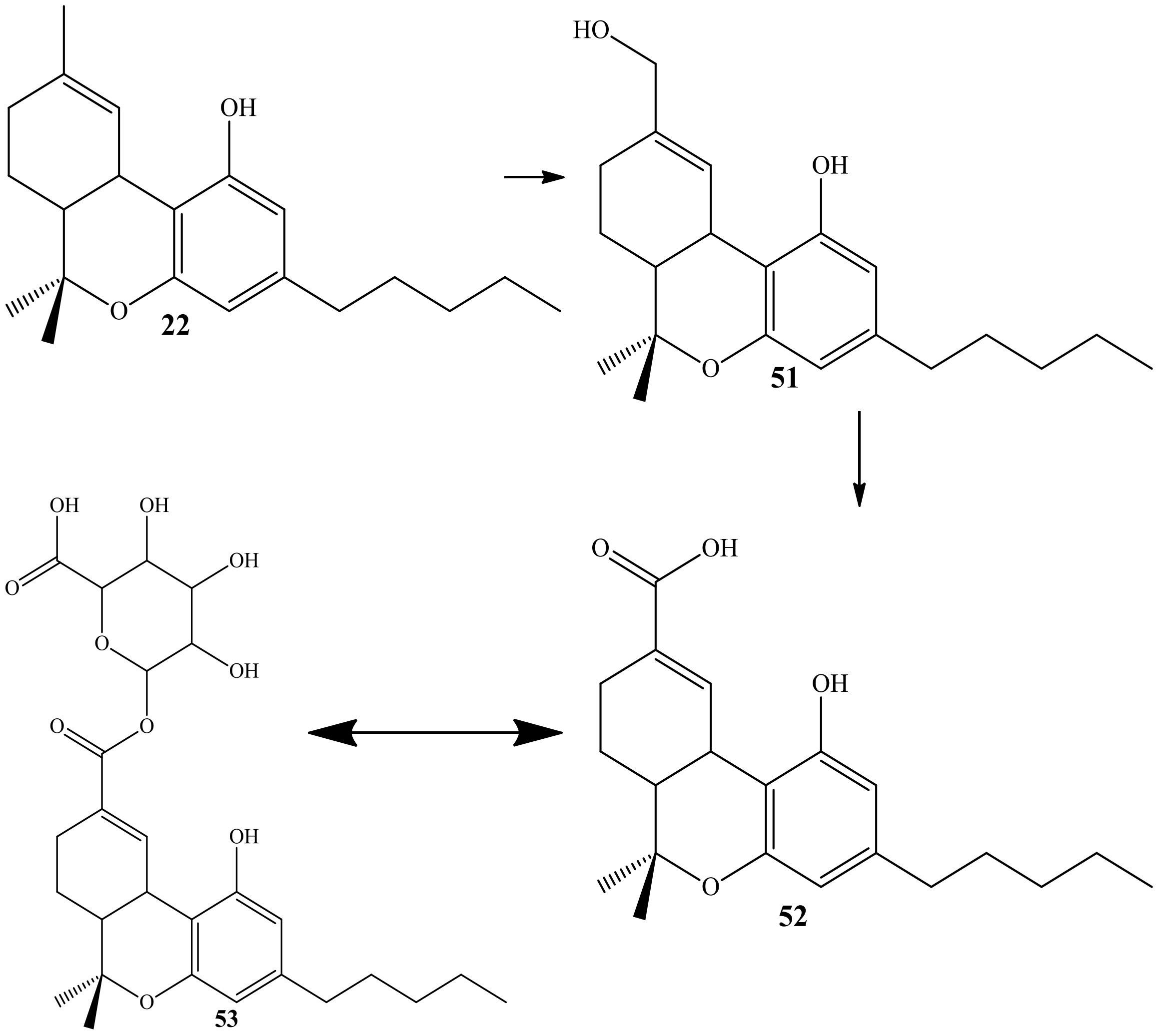


Figure 2.8: Metabolism of Δ9-tetrahydrocannabinol (Δ9-THC)

2.6. Analytical methods for the determination of Phytocannabinoids

Many methods are available for the detection, identification, classification and profiling of the plant and its products. For legal and forensic purposes, field testing techniques such as color tests form part of the preliminary identification of cannabis (United Nations Office on Drugs and Crime, 2009). However due to the non-specificity of such tests additional techniques are required to confirm the results (Goutam et al., 2014). Thin Layer Chromatography (TLC) is a typically simple but effective example of a confirmatory technique for the qualitative and semi-quantitative identification of the cannabinoids (Goutam et al., 2014).

More complex techniques such as Gas Chromatography (GC) with Flame Ionization Detector (FID), GC coupled with mass spectrometry (MS), High Performance Liquid Chromatography (HPLC) and associated tandem techniques, are all available for the quantitative and specific analysis of cannabis. GC-FID has been used in the determination of neutral and acidic cannabinoids of cannabis (Tipparat et al., 2012). However Liquid Chromatography (LC) based methods have been reported to be more efficient compared to GC analyses for the determination of acidic cannabinoids. It was explained that, the high temperatures associated with GC analyses normally results in the partial decarboxylation of these acidic constituents in cannabis, whereas LC methods are conducted at room temperature and cause no decomposition in the analytical sample making the latter more suitable for the evaluation of the total composition of cannabinoids in cannabis (Citti et al., 2016).

HPLC coupled with diode array and quadruple time of flight mass spectrometry method, was employed in the analysis of Cannabis Medicinal Extracts (CMEs) (Citti et al., 2016). Couke et al., (2016) used LC tandem MS in the determination of concentrations of delta-9-THC in exhaled breath. A GC-MS method was also used to analyze cannabis of Albania origin in the determination of the three major cannabinoids (Bruci et al., 2012).

Biologically, molecular techniques such as Polymerase Chain Reaction (PCR) marker for THCA synthase gene and genomic DNA (De-oxyribonucleic acid) marker using Random Amplified Polymorphic DNA (RAPD) have been used to differentiate drug type cannabis from fibre type cannabis (Tipparat et al., 2012).

2.6.1 Gas Chromatography/Mass Spectrometry

GC-MS detection is among the sophisticated established methods for the analysis of cannabis and its product(United Nations Office on Drugs and Crime, 2009; Turner et al., 1980). Derivatization is required (e.g., silylation or methylation) in the analysis of cannabinoid acids, the dominating cannabinoids in the plant, is required (Turner et al., 1980). MS using fragmentation patterns enables the identification of molecules based on their mass-to-charge ratio (Urban, 2016). GC-MS is known to be a very good method for creating chemical fingerprints - a tool for attributing cannabis to an identifiable parameter such as a geographical location or a condition of cultivation (indoor, outdoor) (Ross et al., 1997; Brenneisen R., 1988).

2.7 Legal statuses of cannabis in some countries

Documented benefits of cannabis have and continue to influence the legal status in many countries. For instance cannabis remains illegal according to the federal laws of the United States (US). However it is known that twenty six states in the US have passed laws which either decriminalize cannabis or allow for medicinal use or both (Svrakic et al., 2012). Medicinal cannabis is also legal in Argentina, Chile, Czech Republic, Israel, Italy among others (MacIver, 2017).

Recorded to be the first, Uruguay in December 2013 passed a bill to legalize a regulated market of cannabis. The rationale was that a regulated market would be a disincentive to organized crime groups and subsequently reduce rate of violence (Transform, 2017). The parliament of Canada on June 19, 2018 passed a bill into law to legalize and control the production, distribution and consumption of cannabis in the country.

Notwithstanding all these differential moves to legalize cannabis, many others are of the view that the negative effects associated with the use of the substance far outweigh the positive effects hence it should still remain an illegal entity. Vehemently backing this stand is the fact that there are well researched, acceptable and non - psychoactive alternatives to medicinal cannabis (Clark et al., 2011).

2.8 The use of cannabis in Ghana

Cannabis as mentioned earlier, in any form or use without lawful authority is illegal in Ghana (Narcotic Drugs Law, 1990). Records available at the Forensic Science Laboratory of the Ghana Police Service in Accra, show that cannabis is cultivated in all the regions of Ghana making up about ninety percent of narcotic exhibits submitted at the laboratory by police investigators for examination. The laboratory reported that, in the year 2017 about six thousand tons of cannabis were seized by police across the country involving about 700 male and 40 female suspects. The bulk of the seizures came from the Eastern, Volta and Ashanti regions.

Ghana ranks second in West Africa for the illicit cultivation and exportation of cannabis (UNODC, 2007). The call for a national debate on cannabis was strongly criticized by the then Chief Psychiatrist Dr. Akwasi Osei cautioning that the legalization of the substance would raise the rate of mental ailments and drug related crimes in the country (Jafaru M. Y., 2014). Touching on the prevalence rate of abuse, the newspaper article outlined that the Accra Psychiatric Hospital recorded 4,000 cannabis related out-patient cases in 2013. The health hazards associated with cannabis was also discussed by Appiah (2014) who called for an end to the debate to legalize cannabis.

Taking the discussion on a different approach, Hottor (2015) investigated factors that discouraged non-users of cannabis from the use of the substance. Incidentally the risk of health hazards was rated the topmost motivation for the non-use of cannabis among the sampled population.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Overview

The chapter described the research method, reagents and chemicals used, study area, sample collection, analysis, quality control/assurance and data analysis. The research was designed to determine the percentage content of the three major cannabinoids: delta-9-tetrahydrocannabinol (THC), cannabinol (CBN) and cannabidiol (CBD) in cannabis samples found in Ghana. This was done to determine the potencies of these cannabis samples and also enable classification into drug or fibre type cannabis with reference to the phenotype index. The analysis was carried out using Gas Chromatography coupled with Mass Spectrometry (GC-MS).

3.1 Reagents and Chemicals

Reagents and chemicals used for the analyses included: toluene, methanol, tetracosane, anhydrous sodium sulphate, fast blue BB salt (4-Amino-2,5-diethoxybenzanilide diazotated zinc double salt), ethyl acetate, sodium bicarbonate and petroleum ether.

3.1.1 Reference standards

Reference standards for the three (3) analytes; delta-9-tetrahydrocannabinol (THC), cannabinol (CBN) and cannabidiol (CBD) were obtained from the United Nations Office on Drugs and Crime (UNODC).

3.2 Study Area

Cannabis is cultivated in all the regions of Ghana for recreational use and export (UNODC, 2007). The country is situated on the west coast of Africa, covering a total area of 238 540 sq km with a north-south extent of about 670 km and a maximum east-west extent of about 560 km. It shares borders with Côte d’Ivoire to the west, Burkina Faso to the north and Togo to the east (ADB and ADF, 2012). To the south is the Gulf of Guinea. Ghana has a warm and humid climate with a mean annual rainfall estimated at 1,187 mm. Mean annual temperatures range from 26.1 °C near the coast to 28.9 °C in the extreme north. Annual potential open water evaporation has been estimated as ranging between 1,350 mm in the south to about 2,000 mm in the north (AQUASTAT, 2005). Previously ten (10), the country is currently divided into sixteen (16) administrative regions namely: Greater Accra, Eastern, Ashanti, Western, Central, Brong Ahafo, Volta, Northern, Upper East, Upper West, Oti, Bono East, Ahafo, Bono, North East and Savannah. Figure 3.1 shows the map of Ghana indicating the various towns where samples were taken for the research.

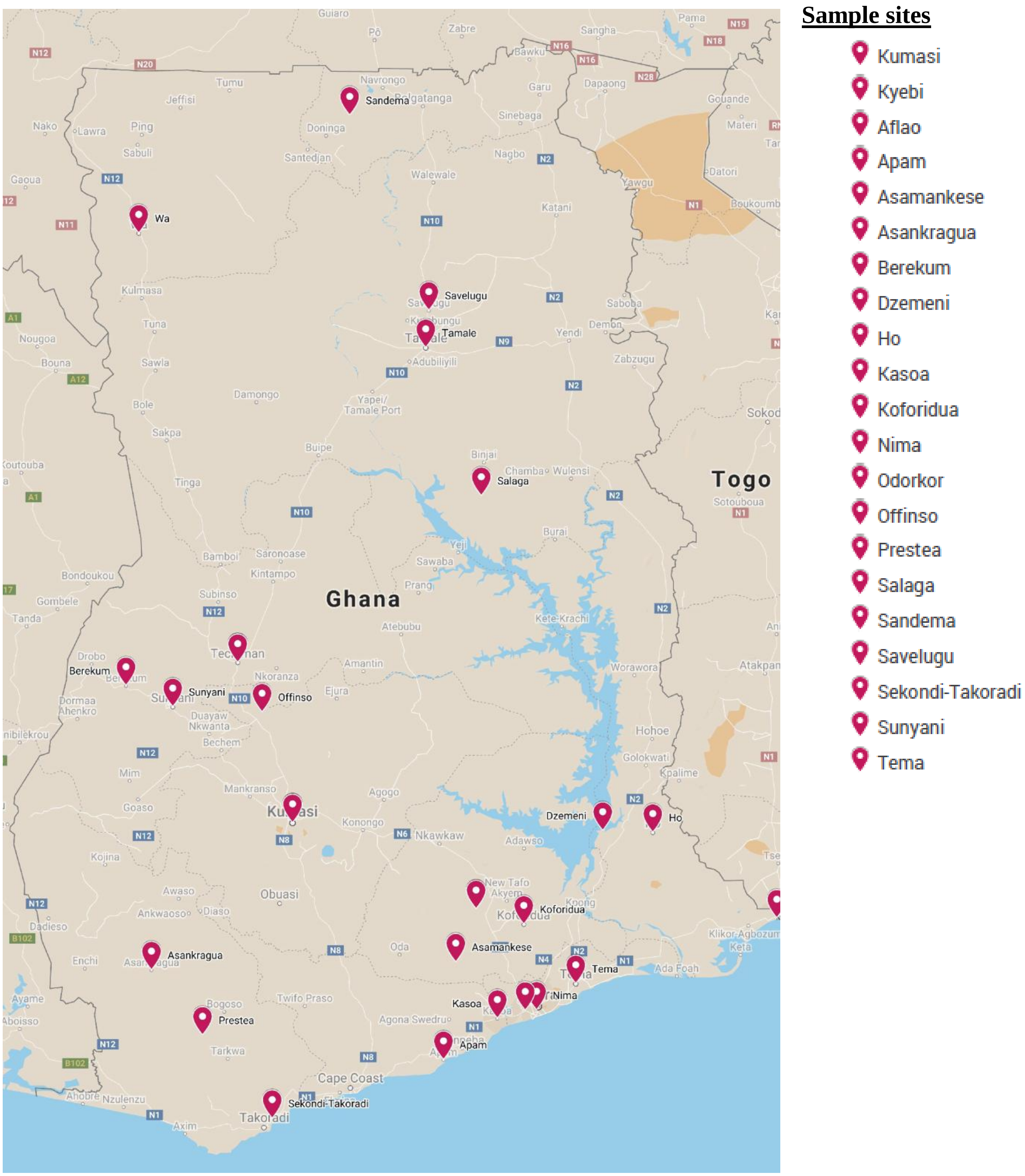


Figure 3.1 Map of Ghana indicating the various towns from which sampling was done

3.3 Sample Collection

Sampling was done in two phases.

3.3.1 Phase 1

In phase 1, thirty (30) cannabis samples were obtained from the Forensic Science Laboratory of the Ghana Police Service for analysis. These samples were plant materials seized by law enforcement officials across the regions of Ghana and submitted to the laboratory for examination. Figure 3.2 shows pictures of such exhibits for examination. The Laboratory stored these exhibit samples in sealed plain polythene bags in closed cabinets. Three samples were randomly picked from each region making a total of thirty samples for the analysis. This mode of selection was to ensure a good representation of the results with respect to the objectives of the research. The samples were given codes for easy identification as indicated in Table 3.1.

Table 3.1: Sample coding with corresponding area of seizure of cannabis.

Sample	Region of arrest	Sample code	Area of arrest
1	Greater Accra	GR 1	Nima
2	Greater Accra	GR 2	Odorkor
3	Greater Accra	GR 3	Tema
4	Ashanti	ASH 1	Kumasi
5	Ashanti	ASH 2	Offinso
6	Ashanti	ASH 3	Kumasi
7	Volta	VR 1	Aflao
8	Volta	VR 2	Aflao
9	Volta	VR 3	Ho
10	Eastern	ER 1	Kibi
11	Eastern	ER 2	Koforidua
12	Eastern	ER 3	Asamankese
13	Brong Ahafo	BA 1	Sunyani
14	Brong Ahafo	BA 2	Berekum
15	Brong Ahafo	BA 3	Techiman
16	Northern	NR 1	Salaga
17	Northern	NR 2	Savelugu
18	Northern	NR 3	Tamale
19	Central	CR 1	Apam
20	Central	CR 2	Kasoa
21	Central	CR 3	Kasoa
22	Western	WR 1	Asankrangua
23	Western	WR 2	Prestea
24	Western	WR 3	Sekondi
25	Upper West	UW 1	Wa
26	Upper West	UW 2	Wa
27	Upper West	UW 3	Wa
28	Upper East	UE 1	Sandema
29	Upper East	UE 2	Sandema
30	Upper East	UE 3	Sandema

3.3.2 Phase 2

Fresh cannabis samples were uprooted from illegal cannabis farms in the Eastern, Ashanti and Volta regions of the country. Figures 3.3a and b show pictures of uprooted cannabis samples from illegal farms in the Ashanti and Volta regions of Ghana respectively. The samples were sent to the laboratory within 24 hours of sampling for analysis. The purpose of analyzing these farm samples was to characterize cannabis grown specifically in the regions in terms of the levels of the three cannabinoids. Again the analysis was to establish if there was any significant variation in the cannabinoids content of the seized samples and the fresh samples. Farm samples were obtained in November 2017, March 2018 and May 2018 from Ashanti, Eastern and Volta regions respectively. The above acquisition times were dependent on the dates communicated by the local police upon identification of an illegal farm. The three regions were selected for this phase of the study because of their prevalent rate of high cannabis seizures by the police. Table 3.2 shows sample coding for phase 2 samples.

Table 3.2: Sample coding with corresponding area of cannabis farms.

Sample	Region of farm	Sample code	Area of farm
1	Ashanti	ASH	Kumasi
2	Eastern	ER	Boti
3	Volta	VR	Dzemeni

3.4 Analysis

3.4.1 Glassware

Glassware used for the experiments were soaked in Klin Kwick bleach for 24 hours, cleaned with brushes and subsequently rinsed with copious amounts of tap water. They were then rinsed with deionized water obtained from an Elga water deionizer in the laboratory. The washed glassware were dried in an oven at 60 ^{o}C for 24 hours.

3.4.2 Sample treatment

The laboratory samples were mostly dry when obtained. To remove any moisture and make them friable to grind, the samples were dried in an oven at a temperature of 40 ^{o}C for an hour. Very high temperatures may degrade THC in the samples. The samples were then ground to fine powder in porcelain mortars with pestles. Figures 3.3c and d show cannabis samples and ground cannabis samples with mortars and pestles. Uprooted fresh cannabis samples were dried in open air in a secured place in the laboratory for three days. The samples were further dried in an oven temperature of 40 ^{o}C for 1 hour to make them friable for easy grinding. The ground samples were stored in black polythene bags to avoid light - which may degrade the THC content - and then refrigerated at 8 ^{o}C until the period of analysis.

Figure 3.2: Cannabis exhibits submitted at the Forensic Science Laboratory.

a) Uprooted cannabis plant from Kumasi b) Uprooted cannabis sample from Dzemeni

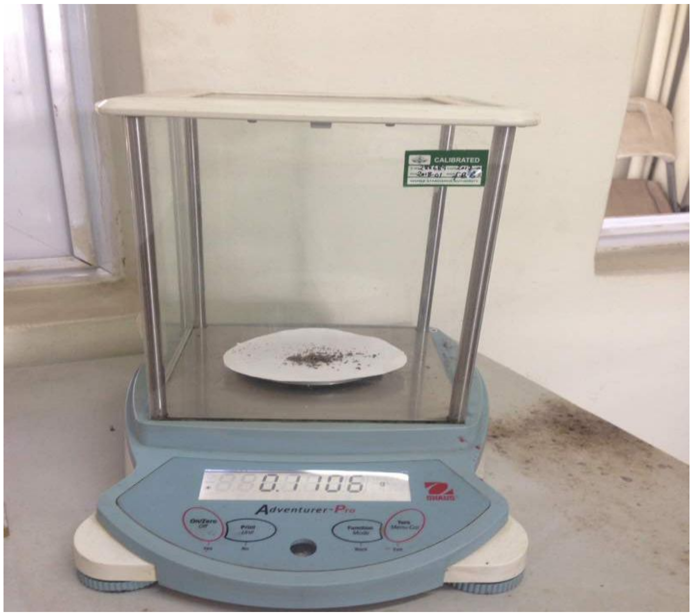
c) Cannabis samples and mortars and pestle d) Cannabis extracts

Figure 3.3: Pictures of fresh cannabis samples, dried samples and extracts.

a) Centrifuge

b) Agilent GC-MS

c) GFL Shaker



d) Weighing scale

Figure 3.4: Pictures of analytical instrument used for the study.

3.4.3 Extraction

The analytes THC, CBN and CBD were readily soluble in ether, methanol, benzene, chloroform, petroleum ether, toluene and ethyl acetate. Toluene was selected as the solvent for extraction and analysis because it is non-polar and compatible with the solubility of the three analytes. It is more stable than the other solvents in terms of volatility thereby ensuring minimum sample loss through evaporation. A mass of 2.00 g sodium sulphate was added to each 100 mL of toluene to remove moisture and then filtered using a Whatman filter paper.

The weight of each ground plant material taken for the analyses was 0.10 g. In the first extraction step, 10 mL toluene was added to each sample in a 15 mL Eppendorf centrifuge tube. The tubes were tightly covered and shaken for 2 hours in a GFL shaker and centrifuged at 5700 rpm for 30 sec in an Eppendorf 5430 centrifuge. The solvent was carefully decanted and the procedure was repeated for four different times with 10 mL toluene per sample. In total, each sample was extracted with 50 mL of the solvent. This was done to ensure that all the three analytes were fully extracted from the plant material. Color test was employed to ensure complete extraction of analytes during the extraction stages of experiments. The test is a well-established method of presumptive drug identification and commonly used by law enforcement officials, border security personnel and forensic laboratories. Two to three drops of the extract were placed in the center of a filter paper folded in quarters and partly opened to form a funnel. A small amount of 1 % Fast Blue BB salt diluted with anhydrous sodium sulphate was added to the center of the filter paper. About three drops of a 10 % w/w aqueous solution of sodium bicarbonate was added to the above. A purple red colored stain at the centre of the filter paper was indicative of a cannabis containing product which meant additional extraction steps were required. The color obtained is a combination of the colors of the different cannabinoids which were the major components of cannabis: THC = red, CBN = purple, CBD = orange (United Nations Office on Drugs, 2009). On the whole, 50 mL Toluene was enough for a full extraction of the cannabinoids from the samples.

The resulting supernatants of each sample were combined to obtain the full extracts of the cannabinoids and 10 mL of the solution was picked with a syringe and then micro-filtered into clean dry beaker with a VWR 0.45 micro-filter. Figures 3.4a, c and d show images of the centrifuge, shaker and weighing scale used in the analysis.

3.4.4 Quantification

Internal standard method was used in the quantification of the analytes. Internal standards were used in most chromatographic procedures to correct systematic errors including reproducibility of injection, instability of the detection system, pipetting errors, evaporation of extraction solvent and extraction deficiency (Whelpton, 2005).

Tetracosane was used as an internal standard in the analysis on the basis of its ability to be completely resolved from both known and unknown substances in a chromatogram; its ability to be eluted near the peak of interest; as well as its chemical stability and similarity to the substance of interest. 100.00 mg tetracosane was weighed into a 100 mL volumetric flask and toluene added to make the mark to obtain a 1.00 mg/mL internal standard stock solution. A working solution of 0.05 mg/mL was prepared by measuring 10 mL of the stock solution into 200 mL volumetric flask and diluting to the mark. 1.00 ml of 0.05 mg/mL internal standard solution was added to 1.00 mL of the filtered sample extract in a 5 mL beaker using a 1 000 µL Brand micro transfer pipette. A volume of 1.0 µL of this mixture was injected into the GC-MS for analysis.

3.4.5 Calibration curve

A four point calibration curve was plotted in the quantification of the analytes. The reference standards of CBN and CBD were solids. A stock solution of each standard was prepared by dissolving 5.00 mg in a beaker using toluene as solvent. The solution was transferred into a 25 mL volumetric flask and toluene added to the mark. The reference standard of THC was a 5 mL amber ampoule of 5 mg/mL methanol solution. The content was carefully transferred into 25 mL volumetric flask and toluene added to the mark.

The concentration of the stock solution for each analyte standard was 0.20 mg/mL. Working standard solutions of each standard of 0.1 mg/mL were prepared by serial dilution. Parameters used in the preparation of the standard solutions for the three analytes are summarized in Table 3.2. The working solution was serially diluted to obtain standard solution concentrations of 0.05 mg/mL, 0.025 mg/mL and 0.0125 mg/mL for each standard to generate a calibration curve. The calculations were done using equation 3.10 below.

$$C_1V_1 = C_2V_2 \tag{3.10}$$

where C_1 = initial concentration

V_1 = initial volume

C_2 = final concentration

V_2 = final volume

Table 3.3: Parameters used in the preparation of standard solutions

Solution	Concentration (mg/ml)	Preparation
Stock (SS)	0.20	5.00 mg in 25 mL Toluene
Working (WS)	0.10	25 mL SS in 50 mL Toluene
Diluted Solution 1 (DS1)	0.05	1.00 mL WS + 1.00 mL Toluene
Diluted Solution 2 (DS2)	0.025	1.00mL WS + 4.00 mL Toluene

Table 3.3 provides a summary of the parameters used in the preparation of the calibrants. In total three (3) calibration curves were plotted for the three standards.

Table 3.4: Parameters used in the preparation of calibrants using 0.05 mg/ml ISTD Solution

Calibrant ID	Concentration of Calibrant (mg/ml)	Preparation
STD 1	0.0125	1 mL DS 2 + 1 mL ISTD Solution
STD 2	0.0250	1 mL DS1 + 1 mL ISTD Solution
STD 3	0.0500	1 mL WS + 1 mL ISTD Solution
STD 4	0.1000	1 mL SS + 1 mL ISTD Solution

3.4.6 Instrumentation

Quantitative determination of the three analytes was carried out using Gas Chromatography coupled with Mass Spectrometry (GC-MS) (Fig. 3.4d). Gas chromatography is considered as a dynamic method of separation for gas (or vapour) mixtures based on the movement of a mobile phase through a stationary phase and on different distribution of mixture components between the mobile and stationary phases (Gras et al, 2018). Mass spectrometry on the other hand is an instrumental technique for the separation of electrically charged species in the gas phase according to their mass to charge ratio (Urban, 2016). The combination of these two analytical techniques in a hyphenated configuration allows for more accurate chemical identification, than either technique used separately (Gras et al., 2018). Also the analytical technique was employed in a similar research by Bruci et al., (2012).

Agilent 7890A Gas Chromatograph coupled with Agilent 5975C Mass Spectrometer using the Chemstation software was used for the analysis. A validated method used at the Forensic Laboratory was adopted for analysis to ensure that results obtained were valid and fit for purpose. The analytical column used was Agilent capillary column HP-5MS (30 m, 0.25 m, internal diameter 0.25 µm thickness) with a stationary phase of 5 % phenyl and 95 % dimethyl polysiloxane. The operating conditions were as follows; the oven temperature was programmed at 80 ^{0}C (hold for 1 min), followed by an increase to 150 ^{0}C at the rate of 25 ^{0}C/min and from 150 ^{0}C/min up to 280 ^{0}C at the rate of 15 ^{0}C/min and hold for 5 min. Split injection mode (50:1) was used. Helium was used as a carrier gas with a flow rate of 1.0 ml/min. The total run time was 17.47 min with the cannabinoids eluting at 12.73 min, 13.32 min and 13.79 min respectively for CBD, THC and CBN. The internal standard eluted at 12.44 min. The operation of the mass spectrometer was carried out in an electron ionization (EI) mode with the electron energy set at 70 eV. The mass spectra of the compounds were obtained in the Total Ion Chromatogram (TIC) mode in a scan range mass to charge ratio of 40 - 600.

3.5 Quality Control/Quality Assurance

The validated method employed in this experiment was verified to make certain that the results obtained were valid and fit for the study (UNODC, 2009). Prior to the analysis of the research samples verification parameters including, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision under repeatable and reproducible conditions; were determined in accordance with international standards (UNODC, 2009).

3.5.1 Limit of Detection and Limit of Quantification

The limit of detection (LOD) was determined by identifying the lowest concentration at which the compound can be detected without any doubt. (Ludwig Huber, 2010).

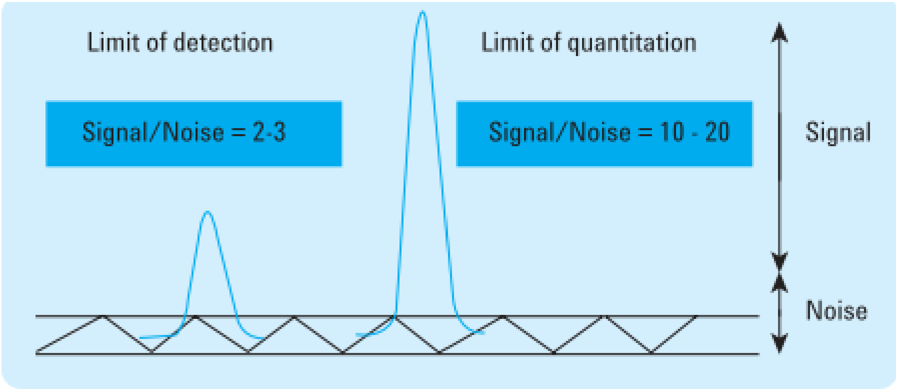


Figure 3.5: LOD and LOQ (Ludwig Huber, 2010)

LOD was calculated by multiplying the signal to noise ratio (S/N) by 3. The limit of quantification was 3 times the LOD. The height of the signal for the least concentration of analyte which offered no signal upon further dilution, was visually measured with a meter rule. The noise around this signal was also measured and the ratio of the signal to noise (S/N) was multiplied by 3 to produce the LOD, whiles the LOD multiplied by 3 produced the LOQ. Figure 3.5 shows an illustration of the LOD and LOQ.

3.5.2 Accuracy and Recovery

To determine accuracy, a sample of known concentration was spiked at three different concentration values of 0.01 mg/mL, 0.03 mg/mL and 0.075 mg/mL and analyzed in duplicates. The concentrations were chosen to represent the low, medium and high levels of the working range. A percentage expression of the difference between the average result and the expected result (bias) should be in the acceptable limit of less than ±15% (UNODC, 2009). Uncertainty associated with bias (U_{bias}) was calculated using equation 3.20 (JCGM, 2008)

$$(U_{bias}) = \sqrt{}(RMS_{bias}^2 + U_{Cref}^2) \qquad [3.20]$$

Where U_{Cref} ----- Uncertainty in the reference standard used in spiking

RMS_{bias} --- Root mean squared bias

The root mean squared (RMS) of the bias

$$RMS_{bias} = \sqrt{}\sum(bias)^2/n \qquad [3.30]$$

n is the number of spiked concentrations

Recovery was obtained by the ratio of the concentration obtained from the spiked samples and the known concentration of the standards.

3.5.3 Precision

Precision was determined under repeatable and reproducible conditions. Six different extracts of an Internal Reference Material (IRM) were analyzed in duplicates for the same day for repeatability and two consecutive days for reproducibility also known as intermediate precision. The relative pooled standard deviation (RSD) should be within the acceptable limit of less than ±15% in both cases (UNODC, 2009). Equations (3.4) and (3.5) were used in the calculation of the uncertainty in the measurement of repeatability and reproducibility (JCGM, 2008).

$$\text{Uncertainty} = \frac{\sqrt{\sum((Sd^2)*(n-1))}}{\sqrt{(n-1)}} \qquad [3.40]$$

$$\text{Relative uncertainty} = \frac{\text{Uncertainty}}{\text{Overall mean}} \times 100 \qquad [3.50]$$

where Sd = standard deviation from the mean.

n-1 = the degree of freedom (df)

n = the number of replicates

3.5.4 Uncertainty of measurement

The overall uncertainty of measurement which is the sum of individual components uncertainties, was calculated at the confidence level of 95 % using equation 3.6 (JCGM, 2008). The components considered were repeatability, reproducibility, accuracy (bias) and glassware (errors associated with glassware used).

$$U = \sqrt{(U_1^2 + U_2^2 + U_3^2 \ldots\ldots)} \qquad [3.60]$$

where U_1, U_2 etc are the individual component uncertainties (UNODC, 2009).

3.6 Data Analysis

The data obtained was analyzed using the Chemstation software of Agilent GC-MS and Microsoft Excel Software. The four-point calibration curves generated for each cannabinoid standard (THC, CBN and CBD) were used in the determination of the contents of the research samples. The concentration ratio of the standard of each analyte and the internal standard was plotted against the response ratio of the standard and the internal standard using

Microsoft Excel. The regression equation of each calibration curve is represented as Equation 3.70.

$$y = mx + c \qquad [3.70]$$

Where, y - ISTD area response ratio of analyte

m - slope

c - y- intercept

x - ISTD concentration ratio of analyte

The area response ratios of the research samples were then substituted into the regression equation for each standard calibration curve to solve for the concentration of that analyte in the sample. Thus the concentration of a cannabinoid of an analyzed sample is equal to the solved x which was ISTD concentration ratio of the analyte in the sample, multiplied by the concentration of the ISTD as shown in equation 3.80. The weight of the cannabinoid is therefore the concentration multiplied by the volume of solvent used in the preparation of the sample solution as shown in equation 3.90.

$$\text{Conc of cannabinoid (mg/ml)} = \text{solved } x * \text{Conc of ISTD (mg/ml)} \qquad [3.80]$$

$$\text{Weight of cannabinoid} = \text{Conc of cannabinoid (mg/ml)} \times \text{Volume of solvent (ml)} \qquad [3.90]$$

The percentage of each cannabinoid in a sample was calculated from equation 3.91:

$$\text{Content (\%)} = \frac{\text{Weight of cannabinoid calculated (mg)}}{\text{Crude weight of sample (mg)}} \times 100 \qquad [3.91]$$

Where the crude weight is the weight of the cannabis sample taken for analysis. Microsoft excel software was used in the creation of tables of values and calculations for comparative analysis.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Overview

The chapter discussed the results of the analytical work as detailed out in chapter three.

4.1 Correlation

Table 4.1 shows the mass-to-charge ratios of the principal ions of the cannabinoids. The chromatographs and mass spectra of the three cannabinoids are shown in appendix A.

Table 4.1 Mass-to-charge ratios of principal ions of cannabinoids

Cannabinoids	m/z ratios of principal ions					
THC	299	231	314	271	243	258
CBN	295	296	238	310	251	223
CBD	231	207	91	232	246	121

Tables 4.2, 4.3 and 4.4 show the calibration tables for THC, CBN and CBD respectively. A four point linear calibration curve was obtained for each standard using the Agilent chemstation software and Microsoft excel spreadsheet for analysis. The calibration curves for THC, CBN and CBD are shown in Figures 4.1, 4.2 and 4.3 respectively.

Table 4.2: The Calibration Table of THC standards analyzed

THC Conc (mg/ml)	ISTD Conc (mg/ml)	Conc Ratio	THC Average Area Response	ISTD Average Area Response	Response ratio
0.0125	0.0250	0.50	756506.00	2229680.00	0.34
0.0250	0.0250	1.00	1908478.50	2007227.00	0.95
0.0500	0.0250	2.00	5071850.00	2086107.50	2.43
0.1000	0.0250	4.00	11946467.50	2165087.50	5.52

Table 4.3: The Calibration Table of CBN standards analyzed

CBN Conc (mg/ml)	ISTD Conc (mg/ml)	Conc ratio	CBN Average Area response	ISTD Average Area Response	Response ratio
0.0125	0.0250	0.50	1388543.01	2199680.00	0.63
0.0250	0.0250	1.00	3504367.43	2007227.00	1.75
0.0500	0.0250	2.00	9251149.13	2086107.50	4.43
0.1000	0.0250	4.00	20428170.89	2265087.50	9.02

Table 4.4: The Calibration Table CBD standards analyzed

CBD Conc (mg/ml)	ISTD Conc (mg/ml)	CONC Ratio	CBD Average Area Response	ISTD Area response	Response ratio
0.0125	0.0250	0.50	815283.00	1418570.00	0.57
0.0250	0.0250	1.00	2361052.00	1325249.00	1.78
0.0500	0.0250	2.00	6590409.00	1520287.00	4.33
0.1000	0.0250	4.00	16742908.00	1649574.00	10.18

The calibration curves plotted for the three cannabinoids were linear with concentration points at 0.0125, 0.0250, 0.0500 and 0.1000 mg/mL. The regression equations for THC, CBN and CBD were $y=1.493x-0.4895$, $y=2.4102x - 0.5616$ and $y=2.7494x-0.9448$ with regression coefficient of 0.9991, 0.9989 and 0.9986 respectively. Linearity was therefore achieved for the three compounds as the regression coefficient of each was greater than 0.99 (UNODC, 2009).

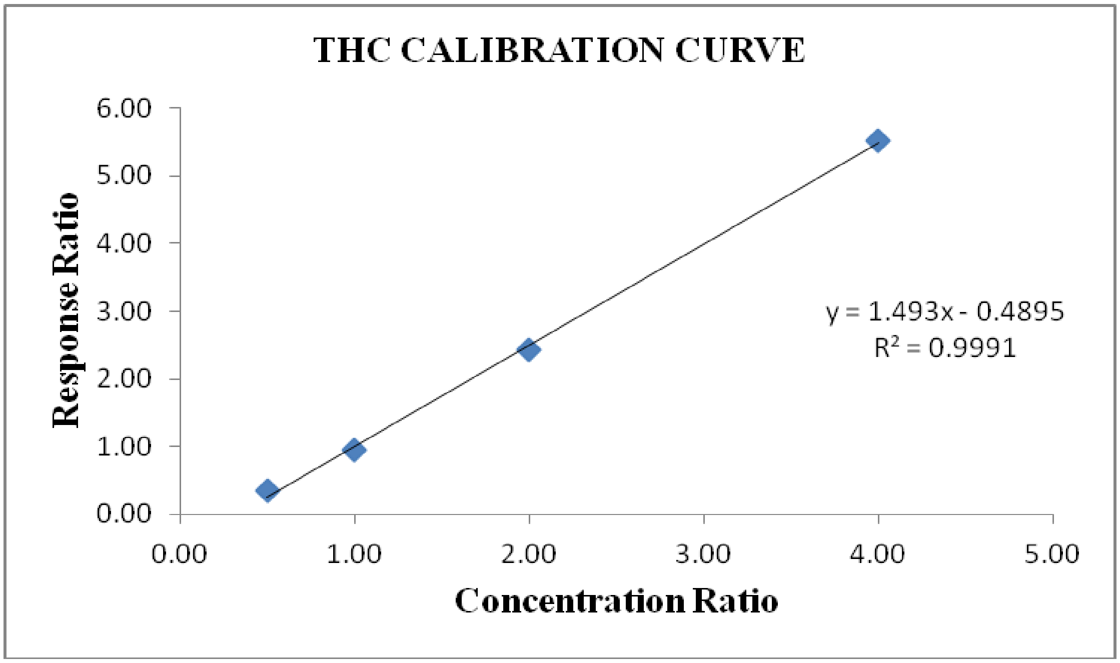


Figure 4.1: THC Calibration Curve plotted for four calibration points

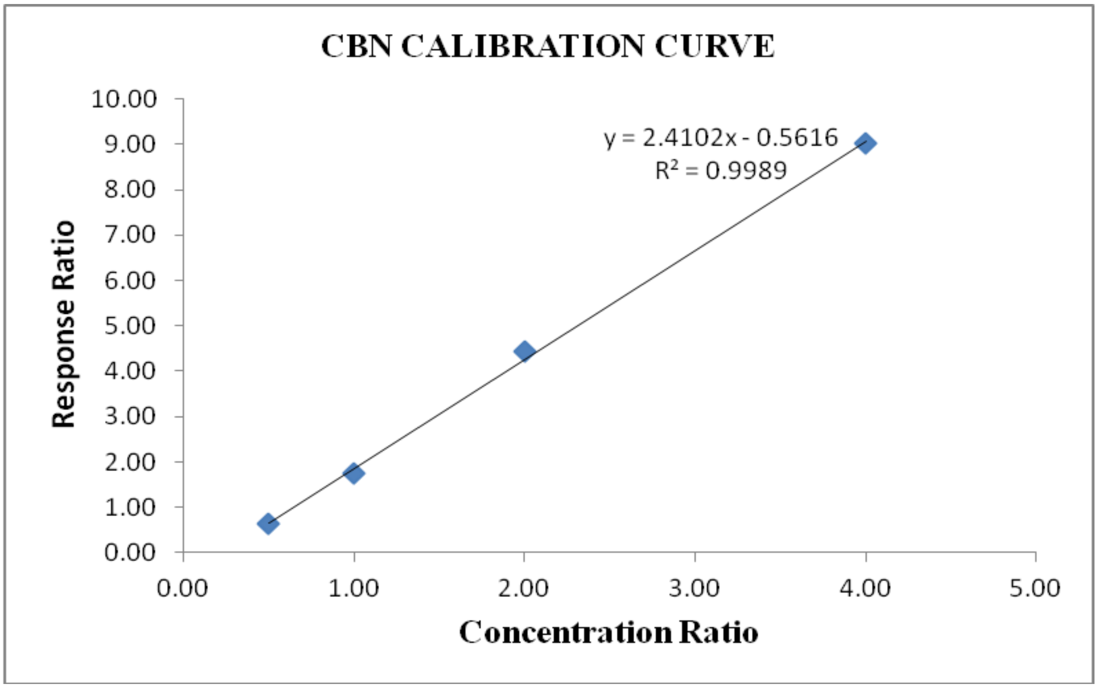


Figure 4.2: CBN Calibration Curve plotted for four calibration points.

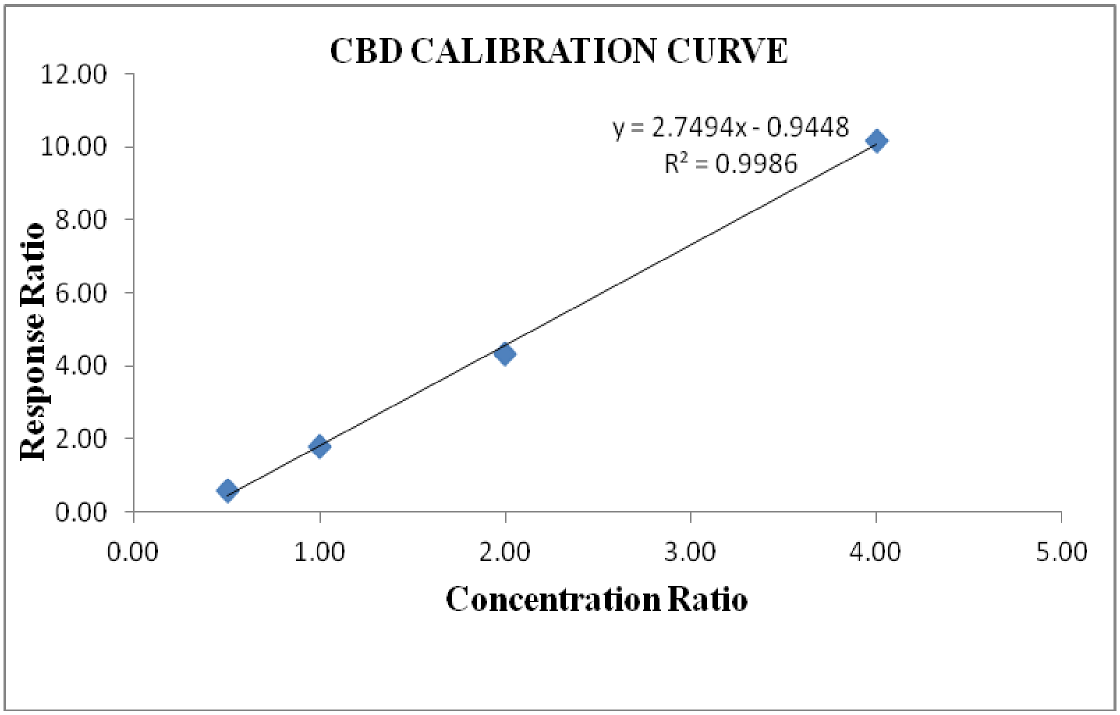


Figure 4.3 CBD Calibration Curve plotted for four calibration points.

The percentage content of THC, CBN and CBD in the various samples were calculated using the equation 3.91.

$$\text{Content (\%)} = \frac{\text{Weight of cannabinoid calculated (mg)}}{\text{Crude weight of sample (mg)}} \times 100 \qquad [3.91]$$

4.2 Method verification

The GC-MS method of analysis obtained from the Forensic Science Laboratory of the Ghana Police Service was verified to ensure that the results of the research were valid and fit for purpose (UNODC, 2009). Parameters determined were limits of detection and quantification, accuracy, recovery and precision under repeatable and reproducible conditions. Results and calculations pertaining to these parameters are shown in Appendix C and D.

4.2.1 Limit of detection and limit of quantification

The limit of detection (LOD) which is 3 times the signal to noise ratio (S/N) was found to be 0.0018 mg/ml, 0.0010 mg/ml and 0.0015 mg/ml respectively for THC, CBN and CBD. The limit of quantification which is 3 times the LOD was found to be 0.0054 mg/ml 0.0030 mg/ml and 0.0045 mg/ml for THC, CBN and CBD respectively.

4.2.2 Accuracy and Recovery

The accuracy of all the analytes in each of the three spiked-concentrations was between 1.82 % and 4.41 % within the acceptable limit of less than 15 % (UNODC, 2009). The recovery for each of the three analytes was between 0.95 and 1.02 which is within the acceptable limit of $0.95 \leq R \leq 1.05$ (Huber L, 2010).

4.2.3 Precision

The relative pooled standard deviation of each analyte for both repeatability and reproducibility was found to be less than 5 % which is within the acceptable limit of less than 15 % (UNODC, 2009).

4.2.4 Measurement Uncertainty

The measurement uncertainty with respect to the glassware used, repeatability, reproducibility and accuracy (bias) was calculated for the three analytes (UNODC, 2009) .

Table 4.5: Calculation of Measurement Uncertainty

Uncertainty	THC	CBN	CBD
Repeatability (%)	1.8067	0.4591	0.5527
Reproducibility (%)	1.8138	0.4195	0.5326
Bias (%)	2.79	3.73	3.37
Certified Reference Material (%)	0.40	0.10	0.10
10 ml Measuring cylinder	1.1547	1.1547	1.1547
100 ml Measuring cylinder	0.5774	0.5774	0.5774
250 ml Measuring cylinder	0.4619	0.4619	0.4619
Combined Relative Uncertainty	4.03	4.02	3.72
Expanded Uncertainty(k=2)	8.06	8.04	7.44

The expanded uncertainty in the measurement at 95 % confidence level (K=2) of THC, CBN and CBD were found to be 8.06 %, 8.04 % and 7.44 % respectively as shown on Table 4.5. The uncertainty associated with a measured content is therefore the combined uncertainty multiplied by the percent content (JCGM, 2008).

4.3 The Cannabinoids concentrations of samples analyzed in phase 1

The results obtained from the analyses of thirty cannabis samples in the determination of the content of the three major cannabinoids; THC, CBD and CBN are shown in Table 4.6 with a graphical representation in Figure 4.4. Generally the contents varied greatly with no particular trend showing, even for samples analyzed from same region. Variations in the cannabinoids content in cannabis have been attributed to genetic factors, environmental conditions of cultivation, time of harvest, parts of the plant harvested and other factors such as drying and storage conditions (Bruci et al., 2012). However information on the above mentioned parameters of the samples analyzed is unknown leaving very little room for deductions with respect to the attribution of the variations in the cannabinoids content to a particular cultivation method or environmental condition. The reason is that, the samples analyzed in this phase of the study were obtained from exhibits submitted by Police Officers to the Forensic Science Laboratory for examination and the only background information known is the area of arrest. More so this may have accounted for the vast variations in the cannabinoids content of samples of the same region since they may have been transported from other regions or even countries into a particular region.

4.3.1 THC Content of Samples Analyzed in phase 1

The potency of cannabis is determined by the content of THC which is known to vary between and within countries (EMCDDA, 2010). Evidently the THC content of the samples analyzed in this study which were obtained from different locations of the country varied considerably**.** The highest THC content of 15.14 % was found in sample UW 3 of the Upper West region with corresponding CBN and CBD content of 0.93 % and 0.86 % respectively. The lowest THC content of 0.93 % was observed for sample VR 3 with corresponding CBN and CBD content of 0.60 % and 0.88 %. Figures 4.5 and 4.6 show graphical representation of the maximum and minimum THC per each region respectively.

Table 4.6: Percentage content of three cannabinoids and classification indices of samples analyzed in phase 1

Sample	Sample code	%THC	% CBN	%CBD	(% THC+ % CBN) / % CBD	Phenotype	Chemotype
1	ER 1	1.96±0.16	2.48±0.20	0.93±0.07	4.76	I	I
2	ER 2	3.54±0.29	0.86±0.07	1.19±0.09	3.68	I	I
3	ER 3	1.81±0.15	0.69±0.06	0.90±0.07	2.79	I	I
4	CR 1	2.72±0.22	0.98±0.08	0.90±0.07	4.13	I	I
5	CR 2	1.10±0.09	1.34±0.11	0.90±0.07	2.71	I	I
6	CR 3	2.01±0.16	0.69±0.06	0.89±0.07	3.03	I	I
7	BAR 1	0.98±0.08	1.37±0.11	0.99±0.07	2.37	I	I
8	BAR 2	2.09±0.17	1.34±0.11	1.19±0.09	2.89	I	I
9	BAR 3	8.32±0.67	0.81±0.07	0.89±0.07	10.31	I	I
10	ASH 1	1.37±0.11	1.07±0.09	1.02±0.08	2.40	I	I
11	ASH 2	3.21±0.26	0.71±0.06	0.97±0.07	4.05	I	I
12	ASH 3	3.82±0.31	0.74±0.06	0.98±0.07	4.63	I	I
13	WR 1	1.30±0.10	1.09±0.09	0.93±0.07	2.58	I	I
14	WR 2	7.07±0.57	1.47±0.12	0.98±0.07	8.72	I	I
15	WR 3	1.91±0.15	0.67±0.05	0.91±0.07	2.84	I	I
16	VR 1	1.43±0.12	2.48±0.20	0.93±0.07	4.21	I	I
17	VR 2	1.57±0.13	2.13±0.17	0.97±0.07	3.83	I	I
18	VR 3	0.93±0.07	0.83±0.07	0.88±0.07	1.99	I	II
19	UER 1	7.28±0.59	1.48±0.12	0.89±0.07	9.83	I	I
20	UER 2	2.67±0.22	1.28±0.10	0.89±0.07	4.45	I	I
21	UER 3	1.40±0.11	1.35±0.11	0.90±0.07	3.07	I	I
22	UWR 1	11.10±0.89	1.03±0.08	1.09±0.08	11.09	I	I
23	UWR 2	6.91±0.56	0.73±0.06	0.97±0.07	7.84	I	I
24	UWR 3	15.14±1.22	0.93±0.07	0.89±0.07	18.07	I	I
25	NR 1	2.01±0.16	1.65±0.13	0.90±0.07	4.06	I	I
26	NR 2	3.01±0.24	1.17±0.09	0.95±0.07	4.39	I	I
27	NR 3	11.06±0.89	1.14±0.09	1.05±0.08	11.62	I	I
28	GR 1	1.59±0.13	1.61±0.13	0.94±0.07	3.38	I	I
29	GR 2	1.86±0.15	1.63±0.13	1.16±0.09	3.01	I	I
30	GR 3	1.77±0.14	3.23±0.26	0.95±0.07	5.28	I	I

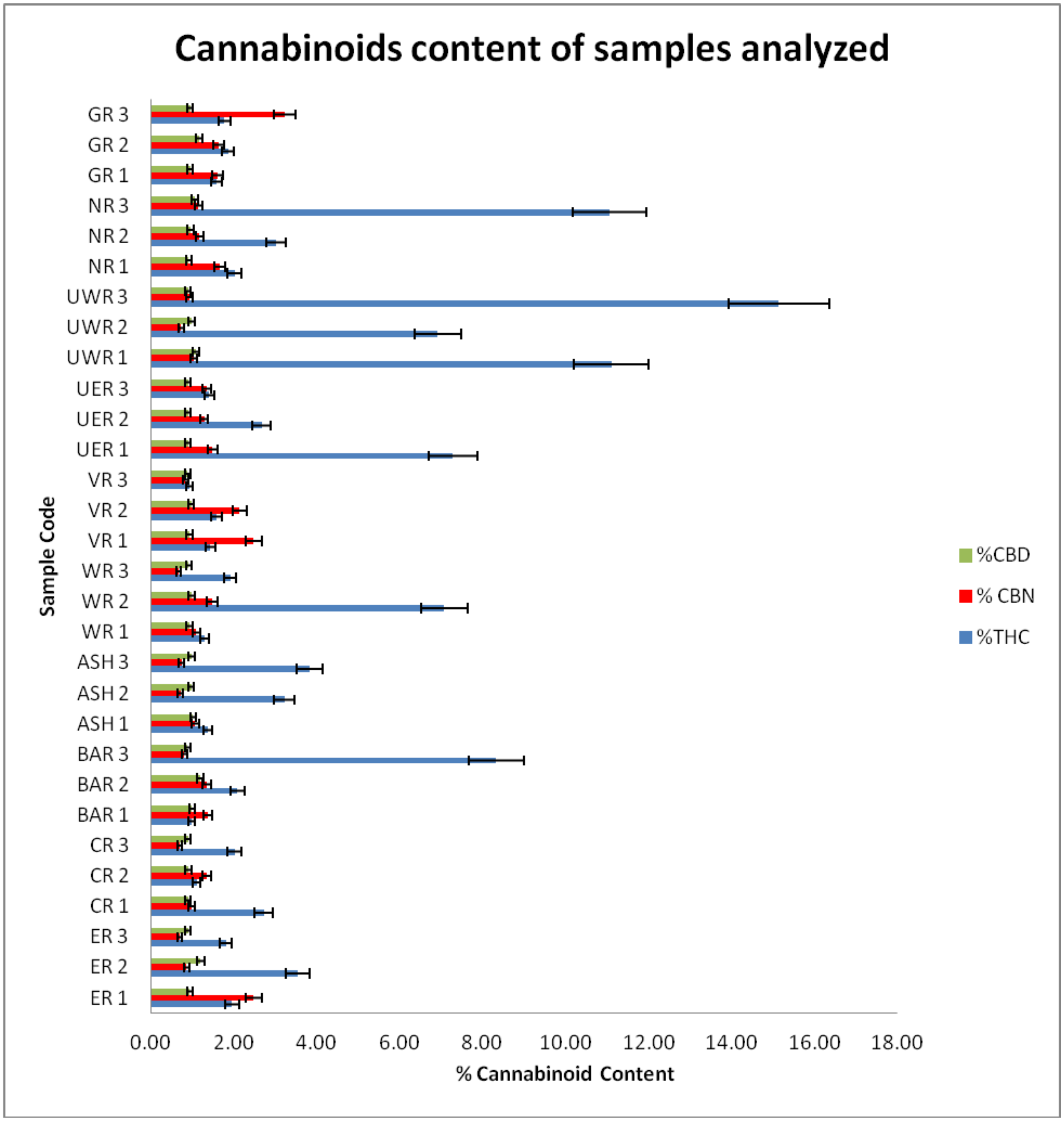


Figure 4.4: Cannabinoids content of the thirty samples analyzed in phase 1 of the experiment

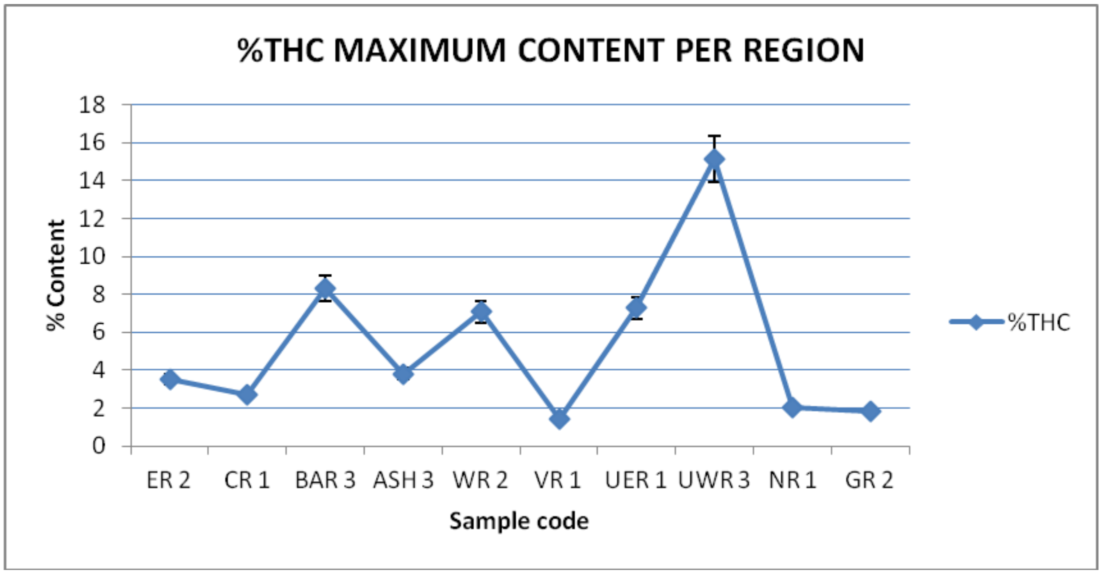


Fig 4.5: Maximum %THC content per Region of the samples analyzed for the study.

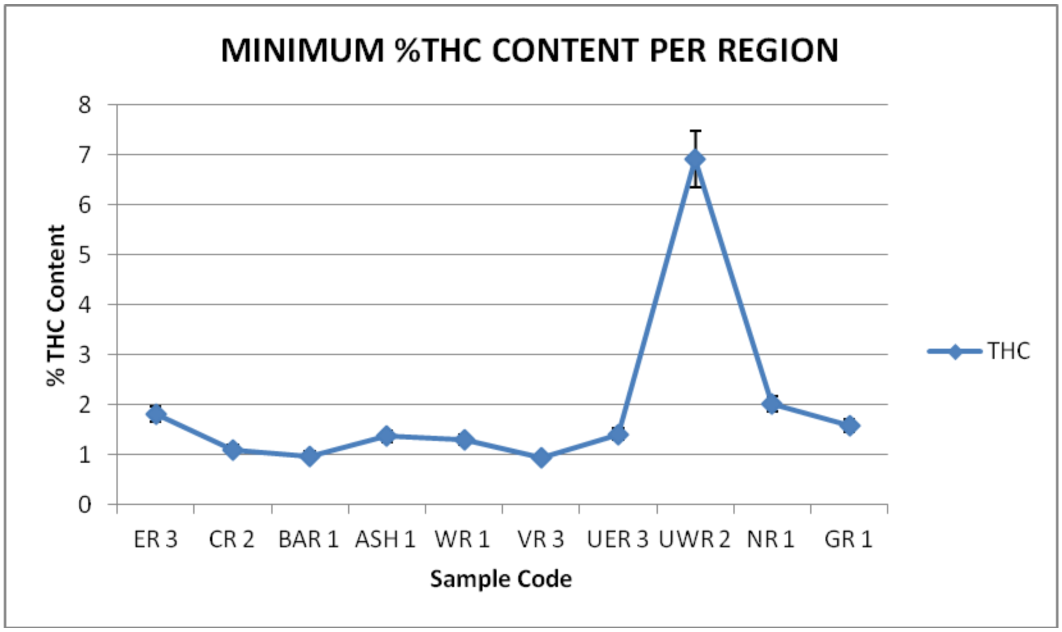


Fig 4.6: Minimum % THC content per Region of the samples analyzed for the study.

The mean THC content was 3.76 % with a standard deviation of 3.60 and median THC concentration was found to be 2.01 %. A graphical representation of the mean THC content per region is shown in Figure 4.7 with a summary of the statistical parameters in Table 4.7.

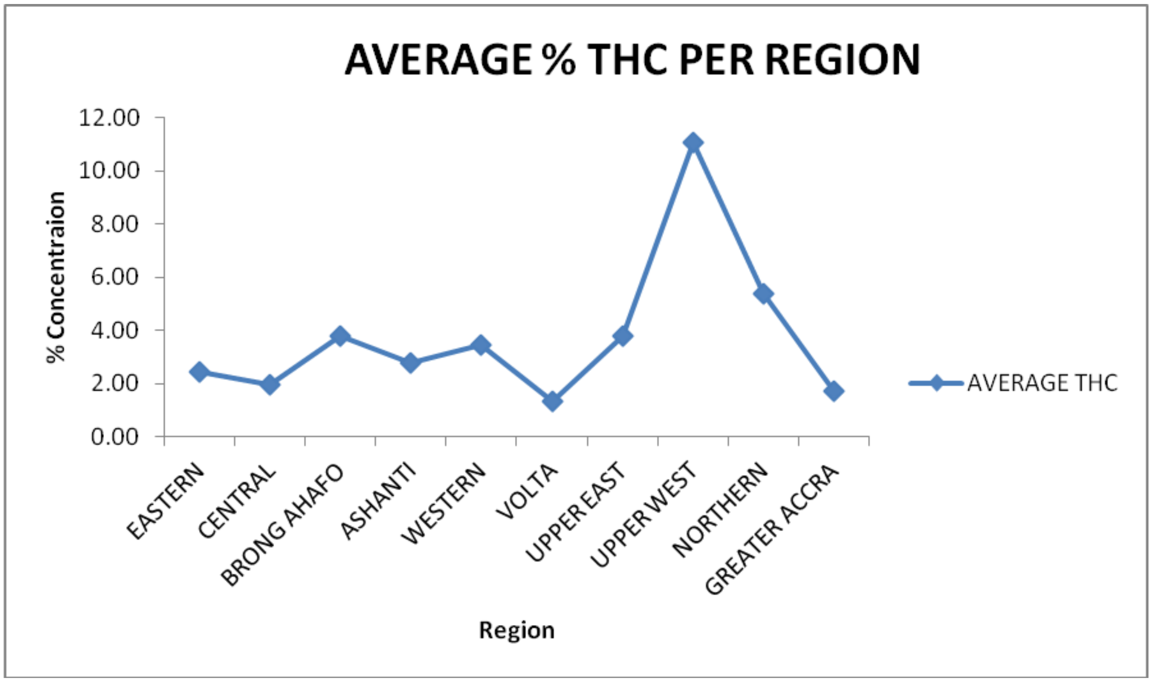


Fig 4.7:%THC Mean per regional samples.

Table 4.7: Sample Statistics

Parameter	**% THC**	**% CBN**	**% CBD**	**Phenotype Index**
MAX	15.14	3.23	1.19	18.07
MIN	0.93	0.67	0.88	1.99
MEAN	3.76	1.30	0.96	5.27
MEDIAN	2.01	1.15	0.94	4.05
STDEV	3.60	0.61	0.09	3.68

A THC content of 0.5 % and above is enough to qualify a cannabis sample as drug type (Gambaro et al., 2002) and even stricter for European countries as the European Union maximum THC content for cannabis grown for fibre is 0.2 % (EMCDDA, 2017). There is therefore a clear indication that the samples analyzed were highly potent as the minimum THC was found to be 0.93 % which is almost two times the limit. The potency of the samples may be due to high THC seed quality, fertile soil and warm climatic conditions (Tefanidou et. al., 2000)

It can be said that the data obtained in this study is a good representation of the national drug market as the Forensic laboratory receives both street level and wholesale seizures (Dujourdy & Besacier, 2017). In a similar study, herbal cannabis samples confiscated by twenty three Police Forces in England reported a mean THC content of 8.4 % in the range 0.3-22 % (Hardwick, 2008) higher than results of this study which was in the range of 0.93 % - 15.14 %. The THC content range of the samples analyzed in this study was also found to be relatively lower than analyzed samples of unknown origin from New South Wales, Australia which had a mean THC content of 14.88 % in the range 0.94 - 39.76 % (Swift et al., 2013).

On the other hand, the THC content range of 0.93 % - 15.14 % of this study was found to be higher than the typical THC range of 0.5 % - 5 % in herbal cannabis reported by the United Nations Office on Drugs and Crime (United Nations Office on Drugs and Crime, 2014). The obtained range was again found to be higher than Greek herbal cannabis of THC range 0.08 - 4.41 % (Tefanidou et al., 2000). Egypt in Africa also reported a relatively higher minimum THC content of 9.55 % (Souleman et al., 2017).

Notwithstanding reported concentrations, no jurisdiction can claim a lead or otherwise in the potency of cannabis as increasing trends in the psychoactive component of the herbal material have been identified over the years (ElSohly et al., 2016). An increase from 5 % to 13 % average THC from 1995 to mid 2016 was reported for herbal cannabis in a study in France (Dujourdy & Besacier, 2017). Examination of the concentration of cannabinoids in illicit cannabis products seized by the United States Drug Enforcement Administration (DEA) from 1995 to 2014 showed an increase in the THC potency from 4 % to 12 % (Elsholy et al., 2016). Also an increase of 0.1 % to 19 % for herbal cannabis in Italy was observed in a study over the period 2010-2012 (Zamengo et al., 2014). Refined methods of cultivation have been mentioned as one of the reasons accountable for the increase in the potency of herbal cannabis with a typical example being the production of unpollinated female cannabis known as sinsemilla which is reported to have very high THC levels (ElSohly et al., 2016). Cultivation of high THC cultivars has also been attributed to the increase in cannabis potency (Small & Marcus, 2016).

THC was found to be higher in samples cultivated in localities with higher temperatures (Bruci et al., 2012). The North of Ghana records very high temperatures compared to the South (AQUASTAT, 2005) and it was observed in this study that the samples from the northern sector of the country were relatively higher in THC levels than those from the southern sector as shown in Figure 4.8. The highest THC was recorded in UWR 3 and if these samples were grown in the North of Ghana then the results show a clear agreement with Bruci et al., (2012).

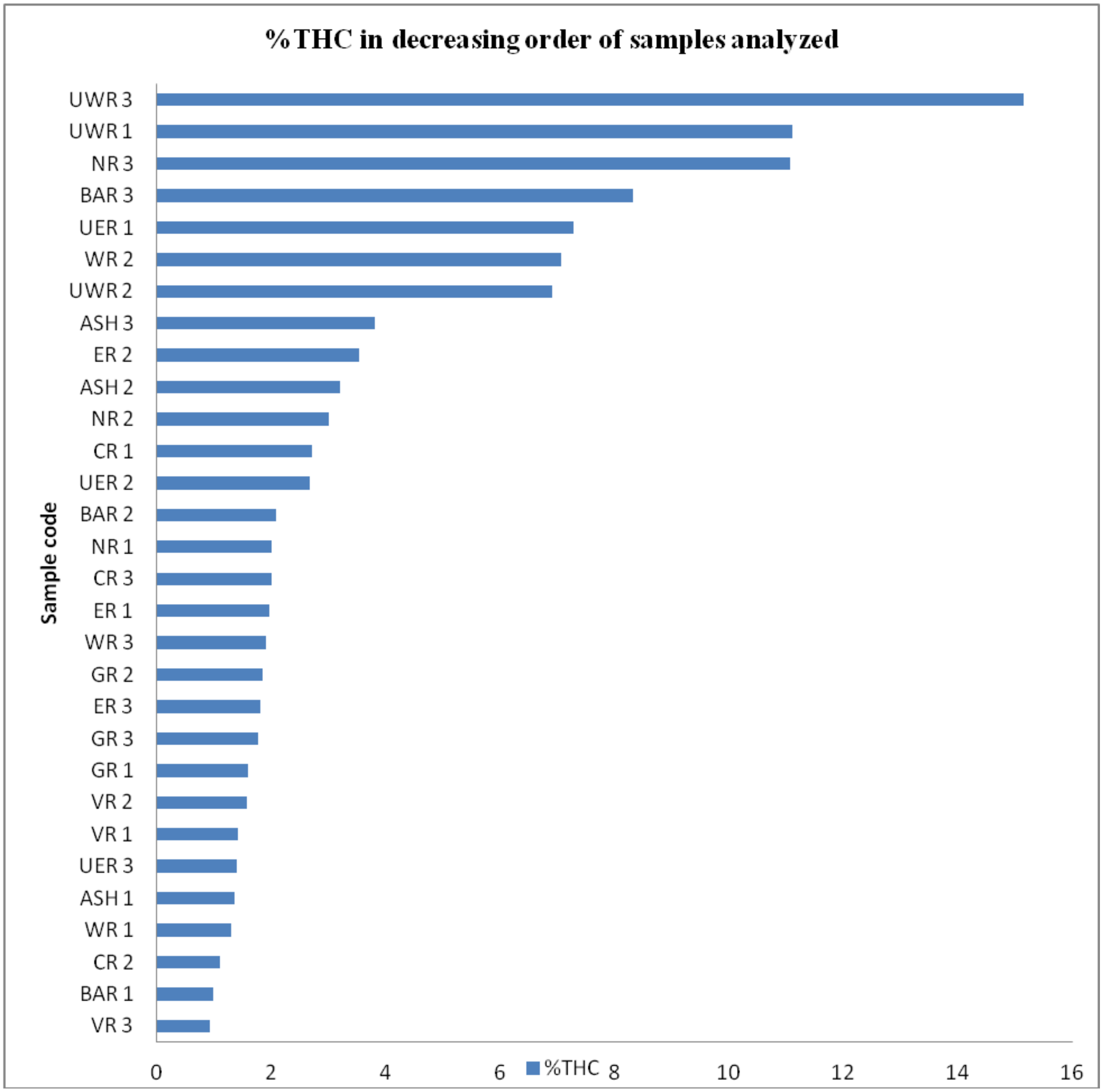


Fig 4.8: %THC in decreasing order of samples analyzed.

4.3.2 CBD Content of samples analyzed

Contrary to THC, CBD is known to have anti-psychotic effect via the inhibition of anandamide (N-arachidonoylethanolamine) - the neurotransmitter produced in the brain that binds to the THC receptors (Dujourdy & Besacier, 2017). Hence the total psychoactive capacity of cannabis is affected by variation in the content of THC and CBD (Zamengo et al., 2014).

Medicinally, CBD has also been reported as an emerging therapeutic agent in numerous pathological conditions such as Alzheimezer's and Parkinson's diseases, epilepsy and cancer (Pisanti et al., 2017). `

Unlike THC and CBN, the CBD content of the samples analyzed were very close to each other with a standard deviation of 0.09 and found in the range of 0.88 % -1.19 %. as shown in Table 4.7. The highest CBD content was found in sample ER 2 and the lowest was found in samples NR 1, VR 3 and CR 3. Compared to other countries, the mean CBD content of 0.96 % of this study was found to be lower than a mean CBD of 4 % reported in a French study (Dujourdy & Besacier, 2017) but higher than a mean CBD content of 0.14 % as reported by Swift et al., (2013) though with a higher range of 0 to 6.5 %.

Generally the reported contents of CBD have not been discussed as much as the THC/CBD ratios of cannabis samples in most literature. This can be attributed to the fact that the ratio of THC to CBD content is a very important index in the classification of cannabis. Thus, THC/CBD ratios are used to differentiate between the two main classes of cannabis which is drug type and fibre type cannabis. Two prominent researches by Fetterman et al. (1971) and Small and Beckstead 1973, are most cited in the classification of cannabis and herein discussed.

4.3.3 CBN content of samples analyzed in phase 1

The maximum CBN content was 3.23 % found in sample GR 3 with corresponding THC and CBD concentration of 1.77 and 0.95 % respectively. The minimum CBN content on the other hand was 0.67 % found in sample WR 3 with corresponding THC and CBD content of 1.91 % and 0.91 % respectively. The mean CBN content was found to be 1.30 %. Just like in the case of CBD, much of the discussion on the CBN has been done relative to the content of THC. CBN is the degradation product of THC, therefore the presence of CBN in cannabis is an indication that the herbal material is not fresh (Dujourdy & Besacier, 2017). Storage conditions such as humidity and temperature play an active role in the degradation process.

A possible pathway shown in the reaction scheme in Figure 4.9; for the decomposition of THC**(I)** to CBN**(II)** was proposed in 1979 (Turner & Elsohly, 1979). The research finding was supported by the isolation of intermediates which included 9,10-dihydroxy-$\Delta^{6a(10a)}$-THC**(VII)** (racemic mixture) and 8,9-dihydroxy-$\Delta^{6a(10a)}$-THC **(VIII)** (racemic mixture). Evidently, the intermediates could be detected only by gas chromatography as their trimethylsilyl (TMS) derivatives whereas direct injection into the GC resulted in CBN (Turner & Elsohly, 1979).

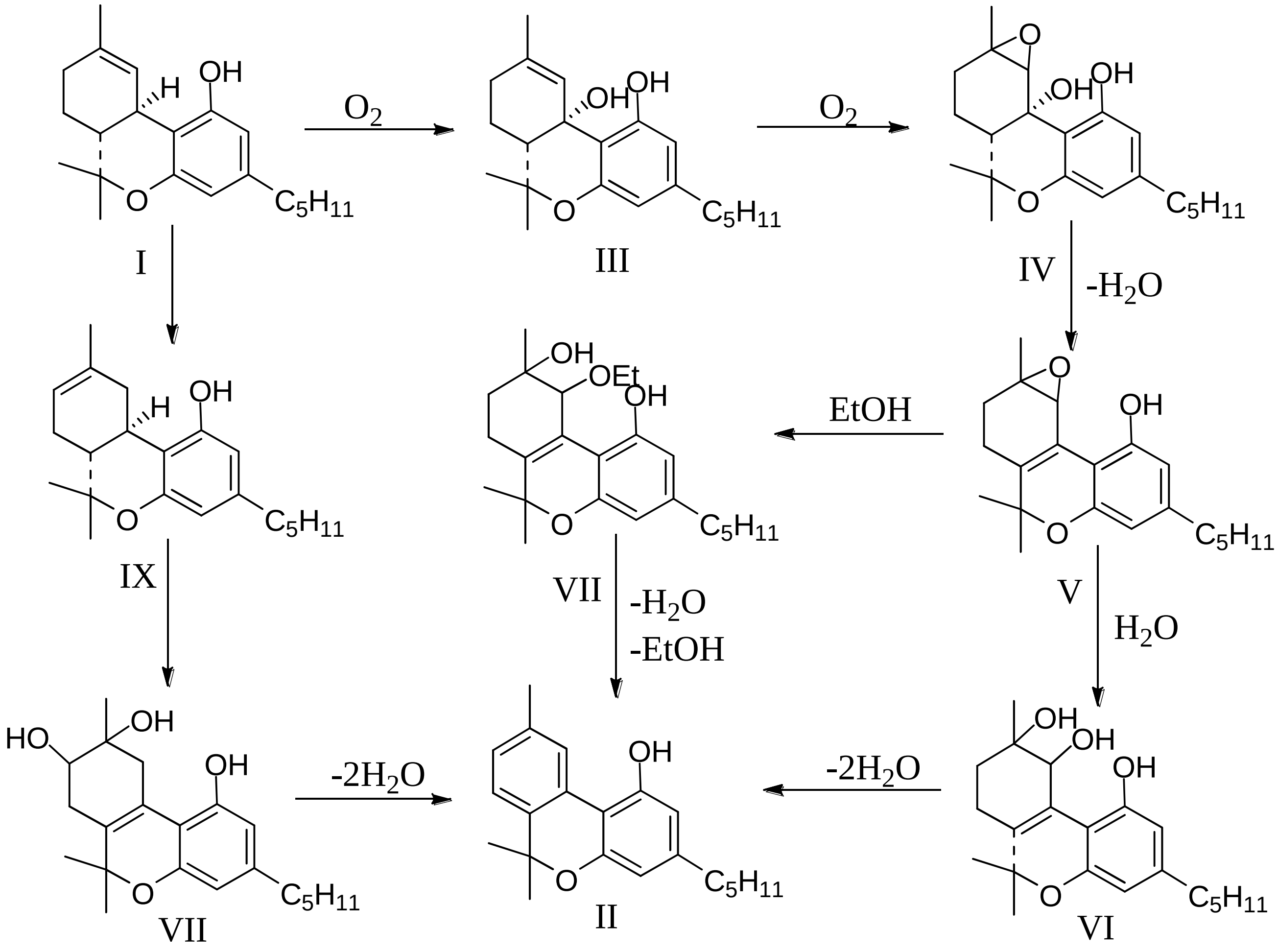


Figure 4.9: Reaction scheme for the decomposition of THC to CBN.

As a result, the content of CBN can be used in the determination of the age or freshness of cannabis plant samples (Dujourdy & Besacier, 2017). Ross et al. (1999) assessed the rate of degradation of THC to CBN to serve as a guide in the estimation of the age of cannabis stored at room temperature. The study reported that a fresh sample less than six months had CBN/THC ratio below 0.013 % and from age 1 up to 2 years CBN/THC ratio was between 0.04% and 0.085%. Consequently, the samples analyzed in this study could be said to be over a year old since the calculated CBN/THC ratios ranged from 0.06 % to 1.82 %. Figure 4.10 shows a graphical representation of the CBN/THC ratios of the samples analyzed. The maximum CBN/THC ratio of 1.82 was found in GR 3 which was expected as most of the cannabis samples used in Accra may have relatively aged since it is a destination of cannabis cultivated in the other regions. The sample UW 3 which recorded the highest THC concentration gave the minimum CBN/THC ratio of 0.06 % an indication that the sample may be relatively fresher than the others.

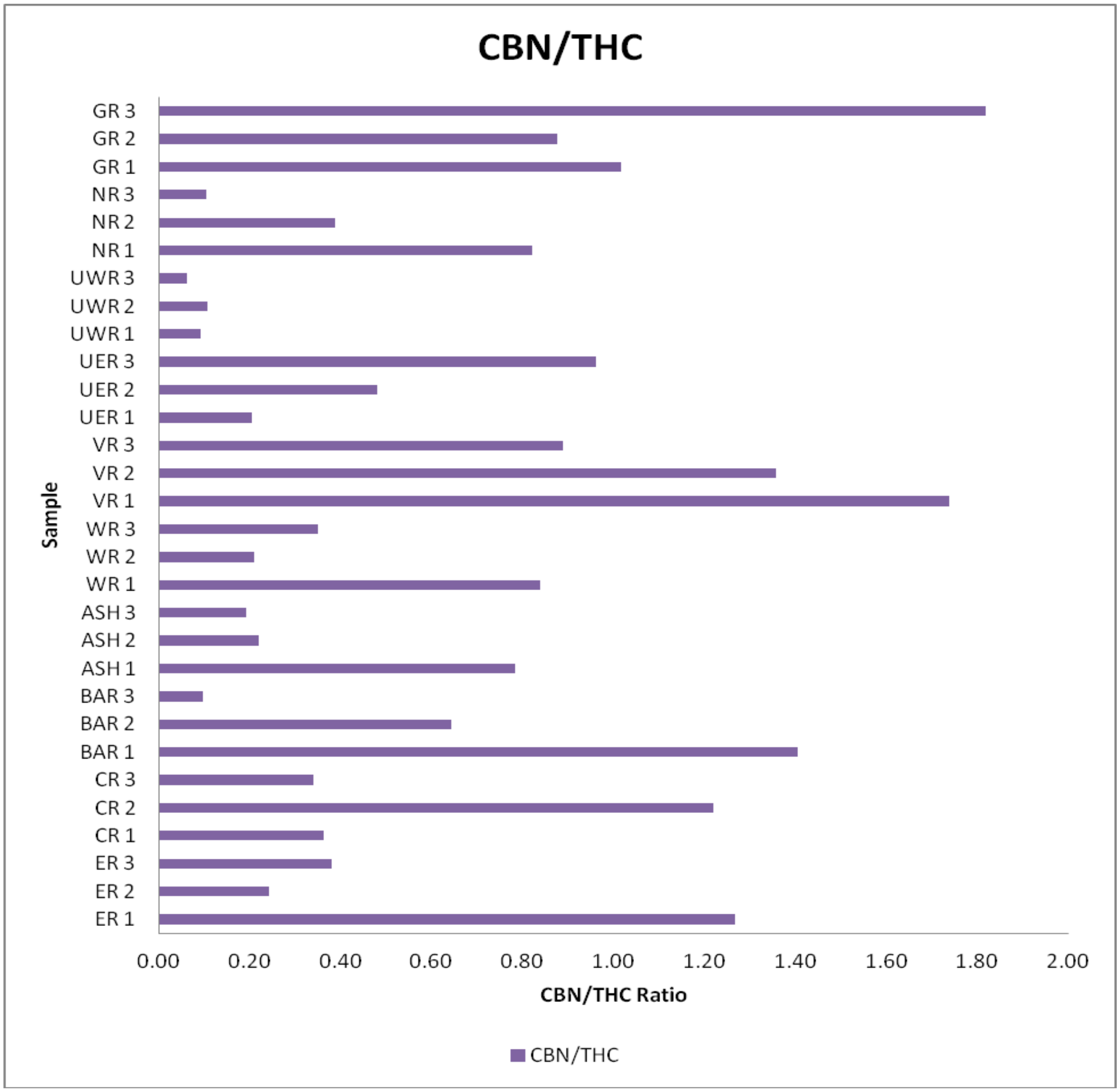


Figure 4.10: CBN/THC content ratio of samples analyzed.

The percentage loss of THC was also found to be proportional to time (Turner & ElSohly, 1979). Ross et al. (1999) showed that on the average, the content of THC in the plant material decreased to about 83.4 % of its original value after one year and 73.2 %, 65.5 % and 58.6 % after two, three and four years, respectively. An earlier study reported that THC decreased at a rate of 3.83, 5.38 and 6.92 per cent over a two years period when stored at -18, 4 and 22 degree celsius respectively (Turner & ElSohly, 1979).

In the present study, the samples were kept at room temperature and analyzed again two months after the first analysis in order to evaluate any change in the THC levels. Table 4.8 summarizes the change in the contents of the cannabinoids two months after the initial analysis with graphical representations in Figure 4.11 and 4.12. Varying degrees of THC loss were observed for all the samples analyzed. By analysis of variance (ANOVA) using the Minitab software, the contents of the two cannabinoids were statistically compared. Twenty three (23) out the 30 analyzed samples showed significant difference ($P < 0.05$) in the THC content after the two months storage. However, no significant difference ($P > 0.05$) was observed in the THC content of samples ER1, CR2, BAR 1, ASH 1, ASH 2, VR3 and UE 1. Considering that the samples were stored under the same conditions, the variability in THC loss may possibly also be attributed to genetic factors of the different varieties of cannabis samples analyzed.

Incidentally, significant difference ($P < 0.05$) was observed for the CBN content of all the samples analyzed including the seven which showed no significant decrease in the THC content. There is therefore the possibility that non-significant change in the THC content of samples resulted in significant increase in the concentration of CBN. The mean THC loss was 11.51 % in the range of 0.17 % - 56.80 % over the two-month storage and this was found to be relatively higher than observations made by Ross et al. (1999) where the maximum percentage THC over a four year storage period was 41.4 %. This difference may be attributed to relatively higher room temperature and humidity in Ghana compared to the US.

Subsequently, Ross et al. (1999) observed that the percentage loss of THC concentration is also a function of the initial concentration; that the higher the initial THC concentration, the faster the degradation and went ahead to attribute this to the high variability in the THC loss of the samples analyzed. However, this observation was not noted in the present study as the sample with the highest THC content lost 5.43 % of the initial concentration of 15.14 % against the highest THC loss of 56.80 % for sample NR 2 of initial THC level of 3.01 %. Interestingly, none of the samples showed results for total degradation of THC as samples with initial low concentrations of THC still showed results for the cannabinoid.

Table 4.8: %THC and %CBN Content of samples two months after initial analysis

Sample	Sample Code	Initial THC	Initial CBN	After Two Months THC	After Two Months CBN	% THC Loss	% CBN Gain
1	ER 1	1.96±0.16	2.48±0.20	1.92±0.15	4.69±0.38	2.04	89.11
2	ER 2	3.54±0.29	0.86±0.07	3.13±0.25	1.36±0.11	11.58	58.14
3	ER 3	1.81±0.15	0.69±0.06	1.61±0.13	0.94±0.08	11.05	36.23
4	CR 1	2.72±0.22	0.98±0.08	2.52±0.20	1.34±0.11	7.35	36.73
5	CR 2	1.10±0.09	1.34±0.11	1.01±0.08	1.63±0.13	8.18	21.64
6	CR 3	2.01±0.16	0.69±0.06	1.80±0.15	0.96±0.08	10.45	39.13
7	BAR 1	0.98±0.08	1.37±0.11	0.97±0.08	1.77±0.14	1.02	29.20
8	BAR 2	2.09±0.17	1.34±0.11	1.89±0.15	2.01±0.16	9.57	50.00
9	BAR 3	8.32±0.67	0.81±0.07	7.91±0.64	1.47±0.12	4.93	81.48
10	ASH 1	1.37±0.11	1.07±0.09	1.34±0.11	1.48±0.12	2.19	38.32
11	ASH 2	3.21±0.26	0.71±0.06	3.13±0.25	1.18±0.09	2.49	66.20
12	ASH 3	3.82±0.31	0.74±0.06	3.60±0.29	1.24±0.10	5.76	67.57
13	WR 1	1.30±0.10	1.09±0.09	1.21±0.10	1.57±0.13	6.92	44.04
14	WR 2	7.07±0.57	1.47±0.12	4.03±0.32	1.81±0.15	43.00	23.13
15	WR 3	1.91±0.15	0.67±0.05	1.74±0.14	1.01±0.08	8.90	50.75
16	VR 1	1.43±0.12	2.48±0.20	1.24±0.10	3.08±0.25	13.29	24.19
17	VR 2	1.57±0.13	2.13±0.17	1.32±0.11	2.80±0.23	15.92	31.46
18	VR 3	0.93±0.07	0.83±0.07	0.92±0.07	1.14±0.09	1.08	37.35
19	UER 1	7.28±0.59	1.48±0.12	7.22±0.58	4.15±0.33	0.82	180.41
20	UER 2	2.67±0.22	1.28±0.10	2.33±0.19	2.05±0.10	12.73	60.16
21	UER 3	1.40±0.11	1.35±0.11	1.29±0.10	2.01±0.16	7.86	48.89
22	UWR 1	11.10±0.89	1.03±0.08	9.19±0.74	1.59±0.13	17.21	54.37
23	UWR 2	6.91±0.56	0.73±0.06	6.62±0.53	1.23±0.10	4.20	68.49
24	UWR 3	15.14±1.22	0.93±0.07	14.31±1.15	1.5±0.12	5.48	61.29
25	NR 1	2.01±0.16	1.65±0.13	1.86±0.15	1.73±0.14	7.46	4.85
26	NR 2	3.01±0.24	1.17±0.09	1.30±0.10	3.04±0.24	56.81	159.83
27	NR 3	11.06±0.89	1.14±0.09	8.17±0.66	2.04±0.16	26.13	78.95
28	GR 1	1.59±0.13	1.61±0.13	1.49±0.12	1.72±0.14	6.29	6.83
29	GR 2	1.86±0.15	1.63±0.13	1.48±0.12	1.87±0.15	20.43	14.72
30	GR 3	1.77±0.14	3.23±0.26	1.48±0.12	4.09±0.33	16.38	26.63

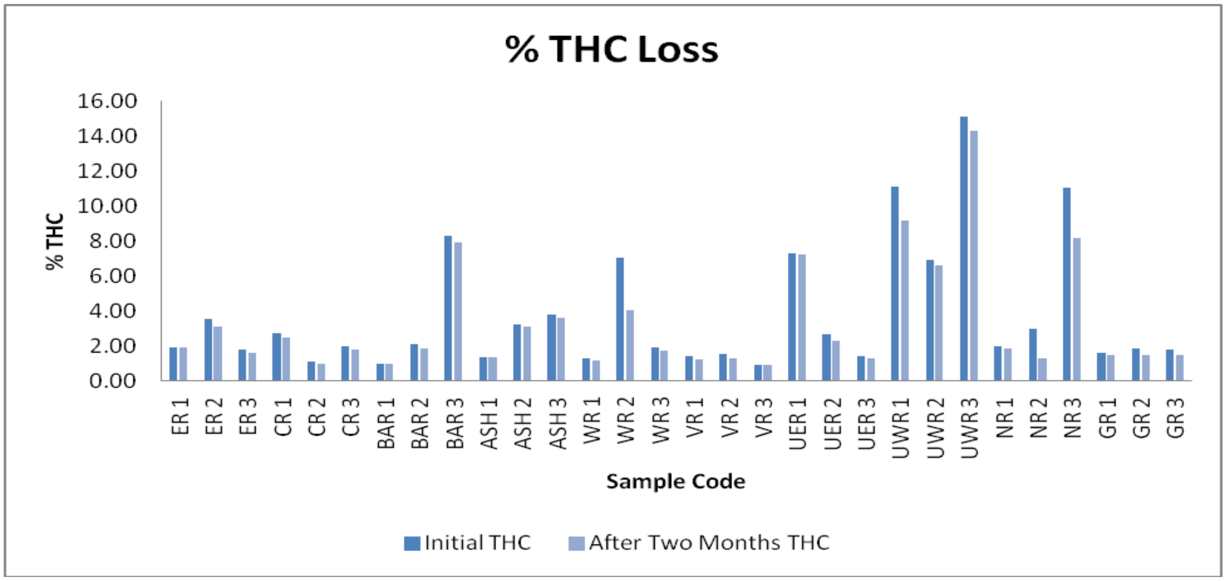


Figure 4.11: %THC Loss in samples re-analyzed after two months storage.

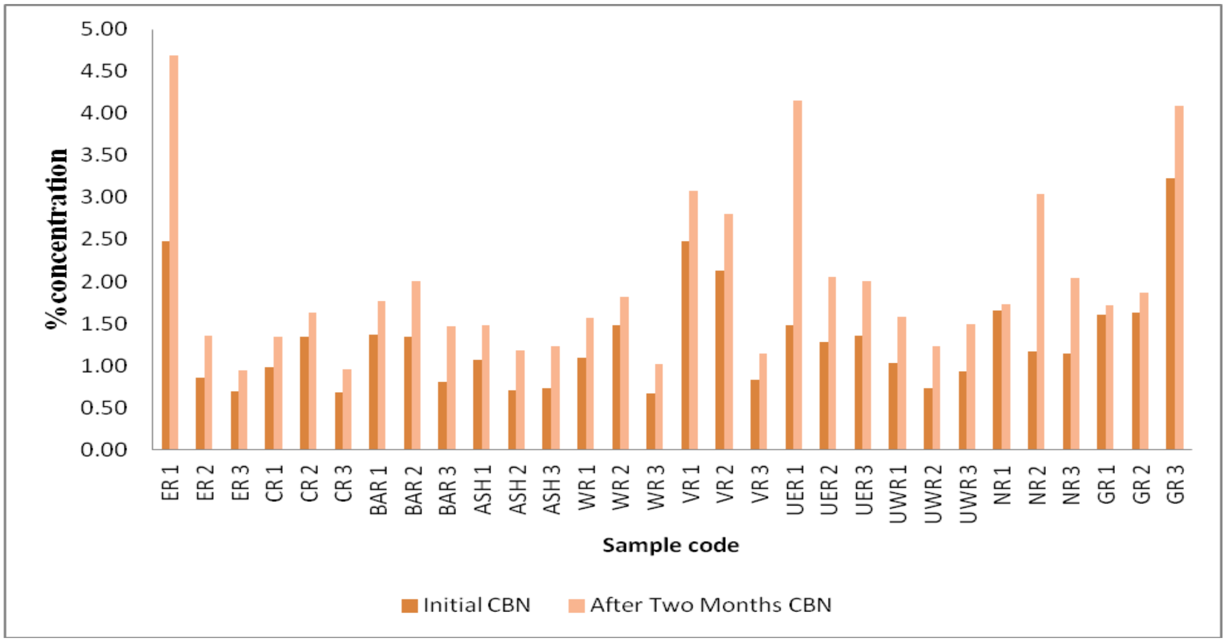


Figure 4.12: %CBN gain in samples re-analyzed after two months storage.

4.4 Phenotype Index of samples analyzed in phase 1

Fetterman et al., (1971), identified drug type and fibre type cannabis using the ratio of the total

THC content and the CBD content, which is known as the phenotype index. The total THC content is represented by the sum of the THC and CBN content; since it is known that CBN is a decomposition product of THC. A phenotype index greater than 1 is an indication of drug type cannabis (phenotype I) and the inverse is true for a fibre type cannabis (phenotype II). One of the objectives of this study was to classify the samples analyzed into drug or fibre type cannabis using the phenotype index. The calculated results showed that the phenotype indices of all the samples analyzed were greater than 1 in the range of 1.99 - 18.07 as presented in Table 4.6 and Figure 4.13; an indication that all the samples were drug type cannabis.

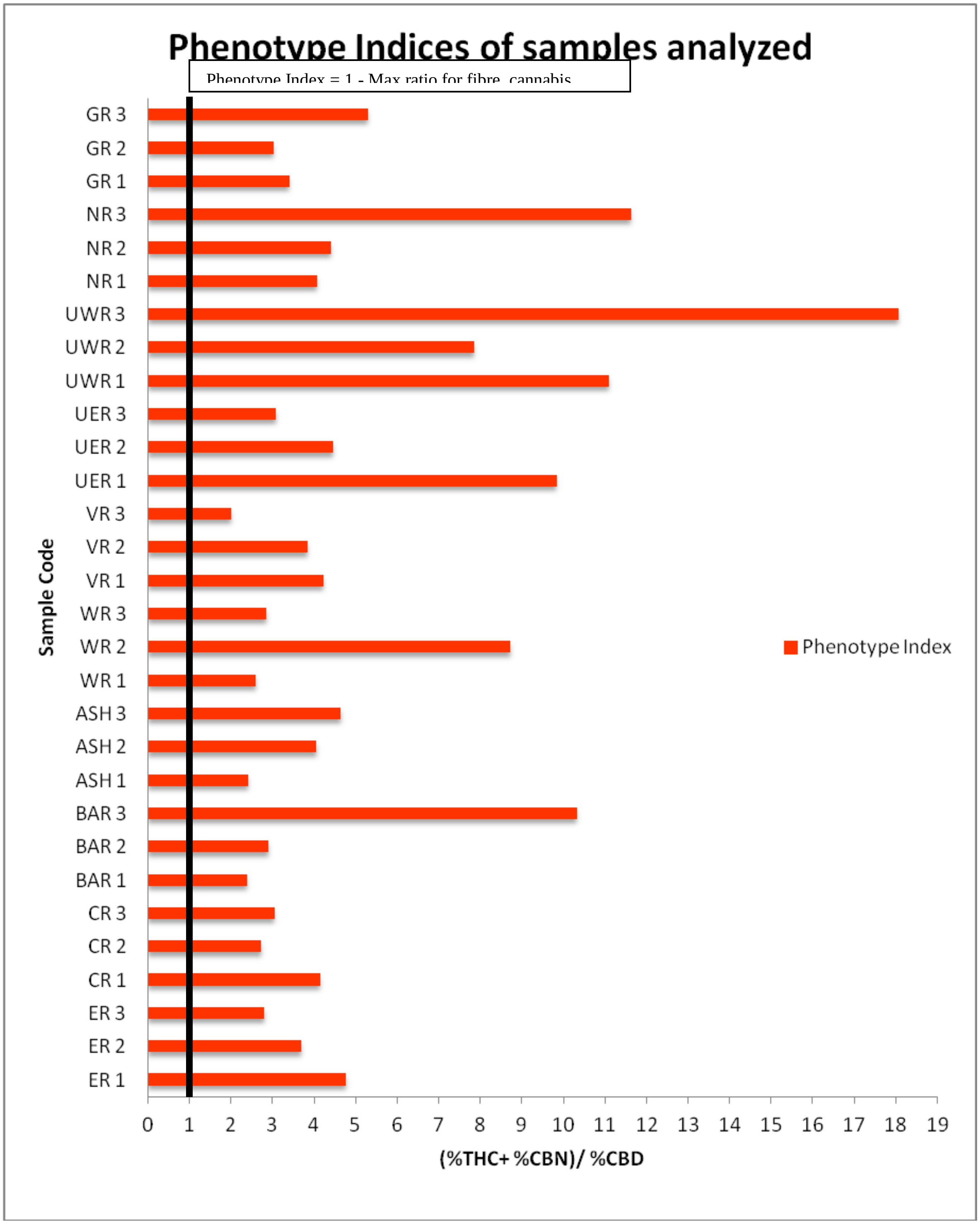


Figure 4.13: Phenotype index of samples analyzed from the different Regions of Ghana.

4.4.1 Plant chemotype of samples analyzed in phase 1

In the other research, Small and Beckstead (1973) classified cannabis by the identification of what is known as three chemotypes. By definition of this classification, plants with values of the total THC to CBD ratio far >1.0 were assigned to chemotype 1 an indication of drug type cannabis, plants with ratios far less than 1 were assigned to chemotype III or fibre type cannabis, and plants with intermediate values were assigned to chemotype II or intermediate type cannabis (Hillig & Mahlberg, 2004). The ratios of the total THC and CDB of the samples analyzed showed drug type cannabis for twenty nine samples and one intermediate type cannabis sample with respect to this classification. None of the samples analyzed therefore showed result for fibre type cannabis.

The THC/CBD chemotype of a plant is said to be determined at a young age and remain stable beyond the seedling stage throughout the life of the plant (Barni, 1984). Hence the deliberate cultivation of hybrids with high THC and low CBD has been employed in the production of high potent cannabis (Aizpurua-Olaizola et al., 2016) and as earlier stated is one of the reasons accounting for the increasing trends in the potency of cannabis.

Again the ratio of THC/CBD was also considered in the chemotaxonomy of cannabis species by Hillig and Mahlberg (2004). The study supported previous researches that, two species of cannabis exist namely cannabis sativa and indica. It investigated the THC/CBD ratios of 157 accessions of cannabis sativa and cannabis indica, and the results showed that the proportion of chemotype I plants in most accessions assigned to C. indica was relatively higher than in most accessions assigned to C. sativa. This attempted to give an indication that the chemotype of cannabis could be used to identify the plant species. The study however conceded that all three chemotypes have been found in both species of cannabis therefore, the chemotype alone of an individual plant is of limited use for chemotaxonomic determination of species members (Hillig & Mahlberg, 2004). Hence deduction of the species of the samples analyzed with respect to the THC/CBD ratios was not considered in the discussion of this study.

4.5 Phase 2 of Study

In phase 2 of the study, fresh farm cannabis samples from the Eastern, Ashanti and Volta regions were analyzed to characterize cannabis grown specifically in the region and also ascertain if there were significant variations between the fresh samples and seized samples analyzed from a particular region. Fresh cannabis samples obtained from Dzemeni in the Volta, Boti in the Eastern and Kumasi in Ashanti recorded THC content of 6.88±0.55, 5.46±0.44 and 4.49±0.36 respectively as indicated in Table 4.9.

CBD content of 1.18 %, 1.31 % and 1.33 % were recorded for the samples VR, ER and ASH respectively. No detectable amounts of CBN were found for samples VR and ER, while detectable CBN amount in sample ASH could not be quantified. The results of CBN was in agreement with Tipparat et al., (2012) since CBN is reported as one of the products of degradation of THC in cannabis and therefore absent in fresh samples.

Table 4.9 Cannabinoids content and indices of fresh samples analyzed in phase 2

	Sample Code	% THC	% CBN	%CBD	(%THC+%CBN)/ %CBD	Phenotype index
1	ASH	6.88±0.55	Not quantifiable	1.33±0.10	5.17	I
2	ER	4.49±0.36	Not detectable	1.31±0.10	3.42	I
3	VR	5.46±0.44	Not detectable	1.18±0.09	4.53	I

The THC percentage content of the seized samples in phase 1 were significantly different ($P < 0.05$) from the fresh cannabis samples collected from farms in the three regions. The recorded fresh sample THC content was about four times the average THC of VR samples in phase 1, almost two times the average THC of ER samples in phase 1 and three times that of ASH samples in phase 1 of this study; as shown in Table 4.10. The significant differences might be on account of decomposition of the seized samples resulting from drying and storage conditions, cultivating conditions and the genetic makeup of the seed (Bruci et al., 2012). The CBD content of phase 1 samples were not significantly different ($P>0.05$) for all the three regions for both the fresh and seized samples.

Table 4.10: Cannabinoids content of phase 2 samples and phase 1 samples from respective regions

	SAMPLE CODE	% THC	% CBN	% CBD
1	**ASH**	**6.88±0.55**	**not quantifiable**	**1.33±0.10**
2	ASH 1	1.37±0.11	1.07±0.09	1.02±0.08
3	ASH 2	3.21±0.26	0.71±0.06	0.97±0.07
4	ASH 3	3.82±0.31	0.74±0.06	0.98±0.07
5	**ER**	**4.49±0.36**	**not detected**	**1.31±0.10**
6	ER 1	1.96±0.16	2.48±0.20	0.93±0.07
7	ER 2	3.54±0.29	0.86±0.07	1.19±0.09
8	ER 3	1.81±0.15	0.69±0.06	0.90±0.07
9	**VR**	**5.46±0.44**	**not detected**	**1.18±0.09**
10	VR 1	1.43±0.12	2.48±0.20	0.93±0.07
11	VR 2	1.57±0.13	2.13±0.17	0.97±0.07
12	VR 3	0.93±0.07	0.83±0.07	0.88±0.07

Although only three fresh cannabis plants were analyzed in this study, the findings showed a good agreement with an in-depth analysis of cannabis, where the THC content of the leaves at different heights was found to increase with height (Bruci et al., 2012). The heights of the fresh plants obtained for this study were; 1.0 m, 0.6 m and 0.3 m for samples ASH, VR and ER respectively and the corresponding THC levels measured were 6.88±0.55, 5.46±0.44 and 4.49±0.36.

Again with most cannabis plants known to reach maturity with height over 1 m (Bruci et al., 2012), there is a possibility of the THC concentration reaching levels above the recorded values. The phenotype indices for the three samples were greater than 1; an indication of very high potent drug type cannabis.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.0 CONCLUSION

Information on the cannabinoids content of cannabis is a good reference in any country for any policy decision on the substance. The identification and measurement of the cannabinoids content are also very essential in the area of forensics, pharmacology and chemotaxonomy.

Notwithstanding the variations in the cannabinoids content of the samples analyzed, the results of the research showed that all the thirty three cannabis samples were high potent drug type cannabis. The content of the samples analyzed varied within and between the regions. The THC content which defines the potency of the substance was found in the range of {(0.93±0.07) - (15.14±1.22)} %, far above the UNODC reported range of 0.5 - 5 % typical THC level for herbal cannabis. The mean THC content was found to be (3.76±0.30) %. Thus globally Ghana can be considered among the producers of high potent cannabis. A phenotype index above 1 qualifies a cannabis sample as drug type. The phenotype indices of all samples analyzed in the study ranged from 1.99 to 18.07 an indication of drug type cannabis.

The CBN content range of {(0.67±0.05) - (3.23±0.26)} % recorded, showed levels of degradation of THC in the seized samples analyzed as the former is a degradation product of the latter. Comparatively fresh samples collected from farms recorded no CBN content and the THC content were higher and significantly different ($P<0.05$) from the seized samples. This was a confirmation of the degradation of THC to CBN and an indication that the recorded potencies of samples analyzed in phase 1 of this study could have been higher if they were stored under very good conditions.

The CBD content which is known to reduce the psychoactive effects of THC in cannabis was found in a very close range of {(0.88±0.07) - (1.19±0.09)} %. with a mean content of 0.96 %. No significant ($P>0.05$) difference was however observed for the CBD content for both seized and fresh samples.

Cannabis cultivated and abused in Ghana have very high potencies and the overall implication on the health and mental well being of users and the entire population must be of primary concern in any debate or policy to legalize the substance.

5.1 RECOMMENDATION

The chemical investigation of the cannabinoids content of cannabis commands a very vast area of study in many countries. The cannabinoid content of cannabis is also a very useful forensic tool in the determination of cannabis plant material from different geographical regions. Therefore it is highly recommended that more researches are conducted on samples found in the country to enhance information available on the cannabis cultivated and abused in Ghana.

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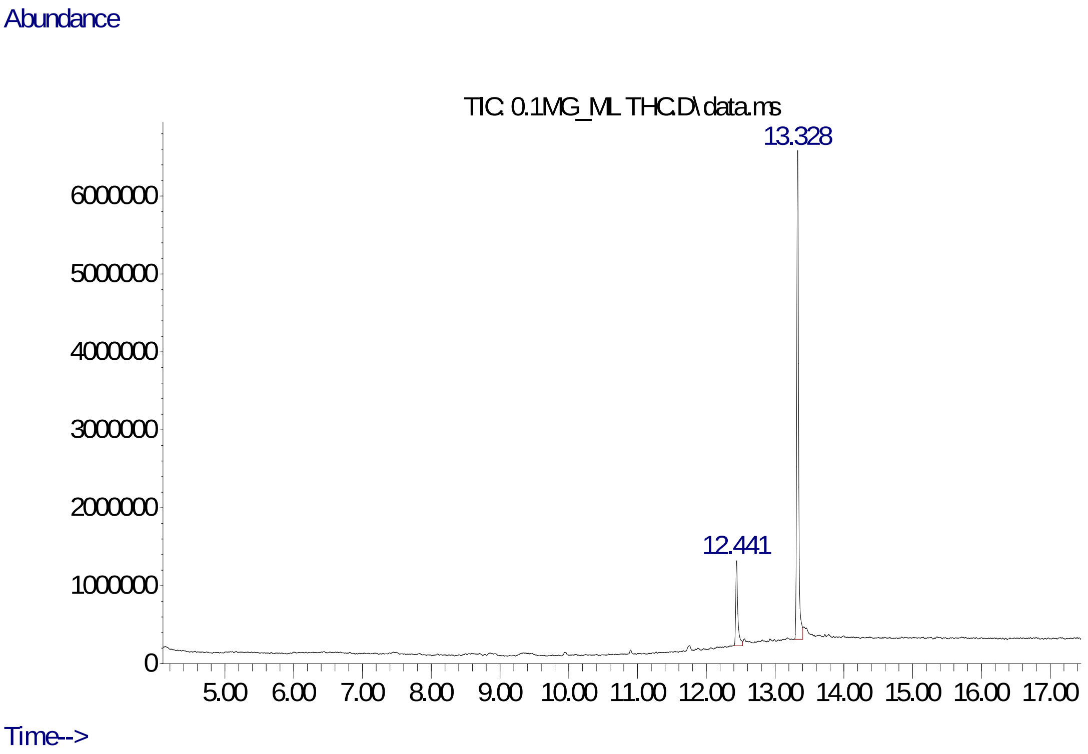
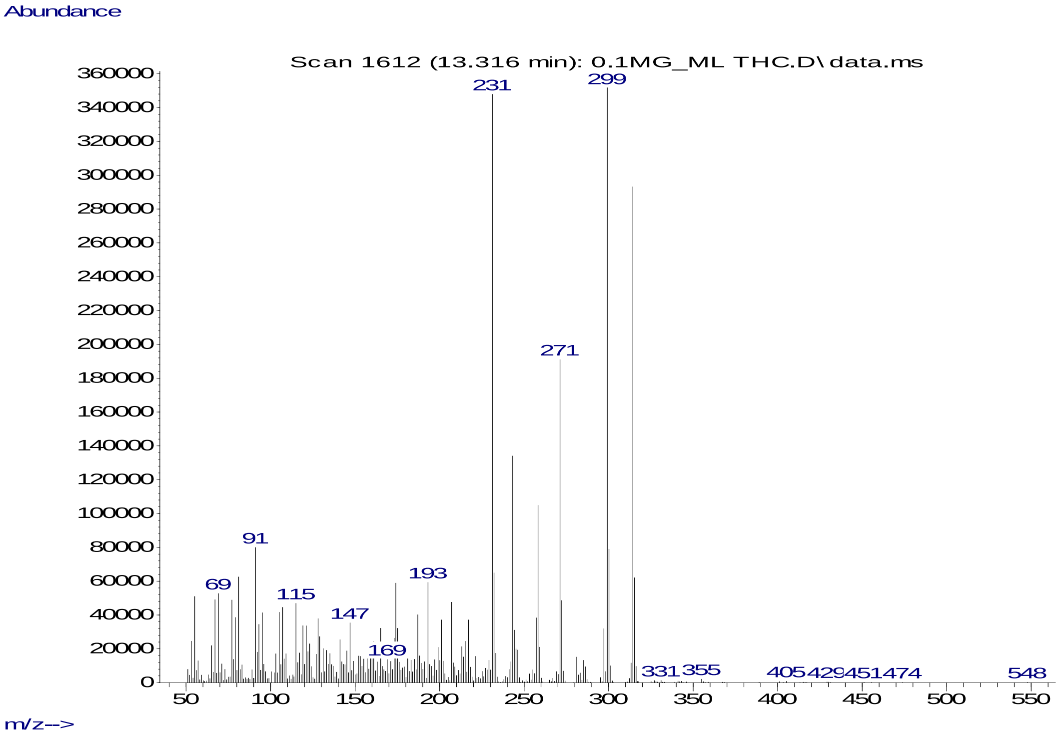
APPENDIX

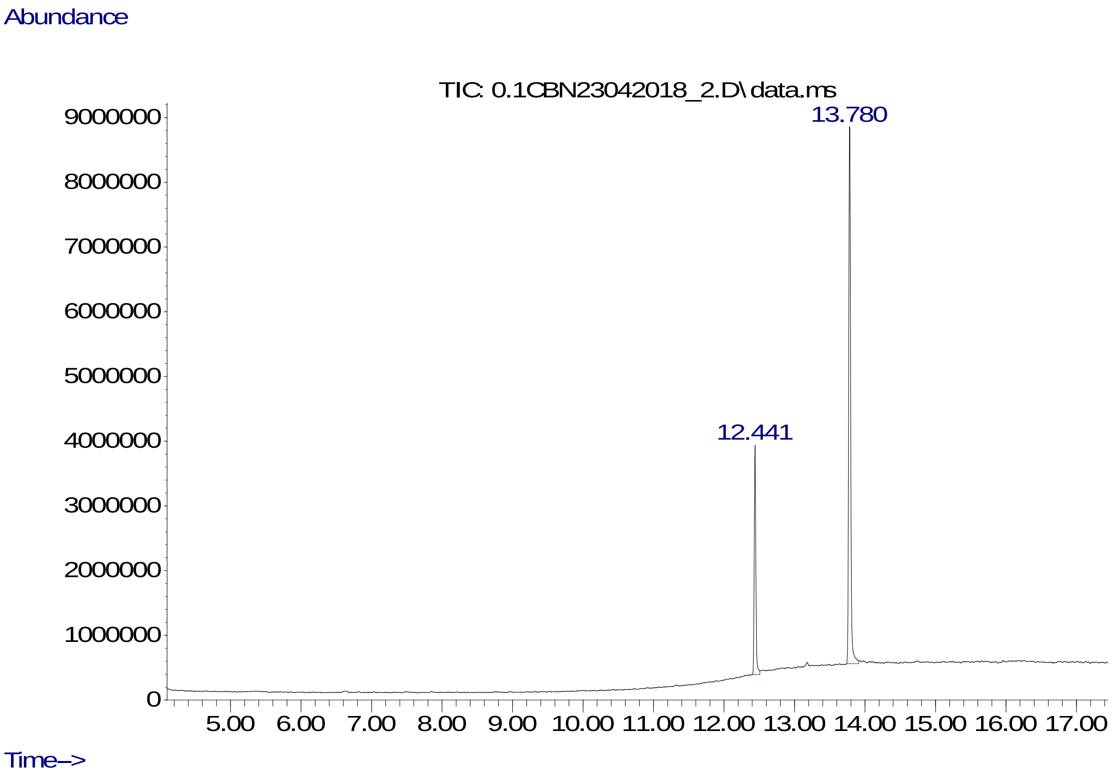
Appendix A - TIC and Mass Spectra of cannabinoids



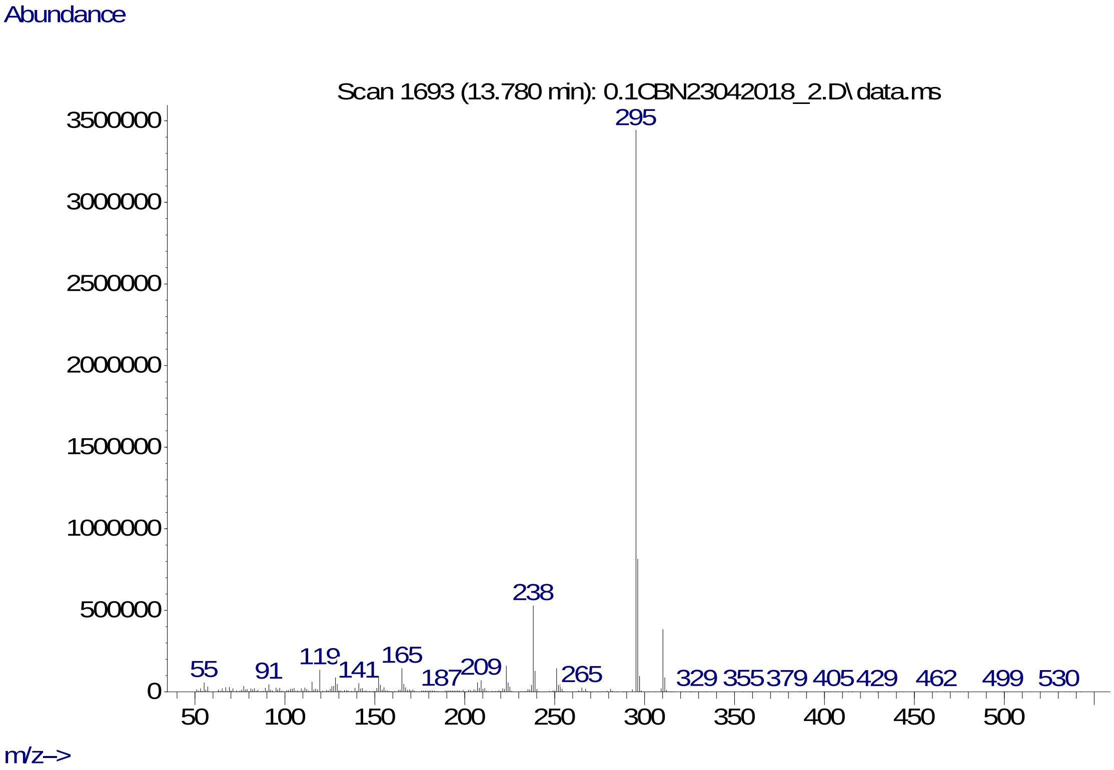
A1: TIC of THC in ISTD Tetracosane solution



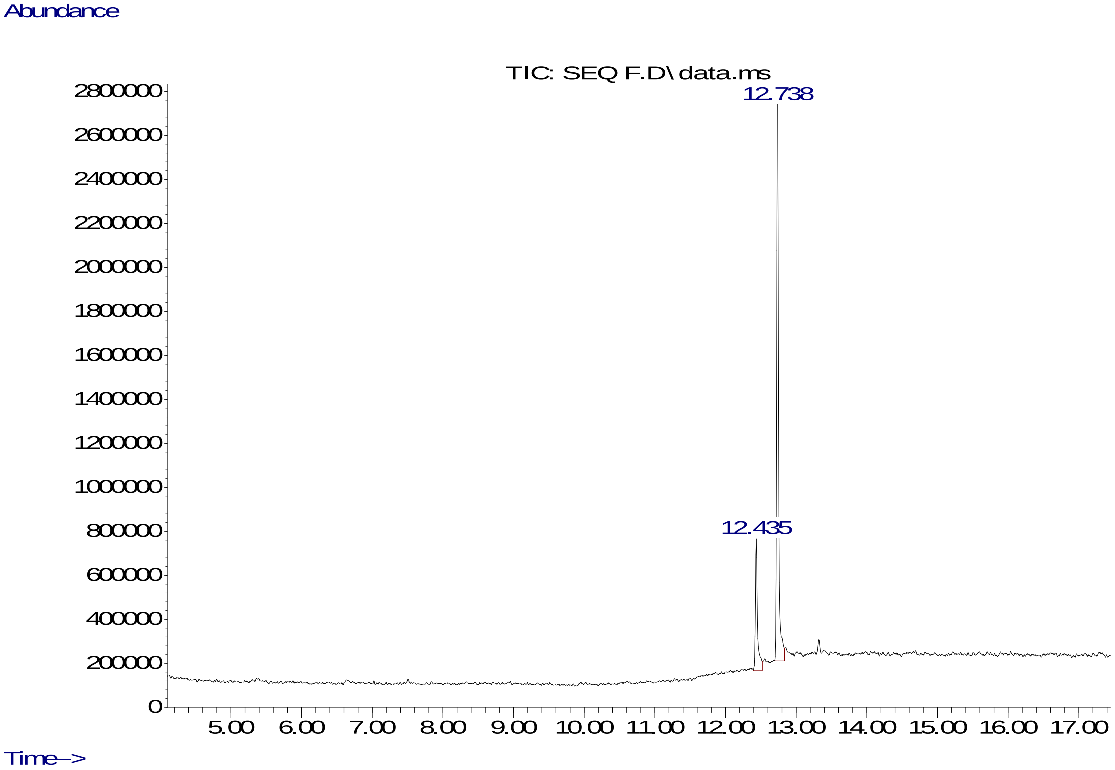
A2: Mass Spectrum of THC



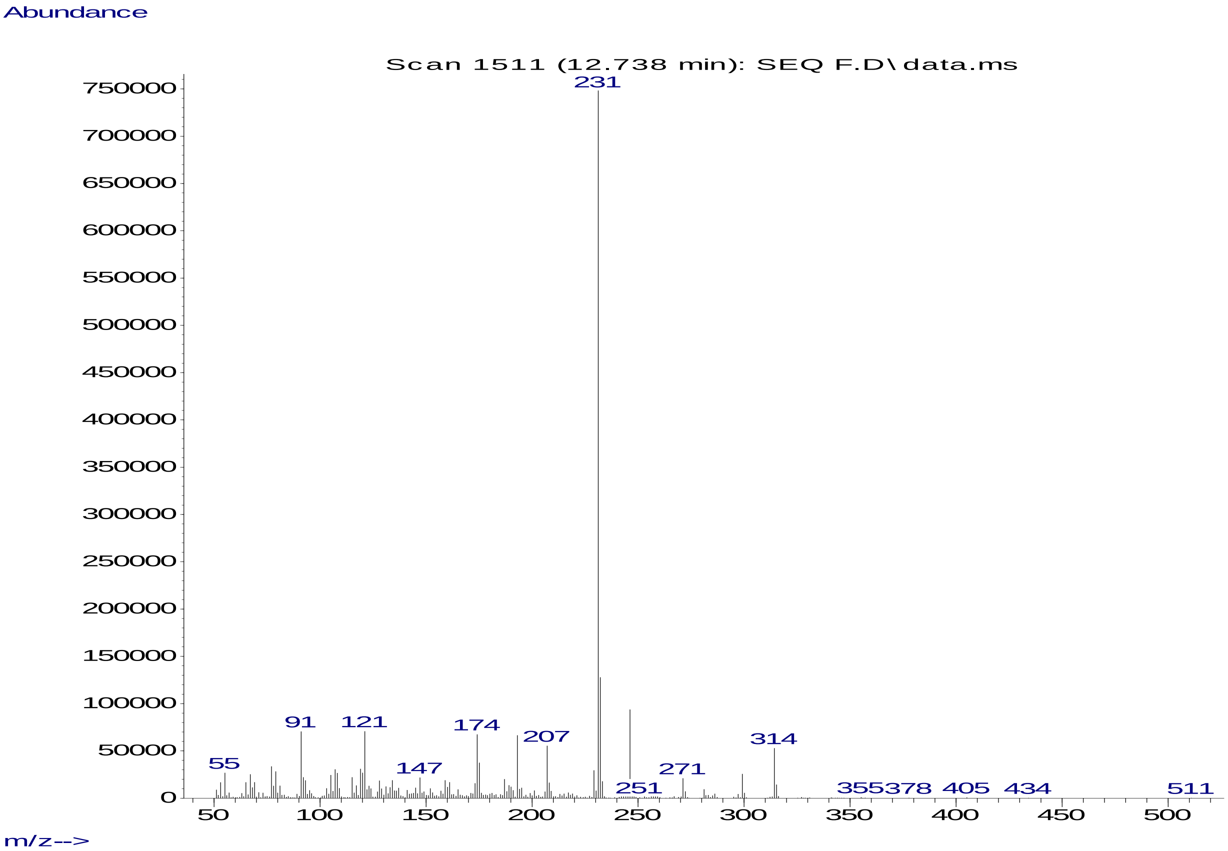
A 3: TIC of CBN in ISTD Tetracosane solution



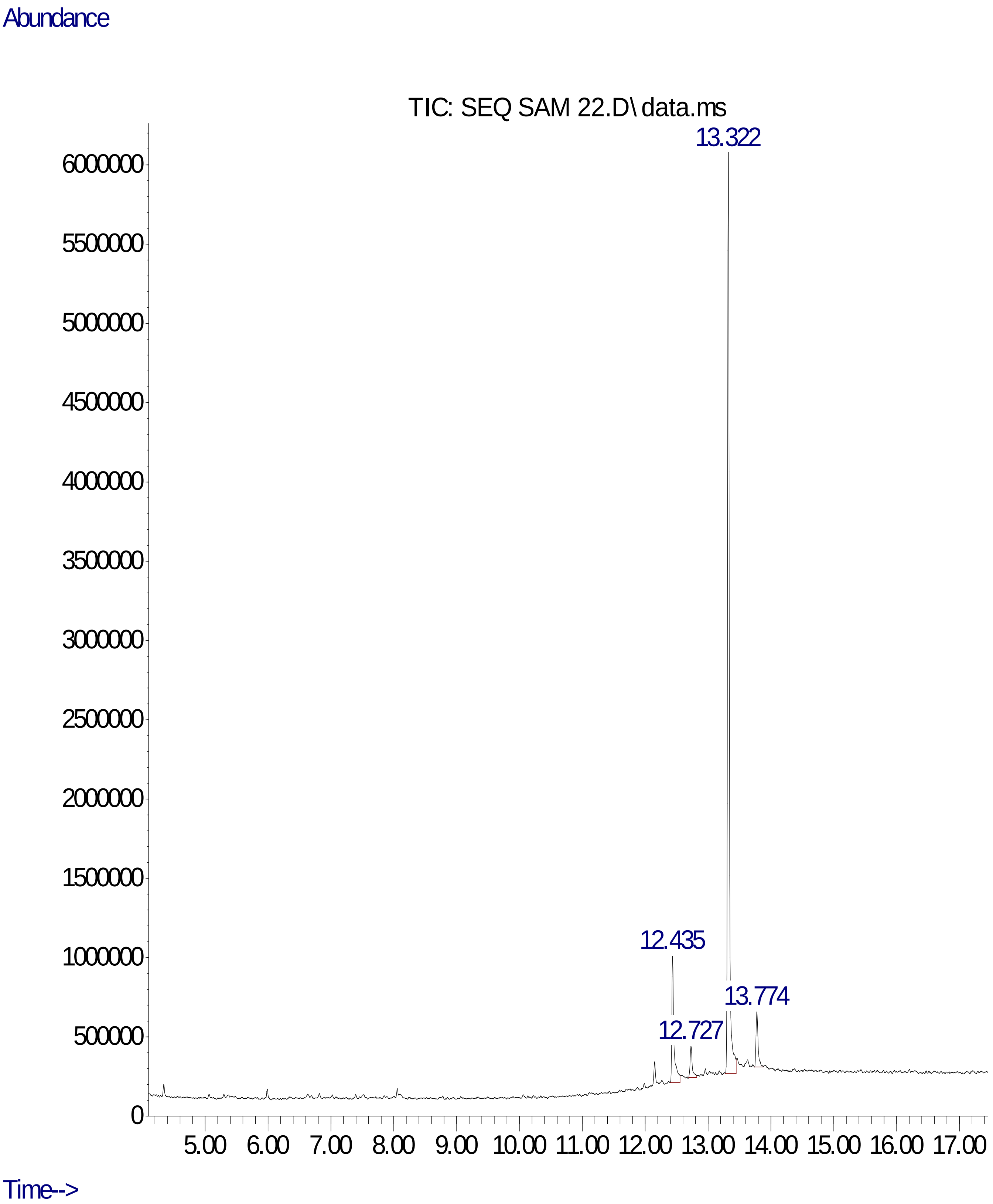
A 4: Mass spectrum of CBN



A 5 : TIC of CBD in ISTD Tetracosane solution



A 6: Mass Spectrum of CBD



A7: TIC of a Sample (WR 3) in ISTD Tetracosane solution

Appendix B - GCMS Results

B1: THC results for February GC-MS run

	Sample code	Average ISTD Area Response	Average THC Area Response	THC/ISTD Ratio	THC Conc Ratio	THC Conc mg/ml	%THC
1	ER 1	1108519	7526500	0.6789690	0.7826316	0.0195658	1.96
2	ER 2	1337097	2170078	1.6229772	1.4149211	0.0353730	3.54
3	ER 3	1393403	8227590	0.5904674	0.7233539	0.0180838	1.81
4	CR 1	1494178	1693165	1.1331749	1.0868553	0.0271714	2.72
5	CR 2	1257016	2094410	0.1666176	0.4394626	0.0109866	1.10
6	CR 3	1545352	1102852	0.7136575	0.8058657	0.0201466	2.01
7	BAR 1	1599542	1486470	0.0929310	0.3901078	0.0097527	0.98
8	BAR 2	1662270	1257181	0.7563037	0.8344298	0.0208607	2.09
9	BAR 3	1965256	8807801	4.4817576	3.3297104	0.0832428	8.32
10	ASH 1	1798573	5871930	0.3264772	0.5465353	0.0136634	1.37
11	ASH 2	1999177	2857215	1.4291956	1.2851277	0.0321282	3.21
12	ASH 3	2049061	3673610	1.7928261	1.5286846	0.0382171	3.82
13	WR 1	2040491	5890210	0.2886663	0.5212099	0.0130302	1.30
14	WR 2	1868948	6980015	3.7347294	2.8293566	0.0707339	7.07
15	WR 3	1914453	1243947	0.6497663	0.7630719	0.0190768	1.91
16	VR 1	1793489	6506830	0.3628029	0.5708660	0.0142716	1.43
17	VR 2	1788389	8036480	0.4493698	0.6288478	0.0157212	1.57
18	VR 3	2112482	1380000	0.0653260	0.3716182	0.0092905	0.93
19	UER 1	1597434	6164719	3.8591385	2.9126848	0.0728171	7.28
20	UER 2	1856763	2046624	1.1022538	1.0661445	0.0266536	2.67
21	UER 3	1910347	6665140	0.3488968	0.5615518	0.0140388	1.40
22	UWR 1	1878034	1152677	6.1376764	4.4388322	0.1109708	11.10
23	UWR 2	1740275	6333465	3.6393472	2.7654703	0.0691368	6.91
24	UWR 3	1757758	1502731	8.5491336	6.0540078	0.1513502	15.14
25	NR 1	1845413	1315780	0.7130003	0.8054255	0.0201356	2.01
26	NR 2	1736309	2271023	1.3079602	1.2039251	0.0300981	3.01
27	NR 3	1825599	1116366	6.1150642	4.4236866	0.1105922	11.06
28	GR 1	2014358	9224900	0.4579573	0.6345997	0.0158650	1.59
29	GR 2	1542881	9541150	0.6183983	0.7420618	0.0185515	1.86
30	GR 3	1596102	9094950	0.5698226	0.7095262	0.0177382	1.77

B2: CBN results for February GC-MS run

	Sample code	Average ISTD Area Response	Average CBN Area Response	CBN/ISTD Ratio	CBN CONC Ratio	CBN CONC mg/ml	% CBN
1	ER 1	1108519	2026850	1.8284305	0.9916316	0.0247908	2.48
2	ER 2	1337097	3529030	0.2639322	0.3425161	0.0085629	0.86
3	ER 3	1393403	1422010	0.1020530	0.2753519	0.0068838	0.69
4	CR 1	1494178	5775010	0.3865008	0.3933702	0.0098343	0.98
5	CR 2	1257016	9185940	0.7307735	0.5362101	0.0134053	1.34
6	CR 3	1545352	1540010	0.0996543	0.2743566	0.0068589	0.69
7	BAR 1	1599542	1214612	0.7593499	0.5480665	0.0137017	1.37
8	BAR 2	1662270	1217756	0.7325862	0.5369621	0.0134241	1.34
9	BAR 3	1965256	4284890	0.2180322	0.3234720	0.0080868	0.81
10	ASH 1	1798573	8437660	0.4691308	0.4276536	0.0106913	1.07
11	ASH 2	1999177	2368400	0.1184687	0.2821628	0.0070541	0.71
12	ASH 3	2049061	3019740	0.1473719	0.2941548	0.0073539	0.74
13	WR 1	2040491	1004916	0.4924873	0.4373443	0.0109336	1.09
14	WR 2	1868948	1607586	0.8601556	0.5898911	0.0147473	1.47
15	WR 3	1914453	1546040	0.0807562	0.2665157	0.0066629	0.67
16	VR 1	1793489	3278436	1.8279655	0.9914387	0.0247860	2.48
17	VR 2	1788389	2670862	1.4934458	0.8526453	0.0213161	2.13
18	VR 3	2112482	4957120	0.2346586	0.3303703	0.0082593	0.83
19	UER 1	1597434	1387500	0.8685805	0.5933866	0.0148347	1.48
20	UER 2	1856763	1248845	0.6725926	0.5120706	0.0128018	1.28
21	UER 3	1910347	1414495	0.7404388	0.5402202	0.0135055	1.35
22	UWR 1	1878034	8012360	0.4266355	0.4100222	0.0102506	1.03
23	UWR 2	1740275	2435610	0.1399555	0.2910777	0.0072769	0.73
24	UWR 3	1757758	5861040	0.3334384	0.3713544	0.0092839	0.93
25	NR 1	1845413	1900682	1.0299494	0.6603391	0.0165085	1.65
26	NR 2	1736309	9766060	0.5624609	0.4663766	0.0116594	1.17
27	NR 3	1825599	9771350	0.5352408	0.4550829	0.0113771	1.14
28	GR 1	2014358	1997355	0.9915591	0.6444109	0.0161103	1.61
29	GR 2	1542881	1551567	1.0056297	0.6502488	0.0162562	1.63
30	GR 3	1596102	4067782	2.5485727	1.290421	0.0322605	3.23

B3: CBD results for February GC-MS run

	Sample code	Average ISTD Area Response	CBD Area response	CBD/ISTD RATIO	CBD CONC RATIO	CBD CONC mg/ml	%CBD
1	ER 1	1108519	89319	0.080575074	0.372945033	0.00932363	0.93
2	ER 2	1337097	493599	0.369157211	0.477906893	0.01194767	1.19
3	ER 3	1393403	56005	0.040192966	0.358257426	0.00895644	0.90
4	CR 1	1494178	61735	0.041317032	0.358666266	0.00896666	0.90
5	CR 2	1257016	56243	0.044743265	0.359912441	0.00899781	0.90
6	CR 3	1545352	56120	0.036315351	0.356847076	0.00892118	0.89
7	BAR 1	1599542	229336	0.143376041	0.395786732	0.00989467	0.99
8	BAR 2	1662270	600238	0.36109537	0.474974674	0.01187437	1.19
9	BAR 3	1965256	57000	0.029003855	0.354187770	0.00885469	0.89
10	ASH 1	1798573	308835	0.171711129	0.406092649	0.01015232	1.02
11	ASH 2	1999177	240065	0.120081914	0.387314292	0.00968286	0.97
12	ASH 3	2049061	280645	0.136962736	0.393454112	0.00983635	0.98
13	WR 1	2040491	152649	0.074809935	0.370848162	0.00927120	0.93
14	WR 2	1868948	249290	0.133385199	0.392152906	0.00980382	0.98
15	WR 3	1914453	100733	0.052617118	0.362776285	0.00906941	0.91
16	VR 1	1793489	135581	0.075596226	0.371134148	0.00927835	0.93
17	VR 2	1788389	213728	0.119508675	0.387105796	0.00967764	0.97
18	VR 3	2112482	58000	0.027455855	0.353624738	0.00884062	0.88
19	UER 1	1597434	57958	0.036281937	0.356834923	0.00892087	0.89
20	UER 2	1856763	58111	0.031296940	0.355021801	0.00887555	0.89
21	UER 3	1910347	80000	0.041877209	0.358870011	0.00897175	0.90
22	UWR 1	1878034	482356	0.256840930	0.437055696	0.01092639	1.09
23	UWR 2	1740275	220356	0.126621367	0.389692794	0.00974232	0.97
24	UWR 3	1757758	57967	0.032977805	0.355633158	0.00889083	0.89
25	NR 1	1845413	90412	0.048992827	0.361458073	0.00903645	0.90
26	NR 2	1736309	175451	0.101048258	0.380391452	0.00950979	0.95
27	NR 3	1825599	382322	0.209422770	0.419808966	0.01049522	1.05
28	GR 1	2014358	189490	0.094069674	0.377853231	0.00944633	0.94
29	GR 2	1542881	503734	0.326489211	0.462387870	0.01155970	1.16
30	GR 3	1596102	153016	0.095868560	0.378507514	0.00946269	0.95

B4: THC results for May GC-MS run

	Sample code	ISTD Area Response	THC Area Response	THC/ISTD Ratio	THC Ccnc Ratio	THC Conc mg/ml	%THC
1	ER 1	1044472	5151692	0.2027435	0.768577	0.0192144	1.92
2	ER 2	2352209	5436850	0.4326419	1.2528795	0.031322	3.13
3	ER 3	743726	5146671	0.1445062	0.6458947	0.0161474	1.61
4	CR 1	1584883	5011585	0.3162439	1.0076761	0.0251919	2.52
5	CR 2	151002	5015351	0.030108	0.4049041	0.0101226	1.01
6	CR 3	894238	4981434	0.1795142	0.7196422	0.0179911	1.80
7	BAR 1	104753	4599846	0.0227732	0.3894526	0.0097363	0.97
8	BAR 2	929794	4719519	0.1970103	0.7564995	0.0189125	1.89
9	BAR 3	6115952	4562933	1.3403554	3.165063	0.0791266	7.91
10	ASH 1	406394	4384768	0.0926831	0.5367245	0.0134181	1.34
11	ASH 2	1854886	4288658	0.4325097	1.2526009	0.031315	3.13
12	ASH 3	2188368	4201189	0.5208925	1.4387877	0.0359697	3.60
13	WR 1	290478	4339633	0.0669361	0.4824859	0.0120621	1.21
14	WR 2	2591651	4292986	0.6036943	1.6132173	0.0403304	4.03
15	WR 3	649640	3866095	0.1680352	0.6954607	0.0173865	1.74
16	VR 1	301556	4074791	0.0740053	0.4973779	0.0124344	1.24
17	VR 2	351289	3998283	0.08786	0.5265641	0.0131641	1.32
18	VR 3	50000	3943647	0.0126786	0.3681875	0.0092047	0.92
19	UER 1	2270009	1877770	1.2088855	2.8881094	0.0722027	7.22
20	UER 2	1061028	3780053	0.2806913	0.9327813	0.0233195	2.33
21	UER 3	319008	3856038	0.0827295	0.5157562	0.0128939	1.29
22	UWR 1	6614643	4180832	1.5821356	3.6743955	0.0918599	9.19
23	UWR 2	4327710	3949719	1.0957007	2.649675	0.0662419	6.62
24	UWR 3	9806365	3836909	2.5557982	5.725507	0.1431377	14.31
25	NR 1	810093	4226893	0.1916521	0.745212	0.0186303	1.86
26	NR 2	357800	4219777	0.0847912	0.5200995	0.0130025	1.30
27	NR 3	5849577	4210492	1.3892859	3.2681396	0.0817035	8.17
28	GR 1	500062	4122673	0.1212956	0.5969993	0.014925	1.49
29	GR 2	505236	4237498	0.1192298	0.5926475	0.0148162	1.48
30	GR 3	482614	4032686	0.1196756	0.5935866	0.0148397	1.48

B5: CBN results for May GCMS run

	Sample code	ISTD Area Response	CBN Area Response	CBN/ISTD Ratio	CBN CONC Ratio	CBN CONC mg/ml	% CBN
1	ER 1	6130267	5151692	1.1899522	1.8741972	0.0468549	4.69
2	ER 2	971311	5436850	0.1786533	0.5440658	0.0136016	1.36
3	ER 3	260421	5146671	0.0505999	0.3756411	0.0093910	0.94
4	CR 1	867931	5011585	0.1731849	0.5368735	0.0134218	1.34
5	CR 2	1303134	5015351	0.2598291	0.6508340	0.0162708	1.63
6	CR 3	276344	4981434	0.0554748	0.3820529	0.0095513	0.96
7	BAR 1	1388449	4599846	0.3018468	0.7060987	0.0176525	1.77
8	BAR 2	1773176	4719519	0.3757112	0.8032503	0.0200813	2.01
9	BAR 3	967766	4562933	0.2120930	0.5880481	0.0147012	1.47
10	ASH 1	941247	4384768	0.2146629	0.5914282	0.0147857	1.48
11	ASH 2	533874	4288658	0.1244851	0.4728201	0.0118205	1.18
12	ASH 3	590745	4201189	0.1406138	0.4940336	0.0123508	1.24
13	WR 1	1056594	4339633	0.2434754	0.6293245	0.0157331	1.57
14	WR 2	1357868	4292986	0.3162992	0.7251074	0.0181277	1.81
15	WR 3	281897	3866095	0.0729152	0.4049917	0.0101248	1.01
16	VR 1	2863954	4074791	0.7028468	1.2335221	0.0308381	3.08
17	VR 2	2465659	3998283	0.6166795	1.1201887	0.0280047	2.80
18	VR 3	438222	3943647	0.1111210	0.4552427	0.0113811	1.14
19	UER 1	1927393	1877770	1.0264266	1.6591169	0.0414779	4.15
20	UER 2	1471904	3780053	0.3893871	0.8212378	0.0205309	2.05
21	UER 3	1446138	3856038	0.3750321	0.8023571	0.0200589	2.01
22	UWR 1	1033652	4180832	0.2472360	0.6342706	0.0158568	1.59
23	UWR 2	553267	3949719	0.1400776	0.4933284	0.0123332	1.23
24	UWR 3	846101	3836909	0.2205163	0.5991271	0.0149782	1.50
25	NR 1	1235291	4226893	0.2922456	0.6934705	0.0173368	1.73
26	NR 2	2913950	4219777	0.6905460	1.2173431	0.0304336	3.04
27	NR 3	1628913	4210492	0.3868700	0.8179271	0.0204482	2.04
28	GR 1	1191342	4122673	0.2889732	0.6891664	0.0172292	1.72
29	GR 2	1411063	4237498	0.3329944	0.7470661	0.0186767	1.87
30	GR 3	4068657	4032686	1.0089199	1.6360908	0.0409023	4.09

Table B6: CBD results for May GCMS run

	Sample code	ISTD Area Response	CBD Area response	CBD/ISTD RATIO	CBD CONC RATIO	CBD CONC mg/ml	%CBD
1	ER 1	178114	5151692	0.0345739	0.3553060	0.0088827	0.89
2	ER 2	304363	5436850	0.0559815	0.4907116	0.0122678	1.23
3	ER 3	157202	5146671	0.0305444	0.3298191	0.0082455	0.82
4	CR 1	157991	5011585	0.0315252	0.3360225	0.0084006	0.84
5	CR 2	167441	5015351	0.0333857	0.3477906	0.0086948	0.87
6	CR 3	148112	4981434	0.0297328	0.3246857	0.0081171	0.81
7	BAR 1	201663	4599846	0.0438413	0.4139232	0.0103481	1.03
8	BAR 2	346084	4719519	0.0733304	0.6004450	0.0150111	1.50
9	BAR 3	148001	4562933	0.0324355	0.3417805	0.0085445	0.85
10	ASH 1	263435	4384768	0.0600796	0.5166324	0.0129158	1.29
11	ASH 2	228470	4288658	0.0532731	0.4735805	0.0118395	1.18
12	ASH 3	229864	4201189	0.0547140	0.4826947	0.0120674	1.21
13	WR 1	173491	4339633	0.0399783	0.3894893	0.0097372	0.97
14	WR 2	151776	4292986	0.0353544	0.3602430	0.0090061	0.90
15	WR 3	130419	3866095	0.0337340	0.3499939	0.0087498	0.87
16	VR 1	129523	4074791	0.0317864	0.3376750	0.0084419	0.84
17	VR 2	176623	3998283	0.0441747	0.4160323	0.0104008	1.04
18	VR 3	127124	3943647	0.0322351	0.3405132	0.0085128	0.85
19	UER 1	128111	1877770	0.0682251	0.5681535	0.0142038	1.42
20	UER 2	127552	3780053	0.0337434	0.3500534	0.0087513	0.88
21	UER 3	128609	3856038	0.0333526	0.3475815	0.0086895	0.87
22	UWR 1	307811	4180832	0.0736243	0.6023045	0.0150576	1.51
23	UWR 2	132660	3949719	0.0335872	0.3490651	0.0087266	0.87
24	UWR 3	128651	3836909	0.0335299	0.3487024	0.0087176	0.87
25	NR 1	128664	4226893	0.0304394	0.3291548	0.0082289	0.82
26	NR 2	184267	4219777	0.0436675	0.4128240	0.0103206	1.03
27	NR 3	314540	4210492	0.0747039	0.6091326	0.0152283	1.52
28	GR 1	154659	4122673	0.0375143	0.3739042	0.0093476	0.93
29	GR 2	370056	4237498	0.0873289	0.6889873	0.0172247	1.72
30	GR 3	134182	4032686	0.0332736	0.3470816	0.0086770	0.87

Appendix C: Precision - Repeatability, reproducibility and uncertainties of measurements

C1: THC - Same Day Trial 1

IRM	ISTD	THC	THC/ISTD	THC CONC RATIO	THC CONC	%THC
1	2049061	3673610	1.79282608	1.528684581	0.038217115	3.821711453
2	2279061	3793610	1.664549567	1.442765952	0.036069149	3.606914881
3	2119061	4024010	1.89895902	1.599771614	0.039994290	3.999429036
4	2070061	4029857	1.946733454	1.631770565	0.040794264	4.079426413
5	2109061	3803610	1.803461351	1.535808005	0.038395200	3.839520011
6	2339066	3990610	1.706069859	1.470575927	0.036764398	3.676439817

C2: THC - Same Day Trial 2

IRM	ISTD	THC	THC/ISTD	THC CONC RATIO	THC CONC	%THC
1	2129061	3833974	1.800781659	1.534013168	0.038350329	3.835032919
2	2409061	3927037	1.630111068	1.419699309	0.035492483	3.549248272
3	2149126	3914010	1.821210111	1.547695988	0.038692400	3.869239971
4	2230201	4119857	1.847303001	1.565172807	0.039129320	3.912932018
5	2117642	3934610	1.858014716	1.572347432	0.039308686	3.930868581
6	2389066	4044610	1.692967042	1.461799760	0.036544994	3.654499401

C3: Relative Standard Deviation and Uncertainty associated with measurement

	THC CONC 1	THC CONC 2	Stdev	RSD	(Stdev)^2	df (n-1)	(sd^2)* (n-1)
	0.0382171	0.0383503	0.0001	0.2460	8.87298E-09	1	8.87298E-09
	0.0360691	0.0354925	0.0004	1.1396	1.66272E-07	1	1.66272E-07
	0.0399943	0.0386924	0.0009	2.3398	8.47459E-07	1	8.47459E-07
	0.0407943	0.0391293	0.0012	2.9460	1.38602E-06	1	1.38602E-06
	0.0383952	0.0393087	0.0006	1.6625	4.17228E-07	1	4.17228E-07
	0.0367644	0.036545	0.0002	0.4233	2.40691E-08	1	2.40691E-08
Mean	0.0384	0.0379			**Sum**	6	2.84992E-06
overall mean	0.0381						

Calculation of uncertainty

$$\text{Uncertainty} = \frac{\text{Sum total of }(sd^{2})*(n-1)}{\text{Sum total of df }(n-1)} \quad \text{---}(B1)$$

$$= \frac{2.84992E-06}{6}$$

$$= 0.000689$$

$$\text{Relative uncertainty} = \frac{\text{Uncertainty}}{\text{Overall mean}} \quad \text{--}(B2)$$

$$= \frac{0.000689}{0.0381}$$

$$=1.8067\%$$

C4: Repeatability CBN Same Day - Trial 1

IRM	ISTD Area	CBN Area	CBN/ISTD	CBN CONC RATIO	CBN CONC (mg/ml)	%CBN
1	2049061	301974	0.1474	0.2942	0.007353870	0.7354
2	2279061	311974	0.1369	0.2898	0.007245115	0.7245
3	2119061	307974	0.1453	0.2933	0.007332743	0.7333
4	2070061	297851	0.1439	0.2927	0.007317703	0.7318
5	2109061	306418	0.1453	0.2933	0.007332239	0.7332
6	2339066	319418	0.1366	0.2897	0.007241701	0.7242

C5: CBN Same Day - Trial 2

IRM	ISTD Area	CBN Area	CBN/ISTD	CBN CONC RATIO	CBN CONC (mg/ml)	% CBN
1	2129061	306574	0.1440	0.2928	0.007318842	0.7312
2	2409061	321974	0.1337	0.2885	0.007211551	0.7212
3	2149126	308474	0.1435	0.2926	0.007314068	0.7314
4	2230201	317851	0.1425	0.2921	0.007303556	0.7304
5	2117642	326851	0.1543	0.2970	0.007426216	0.7462
6	2389066	316630	0.1325	0.2880	0.007199952	0.7200

C6: Relative Standard Deviation and Uncertainty associated with measurement

`	CBN CONC 1	CBN CONC 2	Stdev	RSD	(Stdev)^2	df (n-1)	(sd^2)* (n-1)
	0.007354	0.007319	0.0000	0.3376	6.1348E-10	1	6.1348E-10
	0.007245	0.007212	0.0000	0.3283	5.63271E-10	1	5.63271E-10
	0.007333	0.007314	0.0000	0.1803	1.74378E-10	1	1.74378E-10
	0.007318	0.007304	0.0000	0.1368	1.00069E-10	1	1.00069E-10
	0.007332	0.007426	0.0001	0.9005	4.41584E-09	1	4.41584E-09
	0.007242	0.007200	0.0000	0.4088	8.7149E-10	1	8.7149E-10
Mean	0.0073	0.0073			**Sum**	6	6.73853E-09
overall mean	0.0073						

Calculation of Uncertainty

Uncertainty = $\frac{\text{Sum total of (sd^2)* (n-1)}}{\text{Sum total of df (n-1)}}$

$$= \frac{6.73853E-09}{6}$$

$$= 3.35E-05$$

Relative uncertainty = $\frac{\text{Uncertainty}}{\text{Overall mean}}$

$$= \frac{3.35E-05}{0.0381} \times 100$$

=0.45909%

C7: Repeatability - Same 1 - CBD Trial 1

IRM	ISTD	CBD/ISTD	CBD Conc Ratio	CBD Conc	% CBD
1	2049061	0.13696274	0.39345411	0.00983635	0.98363528
2	2279061	0.13559268	0.39295580	0.00982390	0.98238951
3	2119061	0.14500102	0.39637776	0.00990944	0.99094440
4	2070061	0.14312718	0.39569622	0.00989241	0.98924055
5	2109061	0.13781915	0.39376560	0.00984414	0.98441401
6	2339066	0.11968153	0.38716867	0.00967922	0.96792167

C8: Repeatability - Same Day - CBD Trial 2

IRM	ISTD	CBD/ISTD	CBD Conc Ratio	CBD Conc	% CBD
1	2129061	0.14498504	0.39637195	0.00990930	0.99092988
2	2409061	0.13020924	0.39099776	0.00977494	0.97749440
3	2149126	0.14980601	0.39812541	0.00995314	0.99531354
4	2230201	0.13284991	0.39195821	0.00979896	0.97989553
5	2117642	0.15077242	0.39847691	0.00996192	0.99619227
6	2389066	0.1259915	0.38946370	0.00973659	0.97365925

C9: Relative Standard Deviation and Uncertainty associated with measurement

	CBD CONC 1	CBD CONC 2	Stdev	RSD	(Stdev)^2	df (n-1)	(sd^2)* (n-1)
	0.0098364	0.009909	0.0001	0.5225	2.66085E-09	1	2.66085E-09
	0.0098239	0.009775	0.0000	0.3533	1.19854E-09	1	1.19854E-09
	0.0099094	0.009953	0.0000	0.3111	9.54845E-10	1	9.54845E-10
	0.0098924	0.009799	0.0001	0.6711	4.36645E-09	1	4.36645E-09
	0.0098441	0.009962	0.0001	0.8410	6.93606E-09	1	6.93606E-09
	0.0096792	0.009737	0.0000	0.4179	1.64566E-09	1	1.64566E-09
Mean	0.0098	0.0099			**Sum**	6	1.77624E-08
overall mean	0.0098						

Uncertainty = $\frac{\text{Sum total of (sd^2)* (n-1)}}{\text{Sum total of df (n-1)}}$

$$= \frac{1.77624\text{E-}08}{6}$$

$$= \underline{5.44\text{E-}05}$$

Relative uncertainty = $\frac{\text{Uncertainty}}{\text{Overall mean}}$

$$= \frac{\mathbf{5.44E\text{-}05}}{0.0381} \times 100$$

= 0.5527%

C10: Reproducibility - Intermediate Precision - Day 2 - Trial 1 for THC

	ISTD	THC	THC/ISTD	THC CONC RATIO	THC CONC	%THC
1	2149061	3783610	1.7606	1.507091447	0.037677286	3.7677
2	2389061	3893610	1.6298	1.419468076	0.035486702	3.5487
3	2224061	4114010	1.8498	1.566827799	0.039170695	3.9171
4	2169061	4117757	1.8984	1.599400768	0.039985019	3.9985
5	2214061	3913610	1.7676	1.511799169	0.037794979	3.7795
6	2442066	4080610	1.6710	1.447063834	0.036176596	3.6177

C11: Reproducibility - Intermediate Precision - Day 2 - Trial 2 for THC

IRM	ISTD	THC	THC/ISTD	THC CONC RATIO	THC CONC	%THC
1	2234061	3943974	1.7654	1.510303623	0.037757591	3.7758
2	2529061	4027037	1.5923	1.394377232	0.034859431	3.4859
3	2255126	4004010	1.7755	1.517089740	0.037927244	3.7927
4	2339201	4207757	1.7988	1.532686509	0.038317163	3.8317
5	2222542	4044610	1.8198	1.546759966	0.038668999	3.8669
6	2502066	4134610	1.6525	1.434680772	0.035867019	3.5867

C12: Relative Standard Deviation and Uncertainty associated with measurement

	THC CONC 1	THC CONC 2	Stdev	RSD	(Stdev)^2	df (n-1)	(sd^2)* (n-1)
	0.037677	0.037758	0.0001	0.1506	3.22445E-09	1	3.22445E-09
	0.035487	0.034859	0.0004	1.2610	1.96734E-07	1	1.96734E-07
	0.039171	0.037927	0.0009	2.2809	7.73085E-07	1	7.73085E-07
	0.039985	0.038317	0.0012	3.0123	1.39087E-06	1	1.39087E-06
	0.037795	0.038669	0.0006	1.6165	3.81955E-07	1	3.81955E-07
	0.036177	0.035867	0.0002	0.6077	4.7919E-08	1	4.7919E-08
Mean	0.0377	0.0372			**Sum**	6	2.79379E-06
overall mean	0.0375						

Uncertainty = <u>Sum total of (sd^2)* (n-1)</u>

Sum total of df (n-1)

$$= \frac{2.79379E-06}{6}$$

$$= 0.000682$$

$$\text{Relative uncertainty} = \frac{\text{Uncertainty}}{\text{Overall mean}}$$

$$= \frac{0.000682}{0.0375} \times 100$$

$$= 1.8209\%$$

Overall Relative uncertainty =Average of Day 1 and Day 2 uncertainty

= (1.806721% + 1.820919%)/2

= 1.8138%

C13: Reproducibility - Intermediate Precision - Day 2 - Trial 1 for CBN

	ISTD	CBN	CBN/ISTD	CBN CONC RATIO	CBN CONC	%CBN
1	2149061	301974	0.1405	0.291309598	0.007282740	0.7283
2	2389061	311974	0.1306	0.287189594	0.007179740	0.7180
3	2224061	307974	0.1385	0.290462916	0.007261573	0.7262
4	2169061	297851	0.1373	0.289983381	0.007249585	0.7250
5	2214061	306418	0.1384	0.290430822	0.007260771	0.7261
6	2442066	319418	0.1308	0.287278345	0.007181959	0.7182

C14: Day 2 Trial 2 for CBN

IRM	ISTD	CBN	CBN/ISTD	CBN CONC RATIO	CBN CONC	% CBN
1	2234061	307574	0.1377	0.290131460	0.007253286	0.7253
2	2529061	321974	0.1273	0.285830927	0.007145773	0.7146
3	2255126	308474	0.1368	0.289763473	0.007244087	0.7244
4	2339201	315851	0.1350	0.289032099	0.007225802	0.7226
5	2222542	320851	0.1444	0.292906056	0.007322651	0.7323
6	2502066	315630	0.1261	0.285348831	0.007133721	0.7134

C15: Relative Standard Deviation

`	CBN CONC 1	CBN CONC 2	Stdev	RSD	(Stdev)^2	df (n-1)	(sd^2)* (n-1)
	0.007283	0.007253	0.000021	0.2866	4.33769E-10	1	4.3377E-10
	0.007180	0.007146	0.000024	0.3353	5.76879E-10	1	5.7688E-10
	0.007262	0.007244	0.000012	0.1705	1.5288E-10	1	1.5288E-10
	0.007250	0.007226	0.000017	0.2324	2.82816E-10	1	2.8282E-10
	0.007261	0.007323	0.000044	0.6001	1.91457E-09	1	1.9146E-09
	0.007182	0.007134	0.000034	0.4765	1.16345E-09	1	1.1635E-09
Mean	0.0072	0.0072			**Sum**	6	4.5244E-09
overall mean	0.0072						

$$\textbf{Uncertainty} = \frac{\textbf{Sum total of (sd^2)* (n-1)}}{\textbf{Sum total of df (n-1)}}$$

$$= \frac{4.5244E-09}{6}$$

$$= 2.75E-05$$

$$\text{Relative uncertainty} = \frac{\text{Uncertainty}}{\text{Overall mean}} \times 100$$

$$= \frac{2.75E-05}{0.0072}$$

$$= 0.3799\%$$

Overall Relative uncertainty =Average of Day 1 and Day 2 uncertainty

= (0.459088% + 0.379889 %)/2

= 0.4195%

C16: Reproducibility - Intermediate Precision - Day 2 Trial 1 for CBD

	ISTD	CBD	CBD/ISTD	CBD CONC RATIO	CBD CONC	% CBD
1	2149061	290645	0.1352	0.392828540	0.009820713	0.9821
2	2389061	318024	0.1331	0.392055261	0.009801382	0.9801
3	2224061	318266	0.1431	0.395686801	0.009892170	0.9892
4	2169061	307282	0.1417	0.395164730	0.009879118	0.9879
5	2214061	301669	0.1363	0.393195403	0.009829885	0.9830
6	2442066	279943	0.1146	0.385332682	0.009633317	0.9633

C17: Day 2 Trial 2 for CBD

IRM	ISTD	CBD	CBD/ISTD	CBD CONC RATIO	CBD CONC	% CBD
1	2234061	321782	0.1440	0.396026247	0.009900656	0.9901
2	2529061	325782	0.1288	0.390490797	0.009762270	0.9762
3	2255126	330952	0.1468	0.397015871	0.009925397	0.9925
4	2339201	307282	0.1314	0.391417018	0.009785425	0.9785
5	2222542	331282	0.1491	0.397852423	0.009946311	0.9946
6	2502066	305002	0.1219	0.387975581	0.009699390	0.9699

Table C18: Relative Standard Deviation and Uncertainty associated with measurement

	CBD CONC 1	CBD CONC 2	Stdev	RSD	(Stdev)^2	df (n-1)	(sd^2)* (n-1)
	0.0098364	0.009901	0.0000	0.4608	2.06763E-09	1	2.06763E-09
	0.0098239	0.009762	0.0000	0.4450	1.89913E-09	1	1.89913E-09
	0.0099094	0.009925	0.0000	0.1138	1.27313E-10	1	1.27313E-10
	0.0098924	0.009785	0.0001	0.7689	5.7229E-09	1	5.7229E-09
	0.0098441	0.009946	0.0001	0.7301	5.21946E-09	1	5.21946E-09
	0.0096792	0.009699	0.0000	0.1472	2.03414E-10	1	2.03414E-10
Mean	0.0098	0.0098			**Sum**	6	1.52398E-08
overall mean	0.0098						

Uncertainty = <u>Sum total of (sd^2)* (n-1)</u>

Sum total of df (n-1)

$= \frac{1.52398E\text{-}08}{6}$

= 5.44E-05

Relative uncertainty = $\frac{\text{Uncertainty}}{\text{Overall mean}}$ x100

$= \frac{5.44E\text{-}05}{0.0098}$

= 0.5125%

Overall Relative uncertainty =Average of Day 1 and Day 2 uncertainty

= (0.552754%+0.512502%)/2

= 0.532628

Appendix D: Accuracy, recovery and uncertainties of measurements

D1: THC results for spiked samples

Spike	ISTD Conc	Replicate 1	Replicate 2	Average	ISTD Average response	Area ratio	Conc ratio	THC Conc
1	0.025	1253947	1249877	1251912	1996095	0.6272	0.7479	0.0187
2	0.025	3673610	3773610	3723610	2019422	1.8439	1.5629	0.0391
3	0.025	8907801	8897801	8902801	2026109	4.3940	3.2710	0.0818

D2: THC Results for accuracy and recovery

	Spiked Conc	Known Conc	Bias	Relative Bias	Recovery(%)
Spike 1	0.0187	0.0193	0.0006	3.1160	96.8840
Spike 2	0.0391	0.0393	0.0008	2.0694	99.4448
Spike 3	0.0818	0.0843	0.0025	2.9856	97.0144

D3: CBN results for spiked samples

Cbn	ISTD response	replicate 1	replicate 2	Average	response ratio	conc ratio	conc
spike 1	1996095	2198029	2282635	2240332	1.1224	0.6987	0.0175
spike 2	2019422	6030494	6026547	6028521	2.9853	1.4716	0.0368
spike 3	2026109	15542114	15514721	15528418	7.6642	3.4129	0.0853

D4: CBN results for accuracy and recovery

Spiked Conc	Known Conc	Bias	Relative Bias	Recovery
0.0175	0.0183	0.0008	4.5520	95.4480
0.0368	0.0300	0.0015	3.8397	96.1603
0.0853	0.0750	0.0021	2.4779	102.4779

D5: CBD results for spiked samples

CBD	ISTD response	replicate 1	replicate 2	average	response ratio	Conc ratio	Conc
spike 1	1996095	2064123	2054412	2059268	1.0317	0.7189	0.0180
spike 2	2019422	6284213	6312578	6298396	3.1189	1.4780	0.0370

spike 3	1926109	18412465	18425948	18419207	9.5629	3.8218	0.0956

D6: CBD results for accuracy and recovery

Spiked conc	Known Conc	Bias	Relative Bias (%)	Recovery (%)
0.0180	0.0188	0.0008	4.4062	95.5938
0.0370	0.0388	0.0013	3.3686	95.1346
0.0955	0.0938	0.0017	1.8168	101.8168

D7: Uncertainty associated with bias (U_{bias}) is calculated using equation

	THC	CBN	CBD
Bias (spike 1) (%)	3.1160	4.5520	4.4062
Bias (spike 2) (%)	2.0694	3.8397	3.3689
Bias (spike 3) (%)	2.9856	2.4779	1.8168
∑(bias)2 (%)	22.9057	41.6040	34.0648
RMS_{bias} (%)	2.7632	3.7240	3.3697
U_{Cref} (%)	0.40	0.10	0.10
U_{bias}	2.79	3.73	3.37

D8: Uncertainty associated with glassware used

Glassware	Error (±) ml	Error/Square root of 3 (X)	Relative uncertainty X/10 *100
10 ml measuring cylinder	0.2	0.1155	1.1547 %
100 ml measuring cylinder	1	0.5774	0.5774 %
250 ml volumetric flask	2	1.1547	0.4619 %

Appendix E - GCMS results for regional farm samples from ASH, ER and VR

E1: THC Results for regional farm samples from ASH, ER and VR

SAMPLE	THC Area Response	ISTD Area Response	THC Area Response Ratio	THC Conc Ratio	THC Conc (mg/ml)	%THC
ASH	1446984	1065874	1.36	2.75	0.0688	6.88
ER	899465	1198041	0.75	1.80	0.0450	4.50
VR	1331372	1199396	1.11	2.19	0.0548	5.48

E2: CDB Results for regional farm samples from ASH, ER and VR

SAMPLE	CBD Area Response	ISTD Area Response	CBD Area Response Ratio	CBD Conc Ratio	CBD Conc (mg/ml)	%CBD
ASH	90456	1065874	0.08	0.53	0.0133	1.33
ER	98654	1198041	0.08	0.52	0.0130	1.30
VR	88134	1199396	0.07	0.47	0.0118	1.18

APPENDIX F - ANALYSIS OF VARIANCE (MINITAB) FOR CANNABINOIDS SAMPLES ANALYZED IN THE STUDY

APPENDIX F1 - Comparing cannabinoids for regional samples for February and April GC-MS run

One-way ANOVA:

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.
```

1.   THC versus Eastern Region

```
Factor Information

Factor          Levels  Values
Eastern Region       6  Eastern 1, Eastern 1 (After 2 month), Eastern 2, Eastern 2 (After 2
                        month), Eastern 3, Eastern 3 (After 2 month)


Analysis of Variance

Source          DF   Adj SS   Adj MS  F-Value  P-Value
Eastern Region   5  6.39577  1.27915   685.26    0.000
Error            6  0.01120  0.00187
Total           11  6.40697
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Eastern Region               N    Mean  Grouping
Eastern 2                    2  3.5400  A
Eastern 2 (After 2 month)    2  3.1300    B
Eastern 1                    2  1.9600      C
Eastern 1 (After 2 month)    2  1.9200      C
Eastern 3                    2  1.8100      C
Eastern 3 (After 2 month)    2  1.6100        D
```

```
Means that do not share a letter are significantly different.
```

2. CBN versus Eastern Region

```
Factor Information

Factor          Levels  Values
Eastern Region       6  Eastern 1, Eastern 1 (After 2 month), Eastern 2,
Eastern 2 (After 2
                        month), Eastern 3, Eastern 3 (After 2 month)


Analysis of Variance

Source          DF   Adj SS   Adj MS  F-Value  P-Value
Eastern Region   5  23.7107  4.74213  3092.70    0.000
Error            6   0.0092  0.00153
Total           11  23.7199
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Eastern Region               N    Mean  Grouping
Eastern 1 (After 2 month)  2  4.6900  A
Eastern 1                    2  2.4800    B
Eastern 2 (After 2 month)  2  1.3600      C
Eastern 3 (After 2 month)  2  0.9400        D
Eastern 2                    2  0.8600        D
Eastern 3                    2  0.6900          E

Means that do not share a letter are significantly different.
```

3. THC_CR versus Central Region

```
Factor Information

Factor          Levels  Values
Central Region       6  Central 1, Central 1 (After 2 month), Central 2,
Central 2 (After 2
                        month), Central 3, Central 3 (After 2 month)


Analysis of Variance

Source          DF   Adj SS   Adj MS  F-Value  P-Value
Central Region   5  5.01984  1.00397  1029.71    0.000
Error            6  0.00585  0.00097
Total           11  5.02569
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Central Region              N     Mean  Grouping
Central 1                   2   2.7200  A
Central 1 (After 2 month)   2   2.5200    B
Central 3                   2   2.0100      C
Central 3 (After 2 month)   2   1.8000        D
Central 2                   2   1.1000          E
Central 2 (After 2 month)   2  1.00500          E

Means that do not share a letter are significantly different.
```

4. CBN_CR versus Central Region

```
Factor Information

Factor          Levels  Values
Central Region       6  Central 1, Central 1 (After 2 month), Central 2,
Central 2 (After 2
                        month), Central 3, Central 3 (After 2 month)


Analysis of Variance

Source          DF   Adj SS    Adj MS  F-Value  P-Value
Central Region   5  1.15594  0.231188   792.65    0.000
Error            6  0.00175  0.000292
Total           11  1.15769
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Central Region              N     Mean  Grouping
Central 2 (After 2 month)   2   1.6250  A
Central 2                   2   1.3400    B
Central 1 (After 2 month)   2   1.3400    B
Central 1                   2  0.97500      C
Central 3 (After 2 month)   2  0.95500      C
Central 3                   2   0.6900        D

Means that do not share a letter are significantly different.
```

5. THC_BAR versus Brong Ahafo Region

```
Factor Information

Factor               Levels  Values
Brong Ahafo Region        6  Brong Ahafo 1, Brong Ahafo 1 (After 2 month),
Brong Ahafo 2,
                             Brong Ahafo 2 (After 2 month), Brong Ahafo 3,
Brong Ahafo 3
                             (After 2 month)


Analysis of Variance

Source               DF   Adj SS   Adj MS   F-Value  P-Value
Brong Ahafo Region    5  119.575  23.9151  28698.11    0.000
Error                 6    0.005   0.0008
Total                11  119.580
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Brong Ahafo Region             N    Mean  Grouping
Brong Ahafo 3                  2  8.3200  A
Brong Ahafo 3 (After 2 month)  2   7.910    B
Brong Ahafo 2                  2  2.0900      C
Brong Ahafo 2 (After 2 month)  2  1.8900        D
Brong Ahafo 1                  2  0.9800          E
Brong Ahafo 1 (After 2 month)  2  0.9700          E

Means that do not share a letter are significantly different.
```

6. CBN_BAR versus Brong Ahafo Region

```
Factor Information

Factor               Levels  Values
Brong Ahafo Region        6  Brong Ahafo 1, Brong Ahafo 1 (After 2 month),
Brong Ahafo 2,
                             Brong Ahafo 2 (After 2 month), Brong Ahafo 3,
Brong Ahafo 3
                             (After 2 month)


Analysis of Variance

Source               DF   Adj SS    Adj MS  F-Value  P-Value
Brong Ahafo Region    5  1.68724  0.337448   525.89    0.000
Error                 6  0.00385  0.000642
Total                11  1.69109
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Brong Ahafo Region             N    Mean  Grouping
Brong Ahafo 2 (After 2 month)  2  2.0100  A
Brong Ahafo 1 (After 2 month)  2  1.7700    B
Brong Ahafo 3 (After 2 month)  2  1.4650      C
Brong Ahafo 1                  2  1.3700      C D
Brong Ahafo 2                  2  1.3400        D
Brong Ahafo 3                  2  0.8100          E

Means that do not share a letter are significantly different.
```

7. THC_ASH versus ASHANTI

```
Factor Information

Factor   Levels  Values
ASHANTI       6  Ashanti 1, Ashanti 1 (After 2 month), Ashanti 2, Ashanti 2
(After 2 month),
                 Ashanti 3, Ashanti 3 (After 2 month)


Analysis of Variance

Source   DF   Adj SS   Adj MS  F-Value  P-Value
ASHANTI   5  12.2100  2.44201  5141.07    0.000
Error     6   0.0028  0.00047
Total    11  12.2129
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

ASHANTI                    N     Mean  Grouping
Ashanti 3                  2  3.81500  A
Ashanti 3 (After 2 month)  2   3.6000    B
Ashanti 2                  2   3.2100      C
Ashanti 2 (After 2 month)  2   3.1300      C
Ashanti 1                  2   1.3700        D
Ashanti 1 (After 2 month)  2   1.3400        D

Means that do not share a letter are significantly different.
```

8. CBN_ASH versus ASHANTI

```
Factor Information

Factor   Levels  Values
ASHANTI       6  Ashanti 1, Ashanti 1 (After 2 month), Ashanti 2, Ashanti 2
(After 2 month),
                 Ashanti 3, Ashanti 3 (After 2 month)
```

```
Analysis of Variance

Source    DF    Adj SS    Adj MS  F-Value  P-Value
ASHANTI    5  0.900267  0.180053   348.49    0.000
Error      6  0.003100  0.000517
Total     11  0.903367
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

ASHANTI                     N     Mean  Grouping
Ashanti 1 (After 2 month)  2   1.4800  A
Ashanti 3 (After 2 month)  2   1.2400    B
Ashanti 2 (After 2 month)  2   1.1750    B
Ashanti 1                  2   1.0700      C
Ashanti 3                  2   0.7400        D
Ashanti 2                  2  0.70500        D

Means that do not share a letter are significantly different.
```

9. THC_WR versus WESTERN

```
Factor Information

Factor   Levels  Values
WESTERN       6  Western 1, Western 1 (After 2 month), Western 2, Western 2
(After 2 month),
                 Western 3, Western 3 (After 2 month)


Analysis of Variance

Source    DF   Adj SS   Adj MS   F-Value  P-Value
WESTERN    5  52.8402  10.5680  23927.66    0.000
Error      6   0.0026   0.0004
Total     11  52.8429
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

WESTERN                     N    Mean  Grouping
Western 2                  2  7.0700  A
Western 2 (After 2 month)  2  4.0300    B
Western 3                  2  1.9100      C
Western 3 (After 2 month)  2  1.7400        D
Western 1                  2  1.2950          E
Western 1 (After 2 month)  2  1.2100            F

Means that do not share a letter are significantly different.
```

10. CBN_WR versus WESTERN

```
Factor Information

Factor   Levels  Values
WESTERN       6  Western 1, Western 1 (After 2 month), Western 2, Western 2
(After 2 month),
                 Western 3, Western 3 (After 2 month)


Analysis of Variance

Source   DF   Adj SS    Adj MS  F-Value  P-Value
WESTERN   5  1.76247  0.352493   682.25    0.000
Error     6  0.00310  0.000517
Total    11  1.76557
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

WESTERN                     N     Mean  Grouping
Western 2 (After 2 month)   2   1.8100  A
Western 1 (After 2 month)   2   1.5650    B
Western 2                   2   1.4700      C
Western 1                   2   1.0900        D
Western 3 (After 2 month)   2  1.00500        D
Western 3                   2   0.6700          E

Means that do not share a letter are significantly different.
```

11. THC_VR versus VOLTA

```
Factor Information

Factor  Levels  Values
VOLTA        6  Volta 1, Volta 1 (After 2 month), Volta 2, Volta 2 (After 2
month), Volta 3,
                Volta 3 (After 2 month)


Analysis of Variance

Source  DF    Adj SS    Adj MS  F-Value  P-Value
VOLTA    5  0.705642  0.141128   376.34    0.000
Error    6  0.002250  0.000375
Total   11  0.707892
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

VOLTA                     N     Mean  Grouping
Volta 2                   2   1.5700  A
Volta 1                   2   1.4300    B
Volta 2 (After 2 month)   2   1.3200      C
Volta 1 (After 2 month)   2   1.2400        D
Volta 3                   2  0.92500          E
Volta 3 (After 2 month)   2   0.9200          E

Means that do not share a letter are significantly different.
```

12. CBN_VR versus VOLTA

```
Factor Information

Factor  Levels  Values
VOLTA        6  Volta 1, Volta 1 (After 2 month), Volta 2, Volta 2 (After 2
month), Volta 3,
                Volta 3 (After 2 month)


Analysis of Variance

Source  DF   Adj SS   Adj MS  F-Value  P-Value
VOLTA    5  8.23384  1.64677  6814.21    0.000
Error    6  0.00145  0.00024
Total   11  8.23529
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

VOLTA                     N     Mean  Grouping
Volta 1 (After 2 month)   2  3.07500  A
Volta 2 (After 2 month)   2   2.8000    B
Volta 1                   2    2.480      C
Volta 2                   2   2.1300        D
Volta 3 (After 2 month)   2   1.1400          E
Volta 3                   2   0.8300            F

Means that do not share a letter are significantly different.
```

13. THC_UE versus UPPER EAST

```
Factor Information

Factor      Levels  Values
UPPER EAST       6  Upper East 1, Upper East 1 (After 2 month), Upper East 2,
Upper East 2
                    (After 2 month), Upper East 3, Upper East 3 (After 2
month)
```

```
Analysis of Variance

Source       DF   Adj SS   Adj MS   F-Value  P-Value
UPPER EAST    5  78.4138  15.6828  64894.16    0.000
Error         6   0.0014   0.0002
Total        11  78.4152
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

UPPER EAST                    N     Mean  Grouping
Upper East 1                  2  7.27500  A
Upper East 1 (After 2 month)  2   7.2200  A
Upper East 2                  2   2.6700    B
Upper East 2 (After 2 month)  2   2.3300      C
Upper East 3                  2    1.400        D
Upper East 3 (After 2 month)  2   1.2900          E

Means that do not share a letter are significantly different.
```

14.   CBN_UE versus UPPER EAST

```
Factor Information

Factor      Levels  Values
UPPER EAST       6  Upper East 1, Upper East 1 (After 2 month), Upper East 2,
Upper East 2
                     (After 2 month), Upper East 3, Upper East 3 (After 2
month)


Analysis of Variance

Source       DF   Adj SS   Adj MS   F-Value  P-Value
UPPER EAST    5  11.6091  2.32183  10319.24    0.000
Error         6   0.0014  0.00023
Total        11  11.6105
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

UPPER EAST                    N     Mean  Grouping
Upper East 1 (After 2 month)  2  4.14500  A
Upper East 2 (After 2 month)  2   2.0500    B
Upper East 3 (After 2 month)  2  2.00500    B
Upper East 1                  2  1.47500      C
Upper East 3                  2   1.3500        D
Upper East 2                  2   1.2800          E
```

```
Means that do not share a letter are significantly different.
```

15. THC_UW versus UPPER WEST

```
Factor Information

Factor       Levels  Values
UPPER WEST        6  Upper West 1, Upper West 1 (After 2 month), Upper West 2,
Upper West 2
                     (After 2 month), Upper West 3, Upper West 3 (After 2
month)


Analysis of Variance

Source       DF   Adj SS   Adj MS   F-Value  P-Value
UPPER WEST    5  132.131  26.4263  55634.29    0.000
Error         6    0.003   0.0005
Total        11  132.134
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

UPPER WEST                  N     Mean  Grouping
Upper West 3                2  15.1400  A
Upper West 3 (After 2 month)  2  14.3100    B
Upper West 1                2  11.1000      C
Upper West 1 (After 2 month)  2  9.18500        D
Upper West 2                2   6.9100          E
Upper West 2 (After 2 month)  2   6.6200            F

Means that do not share a letter are significantly different.
```

16. CBN_UW versus UPPER WEST

```
Factor Information

Factor       Levels  Values
UPPER WEST        6  Upper West 1, Upper West 1 (After 2 month), Upper West 2,
Upper West 2
                     (After 2 month), Upper West 3, Upper West 3 (After 2
month)


Analysis of Variance

Source       DF   Adj SS    Adj MS  F-Value  P-Value
UPPER WEST    5  1.11577  0.223153   637.58    0.000
Error         6  0.00210  0.000350
Total        11  1.11787
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

UPPER WEST                    N    Mean  Grouping
Upper West 1 (After 2 month)  2  1.5850  A
Upper West 3 (After 2 month)  2  1.5000    B
Upper West 2 (After 2 month)  2  1.2300      C
Upper West 1                  2   1.030        D
Upper West 3                  2  0.9250          E
Upper West 2                  2  0.7300            F

Means that do not share a letter are significantly different.
```

17. THC_NR versus NORTHERN

```
Factor Information

Factor    Levels  Values
NORTHERN       6  Northern 1, Northern 1 (After 2 month), Northern 2,
Northern 2 (After 2
                  month), Northern 3, Northern 3 (After 2 month)


Analysis of Variance

Source    DF   Adj SS   Adj MS    F-Value  P-Value
NORTHERN   5  164.328  32.8656  281705.43    0.000
Error      6    0.001   0.0001
Total     11  164.329
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

NORTHERN                    N     Mean  Grouping
Northern 3                  2  11.0600  A
Northern 3 (After 2 month)  2   8.1700    B
Northern 2                  2    3.010      C
Northern 1                  2   2.0100        D
Northern 1 (After 2 month)  2  1.85500          E
Northern 2 (After 2 month)  2  1.29500            F

Means that do not share a letter are significantly different.
```

18. CBN_NR versus NORTHERN

```
Factor Information

Factor    Levels  Values
NORTHERN       6  Northern 1, Northern 1 (After 2 month), Northern 2,
Northern 2 (After 2
                  month), Northern 3, Northern 3 (After 2 month)


Analysis of Variance

Source     DF   Adj SS    Adj MS  F-Value  P-Value
NORTHERN    5  4.90504  0.981008  3181.65    0.000
Error       6  0.00185  0.000308
Total      11  4.90689
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

NORTHERN                    N     Mean  Grouping
Northern 2 (After 2 month)  2   3.0400  A
Northern 3 (After 2 month)  2  2.03500    B
Northern 1 (After 2 month)  2    1.730      C
Northern 1                  2   1.6500        D
Northern 2                  2   1.1700          E
Northern 3                  2    1.140          E

Means that do not share a letter are significantly different.
```

19. THC_GR versus GREATER ACCRA

```
Factor Information

Factor         Levels  Values
GREATER ACCRA       6  Greater A 1, Greater A 1 (After 2 month), Greater A 2,
Greater A 2
                       (After 2 month), Greater A 3, Greater A 3 (After 2
month)


Analysis of Variance

Source         DF    Adj SS    Adj MS  F-Value  P-Value
GREATER ACCRA   5  0.278942  0.055788   142.44    0.000
Error           6  0.002350  0.000392
Total          11  0.281292
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

GREATER ACCRA                N     Mean  Grouping
```

```
Greater A 2                   2   1.8600  A
Greater A 3                   2   1.7700    B
Greater A 1                   2  1.58500      C
Greater A 1 (After 2 month)   2  1.48500        D
Greater A 2 (After 2 month)   2   1.4800        D
Greater A 3 (After 2 month)   2   1.4750        D

Means that do not share a letter are significantly different.
```

20. CBN_GR versus GREATER ACCRA

```
Factor Information

Factor         Levels  Values
GREATER ACCRA       6  Greater A 1, Greater A 1 (After 2 month), Greater A 2,
Greater A 2
                        (After 2 month), Greater A 3, Greater A 3 (After 2
month)


Analysis of Variance

Source         DF   Adj SS   Adj MS  F-Value  P-Value
GREATER ACCRA   5  10.9378  2.18755  6250.15    0.000
Error           6   0.0021  0.00035
Total          11  10.9399
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

GREATER ACCRA                 N    Mean  Grouping
Greater A 3 (After 2 month)   2  4.0850  A
Greater A 3                   2  3.2250    B
Greater A 2 (After 2 month)   2   1.870      C
Greater A 1 (After 2 month)   2  1.7200        D
Greater A 2                   2  1.6300          E
Greater A 1                   2  1.6100          E

Means that do not share a letter are significantly different.
```

APPENDIX F2 - Comparing fresh and seized samples

1. THC versus Regions

```
Factor Information

Factor   Levels  Values
Regions      12  Ashanti  2 (seized), Ashanti  3 (seized), Ashanti (fresh),
Ashanti 1
                 (seized), Eastern (fresh), Eastern 1 (seized), Eastern 2
(seized), Eastern 3
                 (seized), Volta (fresh), Volta 1 (seized), Volta 2 (seized),
Volta 3
                 (seized)
```

```
Analysis of Variance

Source   DF   Adj SS   Adj MS  F-Value  P-Value
Regions  11  76.5402  6.95820  3821.44    0.000
Error    12   0.0218  0.00182
Total    23  76.5621
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Regions              N    Mean  Grouping
Ashanti (fresh)      2  6.8800  A
Volta (fresh)        2  5.4600    B
Eastern (fresh)      2  4.4900      C
Ashanti  3 (seized)  2  3.8200        D
Eastern 2 (seized)   2  3.5450          E
Ashanti  2 (seized)  2  3.2100            F
Eastern 1 (seized)   2  1.9600              G
Eastern 3 (seized)   2  1.8100              G
Volta 2 (seized)     2   1.570                H
Volta 1 (seized)     2  1.4300                H I
Ashanti 1 (seized)   2  1.3700                  I
Volta 3 (seized)     2  0.9300                    J

Means that do not share a letter are significantly different.
```

2. CBN versus Regions

```
Factor Information

Factor   Levels  Values
Regions      12  Ashanti  2 (seized), Ashanti  3 (seized), Ashanti (fresh),
Ashanti 1
                 (seized), Eastern (fresh), Eastern 1 (seized), Eastern 2
(seized), Eastern 3
                 (seized), Volta (fresh), Volta 1 (seized), Volta 2 (seized),
Volta 3
                 (seized)


Analysis of Variance

Source   DF   Adj SS   Adj MS  F-Value  P-Value
Regions  11  17.9114  1.62831  2159.09    0.000
Error    12   0.0091  0.00075
Total    23  17.9205
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Regions               N      Mean  Grouping
Volta 1 (seized)      2    2.4800  A
Eastern 1 (seized)    2    2.4800  A
Volta 2 (seized)      2    2.1300    B
Ashanti 1 (seized)    2    1.0700      C
Eastern 2 (seized)    2    0.8600        D
Volta 3 (seized)      2    0.8300        D E
Ashanti  3 (seized)   2    0.7400          E F
Ashanti  2 (seized)   2    0.7050            F
Eastern 3 (seized)    2    0.7000            F
Volta (fresh)         2  0.000000              G
Eastern (fresh)       2  0.000000              G
Ashanti (fresh)       2  0.000000              G

Means that do not share a letter are significantly different.
```

3. CBD versus Regions

```
Factor Information

Factor   Levels  Values
Regions      12  Ashanti  2 (seized), Ashanti  3 (seized), Ashanti (fresh),
Ashanti 1
                 (seized), Eastern (fresh), Eastern 1 (seized), Eastern 2
(seized), Eastern 3
                 (seized), Volta (fresh), Volta 1 (seized), Volta 2 (seized),
Volta 3
                 (seized)


Analysis of Variance
```

```
Source    DF  Adj SS   Adj MS  F-Value  P-Value
Regions   11  1.3002  0.11820     2.27    0.087
Error     12  0.6241  0.05200
Total     23  1.9243
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Regions               N    Mean  Grouping
Eastern 2 (seized)    2   1.690  A
Ashanti (fresh)       2  1.3300  A
Eastern (fresh)       2   1.310  A
Volta (fresh)         2  1.1800  A
Ashanti 1 (seized)    2  1.0200  A
Ashanti  3 (seized)   2  0.9800  A
Ashanti  2 (seized)   2  0.9750  A
Volta 2 (seized)      2  0.9700  A
Volta 1 (seized)      2  0.9300  A
Eastern 1 (seized)    2  0.9300  A
Eastern 3 (seized)    2  0.9000  A
Volta 3 (seized)      2  0.8800  A

Means that do not share a letter are significantly different.
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