Method development and validation for the analysis of medicinal cannabis products, aimed at developing a safety profile for South Africa

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PREFACE

This research project/study was reviewed by the North-West University Health Research Ethics Committee (NWU-HREC) which concluded that this study does not need ethical approval. An attachment of this review can be found in Annexure A at the end of this thesis.

This thesis is comprised of three individual manuscripts, dictating current safety issues regarding cannabis-based products in South Africa. An introduction and supplementary information are given in the first two chapters after which the three research articles follow. Finally, a conclusion encompassing related issues of all three manuscripts, is given.

The first manuscript titled: "*An Assessment of the Potency Related to Cannabis-Based Products in the South African Market*" was published by Elsevier in the journal Forensic Science International. The complete article can be found in Annexure B, including the author guidelines for the journal Forensic Science International in Annexure C, at the end of this thesis.

The second manuscript titled: "*An Assessment of Heavy Metal Contaminants Related to Cannabis-Based Products in the South African Market*" was published by Elsevier in the journal Forensic Science International: Reports. The complete article can be found in Annexure D, including the author guidelines for the journal Forensic Science International: Reports in Annexure E, at the end of this thesis.

The third manuscript titled: "*An Assessment of Solvent Residue Contaminants Related to Cannabis-Based Products in the South African Market*" was submitted to the Journal of cannabis research on the 24th of July 2021. The Article has been assigned to the Editor, and is currently awaiting feedback.

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-Hendrik Jacobus Viviers: Conceptualisation, Methodology, Experimental investigation, Data curation, Writing original draft.

-Anél Petzer: Supervision, Writing - review & editing.

-Richard Gordon: Writing - review & editing.

-Jacques Petzer: Supervision, Writing - proofreading.

ABSTRACT

In the last few years, there has been an increase in the use of cannabis-based products for medicinal purposes. The preparation procedure, however, has not been standardised but is decided by the individual groups of scientists globally. As cannabis-based products are more frequently used for medicinal and recreational uses, standardised quality control methods must be developed, validated and harmonised. This study relates to the three major quality control parameters of interest in cannabis-based products in South Africa. These three quality control tests include cannabis potency, residual solvent residues and heavy metal residues.

For cannabis potency a total of 840 samples were analysed in duplicate (1680 datapoints in total) and reported in an anonymous format. Samples were categorised into 7 different types: Edible, Extract, Infusion, Liquid, Other, Plant material, and Solid. Each category was divided into the following weight by percentage concentration levels: <0.1 wt.-%, 0.1 wt.-% to 1 wt.-% and finally >1 wt.-%. The results indicated that high amounts of tetrahydrocannabinol (THC) are present in most of the cannabis-based products in South Africa. This is of concern due to the health implications of these products, and the current South African legislation related to cannabidiol (CBD) and THC.

For heavy metal residues a total of 310 samples were analysed. The submitted samples were divided into different category classifications and grouped according to relevance for oral or inhalation specification. The results showed an alarming 15% sample failure rate compared to the oral specification limit and a 44% failure rate compared to the inhalation specification limit. It is of the utmost importance for manufacturers to have the appropriate quality control regimes in place, especially for heavy metal residues, in South Africa. Furthermore, it is imperative to ensure regulation is enforced and the South African public is educated about the risks associated with using these products.

Solvent residue analysis employed a total of 279 samples in duplicate and the results are reported in an anonymised format. The results showed an alarming 37% sample solvent residue failure rate with respect to adherence to USP 467 specification.

As seen from the facets discussed above, manufacturers, as well as cultivators should adhere to the current GMP guidelines set forth, to ensure the safety of the consumer as well as the quality of the product produced. With the current findings the safety of consumers is brought into question. With this data South African regulators as well as the public might be informed about the current state of cannabis-based products.

KEYWORDS

Cannabis-based products, Potency, Heavy Metals, Residual Solvents, South Africa, THC, CBD, Impurities, Cannabis.

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LIST OF ABBREVIATIONS

ADI	Allowed daily intake
AOAC	Association of Official Agricultural Chemists
BHO	Butane hash oil
CBC	Cannabichromene
CBD	Cannabidiol
CBDA	Cannabidiolic Acid A
CBE	Cannabielsoin
CBG	Cannabigerol
CBGA	Cannabigerolic Acid A
CBL	Cannabicyclol
CBN	Cannabinol
CBND	Cannabinodiol
CBT	Cannabitriol
CBTV	Cannabitrivarin
CFU	Colony Forming Units
CoA	Certificate of Analysis
CRM	Certified Reference Material
DEA	Drug Enforcement Agency
EPA	Environmental Protection Agency
EPTC	s-Ethyl dipropylthiocarbamate
Eq	Equation
FDA	Food and Drug Administration
FECO	Full Extract Cannabis Oil
GC	Gas Chromatography
GC-FID	Gas Chromatography – Flame Ionisation Detector
GC-MS	Gas Chromatography – Mass Spectrometry
GMP	Good Manufacturing Practice
HPLC	High-Performance Liquid Chromatography
HS GC-MS	Headspace Gas Chromatography – Mass Spectrometry
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantitation
MCC	Medicines Control Council
MCPA	2-Methyl-4-chlorophenoxyacetic acid
MCT	Medium-Chain Triglyceride
MRL	Maximum Residue Limit
MSMA	Monosodium methyl arsonate
MTBE	Methyl Tertbutyl Ether
NCSL	National Conference of State Legislatures
ND	Not Detected
NDoH	National Department of Health
NWU-HREC	North-West University Health Research Ethics Committee
ppb	Parts per billion
ppt	Parts per trillion
PIC/S	Pharmaceutical inspection co-operation scheme
QC	Quality Control
RSD	Relative Standard Deviation
RSO	Rick Simpson Oil
SAHPRA	South African Health Products Regulatory Authority
THC	∆9-Tetrahydrocannabinol
THCA	∆9-Tetrahydrocannabinolic Acid A
THCB	∆9-Tetrahydrocannabinolic Acid B
UNODC	United Nations Office on Drugs and Crime
US EPA	Unites States Environmental Protection Agency
USP	(United States Pharmacopoeia
WHO	World Health Organisation

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CHAPTER 1

1 Introduction

1.1 Introduction

In the last few years, there has been an increase in the use of cannabis-based products for medicinal purposes. The preparation procedure, however, has not been standardised but is decided by the individual groups of scientists globally.

As cannabis-based products are more frequently used for medicinal and recreational uses, standardised methods of analysis must be developed, validated and harmonised. The complexity of cannabis drives the need for advanced analysis of the individual components. There are many analytical challenges in the analysis of cannabis. Since no federal regulations are currently implemented in the United States, laboratories adhere to their own standards.

In South Africa, schedule 7 substances are considered to have no genuine medicinal use and can only be accessed by means of a permit issued by the Director-General of the National Department of Health (NDoH). Medicines and substances categorised as Schedule 4 to Schedule 6 are only available on prescription and can only be obtained from a pharmacy (South African Health Products Regulatory Authority (SAHPRA), 2020:1–8).

The South African Medicines Control Council (MCC) has rescheduled the cannabinoid, cannabidiol (CBD), to a schedule 4 substance (Bowles *et al.*, 2012:1–10). This rescheduling was mainly the result of the international interest and focus specifically on CBD (Medicines Control Council (MCC), 2017:1–32). A myriad of cannabis including the former mentioned CBD products are accessible in United States dispensaries as well as online. However, none of these unregulated products has been approved by the Food and Drug Administration (FDA). Furthermore, United States laws on quality control are inconsistent and in some cases absent. In the United States, controlled clinical trials are proceeding to examine the use of CBD as a possible treatment for several types of neurological disorders.

In a government notice published by the South African Government, in conjunction with the Department of Health, scheduling of cannabis-based products are as follows; tetrahydrocannabinol (THC) is regarded as schedule 7 (Illicit), but when a registered

medicine containing THC is employed for therapeutic use, it is regarded as a schedule 6 substance. The exception to the latter is processed hemp fibre and products thereof, in a form not suitable for ingestion, smoking, or inhaling purposes, and containing not more than 0.1 % THC; or when present in processed products from cannabis seed containing not more than 0.001 % THC, are regarded as industrial hemp (Republic of South Africa, 2020:1–12; South African Health Products Regulatory Authority (SAHPRA), 2020:1–8). As mentioned CBD is listed as a schedule 4 substance, with certain CBD preparations regarded as unscheduled (schedule 0) when adhering to one of two conditions (Republic of South Africa, 2020:1–12)

The MCC briefed Parliament on 23 November 2016 on its research in light of the proposed Medical Innovation Bill, which seeks to legalise the plant for medicinal use. The MCC told members of parliament that it has made progress in its investigation into the medicinal use of cannabis. Briefing the National Assembly committee on health, MCC registrar Dr Joey Gouws said by February 2017, the regulatory body could start the regulation process by issuing permits to allow the controlled cultivation and supply of standardised high quality medicinal cannabis products.

Over the last few decades an increase in cannabis plant potency (THC wt.-%) has been perceived (Brenneisen, 2007:17–49). The constituents as well as their concentrations in medicinal cannabis-based products may vary considerably depending on a number of parameters. Taking this into account it is imperative for South Africa to establish its own cannabis-based testing guidelines, to circumvent the inconsistencies currently experienced in the United States. Additionally, these testing guidelines will ensure that variations during cultivation do not influence the final products consumed by the public. With a set standard or specification, it is the aim that cannabis-based products be tested for adherence to this specification, therefore regulating the products being used in the South African market.

1.2 Background

The following section will provide some insights on the active ingredient composition of cannabis-based products, the medicinal applications of cannabis-based products and finally the analytical quality control, predominantly employed globally to test these products.

1.2.1 Cannabis Active Ingredients

About 483 of the chemical compounds present in the cannabis plant have been identified. These vary depending on a number of factors which may include; strain, growing conditions, climate and nutrients. Terpenes are the most abundant class with about 140 compounds. Terpenes are widespread throughout the plant kingdom. Second is a group of C21 terpenophenolic compounds uniquely found in *Cannabis*. These compounds are labelled as cannabinoids. Cannabinoids are divided into 10 subclasses (Brenneisen, 2007:17–49):

1.2.1.1 Cannabinoids

1.2.1.1.1 Cannabigerol (CBG) type:

CBG was the first identified cannabinoid (Gaoni and Mechoulam, 1964:1646–1647) and its precursor, cannabigerolic acid (CBGA), is considered to be the first biogenic cannabinoid formed in the plant (Shoyama *et al.*, 1975:2189–2192). All cannabinoids are produced from this CBG/CBGA precursor.

1.2.1.1.2 Cannabichromene (CBC) type:

Five CBC-type cannabinoids, mainly present as C5- analogues, have been identified (Brenneisen, 2007:17–49; Eisohly *et al.*, 1982:1319–1323).

1.2.1.1.3 Cannabidiol (CBD) type:

CBD was isolated in 1940 (Adams and Hunt, 1940:196–200) but the structure was only elucidated in 1963 (Mechoulam and Shvo, 1963:2073–2078). Seven CBD-like cannabinoids containing 1 to 5 carbon side-chains have been described. CBD and its matching acid, CBDA, are the most abundant cannabinoids in fibre-type Cannabis plants also known as hemp (Brenneisen, 2007:17–49; Burstein, 2015:1377–1385).

1.2.1.1.4 Δ9-tetrahydrocannabinol (THC) type:

Nine THC-type cannabinoids with 1 to 5 carbon side-chains are currently known. The foremost precursor produced by cannabis plant is tetrahydrocannabinolic acid A (THCA). Tetrahydrocannabinolic acid B (THCB) is present to a much lesser extent. THC is the main psychoactive constituent of phytocannabinoids; the acids are non-psychoactive (Brenneisen, 2007:17–49; Gaoni and Mechoulam, 1964:1646–1647).

1.2.1.1.5 *Δ8-THC type: Δ8-THC and its acid precursor:*

These ∆8 cannabinoids are regarded as the thermal breakdown products of ∆9-THC and ∆9-THCA as result of the more stable 8,9-double-bond (Brenneisen, 2007:17–49). Further thermal breakdown of ∆9 THC to cannabinol (CBN) occurs at different rates depending on temperature (Repka *et al*., 2006:21–32).

1.2.1.1.6 *Cannabicyclol (CBL) type:*

Three cannabinoids characterised by a five-membered ring and C1-bridge instead of the typical ring A, are known: These include cannabicyclol (CBL), CBL acid precursor and the 3 carbon side-chain analogue. CBL is known to be thermally labile, produced from CBC (Brenneisen, 2007:17–49).

1.2.1.1.7 *Cannabielsoin (CBE) type:*

Five cannabielsoin (CBE) type cannabinoids exist, CBE and its respective acid precursors A and B. CBE type cannabinoids are by-products formed from CBD (Brenneisen, 2007:17–49).

1.2.1.1.8 *Cannabinol (CBN) and Cannabinodiol (CBND) types:*

Six cannabinol (CBN) and two cannabinodiol (CBND) type cannabinoids have been identified. They are oxidation by-products of THC and CBD, respectively. Their abundance in cannabis-based products is influenced by the age and storage conditions of these products. CBN was first named in 1896 by Raphael Mechoulam (Mechoulam *et al*., 2007:1678–1692) and its structure elucidated in 1940 (Adams *et al*., 1940:2204–2207).

1.2.1.1.9 *Cannabitriol (CBT) type:*

Nine cannabitriol (CBT) type cannabinoids have been recognised which are characterised by supplementary OH exchange. CBT itself exists as all four probable stereoisomers, whereas only two isomers (9-α- and 9-β-hydroxy) of (cannabitrivarin) CBTV have been identified. CBDA tetrahydrocannabitriol ester (ester at 9-hydroxy group) is the only ester of any naturally occurring cannabinoids (Brenneisen, 2007:17–49; Chan *et al*., 1976:283–284).

1.2.1.1.10 Miscellaneous types:

Eleven cannabinoids of uncommon structures, e.g. with a benzofuran ring (dehydrocannabifuran, cannabifuran), carbonyl function (cannabichromanon, 10-oxo-δ-6a tetrahydrocannabinol) or tetrahydroxy substitution (cannabiripsol) are known (Brenneisen, 2007:17–49).

From 1980 to 1997 a total of 35213 samples of confiscated cannabis products (cannabis plant material, oil etc.) representative of more than 7,717 tons seized in the United States were analysed by gas chromatography (GC) (Mehmedic *et al.*, 2010:1209–1217). THC concentration is usually measured as wt.-% (percentage weight). The cannabinoid mass percentage per mass of sample. The average THC concentration increased from less than 1.5 wt.-% in 1980 to 4.2 wt.-% in 1997. The maximum THC wt.-% found was 29.9 and 33.1% in marijuana and sinsemilla cannabis, respectively. The maximum THC concentrations analysed were 52.9 and 47.0 wt.-%, respectively (Mehmedic *et al.*, 2010:1209–1217). Two studies performed in Switzerland from 1981 to 1985 as well as 2002 to 2003 uncovered average sample THC amounts of 1.4 and 12.9 wt.-%, respectively (Brenneisen, 2007:17–49). Maximum concentrations were 4.8 wt.-% and 28.4 wt.-%, respectively. A myriad of reasons could be postulated for the increase in potency (THC wt.-%) which could include advancement in cultivation, tendencies to prefer indoor cultivation as opposed to outdoor, and the global access to and exchange of genetic seed stemming from high-THC cultivars (Brenneisen, 2007:17–49).

1.2.1.2 Terpenes

The typical scent or aroma of cannabis results from about 140 different terpenes. Isoprene units (C_5H_8) form monoterpenoids (C_{10} skeleton), sesquiterpenoids (C_{15}), diterpenoids (C_{20}) and triterpenoids (C_{30}). The “essential oil” or highly concentrated terpene oil can be obtained through different types of extraction processes. These may include steam distillation or vaporisation, solvent extraction, supercritical fluid extraction etc. Varied yields are associated with several different factors all contributing to recovery. These factors include the cannabis strain, pollination; sex, age and section of the plant; cultivation (indoor, outdoor etc.); harvest time and conditions; drying and storage (Brenneisen, 2007:17–49; Fischedick *et al.*, 2010:2058–2073).

1.2.2 Medicinal Impact of Cannabis

There is no established designation for medicinal cannabis, the term is nonetheless used to refer to the therapeutic use of cannabis-based products and their derivatives (Murnion, 2015:212–215). A 2016 review assessed the current status and prospects for development of CBD and CBD-dominant preparations for medicinal use in the United States, examining its neuroprotective, antiepileptic, anxiolytic, antipsychotic, and anti-inflammatory properties (Mead, 2017:288–291).

1.2.2.1 Cancer

Cannabinoids have been shown to exhibit some anti-cancer effects in laboratory experiments, although there has been little research into their use as a cancer treatment in humans (Fasinu *et al.*, 2016:781–796). Laboratory experiments have suggested that cannabis and cannabinoids exhibit anti-carcinogenic and antitumor effects, specifically a potential effect on breast- and lung-cancer cells. The National Cancer Institute reports that as of November 2013 there have been no clinical trials on the use of cannabis to treat cancer in humans and only one small study using THC that reported potential antitumor activity. While cannabis may have potential for refractory cancer pain, as an antiemetic, and as an antitumor agent, this evidence comes from outdated or small studies and animal experiments. There is no reliable evidence that cannabis use reduces the risk of cancer (Arney, 2015). Cannabis appears to have an emerging therapeutic role, especially in chronic disease and as an adjunct to cancer treatment. Medicinal Cannabis has shown increased use in the chemotherapeutical management of nausea, vomiting and pain. Studies however are limited, particularly studies with standardised dosing methods, while there is an inability to assess biological activities of administered cannabis forms (Stone *et al.*, 2018:480–487).

1.2.2.2 Epilepsy

In December 2015, the Drug Enforcement Agency (DEA) has eased some of the regulatory requirements for conducting FDA-approved clinical trials on CBD (Drug Enforcement Administration (DEA), 2015). Epidiolex is a cannabis-based product which was subjected to clinical trials in the US in 2014 (Cruise, 2016; GW

pharmaceuticals, 2015). Phase 3 trials for Dravet syndrome and Lennox-Gastaut syndrome have begun and was completed in 2015 (GW pharmaceuticals, 2015).

1.2.2.3 Other Conditions

A 2007 review of cannabidiol suggested the possible alleviation of convulsion, inflammation, cough, congestion, nausea as well as inhibition of cancer cell growth (Mechoulam *et al.*, 2007:1678–1692). Preliminary studies have uncovered the possible use of cannabidiol in psychiatric conditions such as anxiety, depression and psychosis (Campos *et al.*, 2012:3364–3378).

1.2.3 Quality Control of Cannabis-based Products

An evaluation of the quality of cannabis-based products has been performed previously (Hazekamp, 2006:1–9), but is limited to the Netherlands. In the Netherlands, the Office for Medicinal Cannabis regulates the manufacture of pharmaceutical-grade herbal cannabis. Approved strains of cannabis are cultivated under precise conditions with stringent quality controls in place to ensure reproducible final products (Hazekamp, 2006:1–9; Murnion, 2015:212–215). According to the WHO a myriad of factors needs to be taken into account to reproducibly cultivate herbal medicines, as well as ensure the safety and efficacy of the product through the implementation of an abundance of control measures (World Health Organisation (WHO), 2010).

To improve the quality of cannabis-based products, scientists and manufacturers must educate customers. Contaminated cannabis may be fatal. For now, customers cannot rely on government regulations to ensure proper analysis of cannabis-based products. The patients need to be their own best advocates. This combination of customer knowledge and advancing analytical methods will increase the safety of products from this industry. The expanding user base requires the assurance that the products being used are safe.

Beyond knowing that a product is safe, medicinal cannabis products should also be assayed for concentration of all actives and not THC exclusively. Proper quality control of these products is thus the starting point to producing safe cannabis-based products. Once quality control analysis is in place, accurate analytical data can guide manufacturers in proper production of a safe medicinal product.

The efficacy of herbal medicines and in this case medicinal cannabis-based products, typically rely on the concentration of two major cannabinoids namely CBD and THC. The WHO has developed a series of technical guidelines for establishing the safety and quality of herbal medications which include medicinal cannabis (World Health Organisation (WHO), 2010).

1.2.3.1 Potency (Cannabinoid Quantitation)

Cannabinoid profiling informs patients about the concentrations of active cannabinoids in medicinal cannabis-based products. Researchers have identified over 113 cannabinoid compounds (Brenneisen, 2007:17–49). An assay to establish the concentration of the active ingredients in a medicinal product is a requirement by the MCC (Medicines Control Council (MCC), 1965:1–392). Potency should be tested on all medicinal cannabis products or derivatives thereof. As is the case with any pharmaceutical product, the active ingredients in cannabis should be clearly labelled. Cannabinoid profiling allows physicians to determine accurate dosage, ensures that providers can verify the quality of their products and helps patients to select the correct treatment for their symptoms.

Association of Official Agricultural Chemists (AOAC) International has published draft methods for the standardisation of medical cannabis potency testing (AOAC International, 2018). The analytes that must currently be evaluated for potency are listed in the AOAC draft include: CBN, CBD, CBDA, THC and THCA. See Table 1 and Figure 1.

Table 1 - (Cannabinoids analytes for potency testing)(AOAC International, 2018)

Potency
Cannabinol (CBN)
Cannabidiol (CBD)
Cannabidiolic acid (CBDA)
Delta-9-tetrahydrocannabinol (Delta-9-THC)
Delta-9-tetrahydrocannabinolic acid (Delta-9-THCA)

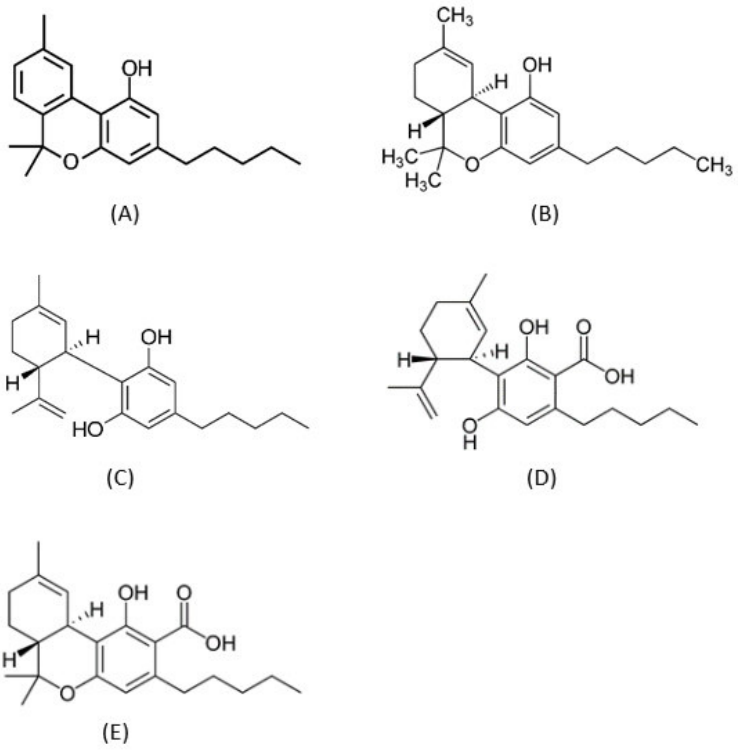

Figure 1 - Cannabinol (A); Delta-9-tetrahydrocannabinol (B); Cannabidiol (C); Cannabidiolic Acid (D); Tetrahydrocannabinolic Acid (E)

1.2.3.2 Residual Solvents Residue Limits

A range of organic solvents are used for manufacturing herbal medicines and can be detected as residues of such processing in the final products. These should be controlled through Good Manufacturing Practice (GMP) quality control. Solvents are classified by ICH (CPMP/ICH 283/95) (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38), according to their potential risk, into:

- Class 1 (solvents to be avoided such as benzene);
- Class 2 (limited toxic potential such as methanol or acetonitrile);
- Class 3 (low toxic potential such as ethanol).

Residual solvents may be analysed by headspace gas chromatography – mass spectrometry (HS GC-MS), liquid injection gas chromatography – mass spectrometry (GC-MS) or gas chromatography – flame ionisation detector (GC-

FID). The use of MS provides additional selectivity for co-eluting solvents. The boiling points of some solvents are too high to be amenable to headspace analysis, thus some class 2 solvents need to be injected in liquid form to be analysed. Current trends in South Africa indicate that Class 3 solvents are employed commonly due to their ease of access to the public and low toxic potential. ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38) provides a guideline which solvents should be included in a solvent working list, and this should be decided by each manufacturer or Quality Control (QC) laboratory. The following tables show solvents that are included in each of the individual classes in ICH document Q3C (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38).

Table 2 - ICH Q3C class 1 solvent list

Solvents Class 1	Limit (ppm)
Benzene	2
Carbon tetrachloride	4
1,2-Dichloroethane	5
1,1-Dichloroethene	8
1,1,1-Trichloroethane	1500

Table 3 - ICH Q3C class 2 solvent list

Solvents Class 2	Limit (ppm)
Acetonitrile	4.1
Chlorobenzene	3.6
Chloroform	0.6
Cumene	0.7
Cyclohexane	38.8
1,2-Dichloroethene	18.7
Dichloromethane	6.0
1,2-Dimethoxyethane	1.0
N,N-Dimethylacetamide	10.9
N,N-Dimethylformamide	8.8

1,4-Dioxane	3.8
2-Ethoxyethanol	1.6
Ethyleneglycol	6.2
Formamide	2.2
Hexane	2.9
Methanol	30.0
2-Methoxyethanol	0.5
Methylbutyl ketone	0.5
Methylcyclohexane	11.8
Methylisobutylketone	45
N-Methylpyrrolidone	5.3
Nitromethane	0.5
Pyridine	2.0
Sulfolane	1.6
Tetrahydrofuran	7.2
Tetralin	1.0
Toluene	8.9
1,1,2-Trichloroethene	0.8
Total Xylenes	21.7

Table 4 - ICH Q3C class 3 solvent list (solvent limits are 5000 ppm)

Solvents Class 3	Solvent Class 3
Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethyl ketone
tert-Butylmethyl ether	2-Methyl-1-propanol
Dimethyl sulfoxide	Pentane
Ethanol	1-Pentanol
Ethyl acetate	1-Propanol
Ethyl ether	2-Propanol

Ethyl formate	Propyl acetate
Formic acid	Triethylamine

1.2.3.3 Terpene Quantitation

Terpenes are the aromatic compounds that provide cannabis with its unique fragrance (Booth and Bohlmann, 2019:67–72). Terpene analysis may be used for differentiation of cannabis strains (Elzinga *et al.*, 2015:1–9). Terpenes may affect the medical and psychological effects of the cannabis plant since essential oils containing similar terpenoids are believed to exhibit certain medicinal properties (Booth and Bohlmann, 2019:67–72). The relationship between cannabinoids and terpenoids is commonly known known as the "entourage effect", ultimately allowing distinction of one strain of medicinal cannabis from another. Terpene analysis is beneficial for patients, producers and breeders alike.

Terpenes are analysed via HS GC-MS or GC-FID because of their volatile nature. As a result of the multitude of terpenoids that needs to be identified in a single run, MS may be employed to provide added selectivity for co-eluting compounds. The Following (Table 5) terpenes are commonly found in the cannabis plant (Brenneisen, 2007:17–49).

Table 5 - Common terpenes found in cannabis (Brenneisen, 2007:17–49)

Terpenes
(-)-alpha-Bisabolol
Camphene
delta-3-Carene
beta-Caryophyllene
Geraniol
(-)-Guaiol
alpha-Humulene
p-Isopropyltoluene (*p*-cymene)
(-)-Isopulegol
d-Limonene
Linalool

beta-Myrcene
Nerolidol
Ocimene
alpha-Pinene
(-)-beta-Pinene
alpha-Terpinene
gamma-Terpinene
Terpinolene

1.2.3.4 Water Content

Water content is usually measured on raw plant material only. The analysis provides insight to processors for prevention of mould and fungus contamination (Farrer, 2016:1–14), since most pathogenic microbial organisms cannot grow at a low water content level. Usually water content can be analysed via near-infrared spectroscopy or Carl Fischer titration. In some instances, laboratories will employ a loss on drying analysis (Andrade-Cetto *et al.*, 2016:1–12), this however is an indication of total volatile loss which would include terpenes as well as water moisture.

1.2.3.5 Pesticides

Medicinal plant materials may contain pesticide residues, which collect in the plant as a result of agricultural practices such as pest control spraying, treatment of growing media during cultivation and administration of fumigants during storage. It is therefore recommended that potentially hazardous contaminants and residues in herbal medicines be tested.

Limits for pesticide residues should be established following the recommendations of the US EPA ((United Stated Environmental Protection Agency (US EPA), 2016). These maximum residue limits (MRLs) have already been established for food and animal feed. The recommendations include an Allowed Daily Intake (ADI) and the analytical methodology for the assessment of specific pesticide residues. Currently, however, there are no standard procedures for assignment of these MRLs in the herbal medicines industry and thus the methods utilised for foods

could be extended. This approach would apply in the case where a botanically identical medicinal plant is used as food, or ingested via an oral route of administration.

GC triple quadrupole MS as well as LC triple quadrupole MS are usually employed in order to analyse for the whole range of pesticides according to the specified MRLs. Triple Quadrupole MS is employed because of the very low (low parts per billion (ppb) to high parts per trillion (ppt) levels) detection limits imposed by the US Environmental Protection Agency EPA (US EPA, 2016), and potentially other statutory bodies in future.

Currently the United States has no Federal regulation regarding minimum pesticide residue limits for medicinal cannabis. Each state thus determines their own MRL. The MRLs and even lists of pesticides to be tested, may differ significantly from state to state (Colorado Department of Public Health and Environment, 2017:1–5; Oregon Department of Agriculture, 2017). Since climate as well as access to different pesticides in South Africa differ from the United States it is recommended that South Africa establishes its own MRL limits for certain pesticides specifically applicable to the local market.

South Africa is the largest consumer of pesticides in sub-Saharan Africa (Dabrowski *et al*., 2014:31–40). Table 6 contains 25 pesticides that are ranked according to their usage in South Africa (Dabrowski *et al*., 2014:31–40). Not all pesticides listed has a maximum residue limit imposed by South Africa, even though they are employed.

Table 6 - South African pesticide maximum residue limits. (Dabrowski *et al.*, 2014:31–40).

Pesticide	SA MRLs mg/kg (ppm)
Glyphosate	0.5
Mancozeb	0.01
Sulphur	50
Copper oxychloride	20
Atrazine	0.05
Terbuthylazine	0.05
Acetochlor	0.02
Metolachlor	0.05
Dichlorophenoxyacetic acid	0.5
Paraquat	0.02
Alachlor	0.05
2-Methyl-4-chlorophenoxyacetic acid (MCPA)	0.1
Ethylene-dibromide	No Limit
Imidacloprid	0.05
Monosodium methyl arsonate (MSMA)	0.05
Potassium-phosphate	No Limit
Cyanamide	No Limit
s-Ethyl dipropylthiocarbamate (EPTC)	0.1
Copper hydroxide	20
Trifluralin	0.05
Copper-carbonate	20
Chlorpyrifos	0.01
Chlorothalonil	No Limit
Terbufos	0.05
s-Metolachlor	0.05

1.2.3.6 Microbiological Contamination

Herbs and herbal materials normally carry a large number of bacteria and moulds, often originating in soil or derived from manure. While a large range of bacteria and fungi form the naturally occurring microflora of medicinal plants, aerobic spore-

forming bacteria frequently predominate (African Organisation of Standardisation, 2014:2–16). Current practices of harvesting, production, transportation, and storage may cause additional contamination and microbial growth (African Organisation of Standardisation, 2014:2–16). Proliferation of microorganisms may result from failure to control the water content levels of herbal medicines during transportation and storage, and the temperatures of liquid forms and finished herbal products (African Organisation of Standardisation, 2014:2–16). The presence of yeast and moulds may indicate poor quality of production and harvesting practices. Microbial contamination may also occur through handling by personnel who are infected with pathogenic bacteria during harvest/collection, post-harvest processing, the manufacturing and packing processes. This should be controlled by implementing best practice guidelines such as GMP.

AOAC International (AOAC International, 2009:2002) has published a standardised method for total yeast and mould counts in foods. The United States Pharmacopoeia (United States Pharmacopoeia (USP), 2012:962–965) has also established limits for microbial contaminants in dietary supplements but to date no monograph addresses medicinal cannabis specifically. For medicinal cannabis, maximum colony forming unit (CFU) limits listed in the Table 7 below may be employed.

Table 7 - Recommended microbial limits for botanical ingredients and products (United States Pharmacopoeia (USP), 2012:962–965)

Material	Recommended Microbial Limit Requirements (cfu)
Dried or Powdered Botanicals	Total Aerobic Microbial Count ≤ 100 000 Total Combined Yeast & Mould Count ≤ 1000 Bile-tolerant Gram-negative Bacteria ≤ 1000 Absence of Salmonella spp. and E. coli in 10 g
Powdered Botanical Extracts	Total Aerobic Microbial Count ≤ 10 000 Total Combined Yeast & Mould Count ≤ 1000 Absence of Salmonella spp. and E. coli in 10 g
Tinctures	Total Aerobic Microbial Count ≤ 10 000 Total Aerobic Microbial Count ≤ 1000
Fluid extracts	Total Aerobic Microbial Count ≤ 10 000 Total Combined Yeast & Mould Count ≤ 1000

1.2.3.7 Heavy Metals Residues

Contamination of herbal materials with toxic substances such as arsenic can result from numerous origins. These include environmental pollution (i.e. contaminated emissions from factories and leaded petrol, contaminated water including runoff water which finds its way into rivers, lakes and the sea, and some pesticides), soil composition and fertilizers. This contamination of the herbal material leads to contamination of the products during various stages of the cultivation and manufacturing process (Khan *et al.*, 2017:203–207; Tchounwou *et al.*, 2012:133–164).

Pesticides that contain arsenic and mercury in their chemical structures were commonly utilised until a few years ago and they are still employed in some countries. As toxic substances, they are likely to be present in many foods due to their abundance in nature, and it is important to note that concomitant ingestion of herbal products would add to the total concentration of toxic metals consumed by people, even if best practice guidelines are followed (World Health Organisation (WHO), 2010).

Inductively coupled plasma mass spectrometry (ICP-MS) is employed to detect heavy metal contamination. ICP-MS is preferentially employed as a result of low residue limits imposed by USP 233/232 (United States Pharmacopoeia (USP), 2017:1–3, 2016). The heavy metals are classified into different classes according to their toxic potential. As shown in Table 8, class 1 is the most dangerous with the lowest allowable limits while class 2 and class 3 are less toxic with class 3 having significantly higher allowable limits (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2015:1–85; United States Pharmacopoeia (USP), 2017:1–3).

Table 8 - Class 1 maximum residue limits for heavy metals as per ICH Q3D (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2015:1–85)

		Oral Limit (µg/g)	Inhalation Limit (µg/g)
Class 1	**Cadmium(Cd)**	0.5	0.3
	Lead(Pb)	0.5	0.5
	Arsenic(As)	1.5	0.2
	Mercury(Hg)	3	0.1
Class 2A	**Cobalt(Co)**	5	0.3
	Vanadium(V)	10	0.1
	Nickle(Ni)	20	0.5
Class 2B	**Thallium(Tl)**	0.8	0.8
	Gold(Au)	10	0.1
	Palladium(Pd)	10	0.1
	Iridium(Ir)	10	0.1
	Osmium(Os)	10	0.1
	Rhodium(Rh)	10	0.1
	Ruthenium(Ru)	10	0.1
	Selenium(Se)	15	13
	Silver(Ag)	15	0.7
	Platinum(Pt)	10	0.1
Class 3	**Lithium(Li)**	55	2.5
	Antimony(Sb)	120	2
	Barium(Ba)	140	30
	Molybdenum(Mo)	300	1
	Copper(Cu)	300	3
	Tin(Sn)	600	6
	Chromium(Cr)	1100	0.3

1.3 Research Questions

With this background in mind, it is pertinent to ask a series of questions to guide the research that has been conducted in this thesis. To narrow down the scope of research,

only South African market cannabis-based products were included in this study. Within this South African market scope:

1.3.1 Potency

What is the current state of the South African cannabis-based product market in terms of cannabinoids concentration or potency? Are South African preparations as potent as preparations sourced globally? Which product types or classes are consumed or purchased the most? Which product category offer the least risk with regards to variability in dosing, as well as consistency in concentration spread between manufacturers. As stated in the introduction cannabis-based product may be classified as unscheduled when they adhere to specification set forth by the South African Government (Republic of South Africa, 2020:1–12, 2019:1–20). The question to pose is how many of the South African products adhere to these imposed regulations?

1.3.2 Impurities

The second question that can be asked addresses the safety of the current cannabis-based products available on the South African market. As mentioned above, the current South African regulation only specifies the concentration of cannabinoids or active ingredients. The other quality control assessments listed previously does not form part of this regulation. It is beyond the scope of this thesis to cover the complete range of quality control measures listed. In addition to cannabis potency, the following two residues will be focused on.

1.3.2.1 Heavy Metal Residues

Heavy metal residues are cultivation or manufacturing processes contributing to a heavy metal content in the cannabis plant, and ultimately carried over into the final products? Additionally, if heavy metal residues are present, are they above maximum pharmaceutical residue limits?

1.3.2.2 Solvent Residues

First and foremost, because of the current mostly unregulated nature of these products, the means used to produce such products are mainly solvent extraction. This does not require sophisticated expensive equipment and is the method of

choice for “home” producers. This begs to ask which solvents are mostly being employed in these preparations and are they below maximum pharmaceutical residue limits?

Each of these questions will be answered in an article format thesis. There is much research that can be carried out and data that can be collected to address these quality control issues. Although all the listed quality control measures are important, the questions asked will only focus on selected tests as a starting point and may be broadened through additional research.

1.4 Aims and Objectives

It is the aim of this project to lead with the development of adequate reliable Quality Control procedures (Potency, Residual Solvents and Heavy Metals) for the analysis of cannabis-based products. After fit for purpose methods have been developed, cannabis-based product samples from the South African market will be analysed and the data interpreted. The mentioned tests and data obtained should serve as a guideline for medicinal cannabis testing depending on the type of product that is produced. Extracted oils should for example have different requirements than dried plant buds. The entire range of tests however should satisfy the quality control needs of a broad variety of medicinal cannabis products.

Samples submitted to a South African contract laboratory, will be analysed and the data interpreted. For this study “Manufacturers” will be defined as any type of user, retailer, reseller, producer, or importer of cannabis-based products. Whether these manufacturers maintain the full value chain or only a portion thereof they are defined as manufacturers for the purpose of this study. All data collected will be presented in an anonymised format. This study will thus attempt to achieve three objectives:

1.4.1 Objective 1

To develop and validate an analytical test method for the measurement of potency (cannabinoid concentration) in cannabis-based products. This method will further be used to gain invaluable data on the current stance of medicinal cannabis products in South Africa with relation to potency (active ingredient concentration).

1.4.2 Objective 2

To develop and validate an analytical test method for the measurement of solvent residues in cannabis-based products. This method will further be used to gain invaluable data on the safety of medicinal cannabis products in South Africa with relation to residual solvent content.

1.4.3 Objective 3

To develop and validate an analytical test method for the measurement of heavy metal residues in cannabis-based products. This method will further be used to gain insights on the safety of cannabis-based products in South Africa with relation to heavy metal content.

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CHAPTER 2

2 Background and literature

2.1 Introduction

Taking into account all the quality control aspects listed in chapter one, this study will focus on three analytical tests to gain insights into the potency, as well as existing residual solvent and heavy metal residues in cannabis-based products in South Africa. To be able to compare this study's prospective data to other countries globally and even in South Africa, one must first ascertain what has been done locally as well as globally. If comparisons are to be drawn, which quality control measures have been implemented locally and globally, and what epidemiological inferences can be made from this data?

Thus, the main focus of this chapter will relate to the three major quality control parameters selected in Chapter 1, namely, Cannabis Potency, Residual Solvent Residues and Heavy Metal Residue. A detailed overview of what has been done globally as well as locally will be presented.

2.2 Cannabis Potency

Potency results will demonstrate how "strong" or potent cannabis-based products are. In pharmaceutical terms it is a cannabinoid assay, to determine the concentrations of active pharmaceutical ingredients, in this case cannabinoids.

A large number of cannabinoids are present in the cannabis plant and many have been identified. The concentration and type of cannabinoid may vary depending on a number of factors which may include strain, growing conditions, climate and nutrients (Brenneisen, 2007:17–49). The cannabinoids of interest in this study are; Δ9-tetrahydrocannabinol (THC), CBD and cannabinol (CBN). Additional to these cannabinoids are their corresponding acids, THCA and CBDA, which will be of interest to this study.

While the cannabinoids of interest in this study will be described in detail, please refer to chapter one for additional information on all other cannabinoids excluding Δ9-tetrahydrocannabinol (THC), CBD and cannabinol (CBN).

2.2.1 CBN

Apart from being a naturally occurring phytocannabinoid, CBN is also a breakdown product of THC (Repka *et al*., 2006a:21–32). According to a previous study (Repka *et al*., 2006b:21–32), as exposure time and temperature increase, increased amounts of THC are converted to CBN. In the published study, a total exposure time of between 75-90 min was employed and the percentage weight measured in the study for CBN was 0.5% at 120 °C and 0.4% at 160 °C, compared to 1.6% at 200 °C (Repka *et al*., 2006b:21–32). From this data it can easily be seen that if incorrect processing methods during extraction or removal of solvent residues are employed, degradation of THC may ensue. It is thus an important part of quality control to measure such breakdown products, not because of safety reasons, but to monitor the manufacturing process and ensure quality.

2.2.2 CBD and CBDA

CBD and its matching acid, CBDA, are the most abundant cannabinoids in fiber-type Cannabis plants also known as hemp (Brenneisen, 2007:17–49; Burstein, 2015:1377–1385). CBD is the cannabinoid researched in most medical trails. Clinical use of THC and additional cannabinoid agonists is frequently restricted by their undesirable psychoactive side effects, and it is for this reason that non-psychoactive cannabinoid compounds, structurally similar to THC, such as CBD are being employed in most clinical trials (Massi *et al*., 2013:303–312). CBD binds mostly to the cannabinoid type I (CB1) and cannabinoid type II (CB2) receptors, which are highly expressed in the central nervous system including the hippocampus (Welty *et al*., 2014:250–252). When CB1 receptors are stimulated they inhibit synaptic transmission which controls seizure activity (Welty *et al*., 2014:250–252). Studies have demonstrated that CBD has low affinity for CB1 receptors, however even at low concentrations synaptic transmission are nonetheless decreased (Welty *et al*., 2014:250–252).

For the analysis of both CBD and CBDA, liquid chromatography should be employed due to decarboxylation of CBDA to CBD at high temperatures. A total CBD (CBD + CBDA) can also be measured using gas chromatography. It should be noted that the decarboxylation of any cannabinoid acid as well as CBDA will have the stoichiometrical

product CO_2. Thus some of the molecules' weight can be attributed to the carboxyl group which produces CO_2 when decarboxylated. total potential CBD would be

$$(CBD + (0.87 \times CBDA)) \quad \textbf{\textit{Eq. 1}}$$

since 13% of the cannabinoid acids' weight can be attributed to CO_2, which is in turn lost to produce CBD from CBDA. It is commonly referred to as total CBD or total potential CBD when the total potential available concentration of CBD is calculated by employing this formula. From this equation it is evident that for the molecular masses of CBD, 314 g/mol, CBDA, 358 g/mol and for CO_2, 44 g/mol, a mass of 44 g/mol CO_2 is lost from CBDA to produce CBD, 314 g/mol. This mass of CO_2 does not contribute to the total potential CBD mass, and should thus not be factored in total potential CBD wt.-%. From the following equation it can be seen why the ratio 0.877 is used.

$$\frac{THC\ Molar\ mass}{THCA\ Molar\ mass} = Ratio \quad \textbf{\textit{Eq. 2}}$$

$$\frac{314 g/mol}{358 g/mol} = 0.87709 \quad \textbf{\textit{Eq. 3}}$$

It would thus be important to distinguish between total potential CBD, CBDA and CBD concentrations.

2.2.3 THC and THCA

THCA is the predominant cannabinoid found in cannabis plant material. THC acid B is present to a much lesser extent. THC is the main psychoactive constituent of phytocanabinoids. The acids are non-psychoactive (Brenneisen, 2007:17–49; Gaoni and Mechoulam, 1964:1646–1647). As mentioned in chapter 1, THC potency or wt.-% has steadily been increasing from the 1980's until now (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126). It is unclear what the cause of this increase might be, but it could be argued that the following factors might play a role: more efficient LED lights, improved nutrient formulations, altered strain genetics etc. The average THC concentration increased from less than 1.5 wt.-% in 1980 to 4.2 wt.-% in 1997. The maximum THC concentrations analysed were 52.9 wt.-% and 47.0 wt.-%, respectively (Mehmedic *et al.*, 2010:1209–1217). Two studies performed in Switzerland from 1981 to 1985 as well as 2002 to 2003 uncovered average sample THC amounts of 1.4 wt.-% and 12.9 wt.-%, respectively (Brenneisen, 2007:17–49). Maximum concentrations

were 4.8 wt.-% and 28.4 wt.-%, respectively. The concentration (wt.-%) of THC has increased four times, and doubled in the United States and Europe, respectively, between 1995 and 2018 as seen in Figure 2 (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126).

In the United Stated the potency of cannabis-based products varies across states, and even though many states monitor the potency of products, the ever-increasing strength of these products may pose definitive health concerns (United Nations Office on Drugs and Crime (UNODC), 2021b:1–95). In Colorado state the average potency of THC in cannabis extracts in 2017 was 69 wt.-%, with some retailers claiming up to 95 wt.-% THC (Colorado Department of Public Health and Environment, 2020:1–53). For plant material in Colorado state, the potency averaged 19.6 wt.-% THC, while overall, 92.9% of all products sold had a potency greater than 15 wt.-% THC (Colorado Department of Public Health and Environment, 2020:1–53). In California, after the year 2022, the state will require a certificate of analysis (CoA) for edible cannabis-based products to certify that the dose of THC per serving does not exceed 10 mg ± 1.2 mg (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126).

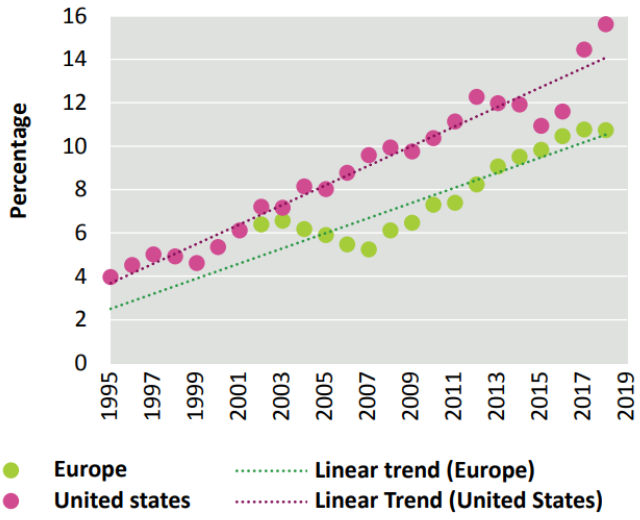

Figure 2 - THC concentration/potency in cannabis plant material plotted annually (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126).

It is clear that as time progresses the potency or concentration of THC in cannabis strains increases globally. It should be noted that the percentages reported was not specific on whether these figures reflect total potential THC or THC alone.

Furthermore, it was not specified whether the reported figures are on a dry weight or wet weight basis. Even without these data, a significant increase in THC concentration is observed, irrespective of the accuracy of the analytical method used.

As with CBDA it should be noted that the decarboxylation of THCA will result in the loss of CO_2. total potential THC is thus calculated as follows:

$$(THC + (0.87 \times THCA)) \qquad \textbf{\textit{Eq. 4}}$$

Although THC is psychoactive, some positive as well as harmful medicinal properties can also be attributed to this cannabinoid. THC is a bronchodilator, analgesic, antioxidant, muscle relaxant and an antipruritic (Russo, 2011:1344–1364). THC medication (Sativex) has also demonstrated marked improvement in subjective sleep parameters in patients with a wide variety of pain conditions including multiple sclerosis, peripheral neuropathic pain, intractable cancer pain, and rheumatoid arthritis (Russo *et al*., 2007:1729–1772). Chronic THC dosing results in cognitive impairment, including amnesia (Puighermanal *et al*., 2009:1152–1160). According to the World Health Organisation (WHO) acute cannabis (Predominantly high-THC strains) use, leads to adverse effects such as impaired cognitive development (learning) as well as impairing psychomotor performance (World Health Organisation (WHO), 2010). Chronic exposure to cannabis (THC predominant strains), in turn, leads to selective impairment of cognitive functioning which include the organisational and integrational skills, increases schizophrenic episodes in affected individuals, epithelial damage of the trachea and bronchi is caused by long term smoking, impairment of foetal development during pregnancy and increased post-natal incidence of cancer (World Health Organisation (WHO), 2010). Additionally, a study found that chronic cannabis is linked to neuropsychological deterioration associated with functioning. This decline was observed among adolescent-onset cannabis consumers, with more persistent use related with greater deterioration (Meier *et al*., 2012).

The United Nations Office on Drugs and Crime (UNODC) released a world drug report in 2021 (United Nations Office on Drugs and Crime (UNODC), 2021b:1–95) that catalogues global illicit drug production and use. It should be noted that the data presented in the World drug report is limited to illicit drug use, in this case illicit cannabis use. Thus the data may

be skewed as a result of medicinal or legal cannabis use not being taken into account, nonetheless inferences can still be made from the data reported by the World drug report.

According to the World drug report published in 2021, cannabis is the most produced illicitly used drug globally, both in size and geographical spread. Cannabis, which includes plant material or herb, resin/extract, or other forms, accounted for 52% of total drug seizures from 2017-2019 (Figure 3). Globally, a total number of 200 million users have been reported for 2019, which equates to 4% of the global population between the ages of 16 and 64. For Africa a higher 9.4% population usage figure is reported. The global number of cannabis users increased by 18% since the year 2010 (United Nations Office on Drugs and Crime (UNODC), 2021b:1–95).

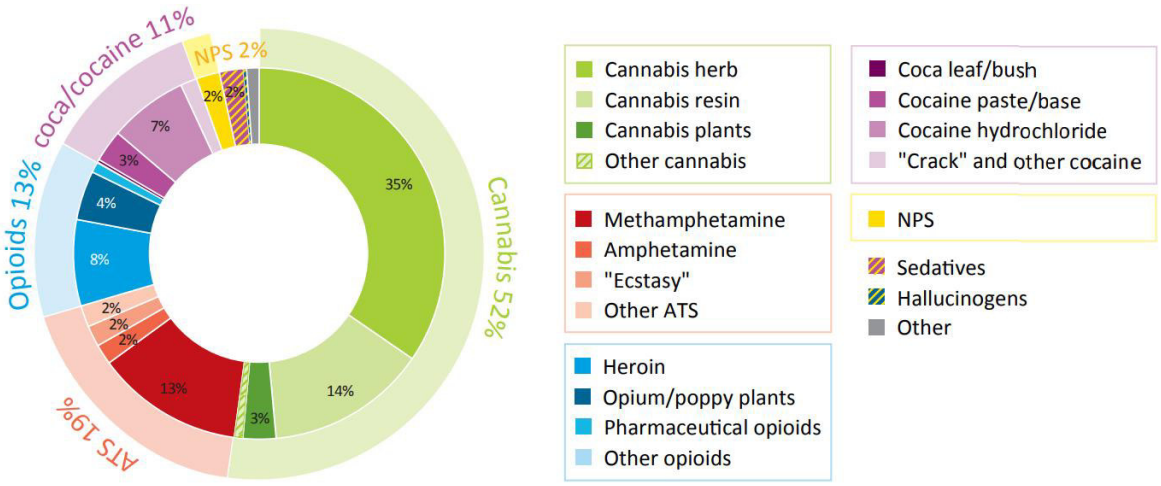

Figure 3 - Global distribution of drug seizure cases by drug types, 2017-2019 (United Nations Office on Drugs and Crime (UNODC), 2021b:1–95)

As seen from the global drug report 2021 (Figure 4), in countries other than Africa, a significant increased number of young adolescents (aged 15-16) are using cannabis compared to older population groups (aged 16-64) (United Nations Office on Drugs and Crime (UNODC), 2021b:1–95). Oceania in the report refers to New Zealand and Australia. Drug production other than cannabis is usually concentrated in a few countries, whereas cannabis production and seizures are reported in all countries globally (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126). Cannabis use in the African region, which includes South Africa, is the third largest among the regions and equally distributed between age groups (Figure 4). For people in South Africa who were admitted to specialist

drug treatment facilities in 2019, cannabis was reported as either the primary or secondary drug for most people who were treated for drug use disorders, in particular among those aged 20 or less (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126).

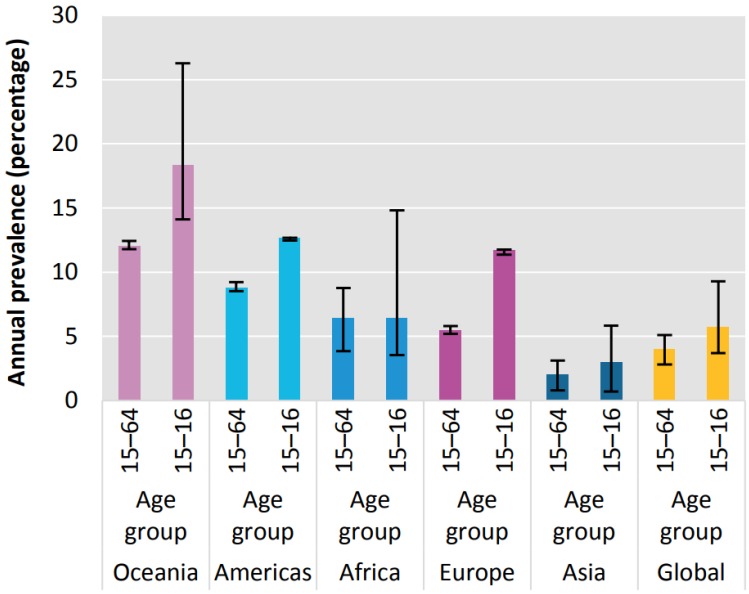

Figure 4 - Global and regional use of cannabis among population aged 15-16 and 16-64, 2019 (United Nations Office on Drugs and Crime (UNODC), 2021b:1–95)

The number of global seizures of cannabis plant material has decreased by 12% since 2019 and increased by 7% for the resin or extract category (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126). The following trends were observed for indoor versus outdoor grown cannabis. The trend for medicinal cannabis is to cultivate indoor, in order to control certain environmental parameters that may impact the quality of the final product. As seen from Figure 5, an increase in both indoor as well as outdoor cultivated cannabis was observed for the years 2012-2019 (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126). It may be postulated that an overall increase in indoor grown cannabis is reported by countries, due to that fact that environmental parameters can be controlled resulting in higher potency plant material being cultivated.

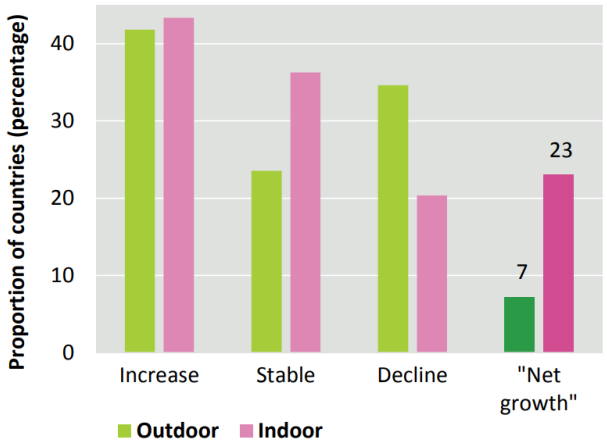

Figure 5 - Reported trends for outdoor vs. indoor cannabis cultivation (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126)

In additional to the increase in cultivation an increase in cannabis resin or extract trafficking was also observed. Year to year fluctuations do occur but a long term upward trend for extract type cannabis seizures have been reported as shown in Figure 6 (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126).

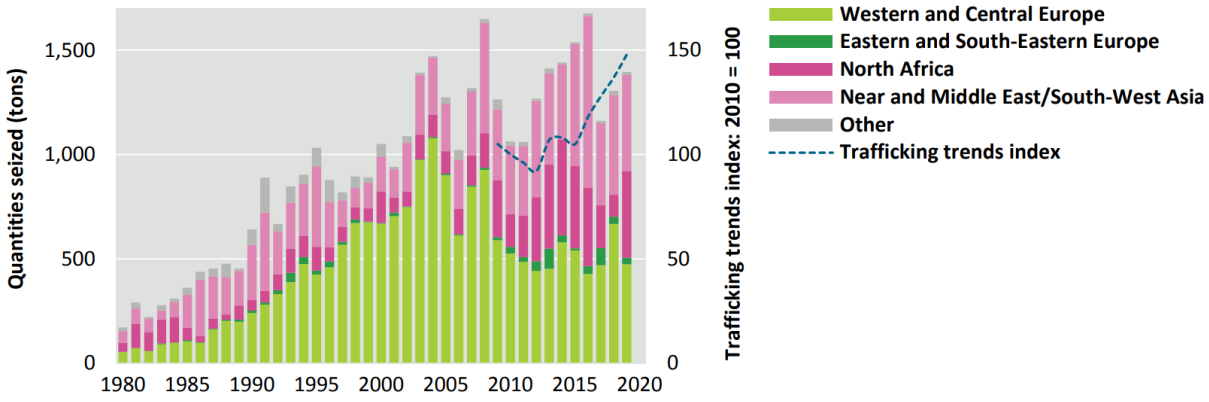

Figure 6 - Cannabis extract/resin trafficking and seizure trends by year (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126)

According to the world drug report, there is a clear dissociation between the perceived risk of consuming cannabis-based products and risk associated with increasing potency/strength of these products over the past few years (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126). It is thus interesting to note that although cannabis-based products have a higher cannabinoid content, a decreased number of

users are perceiving chronic cannabis use as harmful (Figure 7). As mentioned above, cannabis use is associated with significant health related harmful effects. Additionally, chronic cannabis use has been linked to a higher probability of psychotic disorders among users and is further increased fivefold by users who consume cannabis-based products with a THC potency of 10 wt.-% or higher (Forti *et al.*, 2019:427–436; Kuepper *et al.*, 2011:1–8).

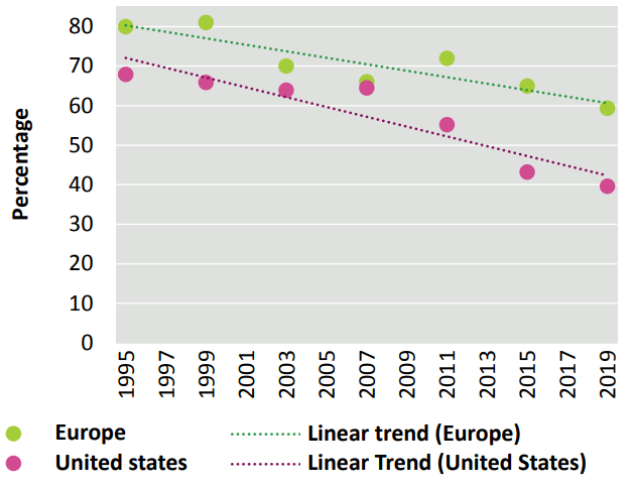

Figure 7 - Perception of risk or harm in using cannabis regularly plotted annually (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126).

In Canada, the use of cannabis and cannabis-based products has also increased tremendously. To monitor the outcome of the "*Cannabis Act"* that was passed by the Canadian government, an annual cannabis survey was established. Increase in recent regular use of cannabis was reported over time before the Act was passed, just after it was passed and one year after it was passed (United Nations Office on Drugs and Crime (UNODC), 2021a:3–126). The most predominant forms or categories of cannabis use, as reported by the Canadian cannabis survey 2020 are: Dried Flower/Plant Material, Edibles, Extracts, Vape Pens, Cannabis oil and Hashish (Government of Canada, 2020). Percentages in Figure 8 may add up to more than 100% as result of the respondents to the survey being able to choose multiple responses (Government of Canada, 2020).

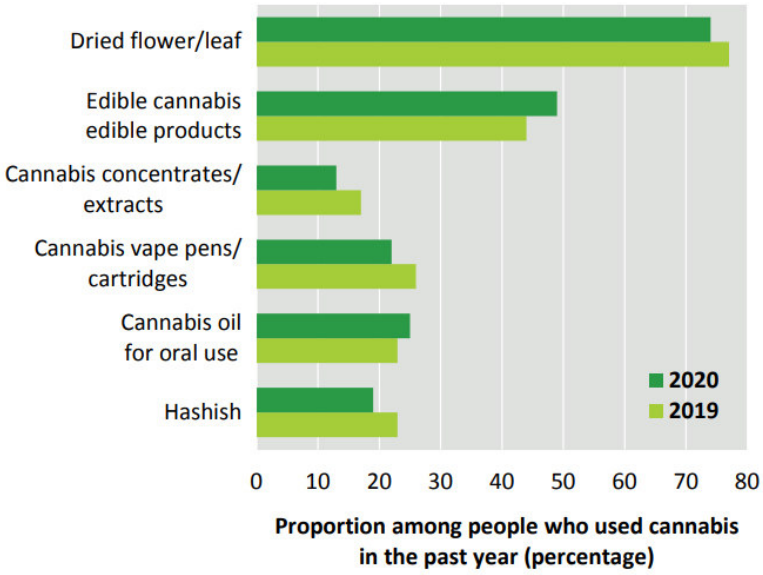

Figure 8 - Cannabis-based product categories used by people in canada in 2020 (Government of Canada, 2020).

In the United States a total of 47 states has permitted the use of cannabis for medicinal purposes, 11 of those states has allowed a restricted low THC potency medicinal use of cannabis-based products (National Conference of State Legislatures (NCSL), 2021:1). The 11 states that have imposed the restriction on the medical use of cannabis, allow the use of cannabis-based products with low potency THC and high potency CBD (National Conference of State Legislatures (NCSL), 2021:1; United Nations Office on Drugs and Crime (UNODC), 2021a:3–126). The following Figure 9, shows the jurisdictions in the United States that allow use of cannabis, medical cannabis or no access at all.

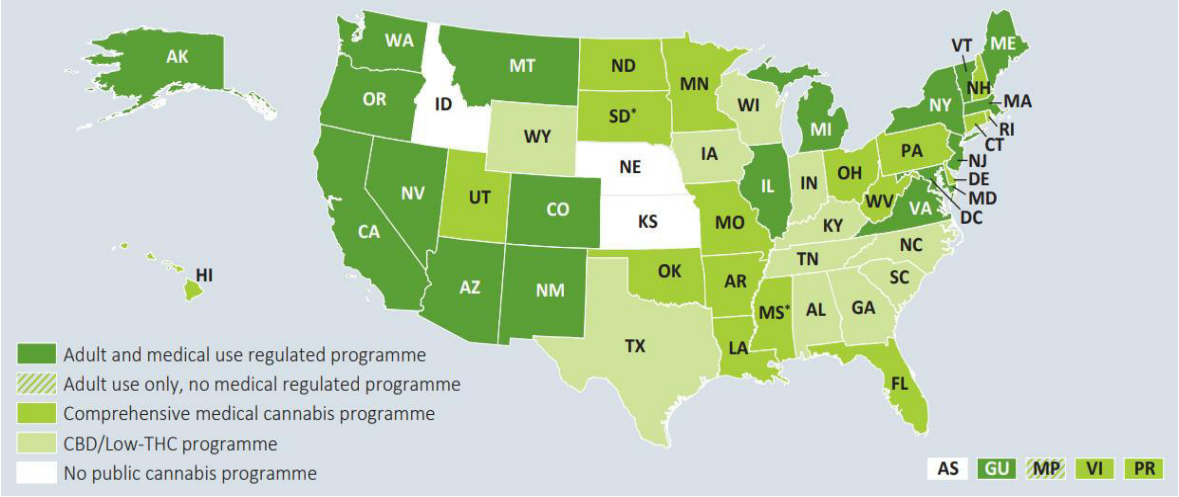

Figure 9 - Cannabis use policy according to state, grouped according to usage profile (National Conference of State Legislatures (NCSL), 2021:1).

As seen from the information listed even though legislature between country and similarly sate differs significantly, a common denominator can be identified: Potency of cannabis-based products are of high concern regardless of country, state, or legislature. It is thus of the utmost importance that potency of cannabis-based products be assessed, and that this assessment be made available to the users, or physicians prescribing these products.

2.3 Trace Elements (Heavy Metals)

Contamination of herbal materials with toxic substances such as arsenic can result from numerous origins. These include environmental pollution (i.e. contaminated emissions from factories and leaded petrol, contaminated water including runoff water which finds its way into rivers, lakes and the sea, and some pesticides), soil composition and fertilizers. This contamination of the herbal material leads to contamination of the products during various stages of the manufacturing process (Khan *et al.*, 2017:203–207; Tchounwou *et al.*, 2012:133–164). During growth these metals accumulate in the biomass of specific plants (Meers *et al.*, 2005:561–572).

Pesticides that contain arsenic and mercury in their chemical structures were commonly utilised until a few years ago and they are still employed in some countries. As toxic substances, they are likely to be present in many foods due to their abundance in nature, and it is important to note that concomitant ingestion of herbal products would add to the total concentration of heavy metals consumed by people, even if best practice guidelines are followed (World Health Organisation (WHO), 2010). A study performed in Nigeria

showed significant amounts of heavy metals in cultivated cannabis plant material (Eboh and Thomas, 2005:349–351). The main metal contaminants of interest found in this study was arsenic (As), mercury (Hg), nickel (Ni) and cadmium (Cd) (Eboh and Thomas, 2005:349–351). These are predominantly class 1 and 2 metal contaminants posing the highest risk to health as seen from Table 9 below. It is also interesting to note that the cannabis leaves contain higher concentrations of metal residues than the seeds with the exception of manganese (Mn) (Eboh and Thomas, 2005:349–351).

As a result of the new regulation imposed by the USP (United States Pharmacopoeia (USP), 2016:1–5) in collaboration with the ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2015:1–85), the detection limits for trace elements are becoming increasingly lower. Heavy metal residues in pharmaceutical end-products, active pharmaceutical ingredients and excipients need to be controlled at a certain limit for safe human consumption (Fischer *et al.*, 2014:121–129). Furthermore, it can be noted in the somewhat unique case of cannabis-based products, there exists an alternative route for administration of these products namely inhalation. The pharmacopoeial guideline stipulates three routes of administration namely parenteral, oral and inhalation (International Community of Harmonisation (ICH), 2015:1–84; United States Pharmacopoeia (USP), 2017:1–3). The latter two routes are almost predominantly employed for cannabis-based products.

Inductively coupled plasma mass spectrometry (ICP-MS) is employed to detect heavy metal contamination. As a result, low residue limits are imposed by USP 232, as well as ICH Q3D (Fischer *et al.*, 2014:121–129; International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2015:1–85; United States Pharmacopoeia (USP), 2017:1–3). The heavy metals are classified into different classes according to their toxic potential, class 1 (Table 9) being the most dangerous while class 2 (Table 10) and class 3 (Table 11) are less toxic with higher allowable limits. The Nigerian study showed that all the class 1 metals tested exceeded both the oral and inhalation limits. These residues include As, Cd, Hg and Pb (Eboh and Thomas, 2005:349–351).

Table 9 - Class 1 heavy metals (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2015:1–85)

Element	ICH Class	Oral (µg/g)	Parenteral (µg/g)	Inhalation (µg/g)
Cd	1	0.5	0.2	0.2
Pb	1	0.5	0.5	0.5
As	1	1.5	1.5	0.2
Hg	1	3.0	0.3	0.1

Table 10 - Class 2A&B heavy metals (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2015:1–85)

Element	USP Class	Oral (µg/g)	Parenteral (µg/g)	Inhalation (µg/g)
Co	2A	5	0.5	0.3
V	2A	10	1	0.1
Ni	2A	20	2	0.5
Tl	2B	0.8	0.8	0.8
Au	2B	10	10	0.1
Pd	2B	10	1	0.1
Ir	2B	10	1	0.1
Os	2B	10	1	0.1
Rh	2B	10	1	0.1
Ru	2B	10	1	0.1
Se	2B	15	8	13
Ag	2B	15	1	0.7
Pt	2B	10	1	0.1

Table 11 - Class 3 heavy metals (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2015:1–85)

Element	USP Class	Oral (µg/g)	Parenteral (µg/g)	Inhalation (µg/g)
Li	3	55	25	2.5
Sb	3	120	9	2
Ba	3	140	70	30
Mo	3	300	150	1
Cu	3	300	30	3
Sn	3	600	60	6
Cr	3	1100	110	0.3

2.4 Residual Solvents

A range of organic solvents are used for manufacturing herbal medicines and can be detected as residues in the final products. Cannabis oil extracts from these solvents have a sticky and viscous nature (Farrer, 2016:1–14; Raber *et al*., 2015:797–803; Romano and Hazekamp, 2013:1–11). The most common example of such an extracted cannabis oil is termed a 'Simpson oil' or 'Rick Simpson oil (RSO)' named after the individual that popularized this type of extraction. Cannabinoids as well as terpenoids and flavonoids are extracted by a solvent, followed by an evaporation step in order to increase the concentration of these compounds in the oil (Romano and Hazekamp, 2013:1–11), and subsequently decrease the amount of solvent present in the final product. Cannabis plant material is usually soaked in the solvent for a period of time, after which the raw plant material is removed, and the solution containing the plant extractables and solvent is boiled (Raber *et al*., 2015:797–803). The solvent evaporation or boiling is usually performed with simple household utensils. These extracts have a very viscous, sticky nature and range in colour from green to black (Raber *et al*., 2015:797–803). These types of cannabis oils are becoming increasingly popular amongst self-medicating patients because of the simplicity and low cost involved in producing these oils (Romano and Hazekamp, 2013:1–11). A study performed in California indicated that the most used solvents are readily available to the public for purchase, and may include acetone, isopropanol and ethanol to name a few (Raber *et al*., 2015:797–803). The high solvent detection rate observed in the reported

study (83.3%) indicates that solvent-based extraction is the method employed by most manufacturers (Raber *et al.*, 2015:797–803).

After solvent evaporation residues are still present within the oil and these solvent residues should be controlled through Good Manufacturing Practice (GMP) and quality control of the final products (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38). It should be noted that the safety limits for these extracts differ considerably between states and published pharmacopoeia's (Griffin, 2020). For the present study, the published and established pharmacopoeial limits will be considered rather than individual American state limits.

In order to ascertain whether a product is safe for chronic human consumption, ICH has predetermined solvent residue limits. Solvents are classified by ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38) according to their potential risk into the following classes:

- class 1 (solvents to be avoided such as benzene);
- class 2 (limited toxic potential such as methanol or acetonitrile);
- class 3 (low toxic potential such as ethanol).

Residual solvents are analysed by headspace gas chromatography-flame ionisation detection (HS GC-FID) or liquid injection GC-FID. The alternative use of mass spectrometry (MS) may provide additional selectivity for co-eluting solvents. The boiling points of some solvents are too high to be amenable to headspace analysis, thus some class 2 solvents need to be injected in liquid form to be analysed. As a result of the viscosity of most of the cannabis oils analysed, liquid injection is not feasible. Currently what is mostly observed in cannabis oil products are class 3 solvents with low toxic potential. Since ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38) provides a guideline of which solvents could be included, a working list of solvents is compiled by each manufacturer or quality control laboratory. From interaction with cannabis oil producers, Table 13 lists the solvents chosen as a class 3 screen for this particular study. This list is by no means exhaustive and is merely a starting point. Few cannabis oil manufacturers employ class 2 solvents,

nonetheless they are present in a limited number of cannabis-based products. Class 2 solvents listed in Table 12 will be employed during this study.

Table 12 - Class 2 residual solvents (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38).

Solvent	Limit (ppm)
Acetonitrile	410
Chlorobenzene	360
Chloroform	60
Cyclohexane	3880
1,2-Dichloroethene	1870
1,2-Dimethoxyethane	100
1,4-Dioxane	380
Hexane	290
Methanol	3000
Methylbutylketone/Hexanone	50
Methylcyclohexane	1180
Methylene chloride	600
Nitromethane	50
Pyridine	200
Tetrahydrofuran	720
Tetralin	100
Toluene	890
Trichloroethylene	80
Xylene*	2170

Table 13 - Class 3 residual solvents (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38).

Solvent	Limit (ppm)
Acetone	5000
Butanone/ Methylethyl Ketone	5000
Diethyl Ether (Ethyl Ether)	5000
Ethanol	5000

Ethyl Acetate	5000
Heptane	5000
Isopropanol	5000
tert-Butylmethyl Ether	5000

2.5 Analytical Methodologies Background

Determining the concentration of active ingredients (Cannabinoids) in any cannabis-based product is of the utmost importance. Cannabinoid quantitation allows doctors to determine accurate dosage and therapeutic monitoring, ensures that providers can verify the quality of their products, and helps patients to select the correct treatment for their symptoms. AOAC International has published draft methods for the standardization of medical cannabis potency testing (AOAC International, 2018). The range of potency analytes currently listed in the AOAC draft are included in Table 14.

Table 14 - Cannabinoids analytes for potency testing (AOAC International, 2018)

Potency
Cannabinol (CBN)
Cannabidiol (CBD)
Cannabidiolic acid (CBDA)
Delta-9-tetrahydrocannabinol (THC)
Delta-9-tetrahydrocannabinolic Acid (THCA)

According to the AOAC guide published, any analytical method capable of measuring the cannabinoids listed in Table 14 and additionally meets a set of method performance requirements, may be employed. The following two sources were employed during the initial development of the potency GC analysis method but was altered in order to optimize throughput and ease of use (Rigdon, 2015; Ruppel *et al.*, 2015:1–6). As seen from literature (Rigdon, 2015), a decarboxylation step can be applied in order to obtain a total potential THC, or alternatively a total potential CBD wt.-%. As a result of the GC inlet temperature being in excess of 200 °C, decarboxylation occurs during injection. The

AOAC has established standard method performance requirements for the measurement of potency. These are listed in Table 15 below.

Table 15 - AOAC method performance requirements for cannabis analysis (AOAC International, 2018)

Parameter	**Performance Limit**		
Limit of Quantitation (LOQ) wt.-%	<= 0.3		
Analytical Range wt.-%	<= 0.3 – 100		
Relative Standard Deviation (RSD) for Specific Range wt.-%	***<=0.3 – 1***	***>1-10***	***>10 - 100***
RSDw (Within Day)	<= 5% RSD	<=4% RSD	<= 2% RSD
RSDb (Between day)	<= 7% RSD	<=5% RSD	<= 3% RSD

For the analysis of all mentioned cannabinoids in Table 14, liquid chromatography should be employed. As a result of the conversion of THCA to THC, and CBDA to CBD at high temperatures, a total potential CBD or total potential THC can also be measured using gas chromatography.

2.5.1 Gas Chromatography

Chromatography refers to a diverse collection of methods that allow for the separation, identification, and determination of compounds within a complex mixture of similar compounds (Skoog *et al*., 2007:947–973) In GC, the components of a gaseous sample are separated as a result of them being partitioned between an inert gas mobile phase and a solid or liquid stationary phase immobilised on the inside of a capillary column. Elution occurs as a consequence of the flow of the gas through the column. In contrast with other chromatographic separations, in GC the mobile phase does not interact with the analyte and only acts as a transport medium (Bishop *et al*., 2010; Skoog *et al*., 2007:947–973). The molecules that strongly interact with the stationary phase have a decreased mass proportion present within the mobile phase at a given time; hence, they will elute later into the detector (Skoog *et al*., 2007:947–973). A detector is placed at the end of the capillary column and the response is plotted as a function of time. As each component elutes at its respective time, the detector measures this response and a series of peaks are obtained. Such a plot is called a chromatogram. The position of the peaks on the time axis of the chromatogram can be used to identify compounds

qualitatively, and the area under the curve is used to quantitatively determine the concentration of the analyte. If the column used does not achieve proper separation, two components may co-elute and form one visible chromatographic peak. GC is perhaps still the best separation tool for the compounds that are amenable to the technique (Dorman *et al.*, 2018:4775–4785).

The wide distribution and popularity of GC-MS renders this type of instrument present in the majority of laboratories. Hence a test method for cannabinoids employing GC-MS will be most beneficial and accepted by a majority of laboratories. The high temperature inlet and column gaseous phase sample introduction has disadvantages as some of the carboxylic acid cannabinoids are heat labile and decarboxylate when exposed to the GC inlet temperature. Thus not all the cannabinoid analytes are amenable to GC. The latter cannabinoids decarboxylate to products that are amenable to GC analysis. An indirect measure of for example total potential THC as well as total potential CBD can be reported by employing GC-MS as analysis technique (Ruppel *et al.*, 2015:1–6).

2.5.2 Liquid Chromatography

High-performance liquid chromatography (HPLC) is an important analytic instrument in modern science, with a high representation of the technique globally. Contemporary HPLC offers increased resolutions allowing for quantitative determination of analytes by separation in complex matrices, also taking into account the techniques compatibility with a wide array of detectors (Gika *et al.*, 2016:93–99).

In contrast to the high temperature inlet of GC, the HPLC technique does not require the sample to pass through a high temperature inlet. Separation and analysis occur at approximately room temperature. The temperature labile cannabinoids can thus be analysed individually by employing HPLC. A total potential THC and CBD concentration can be calculated by taking into account certain ratios of THCA and THC as well as CBDA and CBD, respectively, as seen in Section 2.2.2. (Citti *et al.*, 2018:532–540; Foundation Canna, 2021).

The CO_2 loss as mentioned in Section 2.2.2, is evident for other cannabinoids too, including THCA, CBDA, Cannabigerolic Acid (CBGA) etc. Thus for LC analysis where both the carboxylic acid form as well as the decarboxylated cannabinoid may be

quantitated, these formulas are used to calculate the total potential cannabinoid wt.-%. For GC this is not needed as the measurement occurs after the carboxylic acid form has already been decarboxylated, hence only the decarboxylated form is quantitated even though both might be present in a sample.

2.5.3 Inductively Coupled Plasma

For a sample to be appropriately introduced into the ICP-MS, dissolution of the elements contained in the sample needs to be accomplished through digestion (Gaudino *et al.*, 2007:84–93). Microwave digestion as well as closed or open vessel acid digestion are the two common techniques predominantly employed (Rodushkin *et al.*, 1999:191–200).

The ICP is an incompletely ionised argon gas, produced by radio frequency in a quartz torch (Olesik, 1991:12–21). Samples are aerosolised in a nebuliser spray chamber and introduced into the centre of the plasma. The plasma ionises elements, mostly as singly charged atoms, whereafter charged ions are focused into a MS (Olesik, 1991:12–21). The quadrupole MS separates elements based on their elemental mass to charge ratio. Since the elements are singly charged this ratio is usually the atomic mass of the respective elemental isotope. It should be noted that matrix effects from the sample can cause large variances in measured concentration due to either signal enhancement or suppression (Vanhaecke *et al.*, 1992:737–742). Accurate correction of these fluctuations is usually accomplished by the use of appropriate internal standards (Vanhaecke *et al.*, 1992:737–742). Internal standards are elements not readily found in nature and is usually chosen according to whichever mass most closely resembles the measured element mass (Vanhaecke *et al.*, 1992:737–742). ICP-MS has been successfully employed in the measurement of multiple elements in cannabis plant matrix (Jones and Nelson, 2017:1–7).

2.6 Analytical Methodologies Employed

The following three analytical methods were used as a starting point for the optimisation and analysis of potency, residual solvents and heavy metals.

2.6.1 Potency

For potency both a GC as well as HPLC methods were employed. The GC method starting point was based on an application note released by Perkin Elmer Inc (Ruppel *et al.*, 2015:1–6). The HPLC method was based on an application note released by Quantum analytics (Strull *et al.*, 2016:1–10).

2.6.2 Residual Solvents

For residual solvents a reported analytical method was employed as basis for the development of the final analysis method (Hilliard *et al.*, 2009). Since many of the samples exhibit very high viscosity or are solid in nature, a total-evaporation headspace technique was employed, instead of gas equilibration (Hilliard *et al.*, 2009). Additionally, the USP 467 monograph was also considered as a guide for the development of the analytical technique (United States Pharmacopoeia (USP), 2009:1–10).

2.6.3 Heavy Metals

Heavy metals were also analysed based on a literature method (Jones and Nelson, 2017:1–7). It should be noted that an acid digestion rather than a microwave digestion was employed. This acid digestion was based on the reported procedure (Rodushkin *et al.*, 1999:191–200).

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CHAPTER 3

3 An Assessment of The Potency Related to Cannabis-Based Products In The South African Market

This article was published by Elsevier in the journal Forensic Science international (Viviers *et al.*, 2021:1–8). The complete article can be found in Annexure B, including the author guidelines for the journal Forensic Science International in Annexure C. A Corrigendum (Appendix I) as well as a letter to the editor (Appendix J) was also published, relating to this article.

The Author contributions for this article are as follows:

-Hendrik Jacobus Viviers: Conceptualisation, Methodology, Experimental investigation, Data curation, Writing original draft.

-Anél Petzer: Supervision, Writing - review & editing.

-Richard Gordon: Supervision, Writing - review & editing.

This research project/study was reviewed by the North-West University Health Research Ethics Committee (NWU-HREC) which concluded that this study does not need ethical approval. An attachment of this review can be found in Annexure A at the end of this Thesis.

3.1 Abstract

It was the aim of this study to analyse a portion of the South African cannabis-based products market and provide a detailed overview of their tetrahydrocannabinol (THC) and cannabidiol (CBD) profiles. To date, no data of this kind exists in South Africa. Samples were submitted to a contract laboratory. A total of 840 samples were analysed in duplicate (1680 datapoints in total) and reported in an anonymous format. Samples were categorised into 7 different types: Edible, Extract, Infusion, Liquid, Other, Plant material, and Solid. Each category was divided into the following weight by percentage concentration levels: <0.1 wt.-%, 0.1 wt.-% to 1 wt.-% and finally >1 wt.-%. Both high-performance liquid chromatography (HPLC-UV), as well as, gas chromatography – mass spectrometry (GC-MS) was employed for analysis with the datasets combined. The results indicated that high amounts of THC are present in most of the cannabis-based products in South Africa. This is of concern due to the health implications of these products, and the current South African legislation related

to CBD and THC. Medicines and controlled substance regulators as well as the South African public might be informed about the current state of cannabis-based products in South Africa.

3.2 Introduction

In 2017 South African Medicines Control Council (MCC) rescheduled the cannabinoid, cannabidiol (CBD), to a schedule 4 substance (Medicines Control Council (MCC), 2017:1–32). This rescheduling was mainly the result of the international interest and focus specifically on CBD (Medicines Control Council (MCC), 2017:1–32).

In South Africa, schedule 7 substances are considered to have no genuine medicinal use and can only be accessed by means of a permit issued by the Director-General of the National Department of Health (NDoH). Medicines and substances categorised as Schedule 4 or Schedule 6 are only available on prescription and can only be obtained from a pharmacy (South African Health Products Regulatory Authority (SAHPRA), 2020:1–8).

In a government notice published by the South African Government, in conjunction with the Department of Health, scheduling of cannabis-based products are as follows; cannabis is regarded as schedule 7 (Illicit), but when a registered medicine containing THC is employed for therapeutic use, it is regarded as a schedule 6 substance. The exception to the latter is processed hemp fibre and products thereof, in a form not suitable for ingestion, smoking, or inhaling purposes, and containing not more than 0.1% THC; or when present in processed products from cannabis seed containing not more than 0.001% THC, are regarded as industrial hemp (Republic of South Africa, 2020:1–12; South African Health Products Regulatory Authority (SAHPRA), 2020:1–8). As mentioned CBD is listed as a schedule 4 substance, with certain CBD preparations regarded as unscheduled (schedule 0) when adhering to one of two conditions (Republic of South Africa, 2020:1–12);

1. The preparation contains a maximum total quantity of 600 mg CBD per sales pack and a maximum daily dose of 20 mg CBD with an accepted low-risk claim or health claim.
2. Preparations consist of processed products made from cannabis raw plant material, where only the naturally occurring quantity of cannabinoids found in the source material are contained in the product, and which contain no more than 0.001% of tetrahydrocannabinol (THC) and not more than 0.0075% total cannabidiol (CBD)

A variety of cannabis-based products, aimed at therapeutic use, have been introduced into the South African market with this legislature in mind. Cannabis-based products are either imported, cultivated, or produced by individuals and companies, and are these individuals or companies defined as manufacturers for the purpose of this study. The intent for the manufacturing of these products appears to be therapeutic in nature, but whether these products are employed for the latter remains to be seen.

A myriad of cannabis products, including the former mentioned CBD products, are accessible in dispensaries as well as online globally. United States laws on quality control are inconsistent and in some cases absent (Mead, 2017:288–291). Twenty three European countries as well as Canada and the United States, have approved several cannabinoid-based medicines (Abuhasira *et al*., 2018:2–6). It should be noted that a very limited number of countries have approved the medicinal use of herbal cannabis. These include Canada, Germany, Israel, the Netherlands, and some states in the United States (Abuhasira *et al*., 2018:2–6). In the United States, controlled clinical trials are proceeding to examine the use of CBD as a possible treatment for Dravet syndrome (DS) and Lennox-Gastaut syndrome (LGS) (O'Connell *et al*., 2017:341–348) with some studies also indicating efficacy for epilepsy. CBD effectively and selectively inhibited the growth of different breast tumor cell lines (Massi *et al*., 2013:303–312; Shrivastava *et al*., 2011:1161–1233). CBD has also been shown to decrease proliferation, migration, and invasiveness of glioma, lung cancer, and colon cancer (Massi *et al*., 2013:303–312).

The current regulation in most countries allows for the cultivation of hemp. These strains are specifically engineered to contain trace amounts of THC. Industrial hemp can be defined as all the components of a *Cannabis Sativa* plant with a THC concentration below the regulated hemp limit for that specific country (Bengtsson, 2009:1–40; Rhizo Sciences, 2017). Table 16 below indicates the industrial hemp limits for some countries. It should be noted that the authors are of the opinion that the quoted percentages in the current South African gazette (Republic of South Africa, 2020:1–12) are referring to wt.-%, and will be assuming percentages quoted in any regulation to mean wt.-%.

Table 16 – Legal THC content limit for hemp products in different countries

Country	THC Hemp Limit (wt.-%)	Reference
United States	0.3 wt.-%	(Abernethy, 2019:1; Mead, 2017:288–291; U.S. Food and Drug Administration, 2019)
South Africa	0.2 wt.-%	(Agricultural Research Council, 2014; Republic of South Africa, 2020:1–12)
New Zealand	0.35 wt.-%	(Ministry of Health New Zealand, 2020:1; New Zealand Hemp Industries Association, 1997)
Australia (except Victoria)	1.0 wt.-% (in leaves and flowers)	(Australian Capital Territory Government, 2021:1–53; Government of South Australia, 2021:1; Government of Western Australia, 2021:1; New South Wales (NSW) Government, 2019:1–5; Northern Territory Government, 2020:1–19; Queensland Government, 2020:1; Tasmanian Government, 2020:1)
Australia (Victoria)	0.35 wt.-% (in leaves and flowers)	(Victoria State Government, 2020:1)
Canada	0.3 wt.-% (in leaves and flowers)	(Canada Department of Justice, 2018; Government of Canada, 2018:1)

An evaluation of the quality of cannabis-based products has been performed previously (Hazekamp, 2006:1–9), but is limited to the Netherlands. In the Netherlands, the Office for Medicinal Cannabis regulates the manufacture of pharmaceutical-grade herbal cannabis. Approved strains of cannabis are cultivated under precise conditions with stringent quality control to produce cannabis containing cannabinoid ratios within a specified range (Hazekamp, 2006:1–9; Murnion, 2015:212–215).

According to the World Health Orginisation (WHO), the quality of herbal medicines and cannabis has a direct effect on their safety and efficacy. There is an abundance of control measures for these medicines, plants, and preparations. This is however not a simple task since it involves many areas, including environmental and agricultural practices (World Health Organisation, 2007:1–118). The efficacy of herbal medicines in this case medicinal

cannabis-based products, typically rely on the concentration of two major cannabinoids namely CBD and THC. The WHO has developed a series of technical guidelines for establishing the safety and quality of herbal medications which include medicinal cannabis (World Health Organisation, 2007:1–118).

To improve the quality of cannabis-based products, scientists and manufacturers must standardise analytical processes. This will help educate the regulators, consumers and will allow for the global comparison of products. For now, customers cannot rely on South African government regulations to ensure enforcement of quality control of cannabis-based products. Under the current regulatory system, the patients need to be their own best advocates.

There is no established designation of medicinal cannabis, the term is nonetheless used to refer to the therapeutic use of cannabis-based products and their derivatives (Murnion, 2015:212–215). Therapeutic use of cannabis-based products was the dominating utilisation communicated by manufacturers submitting samples to the laboratory. Whether these products are employed for therapeutic use, the consensus amongst sample submitters is that they aim to either treat disease or export the products. It is thus unknown for which purpose the products are employed and will therefore be designated cannabis-based products.

With this background, the aim of the study is to analyse a segment of the South African cannabis-based products in circulation and provide a detailed overview of their THC and CBD profiles. To date, no data of this kind exists in South Africa. The output of the study will provide a high-level overview of the market and will highlight areas for growth and potential areas to be addressed.

3.3 Materials and Methods

All samples obtained are from a variety of manufacturers in South Africa. Manufacturers are defined as any type of user, retailer, reseller, producer, or importer of cannabis-based products. Whether these manufacturers maintain the full value chain or only a portion thereof they are defined as manufacturers for the purpose of this study. Manufacturers may include cultivators of plants, producers of products, importers, resellers, and pharmaceutical manufacturers. All samples were submitted to a contract laboratory for analysis and the data herein will be represented in an anonymous format. A wide variety of samples were

received, some labelled and sold commercially whilst others had no labelling whatsoever. In total 840 samples were analysed in duplicate (1680 data points in total). Consent was provided to employ the data for research purposes. Sample received were analysed as received by the laboratory irrespective of the moisture or volatile content of the samples.

A Clarus 680 gas chromatograph coupled to an SQ8 mass spectrometer was employed for GC-MS analysis obtained from Perkin Elmer Corporation, Waltham, Massachusetts, United States. A Zebron ZB-Drug-1 (10 m, 0.25 mm x 0.25 μm) capillary column obtained from Phenomenex, Torrance, California, United States, was employed for separation. For HPLC analysis an Agilent Infinity 1260 Quaternary pump, Agilent 1260 Vial sampler, and Agilent 1260 Diode array detector combined with an Agilent Core-shell C_{18} 150 mm x 2.1 mm x 2.7 μm column was employed. All Agilent instrumentation and consumables were obtained from Agilent Technologies, Santa Clara, California, United States.

The samples were categorised into 7 different types and are shown in Table 17.

Table 17 - Sample type categories

Edible	Any sample that is ingested as foodstuffs, be it liquid or solid foodstuffs marketed to humans or animals.
Extract	Any sample prepared or processed in a way to extract cannabinoids from plant material, to up-concentrate the cannabinoid content in the final product. These might include Full extract cannabis oil (FECO), Rosin, Rick Simpson oil (RSO), Hashish, Butane hash oil (BHO), etc.
Infusion	Any sample where raw plant material is added to a carrier medium to produce a certain concentration of cannabinoids. The concentration of cannabinoids in the final product is not more than the initial raw materials added to the carrier medium. For example adding cannabis bud to alcohol, or olive oil.
Liquid	Are any samples that could not be classed as an extract, infusion, edible or solid, and is liquid at room temperature. These are final products usually prepared from extracts or solid samples. The extract or solid isolate is added to a carrier/diluent to produce a final product at a certain cannabinoid concentration. These products can usually be administered

	by employing a dropper bottle. For example, the CBD drops available from vendors in medium-chain triglyceride (MCT) oil.
Other	These are samples that could not be classed in any of the categories above, but was still submitted for a cannabis-related testing panel.
Plant Material	Samples that are plant material, including flowers stems, and leaves, of the cannabis plant.
Solid	Any sample that is not plant material, edible or viscous extract and is solid at room temperature is classed as a solid sample. These usually include pure cannabinoid isolates in solid crystal form of which include extracts that are solid like shatter and DAB, but exclude highly viscous extracts.

Furthermore, each sample type category was divided into the following concentration levels seen in Table 18. These concentration levels were selected because of the dosing requirement to elicit a response. A dose of 15 mg THC is needed to elicit a psychoactive response in an individual (Weinstein *et al*., 2008:441–451). It should also be noted that South African regulations currently stipulate no more than a 20 mg dose of CBD per day (Republic of South Africa, 2020:1–12). A smaller than 0.1 wt.-% limit was chosen to ascertain which samples contained low concentrations of cannabinoids which may not cause a psychoactive response. 0.1 wt.-% to 1 wt.-% was chosen as this would be the average dose needed to either elicit a psychoactive response or adhere to current South African regulations if 1 mL of a product were consumed. Furthermore, if a product contains a higher concentration (> 1 wt.-%) and 1 mL of this product was consumed, more than the regulated quantity of cannabinoids are dosed.

Table 18 - Sample concentration subdivisions

High-CBD. Where CBD concentration is larger than THC concentration	CBD < 0.1 wt.-%
	0.1 wt.-% < CBD < 1 wt.-%
	CBD > 1 wt.-%
High-THC. Where THC concentration is larger than CBD concentration	THC < 0.1 wt.-%
	0.1 wt.-% < THC < 1 wt.-%
	THC > 1 wt.-%

Additional to the concentration subdivisions, data relating directly to manufacturers instead of samples, was also rendered. This would circumvent the statistical implication of one manufacturer submitting numerous samples of the same concentration possibly skewing the results. Manufacturers were then also grouped according to high-CBD (CBD>THC) or high-THC (THC>CBD) type manufacturers.

The following two referenced methods were employed during the initial development of the potency GC analysis method, but were altered in order to optimise throughput and ease of use (Rigdon, 2015; Ruppel *et al.*, 2015:1–6). As seen from literature (Rigdon, 2015), a decarboxylation step can be applied in order to obtain a total potential THC wt.-%, or alternatively a total potential CBD wt.-%. Since the GC inlet temperature is higher than 200 °C, decarboxylation occurs during injection. As a result of this tetrahydrocannabinolic acid A (THCA) and cannabidiolic acid A (CBDA) cannot be measured with ease using gas chromatography. Two analytical techniques were employed for the analysis of manufacturer samples. Firstly, GC-MS and secondly HPLC-UV. A total potential THC and CBD was measured during GC analysis since samples were subjected to a decarboxylation step. As for HPLC analysis, the THCA and THC were added in stoichiometrical ratios to obtain a mathematical total potential THC and CBD. It should be noted that the decarboxylation of THCA, as well as CBDA, will have CO_2 as a by-product. Thus some of the molecules' weight can be attributed to the carboxyl group which produces CO_2 when decarboxylated. Total potential THC is thus (THC+0.87xTHCA) and alternatively, total potential CBD would be (CBD+0.87xCBDA) since 13% of CBDA's and THCA's weight can be attributed to CO_2 which is, in turn, lost to produce THC and CBD. For the remainder of the publication, total potential THC and total potential CBD will be referred to as THC and CBD for ease of explanation. The samples analysed with GC and HPLC were combined to form a large dataset for comparison of THC and CBD concentrations in samples. Total potential THC and total potential CBD was used to be able to combine both datasets, HPLC-UV and GC-MS, since these are the end products produced by GC-MS analysis method.

Since samples submitted to a contract laboratory were analysed, at the start of data collection HPLC instrumentation had not yet been acquired by the laboratory, thus only GC-MS analysis was employed for measurement of THC and CBD. The laboratory acquired HPLC instrumentation to be able to individually measure each cannabinoid at a later stage in the data collection timeline. total Potential CBD, as well as total potential THC, is reported, irrespective of the analytical technique employed the concentration of

cannabinoids can be compared. To increase the dataset size and gain a larger representation of population statistics, the datasets for HPLC analysis as well as GC-MS analysis were pooled.

Validation was performed for both GC-MS, as well as, HPLC techniques. The following performance parameters were determined, Linearity, Precision, Recovery, Limit of detection, and Selectivity. 3 Blanks were analysed and compared to quality control standards for selectivity determination. Appropriate separation and selectivity for analytes were established specifying a UV or MS match of greater than 80% for all analytes. Quality control sample data was employed to determined precision and recovery for both analytical techniques. 60 quality control samples spanning over a period of 1 month was statistically analysed. Validation results for both GC-MS validation as well as HPLC-DAD validation can be viewed in Table 19. Linearity was determined by using 5-point and 6-point calibration curves for GC-MS and HPLC respectively. The Correlation coefficient and linear range can be viewed in Table 19.

Table 19 - Validation results

	GC-MS		***HPLC***		
	CBD	**THC**	**CBD**	**THC**	***Unit***
Linearity	0.98	0.972	0.998	0.998	r^2
Linear range	1.2-100	1.2-100	0.1-100	0.1-100	µg/mL
Repeatability	7.04	6.25	3.87	4.07	%RSD
Recovery/Bias	99.61	98.21	99.65	101.02	%
LOD	0.86	0.203	0.0475	0.0325	µg/mL

To maximize analytical accuracy and minimize bias, two separate lots of each certified reference material (CRM) cannabinoid was employed. The first lot was used to calibrate the analytical instruments whilst a second lot was employed to analyse quality control samples. This ensured that the instrument stayed within calibration. One quality control sample from the first lot of CRM's was run daily to check the calibration and every 10 samples a quality control sample of the second lot of CRM's was run to ensure the instrument did not drift during analysis. If any control sample had a relative standard deviation (RSD) of more than ±8% of the assigned value, the instrument was recalibrated.

3.4 Results

Table 20 shows combined results for both GC-MS as well as HPLC-UV analysis of manufacturer samples grouped according to the categories explained in Table 17 and concentration subdivisions in Table 18:

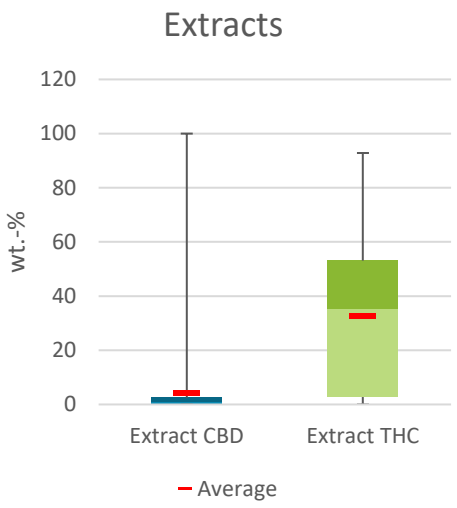
Table 20 - Total sample number and manufacturer distribution

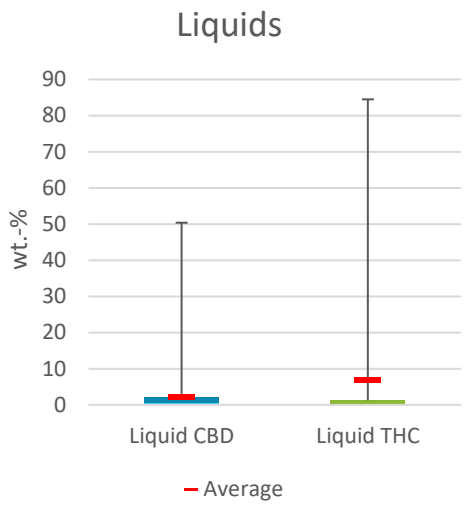
	Edible	**Extract**	**Infusion**	**Liquid**	**Other**	**Plant Material**	**Solid**	***Total***
Total Samples	26	398	129	172	7	100	8	***840***
High-CBD	14	40	43	84	5	36	4	***226***
CBD<0.1 wt.-%	4	6	3	4	2	2	0	***21***
0.1 wt.-% <CBD<1 wt.-%	8	7	16	16	3	14	0	***64***
CBD>1 wt.-%	2	49	24	64	0	20	4	***163***
Manufacturers CBD>THC	6	35	24	28	2	9	3	***107***
Total Manufacturers	16	170	61	72	4	40	6	***369***
Total Samples	26	398	129	172	7	100	8	***840***
High-THC	12	336	86	88	2	64	4	***592***
THC<0.1 wt.-%	6	4	13	5	1	0	0	***29***
0.1 wt.-%<THC<1 wt.-%	4	24	42	36	1	13	1	***121***
Total THC>1 Samples	2	308	31	47	0	51	3	***442***
Manufacturers THC>CBD	10	135	37	44	2	31	3	***262***
Total Manufacturers	16	170	61	72	4	40	6	***369***

Concentration data, instead of concentration ranges, depicted in box and whisker plots (Figure 10-15) and data are shown in Table 20. These plots show the median, first quartile, third quartile, maximum, minimum, and average concentrations of samples grouped by sample type and further subdivided by analyte THC or CBD. The averages in the box and whisker plots are shown with red lines. Units of measure for all values in Table 21 are wt.-%.

Table 21 - Concentration distribution by sample in wt.-%

		Edible	Extract	Infusion	Liquid	Other	Plant material	Solid
CBD	Average	0.56	4.36	0.95	2.18	0.13	1.47	55.95
	1st quartile	0.003	0.00	0.00	0.00	0.02	0.04	0.26
	Median	0.04	1.00	0.03	0.58	0.04	0.18	91.49
	3rd quartile	0.21	2.49	0.59	2.20	0.19	0.64	96.64
	Max	8.80	100.00	19.36	50.38	0.48	13.64	99.41
	Min	0.00	0.00	0.00	0.00	0.00	0.00	0.00
THC	Average	1.20	32.54	1.24	6.90	0.04	6.29	5.96
	1st quartile	0.00	2.74	0.03	0.00	0.00	0.16	0.00
	Median	0.02	35.36	0.29	0.17	0.00	1.44	0.00
	3rd quartile	0.08	53.11	0.90	1.25	0.04	11.08	1.36
	Max	28.40	92.84	38.41	84.49	0.22	30.88	74.18
	Min	0.00	0.00	0.00	0.00	0.00	0.00	0.00





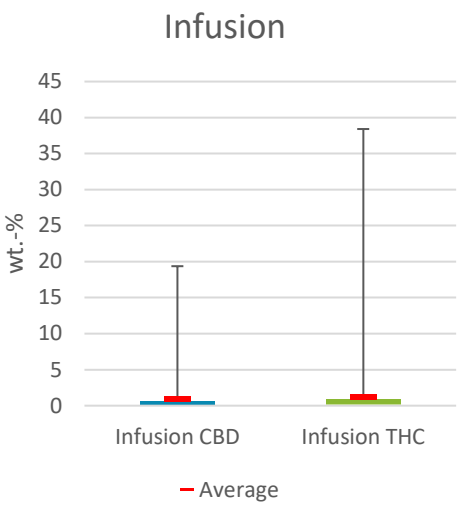
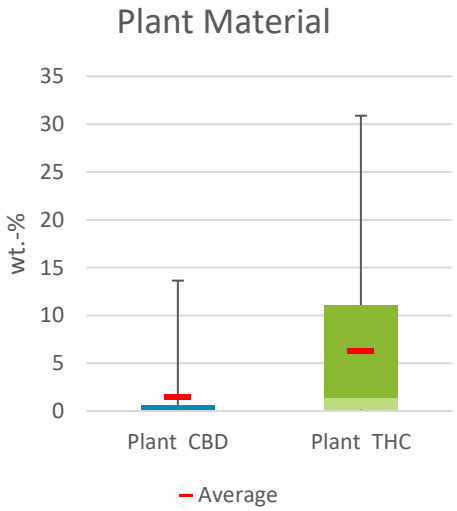
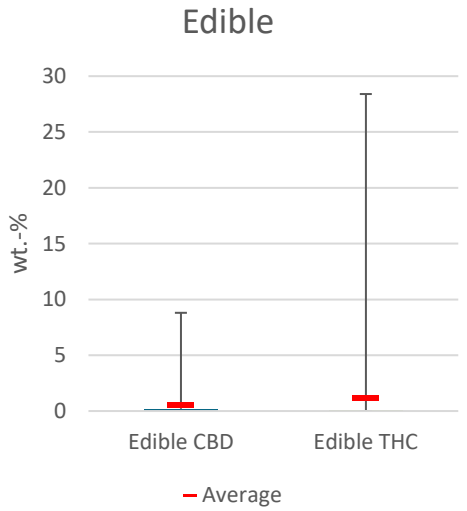


Figure 10 - Concentration distribution of Extracts **Figure 11 - Concentration distribution of Liquids** **Figure 12 - Concentration distribution of Infusions**





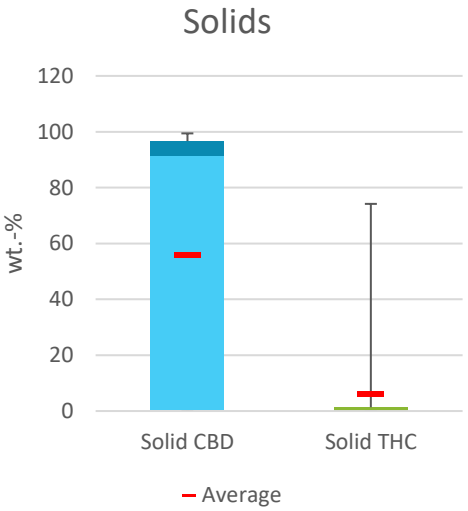


Figure 13 - Concentration distribution of Plant Material **Figure 14 -Concentration distribution of Edibles** **Figure 15 - Concentration distribution of Solids**

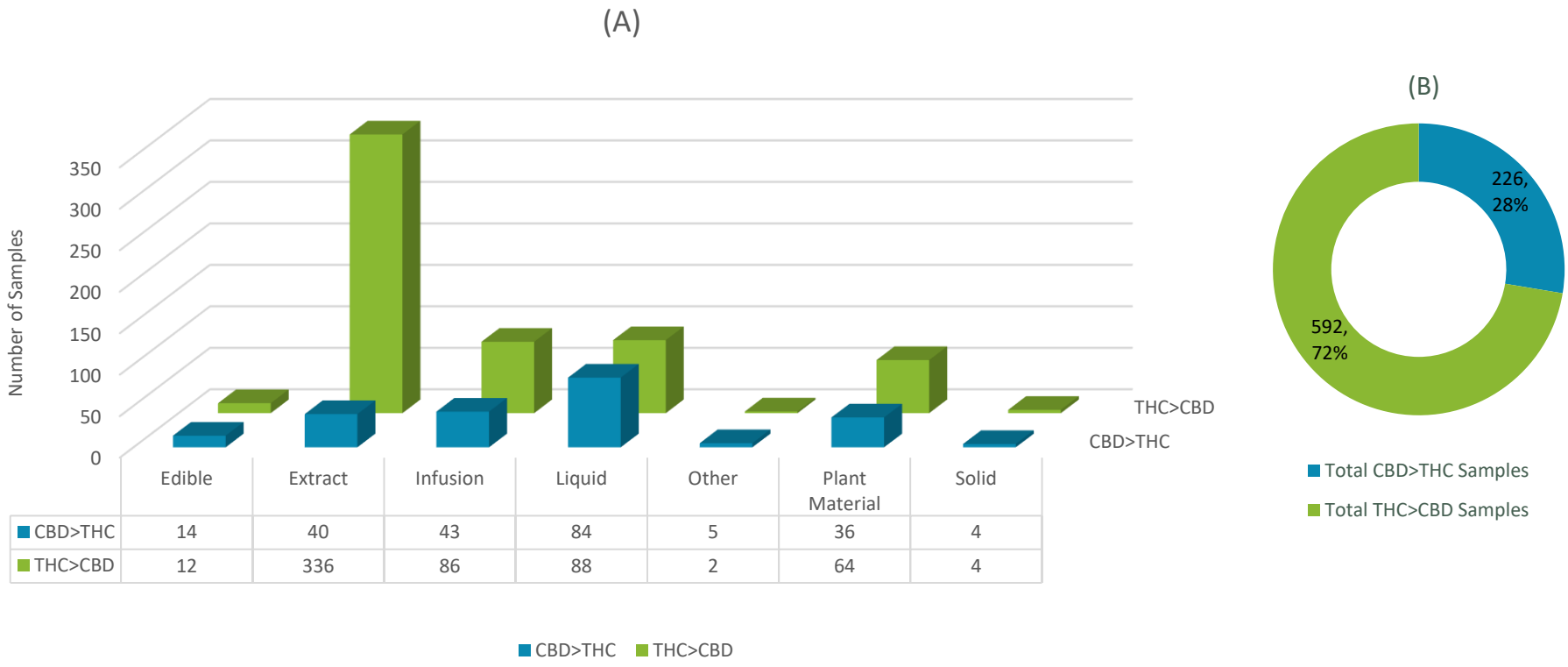
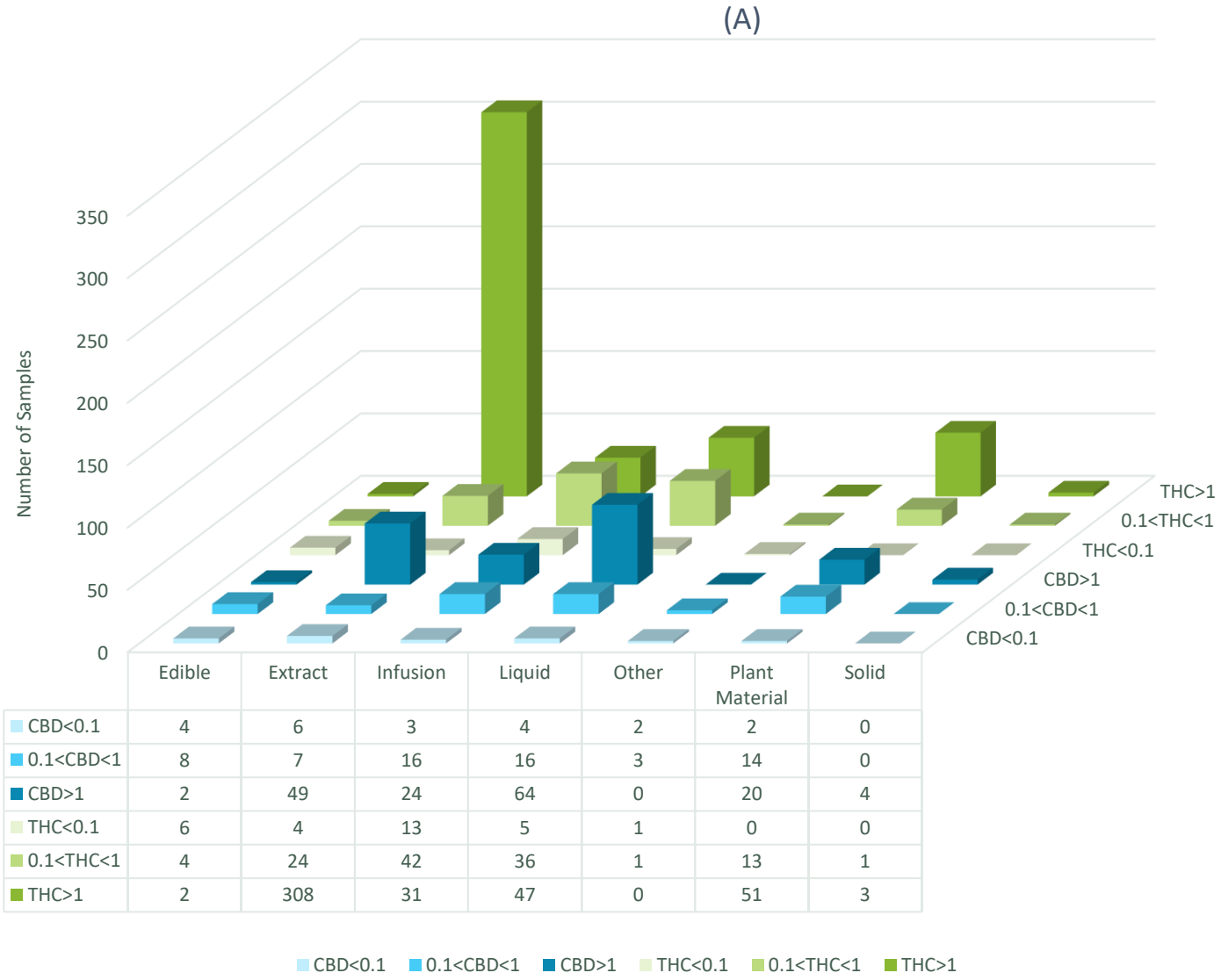


Figure 16 - Total sample proportion by sample type (A), Total sample proportion, groups combined (B).



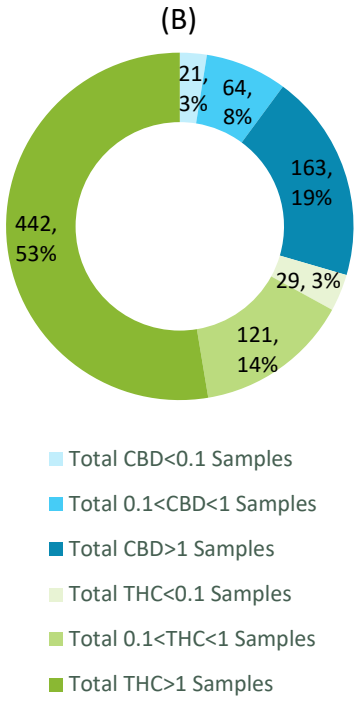


Figure 17 - Total sample proportion grouped by sample type and concentration category (A), Total Sample proportion combined, grouped by concentration category (B)

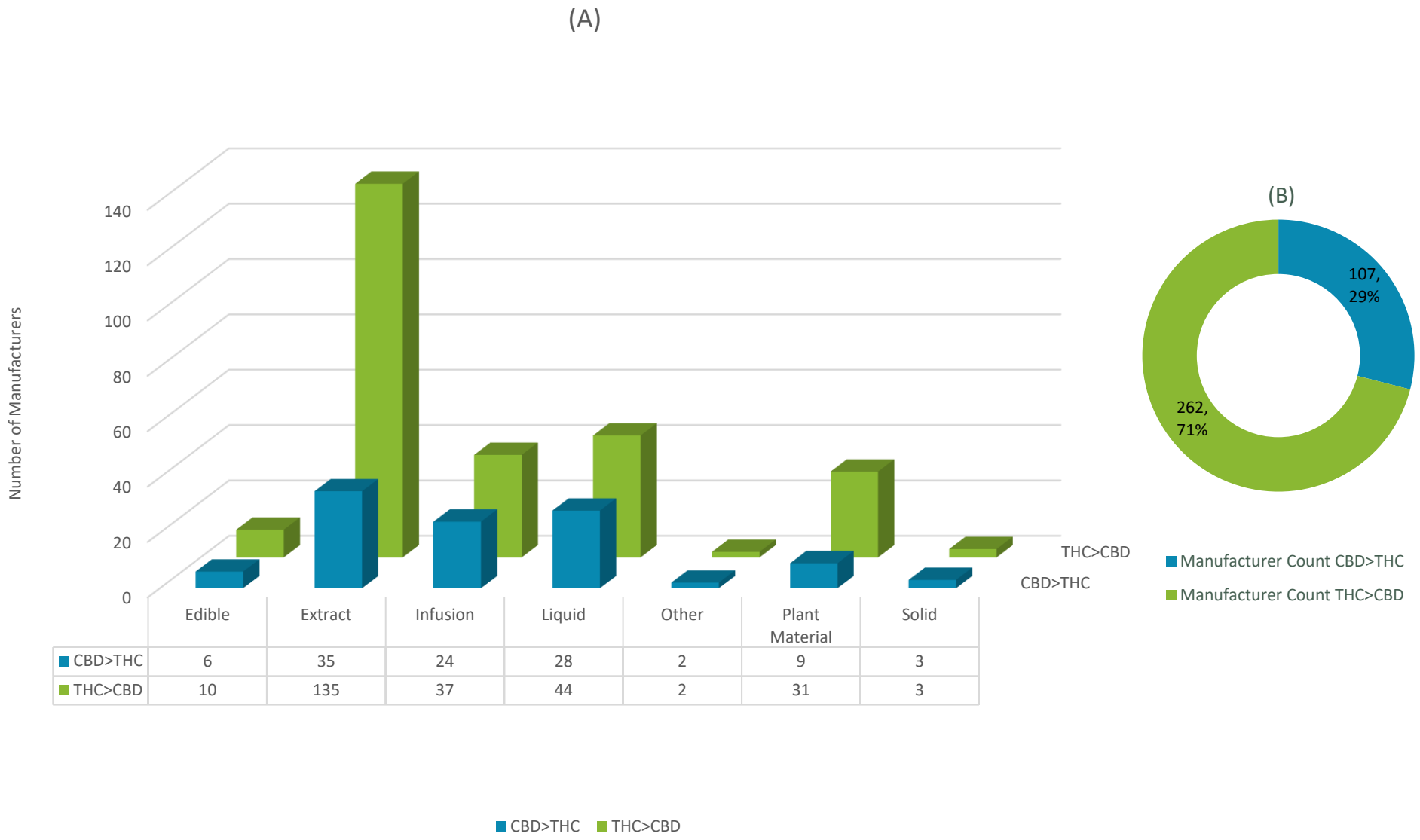


Figure 18 - Total manufacturer proportion grouped by sample type (A), Total manufacturer proportion combined sample type (B)

3.5 Discussion

The data represents an overview of cannabis-based products in South Africa, be it home manufacturers, imported or professional manufactures of these products. This data should not be regarded as a representation of the South African cannabis market since not every product on the market was analysed. Each of the sample type categories will be discussed individually in order of decreasing data set size. Data relating directly to manufacturers instead of samples will also be discussed.

3.5.1 Extract

Extract category type showed the highest number of samples, and the products currently available in South Africa are much more concentrated than one can expect from naturally occurring cannabis plant material. Since no data is available on which products are purchased, the only inference that can be made is that the product type with the highest number of samples is the most purchased or available in the current market. This is especially of concern and may pose the highest risk because dosing is a major concern with these products as seen from literature (Mudan *et al.*, 2019:1086–1087). Furthermore, when extracts are considered alone it can be seen that 336 out of a possible 398 samples contained THC levels that are higher than those of CBD. Since CBD is employed in most medical studies (Massi *et al.*, 2013:303–312; O'Connell *et al.*, 2017:341–348; Shrivastava *et al.*, 2011:1161–1233), it is noteworthy that 84% of all extract samples had higher THC content than CBD. If the concentration data of the extract category is considered, only 4 out of 398 (1%) extract samples adhered to the legal South African industrial hemp THC limit of 0.2 wt.-% (Agricultural Research Council, 2014; Republic of South Africa, 2020:1–12), and even less than 1% of samples adhered to the current South African legislation to have THC absent (<0.001 wt.-%) in products. If the sample were intended for ingestion the South African hemp limit no longer applies and THC concentration of less than 0.001 wt.-% should be present to adhere to the legislation(Republic of South Africa, 2020:1–12). The second concentration category of 0.1 wt.-% to 1 wt.-% as explained, is the concentration cannabinoid necessary to elicit a psychoactive response, or adhere to current South African legislation, if 1 mL of said product was consumed. In the extract type 24 THC and 7 CBD samples were analysed adhering to this concentration category. This means 31 out of 398 (8%) extract type samples had concentrations of cannabinoids to

appropriately dose individuals when 1mL of product is consumed. The remainder of the extract type samples contained high cannabinoid concentrations. A total of 357 samples out of 398 (89.7%) thus contained cannabinoids higher than 1%, in effect overdosing individuals when 1mL of product is consumed. If the manufacturers of this category are considered, a number of 35 CBD>THC and 135 THC>CBD manufacturers were grouped in the extract type. This shows that 79.4% of extract type manufactures produced products where THC concentrations are higher than CBD concentrations.

3.5.2 Liquid

Liquid category type showed the second-highest number of samples analysed. This could be due to the fact that these sample types are prepared from extracts in an attempt to lower the cannabinoid dosage concentration. It can be seen that 84 out of a possible 172 samples contained CBD levels higher than that of THC. The higher ratio of CBD samples could be attributed to internationally purchased CBD isolate being added to liquid dosage forms, where the availability of such high-CBD extracts is currently limited in South Africa, as seen from the extract and solid categories discussed. If the concentration data of the liquid category are analysed, less than 5 out of 172 (2.9%) liquid samples adhered to the legal South African THC limit of less than 0.001 wt.-%. It should be noted that liquid samples cannot be compared to the South African hemp limit, since they are intended for human consumption. 52 out of 172 (30.2%) liquid type samples had the cannabinoid concentration needed to elicit a response if 1 mL was consumed. This is much higher compared to extracts as a result of the possible attempt by manufacturers to control the cannabinoid dosage concentration. This is the category with the highest ratio of CBD to THC combined with acceptable cannabinoid dosage concentration. This category would then pose the least amount of risk since the probability dictates that an individual has a higher chance of purchasing a product with an appropriate dose and CBD concentration. If the manufacturers are considered, a number of 28 CBD>THC and 44 THC>CBD manufacturers were grouped in the extract type. This means 61.1% of manufactures in this category, supplied products where the THC concentration was more than the CBD concentration.

3.5.3 Infusion

The infusion category type showed the third-highest number of samples analysed. This prevalence might be the result of the ease of preparation. In this respect, plant material is simply added to a carrier and then removed in an attempt to extract low concentrations of cannabinoids from the plant material. Since these samples are prepared from raw plant material, it is interesting to note that when comparing the infusion CBD>THC to THC>CBD ratio (0.5) to the plant material CBD>THC to THC>CBD ratio (0.56), they are very similar. This might indeed suggest that the obtainable plant material available in South Africa was employed in the manufacture of these infusions. If the concentration ranges in the infusion category are analysed, only 13 out of 129 (10%) infusion samples adhered to the legal South African industrial hemp THC limit of 0.2 wt.-% (Agricultural Research Council, 2014), and none adhered to the current South African THC legislation of less than 0.001 wt.-%. It should be noted that that infusion are compared to both the South African hemp limit as well as THC limit intended for human consumption since they may contain plant material in large proportions. Since these products are most probably intended for human consumption more weight should be attributed to the South African THC limit for products intended for human use (Republic of South Africa, 2020:1–12). This may be attributed to the fact that none of the plant material samples analysed were absent from THC. 58 out of 129 (45%) infusion type samples had the average cannabinoid concentration needed to elicit a psychoactive response if 1 mL was consumed. The remainder of the infusion type samples contained high cannabinoid concentrations. This is the category with the highest percentage 45% of acceptable cannabinoid dosage concentrations regardless of the specific cannabinoids contained in the products. If the manufacturers are considered, a number of 24 CBD>THC and 37 THC>CBD manufacturers were grouped in the infusion type. This means 60.7% of manufactures that produce infusions, supplied products where the THC has a higher concentration than CBD. The infusion type thus has the second-highest likelihood amongst manufacturers to contain CBD as cannabinoid (39.3%) only exceeded by solids.

3.5.4 Plant Material

The plant material category type showed the fourth-highest number of samples analysed. In this category 36 out of a possible 100 samples contained CBD levels

higher than THC versus 64 out of a possible 100 samples containing THC levels higher than CBD. This may be as a result of the cannabis plants not only being grown for medicinal use but rather recreational use as well. If we break down the plant material category further into its concentration categories, 0 out of 100 (0%) plant material samples adhered to the legal South African industrial hemp THC limit of 0.2 wt.-% (Agricultural Research Council, 2014) or current South African legislation for products intended for human consumption. In the second concentration category of 0.1 wt.-% to 1 wt.-%, a number of 13 THC and 14 CBD samples were analysed. This means 27 out of 100 (27%) plant material type samples had the average concentration needed to elicit a psychoactive response if 1 g was consumed. The remainder of the plant material type samples contained high cannabinoid concentrations. It should also be noted that the predominant form of cannabinoid in the analysed plant material is both THCA and CBDA. If the manufacturers are considered, a number of 9 CBD>THC and 31 THC>CBD manufacturers were grouped in the plant material type. This means 77.5% of manufactures that grow cannabis, produce plants with a higher THC concentration than CBD. This is especially concerning since CBD was the cannabinoid that was amended to a schedule 4 compound whilst THC continued to be an illicit schedule 7 substance in South Africa (Republic of South Africa, 2019:1–20). Since this is the raw material currently available in South Africa it would appear that high-CBD plant material must either be imported or sourced in South Africa with great care.

3.5.5 Edible

The Edible category type showed the third-lowest number of samples analysed. In this category 14 out of a possible 26 samples contained CBD>THC versus 12 out of a possible 26 samples containing THC>CBD. If we break down the edible category further into its concentration categories a possible 6 or less out of 26 (23%) edible samples adhered to the legal South African THC limit of less than 0.001 wt.-% (Republic of South Africa, 2020:1–12). It should be noted that since edibles are intended for human consumption these sample cannot be compared to hemp limit. It should be noted that edible is the category with the highest prevalence of samples adhering to both the legal industrial hemp legislation limit and current South African legislation. A possible cause for this is the increased weight of foodstuffs. A large quantity of foodstuffs can be ingested compared to other dosage forms, and thus the concentration of cannabinoids in these products are low to account for large consumption during a

meal. 12 out of 26 (46.2%) edible type samples had the average concentration needed to elicit a psychoactive response if only 1 g was consumed. Edibles are primarily ingested in quantities of more than 1 g, thus the dosages of cannabinoids administered are of large concern when looking at the edible category. This concern is further amplified by the remainder of edible samples containing high cannabinoid concentrations of > 1 wt.-%. A total of 4 samples out of 26 (15.4%) contained cannabinoids higher than 1%. If the edible manufacturers are considered, a number of 6 CBD>THC and 10 THC>CBD manufacturers were grouped in the edible type. This means 62.5% of manufactures that produce edibles produce products where the THC has a higher concentration than CBD. The edibles type thus has the third-highest likelihood amongst manufacturers to contain CBD as cannabinoid (37.5%) only exceeded by the liquids and solids category.

3.5.6 Solid

The solid category type showed the second-lowest number of samples analysed. This might be as a result of the cost and difficulty obtaining pure solid isolated cannabinoids. In this category 4 out of a possible 8 samples contained CBD levels higher than THC and 4 out of a possible 8 samples contained THC levels higher than CBD. This is interesting since it could be argued that isolated cannabinoids are purchased to compound or formulate cannabis-based products containing predominantly CBD. This is not however the case, and half of the samples contain high-THC. It could be expected that these samples will all fall in the >1 wt.-% range since they are pure isolated cannabinoid forms. Nevertheless a comparison against the South African legal THC limit of less than 0.001 wt.-% for products intended for human use, 0 samples adhered. In the second concentration category of 0.1 wt.-% to 1 wt.-%, only 1 THC and 0 CBD samples were analysed in the solid category. This means 1 out of 8 (12.5%) solid type samples had less than ideal extraction efficiencies. The remainder of the solid type samples contained high cannabinoid concentrations as expected. This is the category with the highest amount of concentrated cannabinoids. If the solid manufacturers are considered, 3 CBD>THC and 3 THC>CBD manufacturers were grouped in the solid type. This means that 50% of manufactures that produce solids, supplied products where the THC has a higher concentration than CBD. The solid type thus has the highest likelihood amongst manufacturers to contain CBD as a cannabinoid (50%). It

should be mentioned that these products have a high concentration of cannabinoids and dilutions will be necessary in order to obtain an acceptable dosage level.

3.5.7 Other

A total of 7 other sample types were analysed because they could not be allocated to any of the above-mentioned categories. These 7 samples showed predominantly average to low concentrations of cannabinoids with only 1 sample being higher than 1 wt.-% .

The combined distribution of all sample types showed that 592 (72%) samples of a possible 840 contained THC concentrations that are higher than that of CBD. 226 (28%) of a possible 840 have CBD concentrations that are higher than that of THC. Even though not all samples may be intended for human consumption or may contain plant material of some sort, a comparison of both the South African legal hemp limit (0.2 wt.-% THC) as well as human use limit (<0.001 wt.% THC) will be provided for interests' sake. If the individual concentrations groups are considered, it is noteworthy that of a possible 840 samples, only 29 (3%) of samples adhered to the legal South African industrial hemp THC limit of 0.2 wt.-% (Agricultural Research Council, 2014), and less than 3% of samples adhered to current South African legislation of less than 0.001 wt.-% THC. A total of 605 samples out of 840 (72%) contained concentrations of cannabinoids higher than 1%.

3.6 Conclusion

In conclusion, it is evident that most of the cannabis-based products in South Africa contain high amounts of THC. This is of concern due to the health implications of these products. Furthermore, a very small portion of all products adheres to the current industrial hemp legislation of 0.2 wt.-% THC, notwithstanding the more stringent < 0.001 wt.-% THC imposed by the South African government for products intended for human use. As seen from the data, the liquid sample type has the greatest chance of containing CBD at a reasonable dosage level, but care must be taken by the public when purchasing these products. Currently, even though the concentration limits and regulations have been imposed by the South African government, enforcement of these limits and regulations are lacking. The publication of these data will assist the South African public to make informed purchases of such medicines and will guide controlled substances and medicines regulators alike.

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CHAPTER 4

4 An Assessment of Heavy Metal Contaminants Related to Cannabis-Based Products in the South African Market

This article was published by Elsevier in the journal Forensic Science international: Reports (Viviers *et al*., 2021:1–6). The complete article can be found in Annexure D, including the author guidelines for the journal Forensic Science International in Annexure E, at the end of this thesis.

The Author contributions for this article are as follows:

-Hendrik Jacobus Viviers: Conceptualisation, Methodology, Experimental investigation, Data curation, Writing original draft.

-Anél Petzer: Supervision, Writing - review & editing.

-Richard Gordon: Supervision, Writing - review & editing.

This research project/study was reviewed by the North-West University Health Research Ethics Committee (NWU-HREC) which concluded that this study does not need ethical approval. An attachment of this review can be found in Annexure A at the end of this Thesis.

4.1 Abstract

South African cannabis-based products that were submitted to a private laboratory for the determination of heavy metal residues, were analysed. Two USP 232/ICH Q3D (Elder and Teasdale, 2014:25–29; United States Pharmacopoeia (USP), 2017:1–3) specifications were considered for both oral as well as inhalation limits. The presence of each heavy metal residue was also determined in order to establish which residues are most prevalent in samples. To date, no data of this kind exist in South Africa specifically relating to cannabis-based medicinal, recreational, or complementary products. A total of 310 samples were analysed in duplicate and are reported in an anonymised format. The submitted samples were divided into different category classifications and grouped according to relevance for oral or inhalation specification. The results showed an alarming 15% sample failure rate compared to the oral specification limit and a 44% failure rate compared to the inhalation specification limit. It is of the utmost importance for manufacturers to have the appropriate quality control regimes in place, especially for heavy metal residues, in South Africa. Furthermore, it is imperative to ensure regulation is enforced and the South African public is educated about the risks associated with using these products.

4.2 Introduction

In the cannabis industry, be it medicinal or recreational, there is an abundance of control measures in place to ensure product safety and efficacy (World Health Organisation, 2007:1–118). Contamination of cannabis plants with toxic heavy metals such as arsenic, cadmium, lead etc. can result from numerous origins. Sources of contamination include environmental pollution such as emissions from factories and automobiles, contaminated water, some pesticides, and naturally occurring metals in soil and fertilisers (Edelstein and Ben-Hur, 2018:431–444). The contamination of the herbal material ultimately leads to contamination of the products during various stages of the manufacturing process (Khan *et al.*, 2017:203–207; Tchounwou *et al.*, 2012:133–164). During growth, metals accumulate in the biomass of specific plants (Meers *et al.*, 2005:561–572). Studies conducted on industrial hemp show that the cannabis plant bioaccumulates heavy metals from the soil, and thus is readily employed for phytoremediation of contaminated soils (Marques *et al.*, 2009:622–654). There have been reported cases of post processing adulteration of cannabis buds, adding heavy metals to increase the weight of the product to purposely

increase the street value (Edelstein and Ben-Hur, 2018:431–444). Pesticides that contain arsenic and mercury as part of their structures were commonly utilised until a few years ago, and are still employed to date. These toxic substances are likely to be present in many foods due to their abundance in nature, and it is important to note that associated ingestion or inhalation of these cannabis products would add to the accumulation of heavy metals consumed by people, even if best practice guidelines are followed (World Health Organisation, 2007:1–118).

As a result of the new regulation imposed by the USP (United States Pharmacopoeia (USP), 2017:1–3) in collaboration with the ICH (International Community of Harmonisation (ICH), 2015:1–84), the detection limits for certain metals have been lowered. Heavy metal residues in pharmaceutical end products, active pharmaceutical ingredients and excipients need to be controlled and should be at a certain limit for safe human consumption (Fischer *et al*., 2014:121–129). Furthermore it can be noted in the somewhat unique case of cannabis-based products, that an alternative route of administration of these products does occur, namely inhalation. The pharmacopoeial guideline stipulates three routes of administration namely: parenteral, oral and inhalation (International Community of Harmonisation (ICH), 2015:1–84; United States Pharmacopoeia (USP), 2017:1–3). Cannabis-based products are predominantly administered through oral and inhalation pathways.

ICP-MS (inductively coupled plasma - mass spectrometry) is employed to detect heavy metal contamination. As a result, low residue limits can be imposed by USP 232 (Fischer *et al*., 2014:121–129; United States Pharmacopoeia (USP), 2017:1–3). Heavy metals are classified into different classes according to their toxic potential, class 1 (Table 22) being the most dangerous, class 2 less toxic and class 3 having the highest limits and being the least toxic. This study will focus on Class 1 and 2 metal residues given they present the greatest health risk to consumers.

Table 22 - Heavy metal residue limits imposed by ICH Q3D R1
(International Community of Harmonisation (ICH), 2015:1–84; United States Pharmacopoeia (USP), 2017:1–3)

		Limit (µg/g)	
		Oral	Inhalation
Class 1	**Cd**	0.5	0.3
	Pb	0.5	0.5
	As	1.5	0.2
	Hg	3	0.1
Class 2A	**Co**	5	0.3
	V	10	0.1
	Ni	20	0.5
Class 2B	**Tl**	0.8	0.8
	Au	10	0.1
	Pd	10	0.1
	Ir	10	0.1
	Os	10	0.1
	Rh	10	0.1
	Ru	10	0.1
	Se	15	13
	Ag	15	0.7
	Pt	10	0.1

It is the aim of this study to analyse a segment of the South African cannabis-based products in circulation and provide a detailed overview of the elemental impurities/heavy metal residues contained in these products. Furthermore, the adherence of these samples to the imposed inhalation as well as oral limits by the ICH and USP will also be evaluated (International Community of Harmonisation (ICH), 2015:1–84; United States Pharmacopoeia (USP), 2017:1–3). To date no data of this kind exist in South Africa. With these data, regulators, medical doctors and the public can gain a better sense of the dangers currently being faced regarding cannabis-based products in South Africa.

4.3 Materials and Methods

A Nexion 350D inductively coupled plasma – mass spectrometer (ICP-MS), which was employed for elemental analysis was obtained from Perkin Elmer corporation (Waltham, Massachusetts, United States). An in-house method was developed for the analysis of these products and validated according to ICH Q2(r1) (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2005:1–17), for linearity, precision, accuracy and stability. A 5 point calibration was employed for all analytes and ranged from 0.0018 µg/mL to 0.5 µg/mL. All calibration curves exhibited a correlation coefficient (r^2) of 0.999 or better. A summary of validation data is listed in Table 23. Certified reference materials (CRM's) were sourced locally and a custom standard mix was prepared according to the stability of the metals as well as the classes analysed. The first CRM 250MUL4A contained Cd (10 µg/mL), Pb (10 µg/mL), As (30 µg/mL), Hg (60 µg/mL) in 5% HNO_3. The second CRM 125AG30-HNO3 contained Ag (30 µg/mL) in 5% HCl. The third CRM 250MUL12A contained Tl (1.6 µg/mL), Co (10 µg/mL), Au (20 µg/mL), Ir (20 µg/mL), Pd (20 µg/mL), Pt (20 µg/mL), Rh (20 µg/mL), Ru (20 µg/mL), Os (20 µg/mL), V (20 µg/mL), Se (30 µg/mL), Ni (40 µg/mL) in 5% HNO_3. Samples were evaluated against a pharmacopoeial limit imposed by both the USP 232 as well as ICH Q3D (International Community of Harmonisation (ICH), 2015:1–84; United States Pharmacopoeia (USP), 2017:1–3). It should be noted that three routes of dose administration are listed in both these guidelines: parental, oral and inhalation. No parenteral samples have been received to date, but oral and inhalation specification limits were considered.

Table 23 - Individual heavy metal residue validation data

	Precision (%RSD)	Bias/Recovery (%)	LOD (µg/mL)	Linearity (r^2)
Cd	2.04	112.71	0.033	0.9994
Pb	6.31	96.47	0.033	0.9999
As	4.25	110.7	0.184	0.9999
Hg	5.03	108.2	0.075	0.9991
Co	2.37	103.9	0.039	0.9997
V	2.49	101.4	0.079	0.9998
Ni	2.53	106.1	0.158	0.9994
Tl	4.72	93.1	0.006	0.9997
Au	5.34	111.7	0.079	0.9991
Pd	2.76	104.0	0.079	0.9998
Ir	5.62	97.65	0.079	0.9999
Os	8.06	91.42	0.079	0.9998
Rh	2.58	101.2	0.079	0.9999
Ru	2.72	99.61	0.079	0.9999
Se	3.99	108.1	0.118	0.9993
Ag	8.59	95.5	0.118	0.9998
Pt	5.91	101.3	0.079	0.9997

A total of 310 samples were submitted to a South African contract laboratory for analysis. Manufacturers are defined as any type of user, retailer, reseller, producer, or importer of cannabis-based products. Whether these manufacturers maintain the full value chain or only a portion thereof they are defined as manufacturers for the purpose of this study. Manufacturers may include cultivators of plants, producers of products, importers, resellers, and pharmaceutical manufacturers. Data of the samples will be presented anonymously. All samples were analysed in duplicate. Class 1 and 2 heavy metal test panels were analysed (See Table 22). It should be noted that the majority of samples were cannabis-based products. Very few samples were of cannabis related cultivation origin which aimed to analyse soil and water. Consent was provided to employ the data for research purposes.

The samples were categorised into 7 different types and are shown in Table 24, the same as reported by a potency study conducted by the same laboratory (Viviers *et al*., 2021:1–8).

Table 24 - Sample type categories

Edible	Any sample that is ingested as foodstuffs, be it liquid or solid foodstuffs marketed to humans or animals.
Extract	Any sample prepared or processed in a way to extract cannabinoids from plant material, to up-concentrate the cannabinoid content in the final product. These might include FECO (Full extract cannabis oil), Rosin, RSO (Rick Simpson oil), Hashish, BHO (Butane hash oil), etc.
Infusion	Any sample where raw plant material is added to a carrier medium to produce a certain concentration of cannabinoids. The concentration of cannabinoids in the final product is not more than the initial raw materials added to the carrier medium. For example adding cannabis bud to alcohol, or olive oil.
Liquid	Are any samples that could not be classed as an extract, infusion, edible or solid, and is liquid at room temperature. These are final products usually prepared from extracts or solid samples. The extract or solid isolate is added to a carrier/diluent to produce a final product at a certain cannabinoid concentration. These products can usually be administered by employing a dropper bottle. For example, the CBD drops available from vendors in medium-chain triglyceride (MCT) oil.
Other	These are samples that could not be classed in any of the categories above, but was still submitted for a cannabis-related testing panel.
Plant Material	Samples that are plant material, including flowers stems, and leaves, of the cannabis plant.
Solid	Any sample that is not plant material, edible or viscous extract and is solid at room temperature is classed as a solid sample. These usually include pure cannabinoid isolates in solid crystal form. These include extracts that are solid like shatter and DAB, but exclude highly viscous extracts.

Approximately 200 mg of each sample was weighed, digested in 10% HNO_3 for 1 hour at 100˚C and diluted 35 times to a total volume of 7 mL. Internal standard Y (Yttrium) and In

(Indium) was used for matrix interference correction. Since large isolate as well as extract sample quantities are scarce, a method needed to be developed to be able to employ as small as possible sample quantity while still being able to reach the USP232/ICHQ3D limits. Analysis started with a blank run, then a 5 point calibration curve, followed by a control standard every 10 duplicates, to avoid instrumental drift. Sample sets were ended by analysing a control standard to ensure all samples within a sample set adhered to bias and variation limits as per USP232/ICHQ3D. The data were grouped into two major groups containing categories applicable to either the inhalation limits and/or categories applicable to the oral specification limits as per USP232/ICHQ3D. All categories were included in the oral specification limit, since all samples needed to be compared to a specification. As for the inhalation specification, the following categories were grouped together for comparison; Extract, Liquid and Plant Material. Since it is not known by the laboratory what the final intended use of the products were, these three categories posed the highest likelihood being dosed in inhalation form. The two major dosage form groups were also subdivided into two different categories.

1. Individual heavy metal residues were grouped together with no relationship to the sample or matrix, in order to determine the number of times this specific metal residue failed to adhere to the USP232/ICHQ3D specification.
2. Secondly samples were grouped together to determine the frequency of failed samples. The frequency of failed samples only considered whether any one of the heavy metal residues in the test panel failed.

Lastly the occurrence of heavy metal residues was determined, without any adherence to a specification limit. This provided valuable data on the metal residues that are predominantly present, in addition to whether they are able to be detected by the test method. Heavy metal residues noted as either present when it was detected in a sample, or not detected (ND). Limits of detection is listed in Table 25 below.

Table 25 - Individual heavy metal residue presence in the dataset

	Present	Not Detected	LOD (µg/mL)
Cd	106	204	0.033
Pb	196	114	0.033
As	92	218	0.184
Hg	73	237	0.075
Co	177	133	0.039
V	50	260	0.079
Ni	194	116	0.158
Tl	117	193	0.006
Au	29	281	0.079
Pd	19	291	0.079
Ir	32	278	0.079
Os	22	288	0.079
Rh	50	260	0.079
Ru	29	281	0.079
Se	56	254	0.118
Ag	50	260	0.118
Pt	21	289	0.079

4.4 Results

Tabulated results of the data are shown in Table 25 above as well as Table 26, Table 27 and Table 28 below.

Table 26 - Individual heavy metal residue failures grouped according to categories applicable to the oral specification limit (USP/ICHQ3D)

	Edible		Extract		Infusion		Liquid		Plant Material		Solid		Other		Total	
	Pass	Fail	Pass	Fail	Pass	Fail	Pass	Fail	Pass	Fail	Pass	Fail	Pass	Fail	Pass	Fail
Cd	28	0	140	1	35	0	44	0	28	0	19	0	15	0	309	1
Pb	10	18	129	12	35	0	43	1	19	9	19	0	15	0	270	40
As	26	2	135	6	35	0	44	0	28	0	19	0	15	0	302	8
Hg	28	0	141	0	35	0	44	0	28	0	19	0	15	0	310	0
Co	28	0	141	0	35	0	44	0	28	0	19	0	15	0	310	0
V	28	0	141	0	35	0	44	0	28	0	18	1	15	0	309	1
Ni	28	0	138	3	35	0	44	0	27	1	19	0	15	0	306	4
Tl	28	0	141	0	35	0	44	0	28	0	19	0	15	0	310	0
Au	28	0	141	0	35	0	44	0	28	0	19	0	15	0	310	0
Pd	28	0	141	0	35	0	44	0	28	0	19	0	15	0	310	0
Ir	28	0	141	0	35	0	44	0	28	0	19	0	15	0	310	0
Os	28	0	141	0	35	0	44	0	28	0	19	0	15	0	310	0
Rh	28	0	141	0	35	0	44	0	28	0	19	0	15	0	310	0
Ru	28	0	141	0	35	0	44	0	28	0	19	0	15	0	310	0
Se	28	0	141	0	35	0	44	0	28	0	19	0	15	0	310	0
Ag	28	0	141	0	35	0	44	0	28	0	19	0	15	0	310	0
Pt	28	0	141	0	35	0	44	0	28	0	19	0	15	0	310	0

Table 27 - Individual heavy metal residue failures grouped according to categories applicable to the inhalation specification limit (USP/ICHQ3D).

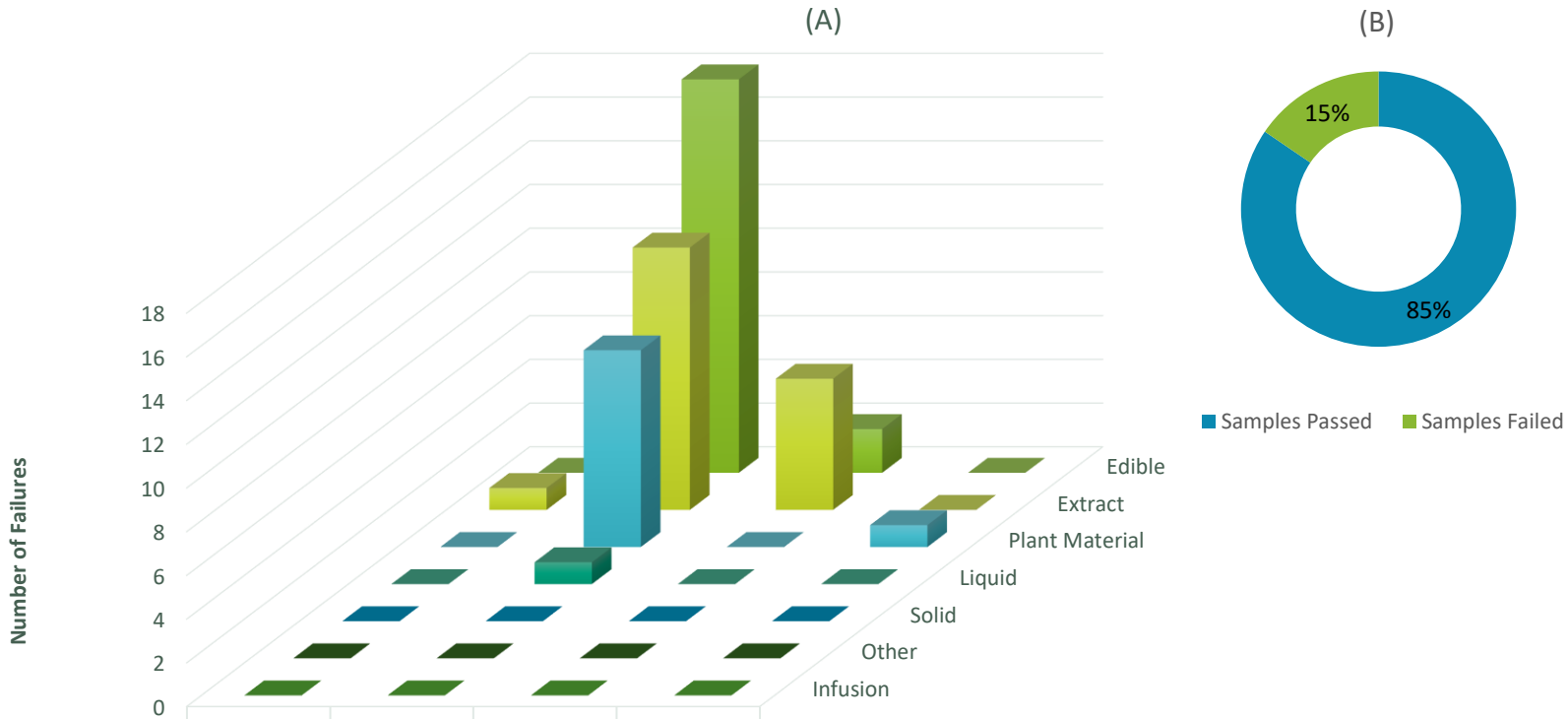
	Extract		Liquid		Plant Material		Total	
	Pass	Fail	Pass	Fail	Pass	Fail	Pass	Fail
Cd	140	1	44	0	28	0	212	1
Pb	129	12	43	1	19	9	191	22
As	130	11	44	0	25	3	199	14
Hg	105	36	37	7	23	5	165	48
Co	137	4	44	0	19	9	200	13
V	129	12	43	1	23	5	195	18
Ni	117	24	43	1	7	21	167	46
Tl	141	0	44	0	28	0	213	0
Au	141	0	44	0	28	0	213	0
Pd	141	0	44	0	28	0	213	0
Ir	137	4	43	1	28	0	208	5
Os	136	5	44	0	28	0	208	5
Rh	139	2	43	1	28	0	210	3
Ru	141	0	44	0	27	1	212	1
Se	141	0	44	0	28	0	213	0
Ag	131	10	43	1	28	0	202	11
Pt	141	0	44	0	28	0	213	0

Table 28 - Number of sample failures, and the occurrence of heavy metal residues in samples.

Oral Passed	Oral Failed	Inhalation Passed	Inhalation Failed	Present	Not Detected
262	48	119	94	273	37

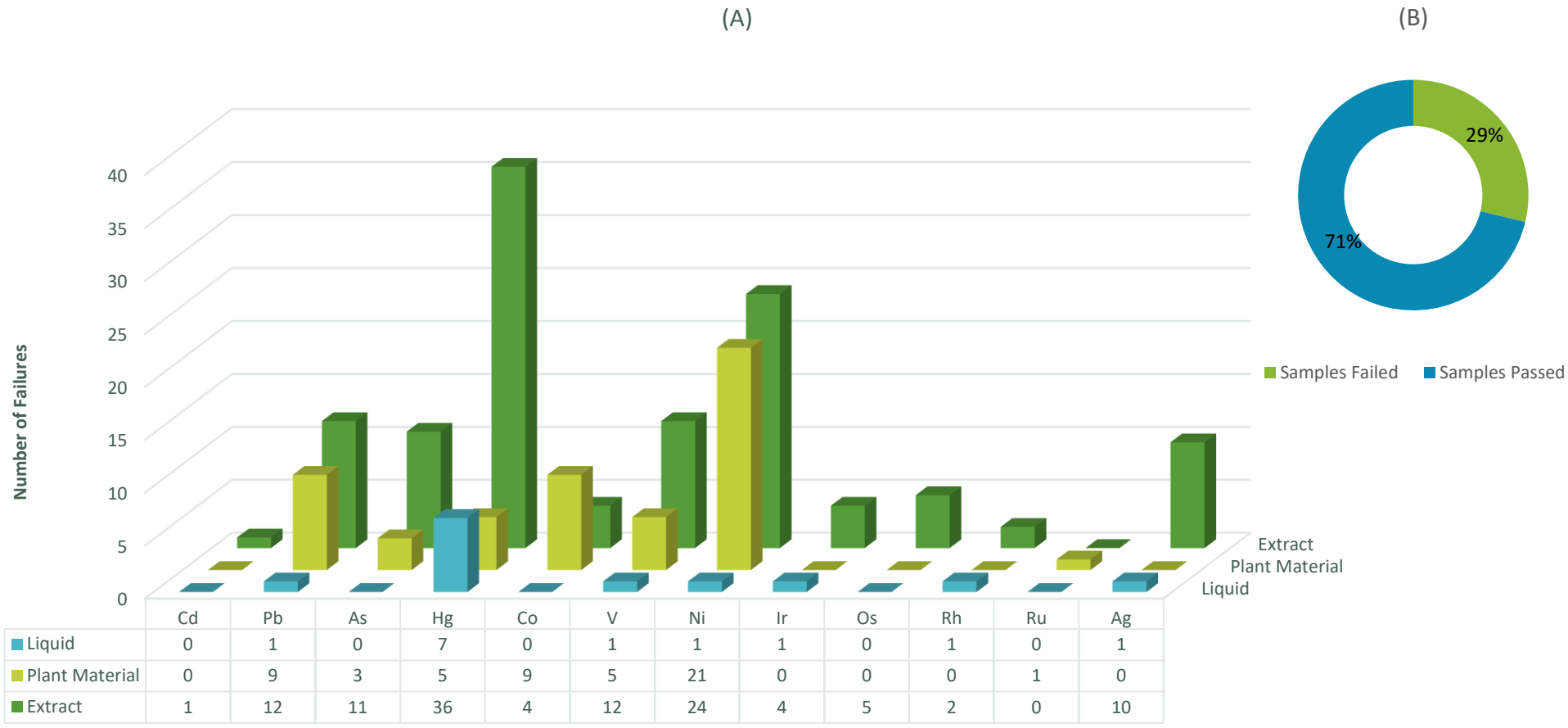
Individual elemental data are grouped within categories according to specification as mentioned above. It should be noted that the applicable categories together with metal residues that failed will be displayed. If a metal was present in the test method but had no failures it will not be displayed on the figures. The presence of each residue is also displayed for the analysed 310 samples, together with the limit of detection for each residue

in Table 25. Additionally, Table 28, shows the number of samples that failed when they are evaluated against the different specification limits. Furthermore, the table also shows the number of samples for which heavy metal residues could be detected, irrespective of the concentration. A visual representation of the data is given in Figure 19, Figure 20 and Figure 21.



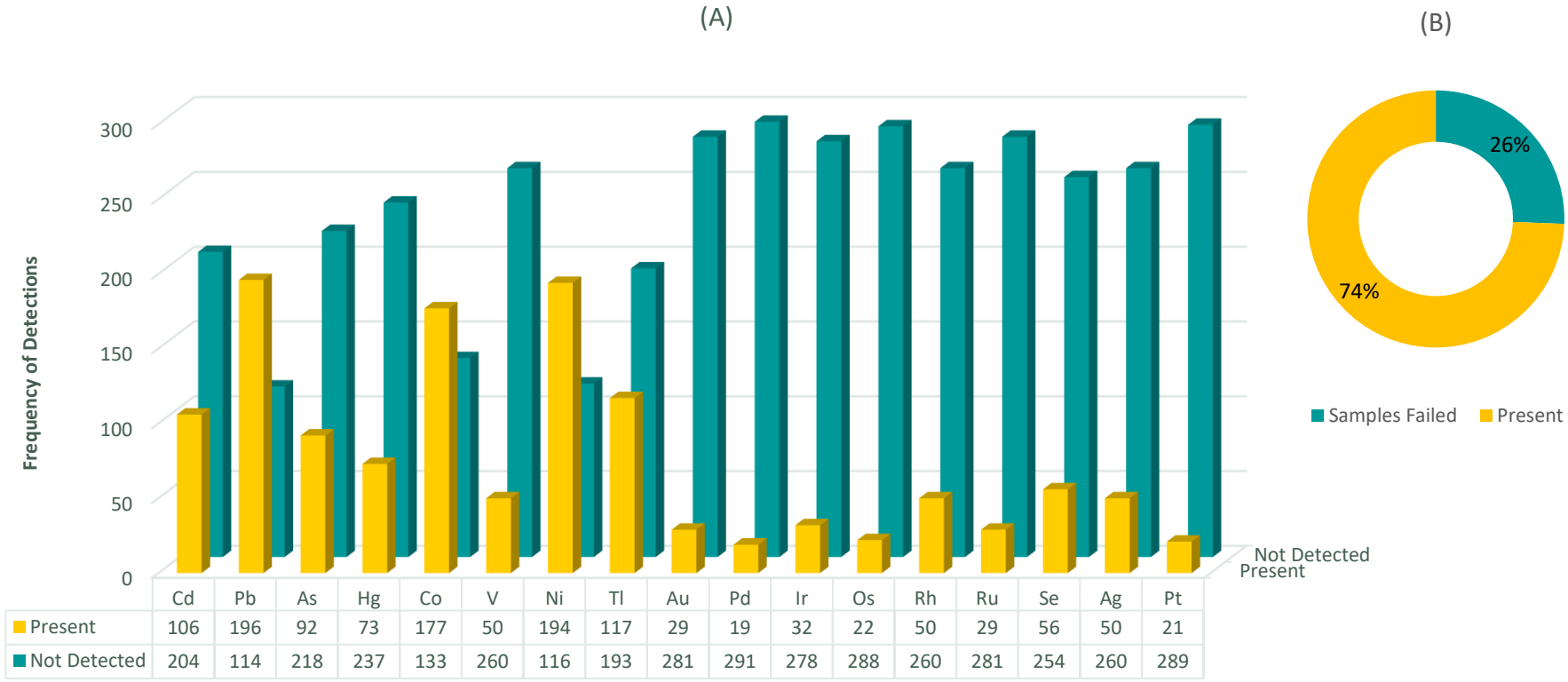
	Cd	Pb	As	Ni
Infusion	0	0	0	0
Other	0	0	0	0
Solid	0	0	0	0
Liquid	0	1	0	0
Plant Material	0	9	0	1
Extract	1	12	6	0
Edible	0	18	2	0

Figure 19 - Number of individual heavy metal residue failures for the oral specification limit, grouped according to category (A); The number of samples failures for the oral specification limit (B).



	Cd	Pb	As	Hg	Co	V	Ni	Ir	Os	Rh	Ru	Ag
Liquid	0	1	0	7	0	1	1	1	0	1	0	1
Plant Material	0	9	3	5	9	5	21	0	0	0	1	0
Extract	1	12	11	36	4	12	24	4	5	2	0	10

Figure 20 - Individual heavy metal residue failures for the inhalation specification limit, grouped according to category (A); The number of samples failures for the inhalation specification limit (B).



	Cd	Pb	As	Hg	Co	V	Ni	Tl	Au	Pd	Ir	Os	Rh	Ru	Se	Ag	Pt
Present	106	196	92	73	177	50	194	117	29	19	32	22	50	29	56	50	21
Not Detected	204	114	218	237	133	260	116	193	281	291	278	288	260	281	254	260	289

Figure 21 - Individual heavy metal occurrences in the dataset (A); The number of samples that contained heavy metals at detectable concentrations (B).

4.5 Discussion

Represented in this data set is a small portion of samples obtained from the South African market. It is by no means a representative sample of the entire South African market, but inferences can be made nonetheless. The dataset will be discussed in two sections, relating to individual heavy metal residues as well as relating to samples.

4.5.1 Individual Heavy Metal Residues

When comparing the individual heavy metal residues that are presented in Figure 19 against the oral limit, it is evident that the following 3 metals are responsible for most failures: lead (Pb), arsenic (As) and nickel (Ni). Detection of Class 1 residues above the USP/ICH oral specification limits were responsible for 91% (49 out of 54) of all residue failures identified in this study. It is further interesting to note, that only four of the seven categories contained heavy metals at concentrations high enough to cause a failure. The categories that did not contain residues at concentrations high enough to fail could be as result of the concentration dilution being performed or alternatively the process removes metal residues to some degree. For example, when Infusions are considered a dilution of concentration cannabinoids is prepared and consequently other residues like heavy metals and solvents are also diluted. As for solids, the process of manufacturing a pure CBD isolate usually entails a distillation or crystallisation of sorts, which would remove metal residues to an extent.

When considering the more stringent inhalation USP/ICH limits, presented in Figure 20, the three metals that were responsible for most failures were mercury (Hg), nickel (Ni) and lead (Pb). Furthermore, only three of the categories were considered for the inhalation limits since products from the other categories had none or low potential for being dosed in an inhalation form. It is evident that a clear increase in the number of residue failures is present when applying to the more stringent inhalation limit. A 346% increase is observed in the number of failures detected when inhalation limit residues are compared to oral limit residues. Note that 46% (85 out of 187) of all failures are attributed to Class 1 residues, which is lower than the samples submitted for comparison with the oral limit. It should however be made clear that 87% (162 out of 187) of failures are attributed to a combination of Class 1 and Class 2A. Next to Class 1, Class 2A residues are the second most dangerous to human health. For samples submitted to

be tested against the inhalation specification limit, a significant number were vape pens or vape pen attachments containing oil. These pens or attachments were manufactured with a transparent glass exterior to be able to view the level of oil, which is contained in a metal housing. Observations made suggests that the metal housing showed characteristics of nickel plating. This could explain the vast number of nickel failures detected, since nickel is most probably leached into the product after production in finished form. The small quantity oil combined with the large surface area of the metal housing could leach enough nickel into products to cause detrimental health effects when inhaled.

If no limit is enforced and only occurrence or presence of a residue in a sample is considered (Figure 21), four main residues make up 52% (684 out of 1313) of all residue occurrences. These residues include lead (Pb), cobalt (Co), nickel (Ni) and thallium (Tl). What is more concerning is that a Class 1 residue was detected in 36% (467 out of 1313) of the samples. This means that, among all residues analysed, a Class 1 residues will be present in a third of all samples. Limits of detection are shown in both Table 25 and Table 23.

4.5.2 Sample Heavy Metal Residues

Considering the sample failures presented in Figure 19 pie chart, a total 15% (48 out of 310) of all samples submitted for heavy metal residue analysis failed when compared against the USP/ICH oral specification limit. These samples included a wide variety of product types; drops, capsules, drinks, edibles and suppositories. The latter was also considered under the oral specification limit although they are not exposed to low pH stomach acids which could decarboxylate certain cannabinoids. A staggering increase in the number of failures for the UPS/ICH inhalation limit is evident compared to the oral limit presented in Figure 20 pie chart. A total of 44% (94 out of 213) of all samples, in the applicable categories, submitted for heavy metal residue analysis failed when compared to the inhalation limit. This is a concern since almost half of all samples grouped in these categories failed. Sample types in this category included vape pens, raw plant material and vape oils. Furthermore, when considering the individual residues, failures are attributed mostly to Class 1 residues when applying the oral limit, and Class 1 as well as Class 2A residues when comparing to the inhalation limit. Class 1 and Class 2A heavy metal residues are considered to be most detrimental to human

health. 88% (273 out of 310) of samples contained heavy metal residues, irrespective of whether they are above or below specification limits as seen in Figure 21 pie chart, this data may be used to focus on the metal residues most predominantly found in the analysed matrices.

4.6 Conclusion

When considering the results obtained it is exceedingly necessary to have an appropriate quality control system in place, especially for heavy metal residues analysis in the current South African market. What is most worrying is the high failure rate in conjunction with the presence of mostly Class 1 and Class 2A residues. Even though published standards and safety limits exist, the enforcement of these limits need to be taken much more seriously. This data should inform the public, who purchase these products, regulators and manufacturers of the potential health endangerment of individuals who consume these products on a daily basis.

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CHAPTER 5

5 An Assessment of Solvent Residue Contaminants Related to Cannabis-Based Products in the South African Market

This article was submitted to the Journal of cannabis research on the 24th of July 2021. The article has been assigned to the Editor, currently awaiting feedback. The Journal of cannabis research guidelines for authors is not available in PDF format and only online instructions are provided at the following web address (Journal of Cannabis Research, 2021).

The Author contributions for this article are as follows:

-Hendrik Jacobus Viviers: Conceptualisation, Methodology, Experimental investigation, Data curation, Writing original draft.

-Anél Petzer: Supervision, Writing - review & editing.

-Richard Gordon: Supervision, Writing - review & editing.

This research project/study was reviewed by the North-West University Health Research Ethics Committee (NWU-HREC) which concluded that this study does not need ethical approval. An attachment of this review can be found in Annexure A at the end of this Thesis.

5.1 Abstract

South African cannabis-based product samples were analysed for solvent residue contaminants as classified by the United States Pharmacopoeia (USP), chapter 467. The origin of these samples ranged anywhere from crude extract, product development samples and market ready final products. To date, no data of this kind exist in South Africa specifically relating to cannabis-based medicinal, recreational, or complementary products. Samples were submitted to a contract laboratory. A total of 279 samples were analysed in duplicate and the results are reported in an anonymised format. The results showed an alarming 37% sample solvent residue failure rate with respect to adherence to USP 467 specification. It is important to ensure regulation is enforced to control product quality. The South African public need to be educated about the risks associated with cannabis-based products.

5.2 Introduction

It is imperative to subject herbal preparations, medicines, and recreational products to quality control. For the pharmaceutical industry as well as cannabis in general there are an abundance of control measures in place to ensure their safety and efficacy (Viviers *et al.*, 2021:1–8; World Health Organisation, 2007:1–118). A range of organic solvents are used for manufacturing herbal medicines and can be detected as residues of such processing in the final products. Medicinal cannabis extracts and other processed forms may thus contain residual solvents. This is especially relevant to extracts which have a sticky and viscous nature that make it difficult to remove solvents (Romano and Hazekamp, 2013:1–11). The most common examples of such cannabis extracts are termed “Rick Simpson oils” or “FECO’s” (full extract cannabis oil).

Cannabinoids as well as terpenoids and flavonoids are extracted by a solvent, followed by an evaporation step in order to increase the concentration of these compounds in the extract (Hazekamp, 2006:1–9; Romano and Hazekamp, 2013:1–11). These types of cannabis oils or extracts are becoming increasingly popular amongst self-medicating patients because of the simplicity and low cost involved in producing the oils (Romano and Hazekamp, 2013:1–11). After solvent evaporation, residues are still present in the extract and the concentrations of the solvent residues should be controlled through good manufacturing processes (GMP) and quality control of the final products (International Conference on

Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38). To ascertain whether a product is safe for chronic human consumption, The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) as well as the United States Pharmacopoeia (USP) have listed predetermined solvent residue limits. Solvents are classified by ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38) as well as the USP (United States Pharmacopoeia (USP), 2009:1–10), according to their potential risks into the following categories:

- class 1 (Solvents to be avoided such as benzene which are potentially carcinogenic);
- class 2 (Toxic potential such as methanol or acetonitrile);
- class 3 (Limited toxic potential such as ethanol).

Residual solvents are primarily analysed by headspace GC-FID (gas chromatography-flame ionisation detection) or liquid injection GC-FID (United States Pharmacopoeia (USP), 2009:1–10). The alternative use of mass spectrometry (MS) detection may provide additional selectivity for co-eluting solvents. In this study, a published analytical method was employed as basis for the development of the final analysis method for residual solvents (Hilliard *et al*., 2009). As a result of the viscosity of most of the cannabis extracts, liquid injection is not feasible. Although ICH and USP (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38; United States Pharmacopoeia (USP), 2009:1–10) provide a guideline that lists the solvents which should be included in a working list, the solvents that will be analysed are ultimately decided by each manufacturer or quality control laboratory.

From interaction with cannabis extract manufacturers Table 29 was compiled and provide a list of the solvents that have been analysed for each class. This list is by no means exhaustive and could be altered to include additional solvents. Although only a few manufacturers employ class 1 and 2 solvents, nonetheless they are included.

Table 29 - Residual solvent limits imposed by ICH and USP (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38; United States Pharmacopoeia (USP), 2009:1–10)

	Solvent		Limit (ppm)
Class 3	2-Butanol		<5000
	Acetone		<5000
	Butanone/Methyl Ethyl Ketone		<5000
	Diethyl Ether		<5000
	Ethanol		<5000
	Ethyl Acetate		<5000
	Isopropanol		<5000
	Methyl Tertbutyl Ether (MTBE)		<5000
Class 2	1.4-Dioxane		<380
	Acetonitrile		<410
	Chlorobenzene		<360
	Cyclohexane		<3880
	Cumene/Isopropyl Benzene		<70
	Methanol		<3000
	Methyl Cyclohexane		<1180
	Methylene Chloride		<600
	Ethylbenzene o-Xylene p- and m-Xylene	Total Xylenes	<2170
	Tetrahydrofuran		<720
	Toluene		<890
	trans-1,2-Dichloroethene cis-Dichloroethene	Total Dichloroethenes	<1870
Class 1	Benzene		<2

An evaluation of the quality of medicinal cannabis-based products has been performed previously (Hazekamp, 2006:1–9), but was only limited to samples in the Netherlands. This study included solvent residue testing but only to extend of comparing different production methods with one another using different types of solvent. It should be noted that due to

the high viscosity of the extracts, significant solvent residues were also reported by this study (Romano and Hazekamp, 2013:1–11). With this background, the aim of this study was to analyse a segment of the South African cannabis-based products in circulation and to provide a detailed overview of the solvent residues contained in these products. Furthermore, the adherence of these samples to the imposed limits by the ICH and USP will also be evaluated (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38; United States Pharmacopoeia (USP), 2009:1–10). To date, no data of this kind exists in South Africa. The output of the study will provide insights to manufacturers, the public, and regulators alike.

5.3 Materials and Methods

An assortment of samples from a variety of manufacturers in South Africa were submitted to the contract laboratory. Manufacturers are defined as any type of user, retailer, reseller, producer, or importer of cannabis-based products. Whether these manufacturers maintain the full value chain or only a portion thereof they are defined as manufacturers for the purpose of this study. Manufacturers may include cultivators of plants, producers of products, importers, resellers, and pharmaceutical manufacturers. The data will be represented in an anonymised format. Solvents from class 1, 2 and 3 were analysed (See Table 29). Depending on the solvents employed in the manufacturing process, the appropriate solvent class was chosen by each manufacturer for each specific sample submission. A screen of all available solvents would be impractical; thus, manufacturers selected the solvent class which was most likely present in the final product. In total 299 samples were analysed in duplicate. Consent was provided to employ the data for research purposes. The majority of samples submitted for residual solvent analysis could be defined as any sample prepared or processed in a way to extract cannabinoids from plant material to up-concentrate the cannabinoid content in the final product. These might include Full extract cannabis oil (FECO), Rosin, Rick Simpson oil (RSO), Hashish, Butane hash oil (BHO), etc.

Obtained from Perkin Elmer corporation (Waltham, Massachusetts, USA), a Clarus 680 gas chromatograph equipped with a SQ8 mass spectrometer and HS40 headspace autosampler was employed for HS GC-MS analysis. A Zebron ZB-624 (30 m, 0.32 mm x 1.8 μm) capillary column obtained from Phenomenex (Torrance, California, USA) was

employed for separation. Two reference methods were employed as a starting point and were altered to achieve more desirable run times (Hilliard *et al*., 2009; United States Pharmacopoeia (USP), 2009:1–10). A limit test procedure was employed for the analysis of all residual solvents. A calibration standard was prepared at the concentration limit of each individual solvent using the first batch of certified reference materials (CRM). A one-point calibration was employed for each USP limit of each individual solvent. A second batch of CRMs were employed to prepare a control standard, again at the limit concentration stipulated by USP monograph 467 (United States Pharmacopoeia (USP), 2009:1–10). Analysis commenced with a blank run, then a calibration standard, followed by a control standard every 10 injections, to avoid instrumental drift. Sample sets were concluded by a control standard to ensure all samples within a sample set adhered to bias and variation limits. Control standard specifications were 15% RSD at the concentration limit, and re-calibration was performed when control standards fell outside these specifications. This method was regarded as quantitative with a very narrow range of 15% across the USP 467 concentration limit. Any values falling outside this window were regarded as semi-quantitative only. Since manufacturers are only interested to know whether the products fall within a certain specified concentration limit, a full quantitative analysis was redundant.

The data was subdivided into three different categories.

1. Individual solvent analytes were grouped together with no relationship to the sample. This was done to determine the number of times this specific solvent failed to adhere to the USP/ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38; United States Pharmacopoeia (USP), 2009:1–10) specification. Furthermore, the number of times a certain solvent was present, irrespective of adherence to a specification limit, was also determined and noted as either present when it occurred or not detected (ND).
2. Secondly, samples were grouped together to determine the frequency of sample failures as well as the frequency by which the test panel solvents are present in a specific sample. The frequency of failed samples only considered whether any one of the solvents in the test panel failed.
3. Lastly with the samples grouped together, the total amount of solvent detected in the sample was summed together and compared to a set adherence limit. This

manner, even if individual solvents within a sample adhered to the USP specification limit, the sum total concentration of solvent present in a sample may exceed the set adherence limit.

5.4 Results

The results of the analyses are shown in Table 30. The results are grouped according to individual solvent analytes and shows the occurrence of each individual solvent across the 298 samples. In Table 31 data shows sample failures as well as whether a solvent was present in the sample irrespective of the safety limit. Table 32 shows sample failures when summed solvents concentrations are considered against a safety limit.

Table 30 - Individual solvent failures, and occurrence in samples.

			USP Limit (ppm)	Passed	Failed	Present	Not Detected
Class 3	2-Butanol		<5000	279	0	0	279
	Acetone		<5000	272	7	106	173
	Butanone		<5000	278	1	9	270
	Diethyl Ether		<5000	279	0	0	279
	Ethanol		<5000	205	74	128	151
	Ethyl Acetate		<5000	278	1	49	230
	IsoOH (Isopropanol)		<5000	236	43	131	148
	MTBE (Tertbutyl methyl ether)		<5000	279	0	9	270
Class 2	1.4 Dioxane		<380	15	0	0	15
	Acetonitrile		<410	15	0	0	15
	Chlorobenzene		<360	15	0	0	15
	Cyclohexane		<3880	15	0	0	15
	Cumene (Isopropyl Benzene)		<70	15	0	1	14
	Methanol		<3000	14	1	6	9
	Methyl Cyclohexane		<1180	15	0	0	15
	Methylene Chloride		<600	15	0	0	15
	Ethylbenzene			14	1	1	14
	o-Xylene	Total Xylenes	<2170	15	0	1	14
	p+m-Xylene			15	0	1	14
	Tetrahydrofuran		<720	15	0	0	15
	Toluene		<890	15	0	0	15
	trans-1,2-Dichloroethene	Total	<1870	15	0	0	15
	cis-Dichloroethene	Dichloroethenes		15	0	1	14
Class 1	Benzene		<2	5	0	2	3
			Total	***2334***	***128***	***445***	***2017***

Table 31 - Number of sample failures, and the occurrence of solvents in samples.

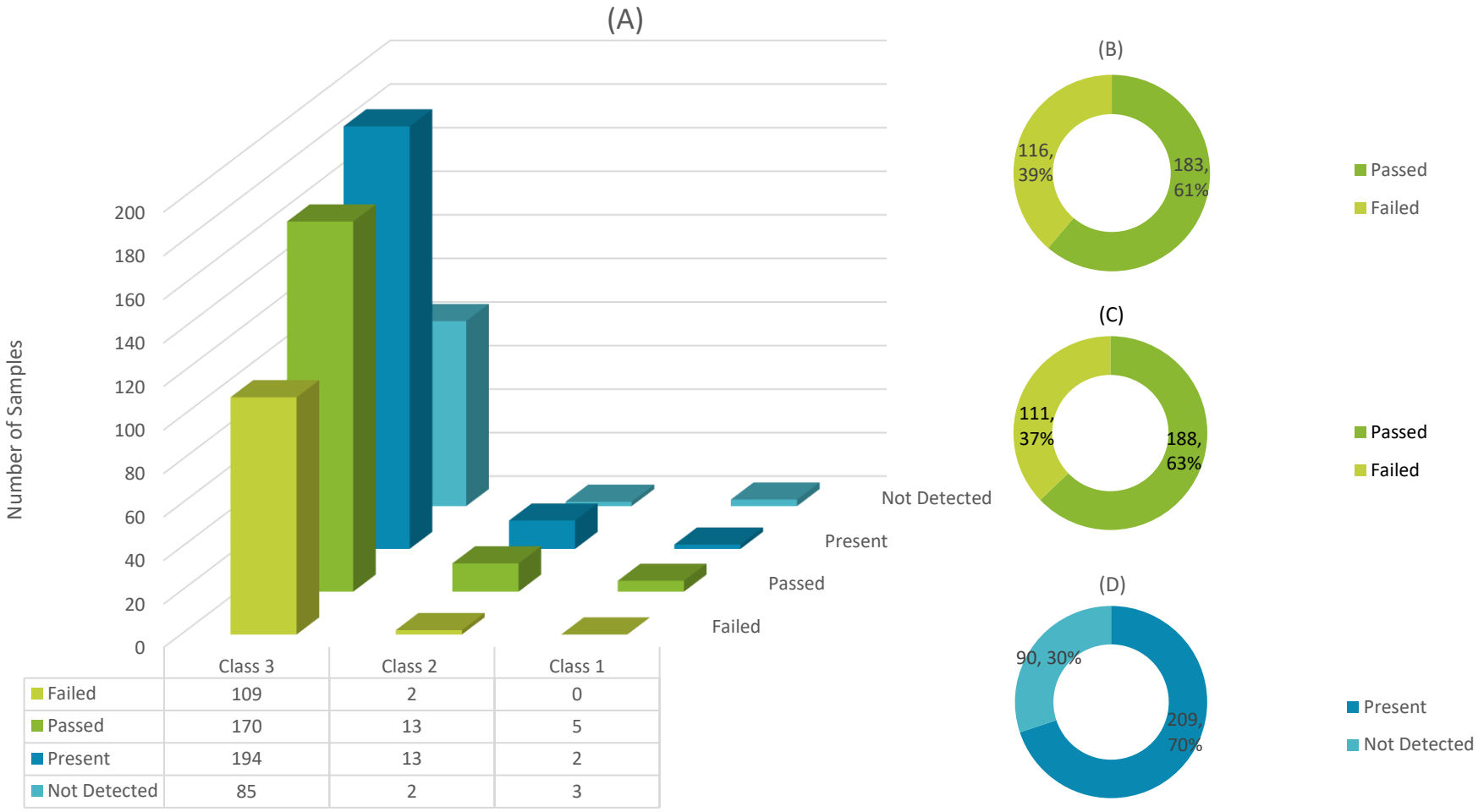
			USP Limit (ppm)	Passed	Failed	Present	Not Detected
Class 3	2-Butanol		<5000	170	109	194	85
	Acetone		<5000				
	Butanone		<5000				
	Diethyl Ether		<5000				
	Ethanol		<5000				
	Ethyl Acetate		<5000				
	IsoOH (Isopropanol)		<5000				
	MTBE (Tertbutyl methyl ether)		<5000				
Class 2	1.4 Dioxane		<380	13	2	13	2
	Acetonitrile		<410				
	Chlorobenzene		<360				
	Cyclohexane		<3880				
	Cumene (Isopropyl Benzene)		<70				
	Methanol		<3000				
	Methyl Cyclohexane		<1180				
	Methylene Chloride		<600				
	Ethylbenzene o-Xylene p+m-Xylene	Total Xylenes	<2170				
	Tetrahydrofuran		<720				
	Toluene		<890				
	trans-1,2-Dichloroethene cis-Dichloroethene	Total Dichloroethenes	<1870				
Class 1	Benzene		<2	5	0	2	3
			Total	***188***	***111***	***209***	***90***

Table 32 - Sample failures when solvent concentrations are summed.

			USP Limit (ppm)	Passed	Failed
Class 3	2-Butanol		<5000	165	114
	Acetone		<5000		
	Butanone		<5000		
	Diethyl Ether		<5000		
	Ethanol		<5000		
	Ethyl Acetate		<5000		
	IsoOH (Isopropanol)		<5000		
	MTBE (Tertbutyl methyl ether)		<5000		
Class 2	1.4 Dioxane		<380	13	2
	Acetonitrile		<410		
	Chlorobenzene		<360		
	Cyclohexane		<3880		
	Cumene (Isopropyl Benzene)		<70		
	Methanol		<3000		
	Methyl Cyclohexane		<1180		
	Methylene Chloride		<600		
	Ethylbenzene o-Xylene p+m-Xylene	Total Xylenes	<2170		
	Tetrahydrofuran		<720		
	Toluene		<890		
	trans-1,2-Dichloroethene cis-Dichloroethene	Total Dichloroethenes	<1870		
Class 1	Benzene		<2	5	0
			Total	***183***	***116***

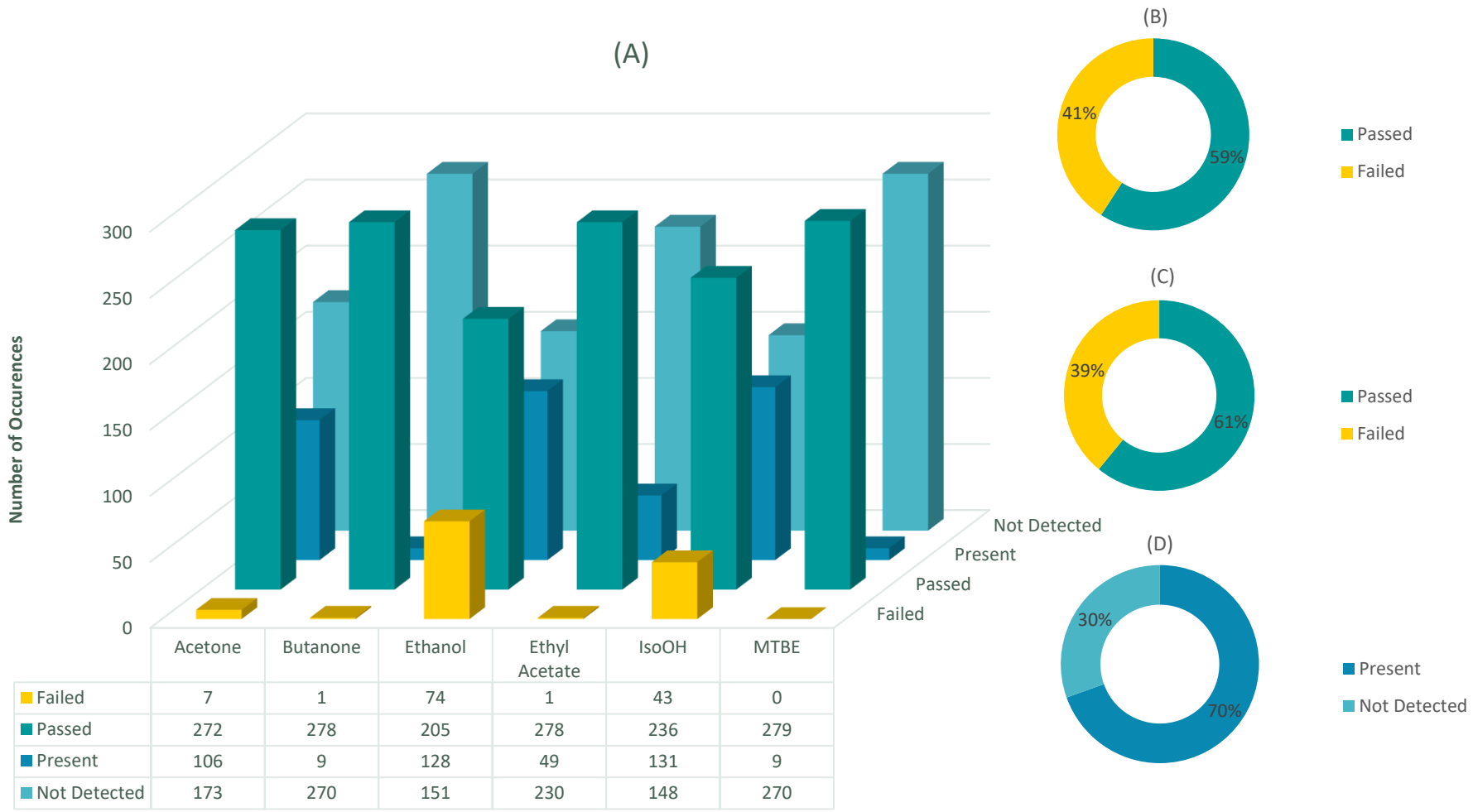
Figure 22 shows the individual solvent failures as well the number of times each solvent occurred or was detected for all three solvent classes. Since most of samples that were analysed contain class 3 solvents, Figure 23 and Figure 24 illustrates the number of failures as well as the occurrence of each individual solvent in class 2 and class 3. It should be noted that for Figure 23 and Figure 24, only solvents which were present are displayed. Additionally Figure 22, Figure 23, Figure 24, pie illustrations B, C and D show the amount

of sample failures, the amount of sample failures if concentrations are summed, and finally the overall occurrence/detected solvents in all samples.



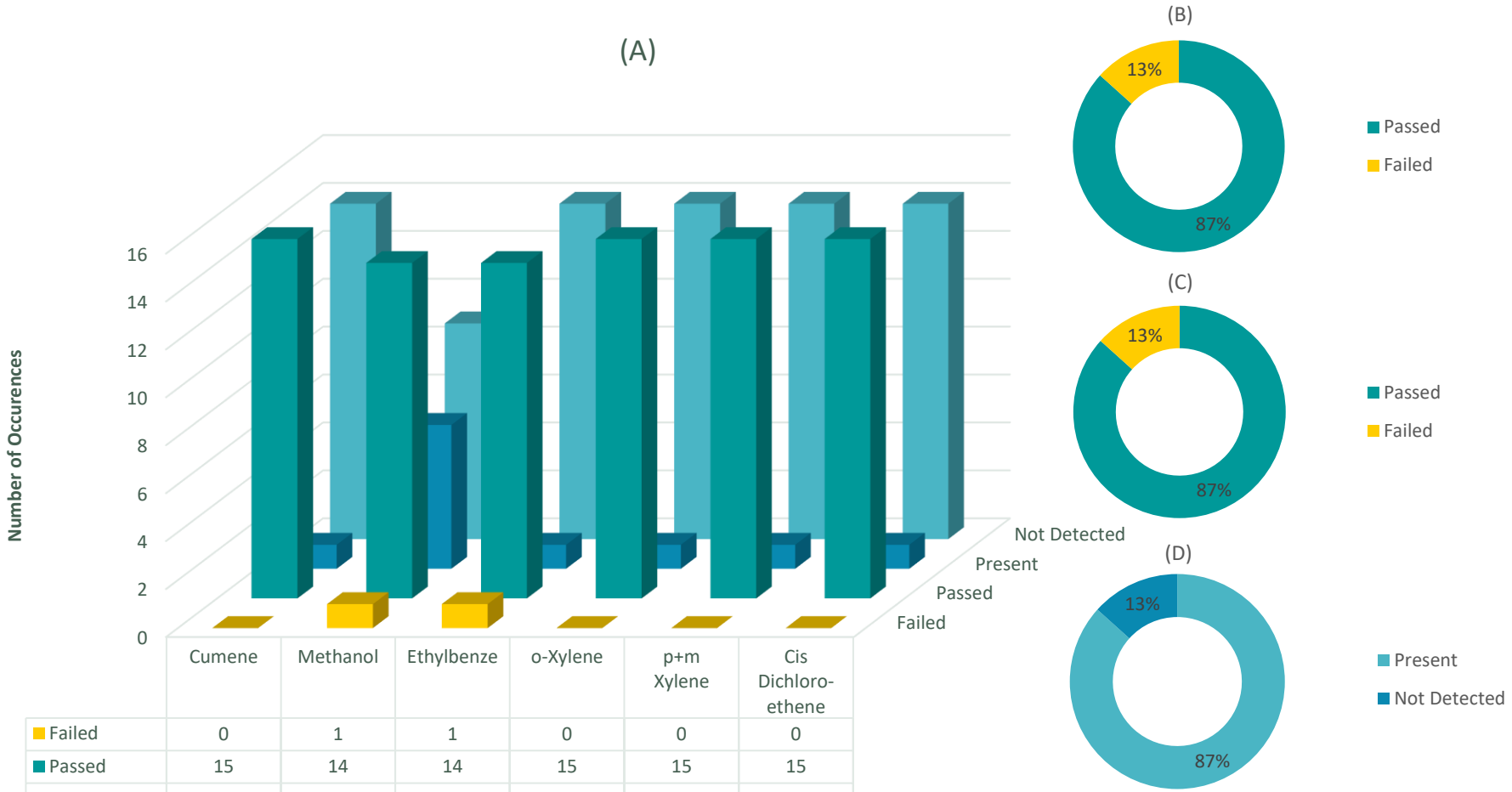
	Class 3	Class 2	Class 1
Failed	109	2	0
Passed	170	13	5
Present	194	13	2
Not Detected	85	2	3

Figure 22 - Number of sample failures, and the number of samples that contained solvent (A), Total percentage of sample failures for all classes, if all solvents present in a sample are summed (B), Total number of sample failures for all classes where one solvent exceeded the limit (C),Total percentage of samples for all classes where any solvent was detected in sample (D) .



	Acetone	Butanone	Ethanol	Ethyl Acetate	IsoOH	MTBE
Failed	7	1	74	1	43	0
Passed	272	278	205	278	236	279
Present	106	9	128	49	131	9
Not Detected	173	270	151	230	148	270

Figure 23 - Number of Individual solvent failure occurrences, and the number of times a class 3 solvent was present (A), Total percentage of sample failures for class 3 solvents, if all solvents present in a sample are summed (B), Total number of sample failures for all class 3 solvents where one solvent exceeded the limit (C),Total percentage of samples for class 3 solvents where any solvent was detected in sample (D).



	Cumene	Methanol	Ethylbenze	o-Xylene	p+m Xylene	Cis Dichloro-ethene
Failed	0	1	1	0	0	0
Passed	15	14	14	15	15	15
Present	1	6	1	1	1	1
Not Detected	14	9	14	14	14	14

Figure 24 - Number of Individual solvent failure occurrences, and the number of times a class 2 solvent was present (A), Total percentage of sample failures for class 2 solvents, if all solvents present in a sample are summed (B), Total number of sample failures for all class 2 solvents where one solvent exceeded the limit (C),Total percentage of samples for class 2 solvents where any solvent was detected in sample (D).

5.5 Discussion

The data represents an overview of cannabis-based products in South Africa that were screened for solvent residues. This data should not be regarded as a representation of the entire South African cannabis market since not every product on the market was analysed. Each solvent class will be discussed individually starting with the solvents that were present in most samples. Solvents that were not detected in any of the samples but were included in the test panel will be omitted from the figures.

5.5.1 Class 3

Class 3 solvents occurred in most of the samples. Solvent residues in this class have the least stringent specification limits (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38; United States Pharmacopoeia (USP), 2009:1–10). This can be viewed in a positive light since most samples analysed fell within this class and can be considered the least dangerous. When considering individual solvent analytes, the most prevalent solvent residue failure was ethanol, followed by isopropanol and acetone (see Figure 23). When considering the presence of these residues in samples, ethyl acetate should also be mentioned. Although ethyl acetate had only 1 residue failure, it was present in almost 50 samples. Additionally, although isopropanol had less failures than ethanol, isopropanol residues were present in more samples than ethanol. A reason for the prevalence of these solvent residues might be the ease of acquiring such solvents from a variety of vendors in South Africa. A variety of manufacturers also do not employ food grade or high purity ethanol, which contains denaturants like ethyl acetate and acetone, since high purity ethanol is taxed at a significant rate in South Africa. Comparing individual class 3 solvent residue failures to summed solvent residue failures, summed solvent residue failures only increased by a small fraction (39% failed individual vs 41% failed summed). It could be argued then that most manufacturers employ a single moderately pure solvent in their production process. A high percentage of Class 3 samples (69.5%) contained detectable concentrations of solvent residues even though these residues were not above the specification limit.

5.5.2 Class 2

Class 2 solvents were second most prevalent in the sample dataset. Solvent residues in this class have more stringent specification limits (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38; United States Pharmacopoeia (USP), 2009:1–10). These solvents should be viewed with concern by informal producers as they are more dangerous to human health. Considering the individual class 2 solvents, only 1 failure occurred for each of methanol and ethylbenzene (See Figure 24), yielding a 15% failure rate. A high percentage of Class 2 samples (87%) contained detectable concentrations of solvent residues even though these residues were not above the specification

limit. Although these solvents pose higher health risks, the lower failure rate of Class 2 solvents is a positive finding since less manufacturers employ these solvents. What should be noted is the much higher percentage of class 2 solvents that are present in final products submitted for this test panel. This might translate to class 3 solvents being easier to remove during manufacturing than class 2 solvents. Alternatively, because of the lower detection limits needed to detect class 2 solvents compared to class 3 solvents, they are detected at a higher frequency in samples.

5.5.3 Class 1

Class 1 solvents occurred the least in the samples. Solvent residues in this class have the most stringent specification limits, and should be avoided (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38; United States Pharmacopoeia (USP), 2009:1–10). These solvents are also recognised as carcinogens (Baker, 1994:1079—1092). The samples were only screened for the presence of benzene in this category and a 0% failure rate occurred, even with an imposed specification limit of 2 ppm (Table 30, Table 31 and Table 32). Benzene was present in only two samples, and it should be noted that in both instances benzene concentrations were below the specified 2 ppm USP/ICH limit (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38; United States Pharmacopoeia (USP), 2009:1–10).

The data of all solvent classes were pooled as shown in Figure 22, which indicates the total number of samples submitted for any class solvent residue analysis that failed. Among the 279 samples, a total of 111 samples had at least one solvent analyte that failed the USP/ICH specification limit (37% failure rate). For summed solvent concentrations the failure rate increased by a marginal 2%, which could be interpreted to mean that manufacturers use a single moderately pure solvent during their production. This is supported by the finding that only high failing concentrations of a single solvent are usually present in a specific sample. The overall presence of any solvent in a sample is displayed in Figure 24. A high percentage of samples (70%) contained detectable concentrations of solvent residues even though these residues might not have been above the specification limit. It should also be noted that not all samples submitted employed solvent extraction as means of manufacturing. A limited number of samples were submitted to obtain a Certificate of Analysis which shows compliance to USP 467 or ICH Q3C, even though no solvent was used in production.

5.6 Conclusion

In conclusion, when assessing solvent residues present in samples against a pharmacopoeial safety limits, it is evident that a large fraction of cannabis-based products in South Africa exceeds these limits. Even though safety limits for solvent residues have been published, adherence by manufacturers is lacking, notwithstanding enforcement of these limits by regulators. With a 37% overall sample solvent

residue failure rate, it is alarming that these products are being distributed in South Africa. It is the aim with the publication of this data to inform the public and regulators alike.

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CHAPTER 6

6 Conclusion

6.1 Introduction

Globally the amount of cannabis users is estimated at 200 million users (4% of the global population) (United Nations Office on Drugs and Crime (UNODC), 2021:1–95). More than double this percentage is reported (9.4% of the population) for the African continent (United Nations Office on Drugs and Crime (UNODC), 2021:1–95). With this high incidence (1 out of every 10 people) of cannabis usage, the quality control of cannabis-based products especially for medicinal purposes need to be strictly regulated.

As seen from Chapters 3-5, it is evident that the safety of cannabis-based products in South Africa is brought into question, notwithstanding additional quality control parameters that were not considered in this study. Data relating to the safety of cannabis-based products have, to date, not been published in South Africa. Although the data cannot be seen as representative of the entire cannabis market in South Africa, ample sample data points from across the county have been evaluated, and conclusions about this data can still be drawn, nonetheless. The results obtained in this study will therefore extend the current knowledge available to the South African public and regulators alike.

6.2 Cannabis Safety in South Africa

Since scheduled pharmaceuticals are regulated in South Africa, it can be assumed that cannabis-based products employed for medicinal purposes should conform to the same regulations. Although the regulations applicable to cultivation as well as cannabis-based product manufacturing are still under development, much is left to be desired. Under current regulations the specification limits only specify the active ingredients of cannabis-based products i.e. cannabinoids. The concentration of active ingredients and the control/regulation thereof should be the starting point. The safety of the end-consumers however not only depends on the concentration of the active ingredients. There are a myriad of quality control and safety factors that need to be considered in conjunction with the concentration of active ingredients. As with the majority of pharmaceuticals manufactured, stringent quality control measures exist not only for the concentration of the active ingredients, but also possible impurities introduced during the manufacturing process (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2006:1–16; World Health Organisation (WHO), 2007:1–118). It is thus imperative that regulation safety limits be broadened to include supplementary quality control parameters to ensure the safety of consumers.

Added to the additional quality control measures that should be included in future regulations, the enforcement of the current regulation should occur to a much greater extent. Currently manufacturers

are able to sell cannabis-based products in retail stores as well as online, without the need to show any certificate of analysis that states the concentration of active ingredients. Current South African law stipulates that cannabis-based products are unscheduled when they adhere to specific conditions (Republic of South Africa, 2020:1–12);

1. The preparation contains a maximum total quantity of 600 mg CBD per sales pack and a maximum daily dose of 20 mg CBD with an accepted low-risk claim or health claim.
2. Preparations consist of processed products made from cannabis raw plant material, where only the naturally occurring quantity of cannabinoids found in the source material are contained in the product, and which contain no more than 0.001% of THC and not more than 0.0075% CBD.

It should thus be of value to retailers to know whether the cannabis-based products currently being sold, adhere to the South African regulation. The alternative, it would seem to be, is that retailers are not aware or not concerned about not adhering to legislation, since the enforcement of such legislation is currently lacking in South Africa. It should be noted that some manufacturers or cultivators regularly submit quality control samples for screening, but the following topics of concern have been identified:

6.2.1 Quality Control Parameters Screened

As mentioned, there are South African manufacturers or cultivators that screen for quality control parameters. The current quality control regime followed by the majority of manufactures or cultivators is lacking to say the least. Globally, different quality control parameters such as, heavy metal, residual solvents, pesticide, and microbiological contaminant screening, to name a few, have been adopted. A guideline published by The South African Health Products Regulatory Authority (SAHPRA), stipulates GMP principles and considerations to be followed for the cultivation and manufacture of cannabis-based products (South African Health Products Regulatory Authority (SAHPRA), 2019:1–30). A list of quality control parameters to screen for is included in this guide as well as specification limits published by the European Pharmacopoeia, where available. It should be noted that residual solvent residue screening did not form part of the quality control test list published in the guide (South African Health Products Regulatory Authority (SAHPRA), 2019:1–30).

The current trend also indicates that the main priority of manufacturers or cultivators is the determination of their products' potency. Although heavy metal screening or residual solvent screening can be offered as a quality control parameter, manufacturers mostly only opt for cannabis potency analysis, which is contrary to what the guideline stipulates (South African Health Products Regulatory Authority (SAHPRA), 2019:1–30). There is a gap in knowledge demonstrated by the public, manufacturers, resellers, and/or cultivators. Predominantly it is falsely accepted by these groups that, through an analysis of cannabis potency, they would acquire a result that would indicate if the product were safe for human consumption. This of course is only part of the equation,

and concentration of active ingredients is an important facet to consider for accurate dosing administration, it is unquestionably not the only parameter to consider.

Awareness of the different safety or quality control parameters should be created, since it is currently up to the consumers or manufacturer to ensure that these have been controlled through the GMP process. Further responsibility also lies with the regulator in this regard. The scope of the current legislation needs to be adapted to include additional quality control parameters with included specification limits, in conjunction with improved enforcement of these regulations by statutory bodies.

6.2.2 Adherence to GMP Principles

South Africa is a Pharmaceutical Inspection Co-operation Scheme (PIC/S) member and has published GMP guidelines that pharmaceutical manufacturers need to adhere to. Currently, cultivation and or manufacturing of cannabis-based products is regulated by SAHPRA, since these products are mostly regarded as scheduled substances (Republic of South Africa, 2020:1–12). The manufacture or cultivation of said products need to adhere to current published guidelines, subsequently audit and finally approval from SAHPRA in order to operate under the legal framework currently set in South Africa. Predominantly there are two facets with regards to the current guidelines (South African Health Products Regulatory Authority (SAHPRA), 2019:1–30) that merit closer attention.

6.2.2.1 Batch Testing.

When considering current GMP guidelines, it is safe to assume that a manufactured batch needs to be qualified against set specification limits before the production batch can be released (South African Health Products Regulatory Authority (SAHPRA), 2019:1–18; World Health Organisation (WHO), 2011:1–54). Furthermore, a unique identification number should be assigned to each production batch to ensure traceability is preserved. Currently seen in the cannabis industry in South Africa is minimal adherence by a large portion of manufacturers/cultivators to these guidelines. Current observations include non-unique batch numbering, “Batch 1” or inconsistent batch testing. A portion of manufactures would test their cannabis-based product only once, irrespective of whether future batches are manufactured. Alternatively, manufacturers may number future batches as “Batch 1” having a valid certificate of analysis (CoA) for “Batch 1” these products are most likely not to be questioned by the uninformed public. Bearing in mind that each manufactured batch or cultivated lot must be qualified, the current sampling regime followed by almost all manufacturers or cultivators further exacerbates the situation.

6.2.2.2 Representative Sampling and Sample Homogeneity

To qualify a manufactured batch or cultivated lot, the reliability of conclusions drawn from such analysis will depend greatly on whether the sample taken is representative of the entire batch or crop (World Health Organisation (WHO), 1998:1–122). It is well known that herbal medicines or medicines of herbal origin are considered to be non-homogeneous in nature (World Health Organisation (WHO), 1998:1–122). The following recommended sampling regimes may be followed:

- Batches larger than 50 units, sample 10% of the batch. Each unit should further be sampled from top, middle and bottom, finally combining the individual samples into a pooled sample which is properly mixed (World Health Organisation (WHO), 1998:1–122).
- Another sampling regime that may be followed is the *r* plan, when materials are herbal medicinal products or are suspected of being non-homogeneous. The following formula is used $r = 1.5\sqrt{N}$, where *N* is the number of sampling units. Each individual sampling unit is subjected to testing and analysis results may be averaged to represent the batch. (World Health Organisation (WHO), 2005:1–35)

The mentioned sampling plans are not followed in the current cannabis-based product industry in South Africa. Numerous accounts have been reported where analysis results do not align with expectations, and when questioned, sampling plans were not followed. Examples include only submitting one cannabis bud for an entire harvest, sampling only from a single point (not top, middle, bottom), mixing non-miscible active ingredients into incompatible diluents etc. It is thus imperative to employ a sampling plan, especially for herbal matrices or products originating from herbal matrices as a result of their non-homogenous nature, to gain meaningful results.

6.2.3 Potency

Numerous studies have shown the benefits of CBD predominant medicinal preparations, as discussed in Chapter 1. Additionally, THC exhibits both positive as well as harmful medicinal properties discussed in Chapter 2. It would seem that the current South African legislative direction is moving towards allowing CBD type preparations and limiting or removing THC from these preparations. With all the adverse effects associated with THC the justification for this legislative direction is understandable. If the aim then, is to limit THC and allow CBD in cannabis-based products, the findings illustrated in Chapter 3 are of concern (Viviers *et al.*, 2021:1–8).

Of all samples analysed for potency, 72% indicated THC concentrations that are higher than that of CBD. What is most concerning is that less than 3% of samples adhered to current South African legislation of less than 0.001 wt.-% THC (Figure 25) (Republic of South Africa, 2020:1–12). If the

aim is to have CBD dominant preparations of cannabis-based products, this objective is not currently being met by industry. In addition, the current legislation stipulates that a single dose pack may not contain more than 600 mg of CBD (Republic of South Africa, 2020:1–12), whereas results indicate that 72% of samples contained cannabinoids at concentrations exceeding 1 wt.-%. This finding then excludes the vast majority of cannabis-based products, and only a few adhere to the current dosing limitations described in the gazette (Republic of South Africa, 2020:1–12).

6.2.4 Heavy Metals

Heavy metals as a quality control parameter for cultivation and manufacture of cannabis-based products, is included in the current South African guideline (South African Health Products Regulatory Authority (SAHPRA), 2019:1–30). A set specification limit for individual heavy metal residues in cannabis-based products has not been set for South Africa. The current pharmaceutical specification limits set forth in USP 232 and ICH Q3D were thus considered (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2015:1–85; United States Pharmacopoeia (USP), 2017:1–3). Two different sets of specifications were considered, differentiated by route of administration, namely, oral specification limits as well as the more stringent inhalation specification limits. With 88% of the samples containing detectable amounts of heavy metal residues, it is evident that screening for these residues should occur regularly as part of the cultivation or manufacturing quality control regime. Of samples likely to be consumed orally, 15% contained metal residue concentrations higher than what is regarded safe for human consumption by USP 232/ICH Q3D (Figure 25). A staggering 44% of samples likely to be administered through inhalation contained metal residues detrimental to human health (Figure 25). The elements detected were As, Pb, Hg and Ni, three of which are class 1 elements posing the highest health risk.

Since cannabis plants tend to bioaccumulate heavy metals, it is imperative that quality control screening of heavy metal residues be performed on each batch of produced product or cultivated crop, especially due to the high failure rates currently seen in the South African market.

6.2.5 Residual Solvents

When compared to potency and heavy metal residue screening, residual solvent contamination screening did not form part of the guideline's required tests (South African Health Products Regulatory Authority (SAHPRA), 2019:1–30). For potency analysis, extracts were the most prevalent sample category type, as described in Chapter 2. Most commonly, easily accessible solvents are employed to produce extracts, and solvent residue contaminants may be present after the production of these extracts. It was thus reasonable to screen for solvent residues due to the high incidence of extract samples. Since residual solvents do not form part of the current required quality control tests as per the guideline, there is no set residual solvent specification for cannabis-

based products in South Africa. The current pharmaceutical specification limits set forth in USP 467 and ICH Q3C were thus considered (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2011:1–38; United States Pharmacopoeia (USP), 2009:1–10).

Of all samples submitted for residual solvent analysis, 70% contained detectable solvent residues. When combining class 1, class 2 and class 3, 37% of the samples contained solvent residues above the allowable specification limit (Figure 25). The main solvent culprits were ethanol, isopropanol, and acetone, all of which are easily accessible to the public. With this in mind, it is recommended that solvent residue analysis forms part of the quality control regime of manufacturers specifically producing extracts.

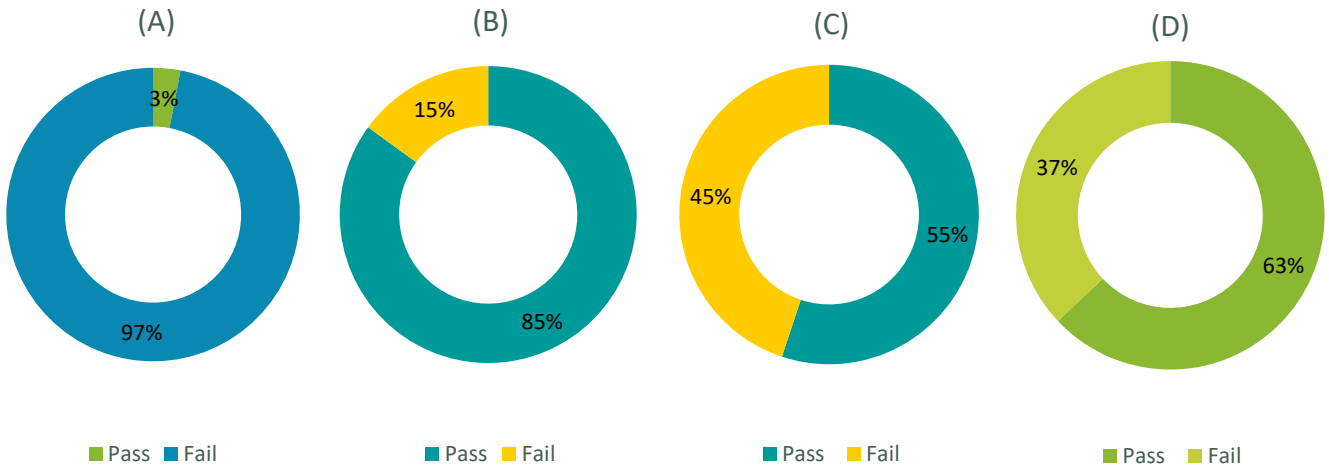

Figure 25 – The number of samples adhering to current legislation with regards to THC concentration (A); The number of sample heavy metal residue failures when compared to the USP232/ICH Q3D oral specification (B); The number of sample heavy metal residue failures when compared to the USP232/ICH Q3D inhalation specification (C); The number of sample solvent residue failures when compared to the USP467/ICH Q3C specification (D).

6.3 Conclusion

As seen from the facets discussed above, manufacturers, as well as cultivators should adhere to the current GMP guidelines set forth, to ensure the safety of the consumer as well as the quality of the product produced. With the current findings the safety of consumers is brought into question. A large number of consumers purchasing cannabis-based products from retailers are concerned about the safety of these products and would thus like to obtain a certificate of analysis (CoA), before administration of these products. It cannot be left to the public consumer to educate themselves on proper GMP principles and everything associated with this. The onus lies with the regulator to first and foremost have proper legislation in place, to enforce regulation more strictly, which will, in turn, educate the industry. Finally, the responsibility also lies with manufacturers and cultivators to adhere to the

published guidelines, as well as have an appropriate, comprehensive quality control regime in place. With all this, the quality of cannabis-based products, as well as the safety of consumers, will most likely be ensured.

6.4 Future Research

Future research that will broaden the current knowledge available to regulators and the public in South Africa, may include the following.

- The assessment of additional quality control tests, to further understand the current South African market. By assessing current cannabis-based product available in the market, additional specification limits can be set for a broader range of quality control parameters. Additional quality control tests that should be considered may include pesticide residue screening as well as microbiological contaminant screening. Although these quality control tests form part of the current guideline, a specification limit for individual contaminants has not been set for South Africa (South African Health Products Regulatory Authority (SAHPRA), 2019:1–30).
- An assessment of the scope and impact of current regulations will be meaningful. To what extent the regulations are currently enforced will provide useful insights to regulators as well as law enforcement agencies. This may provide the knowledge necessary for these bodies to focus their efforts on specific ways to accomplish adherence to regulations by industry.
- A recommendation could be to standardise the sale of cannabis seeds, only making certain genetic strains available to industry. Industry may apply for specific genetic strain of cannabis to be approved. These strains may form part of an approved genetic database. Cannabis-based products or plant material may be screened by law enforcement against the database. All cannabis products with a 'genetic signature' that has not been approved, or part of the regulated database can easily be considered illicit.
- Additionally, all the research conducted on cannabis-based products may also be applied to future or current herbal medicines being manufactured or cultivated in South Africa.

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