Cannabis-Based Products: Analytical Implications and Impact on Regulation.

A dissertation submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy

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DEDICATION

To my everything, Lorianne and Alston, whose unwavering love, support, and encouragement have been the foundation and purpose of my journey.

To the next generation of scientists, may this work serve as a source of inspiration to pursue knowledge, ignite curiosity, and foster research efforts for the betterment of our world.

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Abstract

The human use of cannabis for medicinal and recreational purposes dates back centuries. Research advances and changes in legislation led to the widespread availability of Cannabis based products from a variety of sources.

This project investigated the quality parameters applicable to Cannabis from an analytical perspective and their impact on the regulation of Cannabis. The research question being addressed is to determine the relevance of quality parameters to Cannabis-based products and identify how these parameters inform regulatory sciences in this area.

The objectives of this study involved the development, validation, and implementation of analytical techniques relevant to Cannabis-based products, intended for Cannabis testing and comparing properties of Cannabis in different dosage forms and from different sources, alongside assessment of how the outcomes of this research impact on the establishment of official standards and their adoption in regulatory frameworks.

The initial phase of the study was based on the development and validation of methods for detection and quantification of Cannabinoids (THC, CBD, and CBN) and major terpene compounds in different dosage forms of Cannabis products. The developed analytical procedure based on a rapid liquid extraction and analysis on GC-MS was shown to provide accurate and reliable results by satisfying all the quality criteria included in the extensive validation process conducted.

In the second phase of the study, the validated procedures were applied to analyse Cannabis inflorescence, Cannabis extracts and other dosage forms available on the market. The analytical data indicated a significant difference in the quality traits of cannabis products available on the local market, that included deviations from labelled content exceeding 10% in 28.3% of the products tested, deviations from the 0.2% THC limit enacted in Maltese legislation, inconsistency or absence of Terpene compounds and the presence of adulterants such as semi-synthetic cannabinoids in cannabis based products availiable on the local market.

The final phase was focused on data analysis and the interplay between analytical data and regulatory sciences. The research presented provides pivotal analytical data and regulatory aspects to develop a harmonised regulatory framework for cannabis based products focused on product quality and safety. These are mainly related to the introduction of limits for degradation compounds, enhancing manufacturing quality, product consistency, adherence to legal limits of THC and the regulation of semi-synthetic cannabinoids.

Keywords: Cannabis, GC-MS, cannabinoids, terpenes , regulatory framework.

Contents

List of Tables

List of Figures

List of Appendices

Abbreviations

%E	Percentage Error
ANOVA	Analysis of Variance
CAR	Carboxen
CBD	Cannabidiol
CBN	Cannabinol
CNS	Central Nervous System
CPMs	Cannabis Products for Medicinal Use
CV	Coefficient of Variation
Δ9-THC	Delta-9-tetrahydrocannabinol
DVB	Divinylbenzene
EF	Extraction Efficiency
EMA	European Medicines Agency
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
FID	Flame Ionization Detector
GC-MS	Gas Chromatography-Mass Spectrometry
HHC	Hexahydrocannabinol
HHCP	Hexahydrocannabiphorol
HHC-O	Hexahydrocannabinol-O-acetate
HPLC	High performance Liquid Chromatography
HMPs	Herbal Medicinal Products
ISO/IEC	International Organization for Standardization / International Electrotechnical Commission

k	Coverage Factor
m/z	Mass to Charge Ratio
MCT	Medium Chain Triglycerides
NPS	New Psychoactive Substances
PDMS	Polydimethylsiloxane
Ph. Eur.	European Pharmacopoeia
PSI	Pounds per Square Inch
R^2	Correlation Coefficient
RSD	Relative Standard Deviation
RSDr	Relative Standard Deviation for Reproducibility
RSDx	Relative Standard Deviation between Groups
SD	Standard Deviation
SHS	Static Headspace
SLE	Solid-Liquid Extraction
SOP	Standard Operating Procedure
SPME	Solid Phase Microextraction
THC	Tetrahydrocannabinol
THCP	Tetrahydrocannabiphorol
UV	Ultraviolet

Hypothesis and Research Questions

Aim: The aim of this project is to investigate the quality parameters, applicable to Cannabis products, to increase the scientific knowledge on Cannabis from an analytical perspective and identify how the results from this research impact on the regulation of Cannabis products.

Objectives:

The objectives of this study are:

I. Develop, validate and implement analytical techniques for the identification and quantification of phytochemicals of interest in Cannabis products.
II. Compare Cannabis products from different sources.
III. Assess how the outcomes of this study can be used to spearhead developments in regulatory frameworks.
IV. Dissemination of research findings to inform international fora and competent institutions such as the European Pharmacopeia.

Research question:

Can analytical scientific knowledge impact positively the regulation of Cannabis products?

Hypothesis:

Scientific knowledge related to the analytical implications of Cannabis-based products may inform the establishment of quality standards and impact on the harmonization of regulatory frameworks.

Chapter 1
Introduction

1.1. The introduction of cannabis products for medical use.

In the last decade, the use of cannabis products for therapeutical purposes has gained significant interest. The use of cannabis as a medicine is unconventional, when compared with the usual process for design and development of medicinal products. The medicinal applications were pioneered by patients and care givers, instead of physicians or scientists. The administration of these products has generally been through unconventional means such as smoking.

For centuries, Cannabis was consumed unadulterated, in its botanical form. This has evolved in a myriad of preparations, isolates and plant based preparations, that are available in local and international markets. In general terms, 'Cannabis' is a term that is used to describe an array of products containing one or more Cannabinoids (Dryburgh and Martin, 2020). This proper reference for these products can be challenging mainly because the term 'Cannabis' includes substances scheduled under the 1961 single convention on Narcotic Drugs, and preparations for medicinal and recreational use. However, the distinction of cannabis products that are intended for medicinal use is an important regulatory and medical aspect.

The EMCDDA's definition restricts the use of the term 'medical cannabis' to products that have a marketing authorisation. This includes products that follow quality guidelines and manufacturing standards such as the Australian therapeutic

goods order 93[1]. Products available in European markets and other jurisdictions include capsules, oils and oromucosal sprays. Even though other cannabis-based preparations are used for self-treatment, Cannabis products that do not have a marketing authorisation are defined as 'non-medicinal' cannabis products. This is mainly due to the lack of evidence based medicinal applications and information on the quality traits of these preparations. Apart from the clear definition of the products available in the local markets, this study will also aim to provide essential information on the quality parameters of cannabis products.

For the purpose of this study, 'Cannabis products' refers to any product containing parts or extracts of the Cannabis plant, whilst 'Cannabis products for medicinal use' (CPMs), refers to products that have been approved for medicinal use by regulatory authorities, but do not necessarily have a marketing authorisation.

One of the main factors driving the use of Cannabis products for medicinal purposes is the presence of bioactive cannabinoids such as THC and CBD. The use of cannabis as a treatment has also been driven by the increased public awareness about the risks associated with chronic use of medications such as opioids (Alahyari et al., 2018), leading patients to seek alternative therapies such as Cannabis products as management strategy for chronic pain (Zeng et al., 2021). The access to cannabis products for medicinal use in more than 30 countries has also facilitated the access to patients worldwide.

[1] Australian Federal Government. (2020) Therapeutic Goods order 93 (Standard for Medicinal Cannabis) Version 1.3, Accessed via: https://www.legislation.gov.au/F2017L00286/latest/details

1.2. Considerations for quality attributes and the need for Quality standards.

Medicinal products are regulated by a number of frameworks to ensure that these products meet the specifications for quality, safety and efficacy. Such regulatory provisions are also applicable to HMP's and include Pharmacopoeia monographs, guidance documents issued by the EMA[2] and European directives and regulations [3].

The complexity of the product matrix of HMPs, including CPMs presents specific peculiarities with respect to the quality control of these products. The raw material in these products (Cannabis plant) is a complex and heterogeneous matrix, that can produce a vast array of secondary metabolites. The intrinsic variation within the raw material, and the diverse markets that Cannabis Products originate from makes it of paramount importance to identify and monitor quality traits of these products (Vergara et al., 2017).

Data on the composition and purity of CPMs is essential in the prevention of adverse effects that may result from exposure to substandard Cannabis products (Sarma et al., 2020). The availability of cannabis products or preparations containing cannabinoids prepared according to an established and consistent process will directly enhance the reproducibility and applicability of data from preclinical and clinical studies (Leung et al., 2019).

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[2] European Medicines Agency. Guideline on specifications: test procedures and acceptance criteria for herbal substances, herbal preparations, and herbal medicinal products /traditional herbal medicinal products. vol. 44. 2018

[3] Directive 2004/24/EC of the European Parliament and of the Council of 31 March 2004 amending, as regards traditional herbal medicinal products, Directive 2001/83/EC on the Community code relating to medicinal products for human use. Official Journal of the European Union, L 136, 30 April 2004, pp. 85-90.

Due to the presence of multiple bioactive and medicinal compounds in cannabis-based products it is essential that these major constituents are accurately determined. It is also important that data from testing laboratories is evaluated by regulatory bodies. The latter might be a challenging aspect of cannabis regulation because, across industry standard procedures have not been adopted. In addition, the reliability of results is also put in question due to interlaboratory variation in the quantification of cannabinoids (Jikomes and Zoorob, 2018).

Quality standards and monographs set forth specifications for quality attributes. There are a number of national guidelines and adopted frameworks such as the general guidelines for herbal medicines[4], and monographs for herbal medicinal products that identify limits of defined contaminants that are not specifically addressed to Cannabis based products.

1.3. Defining Cannabis

Cannabis plants are annual dioecious flowering plants, of the *Cannabaceae* family. The classification of these plants from a Taxonomic perspective is still being investigated (Bonini et al., 2018). Schultes and Anderson (Loran C. Anderson, 1980) subdivided the genus into three species, (*Cannabis Sativa Linnaeus, Cannabis Indica Lamarck, and Cannabis Ruderalis Janisch)* based on the geographical distribution, morphological characteristics and metabolomic constitution of these plants. Small and Cronquist (Small and Cronquist, 1976) also

[4] European Medicines Agency. (2011). *Guideline on quality of herbal medicinal products/traditional herbal medicinal products* (EMA/HMPC/201116/2005 Rev. 2).

classified these three species as one species (*Cannabis Sativa L.*) with two subspecies (*C. Sativa spp. Sativa* and *C. Sativa spp. Indica*). Each subspecies was also divided into the wild and domesticated variety. The classification was also revisited by Bocsa and Karus (date), that confirmed the classification of Cannabis plants as one polymorphic species. The classification was reverted to three subspecies (*C. Sativa ssp. sativa, C. Sativa ssp. indica and C. Sativa ssp. Ruderalis*).

To date there is no general consensus on the taxonomical classification of Cannabis plants. The distinction of cannabis species has been an important characteristic in terms of the definition of plant varieties used for industrial purposes and varieties grown for medicinal or recreational use.

The latter definition is essential in the establishment of legal and regulatory framework. Given the lack of consensus in defining Cannabis species, regulating bodies have opted to put a clear distinction between Hemp and Cannabis varieties used for medicinal and recreational purposes.

Hemp is defined as *C. Sativa L.* where the amount of tetrahydrocannabinol in the material is low enough that the material cannot be used to produce narcotics. The 75 hemp varieties registered under the European common agricultural policy (Donald et al., 2002) must have a THC content that is less than 0.2%; however, member states may apply tighter restrictions to align with international obligations.

The definition of such limits has indecisively sparked interest by testing laboratories and regulatory bodies to develop and validate testing procedures that can reliably quantify the cannabinoids of interest in these products (Published

analytical methodology reviewed below). However, the lack of harmonisation in testing procedures across European and global markets has limited the applicability of analytical data.

1.4. Bioactive molecules in cannabis

With more than 600 described metabolites, Cannabis plants are a source of bioactive molecules that exhibit unique molecular structures, biological properties and physiochemical activity. About 20% of the isolated metabolites are classified as Cannabinoids due to their shared molecular structure (Hanuš et al., 2016). The psychoactive cannabinoid, delta-9-THC has been the main focus of research since its isolation in 1964. In the last decade, CBD has also gained significant interest as a pharmacologically active, non-psychoactive metabolite. The presence of THC and CBD present in different ratios in cannabis chemovars determine the potency and pharmacological properties of the plant (Malik et al., 2020). The complex mixture of metabolites in Cannabis plants includes a vast array of volatile secondary metabolites that are pharmacologically active. Among these, Terpene compounds constitute the largest and most important group of volatile secondary metabolites.

The open commercialisation of cannabis products reignited the interest towards cannabis volatile compounds such as terpenes. The presence and variability of different terpene compounds is responsible for the organoleptic properties to cannabis products (Judith K Booth and Bohlmann, 2019) giving rise to a diverse array of cannabis products to appeal to a vast array of consumers.

Terpene compounds are a diverse class of volatile organic compounds produced as secondary metabolites by many plant species, including Cannabis (Sommano et al., 2020). These organic molecules are composed of repeating isoprene units (C_5H_8), connected in a 'head to tail' fashion in accordance with the Isoprene rule, described by Ruzicka, (1953). Isoprene units act as building blocks in the biosynthesis of complex organic molecules (Ruzicka, 1953).

Terpene compounds in Cannabis may be subdivided into two main classes, Terpenes and Terpenoids. Terpenes include all the hydrocarbon isoprene compounds, whilst Terpenoids or Isoprenoids may be distinguished by the presence of additional functional groups, resulting from oxidation or rearrangement of the carbon skeleton (Tholl, 2015). The structural rearrangements and the introduction of additional functionalities gives terpenoids slightly different chemical properties and biological activities compared to their terpene counterparts (Bergman et al., 2019).

Over 200 volatile compounds have been identified in various cannabis strains, these include 58 monoterpene compounds (C_{10}) and 38 sesquiterpene compounds (C_{15}). Terpene compounds that have been found in cannabis also include diterpenes (C_{20}) and triterpenes (C_{30}) (Ross and ElSohly, 1996; Turner et al., 1980; Wanas et al., 2020). Terpenes and terpenoids exhibit diverse biological and pharmacological properties, including antifungal, antiviral, anticancer, anti-inflammatory, antihyperglycemic, antiparasitic, antioxidant, and antimicrobial activities

One of the most prevalent, and the smallest Monoterpene, Myrcene has multiple bioactive capabilities and can act as an analgesic and as a sedative (Koziol et al., 2015). Myrcene has also been associated with its capabilities to act as a muscle relaxant, whereby the consumption of chemovars rich in myrcene (>0.5%) have been reported to cause strong sedative and lethargic effects (Gulluni et al., 2018a; Jansen et al., 2019).

β-caryophyllene is also present in most Cannabis chemovars (Milay et al., 2020). This sesquiterpene is of special note as it is the only terpene described to date that can interact with CB_2 receptors. β-caryophyllene exhibits analgesic, antifungal, and antibacterial properties as well as neuroprotective and gastroprotective properties (Dahham et al., 2015). Its antiviral activity has also been evaluated against HSV type 1 virus, and the compound showed a highly effective therapeutic effect reported to have a high selectivity index (SI >140) (Astani et al., 2011; Francomano et al., 2019).

Other terpene compounds that have been extensively studied are limonene, α-Pinene, β-Pinene, Linalool and terpinol. These compounds are also found in essential oils of other plants and therefore the extracts used in these studies did not directly originate from cannabis plants. The essential oils rich in these terpenes have been shown to have antioxidant, anti-inflammatory, antibiotic and sedative/anxiolytic properties (Erasto and Viljoen, 2008; Kamatou and Viljoen, 2008; Khaleel et al., 2018).

While the primary attention has been on the bioactive properties of cannabinoids, the Terpene compounds may also provide ‘entourage effects’, potentially acting in synergism to enhance or influence the pharmacological effects of Cannabinoids (Koltai and Namdar, 2020). The term ‘Entourage’ refers to the enhanced activity of a compound by an inactive compound, as described by Mechoulam R et al., 1987) as strict inequality, 1+0 >1.

The full relationship of the synergism between Terpene compounds and Cannabinoids has not yet been elucidated. The physiological effects of cannabinoids are mediated by endocannabinoid receptors (CB1 and CB2) (Mackie, 2008). However, experiments using terpene compounds to modify or enhance the receptor-mediated activity of phytocannabinoids and endocannabinoids have shown that terpenoids do not mediate an entourage effect by acting at cannabinoid receptors. The authors concluded that there is no direct effects on the interaction of THC and CBD with endocannabinoid receptors, when terpene compounds are present (Finlay et al., 2020).

During the investigation of anti-inflammatory properties of cannabis extracts rich in terpene and terpenoid compounds, the authors concluded that terpene compounds and Cannabinoids showed a synergistic effect, as isoprene and isoprenoid compounds showed effectiveness against acute inflammation, whilst cannabinoids were effective in inhibiting chronic inflammation (Gallily et al., 2018).

Similarly, during the investigation of antitumor activity of fresh cannabis extracts containing cannabinoids and five of the main terpene compounds (β-caryophyllene, linalool, β-pinene, Humulene and Nerolidol), Blasco-Benito et al. Concluded that the unpurified extract had higher antitumor activity than the purified THC, or any of the Terpene compounds individually, indicating an active synergistic effect (Blasco-Benito et al., 2018). The authors however failed to evaluate pure substances of other cannabinoids present in the crude extract.

1.5. 'Low-THC' products

Cannabis products such as herbal materials and oils marketed as `Low-THC` were introduced to European markets in 2011 (EMCDDA, 2020). Producers and distributors of these products across the EU claim that the products contain low levels of THC. Most of these retailers operate under the assumption that, in accordance with EU regulations, granting payments to hemp farmers (EMCDDA, 2020) products containing less than 0.2% THC were not restricted by legal frameworks controlling Cannabis products. However European courts have ruled against this matter, especially for CBD based products, as extracted CBD cannot be considered as an agricultural product and does not fall under the scope of the Common agricultural practices regulation.

The major Cannabinoid in Low THC products is usually CBD (Grafinger et al., 2020) . The control of this cannabinoid has been the subject of discussion and legal proceedings. Even though this has been controlled as a 'drug' in European countries for centuries, under the realm of EU law, a substance can only be under control if this is included in the Convention on Psychotropic Substances and the Single Convention on Narcotic Drugs. Whilst CBD is absent in the former and included in the latter as being part of an extract from Cannabis plants, the lack of

scientific knowledge on the use of these substances goes against the objective of the Convention of Narctoic Drugs, that is to 'protect the health and welfare of humankind', as to date there has been no reports detailing harmful or psychotropic effects of CBD. Taking the latter considerations, a landmark decision was taken by the European court of justice, overturning a judgement ruled against the owners of a distribution company selling CBD oils in France, showing that the 'risk' was purely based on hypothetical considerations , highlighting a direct knowledge gap on the composition and regulation of Cannabis products in the EU [5].

A *trendspotter* survey conducted by the EMCDDA, in 2019 highlighted the availability and diversity of `Low-THC` products in most European countries. The monitored products were Herbal materials, resin, oils, E-Liquids, crystals, and edible products. However, there is still a major challenge for regulators and producers to establish a legal basis or a regulatory framework applicable to these products.

The exclusion of these products from regulatory frameworks raises concerns relating to the quality characteristics of these products in terms of the actual cannabinoid content and the presence of contaminants (Caulkins and Kilborn, 2019). Independent testing by health authorities and research labs have highlighted the presence of low amounts of THC (<1.5%) and high levels of CBD, that has been reported to go as high as 60% in some products, especially oils (Liebling et al., 2020; Pavlovic et al., 2018).

[5] Court of Justice of the European Union. (2020). *Judgment of the Court (Grand Chamber) of 19 November 2020. A. v B. (Case C-123/20)*.

A study by the Austrian consumer protection department, showed that from a sample of 200 products tested in 2019, 25% of these products exceeded the 0.2% limit, with a range of 0.4 to 1.1% THC[6]. A similar study was conducted on 30 products available in the UK. Analysis of Cannabinoid content in these products revealed that 37% had less than 50% of the claimed CBD content, with one product, containing 0% [7].Further testing also identified the presence of toxic solvents such as dichloromethane and cyclohexane.

(Lachenmeier et al., 2022) also focused on CBD oils available in the US and European markets. The analytical data identified significant deviation (>10%) of cannabinoid content in 64% of products sourced from European retailers (Liebling et al., 2020) and 26% of products in the US market (Miller et al., 2022).

In addition, the source of these products is not consistently provided by manufacturers. An online review of retailers advertising ` low-THC` products in the EU showed that several products do not identify the source or the manufacturing site, whilst reports gathered by the EMCDDA showed that a number of declarations of origin on products marketed in the EU were inaccurate or misleading, in order to hide their true origin (EMCDDA, 2020)

[6] Data submitted to EMCDDA by the Austrian Federal Ministry of Social Affairs, Health, Care and Consumer Protection (2022). Accessed via: https://www.emcdda.europa.eu/data/data-catalogue_en on 13 February 2022

[7] United Kingdom Advisory Council on the Misuse of Drugs. (2023).- Consumer CBD products report. Accessed via: https://www.gov.uk/government/publications/acmd-advice-on-consumer-cannabidiol-cbd-products/consumer-cannabidiol-cbd-products-report

The review of published analytical data has highlighted a major knowledge gap on the quality traits of ‘low-THC’ products (excluding CBD oils). With the constant introduction of cannabis-based products available to consumers, analytical methods have not been expanded to cover different dosage forms and product types. The studies also highlight the need of a regulatory framework for ‘low-THC’ products. The lack of harmonised and thoroughly validated analytical methods, is one of the major hurdles in the establishment of a quality protocol for ‘low-THC’ products. Apart from the development and validation of these methods, in Malta, the use of these methods on samples of `low-THC` products available on the local market will give a snapshot of the market as it stands to date. The analytical data can directly contribute to the regulatory regime and consumer protection.

1.6. Cannabinoid structure-activity relationship and the emergence of semi-synthetic cannabinoids

Δ9-THC has been the primary focus of cannabinoid psychoactivity studies. Following its characterization, researchers identified Δ9-THC as the main psychoactive cannabinoid in Cannabis plants (Pertwee, 2006).

Its interaction with the endocannabinoid system and effects on the central nervous system (CNS) are associated with the 'high' experienced by cannabis users. This interaction results in several physiological effects, including psychoactive effects, CNS depression, changes in cardiovascular state, and ataxia (Bilbao and Spanagel, 2022). These physiological changes are collectively referred to as 'Cannabis/THC-like effects'.

These physiological changes have also been studied at a molecular level, revealing that the interaction of cannabinoids with the endocannabinoid system alters enzyme activity, neurotransmitter functionality, and prostaglandin synthesis (Thomas et al., 2005).

The interaction with cannabinoid receptors and the generation of protein cascade mechanisms have also been studied in terms of the structure-activity relationships of THC.

Early investigations using animal studies identified that the molecular structural requirements mainly included the benzopyran ring structure and the attached aliphatic chain (Edery et al., 1972). Structure-activity relationship studies have also identified that the attached alicyclic ring may be substituted with a heterocycle, allowing for a broad range of possible substituents to be introduced on the molecule while conserving its activity (Bow and Rimoldi, 2016).

The number of carbons on the alkyl side chain in cannabinoids directly relates to cannabinoid receptor affinity (Andersson et al., 2011). Mechoulam et al. (1987) identified that the minimum length for the side chain should not be less than three carbons. Further studies identified a linear relationship between receptor affinity and alkyl chain length, using THC derivatives with variable chain lengths (Andersson et al., 2011).

Based on the understanding of the structural moieties that contribute to the pharmacological activity of THC, numerous molecules have been synthesized

with the aim of producing therapeutic drugs with cannabimimetic effects and improved pharmacological activity by increasing their affinity for CB1 receptors.

The structural modifications of cannabinoids resulted in substances with excessive psychoactivity, rendering them unsuitable for medicinal use (Banister and Connor, 2018). Consequently, many of these substances were produced and sold in the illicit drug market as synthetic cannabinoids, fuelling the wave of New Psychoactive Substances (NPS) over the past decade (Alves et al., 2020). Although the controls imposed on synthetic cannabinoids led to a decrease in the number of such substances available on the illicit market, a new trend of cannabimimetic compounds has been identified in European markets by laboratories since 2022 [8].

Semi-synthetic cannabinoids can be broadly described as either compounds that may be present in cannabis plants in very low concentrations or compounds derived synthetically from cannabinoids found in cannabis. These pseudo-natural cannabinoids have structures that are very similar to THC.

The closest structural derivative in this class is Δ8-THC, which is generally identified in very low quantities in cannabis plants (<0.1%) or in cannabis samples stored for more than two years, where the double bond shifts from Δ9 to the thermodynamically stable position, at the Δ8 (Dalzell HC et al., 1981). This shift does not compromise its pharmacological activity, as Δ8-THC has a comparable

[8] European Monitoring Centre for Drugs and Drug Addiction. (2023). *Hexahydrocannabinol (HHC) and related substances. Technical report*. Luxembourg: Office for Official Publications of the European Communities. https://doi.org/10.2810/852912.

pharmacologic effect to Δ9-THC (Rapaka and Makriyannis, 1987). Over the past two years, cannabis products containing more than 10% Δ8-THC have been identified, originating from semi-synthetic routes based on the acid-catalysed cyclization of CBD (Marzullo et al., 2020)

However, all the regional isomers of tetrahydrocannabinol are regulated under the 1971 Convention on Psychotropic Substances [9]. In the ongoing cycle of regulation and adaptation, new semi-synthetic cannabinoids have emerged in the unregulated cannabis market as uncontrolled alternatives to THC, appearing in the form of pre-rolled cigarettes, resin, and herbal preparations [10]. These include compounds produced by hydrogenating the unsaturated ring in Δ8 and Δ9-THC to produce 9R and 9S-Hexahydrocannabinol (HHC), respectively (Russo et al., 2023). This trend was followed by the introduction of acetylated hydrocannabinols, such as Hexahydrocannabinol -Acetate (HHC-O) (Ujváry, 2023a).

Similar trends were observed with the introduction of butyl, heptyl, and hexyl homologs of THC, in the form of THCB (tetrahydrocannabutol), THCP (tetrahydrocannabiphorol), and THCH (tetrahydrocannabihexol) (Rossheim et al., 2023).

While the uncontrolled sale of semi-synthetic cannabinoids with concentrations often exceeding 50% is an alarming factor for the safety of cannabis users, major

[9] United Nations Office on Drugs and Crime. (1971). Convention on Psychotropic Substances, 1971. Retrieved from https://www.unodc.org/pdf/convention_1971_en.pdf.
[10] European Monitoring Centre for Drugs and Drug Addiction. (2023). *European Drug Report 2023: Trends and Developments*. Luxembourg: Publications Office of the European Union.

concerns have also been raised by the presence of these cannabinoids in products labelled as containing non-psychoactive cannabinoids such as CBD [11], as well as the presence of toxic chemical contaminants, including unknown cannabinoids, solvents, and heavy metals (Kiselak et al., 2020).

1.7. Extraction of phytocannabinoids

The increase in the number of medicinal cannabis preparations available today has increased the demand for analytical techniques for the determination and quantification of the bioactive cannabinoids in these products. Developing robust and standardized techniques for quantifying cannabinoids in botanical and pharmaceutical forms is a critical step forward for an emerging Cannabis-based pharmaceutical industry.

The metabolomic analysis of any products is preliminarily dependent on the ability of the analysis method to selectively extract the compounds of interest from the sample matrix. The technique used for extraction, is typically influenced by the physical characteristics of the matrix whilst the extractant phase is dependent on the physicochemical characteristics of the analytes of interest.

[11] US Cannabis Council. (2021). D8-THC. Washington DC: US Cannabis Council.

The extraction of secondary metabolites from Cannabis based products involves the extraction of a variety of secondary metabolites from a solid complex matrix. The most prevalent technique for the extraction of cannabinoids from plant material is a solid-liquid extraction (SLE) (Citti et al., 2018). The solid matrix comes in contact with a solvent that has a high affinity to the analyte of interest, to obtain a selective extract. Cannabinoid acids and neutral cannabinoids can be extracted using common organic solvents or solvent mixes. The molecular affinity of polar organic solvents like Ethanol, has been described to extract cannabinoids in a selective and efficient manner (Lazarjani et al., 2021a). The extraction of Cannabinoids in Ethanol has also been proposed on a draft of Cannabis Flos monograph issued by the German Pharmacopoeia (Manns et al., 2019).

The extraction of Cannabinoids using a mixture of solvents (9:1 mix of methanol: Chloroform) has been applied for Quality control (Pellegrini et al., 2005). and chemotaxonomic differentiation of cannabis based products (Pandohee et al., 2015). Hexane has also been used by itself to analyse quality control samples (Wianowska et al., 2015) or in combination with isopropanol for chemotype differentiation of Cannabis and the use of THC levels in cannabis as a marker of the age of the plant (Mariotti et al., 2016). Other authors also explored the use of complex procedures that included multiple extracts in different solvents, followed by liquid-liquid fractionation of these extracts. The authors showed that the extraction was exhaustive and included prenylated flavones from cannabis plant material (Peschel and Politi, 2015a).

Omar et al. (Omar et al., 2013) compared the application of Supercritical fluid extraction (SFE) and focused ultrasound extraction (FUSE) for the extraction of Cannabinoids from cannabis cultivars. The authors concluded that the extraction efficiency of FUSE was higher (80%) than that of SFE. However, the use of SFE allowed the selective extraction of cannabinoids, by using ethanol as a co-solvent. The authors also showed that the removal of Terpenes from the extract enhanced the chromatographic separation and analysis of the components in the extract.

The extraction efficiency is also dependent on the physical conditions that the sample matrix is subjected to. The surface contact area between the extracting solvent and the sample matrix and the exposure to heat, and Ultrasound waves have been used to enhance extraction efficiency (Kokosa, 2013). The application of mechanical force (maceration or pulverisation) can assist extraction by increasing the surface area of the sample, and by disruption of cellular membranes (Chen et al., 2019).

Ultrasonication generates shock waves by cavitation. The energy is enough to break chemical bonds and causes cell lysis. The process also causes a localised solvent flow that intensifies analyte extraction (Agarwal et al., 2018). Moreover, the process has a low energy consumption and is not economically demanding. Baranauskaite et al. (Baranauskaite et al., 2020), compared three extraction techniques that involved the use of Maceration, ultrasonication and refluxing, to obtain the best cannabinoid extract from plant material. The authors concluded that Ultrasonication gave a high extraction efficiency and had the lowest processing time and cost.

1.8. Analytical approaches for Terpene compounds

Terpenes are produced in the glandular trichomes alongside Cannabinoids by the combination isoprene units. The number of unsaturated five carbon units is represented in the classification of these compounds such as Monoterpenes (C_{10}), and Sesquiterpenes (C_{10}). The low molecular weight of these compounds makes them volatile at room temperature. Apart from being an important group of secondary metabolites in cannabis plants, Terpenes are responsible for the unique organoleptic properties associated with Cannabis products (Pertwee, 2014).

The organoleptic properties, derived from a combination of Terpene compounds, and their relative abundance, is a defined phenotypic variation between Cannabis Cultivars. However, similarly to Cannabinoids, the production of these volatile secondary metabolites is also affected by the environmental conditions the plant is subjected to during growth (Judith K. Booth and Bohlmann, 2019) The latter combined with the numerous strains produced by genetic recombination techniques, further convolutes the determination of terpene compounds and their role within regulatory frameworks.

Terpene variability has also been associated with medicinal qualities of cannabis varieties (Booth et al., 2017) . One of the most abundant sesquiterpenes, β-Caryophyllene was shown to be responsible for the autoinflammatory response of cannabis products through the interaction with CB2 receptors (Klauke et al., 2014)Terpene compounds including pinene have also been associated with the modulation of THC intoxication and reducing memory loss, through the inhibition of acetylcholinesterase (Miyazawa and Yamafuji, 2005).

To date the scientific knowledge on the possible contribution of terpene compounds on the pharmacologic activity of Cannabis is still limited to a small number of these compounds. However, information on the synergism and pharmacologic effects of these compounds relies on the ability of research labs to accurately extract and quantify these compounds.

1.9. Extraction of volatile compounds from Cannabis products

The extraction efficiency of terpenes from plant materials and the reproducibility of the procedure is directly influenced by the extraction procedure. The latter is mainly distinguished by the physical state of the extractant medium. In solvent-based extractions the polarity of the extraction solvent plays a pivotal role in determining the final composition of the extract. Conversely, the use of solvent free (headspace) techniques does not require an extractant solvent; the extraction efficiency relies on the preferential partitioning of Terpenes in the vapor phase at the set temperature (Zhang and Pawliszyn, 1993)

Solvent-based extraction techniques have been investigated in multiple publications, where extraction efficiency of different solvents or mixtures was evaluated. A study on the terpene composition of Cannabis flowers from the same plant concluded that a mixture of ethanol and hexane (V:V 7:3) produced the highest extraction efficiency (Namdar et al., 2018) Similarly, other studies evaluated the solvent-based extraction protocols in order to propose a standard technique for the evaluation of Terpenes in Cannabis inflorescence (Hazekamp

and Romano, 2013). The study involved extractions in ethanol, Petroleum ether, Naphtha and Olive oil. Olive oil was the best extractant solution, due to its low polarity and volatility, that stabilises the extracted terpene compounds in solution. However, Oil extracts cannot be injected in chromatographic instruments directly, making the procedure only applicable to prepare extracts for non-analytical procedures. A similar evaluation process was undertaken by Ibrahim et al. (Ibrahim et al., 2019) that compared the extraction efficiencies of ethyl acetate, a mix of methanol and Chloroform (1:9 v:v), and two alcohols (methanol and ethanol), identifying ethyl acetate as the solvent with the highest recovery. The use of ethyl acetate allowed the extract to be analysed directly using GC-MS.

Other authors have also evaluated preheating treatment or drying. The evaluation of preheating techniques, during sample preparation showed that if samples are subject to any form of heat, this changed the relative abundances between terpenes, indicating losses by evaporation and degradation. Similarly, the use of vacuum concentrators or rotary evaporators lead to the loss of more than 85% of monoterpenes in the extract, and significant loss (>50%) of sesquiterpene compounds. Sample concentration using a flow of nitrogen, Tarnelli et. al. (2022) investigated the use of a gentle nitrogen to concentrate extracts containing terpenes. The procedure involved the use of a gentle flow of nitrogen gas through the extract, that can volatilise the solvent concentrating the terpene extract(Ternelli et al., 2020a)The procedure showed no alteration in the profile of Monoterpenes and sesquiterpenes.

Solvent free extractions have also been evaluated in analytical procedures used to extract and identify Terpenes from cannabis-based products. Solvent free extraction techniques are mainly centred around the use of Solid phase Micro extraction (SPME) (Vas, 2020).SPME technology is based on the partitioning equilibrium of the analytes of interest between the sample and the extraction phase. The process involves the partitioning of analytes in the extracting phase, followed by selective adsorption of the analytes of interest. The analytes can then be directly discharged into an analytical instrument (Pawliszyn, 2012). The main advantage of using SPME techniques is the ability to integrate sample isolation and extraction from the matrix with analyte enrichment. This technique has been successfully applied for extraction from complex matrices including plant materials and biological samples.

A static-head space SPME technique has been applied for the extraction of terpenes in hemp inflorescences(Pellati et al., 2018). The authors also evaluated the extraction efficiency of Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fibres against the use of Polydimethylsiloxane (PDMS) fibres. The relative peak areas in samples analysed using the DVB/CAR/PDMS were higher. This was also corroborated in another study focusing on the use of HS-SPME techniques for the destination of Cannabis chemovars based on their terpene profile [12].The report concluded that the use of DVB/CAR/PDMS fibres provided a sensitive technique that could be used to produce the required mass spectra for identification.

[12] Thermo Fisher Scientific Center of Excellence. "Terpenes and Residual Solvents in Hemp Products by GC-FID." Thermo Fisher Scientific, 2020.

Despite the results obtained in these studies, the application of DVB/CAR/PDMS fibres may not be the best option for quantitative analysis. The lower number of binding sites in the latter fibres subjects these methods to analyte saturation. Comparatively, Static head space (SHS), are not affected by the nature of the fibre. Therefore, SHS has been used in numerous applications for the analysis of volatile compounds, including its use in analytical testing of residual solvents and terpene compounds in finished cannabis products (Nguyen et al., 2020) SHS was also used in a procedure for the extraction and analysis of terpene compounds in Cannabis samples(Shapira et al., 2019). The authors focused on the advantages of SHS as an extraction technique for the evaluation of terpene content in different chemovars. The described procedure required simple sample preparation (grinding), whilst allowing for an improved chromatographic resolution by minimizing the contamination of the GC inlets and reducing matrix effects. The method was validated using multiple cultivars; however, the authors failed to evaluate the effects of the high temperature used (140 °C) on the terpene profile.

Chapter 2
Methodology

2.1 Methodology

The research focused primarily on the analysis of Cannabis products accessible on the local market. The analytical data was then used to draw informed conclusions on the quality traits of Cannabis products and devise tools to inform regulation. The project was approached in three phases.

Phase 1: Method development

Development and validation of analytical techniques. The development and validation plan was designed on the recommendations of the ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories[13]and the ICH Q2(R1) Scientific guidelines for the validation of analytical procedures[14].

The performance characteristics evaluated during method validation were Precision (Repeatability and Within-lab Reproducibility), Trueness (Bias, Duplicate Bias, residual bias, and selectivity), Measurement Range (Linearity, Limit of detection and Limit of quantification), uncertainty of measurement, sample stability and carryover.

[13] International Organization for Standardization. (2017). General requirements for the competence of testing and calibration laboratories (ISO/IEC Standard No. 17025). Retrieved from https://www.iso.org/obp/ui/#iso:std:iso-iec:17025:ed-3:v1:en

[14] International Council for Harmonisation (2005). Validation of analytical procedures (Harmonised Tripartite Guideline. Q2(R1)) Retrieved from https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf

Phase 2: Analysis of Cannabis products

Sample types analysed are detailed in section 2.2.2, this included cannabis products and dosage forms originating from suppliers authorized to place the respective products on the local market, products from the local market and products seized by local authorities claiming to contain cannabis or extracts of cannabis. The sample preparation and analytical procedure followed is shown in section 2.2 below.

Phase 3: Data analysis and interpretation

Analytical data was analysed using qualitative and quantitative metrics in order to substantiate the research outcomes of the study with the objective of identifying how analytical data can impact the regulatory sciences.

2.2 Methodology: Analysis of Cannabis products using GC-MS

The technique of choice for the analysis of cannabinoids in this research was based on GC-MS. The methodology reflects the use of this technique, including details on reagent and sample preparation steps as well as aspects related to the analytical methodology.

2.2.1 Materials and preparation of reagents

Reference stand solutions were used during the initial establishment of the chromatographic and mass spectrometric conditions and as known quantity samples during method validation. The standards purchased, listed in table 2.1 were certified reference materials, manufactured in accordance with ISO17034, to ensure the reliability and accuracy of the reference standards and the working solutions used. All reference material and standard working solutions were supplied by LGC standards (Wesel, Germany) and stored at -20ºC.

Chemicals and reagents

Chloroform, isopropanol, acetone, and methanol were purchased from Sigma-Aldrich (Supplied by LEVO laboratories, Malta). All solvents were HPLC grade. Diphenylamine (Analytical standard Grade) was purchased from Supelco (Supplied by LEVO laboratories, Malta).

Table 2.1: Certified reference standards (CRM)	
Reference material component	**Concentration and Solvent**
Δ9-THC	5mg/ml in Methanol
CBN	5mg/ml in Methanol
CBD	5mg/ml in Methanol
Alpha-Pinene	2500 ug/ml in Hexane
Beta-Pinene	2500 ug/ml in Hexane
3,7-dimethyl-1,3,6-octatriene	2500 ug/ml in Hexane
4-Isopropyltolun (Cymene)	2500 ug/ml in Hexane
Delta-Limonene	2500 ug/ml in Hexane
3-Carene	2500 ug/ml in Hexane
Camphene	2500 ug/ml in Hexane
Alpha-terpinene	2500 ug/ml in Hexane
Gamma-Terpinene	2500 ug/ml in Hexane
Alpha-terpinolene	2500 ug/ml in Hexane
Linalool	2500 ug/ml in Hexane
Isopulegol	2500 ug/ml in Hexane
Geraniol	2500 ug/ml in Hexane
Trans-Caryophyllene	2500 ug/ml in Hexane
Alpha-humulene	2500 ug/ml in Hexane
Myrcene	2500 ug/ml in Hexane
Nerolidol	2500 ug/ml in Hexane
Guaiol	2500 ug/ml in Hexane
Alpha-bisbolol	2500 ug/ml in Hexane

Preparation of the internal standard extracting solution

The extractant solution contained 0.2 mg/ml of Diphenylamine, that was used as an internal standard. The stock solution was prepared by adding 250 mg of Diphenylamine to a 250 ml volumetric flask. The flask was topped up with a mix of chloroform and methanol (1:9, V:V). The 1.0mg/ml stock was then diluted 5-fold with the same solvent mix to a final concentration of 0.2mg/ml.

Preparation of working standard solution

Individual methanolic stock solutions containing 5 mg/mL of Δ9-THC, CBN and CBD were used to prepare a working mixture of standards, at a concentration of 0.5mg/ml by diluting the standard solutions 10 fold using the internal standard solution containing 0.2 mg/ml of Diphenylamine. The Cannabinoid standard mix was labelled as CN-Mix, and this was stored at -20°C.

CRM solutions containing 2500 ug/ml of Terpenes in Hexane were used to prepare a stock solution of 100 ug/ml by diluting the standard solutions 25 fold using the internal standard solution containing 0.2 mg/ml of Diphenylamine. this was labelled as TERP-Mix, and was stores at -20°C.

2.2.2 Sample collection

The sampling plan for method validation and evaluation was based on the collection of samples, reflecting the products available on the local markets and other products collected from seizures of Cannabis products in Malta. The diversity of product types and dosage forms reflected the products available on the local market.

The samples analysed included:

- Cannabis flowers obtained from suppliers authorized to place the respective products on the local market.
- Extracts from Cannabis flowers in MCT oil obtained from samples at the BioDNA lab using a convenience sampling methodology.
- Other dosage forms such as lollypops, beverages, and gum were obtained from forensic samples.
- Products made available from the local market claiming to contain ‘Cannabis’ or ‘CBD’.

Table 2.2: sample types and products analysed			
Product type		**Sample state at room temperature**	**Number of samples tested**
Inflorescence	Cannabis inflorescence	Solid	112
Other herbal material	Granulate	Solid	9
	Cannabis trim (sugar leaves and fan leaves)	Solid	5
Pre-rolled smoking products	Coarse ground herbal cannabis material rolled in smoking paper.	Solid	28
Resin	Solidified hard resin extracted from cannabis inflorescence	Solid	40
	Oleoresin solidified soft resin extracted from cannabis inflorescence.	Solid	17
Cannabis plants	Whole cannabis plants	Solid	16
Extract in MCT oil	Cannabis extracts diluted in Medium-chain Triglycerides	Liquid	11
Extract in Oilve oil	Cannabis extracts diluted in Oilve oil	Liquid	6
Terpene concentrate	Cannabis volatiles concentrated in an unspecified oil	Liquid	14
Vaping liquid	Cannabis extracts in Propylene/ ethylene glycol	Liquid	36

2.2.3 Sample preparation and evaluation of extraction efficiency

The sample preparation procedures were adjusted to accommodate the diverse group of sample types used in this study based on the physical characteristics of the product. Therefore, two procedures were established to cater for the different states of the sample matrices.

Sample preparation for solid sample matrices

A 0.5g sample was collected from the plant or herbal materials. The samples were homogenised using a pastel and mortar. Samples containing fresh herbal materials were air dried before sampling and homogenisation.
For quantitative analysis, a 10mg of plant material were used.

The sample was prepared in duplicate by weighing the exact amount of homogenised plant material (9.0-11.0mg) followed by the addition of the extractant internal standard solution (900-1100 µL), to prepare a 10mg/ml solution. The samples were then vortexed for 30 seconds and allowed to stand for an additional 5 minutes.

The 10mg/ml extract was then diluted 10-fold, with the internal standard solution and transferred to a 2.5ml vial, fitted with a septum cap.

The sample preparation and extraction were used for the analysis of plant material for the two analytical methods (Analysis of Cannabinoids and Terpenes) using separate injections. 1µL of the extract was injected in a GC-MS using an automatic liquid sampler.

Sample preparation for liquid sample matrices (oils)

Oil samples included extracts in olive oil, extracts in MCT oil and vaporiser liquids. Samples were processed by taking 10 µL of the sample in 1mL of internal standard solution.

The samples were then homogenised by vortexing for 30 seconds and making sure that the sample had dissolved completely in the solvent. Before injection, a 100 µL aliquot was diluted 10-fold with internal standard solution.

The sample preparation and extraction were used for the analysis of Cannabinoids. 1µL of the extract was injected in a GC-MS using an automatic liquid sampler.

Evaluation of extraction efficiency

The extraction efficiency of the methods described were evaluated for extraction efficiency, to determine the ability of the procedure to extract cannabinoids and terpenes from solid and liquid matrices.

The procedure involved the use of blank solid and liquid matrices spiked with a known amount of the analyte of interest using the cannabinoid mix or terpene mix working standard solutions, that were processed and quantified using the developed methods.

The results were compared to blank samples that were spiked with the analyte of interest after the extraction procedure to act as a point of reference as 100% extraction efficiency.

The relative deviation of the concentration of the analytes in the samples spiked before the extraction procedure was calculated against samples spiked after the extraction procedure, to calculate the amount of analyte that is left behind in the sample matrix during the extraction procedure.

For this evaluation 3 samples of each selected matrix were spiked before extraction and three samples were spiked after extraction (N=36), the selected matrices were blank surrogate materials (blanked ground hemp, blanked hemp resin, MCT oil, Olive oil and glycol).

2.2.4 Chromatography and MS analysis

The samples were analysed on an Agilent 7890 gas chromatograph coupled with an Agilent 5975C quadrupole mass detector (Agilent Technologies, California, USA) operating at 70 eV in electron ionisation mode. A J&W 5% phenyl-methylsilicone capillary column (30m x 0.25mm i.d., 0.25µm film thickness, Labopharm, Malta) was installed on the GC.

Chromatographic conditions

For the Analysis of Cannabinoids, Helium was used as carrier gas at a constant flow of 1 mL/min. The GC oven temperature was held at 130°C for 2 min, then increased to 270°C at 15°C/min, followed by a final temperature ramp at 50°C/min, to 310°C (held for 4 min). The injection port was set at 270°C in splitless mode. The mass detector was operated in scan mode (scan range from m/z 50 to 550).

For the analysis of Terpenes, Helium was used as carrier gas at a constant flow of 1 mL/min. The GC oven temperature was held at 40°C for 1 min, then increased to 100°C at 5°C/min, ramped to 240°C at 15°C/min, this temperature was held for 2 minutes, followed by a final temperature of 310°C , reached at a rate of 30°C/min, which was held for 1 minute. The injection port was set at 250°C in pulsed-split mode, with a pulse pressure of 25psi for 0.5 minutes. The mass detector was operated in scan mode (scan range from m/z 40 to 550).

2.3 Method performance characteristics

Accurate and reliable test results are dependent on the use of appropriately validated test methods. To ascertain the reliability and robustness of results in this study, the validation and the implementation of the developed method was based on the the ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories[15] and the ICH Q2(R1) scientific guidelines for the validation of analytical procedures [16].

The criteria shown in table 2.3 were used for all methods developed and validated in this study.

[15] International Organization for Standardization. (2017). General requirements for the competence of testing and calibration laboratories (ISO/IEC Standard No. 17025). Retrieved from https://www.iso.org/obp/ui/#iso:std:iso-iec:17025:ed-3:v1:en

[16] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1). Current Step 4 version dated 27 October 1994, incorporated in November 2005

Table2.3: Summarizing the method performance characteristics used for method validation.

Parameter		Demand
Precision	Repeatability	RSD_r 10 %
	Within-lab reproducibility	RSD_{Rw} 10%
Trueness	Test for bias	Bias < 10 % relative
	Duplicate Bias	<10%
	Residual Bias	<3%
	Selectivity	Test for possible interference
Measurement range	LOD	<0.2%
	LOQ	>LOD≤0.2% W:W
	Linearity	Residual max deviation 10 % relative R^2 >0.99
Measurement uncertainty		Expanded uncertainty < 25 % relative
Sample Stability		24H at room temperature
Carryover		No carryover

2.4 Precision

The method repeatability (RSDr) and within lab reproducibility (RSDrw), were used to evaluate method precision. For repeatability, 10 individually prepared samples (in duplicate) of Mixed analyte standard were analysed on the same day using the same instrument. This was repeated at 3 concentration levels (N= 30 samples in duplicate). The selected levels for Cannabinoids were High (35%) Medium (20%) and Low (5%), whilst for Terpene analysis the selected levels were High (50µg/ml) Medium (25µg/ml) and Low (5µg/ml).

The method repeatability was deemed satisfactory if the %CV was <10%. RSDr, expressed as the percentage coefficient of variation (%CV), was calculated form the sample mean (µ) and the standard deviation (σ)

Reproducibility (RSDrw), was determined by the analysis of 54 duplicate samples, prepared over a period of 3 consecutive days. 6 samples were prepared for each level, High (35% / 50µg/ml) Medium (20% and 25µg/ml) and Low (5% and 5µg/ml) for cannabinoid assays and Terpene compounds, respectively on each day.

Reproducibility, was evaluated using ANOVA (Single factor) $\alpha=0.05$. Where the null hypothesis (HO: That there is no significant difference between the groups of data.) was accepted if the P-Value was > α.

The RSDrw was then be calculated by using the data generated from ANOVA, were the RSDr is the percentage root of the mean square (MS) within groups. This was followed by the between group standard deviation RSDX to calculate RSDrw.

2.5 Bias

The deviation of the data from the reference value, was evaluated at several points of the procedure (Laboratory, sample and duplicate Bias). The deviation was expressed as percentage error (%E) calculated as a percentage deviation from the expected concentration.

During method validation, the laboratory, sample, and duplicate bias was deemed satisfactory when the relative %E was < 10 %.

Test for bias

Method bias was evaluated at Low, Medium, and High concentrations for both cannabinoids and terpenes, by analysing 10 samples of each concentration, individually prepared in duplicate (20 samples for Cannabinoid assays and 20 samples for Terpene assays).

The samples were analysed on the same day on the same instrument.

Duplicate Bias

Duplicate bias was evaluated by preparing 7 duplicate samples, that were individually weighed, and processed. For Cannabinoids, the 7 samples were taken from a reference sample containing 6.5% CBD, 18% THC and 5.5% CBN. For Terpenes, a mixed standard sample was prepared using a mixed standard containing 15 µg/ml of each Terpene compound being evaluated.

Residual Bias

The calibration standard residual bias was calculated using the calibration standards by calculating the %E as a deviation of calibration standards from the calibration curve.

2.6 Target identification and selectivity

Identification of the target compounds was based on the ability of the method workflow to correctly identify the target analytes and the internal standard in mixed extract solutions. 20 samples containing the target analytes (Cannabinoids and terpenes) and 10 blank samples containing the internal standard (Diphenylamine) were analysed.

The identification of analytes in the extracted samples was based on the chromatographic separation of the analytes resulting in specific retention times, and the identification of qualifier ions in the relative mass spectra emerging from mass fragmentation at the analyte specific retention time.

The mass spectra were cross referenced with a mass-spectral databases (Cayman Spectral Library[17] and ENFSI DWG MS library[18]). A mass fragment spectrum match was identified by using the Library match score (Overall score >85).

[17] Cayman Chemical – Mass spectral library in Agilent MSD Format –Version V21022022. Downloaded from https://www.caymanchem.com/forensics/publications/csl

[18] European Network of forensic science institute -Drugs working group Mass spectral library in Agilent MSD Format (Version: 2022.05). Downloaded from https://enfsi.eu/about-enfsi/structure/working-groups/drugs/

Method selectivity was also assessed using the same samples. The method was deemed to have good selectivity if there was no co-elution of the compounds of interest with the solvent, internal standard or any other compounds encountered frequently in test samples.

2.7 Measuring Range

The measuring range of the method was based on the linearity of the calibration curve, and the suitability of the analytical method to quantify the analytes of interest.

Linearity and residual deviation

The linear relationship between the analyte signal and its concentration was studied by preparing spiked samples containing the analytes of interest at the following concentrations;

Cannabinoid concentration (w:w%): 40. 30, 20, 10, 5, 1, 0.5 and 0.1%.
Terpene Concentration (concentration); 50, 40, 30, 25, 10, 5, 1 and 0.5 µg/ml

The 8 calibration levels were prepared and analysed in triplicate. A calibration curve was set up using the acquired data points. Calibration points with sample bias of <20% were accepted and included in the calibration plot, a minimum two data points were included for each calibration level.

The linearity parameter for each calibration curve was satisfied if the Correlation coefficient (R^2) exceeded 0.99.

The residual deviation of the calibration curve was assessed by calculating the deviation of the calibration standards against the calibration curve of the method. An independently prepared set of calibration standards were prepared and analysed. Where the % deviation of the calibration standard.

Working range

The Limits of Detection and Quantification and the Upper limit of Quantification were evaluated by analysing spiked samples at low (0.1-1.0–% for cannabinoids and 0.1-1.0µg/ml for terpenes) and high (30-50% for cannabinoids and 30-50µg/ml for terpenes) concentrations. Once the limit was identified, a minimum of 3 samples were analysed at the proposed concentration. The limit was satisfactory if the analyte response is reproducible and has a signal to noise (S/N) ratio is > 10 for the limits of Quantification. The S/N ratio for the limit of detection was >3.

The lower and higher quantification limits were established at the lowest (LOQ) and Highest concentration that could be confidently quantified (ULOQ), that is when E% and CV% are below 20%.

The Limits of detection (LOD) of the analytes were established at a concentration value giving a S/N >3 for three qualifier ions at a concentration were the precision and bias parameters are satisfied.

2.8 Carry over

To assess sample **Carryover** between subsequent injections, blank samples (n=6) containing the internal standard were injected immediately after control samples containing excess amount of the analytes of interest.

Three spiked solutions containing DPA as an internal standard were prepared. The Cannabinoid mix was tested at a concentration of 75% and 85% (N=6). The terpene mix was tested at a concentration of 70 µg/ml and 80 µg/ml (N=6). Each sample was followed by a blank sample (N=12).

The Carryover of any of the analytes of interest was determined by the presence of qualifier ions from the analytes of interest at the target retention time with a S/N >3.

2.9 Sample stability

The stability of the samples prepared was assessed by preparing identical samples containing the analyte of interest. These samples were then tested immediately after preparation (T=0) and at intervals of 6, 12, 24, 36 and 48 hours. Samples were stored at room temperature (25 ±2°C) in the Autosampler between intervals.

The relative stability was assessed by quantifying changes in the peak area of the analyte over the 48-hour period. Where the relative peak area (%PA) was expressed as the percentage change in the peak area of the analyte.

The analyte was considered as stable in the sample matrix if the relative peak area of the sample remained above 90%.

2.10 Uncertainty of Measurement

The data obtained from validation samples was used to calculate the Uncertainty of Measurement UoM. The Estimation of measurement uncertainty based on validation and quality control data applied to the developed methods was based on the ISO 11352[19] and Nordtest 537 guidelines[20].

The measurand was defined as the quantity of analyte in the sample matrix. Whilst the principal sources of uncertainty in the procedure were the uncertainty in pipetting, method bias, the calibration curve and the response of the instrument.

The sources of uncertainty for sample preparation related to Deviation in pipetting and sample weighing were given a quantitative value from deviations identified during equipment calibrations. Signal and analytical components of uncertainty were derived from the uncertainty of the certified reference standards used, and the previously determined method bias.

The standard uncertainty Uc was calculated as the root of the sum of squares of the uncertainty components in the procedure. The expanded uncertainty (U) was then calculated to account for the distribution of values which could be reasonably attributed to the measurand. The coverage factor (k) chosen for this analytical method was 2.

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[19] International Standards Organisation. Standard 11352:2012, Water quality — Estimation of measurement uncertainty based on validation and quality control data, International Organization for Standardization, 2012.
[20] Nordtest, *Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories (NT TR 537 – Edition 4)*, Nordtest, 2017.

Since the relative uncertainty varies with concentration levels, the expanded uncertainty was calculated at Low, Medium, and High analyte concentrations in order to account for uncertainty of measurement throughout the working range. The selected levels for Cannabinoids were 35% (High), 20% (Medium) and 5% (Low), whilst for Terpene analysis the selected levels were 50µg/ml (High), 25µg/ml (Medium) and 5µg/ml (Low).

Chapter 3
Results

3.1 Samples analysed for validation

The sampling plan for method validation and evaluation was based on the collection of samples, reflecting the products available on the local markets and other products collected from seizures of Cannabis products in Malta.
The samples analysed included:

- Cannabis flowers obtained from suppliers authorized to place the respective products on the local market.
- Extracts from Cannabis flowers in MCT oil obtained from samples at using a convenience sampling methodology.
- Products made available from the local market claiming to contain 'Cannabis' or 'CBD'.

Details of the samples analysed, including their respective sources, are provided in the sample registration log (Appendix 5).

3.2 Sample preparation

The extraction efficiency (%EF) of the developed sample preparation procedures for the quantification of THC, CBD and CBN in liquid and solid sample matrices is shown in table 3.2 below.

For this evaluation three samples of each selected matrix were spiked before extraction and three samples were spiked after extraction, the selected matrices were blank surrogate materials blanked ground hemp, blanked hemp resin, MCT oil, Olive oil and glycol.

Table 3.1: Extraction efficiency for Cannabinoids and terpenes in solid and liquid sample matrices						
Group	Analyte	Solid sample matrices		Liquid sample matrices		
		Ground hemp	Hemp resin	MCT oil	Olive oil	Vape liquid
Cannabinoids	THC	93.4	98.2	96.7	96.3	99.3
	CBD	98.6	98.7	97.5	98.8	97.3
	CBN	97.6	95.9	97.2	94.9	96.6
Terpenes	A-Pinene	97.6	93.9	94.6	95.2	95.6
	Beta-Pinene	94.3	98.3	98.8	94.2	94.3
	3,7-dimethyl-1,3,6-octatriene	97.5	98.3	97.5	95.9	94.2
	4-Isopropyltolune	96.4	96.5	96.3	95.7	98.5
	D-Limonene	97.4	93.5	96.1	98.9	96.6
	3-Carene	96.1	96.7	97.4	98.8	94.5
	Camphene	95.3	96.4	94.1	97.5	95.4
	Alpha-terpinene	97.3	97.9	96.5	96.9	94.2
	G-Terpinene	94.9	98.6	94.8	97.3	96.8
	A-terpinolene	97.2	98.0	97.2	97.2	96.9
	Linalool	98.9	96.6	99.1	96.2	98.2
	Isopulegol	99.5	95.0	98.1	94.3	94.7
	Geraniol	98.8	94.2	98.2	99.4	98.4
	Trans-Caryophyllene	97.7	97.8	95.5	98.3	96.1
	A-humulene	95.2	97.6	95.1	93.8	99.7
	Myrcene	94.1	93.5	97.8	98.5	95.3
	Nerolidol	98.2	96.1	98.2	96.9	99.4
	Guaiol	99.1	95.2	98.1	93.7	94.8
	Alpha-bisbolol	98.7	99.2	99.1	97.6	95.8

3.3 Chromatography and MS analysis

3.3.1 Chromatography

The samples were analysed on an Agilent 7890 gas chromatograph coupled with an Agilent 5975C quadrupole mass detector, the separation of cannabinoids of interest and the internal standard, are shown in the chromatogram (Figure 3.1).

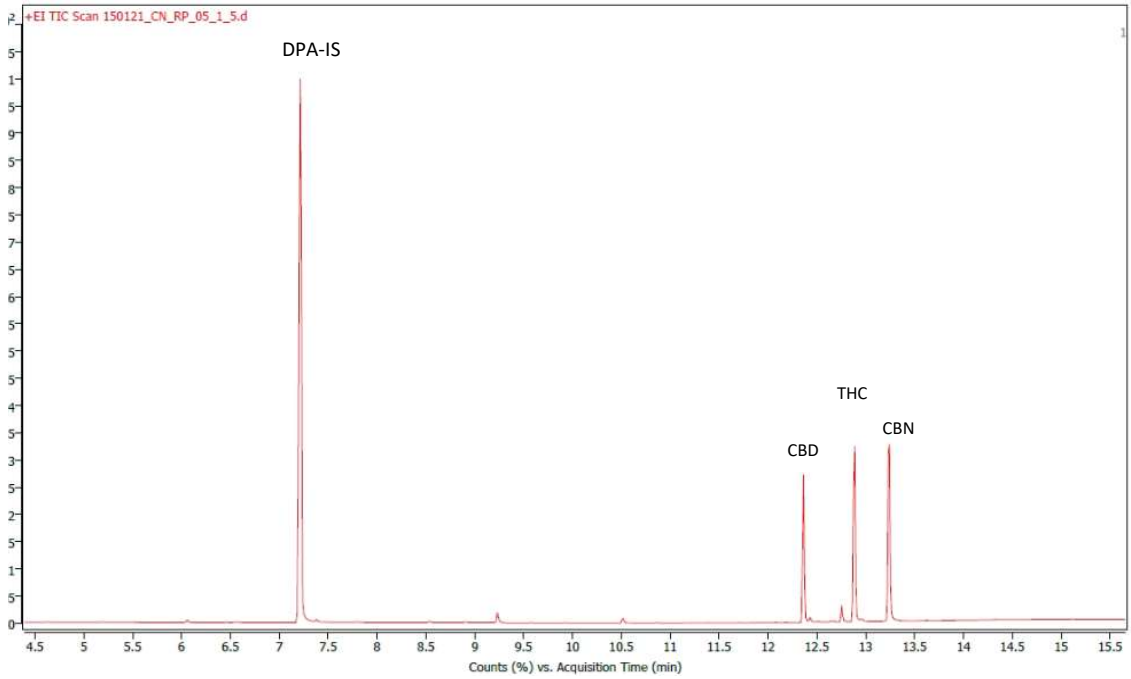

Figure 3.1: GC chromatogram of a spiked sample containing 0.05 mg/ml of THC, CBD and CBN, extracted in the extractant solvent with 0.2 mg/ml of Diphenylamine as an internal standard.

Terpene compounds were analysed using a separate chromatographic program described in section 2.2. The chromatographic separation of ninteen Terpene compounds using GC-MS is shown in Figure 2.

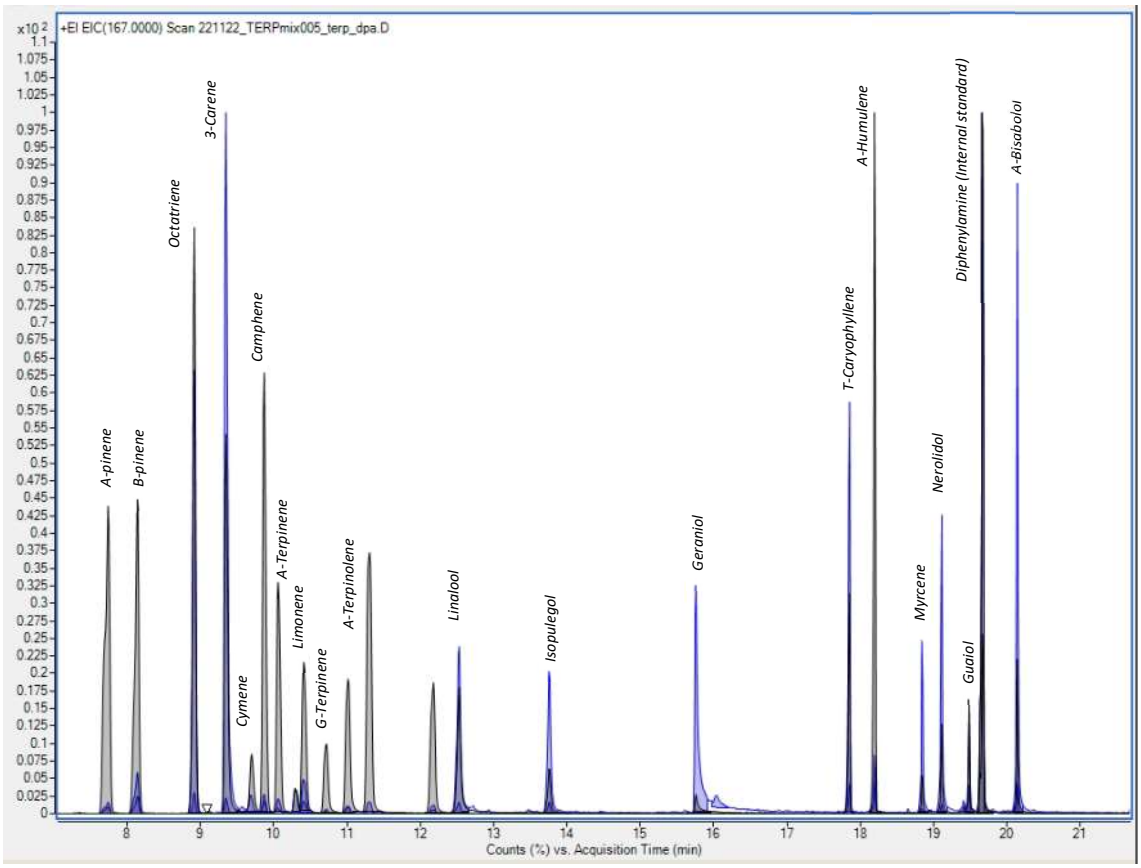

Figure 3.2: GC chromatogram of a spiked sample containing 10 µg/ml of *Alpha-Pinene, Beta-Pinene 3,7-dimethyl-1,3,6-octatriene, 4-Isopropyltolun (Cymene), Delta-Limonene, 3-Carene, Camphene, Alpha-terpinene, Gamma-Terpinene, Alpha-terpinolene, Linalool, Isopulegol, Geraniol, Trans-Caryophyllene, Alpha-humulene, Myrcene, Nerolidol, Guaiol and Alpha-bisbolol* in a solution containing 0.2 mg/ml of Diphenylamine as an internal standard.

3.3.2 **Analyte identification**

The results for target analyte identification included the annotation of specific retention times from chromatograms, the identification of Qualifier ions from mass spectra and the cross-referencing score of the mass spectra with the spectral databases. The analyte identification results for cannabinoids and Terpenes are shown in Tables 3.2 and 3.3 below.

Table 3.2: Target analyte identification criteria for Cannabinoids

Analyte	Retention time (min)	Qualifier ions	Library match score	*Accuracy (%)*
DPA (*Internal standard*)	7.22	169, 168, 167	97.28	100%
THC	12.36	299, 231, 314, 271	96.45	100%
CBD	12.88	231, 232, 246, 314	98.23	100%
CBN	13.24	295, 296, 310, 238	97.84	100%

Table 3.3: Target analyte identification criteria for Terpenes

Analyte	Retention time (min)	Qualifier ions (m/z)	Library match score	*Identification Accuracy %*
Diphenylamine (Internal standard)	19.63	167, 168, 167	99.9	100
Alpha-Pinene	7.74	93, 91, 77, 79, 105	92.8	100
Beta-Pinene	8.137	93, 91, 77, 79, 69	89.4	100
3,7-dimethyl-1,3,6-octatriene	8.918	93, 91, 77, 79, 105	93.2	100
3-Carene	9.69	93, 121, 79, 91, 107	94.5	100
Camphene	9.86	119, 91, 120, 117	90.8	100
Alpha-terpinene	10.07	121, 93, 91, 136, 77	92.3	100
Para-Cymene	10.29	119,91,120,117	92.2	100
Delta-Limonene	10.34	68, 93, 107, 67, 94	92.5	100
Gamma-Terpinene	10.71	93, 136, 121, 91, 77	89.5	100
Alpha-terpinolene	11.01	93, 121, 136, 91, 77	90.3	100
Linalool	12.53	71, 81, 84, 95, 121	94.2	100
Isopulegol	13.75	71, 93, 55, 69, 80	93.4	100
Geraniol	15.75	69, 93, 121, 84, 111	93.6	100
Trans-Caryophyllene	17.85	93, 69, 79, 133, 41	95.6	100
Alpha-humulene	18.19	93, 80, 121, 147, 204	94.7	100
Myrcene	18.84	41, 69, 93, 53, 79	91.4	100
Nerolidol	19.11	41, 69, 93, 71, 107	91.4	100
Guaiol	19.48	161, 59, 107, 189, 204	90.5	100
Alpha-bisabolol	20.14	93, 91, 77, 79, 105	95.7	100

3.3.3 **Mass spectral data**

During the data acquisition, the mass detector was operated in scan mode (scan range from m/z 50 to 550). Reference spectra for the analytes of interest (Cannabinoids and Terpenes) were extracted from the mass fragmentation data at the specific retention time of the analytes. Cannabinoid mass spectra are shown in figure 3.3. An identical exercise was also done for terpene compounds, with mass fragmentation patters being shown in figures 3.4-3.7.

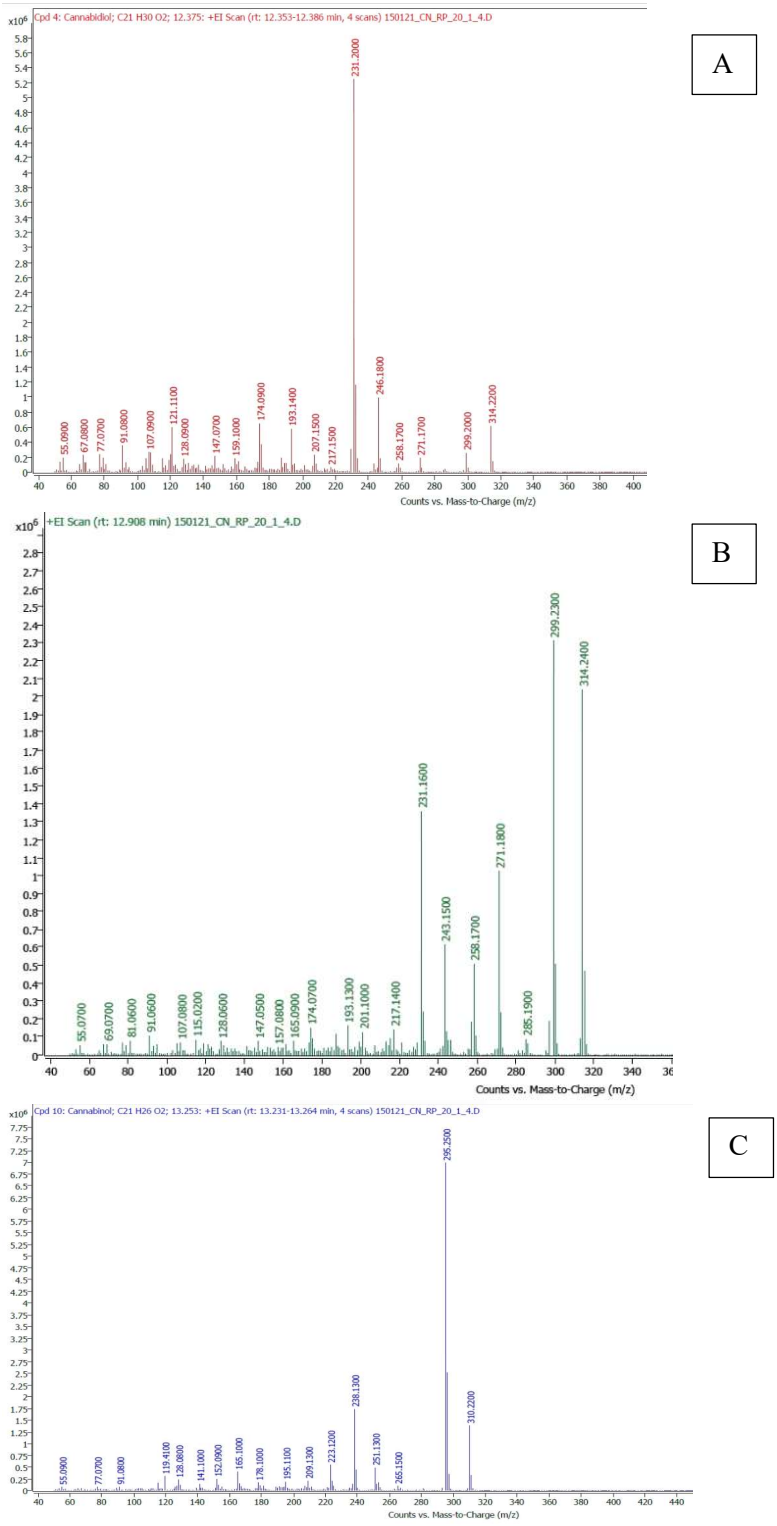

Figure 3.3: Mass spectrum for (A) Cannabinol (B) Tetrahydrocannabinol & (C) Cannabinol from a sample containing 0.2mg/ml of each analyte.

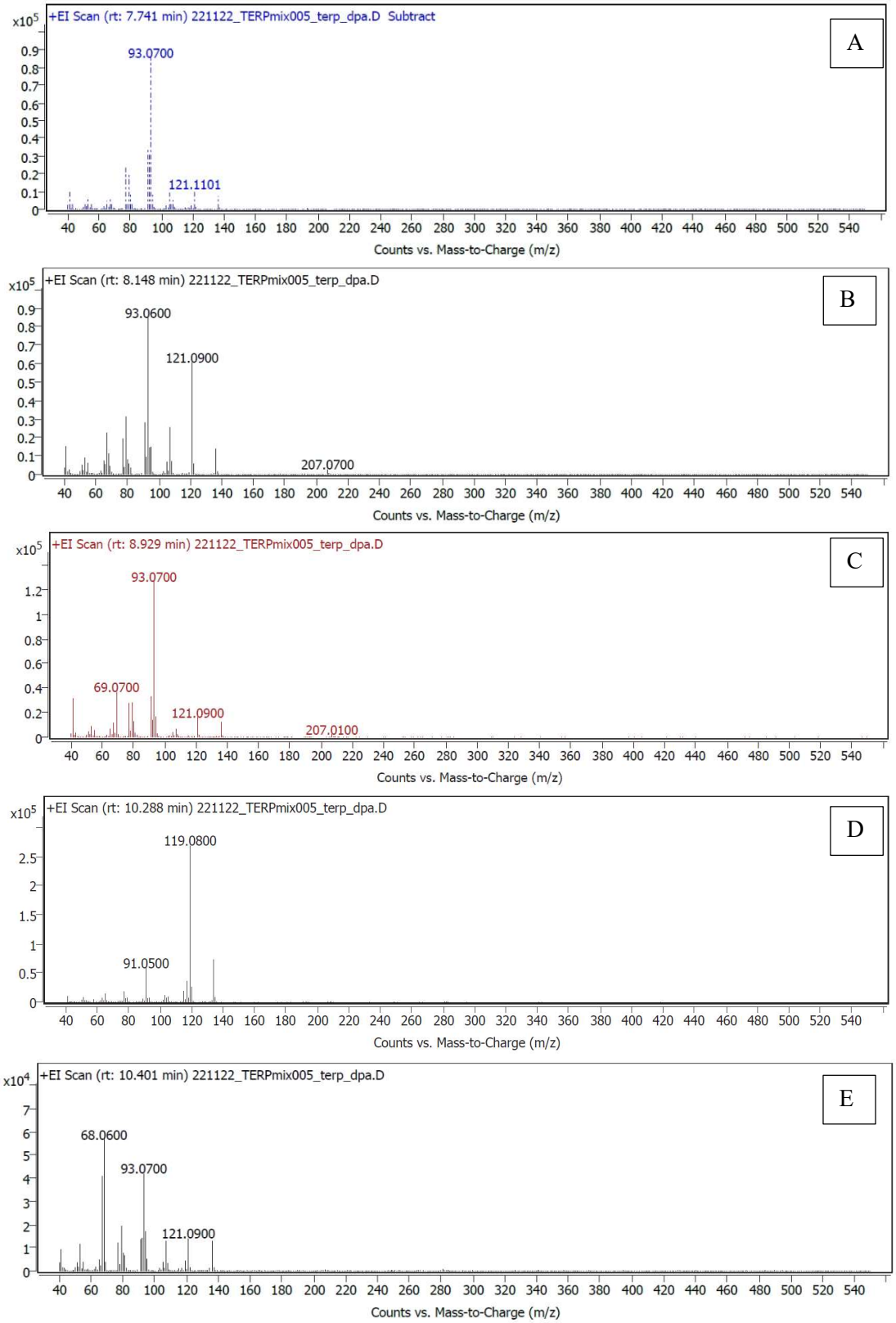

Figure 3.4: Mass spectral fragmentation for (A) Alpha-Pinene, (B) *Beta-Pinene,* (C) 3,7-dimethyl-1,3,6-octatriene, (D) Para-Cymene & (E) D-Limonene

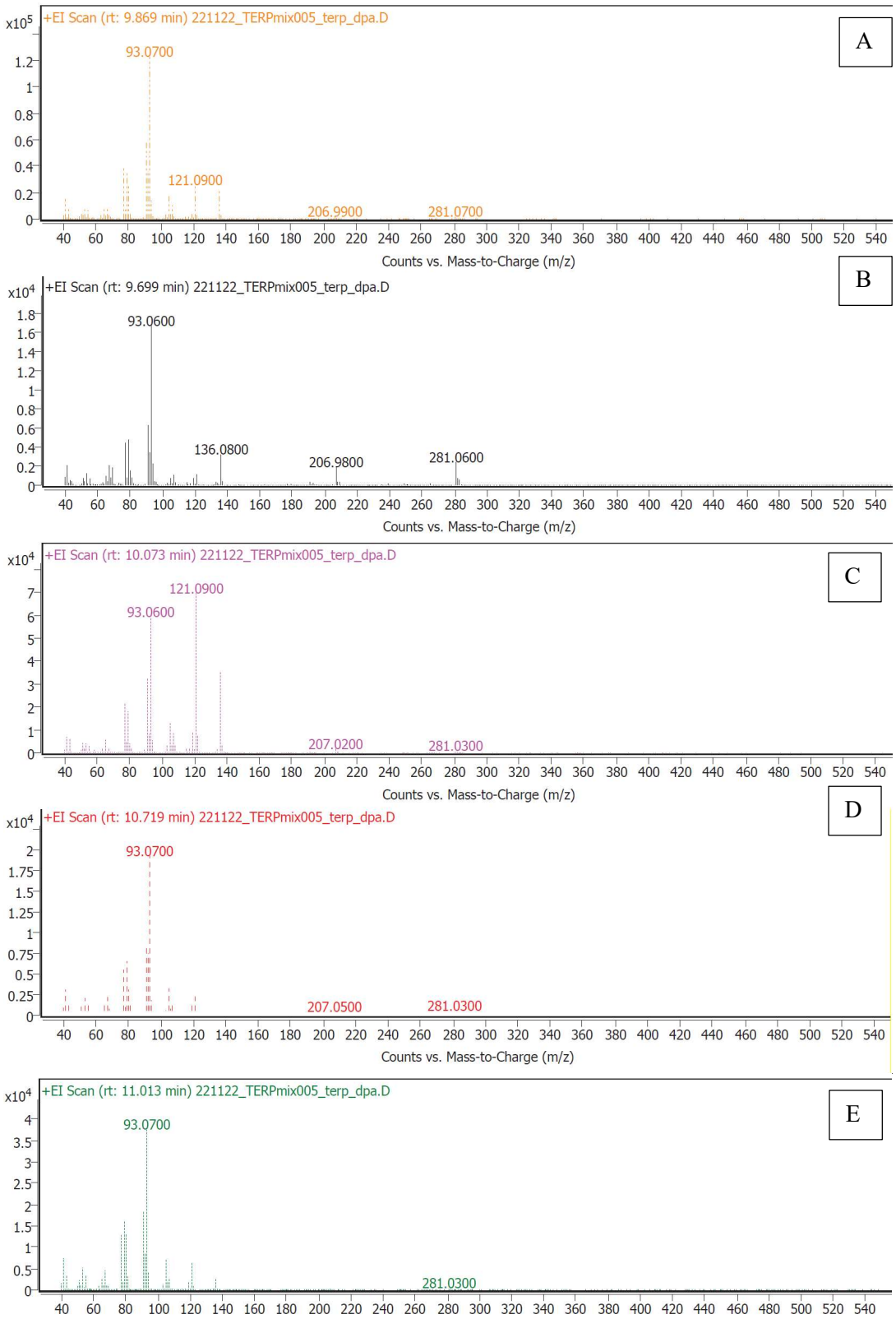

Figure 3.5: Mass spectrum for (A) 3-Carene, (B) Camphene, (C) *Alpha-terpinene, (D)* Gamma-Terpinene & *(E)* Alpha-terpinolene

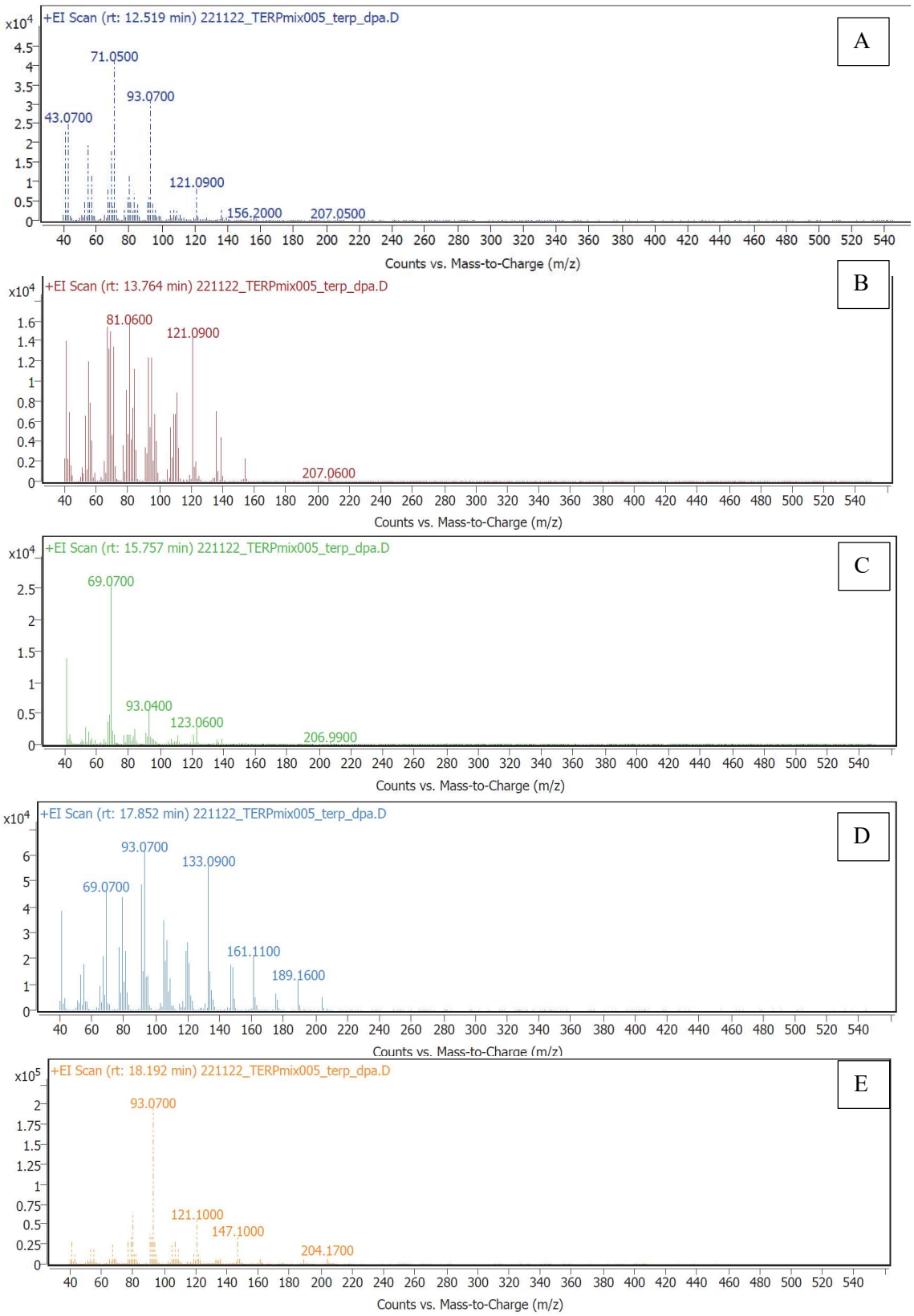

Figure 3.6: Mass spectrum for (A) Linalool, (B) Isopulegol (C) Geraniol, (D) Trans-Caryophyllene & (E) Alpha-humulene.

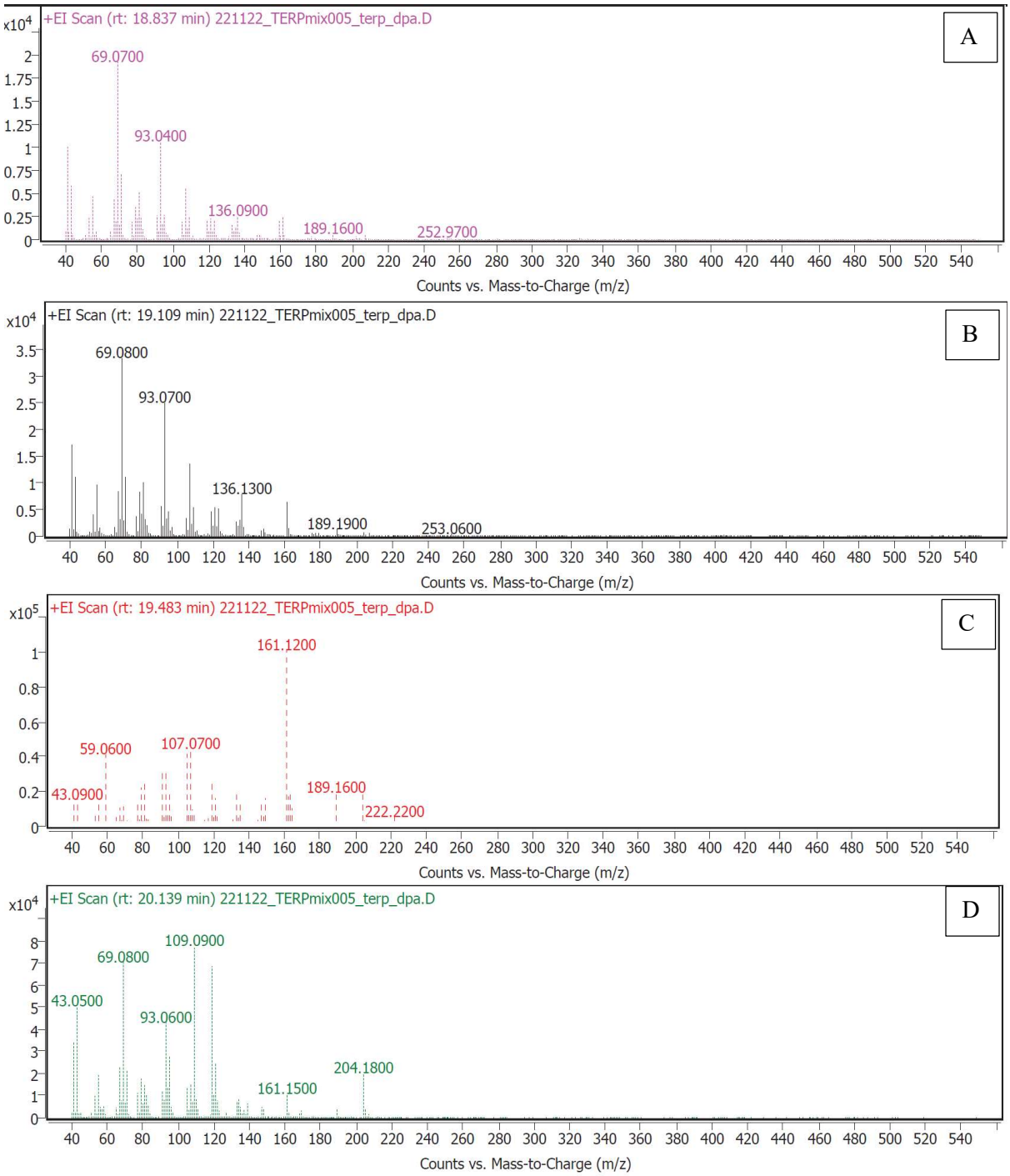

Figure 3.7: Mass spectrum for (A) Myrcene, (B) Nerolidol, (C) Guaiol & (D) Alpha-bisabolol

3.4 Linearity

The linearity parameters for cannabinoids are tabulated in table 3.4. The analytical method was linear with Corelation Coefficients for analytes exceeded 0.99, as shown in the calibration plots in figure. The linear range of the method was established to be within the calibrated concentration range.

32 samples were analysed to build the calibration plot. One of the samples did not fit the quality parameters of the study (duplicate bias exceeding 10%), and was not used for method calibration.

Table 3.4: Linearity parameters for Cannabinoids

Analyte	Calibration levels (data points)	Corelation Coefficient (R^2)	Linear Range	Linear equation
THC	8 (31)	0.998	0.001-0.40 mg/ml	*Y= 0.3702x+0.0012*
CBD	8 (31)	0.996	0.001-0.40 mg/ml	*Y=1.1370x+0.0109*
CBN	8 (31)	0.996	0.001-0.40 mg/ml	*Y = 1.6598x+0.0156*

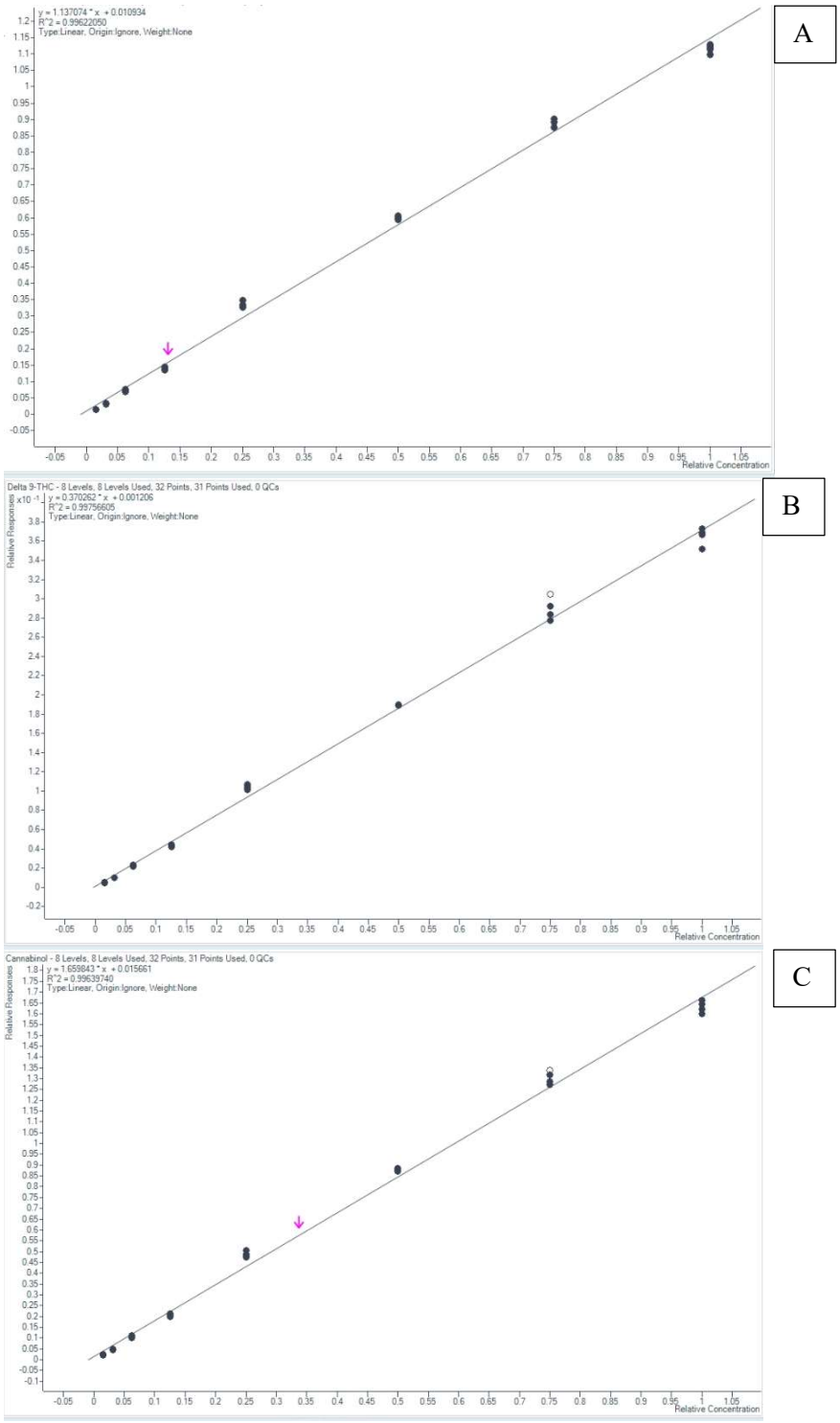

Figure 3.8: Calibration plots compiled from the injection of calibration solutions in quadruplicate containing (w:w%) 40. 30, 20, 10, 5, 1, 0.5 and 0.1%. of (A) Cannabinol (B) Tetrahydrocannabinol & (C) Cannabinol.

The linearity parameters for Terpenes are shown in table 3.5. The analytical method was linear with R^2 Values exceeding 0.99 for all compounds. The minimum number of data points per calibration level was one, from the thirty-two samples injected, the data from five samples was not integrated in the calibration plots shown in figures 3.9-3.13, as these data points showed duplicate bias that exceeded 10%.

Table 3.5: Linearity parameters for Terpenes

Analyte	Calibration levels (data points)	Corelation Coefficient (R^2)	Linear Range	Linear equation
Alpha-Pinene	8 (27)	*0.9994*	0.5-50µg/ml	*y = 0.7247x + 9E-05*
Beta-Pinene	8 (27)	*0.9992*	0.5-50µg/ml	*y = 0.7304x - 0.0036*
3,7-dimethyl-1,3,6- octatriene	8 (27)	*0.9992*	0.5-50µg/ml	*y = 0.8887x - 0.007*
Delta-Limonene	8 (27)	*0.9992*	0.5-50µg/ml	*y = 0.827x - 0.0041*
3-Carene	8 (27)	*0.9997*	0.5-50µg/ml	*y = 0.7906x - 0.0007*
Camphene	8 (27)	*0.9979*	0.5-50µg/ml	*y = 1.1703x - 0.0065*
Para-Cymene	8 (27)	*0.9977*	0.5-50µg/ml	*y = 1.7271x + 0.0223*
Alpha-terpinene	8 (27)	*0.9989*	0.5-50µg/ml	*y = 0.7525x + 0.0002*
Gamma-Terpinene	8 (27)	*0.9973*	0.5-50µg/ml	*y = 0.9489x +0.0074*
Alpha-terpinolene	8 (27)	*0.9992*	0.5-50µg/ml	*y = 0.9515x - 0.0047*
Isopulegol	8 (27)	*0.9992*	0.5-50µg/ml	*y = 0.8083x + 0.0045*
Linalool	8 (27)	*0.999*	0.5-50µg/ml	*y = 0.8349x - 0.0124*
Geraniol	8 (27)	*0.9989*	0.5-50µg/ml	*y = 1.2647x + 0.0022*
Trans-Caryophyllene	8 (27)	*0.9969*	0.5-50µg/ml	*y = 0.6404x - 0.0041*
Alpha-humulene	8 (27)	*0.9992*	0.5-50µg/ml	*y = 0.9515x - 0.0047*
Myrcene	8 (27)	*0.9992*	0.5-50µg/ml	*y = 0.9515x - 0.0047*
Nerolidol	8 (27)	*0.9985*	0.5-50µg/ml	*y = 1.0674x + 0.0018*
Guaiol	8 (27)	*0.9983*	0.5-50µg/ml	*y = 0.446x + 0.0002*
Alpha-bisabolol	8 (27)	*0.9992*	0.5-50µg/ml	*y = 0.9748x + 0.006*

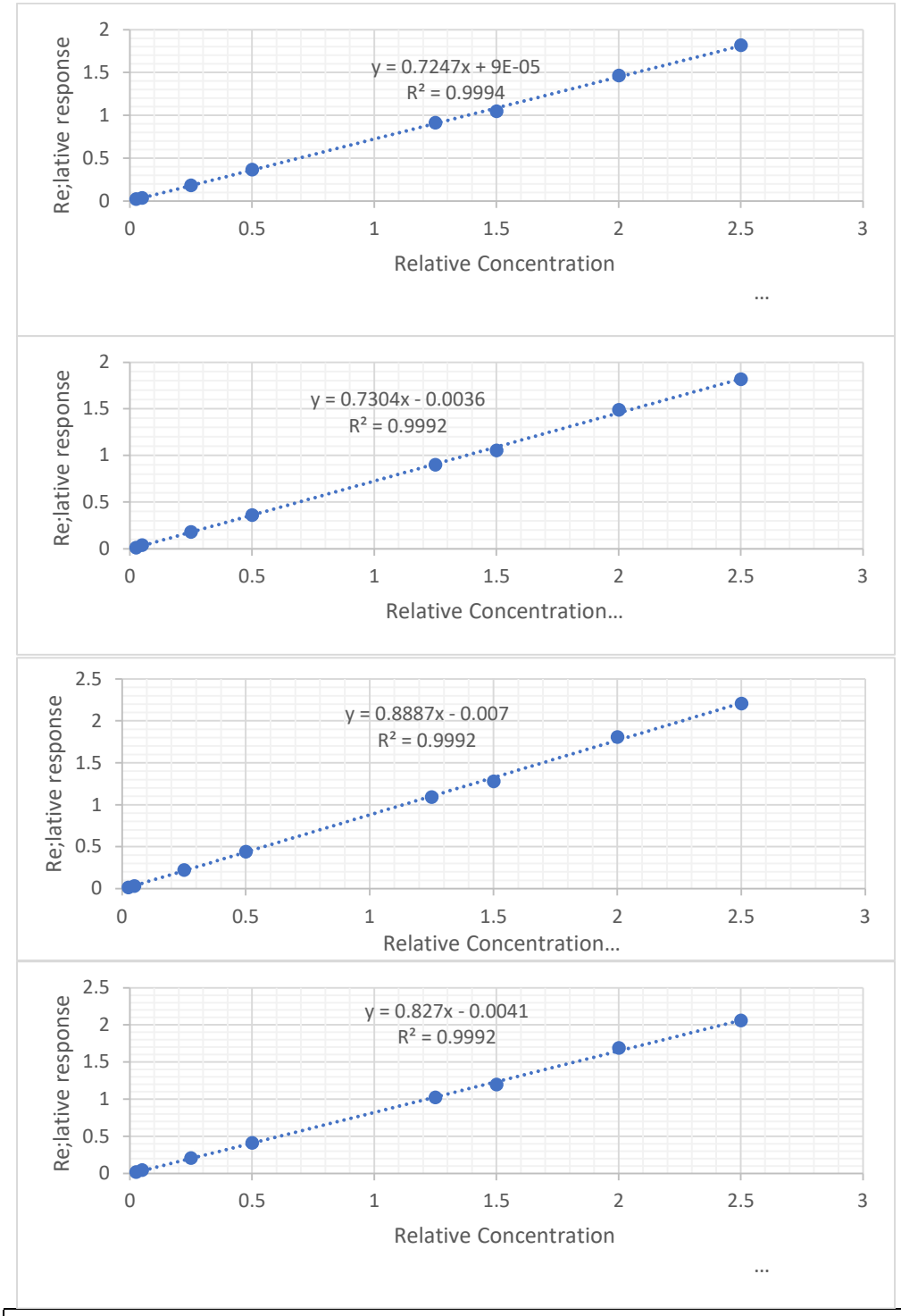

Figure 3.9: Calibration plots compiled from the injection of calibration solutions in quadruplicate containing (w:w%) 50, 40, 30, 25, 10, 5, 1 and 0.5 µg/ml of (A) Alpha-Pinene, (B) Beta-Pinene, (C) 3,7-dimethyl-1,3,6-octatriene & (D) d-Limonene

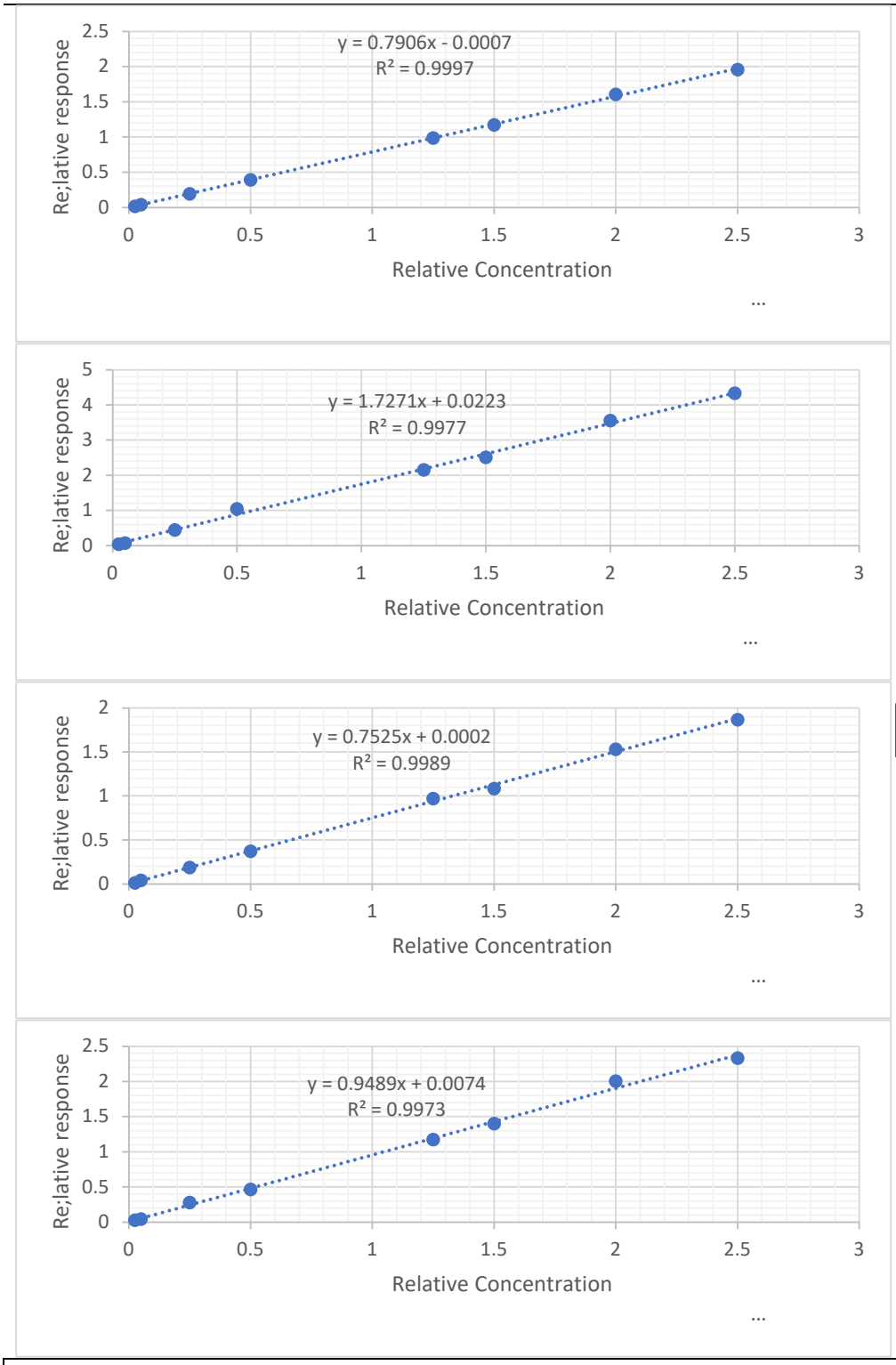

Figure 3.10: Calibration plots compiled from the injection of calibration solutions in quadruplicate containing (w:w%) 50, 40, 30, 25, 10, 5, 1 and 0.5 µg/ml of (A) 3-Carene, (B) Cymene, (C) Alpha-Terpinine & (D) Gamma-Terpinine

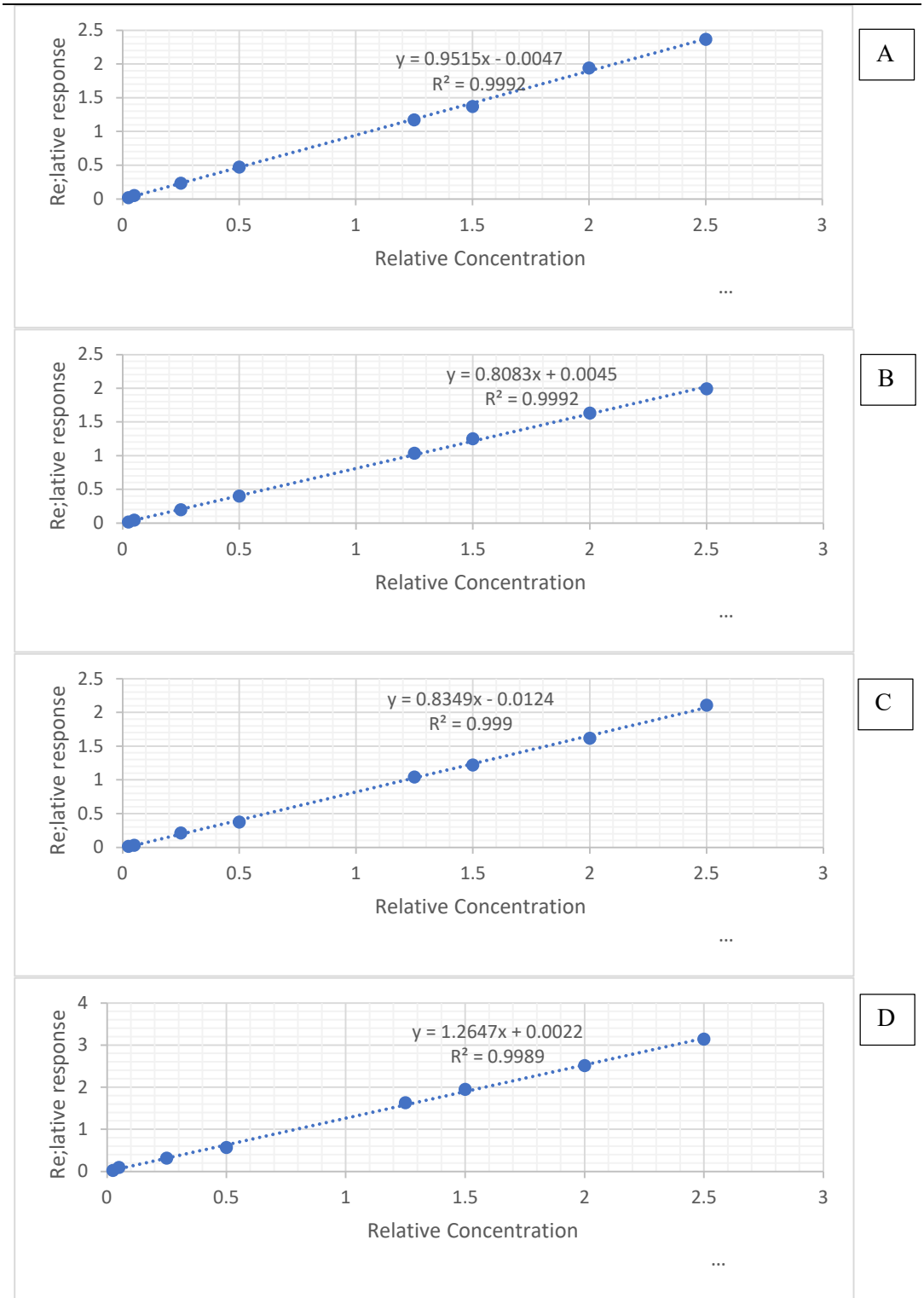

Figure 3.11: Calibration plots compiled from the injection of calibration solutions in quadruplicate containing (w:w%) 50, 40, 30, 25, 10, 5, 1 and 0.5 µg/ml of (A) Alpha-terpinolene, (B) Linalool, (C) Isopulegol & (D) Geraniol.

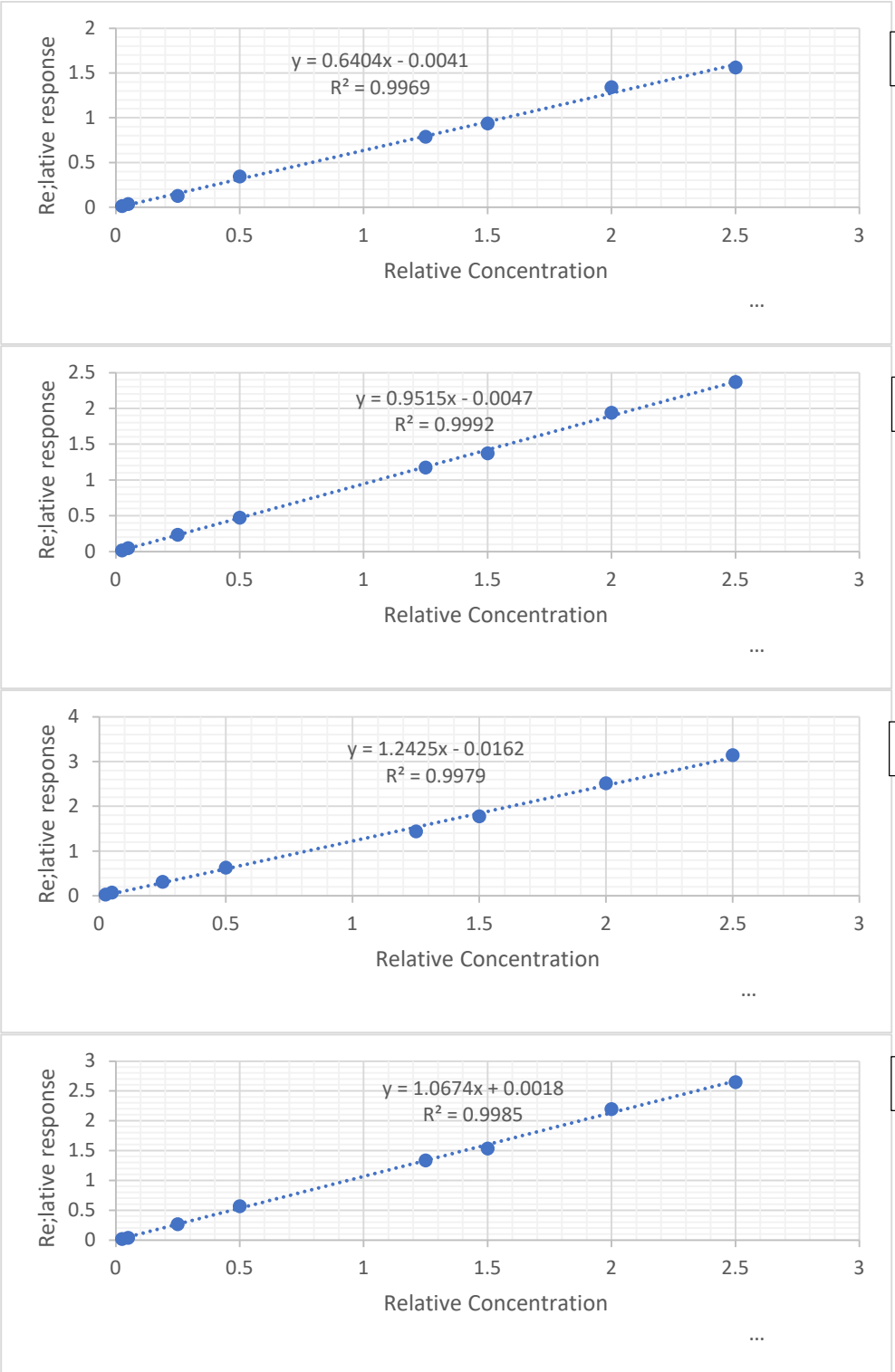

Figure 3.12: Calibration plots compiled from the injection of calibration solutions in quadruplicate containing (w:w%) 50, 40, 30, 25, 10, 5, 1 and 0.5 µg/ml of (A) Trans Caryophyllene (B) Alpha-Humulene, (C) Myrcene & (D) Nerolidol

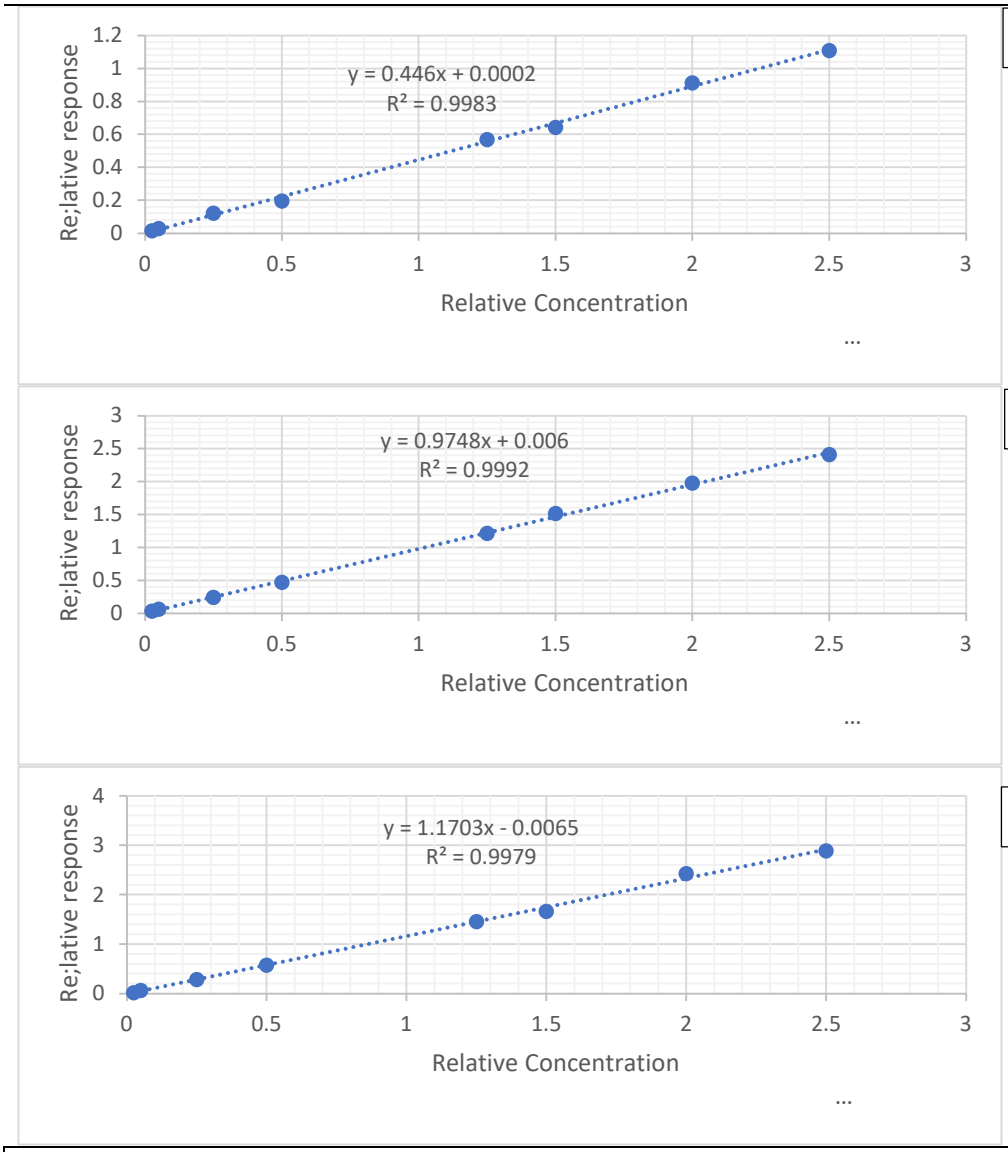

Figure 3.13: Calibration plots compiled from the injection of calibration solutions in quadruplicate containing (w:w%) 50, 40, 30, 25, 10, 5, 1 and 0.5 µg/ml of (A) Guaiol (B) Alpha-bisabolol & (C) Camphene.

3.5 Working range

The working range of the method was based on the linearity of the calibration curve, and the limits of detection. The limits and the working range for Cannabinoids and terpenes are shown in tables

Table 3.8: Limit of detection (LOD), Limit of Quantification (LOQ), Upper Quantification limit (UQL) and Working range for Cannabinoids.

Analyte	**LOD (%w:w)**	**LOQ (%w:w)**	**UQL (%w:w)**	**Working rage (%w:w)**
Cannabidiol (CBD)	0.01	0.1	50	0.01-50
Delta 9-THC (THC)	0.01	0.1	50	0.01-50
Cannabinol (CBN)	0.01	0.1	50	0.01-50

Table 3.9: Limit of detection (LOD), Limit of Quantification (LOQ), Upper Quantification limit (UQL) and Working range for Terpenes				
Analyte	**LOD (µg/ml)**	**LOQ (µg/ml)**	**UQL (µg/ml)**	**Working rage (µg/ml)**
Alpha-Pinene	0.10	0.50	50.00	0.1-50
Beta-Pinene	0.10	0.50	50.00	0.1-50
3,7-dimethyl-1,3,6-octatriene	0.10	0.50	50.00	0.1-50
Delta-Limonene	0.10	0.50	50.00	0.1-50
3-Carene	0.10	0.50	50.00	0.1-50
Camphene	0.10	0.50	50.00	0.1-50
Para-Cymene	0.10	0.50	50.00	0.1-50
Alpha-terpinene	0.10	0.50	50.00	0.1-50
Gamma-Terpinene	0.10	0.50	50.00	0.1-50
Alpha-terpinolene	0.10	0.50	50.00	0.1-50
Isopulegol	0.10	0.50	50.00	0.1-50
Linalool	0.10	0.50	50.00	0.1-50
Geraniol	0.10	0.50	50.00	0.1-50
Trans-Caryophyllene	0.10	0.50	50.00	0.1-50
Alpha-humulene	0.10	0.50	50.00	0.1-50
Myrcene	0.10	0.50	50.00	0.1-50
Nerolidol	0.10	0.50	50.00	0.1-50
Guaiol	0.10	0.50	50.00	0.1-50
Alpha-bisabolol	0.10	0.50	50.00	0.1-50

3.6 Precision

Precision repeatability and reproducibility was assessed at three concentration levels for all analytes (Cannabinoids and Terpenes).

Precision and accuracy results are shown in tables 10-11. The results for method repeatability (RSDr) and within lab reproducibility (RSDrw) evaluated for all analytes at 3 concentration levels within the working range of the method, are shown in tables 10 - 17 below.

Table 3.10: Method repeatability metrics (Average, standard deviation (stDEV), Coefficient of Variation (CV) and Percentage Error (%E)) for cannabinoid analysis.

Analyte	5%				20%				35%			
	Avg	St DEV	% CV	%E	Avg	St DEV	% CV	%E	Avg	St DEV	% CV	%E
Cannabidiol	5.1	0.2	4.2	1.2	20.2	1.1	5.5	0.9	35.2	1.7	4.9	0.7
Delta 9-THC	5.0	0.3	5.2	-0.8	19.7	1.0	5.0	-1.5	35.0	1.9	5.3	0.0
Cannabinol	4.9	0.3	5.3	-1.2	20.0	0.9	4.4	0.2	35.4	1.5	4.3	1.2

Table 3.11: Method repeatability metrics (Average, standard deviation (stDEV),Coefficient of Variation (CV) and Percentage Error (%E)) for cannabinoid analysis

Analyte	5µg/ml				25µg/ml				50µg/ml			
	Avg	St DEV	% CV	%E	Avg	St DEV	% CV	%E	Avg	St DEV	% CV	%E
Alpha-Pinene	5.2	0.5	9.5	3.0	25.3	2.2	8.7	1.2	50.7	0.8	1.6	1.4
Beta-Pinene	5.2	0.2	3.9	4.8	24.9	2.8	7.2	-0.2	50.9	2.1	4.2	1.8
3,7-dimethyl-1,3,6-octatriene	5.2	0.7	13.2	3.8	25.4	2.1	8.3	1.5	51.4	2.3	4.5	2.8
Delta-Limonene	5.1	0.1	1.6	2.4	25.3	2.1	8.3	1.1	50.8	2.0	4.0	1.6
3-Carene	5.1	0.3	6.7	2.4	25.0	2.6	7.4	0.1	50.5	1.3	2.5	1.1
Camphene	5.2	0.7	9.3	4.8	25.4	2.4	9.4	1.6	51.7	2.5	4.8	3.3
Para-Cymene	5.1	0.6	8.9	2.4	25.4	2.3	9.0	1.7	49.7	1.7	3.5	-0.5
Alpha-terpinene	5.1	0.4	8.5	2.8	24.8	2.7	7.9	-0.6	51.0	1.0	1.9	2.1
Gamma-Terpinene	5.2	0.5	8.4	3.4	24.9	1.3	5.2	-0.3	50.9	1.7	3.3	1.8
Alpha-terpinolene	5.2	0.4	7.7	4.4	24.8	1.6	6.5	-0.9	50.4	2.2	4.4	0.7
Isopulegol	5.1	0.6	8.8	2.2	24.9	1.7	6.8	-0.2	49.3	0.6	1.2	-1.5
Linalool	5.1	0.5	9.8	2.6	25.5	3.1	8.2	1.8	50.8	1.1	2.2	1.5
Geraniol	5.2	0.4	7.2	3.4	25.4	3.3	8.0	1.7	51.5	2.2	4.3	3.0
Trans-Caryophyllene	5.1	0.7	7.8	2.4	24.7	1.8	7.3	-1.2	49.6	0.7	1.3	-0.7
Alpha-humulene	5.1	0.3	6.8	2.4	24.9	2.1	8.4	-0.4	50.7	1.5	3.0	1.5
Myrcene	5.1	0.3	5.4	2.0	24.9	2.2	8.8	-0.2	51.7	0.7	1.4	3.3
Nerolidol	5.1	0.4	7.3	2.4	24.8	1.9	7.7	-0.8	49.8	2.1	4.1	-0.4
Guaiol	5.1	0.2	4.9	2.2	25.2	1.3	5.2	0.8	51.0	1.0	2.0	2.0
Alpha-bisabolol	5.1	0.5	9.4	2.0	25.3	2.3	9.1	1.1	51.2	0.9	1.7	2.3

The data sets generated from 54 duplicate samples prepared over a period of 3 days were subject to statistical analysis of variance. The F ratios and P values from samples prepared on the same day, are shown in Tables 3.12-13, whilst data generated over a period of 3 days was used for interday Variation shown in table 3.14-15.

Table 3.12: One way Analysis of variance results showing within lab Interday variation for Cannabinoid analysis

Analtyte	Low (5%)		Mid (20%)		High (35%)	
	F	*P-value*	*F*	*P-value*	*F*	*P-value*
Cannabidiol	0.7474	0.4815	0.1663	0.8475	2.6183	0.0895
Delta 9-THC	1.938	0.16	0.6783	0.5151	1.3117	0.2844
Cannabinol	1.5873	0.2197	0.1547	0.8573	0.118	0.8891

Table 3.13: One way Analysis of variance results showing within lab Interday variation for Terpene analysis

Analtyte	Low (5µg/ml)		Mid (25µg/ml)		High (50µg/ml)	
	F	*P-value*	*F*	*P-value*	*F*	*P-value*
Alpha-Pinene	0.88	0.192	2.09	0.152	1.86	0.747
Beta-Pinene	1.46	0.848	1.07	0.677	0.95	0.798
3,7-dimethyl-1,3,6-octatriene	1.71	0.192	1.56	0.889	1.78	0.394
Delta-Limonene	0.96	0.747	1.52	0.172	1.46	0.152
3-Carene	1.27	0.212	1.32	0.414	1.65	0.808
Camphene	1.77	0.677	1.73	0.828	1.22	0.354
Para-Cymene	1.39	0.798	1.85	0.343	1.88	0.152
Alpha-terpinene	0.90	0.364	1.02	0.758	1.42	0.384
Gamma-Terpinene	1.26	0.566	1.78	0.515	1.27	0.222
Alpha-terpinolene	1.58	0.333	1.61	0.909	2.06	0.212
Isopulegol	0.83	0.505	0.95	0.626	0.80	0.828
Linalool	1.08	0.455	1.53	0.636	2.07	0.869
Geraniol	1.73	0.687	1.31	0.374	2.09	0.293
Trans-Caryophyllene	1.77	0.131	1.51	0.475	1.54	0.394
Alpha-humulene	1.00	0.354	0.94	0.818	1.76	0.354
Myrcene	0.89	0.152	1.74	0.242	1.10	0.192
Nerolidol	1.57	0.394	1.33	0.586	1.48	0.172
Guaiol	1.19	0.303	1.01	0.424	0.89	0.232
Alpha-bisabolol	1.50	0.646	1.12	0.333	0.87	0.273

Table 3.14: One way Analysis of variance results showing within lab variation (Intraday) for Cannabinoid analysis						
Analtyte	Low (5%)		Mid (20%)		High (35%)	
	F	*P-value*	*F*	*P-value*	*F*	*P-value*
Cannabidiol	0.1461	0.7047	0.0031	0.9559	0.7921	0.3797
Delta 9-THC	0.1535	0.6976	1.9261	0.1742	2.9569	0.0946
Cannabinol	0.0418	0.8392	0.0058	0.9398	1.8338	0.1846

Table3.15: One way Analysis of variance results showing within lab variation (Intraday) for Terpene analysis						
Analtyte	Low (5µg/ml)		Mid (25µg/ml)		High (50µg/ml)	
	F	*P-value*	*F*	*P-value*	*F*	*P-value*
Alpha-Pinene	0.1563	0.747	0.9896	0.707	1.8338	0.485
Beta-Pinene	0.1979	0.667	0.6458	0.848	0.2461	0.535
3,7-dimethyl-1,3,6-octatriene	0.3854	0.798	2.8646	0.394	0.7921	0.475
Delta-Limonene	0.1458	0.586	2.3958	0.273	2.9569	0.242
3-Carene	2.1458	0.354	2.5104	0.747	0.0461	1.172
Camphene	1.0833	0.131	0.6250	0.818	0.0535	0.556
Para-Cymene	0.8958	0.495	1.1042	0.465	0.2535	0.485
Alpha-terpinene	1.0521	0.677	2.2188	0.495	3.0569	0.444
Gamma-Terpinene	2.8542	0.747	2.3333	0.253	0.1535	0.172
Alpha-terpinolene	2.1458	0.717	0.3333	0.535	0.0418	0.596
Isopulegol	0.1354	0.545	0.4583	0.707	1.9338	0.717
Linalool	0.4375	0.899	2.9688	0.717		0.313
Geraniol	0.9063	0.394	1.1250	0.596	0.1418	0.828
Trans-Caryophyllene	3.0521	0.111	0.3021	0.263	0.8921	0.444
Alpha-humulene	1.4583	0.455	1.3542	0.475	1.7338	0.283
Myrcene	0.4271	0.798	1.1146	0.778	0.1461	0.586
Nerolidol	3.1146	0.525	0.4896	0.475	0.6921	0.333
Guaiol	2.4375	0.657	2.1042	0.485	2.8569	0.808
Alpha-bisabolol	0.1875	0.242	2.9792	0.172	0.8562	0.121

The RSDrw, calculated by using the data generated from ANOVA, based on the Relative Standard Deviation for Reproducibility (RSDr) and Relative Standard Deviation between groups (RSDx), is shown in tables 3.16 and 3.17 below. The RSDrw (Relative Standard Deviation for within lab Reproducibility) was <10% for all analytes at the three concentrations levels tested.

Table 3.16: Method Reproducibility calculations using parameters from the analysis of variance for Cannabinoid analysis

Analtyte	Low (5%)			Mid (20%)			High (35%)		
	RSDr	**RSDx**	**RSDrw**	**RSDr**	**RSDx**	**RSDrw**	**RSDr**	**RSDx**	**RSDrw**
Cannabidiol	4.65	0.22	4.66	2.06	0.29	2.08	8.29	2.86	8.77
Delta 9-THC	7.41	0.36	7.42	3.77	0.71	3.83	5.71	1.93	6.03
Cannabinol	6.78	0.33	6.78	2.1	0.28	2.12	2.1	0.28	2.12

Table 3.17: Method Reproducibility calculations using parameters from the analysis of variance for Terpene analysis

Analtyte	Low (5µg/ml)			Mid (25µg/ml)			High (50µg/ml)		
	RSDr	**RSDx**	**RSDrw**	**RSDr**	**RSDx**	**RSDrw**	**RSDr**	**RSDx**	**RSDrw**
Alpha-Pinene	5.85	0.30	5.856	2.60	0.34	2.624	2.18	2.40	3.246
Beta-Pinene	6.12	0.66	6.152	5.78	0.36	5.791	6.53	7.18	9.709
3,7-dimethyl-1,3,6-octatriene	4.38	0.32	4.391	3.22	0.45	3.249	4.19	4.61	6.226
Delta-Limonene	7.62	0.48	7.632	2.36	0.65	2.451	3.42	3.75	5.076
3-Carene	5.23	0.59	5.267	7.75	0.55	7.765	5.38	5.91	7.997
Camphene	7.72	0.53	7.734	4.07	0.24	4.078	4.53	4.98	6.728
Para-Cymene	4.18	0.36	4.196	6.24	0.63	6.267	5.66	6.22	8.410
Alpha-terpinene	5.95	0.76	5.996	2.35	0.52	2.409	7.26	7.98	10.786
Gamma-Terpinene	4.44	0.67	4.489	4.31	0.68	4.363	2.50	2.75	3.718
Alpha-terpinolene	5.20	0.75	5.257	3.82	0.64	3.876	4.19	4.61	6.226
Isopulegol	7.40	0.54	7.417	6.23	0.48	6.245	3.43	3.77	5.090
Linalool	4.87	0.34	4.878	6.44	0.41	6.457	7.75	8.51	11.509
Geraniol	7.45	0.64	7.475	3.77	0.62	3.823	4.95	5.43	7.348
Trans-Caryophyllene	6.66	0.62	6.691	6.91	0.59	6.937	4.93	5.41	7.318
Alpha-humulene	7.46	0.64	7.485	4.88	0.41	4.893	6.59	7.25	9.797
Myrcene	7.70	0.38	7.705	3.56	0.55	3.607	5.34	5.87	7.938
Nerolidol	3.62	0.26	3.634	6.47	0.77	6.520	6.38	7.01	9.472
Guaiol	4.16	0.42	4.182	5.27	0.45	5.292	6.84	7.52	10.166
Alpha-bisabolol	4.62	0.56	4.651	6.37	0.34	6.374	2.59	2.85	3.851

3.7 Laboratory and sample bias

The results for Laboratory and samples bias using 20 samples (10 individual samples prepared in duplicate) are shown in tables 3.18 and 3.19 below. In all cases the relative bias was <1% when evaluated at Low , Medium and High concentration.

Table 3.18: Bias results for laboratory and sample bias for Cannabinoid analysis

Analyte	Low (5%)		Mid (20%)		High (35%)	
	Relative Bias	**StDEV**	**Relative Bias**	**StDEV**	**Relative Bias**	**StDEV**
Cannabidiol (CBD)	-0.06	4.19	-0.14	5.39	-0.33	4.97
Delta 9-THC (THC)	0.04	5.02	0.25	4.76	0.01	4.81
Cannabinol (CBN)	0.06	5.13	-0.04	4.36	-0.59	4.67

Table 3.19: Bias results for laboratory and sample bias for Terpene analysis						
Analyte	Low (5µg/ml)		Mid (25µg/ml)		High (50µg/ml)	
	Relative Bias	**StDEV**	**Relative Bias**	**StDEV**	**Relative Bias**	**StDEV**
Alpha-Pinene	-0.07	4.29	0.19	5.32	0.25	3.99
Beta-Pinene	0.03	5.73	0.17	4.70	0.32	5.32
3,7-dimethyl-1,3,6-octatriene	0.13	4.09	0.16	5.32	0.00	4.81
Delta-Limonene	0.21	5.42	0.18	4.50	0.30	4.91
3-Carene	0.31	4.50	0.13	4.81	0.24	5.21
Camphene	-0.04	4.40	0.18	4.81	-0.11	5.32
Para-Cymene	0.11	5.21	0.08	4.81	0.39	4.09
Alpha-terpinene	0.39	4.09	0.21	5.21	0.17	4.60
Gamma-Terpinene	0.17	3.99	0.37	4.09	0.09	4.70
Alpha-terpinolene	-0.01	5.01	-0.07	5.52	0.35	5.52
Isopulegol	0.16	5.32	0.34	5.11	0.25	5.62
Linalool	0.14	4.09	0.01	5.42	0.40	4.81
Geraniol	0.13	5.21	-0.06	4.81	0.12	5.01
Trans-Caryophyllene	-0.11	5.11	0.29	5.62	-0.13	4.60
Alpha-humulene	0.18	4.60	0.10	4.70	-0.11	4.19
Myrcene	0.21	4.09	0.03	4.81	0.11	4.60
Nerolidol	0.26	4.60	-0.09	4.81	0.14	4.60
Guaiol	0.07	4.70	-0.12	4.50	0.19	4.19
Alpha-bisabolol	0.17	5.11	-0.06	4.50	0.02	5.21

3.8 Duplicate Bias

The deviation between individual duplicates was within the allowed specification <10%. The deviation between duplicate samples is shown in tables 3.20 and 3.21.

Table 3.20: Duplicate bias calculated for individual duplicates prepared from a reference sample containing the cannabinoids of interest

Analyte	**Relative Bias**	**StDEV**
Cannabidiol (CBD)	3.76	4.39
Delta 9-THC (THC)	2.73	4.05
Cannabinol (CBN)	2.9	3.93

Table 3.21: Duplicate bias calculated for individual duplicates prepared from a reference sample containing Terpene compounds

Analyte	**Relative Bias**	**StDEV**
Alpha-Pinene	2.34	3.37
Beta-Pinene	2.83	4.38
3,7-dimethyl-1,3,6-octatriene	2.28	4.38
Delta-Limonene	3.07	4.00
3-Carene	2.68	4.68
Camphene	2.99	3.55
Para-Cymene	2.83	4.02
Alpha-terpinene	2.68	3.57
Gamma-Terpinene	3.15	4.63
Alpha-terpinolene	2.68	4.32
Isopulegol	2.28	3.92
Linalool	3.15	4.77
Geraniol	2.99	3.92
Trans-Caryophyllene	2.52	4.63
Alpha-humulene	2.68	4.40
Myrcene	2.76	3.55
Nerolidol	2.28	3.34
Guaiol	2.91	4.70
Alpha-bisabolol	2.99	3.59

3.9 Residual Bias

The residual bias of the calibration curves shown in section 5.1, are shown in tables 3.22 and 3.23 below as the percentage deviation of calibrants used for residual bias.

Table 3.22: Calibration curve bias calculated using 16 calibrant samples at 8 levels of the calibration curve for Cannabinoids

Analyte	Relative Bias	StDEV
Cannabidiol (CBD)	-0.21	4.87
Delta 9-THC (THC)	-0.26	5.35
Cannabinol (CBN)	-0.03	2.87

Table 3.23: Calibration curve bias calculated using 16 calibrant samples at 8 levels of the calibration curve for Terpenes

Analyte	Relative Bias	StDEV
Alpha-Pinene	-1.50	2.96
Beta-Pinene	1.36	3.86
3,7-dimethyl-1,3,6-octatriene	1.89	3.86
Delta-Limonene	-1.19	3.52
3-Carene	1.64	4.12
Camphene	-1.83	3.12
Para-Cymene	-0.17	3.53
Alpha-terpinene	-0.52	3.14
Gamma-Terpinene	1.25	4.07
Alpha-terpinolene	1.13	3.81
Isopulegol	-1.01	3.45
Linalool	-1.73	4.19
Geraniol	0.66	3.45
Trans-Caryophyllene	1.80	4.07
Alpha-humulene	-0.89	3.87
Myrcene	0.96	3.13
Nerolidol	0.82	2.94
Guaiol	1.10	4.13
Alpha-bisabolol	-0.80	3.16

3.10 Target identification and selectivity

The target analyte identification in the blank samples and samples prepared from Certified reference standard solutions were tested using the developed Cannabinoid and Terpene methods.

The chromatograms showed no matrix interference, and no other peaks co eluted at the same as the analytes. Results for target identification and selectivity are shown in tables 3.24 and 3.25.

The quantitative results for Cannabinoid assays in samples originating from investigative sources, Medicinal Cannabis products and 'Low-THC' Products are presented in Appendix 1-3, respectively.

The qualitative and quantitative data , including the statistical evaluation for terpene products is presented in Appendix 4.

Table 3.24: Target analyte identification and selectivity for Cannabinoids

SAMPLE	Analytes	Analytes identified	Retention time deviation	Coeluted peaks or interference
Solvent blanks (N=5)	None	N.A	N.A	0
Standard solutions (N=4)	*CBD / DPA*	*CBD / DPA*	*<0.02 min*	*0*
	THC / DPA	*THC / DPA*	*<0.02 min*	*0*
	CBN / DPA	*CBN / DPA*	*<0.02 min*	*0*
	THC/ CBD/ CBN/ DPA	THC/ CBD/ CBN/ DPA	*<0.02 min*	*0*
Reference samples (N=16)	THC/ CBD/ CBN/ DPA	THC/ CBD/ CBN/ DPA	*<0.02 min*	0
Matrix Blanks (N=5)	DPA	DPA	*<0.02 min*	0

Table 3.25: Target analyte identification and selectivity for Terpenes				
SAMPLE	**Analytes**	**Analytes identified**	**Retention time deviation**	**Coeluted peaks or interference**
Solvent blanks (N=5)	None	N.A	N.A	0
Standard solutions (N=20)	Alpha-Pinene	Alpha-Pinene	*<0.02 min*	*0*
	Beta-Pinene	Beta-Pinene	*<0.02 min*	*0*
	3,7-dimethyl-1,3,6-octatriene	3,7-dimethyl-1,3,6-octatriene	*<0.02 min*	*0*
	Delta-Limonene	Delta-Limonene	*<0.02 min*	*0*
	3-Carene	3-Carene	*<0.02 min*	*0*
	Camphene	Camphene	*<0.02 min*	*0*
	Para-Cymene	Para-Cymene	*<0.02 min*	*0*
	Alpha-terpinene	Alpha-terpinene	*<0.02 min*	*0*
	Gamma-Terpinene	Gamma-Terpinene	*<0.02 min*	*0*
	Alpha-terpinolene	Alpha-terpinolene	*<0.02 min*	*0*
	Isopulegol	Isopulegol	*<0.02 min*	*0*
	Linalool	Linalool	*<0.02 min*	*0*
	Geraniol	Geraniol	*<0.02 min*	*0*
	Trans-Caryophyllene	Trans-Caryophyllene	*<0.02 min*	*0*
	Alpha-humulene	Alpha-humulene	*<0.02 min*	*0*
	Myrcene	Myrcene	*<0.02 min*	*0*
	Nerolidol	Nerolidol	*<0.02 min*	*0*
	Guaiol	Guaiol	*<0.02 min*	*0*
	Alpha-bisabolol	Alpha-bisabolol	*<0.02 min*	*0*
Reference samples (N=10)	Terpene mixture / DPA	Terpene mixture / DPA	*<0.02 min*	0
Matrix Blanks (N=5)	DPA	DPA	*<0.02 min*	0

3.11Carry over

A total of 6 blank samples run immediately after analysing spiked samples containing Cannabinoids and terpenes at high concentration showed no carry over. The results are shown in tables 3.26 and 3.27.

Table 3.26: Sample carryover results for blank samples run immediately after a high concentration cannabinoid samples

Analyte	Qualifier ion Carryover	
	Analyte at 75% (%w:w)	Analyte at 85% (%w:w)
Cannabidiol (CBD)	None	None
Delta 9-THC (THC)	None	None
Cannabinol (CBN)	None	None
Number of samples	3	3

Table 3.27: Sample carryover results for blank samples run immediately after a high concentration Terpene samples

Analyte	Qualifier ion Carryover	
	Analyte at 75% (%w:w)	Analyte at 85% (%w:w)
A-Pinene	None	None
Beta-Pinene	None	None
3,7-dimethyl-1,3,6-octatriene	None	None
Para-Cymene	None	None
D-Limonene	None	None
3-Carene	None	None
Camphene	None	None
Alpha-terpinene	None	None
G-Terpinene	None	None
A-terpinolene	None	None
Linalool	None	None
Isopulegol	None	None
Geraniol	None	None
Trans-Caryophyllene	None	None
A-humulene	None	None
Myrcene	None	None
Nerolidol	None	None
Guaiol	None	None
Alpha-bisabolol	None	None
Number of samples	3	3

3.12 Sample stability

The stability of the samples assessed at room temperature (25 ±2°C) over a period of 36 hours for Cannabinoids and 48 hours for Terpene compounds.

The % relative peak area of the analytes and the internal standard, when compared to the peak areas from the analysis at T=0 hours are shown in table 3.28 and 3.29 below.

The extracted samples were stable showing a relative peak area above 90% for 24 hours, for cannabinoids and Terpene compounds.

Table 3.28: Relative peak area (%PA) for the analytes and internal standard analysed over period of 36h

Time (h)	**Cannabidiol (CBD)**	**Delta 9-THC (THC)**	**Cannabinol (CBN)**	**DPA-IS**
0	100.00	100.00	100.00	100.00
12	94.08	101.01	96.00	99.91
24	92.16	98.00	98.00	97.72
36	85.44	96.00	104.96	78.82
48	78.72	89.00	111.00	66.88

Table 3.29: Relative peak area (%PA) for the analytes and internal standard analysed over period of 48h				
Analyte	**%PA of analyte at T=6 h**	**%PA of analyte at T=12h**	**%PA of analyte at T=24h**	**%PA of analyte at T=48h**
Diphenylamine	99.4	97.3	90.6	85.8
A-Pinene	98.5	98.2	91.8	86.7
Beta-Pinene	98.6	97.5	90.3	89.2
3,7-dimethyl-1,3,6-octatriene	99.3	97.7	93.7	90.1
Para-Cymene	98.1	99.8	92.6	87.2
D-Limonene	98.1	98.8	90.8	89.2
3-Carene	98.2	98.8	92.2	87.5
Camphene	98.1	98.4	93.8	84.1
Alpha-terpinene	98.7	98.7	92.4	86.2
G-Terpinene	98.4	98.1	91.7	86.2
A-terpinolene	99.6	97.2	90.3	89.1
Linalool	99.5	98.8	90.6	90.6
Isopulegol	98.4	99.5	90.7	84.6
Geraniol	98.7	97.7	93.4	86.7
Trans-Caryophyllene	98.4	99.7	90.5	85.8
A-humulene	99.2	99.4	91.2	85.8
Myrcene	98.5	99.7	90.7	87.6
Nerolidol	99.4	96.2	93.4	87.8
Guaiol	99.7	98.2	90.5	89.2
Alpha-bisabolol	99.6	96.4	91.2	88.4

3.13 Uncertainty of Measurement

The relative Expanded uncertainty calculated for all analytes of interest (Cannabinoids and terpene compounds), at three concentration levels are shown in tables 3.30 and 3.31 below. The method uncertainty of Measurement was satisfactory (U_{rel} <20%) for all analytes.

Table 3.30: Results for uncertainty of Measurement Calculated at three concentration levels for CBD, THC and CBN

	U_{rel}		
Concentration level (%W/W)	**5%**	**20%**	**35%**
Cannabidiol (CBD)	*11*	*16.88*	*13.4*
Delta 9-THC (THC)	*8*	*10.4*	*18.4*
Cannabinol (CBN)	*6*	*10.5*	*16.51*

Table 3.31: Results for uncertainty of Measurement Calculated at three concentration levels for Terpenes			
Analyte	**U_{rel} at Concentration level**		
	5 µg/ml	**25 µg/ml**	**50 µg/ml**
A-Pinene	15.8	11.8	7.1
Beta-Pinene	9.4	10.3	7.2
3,7-dimethyl-1,3,6-octatriene	10.7	5.8	8.4
Para-Cymene	16.2	6.1	10.2
D-Limonene	14.1	6.5	9.2
3-Carene	16.2	11.4	7.4
Camphene	9.5	6.1	7.5
Alpha-terpinene	11.5	9.8	7.7
G-Terpinene	12.1	4.4	7.1
A-terpinolene	14.2	4.5	7.2
Linalool	16.3	9.4	6.6
Isopulegol	16.8	5.1	5.8
Geraniol	16.2	12.3	9.2
Trans-Caryophyllene	18.4	11.7	7.3
A-humulene	14.7	4.8	5.1
Myrcene	13.1	10.1	9.7
Nerolidol	10.5	9.6	7.1
Guaiol	11.2	12.7	6.4
Alpha-bisabolol	18.2	9.6	10.6

3.14 Internal standard limits

The peak area of the internal standard (Diphenylamine) calculated by peak integration, was tabulated to calculate the warning limits, and the alarm limits. Results are included in table 3.32 below.

Table 3.32: Deviation limits for internal standard peak area (Mega Counts)

Diphenylamine (DPA-IS)	Mean	stDEV	Lower Alarm limit	lower warning limit	Upper warning limit	Upper alarm limit
Peak area (Megacounts)	17.14	0.38	15.81	16.38	17.90	18.28

The peak area of the internal standard was within limits (Figure 3.14) during the method validation process. The peak areas calculated were within the upper and lower Alarm limits defined for the method.

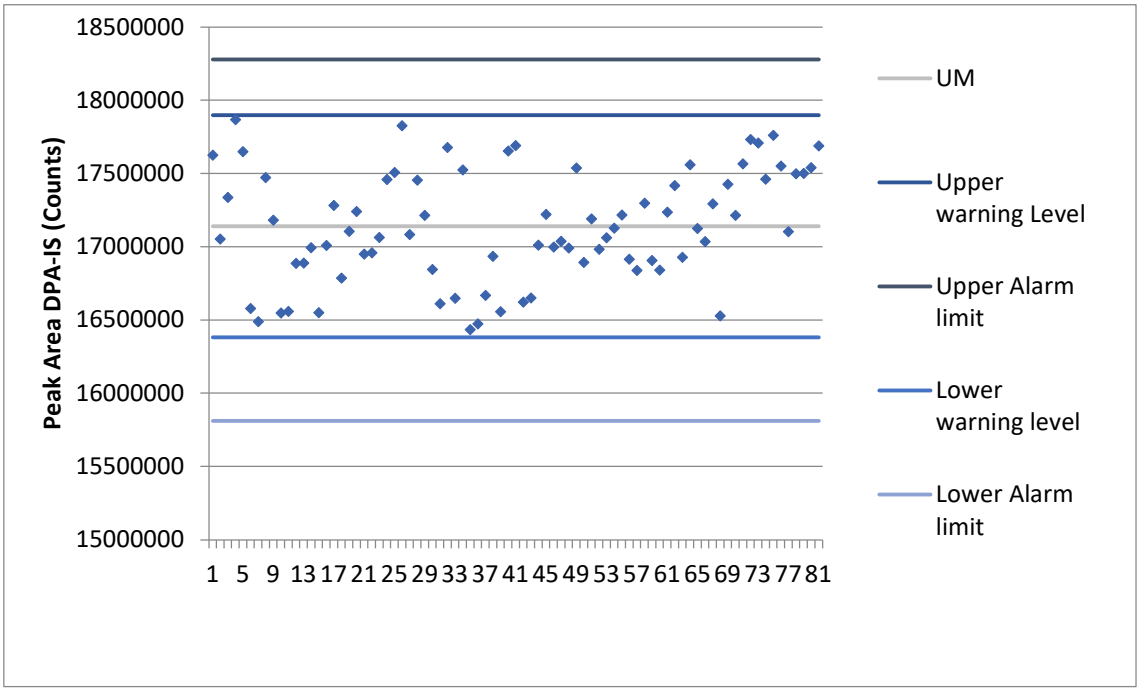

Figure 3.14: Peak area Acceptance levels for internal standard (*Diphenylamine*).

3.15 Method verification

The analytical methods were applied to real samples to verify the applicability of the test methods to real samples. The percentage of samples showing positive identification of Cannabinoids and terpenes, and the percentage of samples where the quantification results could be replicated using the developed assays are shown in table 3.33

Table 3.33: Testing of samples used for the verification of test methods for Cannabinoids and Terpenes.

Sample matrix		Number of samples	Percentage of samples with positive identification of Cannabinoids and Terpenes	Percentage of samples with Quantification results within limits (±10% rel.).
Herbal Cannabis	Medicinal Product	5	100%	100%
	Illicit market	15	100%	100%
	Unregulated	6	100%	100%
	From Cannabis plantation	3	100%	100%
Cannabis extracts	Extract in MCT oil	11	100%	100%
	Extract in Oilve oil	4	100%	100%
	Terpene concentrate in oil	2	100%	100%
	Vaping liquid	3	100%	100%
Cannabis resin	Illicit product	12	100%	100%
	Shatter	1	100%	100%

The identification procedure described also allowed the identification of semi synthetic cannabinoids in 13 samples including Hexahydrocannabinol (HHC), Hexahydrocannabinol-O-Acetate (HHC-0), Hexahydrocannabiphorol (HHCP) and Tetrahydrocannabiphorol (THCP). The chromatograms showing the distinct peaks for semi-synthetic cannabinoids are shown in figures 3.15 and 3.16 below.

The data gathered from the chromatographic analysis and the mass fragmentation of these compounds was compiled in table 3.34 below. The data was then used to identify these compounds through the presence of the mass fragments at the specified retention time.

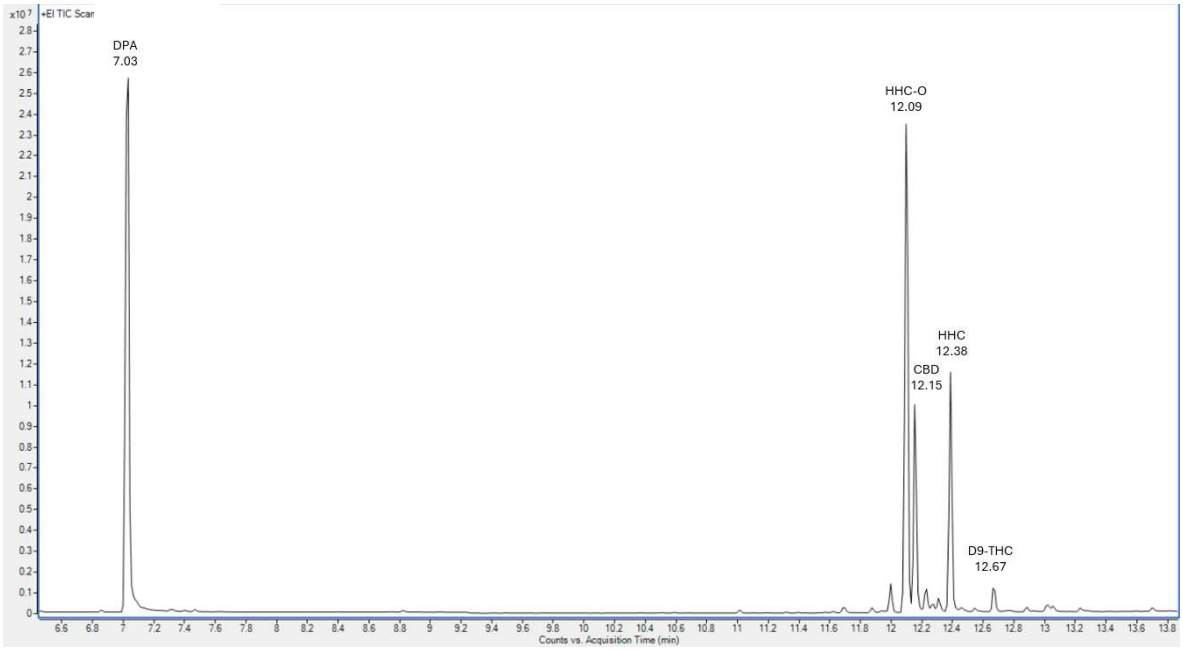

Figure 3.15: GC chromatogram of a sample of Cannabis inflorescence extracted in the extractant solvent with 0.2 mg/ml of Diphenylamine as an internal standard. HHC-O, THC, CBD and HHC were identified using the described methods.

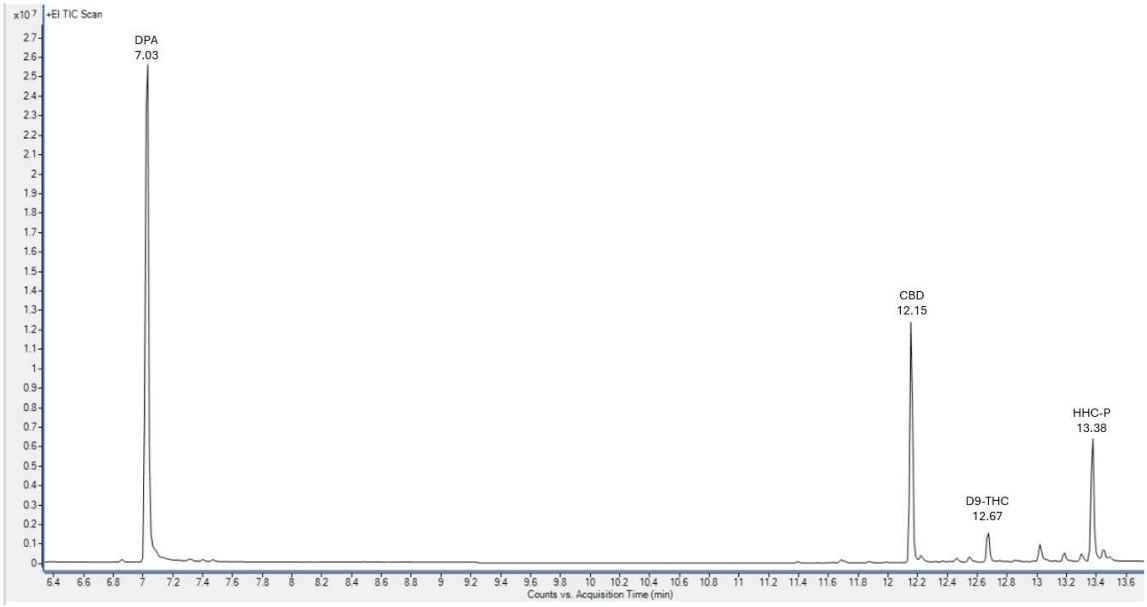

Figure 3.16: GC chromatogram of a sample of resin extracted in the extractant solvent with 0.2 mg/ml of Diphenylamine as an internal standard. THC, CBD and HHC-P were identified using the described methods.

The data gathered from the chromatographic analysis and the mass fragmentation of these compounds was compiled in table 3.34 below. The data was then used to identify these compounds through the presence of the mass fragments at the specified retention time.

Table 3.34: identification criteria extracted from data for the identification of semi-synthetic cannabinoids

Analyte	**Retention time (min)**	**Mass-fragments used for identification (m/z)**				
HHC-O	12.09	273	316	260	358	299
HHC	12.38	193	260	273	316	231
HHC-P	13.38	260	301	344	221	273
THCP	13.87	327	259	299	342	243

3.16 Product compliance and deviation

The evaluation of product compliance for cannabinoid content, using the developed assays was based on the criteria described in table 3.35 blow.

Table 3.35 Evaluation criteria set for product compliance evaluation	
Criterion	**Specification**
% THC (W:W)	<0.2% THC for products labelled as CBD based products or 'Low-THC' products. Concentration within ±10% of labelled content for products labelled as containing THC.
% CBD (W:W)	Concentration within ±10% of labelled content
% CBN (W:W)	<1%
Adulterants	Absence of adulterants including semi synthetic and synthetic cannabinoids
Volatile component	Identification of Terpene compounds in products labelled as full/whole extracts.

The evaluation of terpene compounds could identify 10% of the products that had a Volatile terpene component that was absent or below the LOD (0.1ug/ml), whilst 5%of the products were present at trace levels above the limits of detection but below the limits of quantification (0.1-0.5ug/ml).

The evaluation of the products based on the analytical data produced by the developed assays are shown as a percentage of products that were non-compliant for specific preset criteria in table 3.35. the data is illustrated in in figure 3.17 below.

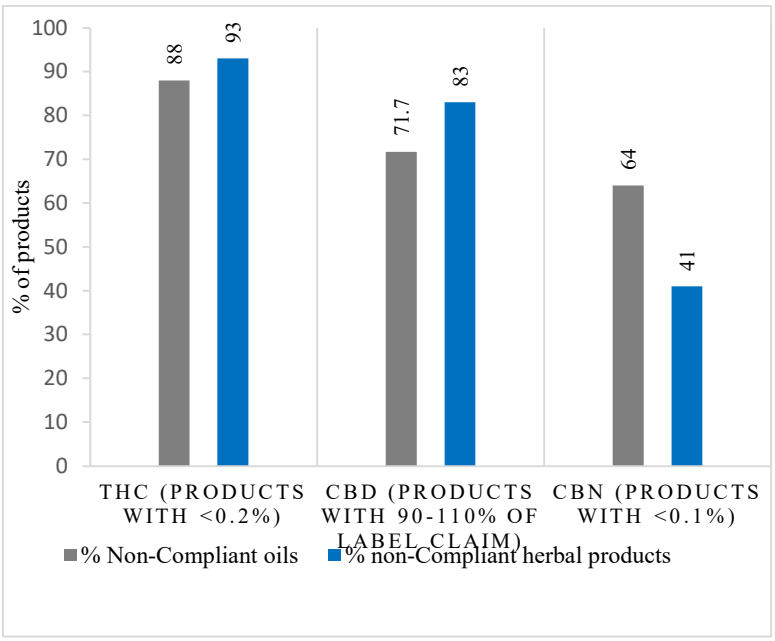

Figure 3.17: Percentage of 'Low-THC' products including oil-extracts (N=34) and inflorescence (n=51) non-compliant with the preset criteria for THC, CBN and CBD.

The quantitative results for Cannabinoid assays in samples originating from investigative sources, Medicinal Cannabis products and ‘Low-THC’ Products are presented in Appendix 1-3, respectively.

The qualitative and quantitative data , including the statistical evaluation for terpene products is presented in Appendix 4.

Chapter 4:
Discussion

4 Discussion

The development and validation of the analytical techniques targeting 22 Cannabinoids and Terpenes identified in Cannabis based products, was successfully concluded. The quality parameters tested for the analytical methods were satisfied for all the analytes. The results presented and discussed below demonstrate the applicability of the analytical methods for the quantification of Cannabinoids and Terpenes in Cannabis products found on the local market.

4.1 Extraction of Cannabinoids and Terpenes from complex matrices

The sample preparation procedure described in this study involves the extraction of plant secondary metabolites from cannabis plant material and cannabis-based preparations including cannabis resin and solidified extracts (*shatter*) and liquid extracts in oils (MCT, olive oil and vaping liquids).

The simple and rapid procedure described can be completed in 10-15minutes. The processing involves the maceration of solid materials followed by a solid-liquid extraction in a mix of methanol and Chloroform (9:1 v:v) for 5 minutes, vortex mixing and a final dilution.

The extraction procedure proved to be a quick and highly efficient method for the extraction of Cannabinoids and terpenes from complex solid matrices. Comparatively, a similar exercise was conducted by Cachia, (2021), the author reported a procedure that involved sample processing times exceeding 13h.

The extraction efficiency (%EF) for Cannabinoids (THC, CBN and CBD) was higher than 93% for herbal materials. The same procedure was also used for the extraction of volatile secondary metabolites from herbal materials that had an extraction efficiency that exceeded the 94% for all the Terpene compounds investigated.

During the optimisation of the extraction procedure, results also showed that any increase in extraction time beyond 5minutes did not enhance the extraction efficiency of the method. The latter highlights the efficiency of the partitioning of the analytes in the extracting solvent.
The high extraction efficiency can be attributed to the increased surface contact between the plant material and the extraction solvent achieved through maceration of the samples and the selection of the optimal solvent mix. Cannabinoids are non-polar molecules that have a high molecular affinity to non-polar matrices. This was also reflected in the relative extraction efficiency of cannabinoids. The Extraction efficiency, expressed as the proportion of the analyte partitioning in the extraction solvent (%EF) for THC was 5% less than that of CBD. The higher polarity of CBD favours the partitioning in the extractant solvent (Huang et al., 2021). This trend was also observed in the extraction efficiency of Terpene compounds. Such as monoterpenoids such as Linalool Isopulegol and geraniol, that showed extraction efficiencies of 98.9%, 99.5% and 99.5% respectively, in the polar extraction solvent.

During the evaluation of the extraction procedure for oils, results showed that the polar solvents investigated (methanol, ethanol, chloroform, acetone, hexane, and ethyl acetate) could not be efficiently separated from the oily matrix. This was not the case with an aqueous solvent, that could be easily partitioned from the oil. However, the partitioning of the hydrophobic cannabinoids in aqueous solvents is minimal <10% (Pandopulos et al., 2022). Furthermore, the extract in an aqueous solvent is not compatible with analytical

instrumentation used in this study, and it would therefore require a second extraction procedure for the extraction of the analytes from the aqueous phase to an organic phase.

Therefore, the analytical technique validated in this study was based on a dilution of the oil in a polar solvent (methanol:chloroform 9:1). The procedure showed to be an efficient approach for the chromatographic separation and identification of the analytes, without extraction.

The results obtained during the evaluation of the extraction efficiency of the methods did not show any deviation between the selected matrices with extraction efficiencies varying <1.8% between different matrices. The extraction efficiency in this study is comparable to published works based on the extraction of cannabinoids from herbal materials that have reported an extraction efficiency of 98% after 3 subsequent extractions with methanol (Kanabus et al., 2023) whilst the efficiency of the proposed sample preparation step for liquid matrices exceeds extraction efficiencies previously reported from olive oil by 13% (Citti et al., 2016).

One of the major advantages of the validated procedures, is the ability to co-extract two groups of plant secondary metabolites in a single extract. This minimizes the volume of sample needed for analysis as well as decreasing sample processing times in a laboratory setting.

Sample stability data for cannabinoids and terpenes generated over a period of 48 hours, also showed that the samples can be prepared in batches and allowed to stand at room temperature for 24 hours before analysis with minimal effects (<10%) on the peak area. Results showed that when the samples are allowed to stand for 48 hours, most terpene compounds show significant reduction in peak areas (>15%) this may be attributed to losses due to evaporation or decomposition.

This trend was also observed for THC and CBD, that have a relative peak areas of less than 90% when compared to the samples analysed at T=0. This was not the case for CBN. The sample showed an increased peak area. The increase in concentration of CBN was associated with the oxidative decomposition of THC into CBN (Repka et al., 2006)

4.2 Chromatographic separation and detection of Cannabinoids and Terpene Compounds.

In this study, two analytical methods were developed and validated. This ties to one of the major objectives of this study, to develop techniques for the detection, identification and quantification of Cannabinoids and terpenes.

Separate chromatographic methods were set to cater for Cannabinoids and Terpene compounds the different physical properties of these molecules. The major difference between the two molecular groups is their molecular size, and hence the volatility and elution times of these compounds. Volatile secondary metabolites like terpenes require low column temperatures to separate into defined components, whilst Cannabinoids require high thermal gradients to elute (Nahar et al., 2020).

The samples were analysed on an Agilent 7890 gas chromatograph coupled with an Agilent 5975C quadrupole mass detector, the chromatographic conditions set for the method allowed the separation of cannabinoids of interest and the internal standard. The same equipment was used for the separation of a mixture of 20 terpene compounds and the internal standard. During the optimisation of the chromatographic methods, the major challenges were the coelution of compounds and asymmetrical or broad chromatographic peaks.

The low temperatures required by Terpene compounds to separate increase the compound's retention time, reducing sensitivity and chromatographic separation (Marcillo et al.,

2022).Any increase in temperature resulted in the coelution of molecules with similar molecular structures such as structural isotopes. α-Pinene and β-pinene, α-Terpinene and γ-terpinene, Limonene and Terpinolene, and Carene and Camphrene showed co-elution at high temperatures. The separation of these compounds was achieved through the use of a pressure pulse (25psi for 30seconds) at the time the sample is introduced in the chromatographic column. The increased pressure accelerates molecules within the column and decreased the retention time of terpenes by 4 minutes, with no peak co-elution. The pressure also decreased the elution time reducing peak width by 40%.

The introduction of an excess amount sample in the column decreases the separation efficiency of the chromatographic column, resulting in decreased chromatographic resolution, peak broadening, and tailing (Myers et al., 2021). Therefore, after the optimisation of the thermal gradients, a split flow was tested.

The introduction of a split flow is a balance between chromatographic resolution and sensitivity (Sun et al., 2022), the method was tested at split ratios of 2:1, 5:1, 10:1 and 50:1. When the sample was split 5:1, 10:1 and 50:1 in the GC-inlet, the sample mixes could be fully resolved, with no coeluting compounds, whilst the shape of the chromatographic peaks was symmetrical. Therefore, a split ratio of 1:5 was used, as the split ratios of 10:1 and 50:1 would inherently reduce method sensitivity.

The chromatographic separation provided one of the key elements for analyte identification, allowing all analytes to elute at a specific retention time with variation of less than 0.02 minutes. The ability of the analytical procedure to selectively identify the target analytes, was

also corroborated by the results obtained during the validation process. All analytes were identified within their respective retention times, in addition the produced chromatograms did not show any peaks eluting at the same retention time as the target analytes.

4.3 Identification, distinction and quantification of Cannabinoids and terpenes using Mass -Fragmentation patterns.

The identification of Cannabinoids and terpenes in the samples and mixtures injected was also based on the detection of specific fragmentation patterns using mass spectrometry. The mass spectrometer was run in scan mode to identify mass fragments with a mass to charge ratio of 50-500.

The Mass spectral identification method was set by the injection of neat standard solutions in order to compile analyte identification characteristics (described in section 5.3.2), this was especially important for structural isomers such as CBD and THC, α-Pinene and β-pinene, α-Terpinene and γ-terpinene. The structural similarity of these isomers results in similar mass fragmentation spectra, that cannot be easily distinguished. Therefore, in unison with resolution of these isomers in the chromatographic column, the identification of qualifier ions and qualifier ion ratios specific to the analyte were used for the identification of Cannabinoids and terpenes.

The identification of Cannabinoids was also facilitated with the presence of reference spectra in common mass spectral liberties, therefore the mass spectral data obtained could be matched to a reference spectrum for identification. This was not the case for terpene compounds.

Results from the Mass spectra of terpenes also showed clear fragmentation similarities between compounds. The mass fragment m/z= 93 is the base peak in most spectra of monoterpenes. The mass fragment is also a common mass fragment amongst other terpene compounds in this study, as shown in section 5.2.3. This mass fragment corresponds to a $C_3H_7^+$ fragment from monoterpenes, the high abundance of this fragments reflects the thermodynamic stability of the fragmentation of monoterpenes to lose 43 mass units. The fragmentation is also verry common in terpenes that have gem dimethyl groups (Talele, 2018) Therefore the molecular fragment was also observed in Carene, terpinene, terpinolene, caryophyllene and terpinolene.

Mass spectra of structural isomers were also closely examined to assess the relative abundances of the most common fragments. This was used to differentiate between the mass spectra of the two isomers. The mass fragmentation of α-Pinene and β-pinene, could be distinguished through the comparative assessment of the fragment abundances of the mass fragments m/z=41 and 121. Similarly, the fragment abundances of terpinene were essential in the molecular distinction of the Alpha and gamma isomers, through the fragments m/z=79 and 121. The variable abundances reflect the changes in thermodynamic stability of the formation of these fragments during ionisation (Silverstein et al., 2014). The minor structural changes favour the formation of one fragment over the other allowing for a clearer distinction of these molecules.

The distinction of structural isomers of monoterpene alcohols (linalool, geraniol and isopulegiol) was assisted by the positioning of the hydroxyl group(Gonçalves et al., 2020). The position of the hydroxyl group on the terminal carbon in geraniol favours the formation of the m/z=69 fragment, whilst in linalool, the position of the hydroxyl group gives a base

peak of m/z 71. The latter mass corresponds to the fragmentation of the molecule at the C-3 position. On the other hand, Isopulegol, that has a cyclic form, has a base peak of 67. This fragmentation results from the loss of a water molecule to form a fragment of m/z 136, that undergoes further fragmentation to give the ions with masses of 93,81 and 55, that are all present in high abundances.

Therefore, the use of Mass spectra for the analysis of Terpenes and cannabinoids was crucial for the differentiation of the compounds targeted in this study. In addition, when the study involves extracts from plant materials and complex matrices containing extracts of natural origin the use of mass spectrometry is essential. When compared to other detection methods such as UV or FID, mass spectrometry allows the laboratory to identify compounds that have not been included in the development of the method. This is especially important for Cannabis products due to the array of Cannabinoids and terpenes that have been described as secondary metabolites of Cannabis plants (Machado et al., 2022; Mudge et al., 2018)

As more information emerges on the use of Cannabinoids and terpenes, the use of mass spectrometry in scan mode can allow retrospective examination of extracts injected, as well as enhance the analytical methodology by the introduction of other cannabinoids and that are relevant for regulatory, monitoring and medicinal purposes.

4.4 Method calibration and linearity relating signal strength to concentration.

The chromatographic signal can be related to the concentration of the sample injected through the use of Calibration plots. However, it is essential to establish a linear corelation between the two variables. This was investigated during method validation, where the linearity of the methods was based on the corelation coefficient (R^2). The results showed that there was a direct and linear corelation between the analyte concentration and the signal in the chromatogram. The R^2 values for terpenes and cannabinoids were all above 0.99. Therefore, calibration plots and the acquired signals can be used to quantify cannabinoids and terpenes of unknown samples.

The range of the method is an essential quality parameter that ensures that the quantified samples are within the calibration range and therefore the signal intensity of the sample is directly relatable to its concentration through the linear equation. The measuring range of the method also was based on the linearity of the calibration curve. The limits were set between the Limits of quantification (0.1% for cannabinoids and 0.5µg/ml for Terpenes) and the Upper Quantification limit (35% for cannabinoids and 50µg/ml for Terpenes). The upper limit of quantification delineates the highest point at which the signal and concentration relationship is linear. Above this limit the molecules reaching the detector does not further increase the signal due to saturation (Wei et al., 2020). Therefore, samples that exceed the upper quantification limits would have to be diluted and analysed within the calibration range. The results showed that all Terpene compounds included had the same limits of detection and quantification. This was also the case for Cannabinoids, which had lower quantification limits, but the limit was the same for all the analytes.

Method calibration is based on the use of prepared standards, that introduces the element of deviation. Therefore, the Residual standard deviation of the calibration plots was calculated. The results for the relative bias show that the maximum deviation from the predicted values was less than 1.73 µg/ml for Terpenes and 0.26% for Cannabinoids. This indicated that the calibration curve is fit for purpose, and that the data points on the linear plots are a true indication of the relationship between the analyte concentration and the relative signal.

4.5 Precision and bias as quality attributes of the analytical methods

During method validation, the precision of the analytical methods was evaluated in terms of variance expressed as a percentage (%CV). The results in section 5.6 showed that the %CV was below the 10% limit described in the method performance characteristics. However, the variance is not indicative of the accuracy of the analytical method. The deviation expressed as relative percentage error %E was calculated from the quantitative data. The results showed that the %E. The deviation form the setpoints at the three concentrations tested (High, medium and low) showed acceptable degrees of deviation (<5% for terpenes and <2% for Cannabinoids). Therefore, the results show that the analytical method satisfies the accuracy and precision parameters.

Method repeatability and reproducibility are also essential criteria that guarantee an accurate and precise result over time. Quantitative data from 54 duplicate samples used for interday variation (1 day) and intraday variation (over 3 days) were subject to statistical analysis of variance (ANOVA, Single factor α=0.05). The resulting P values were less than 0.05 in all cases. Therefore, the null hypothesis was accepted.

These results indicate that there is no significant difference between the analytical data sets acquired in the same day and on different days, demonstrating that the analytical methods satisfy the repeatability parameter, and can produce precise results over time, when the operating conditions are maintained constant.

The method reproducibility (RSD_{rw}) was <10% for all analytes at the three concentrations levels tested. The results are an outcome of the relative standard deviation for reproducibility (RSDr) and the relative standard deviation between groups (RSDx), calculated through the analysis of variance. These results indicate that the method can be used to accurately quantify cannabinoids and terpenes over an extended period, that is usually capped at 5 years, when the analytical method would need to be verified through a revalidation process.

Testing for bias, is a quantitative and directional measure of the deviation of a quantitative result from a setpoint or an expected result. The results presented for laboratory, sample, and duplicate bias showed a relative percentage error that was below the specified limit (<10%)

The results show that the analytical methods have a positive bias of <0.5% for laboratory and sample bias. The duplicate bias was <3%. Both results were within the prespecified limits. The analytical methods can therefore be applied for their intended use.

The deviation between duplicate samples is an essential step in assuring accurate quantification of samples. Quantification samples are prepared in duplicate, and analysed in duplicate. Therefore, the bias between individual duplicates of <3% shows that the analytical methods can produce at least concordant results. This was also reflected in the results for sample and laboratory bias, where the overall bias when considering the average of the

concordant results is <0.5%, that can be attributed to random systematic errors during processing.

4.6 Quantifying Uncertainty of measurement related to quantification results.

Any quantitative value is subject to uncertainty of measurement. The expanded uncertainty of measurement is a quantitative indication of the range around which the quantitative result is expected to lie. The expanded uncertainty was satisfactory for all analytes with uncertainty budgets of less than 20%. The result reflects the combined uncertainty resulting from unexplained variations from analytical equipment including detection instruments and analytical balances, uncertainties in pipetting, uncertainty associated with the calibration curve and the reference standards used to build the curve. The final value is multiplied by a coverage factor (k=2) to account for the distribution of values which could be reasonably attributed to the measurand.

The results for Uncertainty, were calculated at 3 concentration levels to account for variability across the measuring range. The results for the expanded uncertainty of terpenes at higher concentrations is significantly lower (10.6% at 50µg/ml) than that observed at low concentrations (18.4% at 5µg/ml).

4.7 Development of quality parameters through Internal Quality control monitoring

There are many parallels between good practices and laboratory validation strategies as these are indicative of the level of certainty of results. The Validation results are also an important

requisite to set up quality control monitoring parameters. Variation patterns quantified during the validation process can be used as specification limits for quality control checks.

During method validation, the peak area calculated by peak integration and retention time data of the internal standard were used to delineate the quality parameters of the internal standard by applying standard deviation levels ±2 SD for warning levels and ±3 SD for Alarm levels. The use of an internal standard is a key parameter in quality control monitoring of analytical methodologies. Any deviations outside the set parameters in the retention time (dev. 0.02min) and the peak area of the internal standard is a clear indication that the result should be discarded. All the samples tested during validation did not exceed the warning levels. Therefore, Diphenylamine can be used as an internal standard for testing.The peak area of the internal standard also showed that the standard is stable over time (12 months), making it an ideal to use as an internal standard.

4.8 Validation strategy and application of ISO/EC 17025 standard.

The method validation strategy applied in this study was based on the standards set by the ISO17025/2017 standard for testing and calibration laboratories. This was an important aspect of the study, as it is envisaged that one of the applications of the testing methods would be as an assurance of consumer safety and product quality. Therefore, it is crucial that the testing laboratories performing these tests are held to the highest standard to produce accurate and reliable results.

These criteria have also been put in place to prepare the laboratory for the submission of documentation for the extension of scope to include the validated methods in its scope of accreditation.

Further work in this regard will include the formalisation of test methods in the form of a standard operating procedure (SOP), alongside other relevant quality documentation required, that include SOP's detailing the maintenance and calibration of the instrument, sample and batch processing forms and a schedule detailing the participation in proficiency testing schemes.

Product safety is a main goal of any regulatory agency, with laboratory testing of Cannabis products being a fundamental part of such process (Schauer GL, 2021). Studies have highlighted the lack of method standardization and validation (Pruyn SA et al., 2022) in testing procedures. Laboratories also need the ability to keep up to pace with continuous innovation of cannabis-based products, with the introduction of different matrices and compounds.

These challenges were determining factors during the development stages of this study. In order to counteract such challenges, the developed analytical techniques were validated in accordance with ISO/EC17025 standard, whilst the sample preparation procedure was validated on multiple matrices, so that it can be applicable to a multitude of matrices and novel compounds. It is envisaged that, in addition to a robust testing regimen, the application of the developed methods and quality criteria associated with testing are essential to product safety.

4.9 Verification of test methods using samples of known quantities.

Following method validation, verification tests were done to demonstrate that the analytical method is suitable for the analysis of the targeted matrices (Herbal materials, cannabis extracts and resin). The samples used in the verification had known quantities of the analytes of interest.

The results showed that the identification of target analytes was accurate in the method verification samples tested that included samples of herbal materials, cannabis extracts and cannabis resin. Quantitative determinations of the constituents were within the allowed deviation limits of 10%.

These results confirm that the analytical methods can be used for the identification and quantification of different sample matrices from different sources.

4.10 Analytical methods and prospective data as major outcomes

The development and validation of the analytical techniques was concluded successfully with both methods satisfying all method performance characteristics for all the 22 Cannabinoids and Terpenes targeted in this study. The analytical data was also used to set up an internal quality monitoring process to ensure accurate and reliable data is obtained during sample processing.

The developed and validated analytical techniques will be used for the quantification of Cannabinoids and Terpenes in Cannabis products found on the local market. The latter will provide the necessary scientific knowledge, from an analytical perspective that can have significant impacts on the harmonisation and formulation of regulatory frameworks for cannabis products.

The testing procedures can also be applied to the distinction of products containing cannabis. The applicability of the analytical methods to a diverse range of matrices can provide a wide

outlook on cannabis products available in Malta including products licenced for medical use, Cannabis products from the illicit market and unregulated Cannabis based products.

4.11 Regulatory implications of analytical data for cannabinoid concentration in cannabis products

One of the major implications sourced from the data in this study is the identification of Low THC products with variable concentrations of THC. Quantitative data presented in Appendix 3 demonstrates that 88% of products exceeding the 0.2% limit. It has been noted that the amount of THC in these products may vary, for two main reasons. The first reason is the lack of harmonisation on the definition of these products, such as products tested during the implementation of the research methods originating from the US claiming to be 'Hemp based' have shown to contain THC-levels that exceed the 0.2% limit imposed in local legislation. Secondly, the lack of the application of good manufacturing practices and robust analytical methodologies allow these products to deviate outside product intended specifications.

The data also highlights a marked difference in the results obtained from medicinal cannabis-based products. The tested products showed to be in line with required specification for cannabinoid content. The marked differences highlight the need of good manufacturing and laboratory practices in the medicinal cannabis industry, resulting in high quality and safe products.

The latter inherently provides a regulatory challenge. The data collected in this study suggests that there should be a harmonised limit of THC in products marketed as 'Low-THC', 'hemp' or CBD-products. It is also essential that, such products should also be regulated and tested

in the destination country, to ascertain that the products are within legal and stipulated limits. The methods developed and described in this study may be used by control laboratories as a fast, efficient, and low-cost method to test the product at destination markets. The method design, based on full scan mass spectrometry also allows laboratories to identify products containing semi-synthetic and synthetic cannabinoids that may be added to cannabis-based products, giving authorities an essential tool to regulate the cannabis market and ensure the safety of consumers.

The mean percentage of THC in Cannabis inflorescence samples quantified in this study, (presented in Appendix 1) was 14.76% (W:W), which is indicative of a relative increase of 25% in concentration from 11% over the past two years [21]. This trend has also been observed in global cannabis markets, where a similar increases from 9.75% to 14.88% over a period of 9 years were documented (ElSohly et al., 2021; Potter et al., 2018).

Of particular concern is the infiltration of the local market by high-purity THC product. During the analysis of products available on the local market, 6 products containing more than 60% THC content were identified, in oleoresins and solidified extracts (shatter). These findings have also been replicated in by Cuttler et al.,(2021) and El Sohly et al., (2021).

This gradual increases of THC in cannabis products, and the introduction of products with high (60-68%) concentrations of THC represents a potential health risk for consumers, as products containing more than 25% THC have been directly associated with adverse effects

[21] Government of Malta, Ministry for Social Policy and Children's Rights,(2024) National Report on Drugs 2023. Accessed via: https://familja.gov.mt/wp-content/uploads/2023/04/National-Drug-Report-EN.pdf

related to mental health, cardiometabolic and gastrointestinal system (Bero et al., 2023; Karoly et al., 2022).

These results indicate indicates a need for the introduction of regulations to limit the availability of high-THC products, in conjunction with clear and effective labelling practices that indicate the dosage regimen to limit the risk of adverse effects.

4.12 Regulatory implications relating to the presence of semi-synthetic cannabinoids in cannabis products

During the application of the validated methodology, 13 samples contained Semi-synthetic cannabinoids that included HHC, HHC-0, HHCP and THCP. None of the latter originated from medicinal cannabis-based products. The semi-synthetic derivatives of cannabinoids were flagged by early warning systems of synthetic drugs in Europe in 2023 [22], and were also introduced to the local markets due to the lack of legal controls on such substances.

The identification of these compounds highlights the applicability of the validated methods to identify chemically distinct cannabinoids, that may be added as adulterants or replacements in Cannabis products. These findings tie in with reports from the EMCDDA about the identification of these products across Europe [23], which raise regulatory and safety concerns to Cannabis consumers.

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[22] European Monitoring Centre for Drugs and Drug Addiction. (2023). *Hexahydrocannabinol (HHC) and related substances. Technical report*. Luxembourg: Office for Official Publications of the European Communities. https://doi.org/10.2810/852912.

[23] European Monitoring Centre for Drugs and Drug Addiction. (2023). *Hexahydrocannabinol (HHC) and related substances. Technical report*. Luxembourg: Office for Official Publications of the European Communities. https://doi.org/10.2810/852912.

Whilst semi synthetic cannabinoids such as HHC, are promoted as naturally derived products(Ujváry, 2023b) due to their origin from other cannabinoids extracted from hemp, the modifications in the molecular structure of these compounds, especially acetylation, can significantly change the pharmacological properties of the molecule by altering their pharmacokinetic and pharmacodynamic profiles (Graziano et al., 2023).

Additional concerns with cannabis products adulterated with semi synthetic cannabinoids revolve around the mislabelling of products. Two samples in which HHC-O and HHC were identified , mixed with THC and CBD , were marked as products containing HHC-P, and 'no THC'. These claims were also observed on a testing certificate packaged with the product. This trend has also been observed in other European markets (Caprari et al., 2024; Helander et al., 2024). These findings indicate that an analytical certificate of these products does not necessarily portray the real constituents found in the products.

Given the unpredictable risks associated with these molecules, and the influx of these products on the local market, the data collected from the analysis of these products indicates that a robust regulatory framework for cannabis-based products should seek to limit the influx of substances with unknown pharmacologic properties, thereby proactively mitigating the introductions of similar substances in the future.

4.13 Regulatory indications of product conformance

Medicinal cannabis-based products on the local market showed that the deviation in labelled content was <5% whilst the levels of Cannabinol were <1%. The analytical data presented in Appendix 1-3, also showed significant deviation (>±10%) from labelled content in 28.3% of products labelled as containing 'CBD oils or extracts'. Whilst 36% of these products also contained >1% W:W of Cannabinol. It was also noted that a number of products tested did not have the appropriate expiry date on the product. These findings replicate the trends observed in other markets such as the US, where 25% of products significantly deviate from labelled content (Vandrey et al., 2015).

The deviation in labelled content and the presence of degradation products might have resulted either from a lack of control in the production of the products or the absence of studies evaluating the shelf life of such products. The lack of appropriate stability studies and expiry dates on products was also flagged by researchers in Canada, whereby the authors assessed product labels, and identified the manufacturing details and expiry dates as a crucial part of information that should be assessed by manufacturers and included in product labelling (MacCallum et al., 2023).The analytical data indicates essential regulatory measures that should be implemented, mainly the introduction of a framework that regulates product labelling and the evaluation of product stability before it is placed on the market. This measure is already in place for medicinal cannabis-based products in Malta [24].

[24] Malta Medicines Authority, General Guidelines on the production of cannabis for medicinal and research purposes*, (2022) retrieved on 20/11/2023*

4.12. Regulatory indications relating to terpene profile variability

The developed assay allows for the fast and reliable identification of major terpenes. The procedure involves a simple extraction followed by GC-MS analysis, with a run time of 22 minutes. Compared to published analytical methodologies, this method provides equally accurate and reliable results in a more efficient manner.The method features a reduced chromatographic run time, which is 15-50% faster than published methodologies (Dei Cas et al., 2021; Hanuš and Hod, 2020) increasing the number of analyses that can be performed on the same instrument and reducing the burden of instrument maintenance.

One of the major issues faced by care givers and self-treating patients who make use of cannabinoid product is the inconsistency and the lack of reproducibility of the products (Lewis et al., 2018). Whilst to date there isn't enough data to support claims of efficacy of cannabis based products in specific conditions, the use of a consistent mix of compounds is essential in maintaining treatments especially in chronic conditions (Lewis et al., 2018). It is anticipated that the developed assay may be used in various applications related to the regulation of cannabis products and the enhancement of scientific knowledge to further develop condition-targeted, cannabis-based medicinal products as well as its application in quality testing laboratories seeking to assess the quality of terpene compounds in cannabis based products.

During method verification, it was noted that the developed assays can identify the most common terpene compounds found in cannabis-based products. The use of this method has provided important data on the terpene composition of these products. This data can be used to gather analytical information linking the physiological activity of a product to its volatile compound composition. Such an example is Beta-myrcene, that was identified in 90% of the

samples tested. Therefore, the concentration of this terpene compound may be related to more pronounced anxiolytic and sedative physiological response (Koziol et al., 2015). This process was also used, in a limited manner, by Dei Cas et al. (2021). The authors identified a similar application for their method; however, their study was limited to inflorescence samples only, and they noted that the sample pool was not homogeneous due to limited accessibility to samples.

The results for the identification of terpene compounds in CBD oils extracted from hemp varieties that are not regulated as medicinal cannabis products may assist in informing users about their possible physiological effects. Quantitative and qualitative data from 40 samples , which were profiled during this study are presented in Appendix 4, alongside graphical evaluations of terpene concentration in these products. Beta-caryophyllene, alpha-pinene, terpinolene, beta-myrcene, linalool, and limonene were identified in 87% of these oils. This trend was also observed in hemp-based inflorescence marketed as 'Low-THC' products. Conversely, the quantity of the monoterpene myrcene in oils was 67% lower than that identified in hemp inflorescence. The significant depletion of monoterpenes in oils was also the subject of a study evaluating the differences between cannabis inflorescence and cannabis extracts using Supercritical CO_2. The authors findings align with the data in this research, concluding that the extracts have a significantly different chemotypic fingerprint when compared to the starting material (inflorescence) (Sexton et al., 2018).

The physiological effects of hemp extracts were studied by Gulluni et al. (2018b), who concluded that inhalation of extracts containing mainly beta-myrcene, beta-caryophyllene, alpha-pinene, and terpinolene led to several physiological changes such as decreased diastolic

blood pressure, increased heart rate, and increased skin temperature. Therefore, while both hemp-based oils and inflorescence products may have synergistic effects related to the presence of Terpene compounds, the findings of this study suggest that the physiological effects of terpenes may be less pronounced in oil-based extracts, due to the lower concentration of monoterpenes.

This research could not provide statistically significant links between products and their claimed effects due to the limited number of samples from each product type. However, the results across product types indicate that this may be a noteworthy application for the developed assay, in the continued effort to inform consumers and identify false claims by manufacturers.

Whilst, evaluating samples of oils labelled as ‘full spectrum extract’ or ‘whole extract’, it was noted that 10% of these oils were devoid of volatile compounds, 5% had trace amounts of terpene compounds. Therefore, it was clear that the findings of this research contradict the manufacturer’s claims.

The conservation of the metabolome of the plant during production of cannabis products should therefore be an essential part of a validated manufacturing process (Lazarjani et al., 2021b). giving assurance that the extract mirrors the composition of the starting material. The loss of volatile partition of the extract was also noted in the findings of Ternelli et al (2020b) which aimed to provide a protocol for the extraction from cannabis inflorescence to obtain an extract rich in cannabinoids and terpene compounds, which indicated similar losses to this research, that could be associated with several factors such as temperature. These losses have also been associated with raw material inconsistencies (Milay et al., 2020) or issues with

product manufacturing (Lazarjani et al., 2021b) might include product irradiation, that has been shown to reduce the volatile component of Cannabis (Hazekamp, 2016).

The presence of Terpene compounds in Cannabis based products has gained significant interest (Casano et al., 2011; Nuutinen, 2018; Russo, 2011), Terpene profiling plays a crucial role in demonstrating the identity and medicinal effectiveness of various cannabis cultivars or strains (Casano et al., 2011) .

To date, national guidelines for the production of medicinal cannabis-based products [25], do not specify any requirements related to terpene content. The information gathered during this study, and the literature supporting claims of direct and synergistic physiological effects of terpene compounds (discussed in section 1.4) indicate that in order to address concerns on the therapeutic efficacy, consistency and the quality of cannabis based products, quality control and verification testing should include Terpene compounds, especially in products licenced for medicinal use. Guidelines issued by the controllers for cannabis products in New York state [26], have adopted this matter for all cannabis-based products, where a basic terpene profile and any terpenes added to the product should be listed on the packaging.

It was observed that in published pre-clinical studies using cannabis extracts (Baron et al., 2018), the authors assume that the chemical composition of the plant is directly transferable to the extract without verifying the compositions of the extract. Therefore, it is anticipated

[25] Malta Medicines Authority, General Guidelines on the production of cannabis for medicinal and research purposes*, (2022) retrieved on 20/11/2023*

[26] New York State Office of Cannabis Management. (2023, March). Packaging, labelling, marketing, and advertising guidance.

that the introduction of terpene profiling in regulatory frameworks should enhance product efficacy and assist in the development of medicinal products based on cannabis extracts.

4.13. Limitations of the study

Despite the thoroughness of the research project, during its final evaluation a number of limitations inherent to this study have been identified. The points below serve to contextualise the findings of this research and offer insights into avenues for future research.

The major limitation in this project is that the evaluation does not cover all the pillars of quality testing for cannabis products. The research focused on the chemical composition of the products due to temporal and financial constraints, as well as limitations in equipment availability. Other potential aspects such as microbiological contamination and chemical contaminants were not evaluated.

The unavailability of analytical standards for semi synthetic cannabinoids (HHC, HHC-P, THCP and HHC-O) identified during the second phase of the research project limited the applicability of the developed assays in identifying the presence of these adulterants, without allowing for the possibility of generating quantitative data. This was also limited by the inaccessibility to other analytical techniques such NMR, which allows for the quantification of cannabinoids in absence or standards (Barthlott et al., 2021; Moosmann et al., 2012; Peschel and Politi, 2015b).

Insufficient details provided on the products on product manufacturing dates limited data on degradation products such as CBN, and terpene profiles whereby it could not be distinguished

if the degradation product resulted from improper manufacturing processes or product stability in the final packing that led to the degradation of the active cannabinoids.

The samples used in the analysis were derived from a single batch of the product due to the limitations in terms of the availability of samples. Therefore, it was not possible to evaluate inter-batch consistency that is an essential trait of a high-quality cannabis product.

4.14. Further research

Although this study has provided valuable insights on the analysis, and chemical composition of cannabis products, the research identified several aspects that remain unexplored.

Further research endeavours should focus on increasing the scope of this study to several quality aspects that were not included in this study such as the assessment of chemical contaminants including pesticides and heavy metals in cannabis products, and the development and validation of molecular methods for the microbiological testing of cannabis products.

The scope of the project may also be expanded by investigating inter-batch variability by testing the same products from different batches and by expanding the validation parameters to other dosage forms not included in the method verification process.

It is envisaged that these areas would significantly contribute to a wide reaching, harmonised regulatory framework, aimed at enhancing product quality and safety.

4.15. Conclusion

An analytical procedure targeting major Cannabinoid and Terpene compounds in cannabis based products was developed. The analytical procedure based on a rapid extraction and analysis on GC-MS was shown to provide accurate and reliable results by satisfying all the quality criteria included in the extensive validation process conducted.

The developed assay was successfully implemented during the analysis of different cannabis based products available on the local market, that included inflorescence, herbal preparations, and extracts. The analytical data indicated a significant difference in the quality traits of cannabis products available on the local market, that included significant (>10%) deviations from labelled content, deviations from the 0.2% THC limit enacted in Maltese legislation, and terpene profiles that were inconsistent with product claims. Such divergences were not identified in medicinal-cannabis based products. During the application of the analytical technique the data highlighted regulatory and safety aspects relating to the presence of semi-synthetic cannabinoids.

The inferences acquired through quantitative and qualitative analytical data highlight essential regulatory perspectives and quality parameters that are pivotal for the regulation of Cannabis based products. These are mainly related to the introduction of limits for degradation compounds, enhancing manufacturing quality, product consistency, and adherence to legal limits of THC.

The research presented provides pivotal analytical data and regulatory aspects to develop a harmonised regulatory framework for cannabis based products focused on product quality and safety.

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Appendices

Appendix 1: Quantification of Cannabinoids in inflorescence samples from investigative sources.

Table 1: Quantification of cannabinoids in Herbal materials from investigative sources originating from the illicit or unregulated trade of Cannabis products.				
Sample Reference number.[27]	**Type**	**%THC**	**%CBD**	**%CBN [28]**
16	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	15.9	0.8	5.52
100		12.9	3.9	0.05
69		12	1.2	4.16
91		15.5	0.5	5.36
63		22.4	1.4	3.36
72		14.7	0.6	2.4
50		7.9	0.5	0.4
104		17.8	0.4	10.32
60		10.1	0.9	0.35
89		14	0.9	4.48
67		10.4	0.7	4.72
61		11.9	10.8	5.36
25		5.8	1.8	3.1
28		10.1	10.9	0.45
62		11.9	1.9	4.64
86		10.2	10.9	4.16
53		12.9	0.6	4
5		8.7	0.5	0.5
2		9.9	0.7	0.5
110		12.2	5.4	5.12
20		16.6	0.5	11.92
9		15.7	7.6	0.4

[27] Sample reference number refers to a unique number assigned to a sample in chronological order of sample receipt in the sample registration log.

[28] Data entries highlighted in Green are compliant with the 1% W:W specification limit for Cannabinol in Cannabis product in accordance with Table 3.35 Evaluation criteria set for product compliance evaluation shown in pg 91.

19	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	11	0.9	3.92
27		18.1	0.6	11.52
94		15.8	0.5	4.32
18		8.9	0.9	0.05
65		13.9	3.4	4.88
57		22.6	1	2.4
31		15.3	0.8	3.36
4		17.2	0.6	10.96
54		8.7	2.4	0.3
38		9.3	6.8	1.55
26	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	9	2.6	0.15
55		15.8	1	3.6
45		23.8	0.9	4.2
51		10.6	2.9	0.3
76		15.3	0.4	4.08
10		5.4	0.9	0.25
44		10.9	10.5	4.8
42		10.6	10.6	0.1
13		13	3.6	2.56
77		18.9	0.6	3.6
58		13.4	1.2	5.04
108		12	2.8	4.72
52		8.4	2.6	0.1
109		12.2	2.5	3.2
80		20.8	0.5	9.6
101		26.7	1.4	10.16
3		15.1	0.6	0.5
111		10.3	1.03	3.36
49		14.3	0.5	2.72
68		14.8	0.6	4.16
105		17.6	3.5	9.68
33		29.2	1.6	9.92
30		7.4	0.8	0.35
95		15.4	0.4	8.75
71		12.6	1.4	3.12
34		16.7	1.2	11.52
74		15.3	1.6	2.96
47		9.9	0.9	0.25
8		11.3	2.8	0.4
17		18	1	3.7

39	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	6.6	0.5	0.05
78		24.5	0.7	10.8
40		28	0.9	10.08
22		8.6	10.8	0.4
7		12.5	0.7	0.5
56		22.7	0.8	4.72
66		17.2	0.5	9.68
79		28.2	1.5	10.08
14		32.9	0.8	10
29		8.1	4.5	0.25
1		14.2	0.7	10
11		17.8	4.9	0.2
107		15	0.7	9.92

Table 2: Data summary for Quantification of cannabinoids in Herbal materials from investigative sources originating from the illicit or unregulated trade of Cannabis products (%W:W).

	%THC	%CBD	%CBN
Average	**14.55**	**2.30**	**4.25**
ST-DEV	**5.71**	**2.88**	**3.70**
Median	**13.90**	**0.90**	**3.70**
Max	**32.90**	**10.90**	**11.92**
Min	**5.40**	**0.40**	**0.05**

Table 3: Evaluation of product compliance for the presence of CBN [2]

N (samples)	**75**
N <1%limit	**23**
N deviate <1%limit	**52**
% deviating samples	69.3

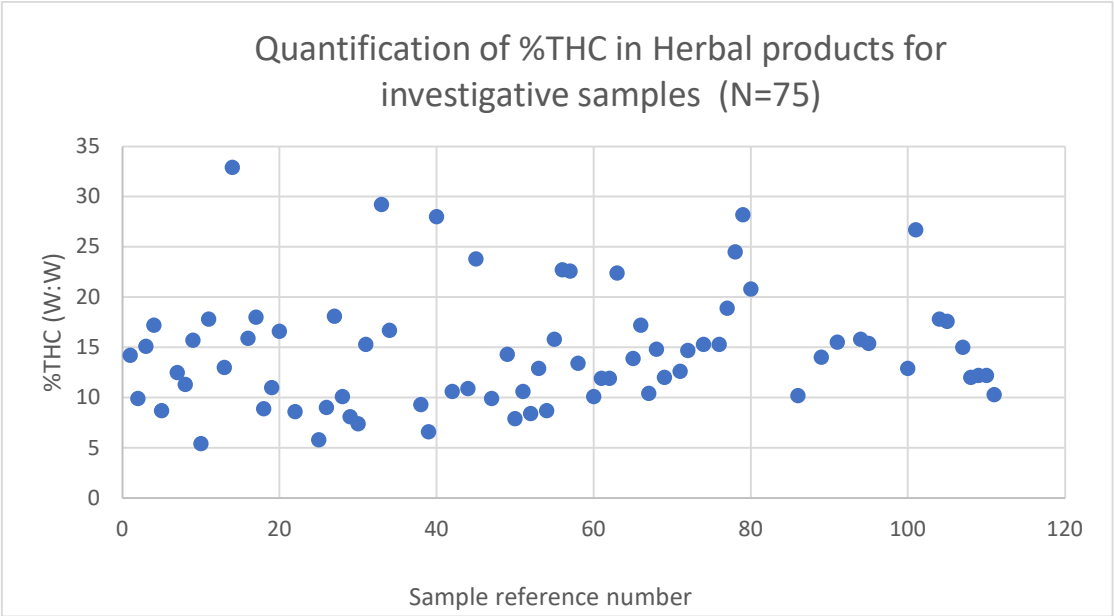

Figure 1: Scatter plot showing %THC concentration in Inflorescence samples from investigative sources (N=75)

Appendix 2: Quantification of Cannabinoids in inflorescence samples from Medicinal cannabis based products.

Table 4 Quantification of Cannabinoids in inflorescence samples from Medicinal cannabis based products.					
Sample Reference number.[29]	**Type**	**%THC**	**%CBD**	**%deviation of THC from labelled content[30]**	**%CBN [31]**
32	Medicinal Cannabis Inflorescence samples from local suppliers. (Solid Matrix)	22	0.8	0	0.8
73		22.8	0	-4	0.4
84		21.2	0	4	0.1
103		22.8	0	-4	0
106		23	0	-5	0.01
15		20.9	0	5	0.5
70		22.6	0	-3	0.8
21		22.7	0	-3	0.8
81	Medicinal Cannabis Inflorescence samples from local investigative authorities.	22.8	0	-4	0
46		21	0	5	0.6
83		23	0	-5	0.01
36		22	0	0	0.05

[29] Sample reference number refers to a unique number assigned to a sample in chronological order of sample receipt in the sample registration log.

[30] Data entries highlighted in Green are compliant with the maximum deviation of ±5% deviation of THC concentrations from labelled content in accordance with Table 3.35 Evaluation criteria set for product compliance evaluation shown in pg 91 and the General Guidelines on the Production of cannabis for medicinal and research purposes, Malta Medicines Authority, 2022.

[31] Data entries highlighted in Green are compliant with the 1% W:W specification limit for Cannabinol in Cannabis product in accordance with Table 3.35 Evaluation criteria set for product compliance evaluation shown in pg 91.

102	(Solid Matrix) Medicinal Cannabis Inflorescence samples from local investigative authorities. (Solid Matrix)	22.1	0	0	0.23
6		22.4	0.3	-2	0
75		21.8	0.6	1	0.12
88		23	0	-5	0.23
37		22.8	0	-4	0.25
43		22.7	1	-3	0.1
87		20.9	1	5	0.0

Table 5: Data summary for quantification of Cannabinoids in inflorescence samples from Medicinal cannabis-based products (%W:W).

	%THC	%CBD	%CBN
Average	22.24	0.19	0.26
ST-DEV	0.73	0.40	0.18
Median	22.25	0.00	0.09
Max	23.00	1.00	0.60
Min	20.90	0.00	0.00

Table 6: Evaluation of product compliance for Medicinal cannabis-based product deviation of THC[4] from labelled content and levels of CBN[5]

N (samples)	19
N within specification for THC and CBN	19
N exceed specifications for THC and CBN	0
% deviating samples	0.0

Appendix 3: Quantification of Cannabinoids in cannabis herbal products and cannabis extracts marked as 'Low THC' Cannabis products.

Table 7: Quantification of cannabinoids in Herbal materials categorized as 'Low-THC'						
Sample Ref. No.[32]	**Type**	**%THC [33]**	**%CBD**	**%CBD-Labelled content**	**%deviation of CBD from labelled content[34]**	**%CBN [35]**
98	Cannabis herbal material (solid matrix from local retail shops	0.01	12	15	20	0.8
99		0.05	5	15	67	0.4
12		0.23	16	15	-7	0.1
82		0.24	5	10	50	5
41		0.33	22	20	-10	0.01
97		0.54	10	12	17	0.5
93		0.6	16.8	15	-12	0.8
23		0.82	5	8	38	0.8
24		0.86	18	20	10	0

[32] Sample reference number refers to a unique number assigned to a sample in chronological order of sample receipt in the sample registration log.

[33] Data entries highlighted in Green are compliant with the maximum limit of 0.2% THC W:W concentrations from labelled content in accordance with Table 3.35 Evaluation criteria set for product compliance evaluation shown in pg 91 and the Limits set forth by the provisions of the Dangerous drugs ordinance (Ch 101), Laws of Malta.

[34] Data entries highlighted in Green are compliant with the maximum deviation of ±10% deviation of THC concentrations from labelled content in accordance with Table 3.35 Evaluation criteria set for product compliance evaluation shown in pg 91 and the General Guidelines on the Production of cannabis for medicinal and research purposes, Malta Medicines Authority, 2022.

[35] Data entries highlighted in Green are compliant with the 1% W:W specification limit for Cannabinol in Cannabis product in accordance with Table 3.35 Evaluation criteria set for product compliance evaluation shown in pg 91.

112	Cannabis herbal material (solid matrix from local retail shops	0.9	8	10	20	0.6
64		1	15	12	-25	3.5
85		1.5	19	25	24	6.4
90		1.3	20	16	-25	2.1
92		1.4	9.4	10	6	0
96		1.5	8	10	20	3.4
59		2.2	22	50	56	12
35		2.1	18	12	-50	4.7
48		3	8	10	20	2.5
122	Cannabis trim (sugar leaves and fan leaves) (solid matrix) from local retail shops	1.2	12	15	20	3.3
123		1.8	13	15	13	0.9
124		0.9	15	22	32	0.6
125		1.1	8	15	47	3.2
126		0.15	12	15	20	0.6
129	Coarse ground herbal cannabis material rolled in smoking paper (solid matrix) from local retail shops	0.48	6	10	40	7.0
131		1.6	8	10	20	0.7
133		1	3	5	40	0.4

Table 8: Data summary for quantification of Cannabinoids in inflorescence samples from Medicinal cannabis-based products (%W:W).

	%THC	%CBD	%CBN
Average	1.36	12.02	3.05
ST-DEV	0.65	5.27	3.03
Median	1.30	12.00	2.50
Max	3.00	22.00	12.00
Min	0.15	3.00	0.00

Table 9: Quantification of cannabinoids in oil-based preparations categorized as 'Low-THC'						
Sample Ref. No.[36]	**Material**	**%THC [37]**	**%CBD**	**%CBD-Labelled content**	**%deviation of CBD from labelled content[38]**	**%CBN [39]**
228	Cannabis extracts diluted in Medium-chain Triglycerides (Liquid matrix) from local retail shops	2.2	26	15	-73	5
229		1.5	9	10	10	0.15
230		3.4	5	2	-150	2
231		0.1	4.8	6	20	1.6
232		1.3	10	6	-67	2.4
233		1.1	12	16	25	1.4
234		1.6	18	19	5	0.05
235		0.89	26	23	-13	3.8
236		0.16	20	21.2	6	0
237		1.4	25	28	11	2.8
238		1.6	6	10	40	4
239	Cannabis extracts diluted in	1.1	5	5	0	0
240		1.5	16	20	20	6.4
241		2.1	12	11	-9	1.3

[36] Sample reference number refers to a unique number assigned to a sample in chronological order of sample receipt in the sample registration log.

[37] Data entries highlighted in Green are compliant with the maximum limit of 0.2% THC W:W concentrations from labelled content in accordance with Table 3.35 Evaluation criteria set for product compliance evaluation shown in pg 91 and the Limits set forth by the provisions of the Dangerous drugs ordinance (Ch 101), Laws of Malta.

[38] Data entries highlighted in Green are compliant with the maximum deviation of ±10% deviation of THC concentrations from labelled content in accordance with Table 3.35 Evaluation criteria set for product compliance evaluation shown in pg 91 and the General Guidelines on the Production of cannabis for medicinal and research purposes, Malta Medicines Authority, 2022.

[39] Data entries highlighted in Green are compliant with the 1% W:W specification limit for Cannabinol in Cannabis product in accordance with Table 3.35 Evaluation criteria set for product compliance evaluation shown in pg 91.

242	Olive oil (Liquid matrix) from local retail shops	0.9	25	22	-14	2.6
243		1.2	8	9	11	0.2
244		3.7	13	10	-30	4

Table 10: Data summary for quantification of Cannabinoids in inflorescence samples from Medicinal cannabis-based products.

	THC	CBD	CBN
Average	1.51	14.16	2.22
ST-DEV	0.92	7.60	1.87
Median	1.40	12.00	2.00
Max	3.70	26.00	6.40
Min	0.10	4.80	0.00

Table 11: Percentage of products exceeding deviation limits for 'Low THC' herbal products and oils based on results shown in tables 7 and 9.

	Herbal products (N=26)	Oils (N=17)
% of samples exceeding 0.2% THC	92%	88%
% of samples exceeding ±10% deviation from labelled content CBD	85%	71%
% of samples exceeding 1.0% CBN	42%	65%

Appendix 4: Quantification of Terpenes in cannabis herbal products and cannabis extracts.

Table 12: Quantitative data for 19 Terpene compounds expressed as %W:W for Cannabis inflorescence (Solid matrix).

Sample number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
3,7-dimethyl-1,3,6-octatriene	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.23	0.05
3-Carene	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.03	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alpha-bisabolol	0.00	0.09	0.07	0.01	0.03	0.06	0.23	0.09	0.04	0.09	0.11	0.10	0.06	0.09	0.05	0.09	0.07	0.30	0.01	0.07
Alpha-humulene	0.44	0.00	0.25	0.23	0.00	0.02	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00
Alpha-Pinene	0.09	0.04	0.03	0.14	0.01	0.11	0.01	0.02	0.01	0.09	0.05	0.02	0.03	0.03	0.01	0.04	0.00	0.04	0.04	0.02
Alpha-terpinene	0.00	0.02	0.00	0.00	0.01	0.01	0.00	0.02	0.00	0.00	0.03	0.01	0.05	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Alpha-terpinolene	0.10	0.11	0.06	0.06	0.02	0.15	0.15	0.03	0.02	0.06	0.12	0.09	0.09	0.00	0.00	0.08	0.06	0.05	0.08	0.03
Beta-Pinene	0.07	0.03	0.05	0.10	0.01	0.10	0.00	0.01	0.01	0.00	0.04	0.00	0.07	0.00	0.05	0.04	0.03	0.00	0.06	0.01
Camphene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Delta-Limonene	0.33	0.05	0.31	0.31	0.03	0.09	0.03	0.08	0.05	0.05	0.05	0.13	0.08	0.10	0.00	0.08	0.07	0.07	0.43	0.00
Gamma-Terpinene	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Geraniol	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00
Guaiol	0.00	0.10	0.00	0.00	0.01	0.00	0.12	0.00	0.01	0.21	0.11	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00
Isopulegol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Linalool	0.06	0.05	0.02	0.16	0.07	0.08	0.15	0.12	0.00	0.06	0.06	0.16	0.07	0.41	0.16	0.00	0.06	0.09	0.08	0.04
Myrcene	0.05	0.06	0.52	0.02	0.03	0.51	0.03	0.05	0.08	0.06	0.06	0.07	0.06	0.09	0.00	0.06	0.87	0.06	0.13	0.04
Nerolidol	0.00	0.04	0.00	0.00	0.01	0.00	0.06	0.00	0.01	0.00	0.04	0.02	0.06	0.00	0.09	0.00	0.00	0.00	0.00	0.00
Para-Cymene	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.12
T-Caryophyllene	0.43	0.42	0.56	0.53	0.26	0.51	0.33	0.70	0.00	0.48	0.47	0.86	0.48	0.53	0.67	0.51	0.08	0.59	0.16	0.18
Total terpene content (%W:W)	1.57	1.00	1.85	1.56	0.53	1.65	1.12	1.13	0.29	1.12	1.13	1.47	1.07	1.46	1.03	1.20	1.74	1.19	1.22	0.57

Table 13: Quantitative data for 19 Terpene compounds expressed as %W:W for Cannabis extracts in oil (Liquid matrix).

Sample number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
3,7-dimethyl-1,3,6-octatriene	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3-Carene	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00
Alpha-bisabolol	0.03	0.01	0.03	0.02	0.08	0.03	0.00	0.00	0.01	0.00	0.03	0.01	0.02	0.02	0.01	0.06	0.00	0.02	0.03	0.02
Alpha-humulene	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.07	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Alpha-Pinene	0.01	0.00	0.03	0.04	0.00	0.01	0.00	0.04	0.00	0.00	0.01	0.01	0.01	0.00	0.03	0.00	0.00	0.01	0.01	0.00
Alpha-terpinene	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.02	0.00
Alpha-terpinolene	0.01	0.01	0.02	0.05	0.05	0.03	0.00	0.02	0.01	0.00	0.02	0.01	0.01	0.00	0.04	0.04	0.01	0.03	0.00	0.00
Beta-Pinene	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.00	0.00	0.01	0.01	0.01	0.02	0.03	0.00	0.00	0.02	0.00	0.02
Camphene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Delta-Limonene	0.03	0.01	0.02	0.03	0.01	0.04	0.00	0.10	0.01	0.00	0.03	0.04	0.02	0.00	0.02	0.01	0.00	0.03	0.03	0.00
Gamma-Terpinene	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Geraniol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Guaiol	0.00	0.00	0.07	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.03	0.00	0.00	0.05	0.00
Isopulegol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Linalool	0.04	0.00	0.02	0.03	0.05	0.05	0.00	0.05	0.02	0.00	0.00	0.03	0.07	0.05	0.02	0.04	0.00	0.02	0.14	0.05
Myrcene	0.02	0.03	0.02	0.17	0.01	0.02	0.00	0.01	0.01	0.00	0.02	0.08	0.02	0.00	0.14	0.01	0.00	0.02	0.03	0.00
Nerolidol	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.02	0.00	0.02	0.00	0.03
Para-Cymene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Trans-Caryophyllene	0.23	0.00	0.16	0.17	0.11	0.28	0.00	0.18	0.08	0.00	0.17	0.08	0.18	0.22	0.14	0.09	0.00	0.16	0.18	0.20
Total terpene content (%W:W)	0.37	0.10	0.37	0.54	0.37	0.48	0.00	0.51	0.17	0.00	0.40	0.34	0.39	0.34	0.45	0.30	0.01	0.35	0.48	0.31

Table 14: Average Concentration and Detection Frequency (% of tested samples) of Individual Terpenes in Herbal Products and Oils				
	Herbal products		**Cannabis extracts in oil**	
	Average conc. (%W:W)	**Frequency (% identification in samples)**	**Average conc. (%W:W)**	**Frequency (% identification in samples)**
3,7-dimethyl-1,3,6-octatriene	0.02	25	0.00	20
3-Carene	0.00	20	0.00	35
Alpha-bisabolol	0.08	95	0.02	85
Alpha-humulene	0.07	30	0.01	25
Alpha-Pinene	0.04	95	0.01	90
Alpha-terpinene	0.01	45	0.00	50
Alpha-terpinolene	0.07	90	0.02	75
Beta-Pinene	0.03	75	0.01	60
Camphene	0.00	0	0.00	0
Delta-Limonene	0.12	90	0.02	80
Gamma-Terpinene	0.00	5	0.00	10
Geraniol	0.01	20	0.00	25
Guaiol	0.04	35	0.01	40
Isopulegol	0.00	0	0.00	0
Linalool	0.10	90	0.03	75
Myrcene	0.14	95	0.03	75
Nerolidol	0.02	40	0.01	50
Para-Cymene	0.01	20	0.00	20
Trans-Caryophyllene	0.44	95	0.13	80
Average Total terpene content	**1.19**		**0.32**	

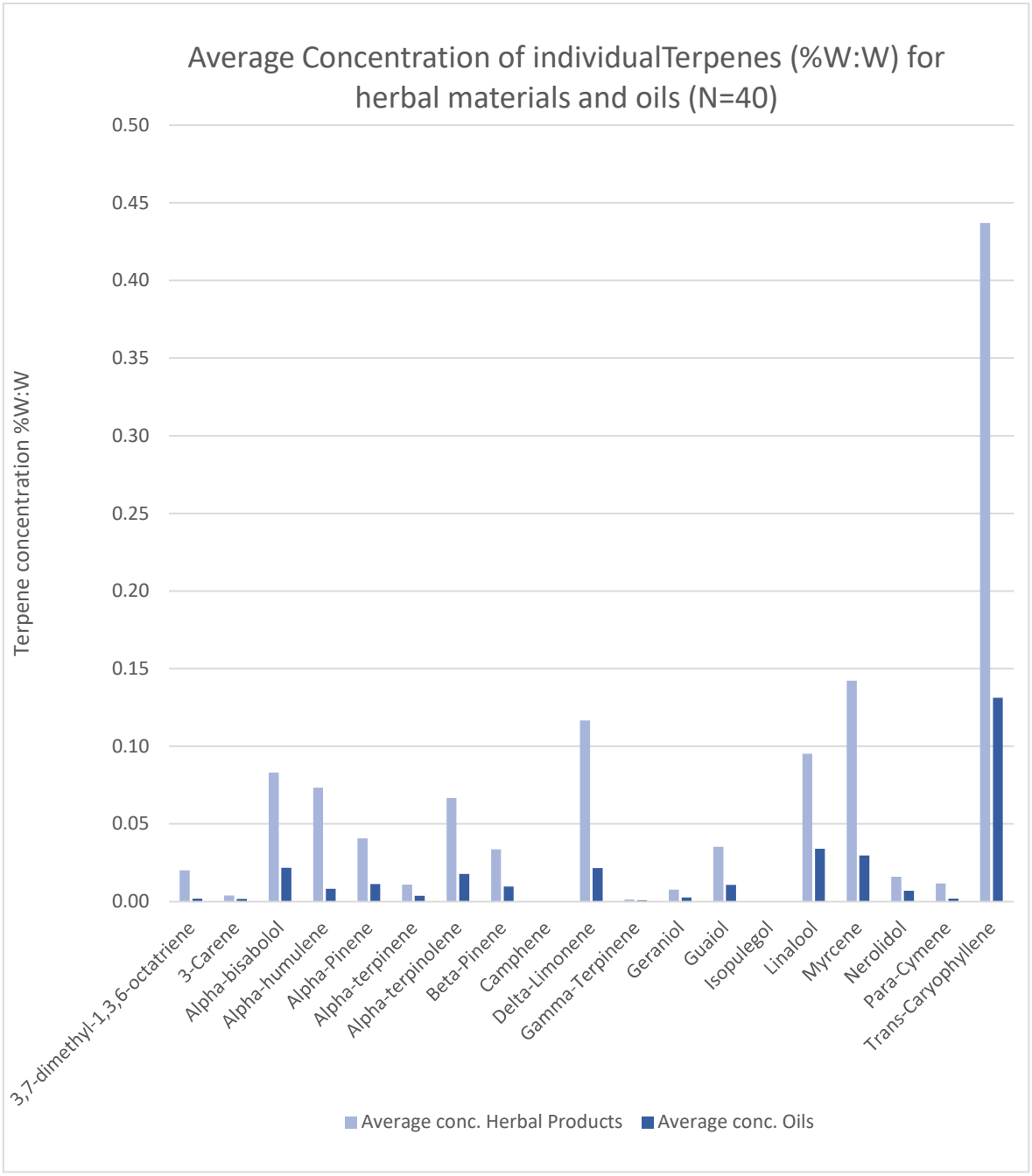

Figure 2: Average Terpene concentration for 19 terpene compounds in Herbal products and oils (%W:W)

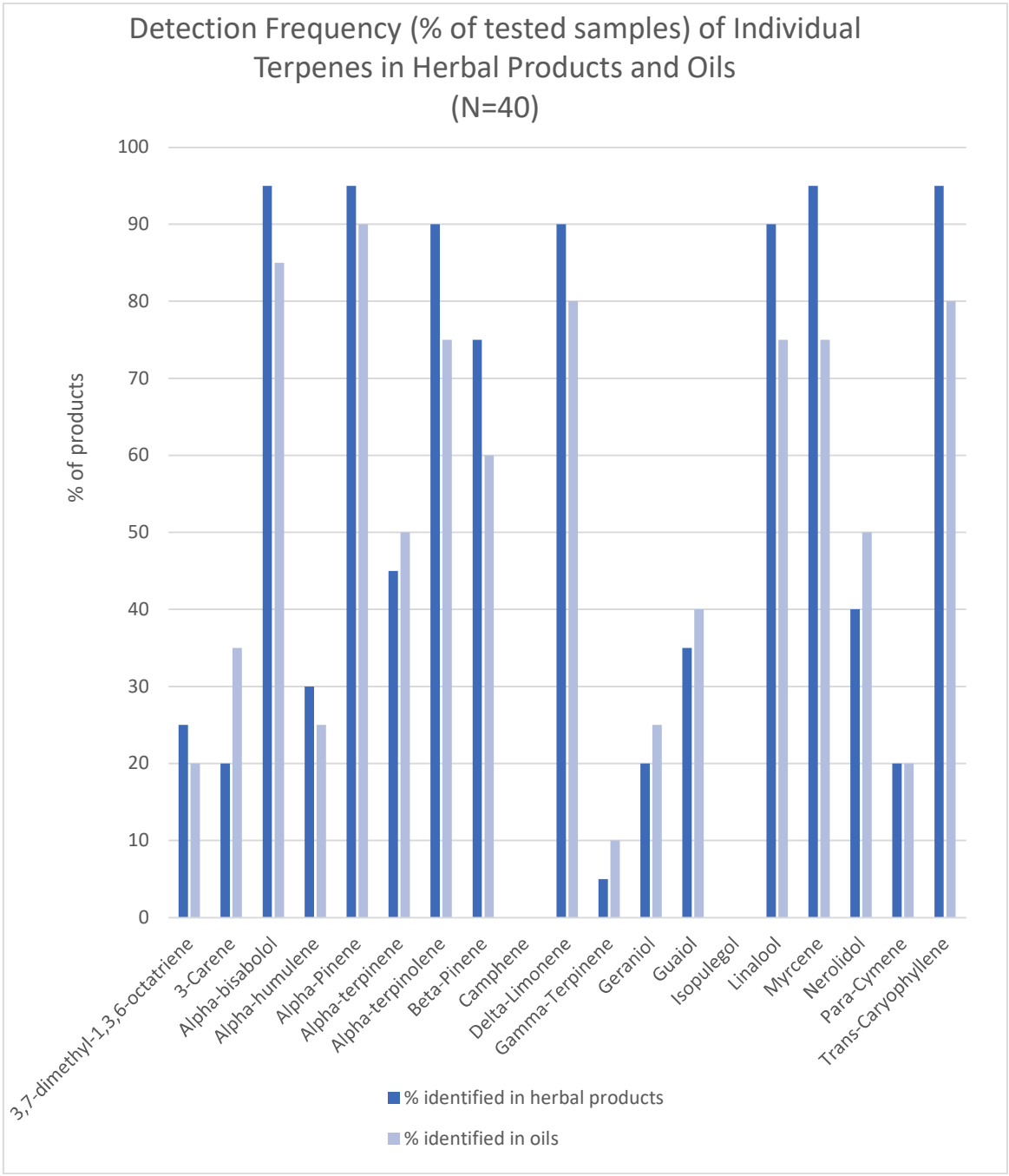

Figure 3: Detection Frequency (% of tested samples) of Individual Terpenes in Herbal Products and Oils (N=40)

Appendix 5: Sample registration log showing sample reference numbers, sample type and source and the date of receipt for samples analysed (N=137)

Table 15: Sample registration log showing sample reference numbers, sample type and source and the date of receipt for samples analysed (N=137)

Sample reference number	Sample type and source	Date received
1	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	09/07/2020
2	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	09/07/2020
3	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	15/04/2020
4	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	18/04/2020
5	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	22/04/2020
6	Medicinal Cannabis Inflorescence samples from local investigative authorities.	24/04/2020
7	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	24/04/2020
8	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	24/04/2020
9	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	01/05/2020
10	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	01/05/2020
11	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	01/05/2020
12	Cannabis herbal material (solid matrix from local retail shops	15/05/2020
13	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	22/05/2020
14	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	02/06/2020
15	Medicinal Cannabis Inflorescence samples from local suppliers.	25/06/2020
16	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	02/07/2020
17	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	06/07/2020
18	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	13/07/2020

19	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	22/07/2020
20	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	23/07/2020
21	Medicinal Cannabis Inflorescence samples from local suppliers.	11/08/2020
22	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	31/08/2020
23	Cannabis herbal material (solid matrix from local retail shops	03/09/2020
24	Cannabis herbal material (solid matrix from local retail shops	03/09/2020
25	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	03/09/2020
26	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	08/09/2020
27	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	10/09/2020
28	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	10/09/2020
29	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	24/09/2020
30	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	24/09/2020
31	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	29/09/2020
32	Medicinal Cannabis Inflorescence samples from local suppliers.	08/10/2020
33	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	28/10/2020
34	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	05/11/2020
35	Cannabis herbal material (solid matrix from local retail shops	07/11/2020
36	Medicinal Cannabis Inflorescence samples from local investigative authorities.	10/11/2020
37	Medicinal Cannabis Inflorescence samples from local investigative authorities.	10/11/2020
38	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	02/12/2020
39	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	14/12/2020
40	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	14/12/2020
41	Cannabis herbal material (solid matrix from local retail shops	17/12/2020
42	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	21/12/2020

43	Medicinal Cannabis Inflorescence samples from local investigative authorities.	21/12/2020
44	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	11/01/2021
45	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	11/01/2021
46	Medicinal Cannabis Inflorescence samples from local investigative authorities.	11/01/2021
47	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	04/02/2021
48	Cannabis herbal material (solid matrix from local retail shops	06/02/2021
49	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	09/02/2021
50	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	17/02/2021
51	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	03/03/2021
52	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	03/03/2021
53	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	09/03/2021
54	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	09/03/2021
55	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	31/03/2021
56	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	31/03/2021
57	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	31/03/2021
58	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	28/04/2021
59	Cannabis herbal material (solid matrix from local retail shops	18/05/2021
60	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	19/05/2021
61	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	19/05/2021
62	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	19/05/2021
63	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	19/05/2021
64	Cannabis herbal material (solid matrix from local retail shops	24/05/2021
65	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	29/05/2021
66	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	04/06/2021

67	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	07/06/2021
68	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	15/06/2021
69	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	21/06/2021
70	Medicinal Cannabis Inflorescence samples from local suppliers.	01/07/2021
71	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	15/07/2021
72	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	15/07/2021
73	Medicinal Cannabis Inflorescence samples from local suppliers.	20/07/2021
74	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	20/07/2021
75	Medicinal Cannabis Inflorescence samples from local investigative authorities.	26/07/2021
76	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	04/08/2021
77	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	04/08/2021
78	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	12/08/2021
79	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	12/08/2021
80	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	12/08/2021
81	Medicinal Cannabis Inflorescence samples from local investigative authorities.	15/09/2021
82	Cannabis herbal material (solid matrix from local retail shops	15/09/2021
83	Medicinal Cannabis Inflorescence samples from local investigative authorities.	15/09/2021
84	Medicinal Cannabis Inflorescence samples from local suppliers.	08/10/2021
85	Cannabis herbal material (solid matrix from local retail shops	08/10/2021
86	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	18/10/2021
87	Medicinal Cannabis Inflorescence samples from local investigative authorities.	25/10/2021
88	Medicinal Cannabis Inflorescence samples from local investigative authorities.	02/11/2021
89	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	06/11/2021
90	Cannabis herbal material (solid matrix from local retail shops	12/11/2021

91	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	12/11/2021
92	Cannabis herbal material (solid matrix from local retail shops	15/11/2021
93	Cannabis herbal material (solid matrix from local retail shops	15/11/2021
94	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	27/11/2021
95	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	29/11/2021
96	Cannabis herbal material (solid matrix from local retail shops	01/12/2021
97	Cannabis herbal material (solid matrix from local retail shops	01/12/2021
98	Cannabis herbal material (solid matrix from local retail shops	01/12/2021
99	Cannabis herbal material (solid matrix from local retail shops	01/12/2021
100	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	14/01/2022
101	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	18/01/2022
102	Medicinal Cannabis Inflorescence samples from local investigative authorities.	18/01/2022
103	Medicinal Cannabis Inflorescence samples from local suppliers.	24/01/2022
104	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	04/02/2022
105	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	14/02/2022
106	Medicinal Cannabis Inflorescence samples from local suppliers.	22/02/2022
107	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	04/03/2022
108	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	04/03/2022
109	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	04/03/2022
110	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Customs).	24/03/2022
111	Cannabis inflorescence samples (Solid Matrix) seized by local authorities (Investigating authorities).	27/03/2022
112	Cannabis herbal material (solid matrix from local retail shops	30/03/2022
122	Cannabis trim (sugar leaves and fan leaves) (solid matrix) from local retail shops	04/04/2022
123	Cannabis trim (sugar leaves and fan leaves) (solid matrix) from local retail shops	04/04/2022

124	Cannabis trim (sugar leaves and fan leaves) (solid matrix) from local retail shops	04/04/2022
125	Cannabis trim (sugar leaves and fan leaves) (solid matrix) from local retail shops	04/04/2022
126	Cannabis trim (sugar leaves and fan leaves) (solid matrix) from local retail shops	04/04/2022
129	Coarse ground herbal cannabis material rolled in smoking paper (solid matrix) from local retail shops	20/05/2022
131	Coarse ground herbal cannabis material rolled in smoking paper (solid matrix) from local retail shops	20/05/2022
133	Coarse ground herbal cannabis material rolled in smoking paper (solid matrix) from local retail shops	20/05/2022
228	Cannabis extracts diluted in Medium-chain Triglycerides (Liquid matrix) from local retail shops	18/06/2022
229	Cannabis extracts diluted in Medium-chain Triglycerides (Liquid matrix) from local retail shops	18/06/2022
230	Cannabis extracts diluted in Medium-chain Triglycerides (Liquid matrix) from local retail shops	18/06/2022
231	Cannabis extracts diluted in Medium-chain Triglycerides (Liquid matrix) from local retail shops	18/06/2022
232	Cannabis extracts diluted in Medium-chain Triglycerides (Liquid matrix) from local retail shops	22/06/2022
233	Cannabis extracts diluted in Medium-chain Triglycerides (Liquid matrix) from local retail shops	22/06/2022
234	Cannabis extracts diluted in Medium-chain Triglycerides (Liquid matrix) from local retail shops	22/06/2022
235	Cannabis extracts diluted in Medium-chain Triglycerides (Liquid matrix) from local retail shops	22/06/2022
236	Cannabis extracts diluted in Medium-chain Triglycerides (Liquid matrix) from local retail shops	22/06/2022
237	Cannabis extracts diluted in Medium-chain Triglycerides (Liquid matrix) from local retail shops	22/06/2022
238	Cannabis extracts diluted in Medium-chain Triglycerides (Liquid matrix) from local retail shops	22/06/2022
239	Cannabis extracts diluted in Olive oil (Liquid matrix) from local retail shops	23/08/2022
240	Cannabis extracts diluted in Olive oil (Liquid matrix) from local retail shops	23/08/2022
241	Cannabis extracts diluted in Olive oil (Liquid matrix) from local retail shops	23/08/2022
242	Cannabis extracts diluted in Olive oil (Liquid matrix) from local retail shops	23/08/2022
243	Cannabis extracts diluted in Olive oil (Liquid matrix) from local retail shops	23/08/2022
244	Cannabis extracts diluted in Olive oil (Liquid matrix) from local retail shops	23/08/2022