Matrix-Specific Challenges in Cannabinoid Determination

Submitted in partial fulfilment

of the requirements of the

Degree of Master of Pharmacy

Michaela Mifsud

Department of Pharmacy

2025

To my younger self, who never believed we would make it this far.

And to my parents, who believed in me so fiercely that we did.

Abstract

Due to an increased interest in medical cannabis over the years, efficient and reproducible extraction and determination of cannabinoids from different matrices has gained importance. The aim of this study was to identify challenges related to the extraction and analysis of cannabinoids from different matrices. The methodology was divided into 2 phases. Phase 1: Literature review was conducted comparing different matrices in which cannabinoids may be extracted from, sample preparation techniques, and analytical methods used in cannabinoid determination. Phase 2: Visits at the Pharmaceutical Synthesis and Technology laboratory were conducted, where the analysis of cannabinoids in medium chain triglyceride (MCT) oil, hemp seed oil and cosmetics was observed and challenges identified. From 49 articles identified, the most common cannabinoid analysed included tetrahydrocannabinol (THC) (n=41). The most common matrix from which cannabinoids were analysed were oils (n=24). The most common sample preparation technique was solid phase extraction (n=12). High performance liquid chromatography was the chromatographic technique most commonly identified (n=35). The most common detector used was the mass spectrometer (n=38). Challenges identified included difficulty with obtaining clear/unwanted peaks on the chromatogram (n=16) and composition of matrices interfering with extraction (n=12). Phase 2: In the laboratory, challenges encountered included inefficient separation of THC from MCT oil, difficulty with obtaining favourable resolution of chromatographic peaks and the matrix effect. Effective quantification of cannabinoids is highly dependent on overcoming challenges posed by sample preparation and analysis. The wide variety of product types such as oils, balms, and emulsions introduces matrix-specific complexities that influence extraction efficiency and measurement accuracy. Lipid-rich oils can co-extract interfering compounds that suppress analytical signals, balms may entrap cannabinoids within waxy

or fatty structures, and emulsions present phase partitioning issues that limit analyte recovery. These matrix effects can compromise both sensitivity and reproducibility, necessitating tailored extraction strategies and rigorous method validation to ensure reliable cannabinoid determination across diverse formulations. By recognizing these matrix-specific issues, analysts can optimize methods for better solvent use, improved analyte recovery, and reduced interference, leading to more accurate and precise results.

Acknowledgements

I am deeply grateful to my supervisor, Dr Janis Vella Szijj, for her invaluable guidance, steadfast support, and encouragement throughout this research.

I would like to extend my sincere gratitude to Prof. Lillian M. Azzopardi, for her support. My thanks are also due to the staff of the Department of Pharmacy for their assistance and to Ms. Karolina Szyrner for kindly welcoming me to her laboratory sessions and providing valuable guidance throughout the course of this study.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

AUP	Area Under the Peak
CB1	Cannabinoid Receptor 1
CB2	Cannabinoid Receptor 2
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBG	Cannabigerol
CBN	Cannabinol
ECS	Endocannabinoid System
FID	Flame Ionization Detector
FLD	Fluorescence Detector
FREC	Faculty Research Ethics Committee
GABA	Gamma-Aminobutyric Acid
GC	Gas Chromatography
GC-MS	Gas Chromatography–Mass Spectrometry
HPLC	High Performance Liquid Chromatography
HPLC-MS	High Performance Liquid Chromatography–Mass Spectrometry
HPLC-UV	High Performance Liquid Chromatography-Ultraviolet
IPA	Isopropanol
LLE	Liquid-Liquid Extraction
MCT	Medium Chain Triglyceride
MS	Mass Spectrometry
O/W	Oil in Water
PPT	Protein Precipitation

RF	Retardation Factor
RSD	Relative Standard Deviation
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
THC	Δ^9-tetrahydrocannabinol
THC-COOH	11-Nor-9-carboxy-THC
THCA	Tetrahydrocannabinolic acid
TLC	Thin Layer Chromatography
U-HPLC	Ultra-High Performance Liquid Chromatography
W/O	Water in Oil

Chapter 1

Introduction

1.1 *Cannabis Sativa* Plant

Cannabis (*Cannabis sativa* L.) is a psychoactive plant containing more than 500 bioactive compounds, with over 100 of these classified as cannabinoids (Lafaye et al, 2017). Among them, Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most widely researched due to their notable pharmacological properties. Interest in cannabis has grown in both scientific and commercial sectors, largely because of the therapeutic potential of these key cannabinoids. Native to tropical regions, *Cannabis sativa* has been used for centuries in traditional medicine and for its psychoactive properties (Pellati et al, 2018; Crippa et al, 2020).

1.2 Cannabinoids

THC is the primary psychoactive component of cannabis and is responsible for its euphoric and intoxicating effects. In addition to its psychoactivity, it also exhibits therapeutic properties such as analgesia, antiemesis, and appetite stimulation (García-Gutiérrez et al, 2020). In contrast, CBD is non-psychoactive yet shares several pharmacological effects with Δ^9-THC, including anti-inflammatory, anxiolytic, and anticonvulsant properties (Stella, 2023). With the increasing global interest in the medicinal use of cannabis, the accurate extraction and quantification of cannabinoids has become an essential focus in both pharmaceutical research and regulatory analysis (Gallo, 2023).

1.2.1 Cannabidiol

CBD is one of the major cannabinoids present in *Cannabis sativa*, and in certain cultivars it can account for as much as 40% of the total cannabinoid fraction. In contrast to THC, CBD does not produce intoxicating or euphoric effects, which has contributed to its

appeal as a therapeutic compound. Structurally, CBD is a 21-carbon terpenophenolic compound that influences multiple biological pathways, most notably the endocannabinoid system (ECS) (Iffland et al, 2017). Although its affinity for the cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2) is relatively weak, CBD indirectly modulates their activity and also acts on other signaling systems, including the serotonin 5-HT1A receptor, transient receptor potential vanilloid 1 (TRPV1) channels, and the peroxisome proliferator-activated receptor gamma (PPAR-γ) (McPartland et al, 2015). Evidence from both animal studies and clinical trials supports a wide range of potential therapeutic actions, such as anti-inflammatory, anxiolytic, anticonvulsant, antipsychotic, and neuroprotective effects. Its best documented clinical application is in epilepsy management, particularly in drug-resistant conditions like Lennox-Gastaut syndrome and Dravet syndrome (McPartland et al, 2015; Iffland et al, 2017).

The oral absorption of CBD is relatively poor, with reported bioavailability ranging from approximately 6% to 19%, largely because of extensive first-pass metabolism. Factors such as co-administration with dietary lipids, the formulation employed, and the route of delivery can significantly affect its uptake. CBD undergoes hepatic metabolism primarily via cytochrome P450 enzymes, notably CYP3A4 and CYP2C19, which raises the potential for clinically relevant drug–drug interactions. With the rapid growth of CBD use in pharmaceuticals, nutraceuticals, and cosmetic applications, robust analytical methods and strict quality assurance practices are critical to guarantee product safety, therapeutic effectiveness, and adherence to regulatory standards (McPartland et al, 2015; Iffland et al, 2017).

1.2.2 Δ^9-tetrahydrocannabinol

THC is the primary psychoactive cannabinoid in *Cannabis sativa*, responsible for the characteristic intoxicating effects of cannabis use. It is a lipophilic terpenophenol with a tricyclic structure, enabling it to readily cross the blood-brain barrier and interact with the ECS. Its pharmacological effects are mainly mediated by partial agonism at CB1 receptors and, to a lesser extent, CB2 receptors, located predominantly in the central nervous system and peripheral tissues, respectively (Pertwee et al, 2005). CB1 activation modulates neurotransmitters such as dopamine, gamma-aminobutyric acid (GABA), and glutamate, contributing to THC's psychoactive, analgesic, antiemetic and appetite-stimulating properties (Howlett et al, 2002; Mechoulam et al, 2013).

Due to its potent psychoactivity, THC has been the focus of both therapeutic exploration and regulatory concern. Clinically, it has shown efficacy in managing nausea and vomiting induced by chemotherapy, chronic pain, and appetite loss associated with conditions like HIV/AIDS and cancer (Whiting et al, 2015).

THC is rapidly absorbed via inhalation and undergoes extensive hepatic metabolism when administered orally, primarily through cytochrome P450 enzymes CYP2C9 and CYP3A4. It is distributed in lipid-rich tissues, contributing to prolonged elimination half-life and to the potential for accumulation with repeated use (Huestis et al, 2007). As the interest in medicinal and recreational cannabis continues to rise, precise quantification and quality control of THC in various formulations remain critical for both efficacy and safety (Pertwee et al, 2005).

1.3 Cannabinoid Receptors

CB1 and CB2 are found in the ECS within the body. When cannabinoids bind to the CB1 and CB2 in the ECS, endocannabinoid levels are altered and thus inhibition of certain neurotransmitters such as glutamate and GABA occurs. Other examples of neuromodulation caused by the binding of cannabinoids to the CB1 and CB2 include a decreased release of acetylcholine and norepinephrine and an increase in the release of dopamine. CB1 and CB2 binding with cannabinoids, therefore, influences the pain pathways of the body, as well as the learning, reward and memory pathways. CB1 is found in highest concentrations in the central nervous system whilst the highest concentrations of CB2 are within the immune cells (Urits et al, 2021).

1.4 Cannabis For Medicinal Use and Recreational Use

Cannabis is used for analgesia (Pantoja- Ruiz et al), Alzheimer's and Parkinson's disease (Namdar et al, 2018), cancer, multiple sclerosis (Filippini et al, 2022) and epilepsy (Kumar, 2025). Medicinal cannabis can be recommended for severe or rare medical conditions, particularly when conventional therapies have proven ineffective. It may be administered in several forms such as oils, capsules, tinctures, or vaporized extracts depending on patient needs and regulatory approval (Černe et al, 2020).

Recreational cannabis is primarily consumed for its psychoactive effects and is subject to different regulatory standards. Both uses require accurate cannabinoid quantification, but medicinal products demand more stringent validation and precision due to their therapeutic context. In Malta**,** medicinal cannabis was legalized in 2018 through the enactment of the *Production of Cannabis for Medicinal and Research Purposes Act.*

Under this framework, registered physicians may prescribe cannabis-based medical products when conventional treatments have proven ineffective.[1]

These products are imported under the control of the Malta Medicines Authority, which ensures compliance with EU-GMP (Good Manufacturing Practice) standards. Dispensing is restricted to licensed pharmacies and only patients with a valid prescription and documented clinical need are permitted access. Products available in Malta include both THC- and CBD-dominant preparations, primarily used for chronic pain management, anxiety disorders, or neurological conditions.[2]

In Malta, medicinal cannabis products authorized for use are available in three main formulations: sublingual oil drops, dried inflorescence, and cannabis extracts prepared in medium chain triglyceride (MCT) oil. These products are designed for administration either through inhalation or oral ingestion, and they differ in their relative concentrations of THC and CBD.[2]

---

1. Production of Cannabis for Medicinal and Research Purposes Act, Cap. 578 (2018 Apr 17) [Internet]. Malta: Government of Malta; [cited 2025 July 8]. Available from: https://legislation.mt/eli/cap/578/eng/pdf
2. Malta Medicines Authority. *Cannabis for Medicinal and Research Purposes* [Internet]. Malta: Malta Medicines Authority; [cited 2025 July 8]. Available from: https://medicinesauthority.gov.mt/cannabisformedicinalandresearchpurposes

An example of a dried flower product is Afghani®, which contains approximately 24% w/w THC and less than 1% w/w CBD. A representative formulation of sublingual oil drops is BCANN oil Medical Cannabis Oil (THC-rich), with 200 mg/mL THC (20%) and 40 mg/mL CBD (4%). For cannabis extracts standardized in MCT oil, Panaxir CBD25 serves as an example, with a composition of less than 1.5 mg/mL THC and 25 mg/mL CBD.[2]

Despite the legal framework, challenges persist. Studies conducted locally highlight that Maltese physicians often feel underprepared to prescribe medicinal cannabis, citing a lack of training, limited clinical guidelines, and stigma surrounding cannabis-based treatments (Camilleri, 2024). Patients similarly report barriers such as high costs, inconsistent product availability, and limited access to diverse formulations . While Malta has taken important regulatory steps in legalizing medicinal cannabis, efforts must now focus on expanding clinician education, enhancing product accessibility, and refining prescription frameworks to optimize therapeutic outcomes.[2]

---

2. Malta Medicines Authority. *Cannabis for Medicinal and Research Purposes* [Internet]. Malta: Malta Medicines Authority; [cited 2025 July 8]. Available from: https://medicinesauthority.gov.mt/cannabisformedicinalandresearchpurposes

1.5 Matrix Variability in Cannabinoid Containing Products

Extraction and analysis of cannabinoids can be carried out from different matrices. These include oils such as cannabis oil, hemp seed oil, olive oil and MCT oil amongst many others. Other matrices include plant materials, cannabis inflorescences, dried medical marijuana, hemp food products, chocolate, biological fluids, cosmetics, and medicines. (Ramos Borges et al, 2020). Cannabinoid containing products differ widely in composition. Lipid based matrices, particularly MCT oil and hemp seed oil, are popular for oral and sublingual administration due to their solubilizing properties and bioavailability enhancement (Millar et al, 2018). Cosmetic products often employ emulsions water in oil and oil in water (W/O and O/W) to deliver cannabinoids through topical application. Each matrix presents unique analytical challenges during sample preparation and analysis, requiring matrix specific method optimization (Duchateau et al, 2025).

1.5.1 Medium Chain Triglyceride Oil

MCT oil is widely utilized as a carrier for cannabinoids due to its high lipid solubility, chemical stability, and compatibility with lipophilic compounds (Stella et al, 2021). Chemically, MCT oil consists of esters formed from medium-chain fatty acids and glycerol, and is particularly rich in fatty acids ranging from C6 to C12. The predominant constituents include caprylic acid (C8) and capric acid (C10), with pharmaceutical-grade MCT oil typically comprising 20%–50% capric acid and 50%–80% caprylic acid (Jadhav & Annapure, 2022). The lipophilic character of MCT oil facilitates strong molecular interactions with cannabinoids, promoting high solubility and effective dispersion within the matrix (McKenzie et al, 2021). Physically, MCT oil is characterized by a low viscosity and a density in the range of 0.93–0.96 g/cm³ While it is insoluble in water, it is miscible

with several organic solvents such as ethanol, methylene chloride, and long-chain hydrocarbons (Jadhav & Annapure, 2022).

Owing to its clear, odourless, and tasteless nature, along with favourable bioavailability, MCT oil is commonly incorporated into cannabinoid-containing pharmaceutical formulations as a delivery vehicle (Ramella et al, 2020; Stella et al, 2021). Analytical challenges can arise during sample preparation. Although methanol and acetonitrile are frequently used extraction solvents, they may preferentially extract polar components of the oil, potentially leading to incomplete recovery of cannabinoids during analysis (Mudge et al, 2017).

1.5.2 Hemp Seed Oil

Hemp seed oil, obtained from *Cannabis sativa*, is a nutrient-dense natural product widely incorporated into nutraceutical and topical applications. It contains a diverse profile of bioactive constituents, including proteins, terpenes, cannabinoids, and significant levels of polyunsaturated fatty acids, notably linoleic acid, α-linolenic acid, and oleic acid (Citti et al, 2018). While this lipid-rich composition offers various health benefits, it also presents analytical challenges during cannabinoid extraction and quantification. The abundance of unsaturated fatty acids increases the susceptibility of hemp seed oil to oxidation, which can reduce the stability and bioavailability of cannabinoid-containing products over time. The oil's characteristic green to yellow hue and nutty aroma reflect the presence of natural pigments and volatile compounds (Pavlovic et al, 2018).

From an analytical perspective, the complex lipid profile of hemp seed oil can complicate sample preparation and instrumental analysis. Co-extraction of lipids during cannabinoid isolation may introduce matrix effects, such as ion suppression in mass spectrometric

detection or baseline instability in UV detection methods. To address these challenges, dilution with polar solvents like methanol or acetonitrile is often employed to disrupt the oil matrix and release cannabinoids. Without adequate sample clean-up steps such as filtration, liquid-liquid partitioning, or solid-phase extraction (SPE) cannabinoid recovery can remain incomplete, compromising analytical accuracy and reproducibility (Mudge et al, 2017; Pavlovic et al, 2018).

1.5.3 Cosmetics

Cosmetic formulations introduce additional analytical complexity due to their diverse compositions and emulsion systems. Two of the most common emulsion types encountered in topical cannabinoid-containing products are W/O and O/W emulsions. In W/O emulsions, water droplets are dispersed throughout a continuous oil phase, while in O/W emulsions, oil droplets are suspended in a continuous aqueous phase. The physical structure and distribution of components in each system directly influence the efficiency of cannabinoid extraction and recovery during analytical procedures (Wang et al, 2022).

Cannabinoids, being highly lipophilic, preferentially localize within the oil phase of these emulsions. As a result, W/O emulsions present a particular challenge because the oil phase is continuous, cannabinoids are more tightly integrated into the matrix, making them less accessible to polar extraction solvents. When standard solvents such as methanol or acetonitrile are used, they tend to extract predominantly from the aqueous phase, potentially leaving a significant fraction of cannabinoids unrecovered. This leads to underestimation of cannabinoid content in the final analysis, particularly when no additional matrix disruption or phase separation techniques are employed (Mudge et al, 2017).

1.5.4 Biological Fluids

Efficient cannabinoid extraction from biological samples requires methods that are reproducible, selective, and capable of minimizing matrix interferences (Citti et al, 2018). Among biological fluids, urine and blood are most frequently examined in drug testing**,** with both THC and CBD measurable in serum, plasma and urine (Nahar et al, 2019). Typically, blood carries higher levels of the unmetabolized compounds compared with other fluids (Knittel et al, 2016). The persistence of THC in blood is influenced by usage frequency. Chronic consumers may test positive for several weeks, whereas in occasional users detection usually lasts only a few days (Hubbard et al, 2020). Analytical difficulties arise because blood contains proteins and lipophilic substances that interfere with measurement (Nahar et al, 2019).

To overcome these challenges, sample preparation strategies such as protein precipitation (PPT), SPE, and liquid–liquid extraction (LLE) are widely used (Klawitter et al, 2017; Ravula et al, 2018; Hubbard et al, 2020). PPT releases cannabinoids bound to plasma proteins, while SPE and LLE concentrate and purify them from the biological matrix (Citti et al, 2018). Since THC is extensively distributed throughout body tissues and demonstrates a large volume of distribution, its concentration in blood is often low, necessitating highly sensitive analytical methods for accurate quantification (Sempio et al, 2020).

Urine testing for cannabinoids generally focuses on THC and its metabolites because there is limited data on CBD excretion in urine (Bergamaschi et al, 2013). Oral fluid is another matrix used for cannabinoid detection, sometimes being easier to identifying THC in comparison to urine and serving as an alternative method for detecting recent cannabis use. Collection of oral fluid samples is faster, less invasive, and safer compared

to urine or blood sampling (Marsot et al, 2016). Due to the small available volume of saliva, highly sensitive analytical methods are necessary (Nahar et al, 2019). THC tends to remain detectable in oral fluids longer than in plasma, likely due to contamination of the mouth during and after inhalation of THC (Marsot et al, 2016).

1.5.5 Plant Material

Analysing cannabinoids in plant material can be challenging due to the complex composition of the cannabis plant and the wide range of compounds it contains. During chromatographic separation, matrix components may co-elute with the target analytes, potentially suppressing or altering their ionization during mass spectrometry (MS) detection. This can result in reduced accuracy and precision of the analysis (Nie et al, 2019).

In sample preparation, polar solvents like methanol and ethanol are effective for extracting a broad spectrum of cannabinoids. They may also dissolve non-target substances such as waxes and chlorophyll leading to co-extraction. This co-extraction can introduce impurities and reduce analytical sensitivity (Addo et al, 2022). Supercritical carbon dioxide extraction provides benefits, including lower solvent usage, but it is costly and demands specialized technical skills (De Aguiar et al, 2023). To ensure accurate results, extraction procedures must be carefully optimized and validated to control the presence of unwanted contaminants, including heavy metals, pesticides, and mycotoxins (Zeger et al, 2022).

The absence of standardized reference materials complicates method validation and quality control in cannabinoid analysis of plant material. Many laboratories depend on external proficiency tests and internal validation procedures to help ensure accuracy.

Variations in matrix composition between calibration standards and sample extracts can cause calibration bias and inaccurate cannabinoid quantification (Vella Szijj, 2024).

1.5.6 Edibles

Cannabinoids are incorporated into a variety of edible products, including chocolates, cookies, brownies, and gummies. The pharmacokinetic and pharmacodynamic profiles of cannabinoids in these forms are not yet fully understood, creating the risk of unintentional overdosing or underdosing by consumers. Edible administration results in a slower onset but extended duration of effects. Following oral ingestion, THC undergoes hepatic metabolism to form 11-hydroxy-THC, a metabolite with greater psychoactive potency, often producing stronger euphoria, sedation, and hallucinations compared with the parent compound. In contrast, inhalation delivers THC directly to the bloodstream via the lungs, bypassing first-pass metabolism. The prolonged effects associated with edibles highlight the need for careful monitoring and analytical evaluation to limit the risk of cannabinoid accumulation and related adverse outcomes (Spindle et al, 2022; Leghissa et al, 2019).

When extracting cannabinoids from edible products, the matrix composition including fat, protein, and carbohydrate content must be considered. Analytical challenges arise from the diversity of edible matrices available, making cannabinoid determination labor-intensive, time-consuming, and often costly (Leghissa et al, 2019; Chambers et al, 2023).

Extraction approaches for edibles include various SPE techniques, supercritical fluid extraction, and pressurized liquid extraction. While these methods can reduce solvent use, they often require high-cost instrumentation and additional clean-up steps, such as SPE, to achieve acceptable purity. Given that cannabis edibles are frequently prepared with

cannabis-infused oils or butter, analysis should also account for possible contamination with pesticides or residual solvents (Vella Szijj, 2024).

1.6 Sample Preparation Techniques

Sample preparation is usually the most time-consuming part on an analytical method. Proper sample preparation ensures that representative results will be obtained from the sample. There is not a single sample preparation technique which works for separation of cannabinoids from all types of matrices (Shi et al, 2022).

During sample preparation there are two types of extraction techniques which can be used: physical and chemical. During physical extraction the force of gravity, filtration and centrifugal forces are used. This technique is cheaper, uses more commonly found instrumentation, which is generally available in most laboratories and easier to carry out than chemical extraction. Examples of chemical extraction techniques include LLE, SPE and solid phase microextraction (SPME) (Wise, 2019).

1.6.1 Physical Extraction Techniques

The three main pieces of equipment which are used for extraction in small laboratories are vortex mixers, sonicators and centrifuges. Vortex mixers are quick and easy instruments which mix samples of liquid fast. Sonicators use ultrasonic waves to promote extraction. Sonication is efficient and is favoured when it comes to cannabinoid extraction when no chemicals are needed. Centrifugation is a technique in which components in a heterogenous mixture are separated using centrifugal forces, allowing the components to separate and settle according to their different densities (Cachia, 2021).

1.6.2 Liquid- Liquid Extraction

LLE is a widely applied technique for analysing samples rich in fatty acids, such as oils. In the method developed by Folch et al., a chloroform–methanol mixture in a 2:1 ratio is used as the extraction solvent. The subsequent addition of a water or saline solution promotes phase separation, allowing the lower organic phase to be collected for further extraction and analysis (Folch et al, 1957).

1.6.3 Solid Phase Extraction

A sample preparation technique which can be used instead of LLE is SPE. This is due to the many disadvantages that come along with LLE. Disadvantages associated with LLE include an increased solvent consumption, high cost and lengthy operation times (Otlës & Kartal, 2016). SPE involves the dispersion of the analyte between two separate phases (solid phase and liquid phase). SPE allows for the purification and enrichment of the analytes which occurs through the process of adsorption. This takes places on a solid adsorbent (Keçili et al, 2020).

SPE isolates and concentrates analytes by passing a liquid sample through a solid sorbent, allowing for high-capacity cleanup and recovery. SPME integrates sampling, extraction, and concentration into a single step using a coated fibre, eliminating solvent use and reducing preparation time, though it generally offers lower analyte capacity than SPE (Chiu & Kuo, 2020).

Carrying out SPME has many advantages. SPME is an easy and quick sample preparation technique which is solvent free. A salting out system is used in order to increase efficacy, allowing the measurement of fatty acids including those which are short chain and those

which are medium chain. Sodium sulfate and sodium chloride are both commonly used salting-out reagents (Chiu & Kuo, 2020).

1.6.4 Protein Precipitation

PPT sample extraction technique is one of the most commonly used techniques in bioanalysis. PPT is used for the extraction of biological fluids such as blood, serum and plasma. PPT is both a simple and quick technique which involves adding an organic solvent, salt or acid to the biological sample (Castro-Perez & Prakash, 2020). The solvent causes the proteins in the biological sample to precipitate out of solution. Following centrifugation, a pellet composed from the proteins will form at the bottom of the tube. This will allow for the supernatant to be further analysed by techniques such as MS (French, 2017).

1.7 Analytical Methods Used for the Analysis of Cannabinoids

Cannabinoids can be analysed using chromatographic techniques. The 2 main chromatographic techniques are High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC).

HPLC and GC are techniques used to separate molecules or atoms. Different types of detectors are used to determine different cannabinoids. These are MS detectors, UV/Visible detectors, diode array detectors, fluorescence detectors (FLD) and flame ionization detectors (FID). The most commonly used detector for the analysis of cannabinoids is the MS detector (Nie et al, 2019).

MS is a widely used analytical technique that ionizes individual atoms or molecules, often inducing fragmentation, and measures them based on their mass-to-charge ratio to generate a characteristic spectrum. Its high selectivity and sensitivity make MS particularly suited for cannabinoid analysis, explaining its frequent application as a detector in this field (Nie et al, 2019).

1.7.1 Gas Chromatography

GC is a routinely applied technique for analyzing volatile and semi-volatile compounds. The sample is vaporized and carried through a separation column by an inert gas, most often helium or nitrogen, where the components are separated and quantified. GC is particularly valued for its sensitivity, rapid analysis time, and ability to achieve high-resolution separation of small molecules. In the context of cannabinoid analysis, GC has traditionally been used for detecting major neutral cannabinoids such as THC, CBD, and cannabinol (CBN), although it poses limitations in detecting their acidic precursors (Cardenia et al, 2018).

Different detectors can be used with GC depending on the analytical requirements. FID are commonly used due to its wide linear range, high sensitivity, and reproducibility, making it effective for routine cannabinoid quantification. For higher specificity, especially when working with complex matrices, gas chromatography coupled to mass spectrometry (GC-MS) is preferred. GC-MS offers improved molecular identification and is routinely used in forensic and pharmaceutical contexts for confirming cannabinoid identity and concentration (Ciolino et al, 2018).

A key limitation in cannabinoid analysis using GC is the thermal instability of acidic cannabinoids such as tetrahydrocannabinolic acid (THCA) and cannabidiolic acid

(CBDA). When exposed to the elevated temperatures typical of GC injection ports and columns, these compounds undergo decarboxylation, converting to their neutral forms (e.g., THCA to THC). This conversion leads to inaccurate profiling and quantification of the native acidic cannabinoids (Wang et al, 2016). To address this, derivatisation of the cannabinoids is required prior to GC analysis. Derivatisation stabilises the molecules by chemically modifying functional groups typically through silylation using reagents like N,O-bis(trimethylsilyl)trifluoroacetamide to render the analytes thermally stable and volatile (Franzin et al, 2025).

While derivatisation allows for more accurate quantification, it also introduces additional sample preparation steps and analytical variability. Derivatised cannabinoids are often unstable and require prompt analysis to prevent degradation. These factors make GC less ideal for high-throughput applications or for accurately quantifying acidic cannabinoids in formulations such as topicals or oils (Hazekamp, 2016). With appropriate sample treatment and method validation, GC remains a reliable tool for cannabinoid profiling, particularly for routine quality control or forensic testing.

1.7.2 High Performance Liquid Chromatography

HPLC is a widely utilized analytical technique for separating, identifying, and quantifying cannabinoids across diverse matrices, such as oils, cosmetics, and plant extracts (Rodriguez et al, 2023). The method is particularly advantageous because it enables the analysis of thermolabile compounds, such as acidic cannabinoids (e.g., THCA and CBDA), without requiring decarboxylation**,** which is often necessary in GC (Mandrioli et al, 2019). This makes HPLC especially suitable for comprehensive cannabinoid profiling, as it preserves the native chemical forms of the analytes (Citti et

al, 2018). HPLC allows for rapid and high-resolution separation of multiple cannabinoids, often within a single run, depending on the complexity of the matrix and optimization of chromatographic parameters (Cardenia et al, 2018).

The accuracy and efficiency of cannabinoid analysis by HPLC are strongly influenced by the choice of mobile phase, stationary phase, and buffer system**.** Common mobile phase combinations include water with organic modifiers such as methanol or acetonitrile, often buffered with formic acid or ammonium acetate to improve peak shape and retention reproducibility. C18 reversed-phase columns are most frequently used due to their hydrophobic interaction capabilities with cannabinoid molecules. The selection of pH and ionic strength of the mobile phase is important, especially for methods relying on UV detection**,** as these parameters can significantly affect analyte ionization and absorption behavior (Hazekamp et al, 2018).

HPLC can be coupled with various detection systems**,** each offering unique advantages depending on the analytical goals. Ultraviolet-visible detectors are the most common, particularly due to the strong chromophores present in cannabinoid structures, which allow for efficient absorption of UV light (McRae et al, 2020). Most cannabinoids show strong absorbance at low wavelengths, particularly between 210–230 nm, where the UV cut-offs of solvents must be considered to avoid signal interference. To maximize selectivity and sensitivity, the detector is typically set at the absorbance maxima of the target analyte CBD and THC, for example, show optimal detection at approximately 220 nm and 228 nm, respectively (Wand et al, 2016). FLD**,** though less commonly used, offer increased sensitivity for certain compounds, while MS provides enhanced specificity and lower detection limits, making it suitable for trace-level quantification and confirmation of cannabinoid identity in complex samples (Lazarjani et al, 2020).

The versatility of HPLC, especially when paired with UV or MS detectors, makes it a robust and reliable platform for cannabinoid analysis**.** It has become the gold standard in both research and regulatory environments for method validation, routine testing, and quality control of cannabis-derived products (Silva et al, 2025).

1.7.3 Thin-Layer Chromatography

Thin-layer chromatography (TLC) can be used for cannabinoid analysis, employing different stationary and mobile phase combinations tailored to specific sample preparation approaches (Cachia, 2021). In TLC, analyte identification is based on the retardation factor (RF), calculated as the ratio of the distance travelled by the analyte to that travelled by the solvent front. RF values are influenced not only by analytical conditions but also by the quality and age of the sample.[3]

---

3. United Nations Office on Drugs and Crime (UNODC). World drug report 2009, United Nations Publications, New York: 2009 [cited 4 April 2024]. Available from: https://www.unodc.org/documents/wdr/WDR_2009/WDR2009_eng_web.pdf

1.8 Challenges

When performing sample extraction techniques different challenges arise when using different matrices. Oils and chocolate have a high fatty acid content which allows for high solvent consumption. Flavonoids are phenolic compounds found in plant materials, which due to their properties, greatly reduce the efficiency of extraction from plant materials (Zhang et al, 2018).

Methanol/chloroform is the most commonly used solvent when it comes to extraction of cannabinoids although, the use of chlorinated solvents is advised against since they are found to be toxic, hazardous to store and expensive to discard. Exposure to chloroform for long periods of time is related to kidney and liver damage. Even though, laboratories take maximum precaution to significantly reduce the risk of exposure, when using chloroform, the risks of spill and inhalation are still very high (Mudge et al, 2017).

1.9 Aims

The project aims to identify challenges related to the extraction of cannabinoids from different matrices.

Chapter 2

Methodology

2.1 Ethics Registration

This Study was registered with the Faculty Research Ethics committee (FREC) (Appendix 1).

2.2 Literature Review

Phase 1 of this study included literature review on different matrices from which cannabinoids may be extracted from. This was done using open access journal articles published between 2013 and 2025 accessed through PubMed. Research articles reporting the analysis of cannabinoids using chromatography were identified. Keywords used in the search included ‘extraction of cannabinoids’, ‘oils’, ‘matrices’, ‘cosmetics’, ‘edibles’ and ‘biological fluids’. Comparison of different matrices from which cannabinoids can be found, the different sample preparation techniques used to clean up the analyte and the analytical methods used for analysis of cannabinoids was carried out followed by identification of challenges related to the extraction of cannabinoids from different matrices. The literature review was conducted using the PRISMA guidelines (Page et al, 2021).

2.3 Laboratory Visits

Phase 2 of this study included visits to the Pharmaceutical Synthesis and Technology Laboratory where identification of challenges in the sample preparation and analysis of cannabinoids in MCT oil, hemp seed oil and cosmetics were carried out during method development, validation and application.

2.3.1 Instrumentation and Materials

The ultrasonic bath and balance utilised during sample preparation were the Labbox Labware Ultrasonic Bath and Sartorius LA230S balance respectively. A liquid chromatography system, Agilent 1260 Infinity Series, was used to perform analysis. The chromatographic system was coupled to a quaternary pump (G7111B) and an autosampler (G7129A) and equipped to a Variable Wavelength Detector (G7114A). The software used to carry out acquisition of data and processing of data was EZ Chrome Open-Lab CDS ChemStation. Avantor ACE 5 C18-AR (250 x 4.6mm; 5 µm) column was used to achieve chromatographic seperation.

2.3.2 Chemicals and Reagents

Chemicals and materials used included HPLC-grade acetonitrile, methanol, and acetic acid, sourced from J.T. Baker (Phillipsburg, NJ, USA). Deionized water was produced using an in-house Barnstead Smart2Pure purification system. Certified reference standards of CBD and CBN (1 mg/mL) and THC (4.98 mg/mL) were obtained from LGC (Wesel, Germany). Standards were stored at −20 °C and brought to room temperature prior to use. Sample filtration was performed using 0.45 µm regenerated cellulose syringe filters.

2.3.3 Standard Preparation

A blank matrix was prepared by diluting 10 µL of cannabinoid-free oil with ethanol to a final volume of 1 mL. A 1 mg/mL THC working solution was obtained by diluting 1 mL of a 5 mg/mL THC stock solution with 4 mL of methanol. Individual standard solutions of CBD, THC, CBN, CBDA, and THCA (each at 1 mg/mL) were transferred into amber HPLC vials for protection from light. A mixed cannabinoid standard solution containing 200 µg/mL of each compound was prepared by combining 200 µL of each 1 mg/mL individual standard and diluting to a total volume of 1 mL.

A calibration series (table 2.1) was generated through serial two-fold dilutions of the mixed standard solution. For each step, 500 µL of the 200 µg/mL standard was mixed with 500 µL of ethanol, resulting in successive dilutions. To ensure matrix consistency across the calibration range, each dilution step was prepared using 500 µL of the standard solution and 500 µL of the blank matrix, yielding 1 mL per calibration level.

Table 2.1: Calibration Levels

Calibration Level	Standard Concentration (µg/mL)	Final Concentration in Calibration Vial (µg/mL)
Level 1	200	100
Level 2	100	50
Level 3	50	25
Level 4	25	12.5
Level 5	12.5	6.25
Level 6	6.25	3.125

2.4 Medium Chain Triglyceride Oil

Seven different products were purchased from different shops around Malta and each product was stored in a refrigerator at 4 °C. Every sample was tested in triplicates and the quantities of CBD, THC and CBN were identified.

Quantification of THC and CBN was performed following a 5-fold dilution in methanol, whereas CBD quantification required a 500-fold dilution in methanol to achieve appropriate analytical concentrations within the calibration curve.

2.4.1 Sample Preparation

A 0.2 mL aliquot of the diluted sample was transferred into a 5 mL volumetric flask and brought to volume with methanol. The prepared solutions were sonicated for 15 minutes at 20 °C to ensure adequate mixing and dissolution. Prior to analysis, each sample was filtered through a 0.45 μm cellulose syringe filter.

2.4.2 Chromatographic Analysis

Chromatographic separation was conducted. Isocratic elution employed a mobile phase of acetonitrile and 0.5% acetic acid in a 75:25 (v/v) ratio at a flow rate of 1.5 mL/min. The column temperature was set to 30 °C, and detection was performed at 220 nm. An injection volume of 10 μL was used, and all measurements were carried out in triplicate to ensure reproducibility and accuracy.

2.5 Hemp Seed Oil

Nine different products were purchased from different shops locally and each product was stored in a refrigerator at 4 °C. Every sample was tested in triplicates and the quantities of CBD, THC and CBN were identified.

Different types of dilutions were analysed. Sample A was prepared by adding 1 mL oil in 100 mL ethanol, sample B was prepared by adding 0.1 mL oil in 10 mL ethanol and sample C was prepared by adding10 µL oil in 990 µL ethanol (microextraction). Each sample was prepared in duplicates, with both samples subjected to duplicate HPLC injections.

2.5.1 Sample Preparation

Commercial hemp seed oil samples containing cannabis-derived compounds were initially diluted by mixing 10 µL of oil with 990 µL of ethanol, resulting in a 1:100 dilution for the analysis of low-concentration constituents. For CBD quantification, a secondary dilution was performed by combining 20 µL of the initial solution with 480 µL of ethanol, yielding a final dilution factor of 1:2500. The diluted samples were vortexed for 1 minute, subjected to 15 minutes of sonication, and filtered using a 0.45 µm syringe filter. A final vortex step of 1 minute was conducted prior to chromatographic injection.

2.5.2 Chromatographic Analysis

The samples for hemp seed oil were analysed under identical chromatographic conditions as those outlined in section 2.4.2.

2.6 Cosmetics

Cannabinoid-containing cosmetics were purchased from shops available locally. Cosmetics tested included a night cream and a scar cream.

2.6.1 Sample Preparation

0.1 g of each sample was precisely weighed into small glass beakers and gently heated on a hot plate at temperatures not exceeding 40 °C. Three millilitres of the appropriate extraction solvent was then added. Once the sample was fully dissolved or adequately dispersed, it was transferred to a 10 mL volumetric flask and diluted to volume with the same solvent. Isopropanol (IPA) was used for W/O formulations, while 95% ethanol was selected for O/W formulations. While IPA can dissolve compounds from both matrices, ethanol was preferred for O/W formulations because it produced a cleaner chromatographic baseline, reducing background signal interference. A lower baseline noise improves signal-to-noise ratio, making it easier to detect and quantify cannabinoids accurately. Even though ethanol has lower solubility for certain components compared to IPA, its ability to enhance precision and reduce analytical artifacts outweighed the solubility limitations for these specific formulations (Monti et al, 2025).

Each solution was vortexed for 1 minute and sonicated for 20 minutes. In instances where complete dissolution was not achieved due to the presence of suspended particles, samples were centrifuged at 10,000 rpm for 10 minutes, filtered through 0.45 µm syringe filters, and then subjected to analytical procedures.

2.6.2 Chromatographic Analysis

Isocratic elution was utilised with a mobile phase consisting of acetonitrile and 0.5% acetic acid in a 75:25 (v/v) ratio, delivered at a flow rate of 1.5 mL/min. The column was

maintained at 30°C, with detection carried out at a wavelength of 220 nm. The injection volume was 10 µL.

2.7 Dissemination of Research

An abstract has been submitted to the 11th Malta Medical School Conference which will take place from the 4th to the 6th of December 2025 at the Mediterranean Conference Centre, Valletta.

Chapter 3

Results

3.1 Literature Review

Two hundred and forty articles were identified from PubMed, of which forty nine studies were eligible to be included in the study. The PRISMA diagram can be seen in figure 3.1 below.

The literature review can be viewed in Appendix 2.

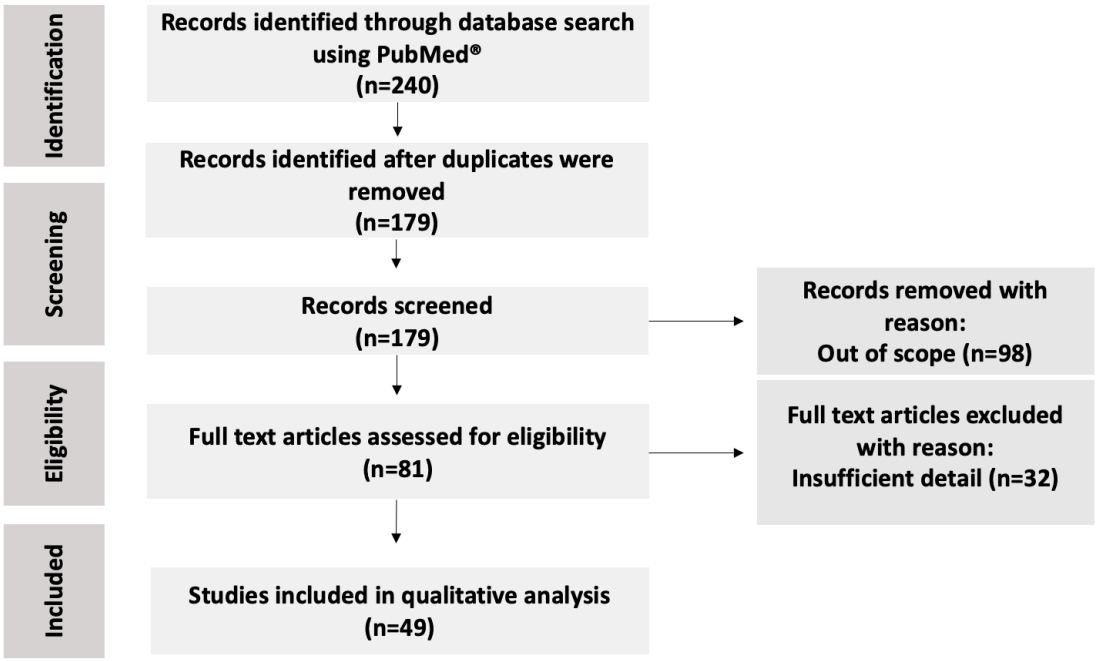

Figure 3.1: PRISMA Diagram Showing Results from Literature Search

3.1.1 Cannabinoids Identified

The most commonly analysed cannabinoids included THC (n=41) and CBD (n=39). Other cannabinoids analysed were CBDA (n=14), THCA (n=11), CBN (n=12), 11-Nor-9-carboxy-THC (THC-COOH) (n=7) and synthetic cannabinoids (n=4).

3.1.2 Matrices in Which Cannabinoids May be Extracted From

The most common matrix in which cannabinoids were found were oils (n=24). The different oils analysed included olive oil (n=1), essential oils (n=2), hemp oil (n=6), cannabis oil (n=4), vape oil (n=3), CBD oil (n=4), oil extracted directly from the *Cannabis sativa plant* (n=1), sesame oil (n=1), MCT oil (n=2) and coconut oil (n=1).

Other analysed matrices included: dried inflorescence (n=4) into cannabis flower (n=4), herbal extract (n=4), plant material (n=3) and dried hemp (n=1).

Biological tissue and fluids were also analysed and these included oral fluids (n=8), urine (n=4), blood (n=4), gastric content (n=1), serum from humans (n=1), serum from rodents (n=1) and umbilical cord tissue (n=1).

Other matrices included commercial hemp products such as edibles (n=7), hemp butter (n=1) and oral dosage froms (n=3). Cannabinoid consisting cosmetics were analysed in the form of hemp oil products (n=1) and CBD containing creams (n=2).

3.1.3 Sample Preparation Techniques

Sample preparation techniques included centrifugation (n=1), solid- liquid extraction (n=2), SPE (n=12), maceration (n=1), vortex with acetonitrile (n=3), hydro-distillation (n=2), sonication (n=1), solid-phase microextraction (n=2), crude extraction (n=1), extraction with methanol (n=7), LLE (n=5), PPT (n=1) and ultrasound assisted extraction (n=1).

3.1.4 Chromatographic Techniques

HPLC was the most commonly used chromatographic technique to analyse cannabinoids (n=35) and included ultra-high performance liquid chromatography (U-HPLC) (n=11). Other chromatographic instrumentation included GC (n=15).

3.1.5 Stationary Phase and Mobile Phase

The most common stationary phase used for the analysis of cannabinoids was the C18 column (n=28). Although the composition of the column was the same for most studies the brands did vary. The most commonly used brands included Agilent (n=5) and Kinetex (n=5).

The mobile phase varied depending on the type of chromatographic technique chosen for analysis. When HPLC was used the most common organic modifier in the mobile phase was acetonitrile (n=14). Other liquids used in the mobile phases in HPLC included methanol (n=10), formic acid (n=10), ammonium formate with HPLC grade water (n=4), methanol with phosphoric acid (n=1) and acetic acid with water (n=1). The most common mobile phase in the case of GC was helium (n=11). Others carrier gases included hexane (n=1).

3.1.6 Detectors

MS was the most common detector used in the analysis of cannabinoids, which was used in 38 out of the 49 studies reviewed. Other detectors used in the analysis of cannabinoids included the diode array detector (n=8), ultra-violet detector (n=6), the FID (n=3) and the FLD (n=1).

3.1.7 Challenges Identified From Literature Review

The challenges identified during the analysis of cannabinoids from different matrices from the literature search can be seen in table 3.1 below. The most common challenges identified from the literature review include difficulty with obtaining clear/unwanted peaks on the chromatogram (n=16) and difficulties in sample preparation leading to high solvent consumption (n=7).

Table 3.1: Challenges Identified from Literature Review (N=49)

Challenges Identified	Number of articles
Difficulty with obtaining clear/unwanted peaks	16
Composition of matrices interfering with extraction	12
Sensitivity	9
Difficulties in sample preparation leading to high solvent consumption	7
Development of a universal sample preparation/ analytical technique	6
Decarboxylation of cannabinoids	4
Finding correct temperature and pH to carry out analysis	4
Small sample leading to quantification problems	3
Improper storage conditions of matrices causing degradation of the cannabinoids within the sample	1
Determination of appropriate column for analysis	1

3.2 Challenges Identified in the Laboratory

In the laboratory challenges were seen during both sample preparation and analysis of cannabinoids using hemp seed oil, MCT oil and cosmetics.

3.2.1 Medium Chain Triglyceride Oil Sample Preparation and Analysis

Table 3.2 below outlines the primary challenges encountered during sample preparation and chromatographic analysis of cannabinoids, extracted from medium-chain triglyceride oil. Table 3.2 summarizes the strategies implemented to mitigate them. These interventions, such as adjusting the sample preparation techniques and modifying instrumental parameters were crucial in enhancing the reliability, selectivity, and efficiency of the analysis.

Table 3.2: Challenges identified during sample preparation and analysis of cannabinoids from MCT oil.

Challenge	Cause of Challenge	Action Taken
Incomplete extraction of CBD from MCT oil during sample preparation	Lipophilic nature of MCT oil causing an interference with CBD.	Diluting the MCT oil samples with methanol at a 1:500 ratio serves to drastically reduce the concentration of the lipid matrix, which can otherwise interfere with chromatographic separation.
Laboratory environmental impact on samples	Laboratory environment factors such as temperature and humidity affecting the stability of the samples.	Ensuring stability of temperature and humidity of the laboratory by keeping tabulated records of the parameters.
Carry over on the chromatogram (figure 3.2)	High concentration of CBD in the samples leading to residual material being retained in the column during chromatographic analysis.	Methanol used between runs to clean the column and effectively remove any remaining analytes.
Determination of ideal wavelength to carry out chromatographic analysis	Ensuring that the wavelength chosen to carry out analysis corresponds to absorbance peaks of MCT oil and not cannabinoids.	Wavelemgth chosen was 220nm to improve selectivty and accuracy of CBD and THC.
Different ranges of concentrations of CBD and THC resulting in the need for separate sample preparation and analysis leading to high solvent consumption	Concentration of CBD in oil commercial sample is much higher when compared to THC so the method of sample preparation and analysis imposes challenges such as high solvent consumption when compared to sample preparation for THC.	Development of an efficient extraction method for sample preparation in order to reduce solvent consumption due to minimal solvents needed.
Obtaining favourable resolution of chromatographic peaks	In MCT-based cannabinoid oils, matrix effects in HPLC-UV stem from lipids and excipients causing baseline noise and peak interference.	Spiking involves adding a known amount of the target analyte or internal standard to the sample to assess and correct for matrix effects. This helps to improve the accuracy of cannabinoid quantification.

Figure 3.2 shows carry over at a retention time of 7 minutes which corresponds to CBD when running a blank with methanol.

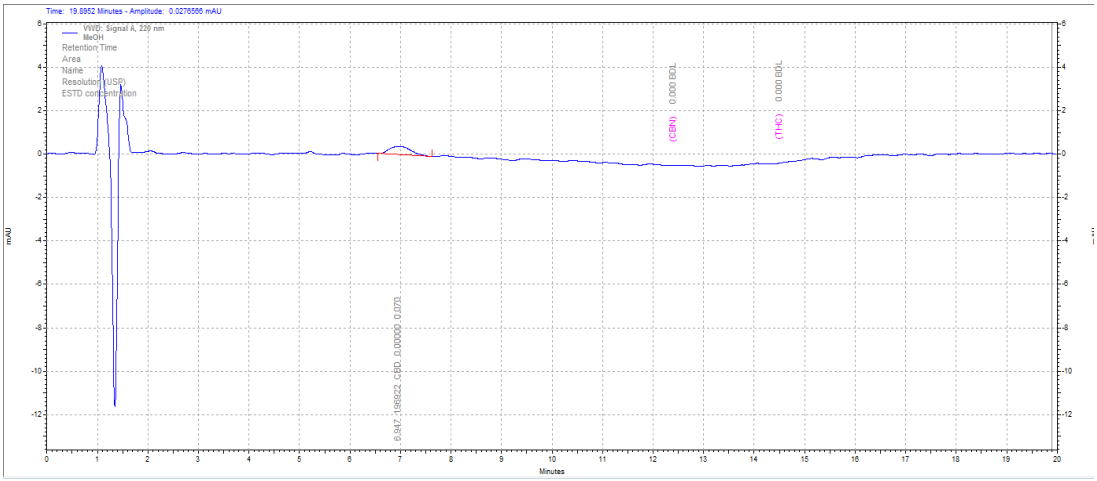

Figure 3.2- Chromatogram Showing Carryover Effect in Methanol

3.2.2 Hemp Seed Oil Sample Preparation and Analysis

The analysis of cannabinoids in hemp seed oil presented challenges during both sample preparation and instrumental evaluation. Table 3.3 highlights key issues such as high cannabinoid concentration, matrix interference, and limitations in solvent and equipment performance. These complications might impact chromatographic resolution, solvent usage, and might impact reproducibility across labs. To overcome these obstacles, a range of strategies including microextraction techniques, dilution adjustments, and optimized mobile phase selection were implemented. This ensured the development of a more robust, reliable, and reproducible analytical method suitable for routine cannabinoid testing in complex oil matrices.

Table 3.3: Challenges identified during sample preparation and analysis of cannabinoids from hemp seed oil.

Challenge	Cause of Challenge	Action Taken
Different ranges of concentrations of CBD and THC resulting in the need for separate sample preparation and analysis leading to high solvent consumption	Concentration of CBD in oil is much higher when compared to THC so the method of sample preparation and analysis imposes challenges such as high solvent consumption when compared to sample preparation for THC.	Development of micoextraction method for sample preparation in order to reduce solvent consumption. The determined dilution technique can be seen in section 3.2.2.1.
Obtaining favourable resolution of chromatographic peaks	In Hemp seed based cannabinoid oils, matrix effects in HPLC-UV stem from lipids and excipients causing baseline noise and peak interference.	Spiking involves adding a known amount of the target analyte or internal standard to the sample to assess and correct for matrix effects. This helps to improve the accuracy of cannabinoid quantification.
Determination of accurate dilution technique	Physical characteristics of the hemp seed oil matrix can interfere with sample preparation, complicating the efficient extraction.	Development of a microextration method. Results obtained can be seen in tables 3.4 and 3.5.
Determining the mobile phase to carry out HPLC	Increasing the proportion of acetonitrile led to a reduction in retention time but compromised chromatographic resolution	Mobile phase chosen included acetonitirle and 0.5% acetic acid (75:25 v/v)
Sourcing oil samples	Limited supply available within pharmacies in Malta	Samples were purchased from retail outlets across Malta.
Chromatographic peak resolution	CBN and CBD are cannabinoids which have similar chemical structures, resulting in similar retention time on the chromatogram	A resolution of a 1:5 ratio was identified in order to obtain separate peaks of CBN and CBD on the chromatogram.
Poor selectivity obtained on the chromatogram during analysis.	Incorrect choice of column used to carry out analysis using HPLC.	Column changed from C18 to a C18 AR (refer to figures 3.3 and 3.4)

3.2.2.1 Determination of an Accurate Dilution Technique

From the 3 samples analysed (A, B and C) the following results were obtained (table 3.4 and table 3.5).

Table 3.4: Summary of Mean and RSD Values for Each Sample Preparation Method.

Sample	AUP (mean ± RSD%)	Rt (mean ± RSD%)	N (mean ± RSD%)	R (mean ± RSD%)	A (mean ± RSD%)
A (100ml)	308,661,437 ± 0.92	7.28 ± 1.09	11,127 ± 0.31	0.94 ± 1.07	0.98 ± 0.51
B (10ml)	328,803,201 ± 2.21	7.12 ± 0.37	10,979 ± 0.32	0.97 ± 1.78	0.99 ± 0.51
C (1ml)	429,672,488 ± 0.04	7.05 ± 0.28	10,200 ± 0.30	1.10 ± 1.05	1.00 ± 2.00

Table 3.5: Chromatographic Evaluation of Dilution Accuracy

Parameter	Chosen sample and explanation
Area under the peak (AUP)	Sample C produced the highest AUP signal, indicating the strongest analyte response, while also showing the lowest relative standard deviation (RSD) , reflecting superior precision and consistency in the measurements.
Retention time (Rt)	Variations in retention time can occur. Sample C demonstrated the most stable retention time, indicating consistent chromatographic performance.
Number of theoretical plates (N)	Sample A showed the highest value
Resolution (R)	Sample C's superior peak separation may be attributed to reduced matrix interferences, allowing clearer resolution of the analytes.
Asymmetry (A)	Sample C displayed the closest to ideal symmetry (1.0)
Overall	Sample C (microextraction) demonstrated the lowest variability across the checked parameters.

Sample C may exhibit enhanced analyte dispersion and a more effective reduction of residual matrix constituents. According to Mandrioli et al. (2019), the miniaturisation of sample preparation, particularly when aligned with green analytical chemistry principles, enhances both the sustainability and analytical robustness of the method by reducing solvent consumption and matrix-induced interferences. From a practical standpoint, the pipetting of 10 µL aliquots proved to be more manageable compared to 1 mL or 100 µL volumes, primarily due to the high viscosity of the oil matrix and the tendency to entrap air bubbles during handling.

3.2.2.2 Poor Selectivity

Table 3.6 demonstrates that the ACE 5 C18-AR column achieved the best separation efficiency within a suitable run time. In contrast, the ACE 5 C18 column led to a prolonged analysis time.

Table 3.6: Chromatographic Column Selection

Type of column	Retention time for CBD (minutes)	AUP for CBD	Symmetry (CBD peak)	Number of theoretical plates for CBD (N)	Resolution (CBD/CBG)	Retention time of THC (minutes)
Avantor ACE 5 C18-AR (250 x 4.6 mm; 5 µm)	5.23	194237135	0.99	9574	1.66	9.4
Avantor ACE 5 C18 (250 x 4.6 mm; 5 µm)	6.9	197813616	1.005	12297	1.7	14.1

The chromatographs comparing CBD hemp seed oil HPLC analysis using AR-C18 column and a C18 column can be seen in figure 3.3 and 3.4 respectively.

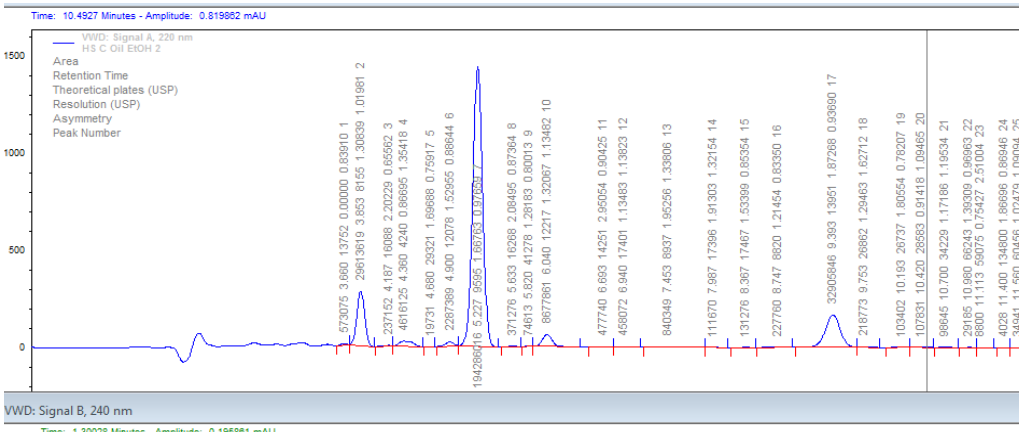

Figure 3.3: Chromatogram of Hemp Seed Oil Using C18-AR Column

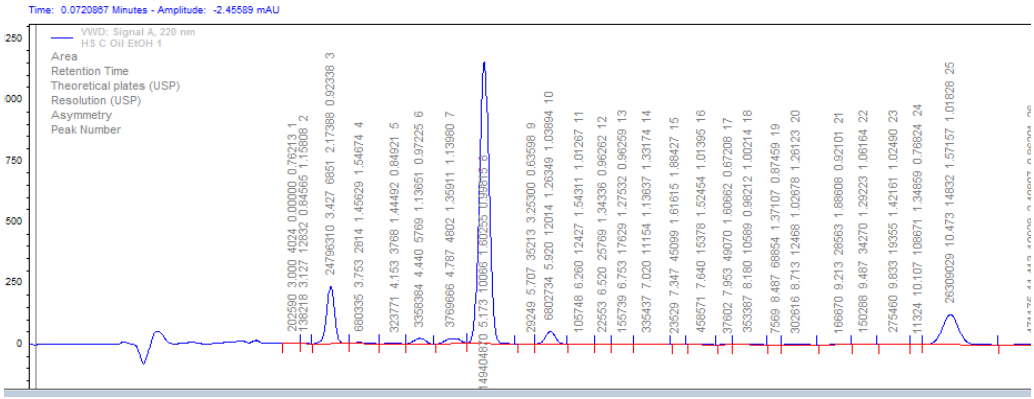

Figure 3.4: Chromatogram of Hemp Seed Oil Using C18 Column

3.2.3 Cosmetics Sample Preparation and Analysis

The preparation and analysis of cosmetic formulations containing cannabinoids introduced analytical and practical challenges due to the complex nature of their matrices. As outlined in Table 3.7, challenges arose from differences in chemical composition, solubility, and the physical characteristics of cosmetic products. Key difficulties included selecting appropriate solvents, determining optimal sample sizes, and minimizing background noise during analysis. Through methodical adjustments such as solvent

optimization, standardized sample preparation, and the adoption of greener extraction techniques these obstacles were addressed to improve the accuracy, consistency, and sustainability of cannabinoid quantification in cosmetic samples.

Table 3.7: Challenges identified during sample preparation and analysis of cannabinoids from cosmetics.

Challenge	Cause of Challenge	Action Taken
Varying physical properties of matrices	Different matrices consist of different chemical and physical properties, resulting in the need for different sample preparation methods and solvents. IPA offers superior solubility, but has high UV absorbance, which can result in increased background noise. Ethanol was a greener option with decreased background noise.	During sample preparation for W/O formulations IPA was used, and for O/W formulations, 95% EtOH was employed.
Specificity	Choosing most appropriate solvent for sample preparation without compromising specificity for W/O matrices.	IPA was chosen as the solvent because it preserves analytical specificity, minimizing interference from non-target compounds in the matrix.
Standardising sample preparation	Diverse physical and chemical properties of different sample matrices result in the need for different sample preparation techniques	Development a robust sample preparation method.
Determining optimal sample mass for sample preparation	A sample size of 0.05g was too small causing challenges when handling the sample and therefore, causing variations in the results obtained from analysis	A sample of 0.1g gives more accurate results with minimal variation.

Chapter 4

Discussion

4.1 Implications of Challenges Identified in Literature Review

CBD and THC are the two most commonly studied cannabinoids of which extraction and quantification have been reported. This is due to the many beneficial pharmacological properties exerted by the two cannabinoids. THC can be used in chronic pain, multiple sclerosis patients, epilepsy, insomnia, and analgesia with a high benefit to risk ratio seen (Whiting et al, 2015).

The most common chromatographic technique used to analyse cannabinoids was HPLC. HPLC allows for accurate, precise and sensitive quantification of cannabinoids (Pourseyes Larzarjani et al, 2020). HPLC facilitates both separation and determination of different cannabinoids to occur rapidly allowing them to accurately be quantified. This technique is appropriate for matrices which are unstable or non-volatile to allow analysis through GC (Ciolino et al, 2018).

GC was the second most common chromatographic technique used, as it allows for all the cannabinoids being analysed to be eluted in a relatively short period of time (between seven to twenty minutes). A major limitation was seen with this method as the high temperatures required in the injection port caused all the acidic cannabinoids to become decarboxylated into their neutral derivatives and therefore not allowing them to be accurately quantified (Tran et al, 2022).

Another advantage of the use HPLC is that it is able to be used in tandem with multiple types of detectors depending on the cannabinoid being analysed. The most common detector used from the studies analysed was the MS. MS allows for high selectivity and sensitivity when analysing cannabinoids regardless of the complex chemical structures of

the cannabinoids. This therefore justifies the common use of this detector in cannabinoid analysis (Nie et al, 2019).

Diode array detector can also be coupled with HPLC especially when it came to the analysis of cannabinoids in oils and plant material. Diode array detectors are inferior when compared to the HPLC coupled with the mass spectrometer (HPLC-MS) instrumentation as it does not allow for the high selectivity required to distinguish between compounds which are very structurally similar (Leghissa et al, 2018).

A common challenge identified was determining the correct temperature and pH in which the extraction process should occur. This is an important factor when it comes to extraction and analysis of cannabinoids as too high of a temperature would cause decarboxylation of the cannabinoid, in turn degrading it. Using a temperature which is too low would not yield the highest concentration of the cannabinoids possible. This would then result in having to carry out the process multiple times to obtain the desired concentration resulting in a loss of both resources and time (Wang et al, 2016).

Obtaining unwanted/ unknown peaks on the chromatogram leaves the analyst uncertain of the results obtained. The analyst would have to decipher what these peaks represent and how to omit them when attempting to carry out the tests again. It is highly possible that the unwanted peaks could be a result of contamination within the system which is not wanted as the results obtained would not be accurate. This challenge was both seen in literature and in the laboratory (Galettis et al, 2021).

Sample preparation is the most time-consuming step when it comes to extraction and analysis of cannabinoids. THC is a highly lipophilic compounds and due to this, challenges arise when attempting to extract it from different matrices. This results in high

solvent consumption of non polar solvents during extraction which can be expensive and time consuming (Antunes et al, 2023).

Literature reports indicate that several sample preparation techniques have been developed to overcome the analytical challenges posed by the high lipophilicity of MCT oil and its strong binding affinity for cannabinoids. These techniques are designed to improve cannabinoid extraction efficiency and analytical recovery. A widely used method includes extensive dilution typically at a 1:500 ratio in methanol combined with a 15-minute sonication step, followed by filtration through a 0.45 μm membrane to facilitate the release of cannabinoids from the lipid matrix (Maggini et al, 2022). Both ultrasonic bath and probe sonication have been applied to enhance solubilisation of cannabinoids during HPLC–MS/MS sample preparation. Organic solvents such as acetonitrile and IPA have also demonstrated effectiveness in disrupting the MCT oil matrix, thereby improving cannabinoid solubility and readiness for chromatographic analysis (Ramella et al, 2020).

A challenge which was experienced during the extraction and analysis of cannabinoids from biological fluids/tissue was limited sample availability. This in turn resulted in the requirement for a high sensitivity analysis due to the low amounts of cannabinoids that needed to be detected. This process is not only time consuming but also leaves no room for error as repetition of tests would not always be an option due to the limited sample available (Concheiro et al, 2013).

4.2 Implications of Challenges Identified in the Laboratory

Determination of CBD concentrations to set up calibration curve was a challenge identified during the analysis of cannabinoids from MCT oil. Concentration of CBD in

commercially available oil is much higher when compared to THC, so the method of sample preparation imposed challenges. Challenges include large amount of solvents needed and an increased risk for pipetting errors due to the need of preparing serial dilutions (Hambidge et al, 2025).

Increased stability of cannabinoids in MCT oil occurs due to the enhanced lipophilic nature of MCT oil which is rich in C8-C10 fatty acids. This strong solubilisation imposed analytical challenges during sample preparation when trying to extract CBD from MCT oil. During sample preparation cannabinoids may not readily partition into typical solvents like methanol or acetonitrile without vigorous dilution, sonication, or prolonged vortexing. This results in increased sample preparation time and high solvent consumption. Without adequate disruption, incomplete extraction can lead to under-quantification of cannabinoids (Maggini et al, 2022).

The impact of environmental conditions, particularly temperature and humidity, on the stability and reliability of chromatographic results is a common challenge identified in both sample preparation and analysis of cannabinoids from different matrices (Phung et al, 2023). These external variables can significantly influence the precision and consistency of the samples, especially when analysing complex matrices like oils and cosmetic formulations. Temperature variations can modify the viscosity of the mobile phase, which in turn affects flow rate, system pressure and retention behaviour of analytes. Even slight temperature changes (less than half a degree Celsius) have been shown to alter peak profiles and selectivity, especially under isocratic or extended gradient conditions (Wang et al, 2014). Uneven heating across the chromatographic column may lead to thermal gradients, which can cause distorted peak shapes and reduced

resolution.[4] Although often underreported, ambient humidity can also interfere with analysis, particularly in UV detection systems, where fluctuations may lead to baseline drift or instability due to refractive index changes or condensation within the detector's optical path. These environmental influences become even more significant when detecting cannabinoids at low concentrations, such as in pharmaceutical or topical products. To address these challenges, it is essential to implement rigorous environmental control measures most notably stable column temperature regulation and controlled humidity conditions to ensure reproducible and accurate quantification of cannabinoids across various matrices (Brandt et al, 1997).

Residual compounds or particulate matter can lead to ghost peaks, baseline instability, or carry-over, which compromise both qualitative and quantitative analysis (Galettis et al, 2021).

Diluting MCT cannabis oil samples with methanol substantially enhances chromatographic separation by altering the retention characteristics of both cannabinoids and the oil matrix. Methanol interacts strongly with the hydrophobic MCT oil, reducing its viscosity and promoting phase disruption, which helps release embedded cannabinoids into solution. As a result, cannabinoids and oil components exhibit distinct retention behaviours. Cannabinoids elute according to their polarity, while lipid residues are

---

4 Thermo Fisher Scientific. The impact of temperature on liquid chromatography: why column thermostatting matters [Internet]. Waltham (MA): Thermo Fisher Scientific; 2016 [cited 2025 July 9]. Available from: https://tools.thermofisher.com/content/sfs/brochures/WP-71499-LC-Temperature-Column-Thermostatting-WP71499-EN.pdf

pushed out or retained separately allowing cleaner, more accurate peak integration and improved resolution on reversed-phase columns. Method development studies demonstrate that using 100% methanol enhances peak symmetry, shifts retention to earlier and more reproducible times, and minimizes matrix interference, supporting more reliable and efficient cannabinoid analysis (Mudge et al, 2017).

During method development a challenge encountered involved selecting an appropriate detection wavelength to specifically identify cannabinoids while minimizing signal interference from the MCT oil matrix. Cannabinoids exhibit strong absorbance in the 200–280 nm range due to their conjugated π-electron systems. Method development studies typically identify an optimal compromise wavelength, with many researchers selecting around 220 nm to maximize cannabinoid detection (Mandrioli et al, 2019). Shallow UV maxima near 220 nm provide excellent sensitivity for both acidic and neutral cannabinoids and correspond to improved peak symmetry and reproducibility. Carefully optimizing UV detection within this region improves selectivity, enhances signal to noise ratio, and helps ensure accurate quantification of cannabinoids in oil-based matrices (Silva et al, 2025).

A significant analytical challenge encountered during the study was the need to quantify cannabinoids present at different concentration levels within the same matrix specifically CBD and THC. In most commercial and pharmaceutical formulations, CBD is present in high concentrations, whereas THC levels are minimal or near trace levels, often below 0.3% w/w. This discrepancy hindered simultaneous quantification with a single dilution factor, as it could drive THC concentrations below the lower limit of quantification while forcing CBD levels beyond the upper calibration limit.

To address this challenge, samples were initially diluted to enable accurate quantification of low-concentration THC. This dilution rendered the CBD concentration too high for the calibration curve. As a result, a second dilution was required for CBD quantification to bring it within the validated analytical range. This two-step approach increased the complexity of the sample preparation workflow and introduced potential risks, including pipetting error, dilution inaccuracies, and increased solvent consumption. Additionally, it posed a practical limitation for routine analysis, where efficiency and reproducibility are critical. This challenge underscores the importance of developing flexible, concentration-adjustable methods or implementing wide-range calibration models to accommodate the dynamic concentration ranges often encountered in cannabinoid-containing products (Hambidge et al, 2025; Mudge et al, 2017).

Another challenge identified in the analysis of cannabinoids from MCT oil included carryover of CBD with methanol, where a small peak was seen at the retention time of CBD when running a blank (figure 3.2). This was a result of the high concentration of CBD in the samples which may lead to residual material being retained in the column. It was imperative to use methanol between runs to clean the column and effectively remove any remaining analytes.

The chemical and physical characteristics of MCT oil contribute to matrix effects that interfere with the chromatographic separation of cannabinoids. The high viscosity of the oil alters the flow dynamics within the HPLC column, leading to peak broadening and reduced peak sharpness. Additionally, lipid residues inherent to the MCT matrix may co-elute with target analytes, thereby complicating the differentiation and identification of individual cannabinoids. Collectively, these matrix-related interferences reduce chromatographic resolution and hinder the accuracy and reliability of quantification.

During method development with hemp seed oil the matrix effect in cannabinoid analysis arises from interactions between the cannabinoids and the lipid components of oil-based formulations. These interactions can interfere with analyte extraction efficiency or cause ion suppression or signal distortion during detection, leading to an underestimation of cannabinoid concentrations by HPLC. As a result, the chromatographic response may show reduced peak intensities for the target compounds (Palermiti et al, 2021).

An additional analytical challenge encountered in the laboratory involved optimizing the ratio of acetonitrile to acetic acid in the mobile phase composition. Increasing the proportion of acetonitrile led to a reduction in retention time, as analytes migrated more rapidly through the HPLC column. However, this acceleration often compromised chromatographic resolution, resulting in insufficient separation of analytes, peak co-elution, and consequently, poorly resolved chromatograms. These factors hinder accurate compound identification and quantification, thereby affecting the overall reliability of the analytical results (Tzimas et al, 2024).

Procuring suitable oil-based samples for analysis presented a logistical challenge, primarily due to the limited availability of cannabinoid-containing formulations and restricted research resources within Malta. This scarcity impacted the consistency and diversity of sample acquisition.

CBN and CBD are cannabinoids which have similar chemical structures. This results in CBN and CBD having close eluting times on the chromatogram during HPLC analysis (Duczmal et al, 2024). A challenge was identified when determining which resolution was ideal to allow for their peaks to be well separated on the chromatogram. A resolution of a 1:5 ratio was identified to ensure no co-elution, accurate quantification of the cannabinoids and ensure better reproducibility and validation.

The chromatographic comparison between the Avantor ACE 5 C18-AR (aromatic) (figure 3.3) and the standard Avantor ACE 5 C18 columns (figure 3.4) reveals key performance differences relevant to cannabinoid analysis. The C18-AR column exhibited shorter retention times for both CBD (5.23 min) and THC (9.4 min) compared to the standard C18 column (6.9 min and 14.1 min, respectively). This indicates that the aromatic modification of the C18-AR column facilitates faster elution of cannabinoids, reducing overall analysis time and solvent use. Both columns provided acceptable resolution for CBD/cannabigerol (CBG) (1.66 and 1.7, respectively) and peak symmetry close to the ideal value of 1.0, though the C18-AR column showed slightly better symmetry for CBD (0.99).

The aromatic C18-AR column offers additional advantages due to π–π interactions between its embedded phenyl groups and the aromatic rings present in cannabinoids. These interactions can enhance selectivity and reduce peak tailing, contributing to improved chromatographic performance, particularly for compounds like CBD and THC While the standard C18 column demonstrated a higher number of theoretical plates (12,297 vs. 9,574), the benefits of faster elution, efficient separation, and improved peak shape with the C18-AR column make it a more suitable choice for high-throughput cannabinoid analysis. Its ability to reduce retention of late-eluting analytes like THC is especially valuable in routine analytical workflows (Kayillo et al, 2006).

Different matrices consist of different chemical and physical properties. This is particularly important in relation to cosmetics. Balms have a water in oil consistency which implements its own challenges such as phase separation. Cannabinoids are lipophilic and so are found in the oil phase of the matrix. This could result in incomplete

recovery when carrying out solvent extraction during sample preparation (Wang et al, 2022).

Identification of the solvent needed for sample preparation of cosmetics was a challenge. The use of standard solvents such as methanol or acetonitrile in cannabinoid analysis can result in the preferential extraction of the aqueous phase in W/O emulsions, potentially leading to an underestimation of cannabinoid concentrations. In contrast, non-polar solvents like hexane or chloroform are more effective at extracting cannabinoids from the lipid-rich oil phase however, they also co-extract lipids and emulsifying agents. This co-extraction introduces matrix effects that can interfere with chromatographic separation and complicate accurate quantification during downstream analysis (Wang et al, 2022).

When selecting the most suitable solvent for sample preparation, it was essential to maintain both extraction efficiency and analytical specificity. The two solvents which were considered were IPA and ethanol. For W/O formulations, IPA was chosen because of its superior specificity and solubility for lipophilic cannabinoids, which enhanced extraction performance. In O/W formulations, 95% ethanol proved more effective, yielding higher extraction efficiency and cleaner chromatographic profiles, thereby supporting its use for accurate cannabinoid quantification.

Ethanol is increasingly preferred in cannabinoid analysis and other chromatographic applications owing to its dual analytical and environmental advantages. Recognized as a “green” solvent, ethanol is biodegradable, less toxic, and environmentally benign compared with more hazardous organic solvents such as IPA, methanol, or chloroform. Its favourable environmental profile aligns with the principles of green analytical chemistry, encouraging safer laboratory practices and reducing ecological impact. IPA,

while advantageous in certain analysis, particularly W/O emulsions, presents analytical challenges. Its higher absorbance in the UV range can generate background noise, potentially obscuring compounds present at low concentrations and compromising clarity in gradient HPLC-UV systems. However, in cosmetic sample analysis, IPA demonstrated strong specificity as it did not produce interfering peaks overlapping with cannabinoid analytes, which ensured cleaner chromatograms and minimized the risk of misidentification or inaccurate quantification(Mudge et al, 2017; Wang et al, 2022).

Standardizing sample preparation poses significant challenges due to the diverse physical and chemical properties of different sample matrices, particularly in cannabinoid-containing products. Variations in formulation types such as oils, balms, or emulsions affect solvent compatibility, homogenization efficiency, and extraction yield. A single preparation method may not be suitable for all matrices, leading to inconsistent analyte recovery and increased matrix interference. Cannabinoids are sensitive to environmental factors like light, heat and oxidation, making analyte stability a concern during preparation. Inadequate filtration and clean-up can introduce lipids or emulsifiers into analytical systems, compromising chromatographic performance. Even when protocols are standardized, minor variations in equipment, analyst technique, and environmental conditions can impact reproducibility. Developing a robust, validated sample preparation method tailored to the specific matrix is essential for achieving accurate, precise, and reproducible analytical results (Dachateau et al, 2025; Wang et al, 2022).

Challenges were identified when deciding what volume of sample mass is ideal for analysis. An increased sample mass (0.5g) resulted in difficulties when dissolving the matrix in the required solvent during sample preparation. This led to the production of inaccurate results as the concentration of cannabinoids present in the sample was

significantly decreased. A sample size of 0.05g was too small causing challenges when handling the sample and therefore, causing variations in the results obtained from analysis. It was agreed that a sample of 0.1g is the ideal amount of matrix needed for sample preparation to give accurate results with minimal variation.

4.3 Limitations

One of the primary limitations of this study was the exclusive use of HPLC coupled with UV detection for the quantification of cannabinoids. While HPLC coupled with an ultra violet (HPLC-UV) detector is a widely accepted and accessible analytical technique for routine cannabinoid analysis, it has inherent constraints in terms of sensitivity, specificity, and compound identification (Nie et al, 2019).

UV detectors measure analytes based on their absorption of ultraviolet light at specific wavelengths, such as 220 nm or 280 nm, where cannabinoids typically absorb. However, this approach lacks molecular selectivity, meaning it cannot confirm the structural identity of the analyte, especially in complex sample matrices. In such cases, co-eluting compounds or impurities with similar absorbance profiles can interfere with accurate quantification, leading to potential errors in peak assignment or misidentification of cannabinoids (Nie et al, 2019).

UV detection is less sensitive than MS based techniques, which limits its ability to detect cannabinoids present at low concentrations (e.g., minor cannabinoids such as CBN, CBG, or Δ^8-THC). Unlike MS, UV detectors do not provide information on the mass-to-charge ratio (m/z) of analytes, making it difficult to distinguish structurally similar or isomeric cannabinoids. This is particularly important in cosmetic formulations and oils, where

cannabinoids may degrade or interact with other components, producing compounds that require high-resolution identification.

While HPLC-UV served as a practical and cost-effective tool for this study, the absence of more advanced detection methods such as HPLC-MS/MS limited the analytical depth, resolution, and confirmatory capability of the cannabinoid quantification.

A further limitation was the restricted diversity of sample matrices, which included only hemp seed oil, MCT oil, cannabinoid containing scar cream, and cannabinoid containing night cream. Although these represent common delivery systems and formulations in the cannabis and cosmetics industries, the narrow range of matrices limits the generalizability and applicability of the results obtained.

Different matrices pose unique analytical and extraction challenges. Oils, such as hemp seed and MCT oil, are relatively non-polar and generally easier to handle in cannabinoid extraction and analysis. Creams, particularly complex topical formulations like scar and night creams contain emulsifiers, waxes, preservatives, and other excipients that can introduce matrix interferences, potentially affecting analyte recovery, retention, and UV detection. These excipients can also co-elute with target cannabinoids or obscure signals, particularly in UV detection, where specificity is already limited.

4.4 Recommendation for Future Work

Future research should aim to incorporate more selective detection technologies, such as HPLC-MS and expand the range of matrices evaluated to include more complex and diverse formulations.

4.5 Conclusion

Recognising the challenges involved in preparing and analysing samples for cannabinoid quantification is crucial for the advancement of reliable analytical techniques. The diverse nature of sample types ranging from oils and balms to emulsions and other complex formulations can significantly impact the efficiency of extraction and the accuracy of measurement. By identifying these matrix-specific challenges, analysts can refine their procedures to ensure better solvent compatibility, improve analyte recovery, and minimize interferences that may arise during chromatographic separation. Addressing such limitations also supports the development of more consistent and reproducible methods, which are essential for ensuring analytical precision across varied product types. Investigating these issues contributes to more effective quantification strategies, enhancing the quality and safety of cannabinoid-containing products.

References

Addo PW, Sagili SU, Bilodeau SE, Gladu-Gallant F-A, MacKenzie DA, Bates J et al. Cold ethanol extraction of cannabinoids and terpenes from cannabis using response surface methodology: optimization and comparative study. *Molecules*. 2022;27(24):8780. doi:10.3390/molecules27248780.

Analakkattillam S, Langsi VK, Hanrahan JP, Moore E. Analytical method validation for assay determination of cannabidiol and tetrahydrocannabinol in hemp oil infused products by RP-HPLC. Sci Rep. 2022; 21;12(1):12453. doi: 10.1038/s41598-022-13737-6.

Antunes M, Barroso M, Gallardo E**.** Analysis of cannabinoids in biological specimens: an update. *Int J Environ Res Public Health*. 2023;20(3):2312. doi:10.3390/ijerph20032312.

Antunes M, Simões S, Fonseca S, Franco J, Gallardo E, Barroso M. Detection and quantification of selected cannabinoids in oral fluid samples by protein precipitation and LC-MS/MS. Forensic Sci Int. 2024;363:112174. doi:10.1016/j.forsciint.2024.112174.

Barnes AJ, Scheidweiler KB, Huestis MA. Quantification of 11-nor-9-carboxy-δ9-tetrahydrocannabinol in human oral fluid by gas chromatography-tandem mass spectrometry. Ther Drug Monit. 2014 ;36(2):225-33. doi: 10.1097/01.ftd.0000443071.30662.01.

Bergamaschi MM, Barnes A, Queiroz RHC, Hurd YL, Huestis MA. Impact of enzymatic and alkaline hydrolysis on CBD concentration in urine. Anal Bioanal Chem2013;405(14):4679-4789. doi: 10.1007/s00216-013-6837-x.

Brandt A, Mann G, Arlt W. Temperature gradients in preparative high-performance liquid chromatography columns. *J Chromatogr A*. 1997;769:109–17. doi:10.1016/S0021-9673(97)00235-5.

Cachia M. Extraction and determination of tetrahydrocannabinol in oil [dissertation]. Msida (Malta): Department of Pharmacy, University of Malta; 2021.

Camilleri G. An investigation of the use, effectiveness, and prescription of medical cannabis from a prescribers' perspective in Malta [dissertation]. Msida (Malta): Department of Clinical Pharmacology & Therapeutics, University of Malta; 2024.

Cardenia V, Toschi TG, Scappini S, Rubino RS, Rodriquez-Estrada MT. Development and validation of a Fast gas chromatography/mass spectrometry method for the determination of cannabinoids in *Cannabis sativa* L. J Food Drug Anal. 2018;26(4):1283-1292. doi: 10.1016/j.jfda.2018.06.001.

Casiraghi A, Roda G, Casagni E, Cristina C, Musazzi UM, Franzè S, et al. Extraction Method and Analysis of Cannabinoids in Cannabis Olive Oil Preparations. Planta Med. 2018;84(4):242-249. doi: 10.1055/s-0043-123074.

Castro-Perez J, Chandra P. Recent advances in mass spectrometric and other analytical techniques for the identification of drug metabolites. In: Ma S, Chowdhury SK, editors. Identification and Quantification of Drugs, Metabolizing Enzymes, and Transporters. 2nd ed. Amsterdam: Elsevier; 2020. p. 39–71.

Černe K. Toxicological properties of Δ9-tetrahydrocannabinol and cannabidiol. Arhiv za Higijenu Rada i Toksikologiju. 2020 1;71(1):1-11. doi: 10.2478/aiht-2020-71-3301.

Chambers MI, Garosi B, Musah RA. DART-MS facilitated quantification of cannabinoids in complex edible matrices—focus on chocolates and gelatin-based fruit candies. ACS Omega. 2023;8(16):14459–14469. doi:10.1021/acsomega.2c08172.

Chiu HH, Kuo CH. Gas chromatography-mass spectrometry-based analytical strategies for fatty acid analysis in biological samples. J Food Drug Anal. 2020;28(1):60-73. doi: 10.1016/j.jfda.2019.10.003.

Christinat N, Morin-Rivron D, Masoodi M. High-Throughput Quantitative Lipidomics Analysis of esterified fatty acids in human plasma. J. Proteome Res. 2016;15(7):2228-2235. doi: 10.1021/acs.jproteome.6b00198.

Ciolino L, Ranieri T, Taylor A. Commercial cannabis consumer products part 1: GC– MS qualitative analysis of cannabis cannabinoids. Forensic Sci Int. 2018;289:429-437. doi: 10.1016/j.forsciint.2018.05.032.

Citti C, Braghiroli D, Vandelli MA, Cannazza G. Pharmaceutical and biomedical analysis of cannabinoids: A critical review. J Pharm Biomed Anal. 2018;147:565–579. doi:10.1016/j.jpba.2017.06.003.

Citti C, Russo F, Linciano P, Strallhofer SS, Tolomeo F, Forni F, et al. Origin of Δ9-Tetrahydrocannabinol Impurity in Synthetic Cannabidiol. Cannabis Cannabinoid Res. 2021;12;6(1):28-39. doi: 10.1089/can.2020.0021.

Concheiro M, Lee D, Lendoiro E, Huestis MA. Simultaneous quantification of Δ(9)-tetrahydrocannabinol, 11-nor-9-carboxy- tetrahydrocannabinol, cannabidiol and cannabinol in oral fluid by microflow-liquid chromatography-high resolution mass spectrometry. J Chromatogr A. 2013;5;1297:123-30. doi: 10.1016/j.chroma.2013.04.071.

Couttas TA, Boost C, Pahlisch F, Sykorova EB, Leweke JE, Koethe D, et al. Simultaneous Assessment of Serum Levels and Pharmacologic Effects of Cannabinoids on Endocannabinoids and *N*-Acylethanolamines by Liquid Chromatography-Tandem Mass Spectrometry. Cannabis. Cannabinoid Res. 2023;8(4):657-669. doi: 10.1089/can.2021.0181.

Correia B, Ahmad SM, Quintas A. Determination of phytocannabinoids in cannabis samples by ultrasound-assisted solid-liquid extraction and high-performance liquid chromatography with diode array detector analysis. J Chromatogr A. 2023;30;1705:464191. doi: 10.1016/j.chroma.2023.464191.

Crippa JAS, Zuardi AW, Hallak JEC, Miyazawa B, Bernardo SA, Donaduzzi CM, et al. Oral Cannabidiol Does Not Convert to Δ8-THC or Δ9-THC in Humans: A Pharmacokinetic Study in Healthy Subjects. Cannabis and Cannabinoid Res. 2020; 5 (1). doi: 10.1089/can.2019.0024.

Dawidowicz AL, Typek R, Olszowy-Tomczyk M. Natural vs. artificial cannabinoid oils: the comparison of their antioxidant activities. Eur Food Res Technol. 2023;249(2):359-366. doi: 10.1007/s00217-022-04121-9.

De Aguiar AC, Vardanega R, Viganó J, Silva EK. Supercritical carbon dioxide technology for recovering valuable phytochemicals from *Cannabis sativa L.* and valorization of its biomass for food applications. Molecules. 2023;28(9):3849. doi:10.3390/molecules28093849.

Dei Cas M, Casagni E, Casiraghi A, Minghetti P, Fornasari DMM, Ferri F, et al. Phytocannabinoids Profile in Medicinal Cannabis Oils: The Impact of Plant Varieties and

Preparation Methods. Front Pharmacol. 2020;13(11)570616. doi: 10.3389/fphar.2020.570616.

De Vita S, Finamore C, Chini MG, Saviano G, De Felice V, De Marino S, et al. Phytochemical Analysis of the Methanolic Extract and Essential Oil from Leaves of Industrial Hemp Futura 75 Cultivar: Isolation of a New Cannabinoid Derivative and Biological Profile Using Computational Approaches. Plants (Basel). 2022 Jun 24;11(13):1671. doi: 10.3390/plants11131671.

Di Trana A, Sprega G, Kobidze G, Taoussi O, Lo Faro AF, Bambagiotti G, et al. QuEChERS extraction and simultaneous quantification in GC-MS/MS of hexahydrocannabinol epimers and their metabolites in whole blood, urine, and oral fluid. Molecules. 2024;29(14):3440. doi:10.3390/molecules29143440.

Duchateau C, Stévigny C, Waeytens J, Deconinck E. Chromatographic and spectroscopic analyses of cannabinoids: a narrative review focused on cannabis herbs and oily products. Molecules. 2025;30(3):490. doi:10.3390/molecules30030490.

Duczmal D, Bazan-Wozniak A, Niedzielska K, Pietrzak R. Cannabinoids—multifunctional compounds, applications and challenges: mini review. Molecules. 2024;29(20):4923. doi:10.3390/molecules29204923.

Ecker J, Scherer M, Schmitz G, Liebisch G. A rapid GC–MS method for quantification of positional and geometric isomers of fatty acid methyl esters. J Chromatogr B. 2012;897;98- 104. doi:10.1016/j.jchromb.2012.04.015.

ElSohly MA, Murphy TP, Khan I, Walker LW, Gul W. Analysis of Cannabidiol, Δ^9-Tetrahydrocannabinol, and Their Acids in CBD Oil/Hemp Oil Products. Med Cannabis Cannabinoids. 2020;13;3(1):1-13. doi: 10.1159/000509550.

Filippini G, Minozzi S, Borrelli F, Cinquini M, Dwan K**.** Cannabis and cannabinoids for symptomatic treatment for people with multiple sclerosis. Cochrane Database Syst Rev. 2022;5(5). doi:10.1002/14651858.CD013444.pub2.

Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J biol Chem. 1957;226(1):497-509.

Franzin M, Di Lenardo R, Ruoso R, Addobbati R. Incomplete Decarboxylation of Acidic Cannabinoids in GC-MS Leads to Underestimation of the Total Cannabinoid Content in Cannabis Oils Without Derivatization. *Pharmaceutics*. 2025;17(3):334. doi:10.3390/pharmaceutics17030334.

Frei P, Frauchiger S, Scheurer E, Mercer-Chalmers-Bender K. Quantitative determination of five cannabinoids in blood and urine by gas chromatography tandem mass spectrometry applying automated on-line solid phase extraction. Drug Test Anal. 2022;14(7):1223-1233. doi: 10.1002/dta.3241.

French D. Advances in Clinical Mass Spectrometry. In: Makowski GS, editors. Advances in Clinical Chemistry, 1st ed. United States: Elsevier; 2017. p.153-198.

García-Atienza P, Martínez-Pérez-Cejuela H, Simó-Alfonso EF, Herrero-Martínez JM, Armenta S. Determination of synthetic cannabinoids in oral fluids by liquid chromatography with fluorescence detection after solid-phase extraction. MethodsX. 2023;5(10)102173. doi: 10.1016/j.mex.2023.102173.

García-Gutiérrez MS, Navarrete F, Gasparyan A, Austrich-Olivares A, Sala F, Manzanares J. Cannabidiol: A Potential New Alternative for the Treatment of Anxiety, Depression, and Psychotic Disorders. Biomolecules. 2020;19(11):1575. doi: 10.3390/biom10111575.

Galant N, Czarny J, Powierska-Czarny J, Piotrowska-Cyplik A. Development and Validation of the LC-MS/MS Method for Determination of 130 Natural and Synthetic Cannabinoids in Cannabis Oil. Molecules. 2022;6;27(23):8601. doi: 10.3390/molecules27238601.

Galettis P, Williams M, Gordon R, Martin J**.** A simple isocratic HPLC method for the quantitation of 17 cannabinoids. Aust J Chem. 2021;74(6):453–462. doi:10.1071/CH20380.

Gallo LL. Constituents of cannabidiol products [dissertation]. Msida (Malta): Department of Pharmacy, University of Malta; 2023.

Guo W, Vrdoljak G, Liao VC, Moezzi B. Major Constituents of Cannabis Vape Oil Liquid, Vapor and Aerosol in California Vape Oil Cartridge Samples. Front Chem. 2021;21(9)694905. doi: 10.3389/fchem.2021.694905.

Hambidge T, Nash R, Corless S, Sanatcumar P, Bowdery P, Griffin J, et al**.** Validated LC-MS/MS methodology for the quantification of CBD, trace level THCA and UK controlled cannabinoids (Δ^9THC, Δ^8THC, CBN and THCV) in food samples. Anal Methods. 2025;17. doi:10.1039/D4AY01946F.

Hazekamp A. The trouble with CBD oil. Med Cannabis Cannabinoids. 2018;1(1):65–72. doi:10.1159/000489287.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, et al. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev. 2002;54(2):161–202. doi:10.1124/pr.54.2.161.

Hsu YH, Fang MC, Huang SC, Kao YM, Tseng SH, Wang DY. Determination of cannabinoids in hemp oil based cosmetic products by LC-tandem MS. J Food Drug Anal. 2021 Sep 15;29(3):502-509. doi: 10.38212/2224-6614.3370.

Huang S, Claassen FW, Van Beek TA, Chen B, Zeng J, Zuilhof H, et al. Rapid Distinction and Semiquantitative Analysis of THC and CBD by Silver-Impregnated Paper Spray Mass Spectrometry. Anal Chem. 2021;93(8):3794-3802. doi: 10.1021/acs.analchem.0c04270.

Huang S, Righetti L, Claassen FW, Krishna A, Ma M, van Beek TA, et al. Ultrafast, selective, and highly sensitive nonchromatographic analysis of fourteen cannabinoids in cannabis extracts, Δ8-tetrahydrocannabinol synthetic mixtures, and edibles by cyclic ion mobility spectrometry-mass spectrometry. *Anal Chem*. 2024;96(25):10170-81. doi:10.1021/acs.analchem.4c01394.

Huang S, Van Beek TA, Beij E, Ma M, Chen B, Zuilhof H, et al. Rapid analysis of Δ8-tetrahydrocannabinol, Δ9-tetrahydrocannabinol, and cannabidiol in Δ8-tetrahydrocannabinol edibles by Ag(I) paper spray mass spectrometry after simple extraction. Food Chem. 2025;479:143829. doi:10.1016/j.foodchem.2025.143829.

Hubbard JA, Smith BE, Sobolesky PM, Kim S, Hoffman MA, Stone J et al. Validation of a liquid chromatography tandem mass spectrometry (LC-MS/MS) method to detect cannabinoids in whole blood and breath. Clin Chem Lab Med. 2020;58(5):673-681. doi: 10.1515/cclm-2019-0600.

Huestis MA. Human cannabinoid pharmacokinetics. *Chem Biodivers*. 2007;4(8):1770–1804.

Iffland K, Grotenhermen F. An update on safety and side effects of cannabidiol: a review of clinical data and relevant animal studies. Cannabis Cannabinoid Res. 2017;2(1):139–154. doi:10.1089/can.2016.0034.

Jadhav HB, Annapure US. Triglycerides of medium-chain fatty acids: a concise review. J Food Sci Technol. 2023;60(8):2143-2152. doi:10.1007/s13197-022-05499-w.

Jensen TL, Wu F, McMillin GA. Detection of *in utero* Exposure to Cannabis in Paired Umbilical Cord Tissue and Meconium by Liquid Chromatography-Tandem Mass Spectrometry. Clin Mass Spectrom. 2019;4(14):115-123. doi: 10.1016/j.clinms.2019.01.002.

Kayillo S, Dennis GR, Shalliker RA**.** An assessment of the retention behaviour of polycyclic aromatic hydrocarbons on reversed phase stationary phases: selectivity and retention on C18 and phenyl-type surfaces. J Chromatogr A. 2006 Sep 8;1126(1–2):283–297. doi:10.1016/j.chroma.2006.04.054.

Keçili R, Büyüktiryaki S, Dolak I, Hussain CM. The use of magnetic nanoparticles in sample preparation devices and tools. In: Hussain CM, editors. Handbook of Nanomaterials in Analytical Chemistry, 1st ed. United States: Elsevier;2020. p.75-95.

Klawitter J, Sempio C, Mörlein S, De Bloois E, Klepacki J, Henthorn T, et al. An Atmospheric Pressure Chemical Ionization MS/MS Assay Using Online Extraction for the Analysis of 11 Cannabinoids and Metabolites in Human Plasma and Urine. Ther Drug Monit. 2017; 39(5): 556-564. doi:10.1097/FTD.0000000000000427.

Knittel JL, Holler JM, Chmiel JD, Vorce SP, Magluilo J Jr, Levine B, et al. Analysis of Parent Synthetic Cannabinoids in Blood and Urinary Metabolites by Liquid Chromatography Tandem Mass Spectrometry. J Anal Toxicol. 2016;40(3):173-186. doi: 10.1093/jat/bkv137.

Kumar U. Cannabinoids: role in neurological diseases and psychiatric disorders. Int J Mol Sci. 2024;7;26(1):152. doi:10.3390/ijms26010152.

La Maida N, Pellegrini M, Papaseit E, Pérez-Mañá C, Poyatos L, Ventura M, et al. Determination of the Synthetic Cannabinoids JWH-122, JWH-210, UR-144 in Oral Fluid of Consumers by GC-MS and Quantification of Parent Compounds and Metabolites by UHPLC-MS/MS. Int J Mol Sci. 2020;21(24):9414. doi: 10.3390/ijms21249414.

Li D, Ilnytskyy Y, Ghasemi Gojani E, Kovalchuk O, Kovalchuk I. Analysis of anti-cancer and anti-inflammatory properties of 25 high-THC cannabis extracts. Molecules. 2022;27(18):6057. doi:10.3390/molecules27186057.

Li L, Duffy BC, Durocher LA, Dittmar MA, Acosta RA, Delaney ER, et al. Potency Analysis of Medical Marijuana Products from New York State. Cannabis Cannabinoid Res. 2019;4(3):195-203. doi: 10.1089/can.2018.0037.

Lafaye G, Karila L, Blecha L, Benyamina A. Cannabis, cannabinoids and health. Dialogues Clin Neurosci. 2017;19(3):309-316. doi: 10.31887/DCNS.2017.19.3/glafaye.

Lazarjani MP, Torres S, Hooker T, Fowlie C, Young O, Seyfoddin A. Methods for quantification of cannabinoids: A narrative review. J Cannabis Res. 2020;2(1):35. doi:10.1186/s42238-020-00040-2.

Leghissa A, Hildenbrand ZL, Schug KA. A review of methods for the chemical characterization of cannabis natural products. J Sep Sci. 2018;41(1):398-415. doi: 10.1002/jssc.201701003.

Leghissa A, Hildenbrand ZL, Schug KA. The imperatives and challenges of analyzing Cannabis edibles. Curr Opin Food Sci. 2019;28:18–24. doi:10.1016/j.cofs.2019.02.010.

Lindekamp N, Triesch N, Rohn S, Weigel S. Quantification of sixteen cannabinoids in hemp seed edible oils and the influence of thermal processing on cannabinoid contents and profiles. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2024;41(5):550–562. doi:10.1080/19440049.2024.2319270.

Maggini V, Calvi L, Pelagatti T, Gallo ER, Civati C, Privitera C, et al. An optimized terpene profile for a new medical cannabis oil. *Pharmaceutics*. 2022;14(2):298. doi:10.3390/pharmaceutics14020298.

Mandrioli M, Tura M, Scotti S and Gallina TT. Fast Detection of 10 cannabinoids by RP-HPLC-UV method in cannabis sativa L. Molecules. 2019;24(11):2113. doi:10.3390/molecules24112113.

Marsot A, Audebert C, Attolini L, Lacarelle B, Micallef J, Blin O. Comparison of Cannabinoid Concentrations in Plasma, Oral Fluid and Urine in Occasional Cannabis Smokers After Smoking Cannabis Cigarette. J Pharm Pharm Sci. 2016;19(3):411-422. doi: 10.18433/J3F31D.

Mastellone G, Marengo A, Sgorbini B, Rubiolo P, Anderson JL, Cagliero C. Ultrasound-assisted dispersive solid-liquid microextraction with eutectic solvents for the

determination of cannabinoids in different hemp products. J Chromatogr B Analyt Technol Biomed Life Sci. 2024;1;1232:123967. doi: 10.1016/j.jchromb.2023.123967.

McPartland JM, Duncan M, Di Marzo V, Pertwee RG. Are cannabidiol and Δ⁹-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br J Pharmacol. 2015;172(3):737–753. doi:10.1111/bph.12944.

McRae G, Melanson JE. Quantitative determination and validation of 17 cannabinoids in cannabis and hemp using liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2020;412(27):7381-7393. doi: 10.1007/s00216-020-02862-8.

Mechoulam R, Parker LA. The endocannabinoid system and the brain. Annu Rev Psychol. 2013;64:21–47. doi:10.1146/annurev-psych-113011-143739.

Meepun N, Sommart U, Bualuang O. Green solvent-based extraction of *Cannabis sativa* L. by-products: enhancing antioxidant potential and cannabinoid enrichment using MCT oil. J Cannabis Res. 2025;7(1):50. doi:10.1186/s42238-025-00309-4.

Meng Q, Buchanan B, Zuccolo J, Poulin MM, Gabriele J, Baranowski DC. A reliable and validated LC-MS/MS method for the simultaneous quantification of 4 cannabinoids in 40 consumer products. PLoS One. 2018;13(5):196396. doi: 10.1371/journal.pone.0196396.

Millar SA, Stone NL, Yates AS, O'Sullivan SE. A systematic review on the pharmacokinetics of cannabidiol in humans. Front Pharmacol. 2018;9:1365. doi:10.3389/fphar.2018.01365.

Montero L, Meckelmann SW, Kim H, Ayala-Cabrera JF, Schmitz OJ. Differentiation of industrial hemp strains by their cannabinoid and phenolic compounds using LC × LC-

HRMS. Anal Bioanal Chem. 2022;414(18):5445-5459. doi: 10.1007/s00216-022-03925-8. Epub 2022 Mar 17.

Monti MC, Stoll A, Schlotterbeck G, Duthaler U. Miniaturized one-step extraction for the analysis of Δ9- and Δ8-tetrahydrocannabinol isomers, phytocannabinoids, and hexahydrocannabinol in whole blood using liquid chromatography–tandem mass spectrometry. J Chromatogr A. 2025;7;1760:466276. doi:10.1016/j.chroma.2025.466276.

Mudge EM, Murch SJ, Brown PN. Leaner and greener analysis of cannabinoids. Anal Bioanal Chem. 2017;409:3153-3163. doi: 10.1007/s00216-017-0256-3.

Nahar L, Onder A, Sarker SD. A review on the recent advances of HPLC, UHPLC and UPLC analyses of naturally occurring cannabinoids (2010-2019). Phytochem Anal. 2020;31(4):413-457. doi: 10.1002/pca.2906.

Namdar D, Koltai H. Medical Cannabis for the Treatment of Inflammation. Natural Product Communications. 2018;13(3):249-254. doi: 10.1177/1934578X1801300304.

Nie B, Henion J, Ryona I. The role of mass spectrometry in the cannabis industry. J Am Soc Mass Spectrom. 2019;30(5):719-730. doi: 10.1007/s13361-019-02164-z.

Ötles S, Kartal C. Solid-Phase Extraction (SPE): Principles and Applications in Food Samples. Acta Sci Pol Technol Aliment. 2016;15(1):5-15. doi: 10.17306/J.AFS.2016.1.1.

Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. Published 2021 Mar 29. doi:10.1136/bmj.n71.

Palermiti A, Cafaro A, Barco S, Bucchioni P, Franceschini P, Cusato J, et al. Analysis of cannabinoids concentration in cannabis oil galenic preparations: harmonization between three laboratories in Northern Italy. Pharmaceutics (Basel). 2021;14(5):462. doi:10.3390/ph14050462.

Pantoja-Ruiz C, Restrepo-Jimenez P, Castañeda-Cardona C, Ferreirós A, Rosselli D. Cannabis and pain: a scoping review. Braz J Anesthesiol. 2022;72(1):142–51. doi:10.1016/j.bjane.2021.06.018.

Paul R, Williams R, Hodson V, Peake C. Detection of cannabinoids in hair after cosmetic application of hemp oil. Sci Rep. 2019;9(1):2582. doi: 10.1038/s41598-019-39609-0.

Pavlovic R, Nenn G, Calvi L, Panseri S, Borgonovo G, Giupponi L, et al. Quality Traits of "Cannabidiol Oils": Cannabinoids Content, Terpene Fingerprint and Oxidation Stability European Commercially Available Preparations. Molecules. 2018;23(5):1230. doi: 10.3390/molecules23051230.

Pellegrini M, Marchei E, Papaseit E, Farré M, Zaami S. UHPLC-HRMS and GC-MS Screening of a Selection of Synthetic Cannabinoids and Metabolites in Urine of Consumers. Medicina (Kaunas). 2020;56(8):408. doi: 10.3390/medicina56080408.

Pellati F, Borgonetti V, Brighenti V, Biagi M, Benvenuti S, Corsi L. *Cannabis sativa L.* and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation and Cancer. Biomed Res Int. 2018; 15 pages. doi:10.1155/2018/1691428.

Pertwee RG. Pharmacological actions of cannabinoids. *Handb Exp Pharmacol*. 2005;(168):1–51. doi:10.1007/3-540-26573-2_1.

Phung E, Lee D, Swart C, Ke Y, Moore-Bollinger K, Bynum N,et al. Evaluation of the long-term stability of select phenylacetylindole, cycloalkylindole, quinolinyl, and carboxamide synthetic cannabinoids using LC-MS-MS. J Anal Toxicol. 2023;47(8):685-693. doi: 10.1093/jat/bkad073. PMID: 37697731.

Pieracci Y, Ascrizzi R, Terreni V, Pistelli L, Flamini G, Bassolino L, et al. Essential Oil of *Cannabis sativa* L: Comparison of Yield and Chemical Composition of 11 Hemp Genotypes. Molecules. 2021 Jul 3;26(13):4080. doi: 10.3390/molecules26134080.

Pino S, Espinoza L, Jara-Gutiérrez C, Villena J, Olea AF, Díaz K. Study of Cannabis Oils Obtained from Three Varieties of *C. sativa* and by Two Different Extraction Methods: Phytochemical Characterization and Biological Activities. Plants (Basel). 2023;12(9):1772. doi: 10.3390/plants12091772.

Pourseyed Lazarjani M, Torres S, Hooker T, Fowlie C, Young O, Seyfoddin A. Methods for quantification of cannabinoids: a narrative review. J Cannabis Res. 2020;2(1):35. doi:10.1186/s42238-020-00040-2.

Qamar S, Manrique YJ, Parekh HS, Falconer JR. Development and Optimization of Supercritical Fluid Extraction Setup Leading to Quantification of 11 Cannabinoids Derived from Medicinal Cannabis. Biology (Basel). 2021;10(6):481. doi: 10.3390/biology10060481.

Ramella A, Roda G, Pavlovic R, Dei Cas M, Casagni E, Mosconi G, et al. Impact of Lipid Sources on Quality Traits of Medical Cannabis-Based Oil Preparations. Molecules. 2020; 25(13):2986. doi.org/10.3390/molecules25132986.

Ramos Borges G, Birk L, Scheid C, Morés L, Carasek E, Kitamura RO, et al. Simple and straightforward analysis of cannabinoids in medicinal products by fast-GC–FID. Forensic Toxicol. 2020;38:531-535. doi: 10.1007/s11419-020-00522-1.

Ravula A, Chandasana H, Setlow B, Febo M, Bruijnzeel AW, Derendorf H. Simultaneous quantification of cannabinoids tetrahydrocannabinol, cannabidiol and CB1 receptor antagonist in rat plasma: An application to characterize pharmacokinetics after passive cannabis smoke inhalation and co-administration of rimonabant. J Pharm Biomed Anal. 2018;160: 119-125. doi:10.1016/j.jpba.2018.07.004.

Rochfort S, Isbel A, Ezernieks V, Elkins A, Vincent D, Deseo MA, et al. Utilisation of Design of Experiments Approach to Optimise Supercritical Fluid Extraction of Medicinal Cannabis. Sci Rep. 2020;10(1):9124. doi: 10.1038/s41598-020-66119-1.

Rodriguez ET, Vella Szijj J, Cachia M, Falzon P, Axisa K, Serracino-Inglott A, et al. An efficient HPLC-UV method for determination of tetrahydrocannabinol in oil. Asian J Pharm Clin Res. 2023;16(3):110-5. doi:10.22159/ajpcr.2023v16i3.47462.

Scheidweiler KB, Himes SK, Chen X, Liu HF, Huestis MA. 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol quantification in human oral fluid by liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2013l;405(18):6019-27. doi: 10.1007/s00216-013-7027-6.

Sempio C, Huestis MA, Mikulich-Gilbertson SK, Klawitter J, Christians U, Henthorn TK. Population pharmacokinetic modelling of plasma Δ9-tetrahydrocannabinol and an active and inactive metabolite following controlled smoked cannabis administration. Brit J Clin Pharmacol. 2020;86(3); 611-619. doi: 10.1111/bcp.14170.

Shao M, Yu XQ, Huang LJ, Yao H, Li SC. [Determination of eight cannabinoids in foods with enhanced matrix removal-lipid adsorbent by ultra performance liquid chromatography-tandem mass spectrometry]. Se Pu. 2023;41(5):426-433. doi: 10.3724/SP.J.1123.2022.08010.

Shi N, Bu X, Zhang M, Wang B, Xu X, Shi X, et al. Current Sample Preparation Methodologies for Determination of Catecholamines and Their Metabolites. Molecules. 2022;27(9):2702. doi: 10.3390/molecules27092702.

Silva EMP, Vitiello A, Miro A, Ribeiro CJA. Recent HPLC-UV approaches for cannabinoid analysis: from extraction to method validation and quantification compliance. Pharmaceuticals (Basel). 2025;18(6):786. doi:10.3390/ph18060786.

Stella B, Baratta F, Della Pepa C, Arpicco S, Gastaldi D, Dosio F. Cannabinoid Formulations and Delivery Systems: Current and Future Options to Treat Pain. Drugs. 2021;81(13), 1513–1557. doi.org/10.1007/s40265-021-01579-x.

Stella N. THC and CBD: Similarities and differences between siblings. Neuron. 2023;111(3):302-327. doi: 10.1016/j.neuron.2022.12.022.

Spindle TR, Cone EJ, Herrmann ES, Mitchell JM, Flegel R, LoDico C, et al. Pharmacokinetics of Cannabis Brownies: A Controlled Examination of Δ9-

Tetrahydrocannabinol and Metabolites in Blood and Oral Fluid of Healthy Adult Males and Females. J Anal Toxicol. 2020;44(7):661–671. doi:10.1093/jat/bkaa067.

Taiti C, Masi E, Cicaloni V, Vinciguerra V, Salvini L, Garzoli S. SPME-GC-MS and PTR-ToF-MS Techniques for the Profiling of the Metabolomic Pattern of VOCs and GC-MS for the Determination of the Cannabinoid Content of Three Cultivars of Cannabis sativa L. Pollen. Molecules. 2022;27(24):8739. doi: 10.3390/molecules27248739.

Tanase Apetroaei V, Istrati DI, Vizireanu C. Plant-derived compounds in hemp seeds (*Cannabis sativa* L.): extraction, identification and bioactivity – a review. Molecules. 2024;30(1):124. doi:10.3390/molecules30010124.

Tan J, Rylski A, Bergqvist A, Stephanson NN. Automated and fully validated high-throughput LC-MS/MS assay for analyzing multiple drugs of abuse in oral fluids using novel features in sample preparation and chromatographic conditions. J Mass Spectrom. 2025;60(5):5132. doi:10.1002/jms.5132.

Tran J, Elkins AC, Spangenberg GC, Rochfort SJ. High-Throughput Quantitation of Cannabinoids by Liquid Chromatography Triple-Quadrupole Mass Spectrometry. Molecules. 2022;27(3):742. doi: 10.3390/molecules27030742.

Tzimas PS, Petrakis EA, Halabalaki M, Skaltsounis LA. Extraction solvent selection for *Cannabis sativa* L. by efficient exploration of cannabinoid selectivity and phytochemical diversity. Phytochem Anal. 2024;35(1):163–83. doi:10.1002/pca.3282.

Urits I, Charipova K, Gress K, Li N, Berger AA, Cornett EM, et al. Adverse Effects of Recreational and Medical Cannabis. Psychopharmacol Bull. 2021;12;51(1):94-109.

Vella Szijj J. Challenges of extracting and determining cannabinoids in different matrices. Cannabis Cannabinoid Res. 2024;9(6):1470-1477. doi:10.1089/can.2024.0087.

Ververi C, Gentile C, Massano M, Salomone A, Vincenti M. Quantitative determination by UHPLC-MS/MS of 18 common drugs of abuse and metabolites, including THC and OH-THC, in volumetric dried blood spots: a sustainable method with minimally invasive sampling. J Chromatogr B Analyt Technol Biomed Life Sci. 2024;1247:124337. doi:10.1016/j.jchromb.2024.124337.

Wakshlag JJ, Schwark WS, Deabold KA, Talsma BN, Cital S, Lyubimov A, et al. Pharmacokinetics of Cannabidiol, Cannabidiolic Acid, Δ9-Tetrahydrocannabinol, Tetrahydrocannabinolic Acid and Related Metabolites in Canine Serum After Dosing With Three Oral Forms of Hemp Extract. Front Vet Sci. 2020;7:505. doi: 10.3389/fvets.2020.00505.

Wang DY, Chang-Chien GP, Lee CH, Hsieh **YC.** Determination of cannabinoids in hemp oil–based cosmetic products by LC–MS/MS. J Chromatogr A. 2022;1676:463371. doi:10.1016/j.chroma.2022.463371.

Wang M, Wang YH, Avula B, Radwan MM, Wanas AS, Van Antwerp J, et al. Decarboxylation study of acidic cannabinoids: a novel approach using ultra-high-performance supercritical fluid chromatography/photodiode array-mass spectrometry. Cannabis Cannabinoid Res. 2016;1(1):262–271. doi:10.1089/can.2016.0020.

Whiting PF, Wolff RF, Deshpande S, Di Nisio M, Duffy S, Hernandez AV, et al. Cannabinoids for Medical Use: A Systematic Review and Meta-analysis. JAMA. 2015;313(24):2456-73. doi: 10.1001/jama.2015.6358.

Wise A. An overview of extraction terminology, specific types of extraction methodologies, and clarification on some common misconceptions. Cannabis Sci Tech. 2019; 2(1):20-26.

Wongumpornpinit V, Temkitthawon P, Khumpirapang N, et al. Pilot-scale preparation of broad-spectrum CBD: extraction optimization and purification using centrifugal partition chromatography. Med Cannabis Cannabinoids. 2025;8(1):65-79. doi:10.1159/000546263.

Zeger VR, Bell DS, Herrington JS, Anderson JL. Selective isolation of pesticides and cannabinoids using polymeric ionic liquid-based sorbent coatings in solid-phase microextraction coupled to high performance liquid chromatography. J Chromatogr A 2022;1680;463416. doi.org/10.1016/j.chroma.2022.463416.

Zhang S, Alam MM, Chandler BD, Lanorio JP, Deskins C, Song LG, et al. Potency analysis of semi-synthetic cannabinoids in vaping oils using liquid chromatography diode array detector with electrospray ionization time-of-flight mass spectrometry for confirmation of analyte identity. Molecules. 2025;30(12):2597. doi:10.3390/molecules30122597.

Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med. 2018;13. doi: 10.1186/s13020-018-0177-x.

Zheljazkov VD, Maggi F. Valorization of CBD-hemp through distillation to provide essential oil and improved cannabinoids profile. Sci Rep. 2021;11(1):19890. doi: 10.1038/s41598-021-99335-4.

Zughaibi TA, Alharbi H, Al-Saadi A, Alzahrani AE, Al-Asmari AI. 11-Nor-9-Carboxy Tetrahydrocannabinol Distribution in Fluid from the Chest Cavity in Cannabis-Related Post-Mortem Cases. Toxics. 2023;11(9):740. doi: 10.3390/toxics11090740.

List of Publications and Abstracts

Matrix-Specific Challenges in Cannabinoid Determination

Michaela Mifsud, Janis Vella Szijj

Department of Pharmacy, Faculty of Medicine and Surgery, University of Malta

Over recent years, interest in medical cannabis has grown substantially, highlighting the need for efficient and reproducible methods for extracting and quantifying cannabinoids from various matrices. The aim of this study was to identify challenges associated with the extraction and analysis of cannabinoids across different matrices.

The methodology was conducted in two phases. Phase 1 consisted of a literature review, focusing on matrices from which cannabinoids are extracted from, sample preparation techniques employed, and analytical methods used for cannabinoid determination. Phase 2 involved laboratory visits at the Pharmaceutical Synthesis and Technology Laboratory, where the analysis of cannabinoids in medium-chain triglyceride (MCT) oil, hemp seed oil and cosmetics was observed, and specific challenges were identified.

From the 49 articles reviewed, tetrahydrocannabinol (THC) was the most commonly analysed cannabinoid (n = 41). Oils were the most frequent matrix studied (n = 24). Solid-phase extraction was the most widely reported sample preparation technique (n = 12). High-performance liquid chromatography was the most frequently identified chromatographic method (n = 35), and mass spectrometry was the most common detection technique (n = 38). Key challenges reported included difficulties in obtaining clear chromatographic peaks (n = 16) and chemical and physical properties of matrices interfering with extraction (n = 12).

In Phase 2, laboratory observations revealed additional challenges, such as inefficient separation of THC from MCT oil, limited resolution of chromatographic peaks, and matrix effects.

Accurate quantification of cannabinoids relies heavily on addressing the challenges inherent in sample preparation and analysis. The diversity of product formulations including oils, balms, and emulsions introduces matrix-specific complexities that affect both extraction efficiency and measurement accuracy. For example, lipid-rich oils may co-extract interfering compounds that suppress analytical signals, balms can trap cannabinoids within waxy or fatty structures, and emulsions often present phase-partitioning issues that hinder analyte recovery. These matrix effects compromise sensitivity and reproducibility, underscoring the need for tailored extraction strategies and rigorous method validation.

By accounting for these matrix-specific challenges, analytical methods can be optimized to improve solvent efficiency, enhance analyte recovery, and minimize interference, ultimately enabling more accurate and precise cannabinoid determination across diverse formulations.

Appendix 1

FREC Approval

The status of your REDP form (MED-2024-00088) has been updated to Acknowledged

form.urec@um.edu.mt Fri, 22 Mar, 08:44

to me

Dear Michaela Mifsud,

Please note that the status of your REDP form (MED-2024-00088) has been set to *Acknowledged*.

This status change was accompanied by the following explanation/justification: *Dear applicant, Your research ethics application has been received. This does not mean that your application has FREC ethical approval and may be subject to an audit review. The FREC number generated by submission for records only cannot be used as proof of ethical approval. As indicated in the Research Ethics Review Procedures, submissions which have no self-assessment issues are kept for record and audit purposes only, so research may commence. Kindly note that FREC will not issue any form of approval as the responsibility for the self-assessment part lies exclusively with the researcher. Please note that SCPD generally requires review. If you have any questions or doubts or require any further clarification you can contact the MED FREC secretary. Regards, MED FREC*

You can keep track of your applications by visiting: https://www.um.edu.mt/research/ethics/redp-form/frontEnd/.

This email has been automatically generated by URECA. Please do not reply. If you wish to communicate with your F/REC please use the respective email address.

Appendix 2

Literature Review Results

Author, Date	Cannabinoid studied	Matrix	Sample Preparation Technique	Chromatographic Instrumentation	Stationary Phase	Mobile phase	Detector	Challenges identified
Shaozhong et al, 2025	CBC, CBD, CBDV, CBG, CBN, CBN-O-acetate, CBT, THC	Vape oil	Methanol extraction, diluted to 50 µg/mL	LC	Agilent Poroshell 120 EC-C18 column (150 mm × 2.1 mm, 2.7 µm)	Solvent A: 0.02% (v/v) formic acid in water Solvent B: acetonitrile. Isocratic at 70% B (v/v)	DAD	Separation of isomeric/semi-synthetic cannabinoids optimized via column selection and flow programming
Duchateau et al, 2025	THC, CBD, CBC, CBN, CBDV, CBG, THCV, CBDA, THCA	Cannabis herbs (flowers, plant materials) and CBD oils	Solvent-based extraction methods with apolar solvents (e.g., hexane, dichloromethane), polar solvents (e.g., methanol, ethanol).	GC	Reversed-phase C18 columns	N/A	MS, FID	GC often led to decarboxylation of acidic cannabinoids, necessitating derivatization for accurate quantification.
Tan et al, 2025	THC, HHC	Human Oral Fluid	salting-out assisted liquid–liquid extraction	UHPLC	Kinetex PS C18 column	N/A	MS	Designing a SALLE system to allow direct injection of acetonitrile extracts without evaporation, a novel feature enhancing throughput.
Meepun et al, 2025	CBD, THC	*Cannabis sativa* by-products (leaves, stems, roots, flowers)	Extraction using green solvents: optimized by ultrasound-assisted extraction	HPLC	C18 reverse-phase column	Gradient of acetonitrile and water (acidified, usually with formic acid; specified in methods)	UV	Low cannabinoid content in hemp by-products compared to flowers.

Author, Date	Cannabinoid studied	Matrix	Sample Preparation Technique	Chromatographic Instrumentation	Stationary Phase	Mobile phase	Detector	Challenges identified
Huang et al, 2025	THC, CBD, and other THC isomers	Δ^8-THC edibles (brownies) and vape oil samples	Simple methanol extraction prior to analysis; extraction	N/A	N/A	N/A	MS	Achieving clear differentiation between isomeric cannabinoids within a very short time (under 1 minute)
Huang et al, 2025	*Δ^3-THC, Δ^8-THC, Δ^9-THC, CBD, Δ^8-iso-THC, Δ(4)8-iso-THC, 9α- & 9β-hydroxy-HHC, 8-hydroxy-iso-THC, THCA, CBDA, Δ^8-THCV, Δ^8-iso-THCV, and Δ^9-THCV*	Cannabis extracts, edibles, and acid-treated CBD mixtures	Samples were mixed with methanol and $AgNO_3$ solution	N/A	N/A	N/A	MS	Differentiating isomeric cannabinoids unresolved by chromatography
Wongumpornpinit *et al, 2025*	CBD, THC	Ethanolic cannabis extracts derived at pilot scale	N/A	HPLC	N/A	Hexane/ 0.1% formic acid in acetonitrile/20 mM ammonium formate	N/A	Balancing extraction yield, appearance (colour), and selectivity
Ververi et al, 2025	THC	Volumetric dried blood spots	Whole blood was spiked onto DBS cards, then the spots were extracted	U-HPLC	N/A	N/A	MS/MS	Limit of detection for THC and THC-OH was 1 ng/mL (higher than most analytes at 0.5 ng/mL)
Antunes et al, 2024	Δ^9-Tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-THC (THC-OH), 11-nor-9-carboxy-Δ^9-THC (THC-COOH), CBN, and CBD	Human oral fluid	Protein precipitation using methanol:acetonitrile (80:20, v/v); centrifugation, evaporation under nitrogen or vacuum, then reconstitution in 100 µL methanol	LC	N/A	N/A	MS/MS	Observed ion suppression for THC, CBN, and CBD, though sensitivity remained adequate – with limits of detection (LOD) and quantitation (LOQ) between 0.05 and 0.5 ng/mL.

Author, Date	Cannabinoid studied	Matrix	Sample Preparation Technique	Chromatographic Instrumentation	Stationary Phase	Mobile phase	Detector	Challenges identified
Lindekamp et al, 2024	CBD, THC, THCA	commercial hemp seed edible oils	N/A	UHPLC	N/A	N/A	MS/MS	Significant impact of thermal processing on cannabinoid content, notably increasing THC while degrading cannabinoid acids
Tanase et al, 2024	THC, CBD	Hemp seed oil products	mechanical pressing (hot/cold), steam distillation, Soxhlet maceration, microwave-assisted extraction, ultrasound-assisted extraction, supercritical CO_2 extraction;	HPLC, GC	N/A	N/A	UV, MS	Extremely low cannabinoid levels in seeds often below regulatory thresholds. Extraction method variability affects yield and integrity of bioactive compounds, especially when involving heat
Di Trana et al, 2024	THC, THC-OH, THC-COOH, CBD	Whole blood, urine and oral fluid	N/A	LC	N/A	N/A	MS/MS	Designing a universal method that worked consistently across three very different biological matrices.
Correia et al, 2023	THC	Cannabis herbal samples	Solid-liquid extraction	HPLC	Kinetex 2.6 µm C18 150 mm x 2.1 mm reverse phase column	ACN and methanol	DAD	High sample consumption during sample preparation. Optimisation of SLE approach.
Montero et al, 2023	CBD, CBDA	Dried hemp inflorescence	Freeze dried followed by extraction with acetone and water	HPLC	C18 column	Water and formic acid	DAD	Chemical composition of matrix interfering with extraction and difficulty in obtaining peaks.
García-Atienza et al, 2023	Synthetic cannabinoids	Oral fluids	SPE	HPLC	Scharlau Kromaphase 100 C18 column	Water and acetonitrile	Fluorescence	Determination of proper recovery time On chromatogram

Author, Date	Cannabinoid studied	Matrix	Sample Preparation Technique	Chromatographic Instrumentation	Stationary Phase	Mobile phase	Detector	Challenges identified
Pino et al, 2023	THC, CBD	Oils from cannabis	SLE, super critical fluid extraction	HPLC	C18 column (Chromolith)	Water and ACN	DAD, UV	SLE uses toxic organic solvents.
Zughaibi et al, 2023	THC-COOH	Blood, urine, gastric content	SPE	U-HPLC	C18 column (Raptor Biphenyl)	N/A	MS	Small sample sizes provided difficulty in obtaining enough analyte to be detected.
Chambers et al, 2023	THC, CBD	Edibles (chocolates and gelatin based)	N/A	HPLC	N/A	N/A	MS, DAD	Low amounts of cannabinoids present in matrix resulting in the need for a highly sensitive technique.
Couttas et al, 2023	CBD, THC	Human serum, rodent serum	SPE	LC	C18 column (Phenomenex)	Formic acid, water, methanol	MS	Matrix Variability across 2 collection devices. Maintaining chromatographic integrity when injecting extracted acetonitrile.
Mastellone et al, 2023	CBD, CBDA, THC	Freeze dried aerial parts, seedless dried inflorescence and CBD oil (coconut and MCT)	Liquid based microextraction	U-HPLC	C18 column (Ascentic express)	Water, formic acid	MS, DAD	Viscosity of oil may lead to problem once directly injecting the sample in the GC.
. Dawidowicz et al, 2022	CBGA, CBDA, THCA, CBG, CBD, THC	Hemp extracts, Cannabinoids oils	Maceration with dichloromethane	HPLC	SYNERGI 4u Polar-RP column (250 × 4.6 mm, 5 µm)	1. ACN with 0.1% formic acid 2.Water with 0.1% formic acid	MS	Variability in Oil Composition

Author, Date	Cannabinoid studied	Matrix	Sample Preparation Technique	Chromatographic Instrumentation	Stationary Phase	Mobile phase	Detector	Challenges identified
Galant, 2022	THC, CBD, CBDA, THCA and 117 synthetic cannabinoids	Dietary supplements, food products	Vortex in acetonitrile and freezing	HPLC	C18 Column (Kinetex)	Water and methanol	MS	Technique used was not sensitive enough, difficulty in obtaining peaks
Analakkattillam, et al, 2022	CBD, THC	Hemp oil, Tablets and tinctures, soft gel capsules	N/A	HPLC	C18 column (Solas)	ACN and water	UV	Validating across diverse matrices (oils, tablets, tinctures). Achieving reliable separation and detection via tuning HPLC parameters.
Dongping Li et al, 2022	THC, CBD, CBG, CBN	Cannabis sativa flowers	Ethyl acetate extraction	HPLC	C18 column (Kinetex)	Ammonium formate and HPLC grade water	FID	Variability in extract composition. This variability complicates sample preparation and standardization for reliable comparative analysis.
De vita et al, 2022	CBD, CBDA	*Cannabis sativa* inflorescence	Crude extraction	GC	Rtx®-5 Restek capillary column (30 m × 0.25 mm i.d., 0.25 mm	Helium	MS	Complex plant matrix: Diverse phytochemicals needed to be disentangled for analysis
Frei et al, 2022	THC, CBD, OH-THC, CBN, THC-COOH	Blood and urine samples	SPE	GC	C18 column	Helium	MS	THC-COOH stability during precipitation from blood samples.
Taiti et al, 2022	CBD, THC, CBG	Food sample (chocolate and gelato)	SPME	GC	C18 Column	Water and formic acid	MS	SPME selection bias: Fiber affinity variations may skew volatile compound detection.

Author, Date	Cannabinoid studied	Matrix	Sample Preparation Technique	Chromatographic Instrumentation	Stationary Phase	Mobile phase	Detector	Challenges identified
Zheljazkov et al, 2021	THC, CBD	Essential oil	N/A	HPLC, GC	HP-5MS (30 m l. × 0.25 mm i.d., 0.1 mm f.t., Folsom, CA, USA) made up of 5% phenylmethylpolysiloxane	Helium	MS, UV	The thermal nature of distillation caused in situ decarboxylation of cannabinoid acids which altered the natural acid-to-neutral cannabinoid balance, Potentially skewing quantitative results.
Pieracci et al, 2021	CBD, THC	Essential oils extracted from plant material	Hydro distillation	GC	Agilent HP-5MS capillary column	Hexane	MS	Separation of THC from oil
Huang et al, 2021	THC, CBD	Cannabis flowers and CBD oils	Vortex using methanol	U-HPLC	Nucleosil SA, 100 A, 5 µm, 2.1 × 250 mm; Grace	methaanol	Silver impregnnted paper spray MS	Difficult to distinguish CBD and THC by MS/MS analysis
Guo et al, 2021	THC, CBN, CBT, CBG, THCV, CBC, THC and CBD	Vape oil	SPME	GC	DB-5MS column (30 m × 0.25 mm	Helium	MS	Separation of THC from oil
Yun-Hua Hsu et al, 2021	CBD, CBN, THC	Hemp seed oil-based cosmetics	Ultrasound asssited extraction using methanol	HPLC	shiseido CAPCELL PAK C18 column (2.7 µm, 4.6 × 150 mm) and Xbridge BEH Shield RP18 column	Methanol and acetonitrile	MS	Coeluting matrix compounds. This resulted in notable errors in quantification.
Citti et al, 2021	CBD	Synthetic and extracted CBD samples	N/A	U-HPLC	Poroshell 120 EC-C18 (3.0×100 mm, 2.7 µm	Formic acid	MS	The retention time for the peaks was not in the usual position for the synthetic CBD samples.

Author, Date	Cannabinoid studied	Matrix	Sample Preparation Technique	Chromatographic Instrumentation	Stationary Phase	Mobile phase	Detector	Challenges identified
Qamar et al, 2021	CBD, THCV, CBDA, CBGA, THC, CBC	Flower material	Super critical fluid extraction	U-HPLC	C18 column	Methanol and phosphoric acid	DAD	PH of mobile phase influencing the retention time and operating pressure of the column
Dei Cas, 2020	CBD, THC, CBDA, THCA	Cannabis oil	Centrifugation and vortex	GC	capillary column Rxi-5ms (30 m × 0.25 mm, i.d. 0.25 mm, Restek	Helium	MS	Decarboxylation of cannabinoids resulting in different ratios of cannabinoids present
ElSohly, et al 2020	THC, CBD, THCAA, CBDA	Hemp Oil products and hemp butter	Vortex using hexane	GC	10-m × 0.18-mm DB-1 (0.4 µ film) column	helium	MS	Samples were decarboxylated, difficulty in obtaining peaks
Joseph J. Waks hlag et al, 2020	CBD, CBDA, THC, THCA, CBN, CBG	Oral dosage forms of Hemp	N/A	HPLC	Waters Atlantis T3 HPLC column (3 µm 2.1 × 50 mm)	Formic acid & water	MS	Method Sensitivity and Quantitation Limit Constraints. The assay's sensitivity was insufficient to consistently capture trace-level metabolites, especially in low-THC and THCA dosing conditions
Rochfort et al, 2020	CBD, THC	Dried plant inflorescence	Super critical carbon dioxide extraction	U-HPLC	N/A	ACN and methanol	MS	Recovery time for THC is not as reproducible as that of CBD
La Maida et al, 2020	Synthetic cannabinoids	Oral fluids	Liquid-liquid extraction	U-HPLC, GC	C18 column (Agilent technologies)	Helium, ammonium formate, formic acid	MS	Insufficient sensitivity to detect low-level metabolites
Pellegrini et al, 2020	Synthetic cannabinoids	Urine samples	Liquid-liquid extraction	GC, U-HPLC	C18 column (Agilent technologies)	Helium, ammonium formate, water	MS	Large amount of different synthetic cannabinoids and matrices

Author, Date	Cannabinoid studied	Matrix	Sample Preparation Technique	Chromatographic Instrumentation	Stationary Phase	Mobile phase	Detector	Challenges identified
								results in the use of multiple sample preparation and analysis techniques.
Shao et al, 2020	CBD, THC, CBN, CBG, CBDA	Food (chocolate, biscuit, fondant, beverage)	Enhanced matrix removal-lipid adsorbent (EMR-Lipid)	U-HPLC	C18 column (acquity)	acetonitrile	MS	Oils and adhesives in foods interfere with extraction and cause variability.
Paul et al, 2019	THC, CBN, CBD, THC-OH and THC-COOH	Hemp oil	Liquid-Liquid phase extraction, solid phase extraction	GC	Varian VF-5ms GC column	Helium gas	MS	The peak present on the chromatogram for THC did not meet the accepted criteria
Jensen et al, 2019	THC, THCA, CBD, CBN, 11-OH-THC	Umbilical cord tissue, meconium	SPE	HPLC	C18 column (Agilent technologies)	Water and Methanol	MS	Multiple matrix types: Meconium vs. umbilical cord tissue require distinct preparation strategies. High sensitivity requirements. Matrix effects & recovery. Much lower analyte levels in cord tissue posed detection and quantification challenges.
Li et al, 2019	CBDA, CBD, THC, THCA, CBC	MCT oil, sesame oil, hemp oil, ethanol propylene glycol tincture	N/A	HPLC	120 Poroshell column	Ammonium formate, formic acid, methanol, ACN and water	DAD	Decarboxylation of cannabinoids.

Author, Date	Cannabinoid studied	Matrix	Sample Preparation Technique	Chromatographic Instrumentation	Stationary Phase	Mobile phase	Detector	Challenges identified
Meng et al, 2018	THC, CBD, CBDA, THCA	Oils, plant material and cosmetic cream	SPE	HPLC	C18 column (Agilent eclipse)	Water, acetonitrile, and formic acid.	MS, UV	Extraction of acidic cannabinoids from the different matrices. Separation of THC from oil. The CBDA and THCA-A analytes exhibited overlapping fragmentation in the mass spectrometer, complicating accurate peak identification and quantification, particularly when distinguishing between these two acidic cannabinoids
Casiraghi et al, 2017	THC	Olive oil	SPE	GC	Dimethyl polysiloxane	Helium	MS, FID	Determining the correct temperature necessary for analysis to yield maximum amount of THC without degrading the cannabinoids
Concheiro et al, 2014	THC-COOH, THC, CBN, CBD	Oral fluid	SPE	HPLC	C18 column (Speware corporation)	Ammonium acetate and ACN	MS	Small sample size. Highly viscous oral fluid leading to difficulty during sample extraction.
Barnes et al, 2014	THC-COOH	Oral fluid	SPE	GC	HP 5-MS capillary columns	Helium	MS	Small concentrations and so a method with high sensitivity was needed.
Shedweiler et al, 2013	THC	Oral fluid	SPE	HPLC	C18 column (Kinetex)	Acetic acid in methanol and water	MS	The devices used for oral fluid collection required the use of buffers. The buffers

Author, Date	Cannabinoid studied	Matrix	Sample Preparation Technique	Chromatographic Instrumentation	Stationary Phase	Mobile phase	Detector	Challenges identified
								caused challenges to arise upon HPLC analysis. Difficulty in obtaining peaks