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MINI-REVIEW

# Challenges of Extracting and Determining Cannabinoids in Different Matrices

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**Abstract**

**Introduction:** Accurate and precise analysis of cannabinoids is important for elucidating their therapeutic potential and developing therapies, which are targeted toward different medical conditions. A wide range of cannabis products are present on the market and are available in different dosage forms, including dried flowers, extracts, and consumables. The aim of this article is to provide an updated narrative review of literature on challenges of analyzing cannabinoids in plant material, oils, and edibles.

**Method:** Literature search was conducted to identify sample preparation and analytical techniques for determination of cannabinoids in plant material, oils, and edibles and associated challenges.

**Results:** Challenges related to determination of cannabinoids in plant material include matrix complexity, co-extraction of unwanted compounds during sample preparation, and differences in matrix composition between calibration standards and sample extracts. During analysis of cannabinoids in oil, the unique properties of carrier oils need to be taken into consideration. Analysis of cannabinoids in edibles can be considered to be challenging due to the wide range of matrix types that are available on the market, rendering analysis resource-intensive, time-consuming, and impractical.

**Discussion:** Analysis of cannabinoids in plant material, oils, and edibles requires a multifaceted approach that includes regulatory guidance, method development, and technological innovation. In the face of an evolving analytical landscape where novel cannabinoids are being identified and require determination, there is a need for the development and validation of standardized accurate and precise analytical methods, which are specifically tailored for each matrix.

**Keywords:** analysis; cannabinoids; edibles; oils; plant material; sample preparation

## Introduction

More than 500 compounds have been identified in *Cannabis sativa*, which is increasingly researched due to its numerous therapeutic properties, including analgesic, anticonvulsant, anti-inflammatory, and anxiolytic effects.[1] Biologically active components present in the cannabis plant, which are responsible for exerting these effects include cannabinoids, terpenes, and flavonoids.[2,3] Cannabinoids like delta-9-tetrahydrocannabinol (Δ−9-THC) and cannabidiol (CBD) interact with the endocannabinoid system,[4,5] influencing mood, appetite, and pain perception.[6] Terpenes and flavonoids contribute to the aroma of the plant and exert antioxidant and anti-inflammatory effects. Analysis of different compounds in cannabis is important for determining their therapeutic benefits and formulating treatments tailored to specific medical needs. Different analytical methods have been developed for determination and quantification of cannabinoids with gas chromatography (GC), high-performance liquid chromatography (HPLC), and ultrahigh-performance liquid chromatography (UHPLC) being the most common techniques. Liquid chromatographic methods are often preferred due to their ability to determine both acidic and neutral cannabinoids without the need for derivatization.[7] HPLC can be coupled to different detectors, such as ultraviolet (UV),[8,9] diode array detectors (DAD),[10–12] and mass spectrometry (MS)[13–15] detectors. MS provides greater sensitivity and

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selectivity whereas UV and DAD are used for analysis of cannabis products where cannabinoids are present in higher concentrations.[16]

With increasing acceptance and regulations, a wide range of cannabis products are present on the market. These products are available in different dosage forms, including plant material, oils, edibles, capsules, topicals, and beverages, with plant material, oils, and edibles being commonly consumed.[17]

The development, validation and application of analytical procedures for determination of cannabinoids brings about diverse challenges. Standardized and consistent methods for analysis of cannabinoids are lacking. There is no single method that can provide accurate and precise determination of all aspects of complex and diverse cannabis products and consequently laboratories find it problematic to apply techniques that meet specific needs and yield dependable outcomes. Cannabis testing laboratories often need to incorporate standards and protocols from different sectors, including food, health care, cosmetic, and pharmaceutical industries to meet analytical needs.[18]

When ensuring reliability of test results during method validation, laboratories must have access to traceable standards and materials, which are comparable to samples being tested. Only about 20% of phytocannabinoids currently have available reference materials.[19] Acquiring these materials in terms of availability, accessibility, and expense is another challenge which analysts often face. Regulation of cannabis analysis is still in its infancy in many countries, leading to absence of clear guidelines and standardization. Laboratories face difficulties in understanding their regulatory obligations and responsibilities due to lack of established guidelines.[20]

The aim of this article is to provide an updated narrative review of literature on challenges of analyzing cannabinoids in plant material, oils, and edibles.

## Method

Literature search was conducted in March 2024 on PubMed, Google Scholar, and Research Gate to identify original and review articles describing sample preparation and analytical techniques for determination of cannabinoids in plant material, oils, and edibles and associated challenges. Keywords used included: 'analysis of cannabinoids in plant material,' 'analysis of cannabinoids in oils,' 'analysis of cannabinoids in edibles.' Information from 56 articles published between 2009 and 2024 were included in the narrative review.

## Results

### Analysis of cannabinoids in plant material

Determination of cannabinoids can be conducted using different parts of the cannabis plant, including male and female inflorescences, leaves, and roots.[12,21–41] Cannabinoids can be extracted from plant samples using extraction with sonication or microwave-assisted extraction. Ethanol and methanol are commonly used extraction solvents. Cosolvents, such as acetonitrile or hexane, can be used to change the polarity of the extraction solvent. For large-scale extractions, supercritical fluid extraction can be employed (Table 1). The use of supercritical carbon dioxide extraction using ethanol leads to lower solvent consumption and toxicity and increased stability of thermo-labile components.[42] Cannabinoids are highly soluble in supercritical carbon dioxide, with their solubility being in the order 1–2 kg per kg of carbon dioxide.[43,44]

Analysis of cannabinoids is then carried out using LC or GC (Table 2), which usually requires derivatization for determination of acidic cannabinoids as synthesized in the plant. During GC analysis, derivatization enhances the volatility of cannabinoids, which are typically nonvolatile molecules with high boiling points. Derivatization makes cannabinoid more amenable to GC analysis, which requires compounds to be in the gas phase.[45] Sensitivity and selectivity can be enhanced through derivatization, which can enhance detectability of cannabinoids and introduce specific functional groups that improve sensitivity of analysis.[46]

HPLC-UV is a nonselective technique, which relies on the retention time for identification of analytes and misidentification of an interfering substance can occur. Terpenes might coelute with cannabinoids, generating a false-positive result during cannabinoid quantification. Although plant samples usually have a cannabinoid concentration of about 20–30% and a terpene concentration of about 1–3%, analysis should ideally be carried out at wavelengths of 228 and 270 nm since terpenes do not absorb well at these wavelengths.[47]

Coupling of a liquid chromatographic technique to a UV or DAD detector is useful for determination of major cannabinoids, such as Δ–9-THC or CBD, since these strongly absorb UV radiation.[48] The use of a more sensitive and selective detector such as MS would be required for the determination of minor cannabinoids, such as cannabigerol, cannabidivarin, and tetrahydrocannabivarin, which are usually present in smaller

**Table 1. Sample Preparation Techniques for Extraction of Cannabinoids from Plant Material**

| Sample preparation technique | | References | Comments |
|---|---|---|---|
| Solvent Extraction | Extraction Solvent | | |
| | Methanol | 12,23–27,32 | Extraction yield: ~4–62 mg/g[32] |
| | Ethanol | 28,31 | Recovery: 98.5–105%[28] |
| | Methanol and Chloroform | 24,32,38 | Extraction yield: ~4–61 mg/g[32] |
| | | | Recovery: 70.5–116.2%[38] |
| | *n*-hexane, Dichloromethane and Methanol | 22 | Percentage yield: |
| | | | *n*-hexane: 10.5%w/w[22] |
| | | | Dichloromethane: 3.8% w/w[22] |
| | | | Methanol: 6.7%w/w[22] |
| | Ethanol and Tribenzylamine | 12 | Mean recovery: 100.53 ± 3.12%[12] |
| | Ethyl acetate and Isopropanol | 33 | Recovery: 80–120%[33] |
| | Acetonitrile and methanol | 39 | Recovery: 96.10–107.57%[39] |
| Supercritical fluid extraction | | 21,23,35 | Recovery: 92–104%[21] |
| | | | Extraction yield: ~1–18 mg/g[23] |
| Ultrasound-assisted extraction | | 23,29,30,34 | Extraction yield: ~1–17 mg/g[23] |
| Microwave-assisted extraction | | 23 | Extraction yield: ~1–21 mg/g[23] |
| Dynamic maceration | | 23 | Extraction yield: ~1–23 mg/g[23] |

concentrations in plant material.[49] The use of MS as a detection technique is more expensive, requiring more skilled expertise to operate and interpret, when compared with other detection techniques such as UV or DAD, which are more readily available in most analytical laboratories.[50]

The use of MS as a detection technique in cannabinoid analysis carries with it various advantages including higher sensitivity and lower detection limits compared to UV detection and this is useful in analysis of trace amounts of cannabinoids in complex matrices like biological samples. MS provides accurate molecular weight information and structural details of cannabinoids which can help differentiate between similar cannabinoids.[7]

There is a need to develop and validate efficient analytical methods to determine Δ–9-THC in CBD products as a result of a consideration being discussed at the United Nations Narcotics Board with respect to the reclassification of CBD as a narcotic limiting the amount of Δ–9-THC present in CBD products to less than 0.2 (w/w) using instrumentation, which is readily available in most laboratories.[51]

Analysis of cannabinoids in plant material can be challenging due to the complex nature of cannabis matrices and diversity of compounds present. Matrix components may coelute with target analytes during chromatographic separation or suppress or change ionization of analytes during MS detection, leading to decreased analytical accuracy and precision.[52]

During sample preparation, the use of polar solvents, such as methanol and ethanol, can effectively lead to extraction of a wide range of cannabinoids but can also lead to coextraction of unwanted compounds such as waxes and chlorophyll, leading to sample impurities and decreased sensitivity of analysis.[53] Although use of supercritical carbon dioxide extraction offers advantages, such as reduced solvent consumption, it may be expensive and requires skilled expertise to operate.[42,54] Extraction methods need to be properly optimized and validated to achieve control of coextracted constituents such as heavy metals, pesticides, or mycotoxins.[55]

Winterization, which involves the removal of fats, lipids, and waxes found in plant material, can also be considered during sample preparation. During winterization, extract is attained, dissolved in a solvent, and subjected to cold treatment, by placing in freezer or cold bath. Cold temperatures cause fats, lipids, and waxes to solidify, hence separating from solution.[56] Issues related to winterization include it being a time-consuming process and it not being able to adequately remove the matrix before analysis.[57]

The lack of standardized reference materials makes method validation and quality assurance of analysis of cannabinoids in plant material challenging. Laboratories commonly rely on external proficiency testing and in-house validation protocols to ensure reliability of their results. Differences in matrix composition between calibration standards and sample extracts can lead to calibration errors and inaccurate determination of cannabinoid concentration.

### Analysis of cannabinoids in oil preparations

Cannabis oil preparations are the most popular pharmacological form of medicinal cannabis. Cannabinoid extracts may be combined with lipid sources such as

**Table 2. Chromatographic Analysis of Cannabinoids in Plant Material**

| Chromatographic technique | Cannabinoids analyzed | Limit of quantification |
|---|---|---|
| HPLC-UV[31] | CBDV | 0.205 μg/mL |
| | CBDA | 0.035 μg/mL |
| | CBGA | 0.040 μg/mL |
| | CBG | 0.313 μg/mL |
| | CBD | 0.310 μg/mL |
| | THCV | 0.311 μg/mL |
| | CBN | 0.023 μg/mL |
| | Δ–9-THC | 0.654 μg/mL |
| | Δ–8-THC | 0.684 μg/mL |
| | THCA | 0.075 μg/mL |
| | CBC | 0.082 μg/mL |
| HPLC-DAD[33] | CBDA | 0.47 μg/mL |
| | THCV | 0.53 μg/mL |
| | CBD | 2.74 μg/mL |
| | CBG | 0.84 μg/mL |
| | CBN | 0.43 μg/mL |
| | THCA | 0.69 μg/mL |
| | Δ–9-THC | 0.25 μg/mL |
| | CBC | 0.93 μg/mL |
| HPLC-DAD[38] | CBD | 0.5 μg/mL |
| | CBG | 0.5 μg/mL |
| | CBDA | 0.5 μg/mL |
| | CBN | 0.5 μg/mL |
| | Δ–9-THC | 0.5 μg/mL |
| | CBGA | 0.5 μg/mL |
| | CBC | 0.5 μg/mL |
| | THCA | 0.5 μg/mL |
| HPLC-DAD[41] | Δ–9-THC | 1 μg/mL |
| | THCA | 10 μg/mL |
| | CBD | 1 μg/mL |
| | CBDA | 10 μg/mL |
| | CBN | 1 μg/mL |
| UHPLC-DAD[21] | CBD | 2.50 μg/mL |
| | Δ–9-THC | 2.50 μg/mL |
| | CBDA | 0.50 μg/mL |
| | CBN | 0.05 μg/mL |
| | CBC | 0.33 μg/mL |
| | THCA | 0.50 μg/mL |
| HPLC-UV/DAD[23] | CBDA | 2.5 μg/mL |
| | CBGA | 5.5 μg/mL |
| | CBG | 1.8 μg/mL |
| | CBD | 2.3 μg/mL |
| Fast HPLC-DAD[24] | CBD | 15.13 μg/mL |
| | CBN | 15.13 μg/mL |
| | Δ–9-THC | 15.13 μg/mL |
| | THCA | 15.13 μg/mL |
| UHPLC-UV-MS/MS[34] | CBDA | 5.0 μg/g |
| | CBGA | 20.0 μg/g |
| | CBDV | 5.0 μg/g |
| | THCA | 5.0 μg/g |
| | CBG | 5.0 μg/g |
| | CBD | 5.0 μg/g |
| | CBN | 5.0 μg/g |
| | Δ–9-THC | 5.0 μg/g |
| | Δ–8-THC | 5.0 μg/g |
| HPLC-MS/MS[35] | CBD | 0.0014 μg/mL |
| | Δ–9-THC | 0.0016 μg/mL |
| | CBC | 0.0021 μg/mL |
| | CBDV | 0.0011 μg/mL |
| | THCV | 0.0009 μg/mL |
| | CBG | 0.0009 μg/mL |
| | CBN | 0.0013 μg/mL |
| | CBGA | 0.0022 μg/mL |
| | CBDA | 0.0009 μg/mL |
| | THCA | 0.0030 μg/mL |
| UHPSFC/PDA-MS[39] | CBD | 10.0 μg/mL |
| | Δ–8-THC | 10.0 μg/mL |
| | THCV | 10.0 μg/mL |
| | Δ–9-THC | 10.0 μg/mL |
| | CBN | 10.0 μg/mL |
| | CBG | 10.0 μg/mL |
| | THCA | 5.0 μg/mL |
| | CBDA | 5.0 μg/mL |
| | CBGA | 5.0 μg/mL |
| GC-FID[41] | THCV | 100 μg/g |
| | CBD | 100 μg/g |
| | CBC | 100 μg/g |
| | Δ–8-THC | 100 μg/g |
| | Δ–9-THC | 100 μg/g |
| | CBG | 100 μg/g |
| | CBN | 100 μg/g |
| Fast GC-MS[39] | THCV | 196.29 μg/g |
| | CBD | 12.45 μg/g |
| | CBC | 7.18 μg/g |
| | Δ–8-THC | 12.42 μg/g |
| | Δ–9-THC | 34.53 μg/g |
| | CBG | 82.93 μg/g |
| | CBN | 99.68 μg/g |
| | CBDA | 43.05 μg/g |
| | THCA | 24.54 μg/g |
| | CBGA | 45.25 μg/g |

CBC, Cannabichromene; CBD, Cannabidiol; CBDA, Cannabidiolic acid; CBDV, Cannabidivarin; CBG, Cannabigerol; CBGA, Cannabigerolic acid; CBN, Cannabinol; DAD, diode array detectors; GC-FID, Gas Chromatography with Flame Ionization detection; HPLC, High Performance Liquid Chromatography; THCA, Tetrahydrocannabinolic acid; THCV, Tetrahydrocannabivarin; Δ–8-THC, Δ–8-Tetrahydrocannabinol; Δ–9-THC, Δ–9-Tetrahydrocannabinol; UHPSFC/PDA-MS, Ultrahigh-Performance Supercritical Fluid Chromatography with Photodiode Array Detection and Mass Spectrometry; UV, ultraviolet.

medium-chain triglyceride (MCT) oil, olive oil, and hempseed oil. MCT oils are bioavailable, tasteless, odorless, and stable making them suitable carrier oils for many cannabinoid-containing oil formulations.[58,59] Olive oil is a good source of antioxidants. Unlike MCT oil, olive oil contains long-chain fatty acids, which take longer to break down and be digested, which can lead to a longer time for absorption of cannabinoids to occur. Hempseed oil is high in unsaturated fatty acids, which tend to make products susceptible to oxidation and reduce bioavailability.[60] Efficient sample preparation procedures are needed for analysis of cannabinoids in oil since oil cannot be directly introduced into the chromatographic system due to its high viscosity.

When extracting cannabinoids for oil preparations, extraction techniques should be efficient and cost-effective with good yield. In case of medicinal cannabis preparations, where high purity of one cannabinoid is often sought, issues related to coextraction of

cannabinoids should be considered. Isolation of CBD from coextracted Δ–9-THC, which is psychoactive, can be problematic, especially since Δ–9-THC can be generated with CBD during extraction due to thermal conversion.[61,62]

Dilution combined with vortex mixing is a common method for preparing oil samples containing cannabinoids for analysis.[63–65] Oil samples can be diluted in isopropanol, methanol, and ethanol. Solid-phase extraction and microwave-assisted extraction can also be used during sample preparation of oils.[66–68]

The unique properties of different carrier oils need to be considered since these can affect extraction efficiency and matrix complexity. MCT oil is relatively less viscous and readily soluble in organic solvents, whereas olive oil contains high levels of antioxidants and polar compounds, which may interfere with extraction. Hempseed oil and sunflower oil have distinct lipid profiles, requiring tailored extraction methods to ensure efficient recovery of cannabinoids.[59,69]

MCT oil is composed of medium-chain fatty acids, namely caprylic acid and capric acid, which can coelute with cannabinoids during chromatographic separation, leading to peak overlap and inaccurate quantification during HPLC-UV analysis.[60] To avoid analyte coelution, chromatographic method optimization needs to be undertaken and this might include using chromatographic columns other than $C_{18}$ columns, which are commonly used for separation of cannabinoids and which might not provide desired selectivity.[70]

Issues related to the analysis of cannabinoids in olive oil are related to quality of olive oil, which varies extensively from product to product. These issues are particularly significant if the cannabinoid-containing oil is not of medicinal grade. The source of olives, age of oil product, and method of processing, all influence the quality of the oil.[71] Different quality characteristics of different types of olive oil might lead to difficulty in peak identification during method application to determine unknown concentrations of cannabinoids using HPLC-UV. The presence of pesticides, impurities of residual solvents, which may originate from environmental sources during processing and cultivation or from the olive oil itself from processing, can also lead to issues in peak separation and identification.

### Analysis of cannabinoids in edibles

Cannabinoids can be found in different edible preparations, including chocolate, cookies, brownies, and gummies. The pharmacokinetics and pharmacodynamics of cannabinoids in edible products remain poorly elucidated, leading to potential overdosing or underdosing by consumers. When administered in edible products, cannabinoids exhibit a prolonged duration of action.[72] When ingested orally, Δ–9-THC is metabolized in the liver to 11-hydroxy THC, which is more psychoactive inducing greater euphoria, sedation, and hallucinations when compared with the parent compound. Δ–9-THC is rapidly absorbed through the lungs and into the bloodstream, bypassing the liver when administered *via* inhalation. The prolonged duration of action of cannabinoids necessitates careful monitoring and analysis to help mitigate risks of cannabinoid accumulation and associated adverse effects.[73]

When choosing appropriate extraction methods for cannabinoids from edibles, the composition of the edible needs to be taken into consideration, according to the amount of proteins, fats, and carbohydrates.[73,74] Analysis of cannabinoids in edibles can be considered to be challenging due to the wide range of matrix types that are available on the market, rendering analysis resource-intensive, time-consuming and impractical.[75]

Microwave-assisted extraction and solid-phase dispersion can be used for the analysis of edibles containing a high-fat content such as chocolates. Although the use of microwave-assisted extraction leads to reduced solvent consumption and extraction time, temperature must be controlled since thermal degradation of cannabinoids can occur leading to the formation of degradation products, which can affect accuracy and reliability of results. Further sample cleanup might be necessitated, particularly on sample matrices containing more than 30% water due to restricted solvent diffusion.[73,76,77]

Sample preparation for edibles containing a high amount of carbohydrates, such as gummy bears and brownies can be carried out using solvent extraction[78] and QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), which is a combination of solvent extraction and dispersive solid-phase extraction.[79], If QuEChERS is utilized for determination of trace cannabinoids in edible products, its use must be followed with chromatographic instrumentation coupled to a more sensitive detector, such as HPLC-MS or GC-MS, especially since some parts of the matrix, such as sugars, pigments, and fats, can be coextracted. HPLC-UV would be less suited for analysis of QuEChERS extracts.[73]

Different types of solid-phase extraction and supercritical fluid extraction have been used for separating

cannabinoids from food products. Pressurized liquid extraction can also be performed during sample preparation of edibles. The use of the technique leads to reduced solvent consumption but requires expensive equipment and further clean-up using techniques, such as solid-phase extraction is required.[73] Since edibles are commonly prepared using cannabis oil and butter, consideration of the presence of pesticides and solvents in samples needs to be made.

## Discussion and Future Directions

Addressing challenges related to the analysis of cannabinoids in flowers, oils, and edibles requires a multifaceted approach that includes regulatory guidance, method development, and technological innovation. In the face of a changing landscape where novel cannabinoids are being identified and require determination, there is a need for the development and validation of standardized accurate and precise analytical methods, which are specifically tailored for each matrix, with minimal matrix interferences.

Analysis of chiral cannabinoids, which is an important emerging issue, presents several challenges. The difficulty in separating enantiomers requires specialized and often expensive chiral chromatography techniques. Achieving accurate resolution and selectivity can be complex. Obtaining pure enantiomeric standards can be challenging and costly and potential interconversion between enantiomers under certain conditions can further complicate analysis.

Investment in research and development to explore alternative sample preparation and analytical techniques can help overcome issues related to matrix complexity and variability. Extraction efficiency and selectivity can be improved by application of techniques, such as supercritical fluid extraction, microwave-assisted extraction, and solid-phase microextraction. Newer analytical techniques, such as supercritical fluid chromatography, can be considered for separation and determination of cannabinoids since many of them are optically active.

Collaboration between regulatory agencies, industry stakeholders, and researchers is essential to establish standardized protocols and guidelines for cannabinoid analysis, including proficiency testing programs, availability and identification of reference material, and quality control measures to ensure interlaboratory reliability of results. This will lead to increased safety, quality, and efficacy of cannabinoid-containing products.

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## Abbreviations Used

| | |
|---|---|
| Δ-8-THC | = delta-8-tetrahydrocannabinol |
| Δ-9-THC | = delta-9-tetrahydrocannabinol |
| CBC | = cannabichromene |
| CBD | = cannabidiol |
| CBDA | = cannabidiolic acid |
| CBDV | = cannabidivarin |
| CBG | = cannabigerol |
| CBGA | = cannabigerolic acid |
| CBN | = cannabinol |
| DAD | = diode array detector |
| GC | = gas chromatography |
| GC-FID | = gas chromatography with flame ionization detection |
| HPLC | = high performance liquid chromatography |
| MCT | = medium chain triglyceride MS |
| QuEChERS | = quick easy cheap effective rugged and safe |
| THCA | = tetrahydrocannabinolic acid |
| THCV | = tetrahydrocannabivarin |
| UHPLC | = ultra high performance liquid chromatography |
| UHPSFC/PDA-MS | = ultrahigh-performance supercritical fluid chromatography with photodiode array detection and mass spectrometry |
| UV | = ultaviolet |