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Analysis of Cannabinoids in Oil

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Abstract

Introduction: Cannabinoids are bioactive molecules found abundantly in the cannabis plant, with two major cannabinoids being Δ-9-tetrahydrocannabinol (THC) and cannabidiol.

Method: The study was divided into three phases: (1) systematic literature search on the analysis of cannabinoids in oils, (2) development and validation of a rapid and efficient high-performance liquid chromatography (HPLC)-ultraviolet (UV) method for the determination of THC in medium-chain triglyceride (MCT) oil, and (iii) green assessment of methods for the determination of cannabinoids in oil.

Results: Articles identified describe the analysis of cannabinoids in olive oil and hemp oil. Regarding the developed and validated method for analysis of THC in MCT oil, separation was achieved using an ACE C_{18}-AR (250 × 4.6 mm; 5 μm) column with acetonitrile and 0.5% acetic acid (70:30, v/v) as the mobile phase at a flow rate of 2 mL/min. The analysis was conducted in isocratic mode with UV detection set at 220 nm. Injection volume was 10 μL. The method was validated in the linear range of 0.03125–0.5%. The method developed in this study was found to have equivalent greenness to other HPLC-UV methods reported in the literature.

Discussion: The method has acceptable accuracy, precision, and stability, is relatively green, and can be successfully applied to determine concentrations of THC in commercially available cannabinoid-containing oils where the allowed limit of THC is 0.2–0.3%.

Keywords: cannabinoids; THC; MCT oil; HPLC-UV; method validation; greenness assessment

Introduction

Cannabinoids are bioactive molecules found abundantly in the cannabis plant, with two major cannabinoids being Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC exerts the majority of cannabis' adverse effects and has analgesic, antispasmodic, anti-inflammatory, and psychoactive properties. CBD has anticonvulsive, anxiolytic, anti-

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inflammatory, and analgesic effects.[1–3] Cannabis is used for the management of pain, epilepsy, anxiety, sleep disorders, and chemotherapy-induced nausea and vomiting.[4,5]

The Single Convention on Narcotic Drugs of 1961 classified cannabis, cannabis extracts, cannabis tinctures, and cannabis resin as Schedule I drugs.[6] In 2020, the European Court of Justice issued a statement exempting CBD from the list of narcotic drugs since its potential for abuse is low and it does not produce psychostimulant effects.[7] CBD-containing products are not considered to be narcotics, provided the THC content does not exceed 0.2–0.3%.[8]

Different cannabis-based products are available on the market,[9] with oil preparations being common. Cannabinoid extracts can be formulated in lipid sources such as olive oil, hempseed oil, sunflower oil, and medium-chain triglyceride (MCT) oil.[10] The use of MCT oil in CBD-rich oils is popular due to MCT oil being a lipid base, which efficiently enhances the absorption of cannabinoids when compared with other lipid sources. MCT oils are tasteless and odorless and are easily broken down by the body. MCT oil helps preserve the integrity and potency of CBD products and extends their shelf-life.[11,12]

Chromatographic methods are used for the analysis of cannabinoids, with the most commonly used techniques being gas chromatography (GC), high-performance liquid chromatography (HPLC), and ultrahigh-performance liquid chromatography (UHPLC).[13,14] GC provides good resolution of major cannabinoids present in a sample, but its use requires chemical derivatization of cannabinoids to avoid decarboxylation due to the use of elevated temperatures.[14] Liquid chromatography can be used to determine cannabinoids without the need for derivatization and can distinguish between neutral and acidic cannabinoids.[15] HPLC and UHPLC can be coupled with different detectors such as ultraviolet (UV) and mass spectrometry (MS) detectors.[16] UV detectors are commonly found in analytical laboratories. UV detectors are simpler and cheaper to use and require the need for less-skilled expertise when compared with MS detectors.[17,18]

Although the analysis of cannabinoids in different oils is described in literature,[19–24] there is a need for efficient methods of determination of THC in MCT oil, which is commonly used as a carrier oil in cannabinoid-containing oils, using instrumentation that is readily available in most laboratories such as HPLC-UV. This will help in classifying CBD products containing <0.2–0.3% as being non-narcotic. This article is the first describing the development and validation of an efficient HPLC-UV method for the determination of THC in MCT oil using a mixed aromatic functionality stationary phase.

Method

The study was divided into three phases: Phase 1 involved a systematic literature review of methods of analysis for the determination of THC in oil preparations. Phase 2 involved the development and validation of an HPLC-UV method for the determination of THC in MCT oil. Phase 3 involved Green Assessment of identified methods describing analysis of cannabinoids in oil.

Phase 1: Systematic literature review of methods of analysis for the determination of THC in oil preparations

A literature search about different analytical methods describing the determination of THC and other cannabinoids in oil using HPLC was carried out. Google Scholar and PubMed were used to identify open-access journal articles published in English between January 2010 and May 2023. Keywords included CBD oil THC oil AND sample preparation, cannabis oil AND HPLC, and cannabis oil analysis.

Systematic literature search using the same inclusion criteria was performed independently and checked by another author.

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis method was utilized for reporting. The analysis of cannabinoids in matrices other than oils was excluded. The cannabinoids analyzed, sample preparation technique, detector used, stationary phase, and mobile phase described in each study were identified and compared. Using the inclusion criteria, no article describing the determination of THC in MCT oil was identified, and a method for the determination of THC in this carrier oil was developed and validated.

Phase 2: Development and validation of an HPLC-UV method for the determination of THC in MCT oil

Instrumentation and reagents. All liquids used were HPLC grade. Acetonitrile (ACN) and methanol were obtained from Honeywell, orthophosphoric acid was obtained from Fisher Chemical, and acetic acid was obtained from Carlo Erba Reagents. Deionized water was produced using a Thermo Scientific Barnstead Smart2Pure Water Purification System from Fisher

Scientific. Standard of THC (98.62% purity) was obtained from LoGiCal.

The analysis was conducted using an Agilent 1260 Infinity Series liquid chromatography system having a quaternary pump and UV-visible detector with OpenLab CDS ChemStation software. A Sartorius LA230S balance and Labbox Labware Ultrasonic Bath were used for the preparation of standards, and Hanna Portable pH meter HI 8010 was used for mobile phase preparation. Separation was carried out on an InfinityLab Poroshell 120 EC C_{18} column (150 × 3.0 mm; 3 μm) and an ACE C_{18}-AR (250 × 4.6 mm; 5 μm).

Sample preparation. Samples of THC in MCT oil were prepared in varying concentrations, and these were diluted in methanol in a ratio of 1:500. All samples were vortex-mixed for 5 min, sonicated for 15 min, and filtered using 0.45 μm nylon syringe filters prior to analysis.

Method development. Analysis was initially conducted using parameters based on the German Pharmacopoeia monograph for cannabis extracts[25] using the InfinityLab Poroshell 120 EC C_{18} column. The monograph describes the use of ACN and 85% orthophosphoric acid in the mobile phase. Results attained were poor in selectivity, with chromatographic peaks for THC and blank MCT oil eluting at the same time.

Different mobile phase ratios of ACN and orthophosphoric acid and flow rates (0.5–2 mL/min), detector wavelengths (225, 254, 275, and 306 nm), and column temperatures (30°C–50°C) were then tested to determine whether improvement in selectivity could be attained. No improvement in selectivity was obtained, and the type of column was subsequently changed.

Chromatographic conditions. Analytical conditions made use of an ACE C_{18}-AR column at a temperature of 25°C and ACN and 0.5% acetic acid (70:30, v/v) as the mobile phase at a flow rate of 2 mL/min. The analysis was conducted in isocratic mode with UV detection set at 220 nm. Injection volume was 10 μL.

Method validation. Developed method was validated for specificity, accuracy, precision, linearity, limit of detection, limit of quantification, and stability according to the International Council on Harmonization guidelines.[26]

Specificity. The specificity of the method was confirmed by analyzing blank MCT oil samples in triplicate.

Linearity. Calibration standards of THC in oil were prepared at five concentration levels ranging from 0.03125% to 0.5%, before diluting in methanol in the ratio of 1:500. The area under the peak for THC was plotted against the concentration of THC in oil. Least-squares linear regression analysis of the calibration data was done using the equation $y = mx + c$.

Accuracy. The accuracy of the method was determined by finding the percentage bias. The percentage bias was calculated as measured concentration-theoretical concentration/theoretical concentration × 100. The method was considered to be accurate if the percentage bias was ±15% or less.[27] Three concentrations of 0.5%, 0.125%, and 0.03125% of THC in oil diluted in methanol in a ratio of 1:500 were analyzed in triplicate.

Precision. Intraday precision was assessed by analyzing standards of each concentration in triplicate on the same day. Interday precision was assessed by analyzing each concentration once for 3 days. Intraday and interday precisions were expressed as the percentage relative standard deviation (RSD).

Stability. Stability of THC in MCT oil was assessed by analyzing each concentration of THC in oil after 8 days following storage at 4°C. The analysis was performed in triplicate. Results were expressed in terms of percentage RSD.

System suitability and robustness. A system suitability test was performed for the standard mixture of 0.5% THC in oil. Five replicate injections were performed, and RSD values for retention time, area of peak, and height of peak were calculated. The number of theoretical plates and capacity factor were calculated. Method robustness was assessed by varying chromatographic parameters to show the reliability of the proposed analytical method during normal usage. The parameters varied were flow rate (+0.1 mL/min and −0.1 mL/min), detector wavelength (+2 nm and −2 nm), and column temperature (+5°C and −5°C). The system suitability standard solution was analyzed in duplicate with each variation in parameter. Acceptable criteria included the number of theoretical plates ≥2000 and capacity factor between 1 and 10.

Method application. The validated method was applied to determine concentrations of THC in two commercially available CBD oils to check whether the concentration of THC present in the product matched the concentration stated on the label. The analysis of each oil was carried out in triplicate.

Phase 3: Green assessment of methods for the determination of cannabinoids in oil

Green assessment of the methods identified through the literature review and the developed and validated HPLC method was conducted using the Modified National Environmental Methods Index (NEMI) metric tool.[28] The Modified NEMI was selected as a metric tool for this study since it was developed as an improvement over the metric tool NEMI, which is one of the oldest tools used for greenness assessment according to Green Analytical Chemistry.[29] The Modified NEMI includes categories related to energy consumption and environment, which are not considered in the metric NEMI, provides a simple method to assess the greenness of analytical techniques, and is easy to interpret.[30]

The selected tool assesses methods for five categories, namely, (1) health, (2) safety, (3) quantity of waste produced, (4) environment, and (5) energy usage.[28–30] The greenness assessment result is presented as a pentagonal pictogram, where each segment of the pentagon represents one of the assessed categories (Fig. 1). Each category is attributed a color, green, yellow, or red, according to the established criteria. The category health is assigned the color green if the chemical is listed in the NFPA health hazard with a score of 0 or 1, yellow if chemical substances with a score of 2 or 3 are used, and red for carcinogenic chemicals with a score of 4. A green color is attributed to safety if the NFPA flammability scores of the chemicals are 0 or 1, yellow if the chemicals have a score of 2 or 3, and red if the NFPA flammability score is 4. The waste category is assessed according to the amount of waste generated when analyzing one sample. A green color indicates that the total amount of waste generated during the analysis of one sample is <50 g or mL, yellow if between 51 and 250 g or mL, and red if the amount of waste generated exceeds 250 g or mL. The category environment is green if the environmental risk is < 50 g, yellow if between 50 and 250 g, and red if more than 250 g. The energy category is determined according to the amount of energy used per sample by the instrument used. A green color is assigned if the method used ≤0.1 kWh per sample, yellow if ≤1.5 kWh of energy was used for each sample, and red if the energy usage per sample was >1.5 kWh.

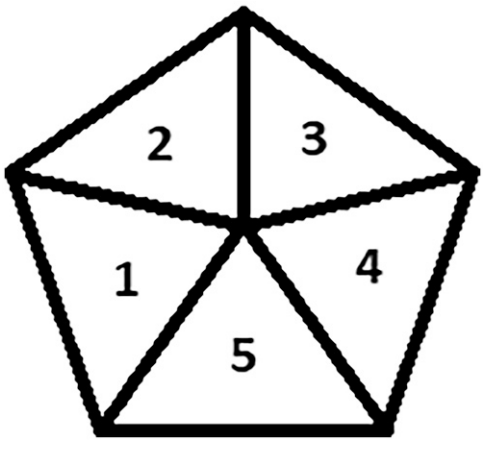

FIG. 1. Pictogram used for the Modified National Environmental Methods Index (NEMI).

Results

Phase 1: Systematic literature review results

A total of 124 studies were identified, of which 7 were considered for the comparative analysis (Fig. 2). HPLC using MS, diode array detector (DAD), and UV were commonly used detection techniques for the analysis of cannabinoids in oil. Cannabinoids were analyzed in olive oil and hemp oil. All studies used a C18 column with particle sizes ranging from 2.1 to 5 μm. Table 1 summarizes different analytical techniques for the analysis of cannabinoids in oil.

Phase 2: Method development, validation, and application

Method was linear with acceptable specificity, accuracy, and precision.

Specificity

The absence of peaks at the retention time of THC, 13.21 min (Fig. 3) when analyzing blanks, confirmed the specificity of the method.

Linearity

The calibration curve for THC in oil was linear. The calculated coefficient of determination (r^2) was 0.9957, indicating good linearity between peak area and concentration. The mean equation of the regression line derived from the five concentration standards was $y = 6 \times 10^6 x + 448143$.

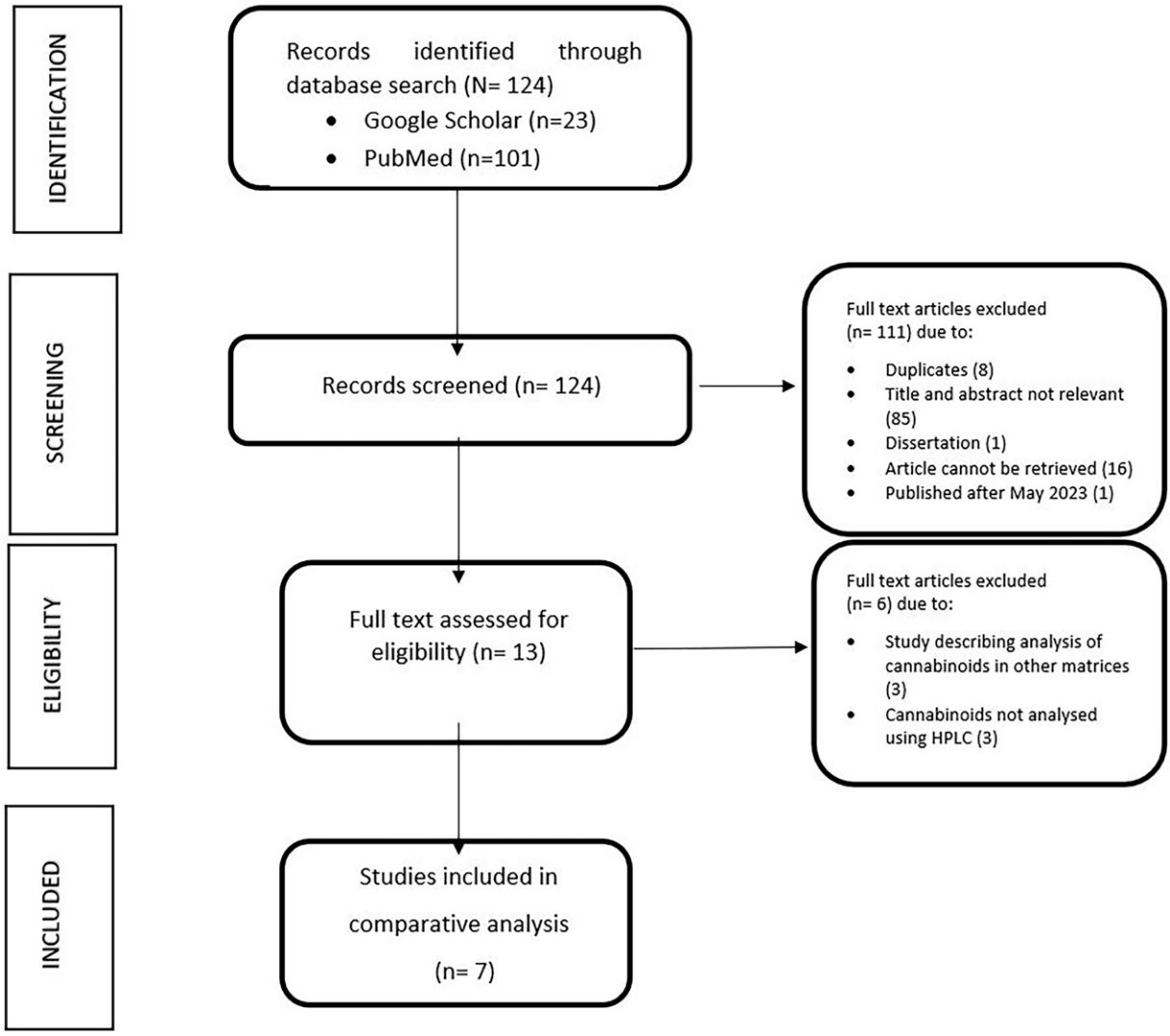

FIG. 2. Literature search on the analysis of cannabinoids in oil.

Accuracy

The method was found to have acceptable accuracy. The percentage bias was within the ±15% limit (Table 2).

Precision

Intraday precision percentage RSD values ranged between 8.35% and 13.68%, and interday precision RSD values ranged between 3.80% and 12.50%.

Stability

Stability percentage RSD values ranged between 0.53% and 12.65% indicating that THC was stable in MCT oil after 8 days following storage at 4°C.

Limit of detection and limit of quantification

The limit of detection was 0.01% THC in oil, and the limit of quantification was 0.03125% THC in oil with a percentage RSD of 13.08%.

System suitability and robustness

The RSD values for retention time, area of peak, and height of peak were all less or equal to 1%. Table 3 displays robustness results for parameter changes. Variation in flow rate and detector wavelength are critical parameters, which lead to poor efficiency (lower number of theoretical plates).

Table 1. Analysis of Cannabinoids in Oil ($n = 7$)

Method	Author	Type of oil	Detector used	Stationary phase	Mobile phase	Retention time of THC	Run time	Limit of quantification	Linearity range
1	Deidda, R et al.[15]	Olive oil	DAD	Poroshell 120 SB-C18 column (2.7 µm, 150 mm × 2.1 mm i.d.)	ACN/5 mM K2HPO4 (pH 3.45)	≈ 4 min	6 min	n/a	1–40 µg/mL
2	Citti, C et al.[31]	Hemp oil	UV and MS	Poroshell 120 EC-C18 (3 × 100 mm, 2.7 µm)	0.1% (v/v) formic acid in both water (A) and ACN (B)	10.7 min	15 min	1 µg/mL	1–10 µg/mL
3	Bongiorno P et al.[32]	Olive oil	UV	Poroshell 120 EC-C18 (3.0 × 50 mm, 2.7 µm) column	0.1% (v/v) formic acid (A) and 0.05% (v/v) formic acid in methanol (B)	7.5 min	≈9.5 min	0.50 µg/mL	1–250 µg/mL
4	Piani B et al.[33]	Hemp oil	DAD	Poroshell 120 EC-C18 (4.6 × 150 mm, 2.7 µm) column	0.1% formic acid in water (A) and 0.1% formic acid in ACN (B)	≈22.5 min	≈37 min	0.38 µg/mL	0.5–100 µg/mL
5	Citti, C et al.[34]	Olive oil	DAD and MS	Poroshell 120 EC-C18 (2.1 × 100 mm, 2.7 µm)	0.1% formic acid in water (A) and 0.1% formic acid in ACN (B)	5.9 min	10 min	0.10 µg/mL	0.1–10 µg/mL
6	Ternelli M et al.[35]	Olive oil	MS/MS	Kinetex EVO C18 (100 × 2.1 mm, 5 µm) column	2.0 mM ammonium acetate (A) and ACN (B)	11.0 min	20 min	n/a	n/a
7	Calvi, L et al.[36]	Olive oil	MS	Synergi Hydro RP C18 (150 × 2 mm i.d., 4 µm)	0.1% formic acid in water (A) and ACN (B)	11.38 min	20 min	0.0001 µg/mL	0.001–1 µg/mL

ACN, acetonitrile; DAD, diode array detector; MS, mass spectrometry; THC, Δ-9-tetrahydrocannabinol; UV, ultraviolet.

Method application

Difference between analyzed concentration of THC and concentration indicated on CBD oil product label was noted for one of the two commercially available oils, which were analyzed (Table 4).

Phase 3: Green assessment of methods for the determination of cannabinoids in oil

The method developed in this study was found to have equivalent greenness to other HPLC-UV methods identified in the literature (Fig. 4). The HPLC-UV methods were assigned a green color for categories related to the quantity of waste produced and environment, and a yellow color for health, safety, and energy categories, respectively. The HPLC-MS methods for the analysis of cannabinoids developed by Citti et al.,[31] Ternelli et al.,[35] and Calvi et al.[36] were found to be less green compared with the methods using HPLC-UV, with the field related to energy consumption being assigned the color red due to the use of MS detector.

Discussion

This article describes the analysis of THC in MCT oil using HPLC-UV and a mixed aromatic functionality stationary phase. A number of HPLC-UV methods describe the analysis of cannabinoids in different types of oils such as olive oil and hemp oil.[31,36–43] Liquid chromatography methods commonly make use of a C_{18} column for the determination of cannabinoids. Although the German Pharmacopoeia monograph includes the use of a C_{18} column for the analysis of cannabinoids,[44] the use of a C_{18} column for the analysis of THC in MCT oil led to poor method selectivity despite modification of other analytical method parameters. The use of wavelengths other than those mentioned in this article would have led to lower concentrations of THC not being successfully quantified since neutral cannabinoids such as THC have maximum UV absorption occurring around 220 nm. Although changing the amount of ACN in the mobile phase might affect peak separation,[45] it did not lead to the separation of the analyte and oil peaks.

The use of a column with a mixed aromatic functionality stationary phase (C18-AR) led to the improved method selectivity. The use of such a column has been described by Ciolino et al., who also reported improved separation compared with C_{18} columns when analyzing different cannabinoids in various matrices using HPLC coupled to a photodiode array detector. The method described in this article made use of a UV detector, which is cheaper than a photodiode array detector and

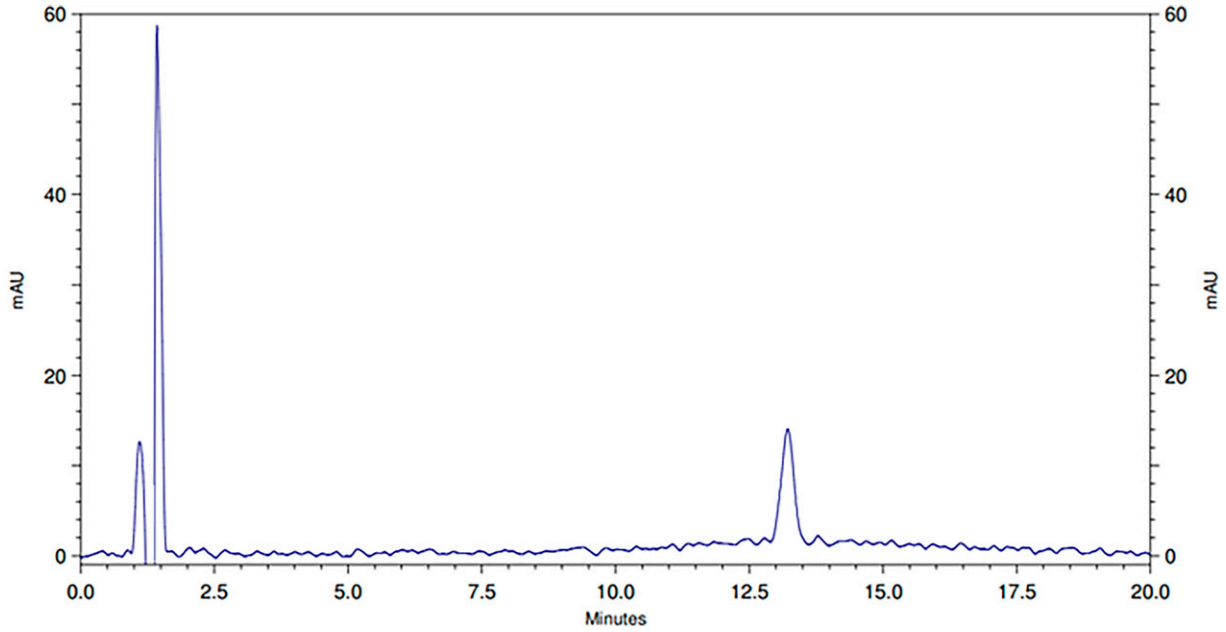

FIG. 3. Chromatogram of THC (retention time 13.21 min) in MCT oil (0.25%). THC, Δ-9-tetrahydrocannabinol; MCT, medium-chain triglyceride.

easily available in most laboratories. The use of acetic acid in the mobile phase also helps improve resolution and peak shape.[46]

The developed and validated method presented in this article is efficient with a relatively simple sample preparation technique. Other sample preparation techniques utilized for the analysis of cannabinoids include solvent extraction[31,38] and solid phase extraction,[36,37] which usually include a larger number of steps leading to a greater probability of error. The analysis of THC can be reliably carried out using instrumentation, which is commonly available in most analytical laboratories.

There was no difference in greenness between the methods using HPLC-UV for the analysis of cannabinoids when using the Modified NEMI as a greenness assessment metric tool. The HPLC-UV methods were rated green for the categories related to the amount of waste produced and environment. The methods were rated yellow in the health and safety categories, respectively, due to the nature of the solvents used during the sample preparation and analysis of cannabinoids, as per NFPA score. Another field that was yellow was that related to the energy category due to the associated energy consumption when using HPLC-UV for analysis. The methods developed by Citti et al.,[31] Ternelli et al.,[35] and Calvi et al.[36] achieved the same result for the health, safety, quantity of waste produced, and environment categories, respectively, as per the other methods; however, they were found to be less green compared with the

Table 2. Accuracy Values for the Determination of THC in MCT Oil

Concentration (%)	Mean calculated quantity of three replicates (%)	Bias (%)
0.03125	0.029687	−5.0016
0.125	0.121512	−2.7904
0.5	0.509303	1.8606

MCT, medium-chain triglyceride.

Table 3. Robustness Results for HPLC Parameter Changes

Parameter change	Retention time (minutes)	Number of theoretical plates	Capacity factor
Method conditions	13.21	2312.42	8.59
Flow rate +0.1 mL/min	14.25	1128.72	8.50
Flow rate −0.1 mL/min	15.85	1755.93	8.91
Detector wavelength +2 nm	14.63	1460.09	8.76
Detector wavelength −2 nm	14.79	1021.56	8.70
Column temperature +5°C	15.02	2604.78	8.39
Column temperature −5°C	15.64	2543.90	8.78

HPLC, high-performance liquid chromatography.

Table 4. THC Concentrations Analyzed in Commercial Products

Oil A		Oil B	
THC concentration on label	THC concentration analyzed	THC concentration on label	THC concentration analyzed
0%	THC not detected	0.2%	0.49%

other methods due to a higher energy consumption attributed to the application of an HPLC-MS.

A limitation of the developed method can be considered to be the relatively long retention time of THC. Other studies report a relatively longer retention time for THC.[19,33] Modification of the mobile phase composition to include a larger proportion of organic solvent to shorten retention time would lead to higher solvent consumption, rendering the method less green. An increase in mobile phase flow rate can result in an increased risk for column back pressure.

Robustness studies indicated sensitivity of method to changes in experimental conditions. Maintaining strict control over flow rate and detector wavelength is recommended. Column temperature variations are less critical with regard to efficiency but should be monitored to ensure optimal performance. Although the method can be successfully and effectively applied using the described

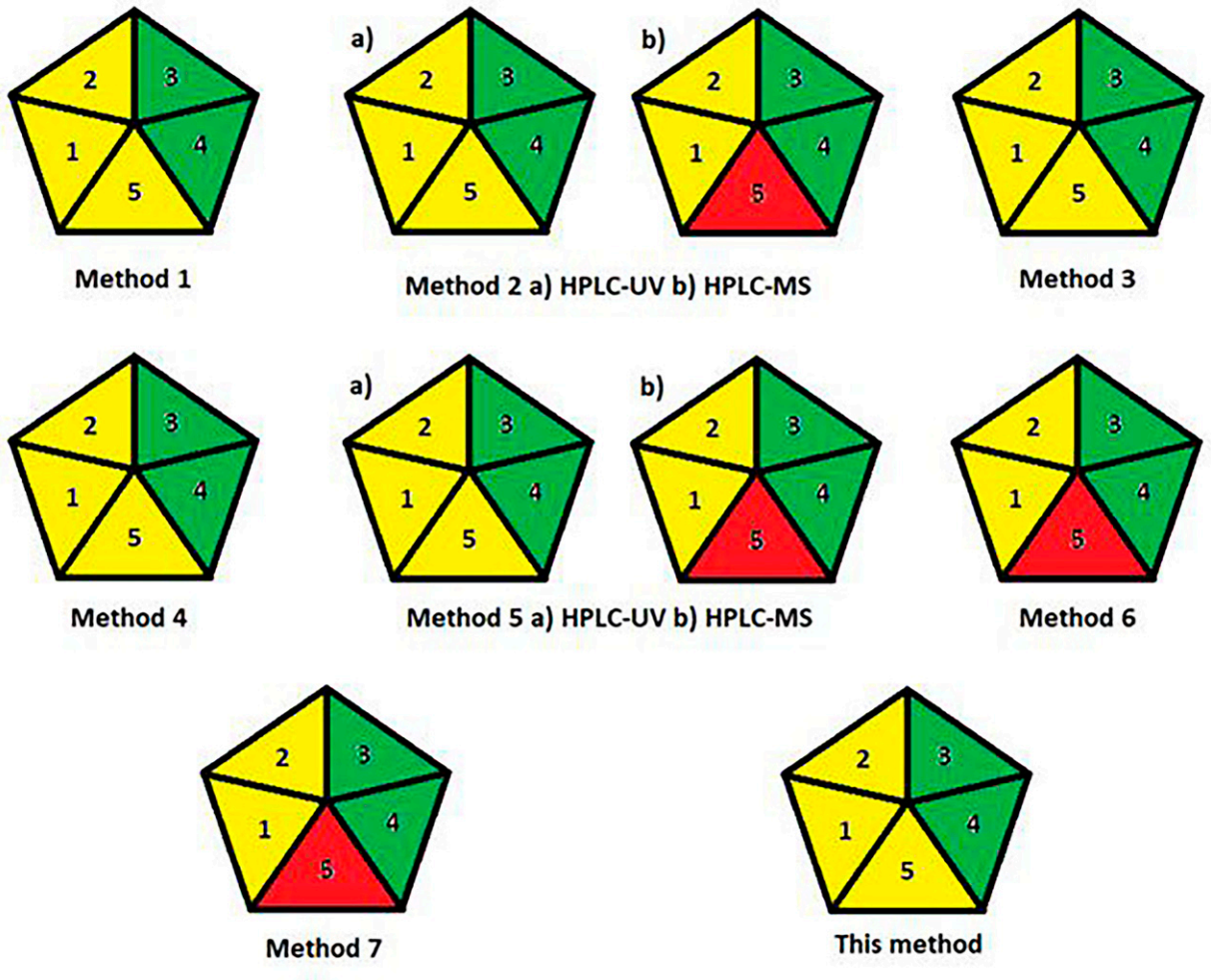

FIG. 4. Greenness assessment of the analytical methods of cannabinoids identified in literature and of the analytical method developed in this study. (1) Health, (2) safety, (3) quantity of waste produced, (4) environment, and (5) energy usage.

parameters, potential areas for further research can aim at improving the robustness of the method to help mitigate sensitivity issues identified.

The method has acceptable accuracy, precision, stability, and linearity. Although other HPLC-UV methods for the determination of cannabinoids[15,32,33] report lower limits of quantification, the range of concentrations that can be analyzed using the developed method is appropriate for the intended use of the method, which is to determine concentrations of THC in commercially available cannabinoid-containing MCT oil to ensure that they are within the legal limits. Also, other methods describe the analysis of cannabinoids in other types of oil and not MCT oil. This developed method can be used as a standard method by laboratories involved in the analysis of cannabis in MCT oil, a commonly used carrier oil. This can help ensure that commercial CBD products, particularly CBD oils, comply with regulations stating that THC content should not exceed the allowed limits of 0.2–0.3%.[8] Future work could also involve adapting the developed method for the determination of other cannabinoids.

Conclusion

Although literature describes the analysis of cannabinoids in oils such as olive oil and hemp oil, literature about the determination of THC in MCT oil, a commonly used carrier oil in cannabinoid-containing preparations, is scarce. The method described for the determination of THC in MCT oil is efficient and simple to perform with a relatively short analytical run time. The method makes use of a UV detector, which is readily available in most analytical laboratories, and a mixed aromatic functionality stationary phase, which leads to improved selectivity. The method can be successfully used to determine concentrations of THC in commercially available CBD preparations in MCT oil to ensure that concentrations in products match those stated on the product label. This can help increase the reliability of use and ensure the quality of CBD products.

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L.L.G. Constituents of Cannabidiol Products [Pharm D. Dissertation]. Malta: University of Malta; 2023.

Authors' Contributions

J.V.S.: Conceptualization, methodology, writing—original draft, and supervision. L.L.G.: Investigation. P.I.B.: Investigation. K.S.: Investigation. N.S.B.: Methodology and Investigation, writing—reviewing and editing. S.R.: Writing—reviewing and editing. A.S.I.: Writing—reviewing and editing. L.M.A.: Writing—reviewing and editing.

Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

ACN = Acetonitrile
CBD = Cannabidiol
GC = Gas Chromatography
HPLC = High Performance Liquid Chromatography
MCT = Medium Chain Triglyceride
MS = Mass Spectrometry
NEMI = National Environmental Methods Index
RSD = Relative Standard Deviation
THC = Δ-9-tetrahydrocannabinol
UHPLC = Ultra-High Performance Liquid Chromatography
UV = Ultraviolet