

HPLC-DAD Method for Cannabinoid Quantification in Brazilian Cannabis Oils and Extracts

Maria Vitória dos Santos Villa Bande

University of Brasilia - UnB

Fernanda Vasconcelos de Almeida

University of Brasilia - UnB

Claudete da Costa-Oliveira

State University of Campinas - UNICAMP

João Gabriel Gouvea

State University of Campinas - UNICAMP

Ana Cristi Basile Dias

acbdias@unb.br

University of Brasilia - UnB

Research Article

Keywords: Cannabis extracts, medicinal oils, Cannabinoids, HPLC-DAD, Method validation

Posted Date: April 28th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9224291/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Cannabis sativa L. is one of the most versatile plants used by different cultures worldwide as a source of food, fiber, and medicine. Due to the variability of *Cannabis*, research on this plant and the standardization of medications and *Cannabis*-based products prove to be challenging. This study aimed to optimize and validate a high-performance liquid chromatography method coupled with photodiode array detection (HPLC-DAD) for the determination of cannabinoids in *Cannabis* extracts and medicinal oils produced in Brazil.

Method

The analytical procedure is distinguished by its simple sample preparation for *cannabis* oils and extracts, consisting only of dilution with anhydrous alcohol, while cannabinoid quantification was performed using conventional HPLC-DAD. Its performance was evaluated through validation parameters, including linearity, sensitivity, precision, and accuracy, and was applied to quantify eight cannabinoids in 15 different samples.

Results

The method demonstrated excellent linearity ($R^2 > 0.999$) for all analytes. It showed adequate sensitivity, with LOQ ranging from 0.11 to 0.34 $\mu\text{g/mL}$, as well as high precision, with RSD values below 5% for both intraday and interday analysis. Accuracy was confirmed through recovery studies, with an excellent recovery (103.2% to 103.4%) for CBD in a concentrated *cannabis* extract, while Δ^9 -THC recovery ranged from 86.1% to 90.6% in the medicinal oil matrix.

Conclusion

The results are promising for broadening the application of this chromatographic method to the determination of cannabinoids in *Cannabis* products produced by Brazilian *Cannabis* producers' associations. The combination of simple sample preparation and reliable validation parameters addresses the demand for quality control.

Introduction

Cannabis sativa L. is a plant widely recognized for its historical significance and social and pharmacological potential [1]. Its widespread cultivation and global distribution have contributed to substantial genetic, morphological, and chemical variability. As a result of continuous crossbreeding

aimed at developing specific chemical profiles for different applications, cannabis is generally regarded as a single species, *Cannabis sativa L.*, encompassing multiple subspecies or chemotypes[1–3].

The plant's rich profile of active compounds makes the study of its chemical composition highly relevant. Studies have shown that the medicinal effects of cannabis are determined by the specific concentration, proportion, and interaction of its various constituents [4]. More than 500 bioactive compounds have been identified and isolated in cannabis, including cannabinoids or phytocannabinoids – a unique class of terpene-phenolic compounds characteristic of the plant [5]. This class is often divided into eleven subclasses, including cannabigerol (CBG), Δ^9 -tetrahydrocannabinol (Δ^9 -THC), cannabidiol (CBD), cannabichromene (CBC), cannabinol (CBN), Δ^8 -tetrahydrocannabinol (Δ^8 -THC), cannabicyclol (CBL), cannabinodiol (CBND), cannabielsoin (CBE), cannabitriol (CBT) and miscellaneous[5–8].

Cannabinoids are biosynthesized in the plant as their acidic precursors (e.g., CBDA, THCA) and typically undergo photolytic or thermal decarboxylation processes, being converted into their neutral forms [5, 9]. Acidic cannabinoids are largely considered pharmacologically less active because their polar nature limits their ability to readily cross the blood-brain barrier [10–12].

Given their therapeutic potential and high abundance in the plant, CBD and Δ^9 -THC are the most extensively researched cannabinoids. CBD is known for its antioxidant and anti-inflammatory properties, in addition to having anxiolytic and anticonvulsant effects that are being explored for treating a wide range of diseases [1]. In contrast, although Δ^9 -THC also offers therapeutic benefits, such as antiemetic and appetite-stimulating properties, it is the plant's main euphorogenic component and is therefore primarily associated with the recreational use of cannabis [6, 10].

Although the therapeutic use of cannabis dates back thousands of years, legislative, sociocultural, and economic changes in various countries over the last 20 years have led to the emergence of new studies investigating the potential of its bioactive compounds for treating numerous diseases [11, 12]. In addition, public interest in the therapeutic properties of cannabis has also grown significantly as an alternative for treating diseases, aiming to minimize the adverse effects of conventional synthetic drugs [13].

In Brazil, the use of cannabis is illegal; however, following the growing evidence for the efficacy of treatments with cannabis-derived products, the Brazilian Health Regulatory Agency (ANVISA) established relatively restrictive regulations concerning the prescription, importation, and manufacture of these products for therapeutic use [14, 15], which have recently been updated and expanded through new regulatory resolutions issued in 2026 [16, 17]. Facing these challenges, cannabis civil associations – formed by patients, family members, along with activists, lawyers, and researchers – have emerged in the country, acting to support research and provide aid, information, and assistance to patients and their families in accessing these products [18, 19]. More recently, the publication of RDC 1014/26 has shed light on the role and operation of non-profit patient associations in Brazil by introducing a specific

regulatory framework for their activities [17], although many associations still depend on judicial authorization for cannabis cultivation and the supply of cannabis-derived products to patients.

With the new regulatory framework recently issued by ANVISA, which establishes more specific criteria for cultivation, research, manufacturing, and quality control, an even greater level of scientific rigor is now required. In this context, there is a growing demand for robust, precise, and reliable analytical methods capable of evaluating cannabis-derived products at different stages of medicinal oil production, in order to ensure quality control in compliance with regulatory standards and, ultimately, patient safety. A wide variety of cannabis-based products has been developed for specific applications. Brazilian association products include creams, soaps, gummies, ointments, sprays, and oils, the main therapeutic form. These oils are typically formulations prepared by the dilution of cannabis crude extracts or isolated cannabinoids into an oily excipient [20]. However, the inherent complexity of the lipid matrix in these formulations presents a significant analytical challenge. Sample preparation of oily matrices is often laborious, involving multiple extraction steps or the use of toxic organic solvents [21].

A variety of analytical techniques have been developed for the identification and quantification of cannabinoid and other cannabis bioactive compounds [5, 22, 23]. Liquid Chromatography (LC) coupled with Diode Array Detector (DAD) is a suitable and widely utilized method in this context [1, 9, 10, 12, 24–26]. LC provides a separation profile without heating the samples, allowing accurate quantification of both neutral and acidic cannabinoid forms [9, 12, 24]. This capability is critical for relating dose to pharmacological effect. Furthermore, DAD is a relevant detection system because cannabinoids are chromophores in the ultraviolet (UV) region, and this detector is cheaper, requires less operator expertise, and is widely available [12].

Therefore, this study aimed to optimize and validate an HPLC-DAD method for the determination of eight neutral cannabinoids in medicinal oils and extracts produced by Brazilian associations. To address the challenges of complex lipid matrices, the method focused on simple sample preparation involving dilution with anhydrous alcohol.

Materials and Methods

Chemical and Reagents

HPLC-grade methanol and acetonitrile $\geq 99.9\%$ and reagent-grade formic acid ($\geq 95\%$) were purchased from Sigma-Aldrich (Darmstadt, Germany). HPLC-grade anhydrous alcohol mixture, composed of ethanol (89–91%), methanol (4–6%), and isopropanol (4–6%), was purchased from Tedia (Rio de Janeiro, Brazil). Cannabinoid reference standards CBN (C-046-1ML), CBG (C-212-1ML) and Δ^9 -THC (T-005-1ML) were purchased from Supelco (Sigma-Aldrich, Germany), while CBD (LGCAMP1088.02-01) was obtained from LGC Standards (Germany); all standards were supplied as $1000 \mu\text{g mL}^{-1}$ methanolic solutions. Additionally, a $500 \mu\text{g mL}^{-1}$ standard mixture of eight neutral cannabinoids – CBD, Δ^9 -THC, Δ^8 -THC, CBG, CBN, CBC, CBDV and THCV – in methanol was purchased from Supelco (C-219-1ML). Ultrapure type I

water, obtained from an Arium® Mini system (Sartorius, Germany), was used to prepare aqueous solutions.

Cannabis Samples

A total of 15 cannabis samples, including six medicinal oils and nine concentrated extracts, were kindly donated by three Brazilian cannabis associations: APEPI (Rio de Janeiro), Abrapango (Brasilia, Federal District) and Flor do Amor (Brasilia, Federal District). Among the six oil samples, two were Δ^9 -THC strains (Purple Wreck from APEPI and Rainbow from Abrapango), two were CBD strains (Doctor and Harle Tsu from APEPI), one was a mixed CBD and Δ^9 -THC strain (Schanti from APEPI), and one was a CBG strain (O-CBG from APEPI). The nine concentrated extract samples comprised four Δ^9 -THC strains (OG Mix, HTHC and Distilled Extract from Abrapango, and R-Purple Wreck from APEPI), three CBD strains (R-Doctor from APEPI, Fenoqueen from Flor do Amor, and HCBD from Abrapango), one CBG strain (R-CBG from APEPI), and one mixed CBD and Δ^9 -THC strain (R-Schanti from APEPI).

Sample preparation

Sample preparation was carried out following the recommendations described by AOAC International, with adaptations [27]. Each medicinal oil sample was thoroughly homogenized, and a 0.5 g aliquot ($n = 3$) was weighed into a 25 mL volumetric flask. Anhydrous alcohol was added, and the sample was vortexed (KASVI, Parana, Brazil) for 1 min and sonicated (Eco-sonics, Ultronique, São Paulo, Brazil) for 2 min. After solubilization, the volume was adjusted to the 25 mL mark with anhydrous alcohol. The solution was then filtered through a 0.22 μm PVDF filter and diluted with methanol. For the cannabis extracts, approximately 0.05 g of each sample ($n = 3$) was weighed into a 10 mL beaker, and 3 mL of methanol was added. The sample was manually dissolved and quantitatively transferred to a 25 mL volumetric flask, which was brought to volume with methanol. The solution was then filtered through a 0.22 μm PVDF filter and diluted with methanol for subsequent HPLC-DAD analysis. Sample dilutions were performed to adjust minor and major cannabinoid concentrations to the analytical curves, ranging from 10-fold to 200-fold.

HPLC-DAD and chromatographic conditions

Chromatographic runs were carried out on an HPLC (Shimadzu, Prominence) equipped with a LC-20AD quaternary pump, a DGU-20A5R degasser module, a SIL-20AHT autosampler, a CTO-20A column oven and a SPD-M20A diode array detector. Shimadzu LabSolutions software was used for instrument control and data acquisition. The separations were achieved using a Poroshell 120 EC-C18® (Agilent) 150 x 3 mm, 2.7 μm column, which was maintained at 30°C in the column oven. Different mobile phase compositions were preliminarily tested, and trials were performed with different solvents (methanol and acetonitrile), their acidification with formic acid, and organic phase composition ranging from 50 to 80%.

The optimal separation was achieved with an isocratic elution using a mobile phase composed of 75% acetonitrile and 25% ultrapure water with 0.1% (v/v) formic acid at a flow rate of 1 mL min⁻¹. The total runtime was 10 min, and the injection volume was 10 µL. The UV spectra were recorded from 200 to 400 nm, and detection was set at 210, 220 and 280 nm. The wavelength of 210 nm was used for quantification.

Method Validation (dup: abstract ?)

The proposed method was validated according to the guidelines established by ANVISA Resolution of the Collegiate Board (RDC) No. 166/2017, following the requirements for quality control of phytotherapeutic products [28]. The analytical curve was constructed from a 25 µg mL⁻¹ stock solution, prepared by diluting the cannabinoid standard mixture in methanol, and was subsequently used to prepare six working solutions with individual cannabinoid concentrations of 0.5, 1.0, 2.5, 5.0, 7.5 and 10 µg mL⁻¹. Figures of merit, including limit of detection (LOD), limit of quantification (LOQ), linear range, linearity, precision and accuracy were determined. All samples were injected three times to assess instrument precision, and system suitability was verified by analyzing two calibration standards at the beginning of each sample set.

Linear range and linearity were evaluated from calibration curves, considering the determination coefficient (R^2) greater than 0.99. The limits of detection and quantification were determined as $LOD = 3.3 * s/b$ and $LOQ = 10 * s/b$, respectively, where s is the standard deviation of the intercept and b is the slope of the calibration curve. Method precision was evaluated using the CBD-rich oil from APEPI; this sample was selected because APEPI is one of the largest cannabis associations in Brazil, and their oils are standardized to an acceptable concentration level, as evidenced by the provision of certificates of analysis. Intra-day repeatability was determined from the relative standard deviation (RSD) of three sample replicates of the same batch prepared on the same day ($n = 3$). Intermediate precision, or inter-day repeatability, was determined from the RSD of the same sample prepared on three different days.

The accuracy of the method was assessed through a recovery study. Due to reagent limitations, specifically the high cost of certified cannabinoids reference materials, coupled with bureaucratic import procedures and slow delivery times, this study was performed only for CBD and Δ^9 -THC. For the recovery study, a Δ^9 -THC-rich extract and a CBD-rich oil were spiked at three different concentration levels of the standards. The final concentrations added to the samples were 0, 1.0, and 2.5 µg mL⁻¹. CBD was spiked into the Δ^9 -THC-rich extract and Δ^9 -THC was spiked into the CBD-rich oil. Recovery was calculated as $R (\%) = (C_{\text{measured}} - C_{\text{sample}}) / (C_{\text{spiked}}) * 100$. Where C_{measured} is the analyte concentration measured after spiking, C_{sample} is the analyte concentration measured in the non-spiked sample (original matrix) and C_{spiked} is the analyte concentration added.

Results and Discussion

Chromatographic Separation

To optimize chromatographic separation, a variety of mobile phases were evaluated. Assays using acetonitrile resulted in significantly reduced cannabinoid retention times and demonstrated superior chromatographic efficiency, as evidenced by peaks with greater intensity and narrower widths compared to those obtained using methanol. In addition, runs performed with methanol exhibited higher system back pressure, attributable to the higher viscosity of this solvent [26, 29].

Among the eight neutral cannabinoids, the CBD/CBG pair was identified as critical in the chromatographic separation due to their structural similarities (closed and open ring structures) and similar polarity profiles [30]. Different proportions of acetonitrile were evaluated to enhance the resolution achieved between CBG and CBD. The best condition for maximizing chromatographic separation was obtained using 75% acetonitrile in the mobile phase. This condition allowed an isocratic and rapid analysis while achieving a resolution of 1.3 between these cannabinoids, which is considered satisfactory for quantitative purposes. The chromatographic separation profile of the eight neutral cannabinoids studied is shown in Fig. 1.

Method Validation

Linearity, Limits of Detection (LOD) and Quantification (LOQ)

The calibration curves were constructed using five mixed-standard dilutions ranging from 0.5 to 10 $\mu\text{g mL}^{-1}$ for the eight target cannabinoids, with each concentration level analyzed in triplicate. Coefficient of determination (R^2), LOD and LOQ of the working standards are reported in Table 1. The R^2 values were greater than 0.999 for all cannabinoids, indicating excellent linearity across the working range (Figure S1 and S2). The LOD values ranged from 0.04 to 0.11 $\mu\text{g mL}^{-1}$, while the LOQ values ranged from 0.11 to 0.34 $\mu\text{g mL}^{-1}$. Cannabinol (CBN) exhibited the lowest LOD and LOQ values, whereas Δ^8 -THC showed the highest values. These limits demonstrate that the proposed method has adequate sensitivity, since dilution of cannabis samples is recommended to avoid potential matrix interference and prevent column damage [10].

Table 1
Correlation coefficient R^2 , LOD and LOQ for cannabinoid standards

Cannabinoid	R^2	LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)
CBDV	0.99996	0.07	0.21
CBG	0.99988	0.10	0.32
CBD	0.99997	0.07	0.21
THCV	0.99984	0.09	0.28
CBN	0.99973	0.04	0.11
Δ^9 -THC	0.99998	0.05	0.16
Δ^8 -THC	0.99982	0.11	0.34
CBC	0.99981	0.08	0.24

Precision

The precision of the method was evaluated by assessing intraday and inter-day repeatability, expressed as relative standard deviation (RSD), following the analysis of the CBD-rich oil sample (Doctor) from APEPI). Only CBG, the neutral form of the main precursor cannabinoid (CBGA), as well as CBD, Δ^9 -THC, and CBC were present in the sample and therefore included in the evaluation. The results, reported in Table 2, demonstrated high repeatability of the method, with RSD lower than 5% for all cannabinoids studied.

Table 2
Intra and Inter-day RSD (%) values

Intra-day RSD (%)	CBG	CBD	Δ^9 -THC	CBC
	2.1	1.0	1.8	3.5
Inter-day RSD (%)	2.3	2.8	3.2	3.9

Recovery

Accuracy of the method was assessed through the recovery of the main pharmacological markers – CBD and Δ^9 -THC – spiked into a Δ^9 -THC-rich concentrated extract and a CBD-rich oil sample, as presented in Table 3. The recoveries of CBD when spiked into the Δ^9 -THC-rich concentrated extract averaged 103.2% and 103.4% at the 1 and 2.5 $\mu\text{g mL}^{-1}$ concentration levels, respectively. These results were within $\pm 5\%$, demonstrating excellent accuracy for CBD in this matrix. Δ^9 -THC recoveries when spiked into the CBD-rich oil matrix averaged 90.6% and 86.1%. These results are considered satisfactory, and the lower recovery for Δ^9 -THC, varying up 14% from the predicted concentration, may be attributed

to the higher complexity of the medicinal oil matrix, which is composed of olive oil and CBD-rich cannabis extract.

Table 3
Spike recoveries (%) of CBD and Δ^9 -THC

Spiked Concentration ($\mu\text{g mL}^{-1}$)	CBD	Δ^9 -THC
1.0	103.2 \pm 1.4%	90.6 \pm 0.9%
2.5	103.4 \pm 0.4%	86.1 \pm 0.3%

Despite most cannabis-based therapeutic products being formulated as oils, i.e., cannabis extracts diluted in an oily vehicle such as Medium-Chain Triglycerides (MCT), extra virgin olive oil (EVOO), or hemp-seed oil [20], a significant challenge remains in the sample preparation of these products. This challenge is more significant for oils diluted in EVOO, which is the main oily vehicle diluent used in Brazil due to its accessibility. These complex matrices exhibit low miscibility in the typically used chromatographic solvents for analyzing cannabinoids, such as methanol or acetonitrile. Most methods proposed involve plant material, such as inflorescences or crude cannabis extracts. For oil formulations, some studies report the use of organic solvents like tetrahydrofuran (THF), n-hexane, or dichloromethane [12, 26, 31], or the employment of liquid-liquid extraction (LLE) methods, sometimes involving fat-freezing [21, 25, 32–34].

A method proposed by the AOAC [27] states that simple dilution with ethanol is sufficient to prepare these samples, although specific details are not presented. However, during preliminary tests, it was observed the formation of emulsions and incomplete dissolution of the samples using HPLC-grade ethanol. In this work, the use of anhydrous alcohol, a commercial mixture composed of ethanol (89–91%), methanol (4–6%) and isopropanol (4–6%), allowed complete sample dissolution assisted by vortex and sonication in only three minutes. This demonstrates a rapid and effective preparation technique utilizing a less toxic solvent system, ideal for routine quality control analysis.

Sample Analysis

The proposed method was applied in the analysis of five Cannabis medicinal oils and nine concentrated extracts produced by three Brazilian cannabis associations. The concentration of each cannabinoid is reported on a weight-by-weight basis (% w/w), calculated using the initial weighed mass and sample dilution factor. The resulting data are summarized in Table 4, and chromatograms of selected samples, such as Harle Tsu, O-CBG, Purple Wreck, and Schanti, are provided in the Supplementary Material (Figure S3).

Table 4
Average content (\pm SD) of cannabinoids in 15 samples (n = 3).

Cannabis Oils							
Sample	CBDV	CBG	CBD	THCV	CBN	Δ^9 -THC	CBC
O-CBG	ND*	2.884 \pm 0.047	☒ LOQ**	ND	ND	0.038 \pm 0.002	0.164 \pm 0.006
Schanti	ND	0.056 \pm 0.001	1.277 \pm 0.019	ND	ND	0.642 \pm 0.008	0.047 \pm 0.001
Purple Wreck	ND	0.019 \pm 0.002	☒ LOQ	0.041 \pm 0.001	☒ LOQ	0.818 \pm 0.003	ND
Harle Tsu	0.174 \pm 0.001	0.109 \pm 0.001	4.080 \pm 0.018	ND	ND	0.117 \pm 0.002	0.155 \pm 0.008
Doctor	0.048 \pm 0.001	0.132 \pm 0.003	3.539 \pm 0.040	ND	ND	0.142 \pm 0.004	0.156 \pm 0.006
Rainbow	ND	0.016 \pm 0.001	ND	☒ LOQ	☒ LOQ	0.688 \pm 0.013	☒ LOQ
Cannabis Extracts							
Sample	CBDV	CBG	CBD	THCV	CBN	Δ^9 -THC	CBC
R-Doctor	0.803 \pm 0.027	2.145 \pm 0.062	42.939 \pm 1.674	ND	ND	2.228 \pm 0.052	2.624 \pm 0.053
R-CBG	ND	45.676 \pm 0.617	ND	ND	☒ LOQ	0.635 \pm 0.007	2.956 \pm 0.072
R-Schanti	ND	1.257 \pm 0.039	30.462 \pm 0.142	ND	ND	32.976 \pm 1.055	1.432 \pm 0.195
R-Purple Wreck	ND	2.466 \pm 0.034	2.930 \pm 0.063	2.467 \pm 0.022	ND	42,241 \pm 0.486	1.076 \pm 0.069
OG Mix	ND	3.850 \pm 0.032	ND	0.327 \pm 0.011	0.243 \pm 0.019	42.300 \pm 1.323	☒ LOQ
Fenoqueen	1.271 \pm 0.005	0.787 \pm 0.009	55.519 \pm 0.573	ND	ND	2.625 \pm 0.005	2.556 \pm 0.017
Distilled Resin	☒ LOQ	1.116 \pm 0.013	0.456 \pm 0.008	ND	2.273 \pm 0.051	21.672 \pm 0.442	☒ LOQ
* Content below the LOD							
** Content below the LOQ							
Note: Δ^8 -THC content is not reported as this cannabinoid was not detected in any of the analyzed samples.							

Cannabis Oils							
HCBD	0.647 ± 0.008	1.611 ± 0.017	52.840 ± 0.835	ND	ND	2.394 ± 0.081	2.754 ± 0.043
HTHC	ND	1.412 ± 0.026	⊠ LOQ	0.511 ± 0.014	1.789 ± 0.026	29.939 ± 0.906	1.022 ± 0.038
* Content below the LOD							
** Content below the LOQ							
Note: Δ^8 -THC content is not reported as this cannabinoid was not detected in any of the analyzed samples.							

Analysis of the sample data (Table 4) revealed that all samples contained levels of Δ^9 -THC and CBG above the method's LOQ. In addition, CBD and CBC were identified in most samples. Cannabigerol (CBG) is the neutral form of cannabigerolic acid (CBGA), the main precursor of major phytocannabinoids in the cannabis plant. Therefore, it is expected to be present as a minor component in cannabis-based samples [1, 25, 35]. Since CBD, Δ^9 -THC and CBC are the main neutral cannabinoids derived from CBGA [7], their presence is also anticipated. All samples exhibited the expected chemotype.

Additionally, it was observed that respective "varins" (CBDV and THCV) were identified in samples in which their corresponding cannabinoid was the major component. Specifically, CBDV was detected at significant levels in CBD-rich chemotypes, and similarly, THCV was observed in Δ^9 -THC-rich samples. The literature reports that CBDV is found in landrace cannabis strains characterized by high CBD and low Δ^9 -THC contents [36], the present results corroborate this observation. Varins are structural variants of phytocannabinoids containing 19 carbon atoms instead of 21, in which the *n*-pentyl side chain is replaced by an *n*-propyl chain during biosynthesis.

Higher standard deviations (SD) were observed in cannabinoid concentrations among replicates of the concentrated extract samples when compared to those of the cannabis medicinal oils. This effect can be attributed to the greater heterogeneity of the concentrated extracts, which are semi-solid and viscous, whereas the oils are easily homogenized. Despite this, SDs did not exceed 5% for any sample. Based on the results obtained, the suitability of the proposed chromatographic method, applied across 15 different samples, is demonstrated.

Conclusion

In conclusion, the proposed HPLC-DAD method was successfully validated in accordance with ANVISA requirements, demonstrating excellent linearity, sensitivity, precision, and accuracy for the identification and quantification of eight neutral cannabinoids – CBD, Δ^9 -THC, Δ^8 -THC, CBG, CBN, CBC, CBDV and THCV – in cannabis concentrated extracts and oils. By employing a simple anhydrous alcohol dilution procedure, this study establishes an effective methodology for preparing cannabis medicinal oil

samples. This approach replaces time-consuming steps such as liquid-liquid extraction and matrix clean-up, as well as the use of additional organic solvents, resulting in a procedure that is significantly faster, more cost-effective, and environmentally friendly. This method meets the practical needs of Brazilian cannabis associations, particularly smaller ones, by providing a simple and robust tool for routine quality control, ensuring product safety and dosage accuracy in compliance with regulatory requirements.

Declarations

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics, Consent to Participate, and Consent to Publish declarations

not applicable.

Funding

Declaration: CAPES (Finance code 001) for a master fellowship, FAPDF (Process 00193–00002054/2023-58) for research funding, and APEPI for sample donation.

Author Contribution

Maria V. S. V. Bande: conceptualization, methodology, investigation, formal analysis, data curation, writing – original draft. Ana C. B. Dias: conceptualization, resources, writing– review & editing, supervision, project administration, funding acquisition. Fernanda V. de Almeida: conceptualization, resources, writing– review & editing, supervision, project administration, funding acquisition. Claudete C. Oliveira: conceptualization, supervision. João G. Gouveia: conceptualization, supervision

Acknowledgement

The authors thank CAPES (Finance code 001) for a master fellowship, FAPDF (Process 00193–00002054/2023-58), and APEPI for sample donation.

Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information. The original results reported in the manuscript are derived from the analytical curves, absorption spectrum and chromatograms presented in the supplementary material.

References

1. Brighenti V, Pellati F, Steinbach M, Maran D, Benvenuti S. Development of a new extraction technique and HPLC method for the analysis of non-psychoactive cannabinoids in fibre-type *Cannabis sativa* L. (hemp). *J Pharm Biomed Anal*. 2017;143:228–36. <https://doi.org/10.1016/j.jpba.2017.05.049>.
2. Balant M, Gras A, Ruz M, Vallès J, Vitales D, Garnatje T. Traditional uses of Cannabis: An analysis of the CANNUSE database. *J Ethnopharmacol*. 2021;279. <https://doi.org/10.1016/j.jep.2021.114362>.
3. UNODC. Recommended Methods and Analysis of Cannabis for the Identification and Cannabis Products. 2022.
4. Hourfane S, Mechqoq H, Bekkali AY, Rocha JM, El Aouad N. A Comprehensive Review on Cannabis sativa Ethnobotany, Phytochemistry, Molecular Docking and Biological Activities. *Plants*. 2023;12:1–43. <https://doi.org/10.3390/plants12061245>.
5. 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. <https://doi.org/10.1186/s42238-020-00040-2>.
6. Fordjour E, Manful CF, Sey AA, Javed R, Pham TH, Thomas R, et al. Cannabis: a multifaceted plant with endless potentials. *Front Pharmacol*. 2023;14:1–36. <https://doi.org/10.3389/fphar.2023.1200269>.
7. dos Santos NA, Romão W. Cannabis – A state of the art about the millenary plant: Part I. *Forensic Chem*. 2023;32. <https://doi.org/10.1016/j.forc.2023.100470>. November 2022.
8. Sainz Martinez A, Lanaridi O, Stigel K, Halbwirth H, Schnürch M, Bica-Schröder K. Extraction techniques for bioactive compounds of cannabis. *Nat Prod Rep*. 2023;40:676–717. <https://doi.org/10.1039/d2np00059h>.
9. Song L, Carlson S, Valenzuela G, Chao M, Pathipaka SB. Development of a validated method for rapid quantification of up to sixteen cannabinoids using ultra-high-performance liquid chromatography diode-array detector with optional electrospray ionization time-of-flight mass spectrometry detection. *J Chromatogr A*. 2022;1670:462953. <https://doi.org/10.1016/j.chroma.2022.462953>.
10. 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;1705:464191. <https://doi.org/10.1016/j.chroma.2023.464191>.

11. Charitos IA, Gagliano-Candela R, Santacroce L, Bottalico L. The Cannabis Spread throughout the Continents and its Therapeutic Use in History. *Endocr Metab Immune Disord - Drug Targets*. 2020;21:407–17. <https://doi.org/10.2174/1871530320666200520095900>.
12. Hall DR, Sinclair JS, Bhuyan DJ, Khoo C, Li CG, Sarris J, et al. Quality control of cannabis inflorescence and oil products: Response factors for the cost-efficient determination of ten cannabinoids by HPLC. *Talanta Open*. 2022;5:100112. <https://doi.org/10.1016/j.talo.2022.100112>.
13. Licitra R, Marchese M, Naef V, Ogi A, Martinelli M, Kiferle C, et al. A Review on the Bioactivity of Cannabinoids on Zebrafish Models: Emphasis on Neurodevelopment. *Biomedicines*. 2022;10:1–28. <https://doi.org/10.3390/biomedicines10081820>.
14. Brazil. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução da Diretoria Colegiada – RDC nº 17, de 06 de maio de 2015.
15. Brazil. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução da Diretoria Colegiada - RDC nº 327, de 22 de novembro de 2019.
16. Brazil. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução da Diretoria Colegiada - RDC nº 1012, de 30 de janeiro de 2026.
17. Brazil. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução da Diretoria Colegiada - RDC nº 1014, de 30 de janeiro de 2026.
18. Rodrigues APLdaS, Lopes I da, Mourão S. Sobre ativismos e conhecimentos: a experiência de associações canábicas no Brasil. *Cien Saude Colet*. 2024;29. <https://doi.org/10.1590/1413-81232024292.18462022>.
19. Policarpo F. O debate em torno da maconha no Brasil: um breve panorama das controvérsias e disputas atuais. In: Pinto JB, editor. *Drogas e sociedade: estudos comparados Brasil e Portugal*. 1st edition. Rio de Janeiro: Letra Capital Editora; 2019. pp. 109–23.
20. Gouvêa-Silva JG, Costa-Oliveira C, Da, Ramos YJ, Mantovanelli DF, Cardoso MS, Viana-Oliveira LD, et al. Is There Enough Knowledge to Standardize a Cannabis sativa L. Medicinal Oil Preparation with a High Content of Cannabinoids? *Cannabis Cannabinoid Res*. 2023;8:476–86. <https://doi.org/10.1089/can.2022.0076>.
21. Madej K, Chmiólek A, Szlachta K, Piekoszewski W. Hplc-dad analysis of hemp oil supplements for determination of four cannabinoids: Cannabidiol, cannabidiolic acid, cannabinol and delta 9-tetrahydrocannabinol. *Separations*. 2021;8. <https://doi.org/10.3390/separations8120227>.
22. Deidda R, Dispas A, De Bleye C, Hubert P, Ziemons É. Critical review on recent trends in cannabinoid determination on cannabis herbal samples: From chromatographic to vibrational spectroscopic techniques. *Anal Chim Acta*. 2022;1209. <https://doi.org/10.1016/j.aca.2021.339184>.
23. Nahar L, Onder A, Sarker SD. A review on the recent advances in HPLC, UHPLC and UPLC analyses of naturally occurring cannabinoids (2010–2019). *Phytochem Anal*. 2020;31:413–57. <https://doi.org/10.1002/pca.2906>.
24. Citti C, Ciccarella G, Braghiroli D, Parenti C, Vandelli MA, Cannazza G. Medicinal cannabis: Principal cannabinoids concentration and their stability evaluated by a high performance liquid

- chromatography coupled to diode array and quadrupole time of flight mass spectrometry method. *J Pharm Biomed Anal.* 2016;128:201–9. <https://doi.org/10.1016/j.jpba.2016.05.033>.
25. Carvalho VM, Aguiar AFL, Baratto LC, Souza FLC, Rocha ED. Cannabinoids quantification in medicinal cannabis extracts by high performance liquid chromatography. *Quim Nova.* 2020;43:90–7. <https://doi.org/10.21577/0100-4042.20170457>.
26. Silva Sofrás FM, Alonso R, Retta DS, Di Leo Lira P, Desimone MF, van Baren CM. Development and Validation of a Simple, Fast, and Accessible HPLC-UV Method for Cannabinoids Determination in Cannabis sativa L. Extracts and Medicinal Oils. *Curr Pharm Des.* 2023;29:1918–28. <https://doi.org/10.2174/1381612829666230809094304>.
27. Vaclavik L, Benes F, Fenclova M, Hricko J, Krmela A, Svobodova V, et al. Quantitation of Cannabinoids in Cannabis Dried Plant Materials, Concentrates, and Oils Using Liquid Chromatography–Diode Array Detection Technique with Optional Mass Spectrometric Detection: Single-Laboratory Validation Study, First Action 2018.11. *J AOAC Int.* 2019;102:1822–33. <https://doi.org/10.5740/jaoacint.18-0426>.
28. Brazil. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução da Diretoria Colegiada - RDC n° 166, de 24 de julho de 2017.
29. Collins CH, Braga GL, Pierina SB. *Fundamentos de Cromatografia.* Campinas: Editora da Unicamp; 2006.
30. Ciolino LA, Ranieri TL, Taylor AM. Commercial cannabis consumer products part 2: HPLC-DAD quantitative analysis of cannabis cannabinoids. *Forensic Sci Int.* 2018;289:438–47. <https://doi.org/10.1016/j.forsciint.2018.05.033>.
31. Deidda R, Avohou HT, Baronti R, Davolio PL, Pasquini B, Del Bubba M, et al. Analytical quality by design: Development and control strategy for a LC method to evaluate the cannabinoids content in cannabis olive oil extracts. *J Pharm Biomed Anal.* 2019;166:326–35. <https://doi.org/10.1016/j.jpba.2019.01.032>.
32. Wilson WB, Abdur-Rahman M. Determination of 11 Cannabinoids in Hemp Plant and Oils by Liquid Chromatography and Photodiode Array Detection. *Chromatographia.* 2022;85:115–25. <https://doi.org/10.1007/s10337-021-04114-y>.
33. Madej K, Kózka G, Winiarski M, Piekoszewski W. A simple, fast, and green oil sample preparation method for determination of cannabidioloic acid and cannabidiol by hplc-dad. *Separations.* 2020;7:1–10. <https://doi.org/10.3390/separations7040060>.
34. Mudge EM, Murch SJ, Brown PN. Leaner and greener analysis of cannabinoids. *Anal Bioanal Chem.* 2017;409:3153–63. <https://doi.org/10.1007/s00216-017-0256-3>.
35. Jin D, Dai K, Xie Z, Chen J. Secondary Metabolites Profiled in Cannabis Inflorescences, Leaves, Stem Barks, and Roots for Medicinal Purposes. *Sci Rep.* 2020;10:1–14. <https://doi.org/10.1038/s41598-020-60172-6>.
36. Walsh KB, McKinney AE, Holmes AE. Minor Cannabinoids: Biosynthesis, Molecular Pharmacology and Potential Therapeutic Uses. *Front Pharmacol.* 2021;12:1–18.

Figures

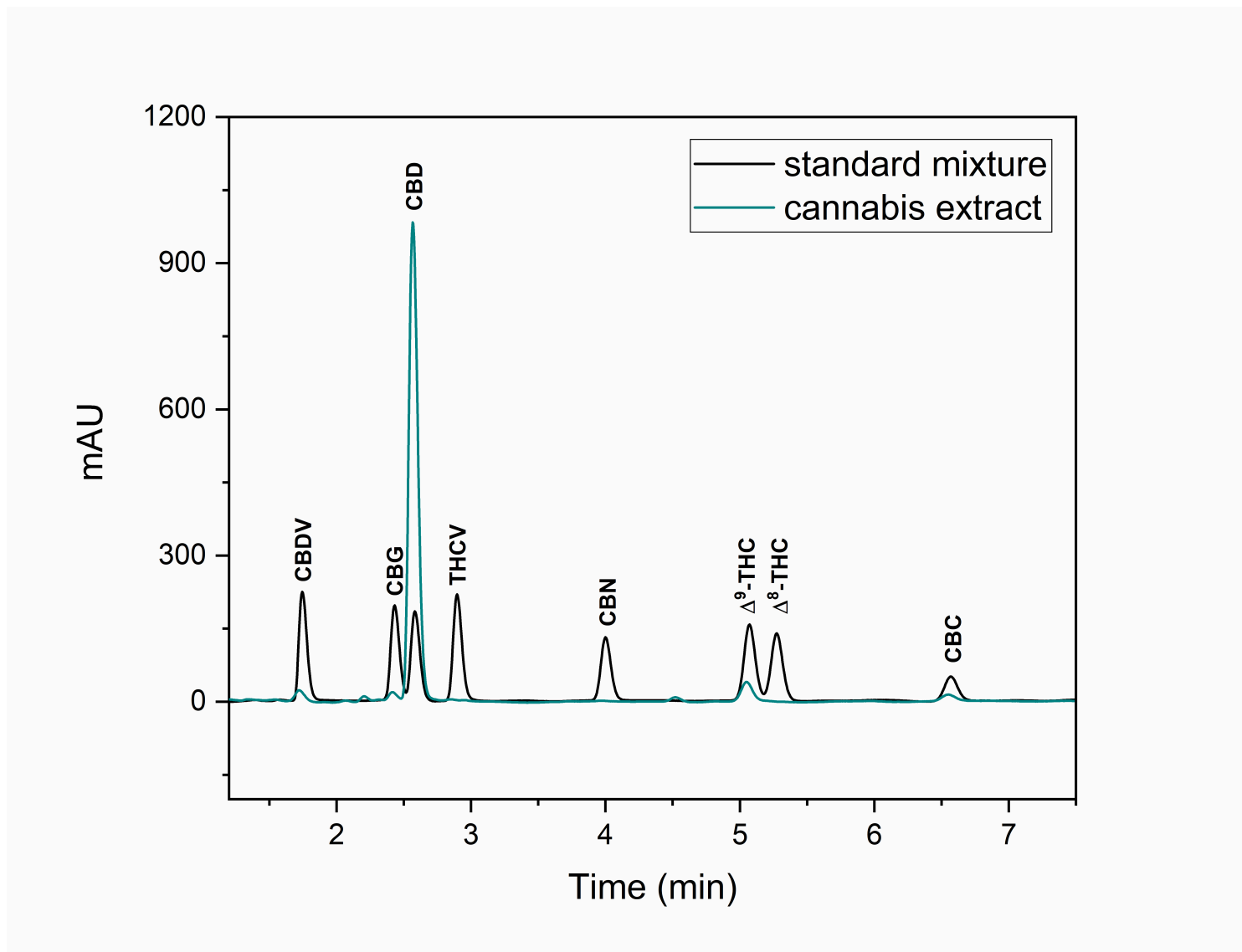


Figure 1

Overlaid chromatogram of 8 neutral cannabinoids standard mixture (black) and CBD-rich oil sample (green)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation.docx](#)