Quantification of Cannabinoids in Cannabis Oil Using GC/MS: Method Development, Validation, and Application to Commercially Available Preparations in Argentina

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ABSTRACT

The medicinal use of cannabis oil is increasing all over the world. Few analytical methods for the quantification of cannabinoids have been validated using internationally accredited guidelines. This work describes the development and validation of a selective and sensitive gas chromatography-mass spectrometry method for the qualitative analysis of the main cannabinoids, namely cannabidiolic acid, tetrahydrocannabinolic acid, cannabigerol, and cannabichromene as well as quantitative determination of cannabidiol, Δ^9-tetrahydrocannabinol, and cannabinol, present in cannabis oils. The method was fully validated according to Food and Drug Administration and International Conference on Harmonization guidelines. A linear range of 0.1–30 µg/mL was obtained for CBD and Δ^9-THC and 0.034–11.7 µg/mL for CBN, presenting determination coefficients above 0.99. The lower limits of quantification ranged from 0.034 to 0.1 µg/mL. The intra- and inter-day precision, calculated in terms of relative standard deviation, were 3.9–13.8 and 4.7–14.1 %, respectively. Extraction efficiency at lower limits of quantification was 95–103 %. Verification of method validity was performed with authentic cannabis oil samples. To our knowledge, this is the first method available in Argentina, validated according to international guidelines, for quantification of CBD, Δ^9-THC, and CBN in cannabis oil. The primary application of this method is to differentiate between cannabis oils with high or low content of Δ^9-THC, CBD, or mixed Δ^9-THC/CBD. This is of fundamental importance for the patient and so that the physicians can carry out a suitable therapy.

ABBREVIATIONS

CBC	cannabichromene
CBD	cannabidiol
CBDA	cannabidiolic acid
CBG	cannabigerol
CBN	cannabinol
FDA	Food and Drug Administration
GC-MS	gas chromatography-mass spectrometry
ISTD	internal standard
ICH	International Conference on Harmonization
LLOQ	lower limits of quantification
MSTFA	N-methyl-N-trimethylsilyltrifluoroacetamide
THCA	tetrahydrocannabinolic acid
Δ^9-THC	trans-Δ^9-tetrahydrocannabinol
WADA	World Anti-Doping Agency

Introduction

The medicinal use of *Cannabis sativa L.* (Cannabaceae) products is increasing all over the world. The most common therapeutic indications of cannabis and cannabinoids are for the treatment of pediatric resistant epilepsy, chronic noncancer pain, multiple sclerosis, dyskinesias of Huntington's and Parkinson's diseases, and tics of Tourette syndrome [1].

Cannabis oil extracts are prepared from dried *Cannabis sativa L.* inflorescences and incorporated in common edible oils (e. g., olive or sunflower) or even obtained using these oils as extraction media.

Cannabinoids are terpene phenolic compounds typical of the cannabis plant. Δ^9-THC is the most psychoactive constituent in cannabis. It has many diverse pharmacological effects with therapeutic value in the treatment of different medical conditions [2–5].

Other non-psychotropic cannabinoids, mainly CBD and CBN, are increasingly researched, showing partially distinctive effects [6–11]. Thus, quantification of these cannabinoids is also important to understanding the pharmacological properties of cannabis oil. Δ^9-THC and CBD are present in the plant as THCA and CBDA, respectively [12, 13]. Decarboxylation is temperature-dependent [14, 15], and preheating of cannabis samples has been recommended to potentiate the final cannabis oil extract [16, 17].

Medical cannabis oils generally possess high levels of the therapeutic CBD and lower levels (generally less than 0.3 %) of the psychotropic Δ^9-THC. The FDA has issued warning letters to firms that market unapproved new drugs that allegedly contain CBD. As part of these actions, the FDA has determined the cannabinoid content of some cannabis oil products (not approved by the FDA), and many were found to contain levels of CBD that are very different from the label claim [18].

In Argentina, cannabis and its derivatives are schedule IV-controlled substances (prohibited use) [19], but new regulations have allowed production for medical purposes through licensed producers [20]. Production of commercial cannabis oil in Argentina must take place in a facility using good manufacturing practices, and products must be tested for the presence and content of Δ^9-THC and CBD, using validated analytical methods.

Several methods, based on GC [21–24] or LC [24–26] have been published for the determination of Δ^9-THC, CBD, and other cannabinoids in cannabis oil. GC, one of the most used techniques for the quantitative analysis of cannabinoids in plant materials, has been in use for a long time. The high temperature of the injection port transforms the acid cannabinoids into the neutral cannabinoids. Since the cannabis oils contain the acidic and neutral forms, a derivatization step is required to prevent the decarboxylation [27, 28], and trimethylsilyl derivatives have been shown to be suitable for analysis [28, 29]. Thereby, the value of total cannabinoids can be measured by determining the acid and neutral form separately. In contrast to GC, LC-based techniques allow the direct analysis of cannabinoid (neutral and acid) in the extracted sample.

The main goal of the present work is the development, optimization, and validation of a method with a simple derivatization coupled to GC-MS for the determination of Δ^9-THC, CBD, and CBN in cannabis oil.

The GC-MS validated method, according to FDA [30] and ICH [31] guidelines, has proven to be very accurate, highly reproducible, and sensitive to determine the target cannabinoids, with only 10 µL of sample tested. In addition, the method was successfully applied to the quantitative analysis of 10 different cannabis oils. The application of this method to differentiate between cannabis oils with high or low content of Δ^9-THC, CBD, or mixed Δ^9-THC/CBD will provide physicians with essential information so that they can carry out a suitable therapy with patient's pathology.

Results and Discussion

An accurate and robust analytical method has been developed for the quantification of 3 cannabinoids relevant to the health and safety of cannabis oil users. This process was optimized for dilution solvent and sonication time. Dilution solvents had been selected according to the existing literature [23, 25, 32, 33] and to the physical-chemical properties of the studied analytes. The solvents evaluated were methanol, ethanol, diethyl ether, and petroleum ether. To evaluate the effect of different sonication times, the samples were sonicated at various times (5, 10, 20, and 30 min). These experiments were performed in triplicate using cannabis oil, and the relative peak areas obtained for each cannabinoid were compared to establish the best dilution solvent and sonication time. An initial prescreening (methanol and 5 min sonication time) of cannabinoids was made in full scan mode. Mass spectrometric identification criteria were according to the WADA [34, 35]. The cannabinoids identified were Δ^9-THC, CBD, CBN, CBC, CBG, THCA, and CBDA.

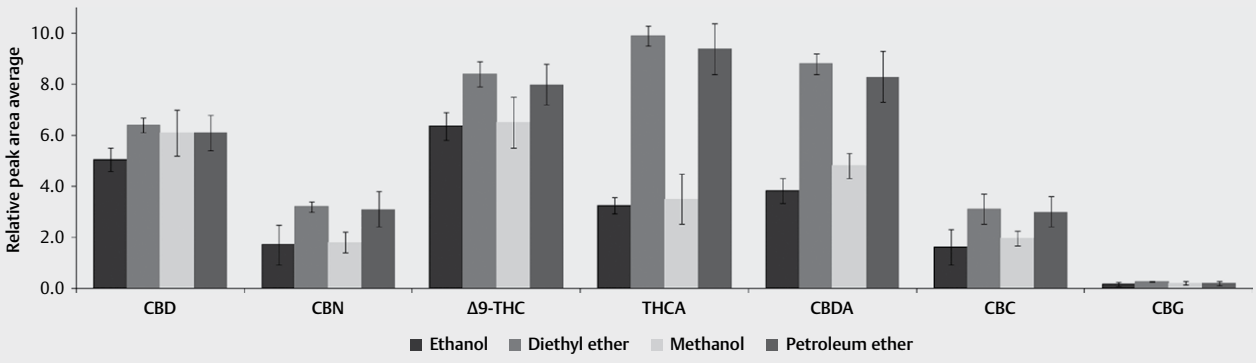
The statistical analysis of these data indicated that dilution solvent was the most significant factor (▶ **Fig. 1**). Regarding diethyl ether, the recoveries obtained for Δ^9-THC, CBN, CBC, THCA, and CBDA were significantly greater when compared to using methanol: [$F(1.4) = 10.85$, $p < 0.05$], [$F(1.4) = 36.74$, $p < 0.05$], [$F(1.4) = 8.37$, $p < 0.05$] [$F(1.4) = 102.94$, $p < 0.05$], and [$F(1.4) = 107.49$, $p < 0.05$], respectively. Subsequently, the recoveries obtained with diethyl ether for Δ^9-THC, CBN, CBC, THCA, and CBDA were significantly greater when compared to using ethanol: [$F(1.4) = 10.53$, $p < 0.05$], [$F(1.4) = 10.79$, $p < 0.05$], [$F(1.4) = 16.94$, $p < 0.05$] [$F(1.4) = 157.39$, $p < 0.05$], and [$F(1.4) = 184.72$, $p < 0.05$], respectively. These results were obtained using Fisher's test, which evaluates the intra- and inter-group study variance.

Diethyl ether and petroleum ether extractions resulted in similar recoveries, with no significant differences between them. Lower standard deviations and associated errors are observed with diethyl ether, which makes diethyl ether the most promising option (▶ **Fig. 1**).

Another relevant parameter, also studied, was the sonication time (5–30 min) that might result in a greater recovery of the target analytes, as well as influence signal intensity. All studied times of extraction resulted in similar recoveries, with no significant differences between them. The sonication appears not to depend on the time of exposure to the solvent mixture. In order to make the process faster, 5 min was chosen.

The data were used to develop an optimized sample preparation protocol using 10 µL of cannabis oil with 10 mL of diethyl ether, with sonication for 5 min and vortexing for 30 sec (3 cycle sonication/vortexing).

Full method validation was conducted according to the FDA and ICH guidelines using olive and cannabis oil as matrix. The selectiv-



▶ **Fig. 1** Relative peak areas and SDs obtained for different dilution solvents for cannabidiol (CBD), cannabinol (CBN), Δ^9-tetrahydrocannabinol (Δ^9-THC), tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), cannabichromene (CBC) and cannabigerol (CBG).

ity of the method was evaluated by analyzing of blank samples. No interfering substances were detected at the selected retention times and m/z windows for all cannabinoids. Using the above-mentioned criteria for positivity, all the analytes (Δ^9-THC, CBD, and CBN) were successfully and unequivocally identified in all the spiked samples at the LLOQ. Therefore, the method was considered selective for Δ^9-THC, CBD, and CBN determination.

The calibration model was evaluated from a set of 9 calibration points. The homoscedasticity assumption was tested in a linear regression analysis. The residual plot clearly showed that error was not randomly distributed around the concentration axis. The F-test also revealed a significant difference between the variances. There was evidence that variances were significantly different, thus homoscedasticity was not met.

Weighted least squares regressions had to be adopted in order to compensate for heteroscedasticity. Six weighting factors were evaluated for each compound ($1/x^{0.5}$, $1/x$, $1/x^2$, $1/y^{0.5}$, $1/y$, $1/y^2$). The weighting factor that resulted in the lower sum of relative errors and, simultaneously, a mean R^2 value of at least 0.99 was chosen. This factor was $1/x^2$ for all analytes. The method obtained linear relationships by means of these weighted least squares regressions. Calibrators' accuracy [mean relative error (bias) between the measured and spiked concentrations] was within a ± 15 % interval for all concentrations. Calibration data are shown in ▶ **Table 1**.

The LLOQs of the compounds were 0.1 µg/mL for Δ^9-THC, CBD; and 0.04 µg/mL for CBN (▶ **Table 1**), and the upper limit of quantification ranged from 11.7 (CBD) to 30.0 (Δ^9-THC, CBD) µg/mL with a minimum of 9 calibration points.

These limits were considered satisfactory, especially when compared to those obtained by other authors. Citti et al. [36] used 100 µL (10 times higher) of cannabis oil, and the reported LLOQs for Δ^9-THC, CBD, and CBN were greater than that presented herein. Bettiol et al. [37] and Deida et al. [38] applied 40 µL (4 times higher) of cannabis oil and reported LLOQs of 1.0 µg/mL for Δ^9-THC, CBD, and CBN. The mentioned papers report higher LLOQs than ours when analyses were carried out using a LC-DAD or LC-MS system. A GC-MS analytical method reported by Ciolino et al. [23] presented a LLOQs of 0.3 µg/mL for Δ^9-THC, CBD, and CBN; however, this work, unlike ours, started from gravimetrically measured samples.

Both intra-day and inter-day precisions for the entire extraction and analysis process were determined by extracting 2 cannabis oils over the course of 5 days, and 5 times on a single day. These oils were chosen to obtain a broad representation of analyte profiles. Cannabis oil A contains a high concentration of CBD and a low concentration of Δ^9-THC and CBN, while cannabis oil B contains a low concentration of CBD and a high concentration of Δ^9-THC and CBN.

Intra-day precision was evaluated by analyzing, on the same day, 5 replicates of the cannabis oils. The obtained % RSD were lower than 13.8 % at all studied concentration levels. (▶ **Table 2**). The evaluation of inter-day precision was made within a 5-day period. The obtained % RSD were lower than 14.1 % for all cannabinoids at the tested concentrations. (▶ **Table 2**).

The recovery was evaluated at LLOQ for Δ^9-THC, CBD, and CBN. The recoveries for the compounds were between 95 % and 103 % (▶ **Table 1**). According to our results, the method can be considered a powerful technique, revealing a fast and efficient extraction of the target analytes with a lower sample volume.

After validation of this analytical method, in order to demonstrate the applicability, it was successfully applied to routine analysis of 10 cannabis oils.

In controlled/regulated production cannabis oils (cannabis oil #1 and #2), CBD was detected at levels consistent with the product labeling, and Δ^9-THC levels were very low, as expected for products derived from hemp oil (▶ **Table 3**). In addition, cannabis oil #1 and #2 showed high ratios of CBD to Δ^9-THC and CBN (▶ **Table 3**).

Uncontrolled/unregulated production cannabis oils had CBD, Δ^9-THC, and CBN concentrations that differed notably. Our analysis revealed that 2 preparations (samples oils #3 and #7) exhibited high levels of Δ^9-THC and low or undetectable concentration of CBD, while in another 3 (samples oil #6, #8, and #9), the CBD content was not detectable and Δ^9-THC ranged from 1.3 to 4.3 mg/mL (▶ **Table 3**).

CBD appears not to have adverse consequences at high doses; however, Δ^9-THC concentrations observed in cannabis oils (especially oil # 3, # 4, # 7, # 8) could be enough to produce intoxication, especially among children [39].

▲ **Table 1** Linearity and lower limit of quantification (LLOQ) data

Compound	Linearity data (n = 6)					LLOQ (n = 10)				
	IS	Weight	Lineal range (µg/mL)	Slope (mean ± SD)	y-Intercept (mean ± SD)	R2 (range)	Nominal conc. (µg/mL)	Mean calculated conc. ± SD (µg/mL)	CV[a] (%)	RE[b] (%)	Recovery ± S.D. (%)
CBD	Δ^9-THC-d_3	$1/X^2$	0.1 – 30	0.236 ± 0.031	0.077 ± 0.015	0.9936–0.9970	0.1	0.109 ± 0.011	10.4	8.6	103 ± 2
Δ^9-THC	Δ^9-THC-d_3	$1/X^2$	0.1 – 30	0.129 ± 0.029	0.030 ± 0.011	0.9924–0.9990	0.1	0.101 ± 0.020	13.2	1.1	100 ± 1
CBN	Δ^9-THC-d_3	$1/X^2$	0.04 – 11.7	0.770 ± 0.027	-0.018 ± 0.001	0.9911–0.9953	0.04	0.041 ± 0.008	12.8	2.6	95 ± 4

Mean values and standard deviations. [a] Coefficient of variation (%). [b] Relative error (%) = [(spiked concentration − nominal concentration/nominal concentration) × 100]. CBD cannabidiol, Δ^9-THC Δ^9-tetrahydrocannabinol, CBN cannabinol and Δ^9-THC-d_3 Δ^9-tetrahydrocannabinol-d_3.

Interestingly, the web site from the CBD oil #4 producer reports a CBD/Δ^9-THC ratio of 1:1, while we found a CBD/Δ^9-THC ratio of 0.25:1.

Finally, in the cannabis oil #10, CBD, Δ^9-THC, and CBN were not detected, which would indicate that it was falsely sold as cannabis oil.

CBN was quantifiable in most samples (except oil #5, #8, and # 10). CBN is formed by Δ^9-THC oxidation during plant aging or inappropriate storage conditions [40]. Therefore, its determination may assist in the evaluation of the quality of cannabis oils.

Taken together, the results highlighted the extreme variability of the uncontrolled/unregulated production of cannabis oils, and these results are in agreement with those obtained from products available on the United States and Italy markets [41, 42]. Bonn-Miller et al. [41] reported that 26 % of tested products contained less CBD than declared on the label, while Pavlovich et al. [42] reported 9 out of 14 tested samples had concentrations that differed notably from the declared amount.

In conclusion, a GC-MS method was developed, optimized, validated, and applied for the simultaneous detection and quantification of CBD, Δ^9-THC, and CBN in cannabis oil. The analyses were carried out using small sample volumes (10 µL of cannabis oil), and the method was successfully applied to real samples derived from Argentina's market.

The issues of variability of cannabinoid content in preparations and inaccurate label claims in the global market justify the need to have a method like the one that has been developed and validated to provide concentration data for each preparation.

To our knowledge, this is the first method available in Argentina validated according to international guidelines for quantification of CBD, Δ^9-THC, and CBN in cannabis oil.

In addition, CBD and Δ^9-THC concentration data in medicinal cannabis oil are crucial for physicians to be able to properly adapt the prescribed dose to the available preparation.

Further studies are needed to evaluate the impact of different cannabis oils on cannabinoids pharmacokinetics and clinical outcomes.

Materials and Methods

Reagents and standards

Analytical standards: Δ^9-THC (purity 99.4 %), CBD (purity 99.8 %), CBN (purity 99.5 %), and ISTD: Δ^9-tetrahydrocannabinol-d_3 (Δ^9-THC -d_3, purity 98.8 %) were purchased from Cerilliant (Round Rock, TX, USA) as 1.0 and 0.1 mg/mL in methanol solutions.

Methanol, ethanol, diethyl ether, petroleum ether, and ethyl acetate were provided from Merck Chemistry (Buenos Aires, Argentina); all chemicals were analytical ACS or chromatography grade. MSTFA was acquired from Thermo Fisher Scientific.

Calibrators and internal standard

A working solution (A) was prepared by proper dilution of stock solutions (1 mg/mL) with methanol to the final concentrations of 10.0 µg/mL for Δ^9-THC and CBD. Additionally, working solutions of 400 and 4.0 µg/mL for CBN were prepared. As ISTD (Δ^9-THC -d_3) stock

▶ **Table 2** Intra-day and inter-day precision data.

Cannabis Oil	Compound	Intra-day (n = 5)	Inter-day (n = 15)		
		Measured ± SD (µg/mL)	RSD[a] (%)	Measured ± SD (µg/mL)	RSD[a] (%)
A	CBD	23.60 ± 0.81	4.5	22.45 ± 0.71	4.7
	Δ^9-THC	0.42 ± 0.09	3.9	0.52 ± 0.10	10.7
	CBN	0.18 ± 0.04	13.8	0.17 ± 0.04	12.5
B	CBD	0.11 ± 0.04	11.3	0.10 ± 0.06	11.7
	Δ^9-THC	28.20 ± 2.51	10.3	25.81 ± 3.74	10.9
	CBN	4.81 ± 0.43	12.9	4.27 ± 0.61	14.1

Mean values and standard deviations. [a] Relative standard deviation (%). CBD cannabidiol, Δ^9-THC Δ^9-tetrahydrocannabinol and CBN cannabinol.

▶ **Table 3** Cannabinoid content of tested authentic samples.

Cannabis Oil	Declared CBD * (mg/mL)	CBD (mg/mL)	Δ^9-THC (mg/mL)	CBN (mg/mL)	CBD / Δ^9-THC ratio
# 1	20	22.0	1.1	1.1	20
# 2	20	22.0	1.0	1.0	22
# 3	Not declared	0.4	29.0	3.4	0.01
# 4	Not declared	1.5	6.1	0.3	0.25
# 5	Not declared	0.3	2.0	ND	0.15
# 6	Not declared	ND	1.3	0.6	–
# 7	Not declared	ND	10.3	2.6	–
# 8	Not declared	ND	4.3	ND	–
# 9	Not declared	ND	2.4	0.2	–
# 10	Not declared	ND	ND	ND	–

* CBD declared on labels. ND: Not detected. CBD cannabidiol, Δ^9-THC Δ^9-tetrahydrocannabinol and CBN cannabinol.

solutions of 0.1 mg/mL was used. All primary and working solutions were stored at − 20 °C into amber glass vials.

Working calibrators (0.1, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, 20.0, and 30.0 µg/mL Δ^9-THC and CBD; and 0.04, 0.80, 1.50, 2.40, 3.20, 3.90, 5.90, 7.80, and 11.70 µg/mL CBN) were made daily by adding of each standard to 1.0 mL (final volume) of ethanol. For the 0.1 µg / mL Δ^9-THC and CBD and 0.04 µg / mL CBN points, working solution (A) and 4.0 µg / mL CBN were used, respectively. For the rest of the points, stock solutions and a working solution of 400 µg / mL CBN, were used.

Tested material

The cannabis oil extracts used in this study were obtained from subjects who attended the Analytical Toxicology Advisory Laboratory (CENATOXA) with a request for quantification of Δ^9-THC and CBD.

Eight samples were obtained from uncontrolled/unregulated production cannabis oil (n = 8) and 2 samples from controlled/regulated production cannabis oil (n = 2).

Gas chromatography and mass spectrometry analysis

The samples were analyzed using an HP 6890 N gas chromatograph, combined with an HP 5973 quadrupole mass spectrometer and an HP 6890 Series injector (all from Hewlett-Packard). Data were acquired and analyzed using Agilent Enhanced ChemStation G1701DA software (Agilent Technologies). The separation of the analytes was achieved using a capillary column (30 m × 0.25 mm I.D., 0.25 µm film thickness) with 5 % phenylmethylsiloxane (HP-5 MS), supplied by J & W Scientific. Carrier gas (helium) was set at a constant flow rate of 1.0 mL/min. The volume of injection was 2 µL on split mode (split ratio of 1:10); the injection port and transfer line temperatures were set at 280 °C and 280 °C, respectively. The oven temperature started at 60 °C, followed by a temperature ramp of 10 °C/min to 300 °C held for 2 min. Total separation run time was 26.0 min. The ion source was maintained at 220 °C and the quadrupole at 150 °C. The mass spectrometer was operated with a filament current of 300 mA and an electron energy of 70 eV in the positive electron ionization mode. Selected ion monitoring mode was used with a dwell time of 80 ms. Three ions for each analyte and 1 ion for ISTD were chosen based on selectivity and abundance in order to maximize the signal-to-noise ratio in matrix extracts (▶ **Table 4**). Agilent Enhanced ChemStation G1701DA software (Agilent Technologies) was used for data acquisition, data processing, and instrument control. The mass spectra were obtained by collecting the data at rate of 1.38 scan/s over the m/z range of 50–600. Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds analyzed under the same conditions when available and through the National Institute of Standards and Technology (NIST 1998); Pfleger/Maurer/Weber: Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites (2011), and Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG 2019), MS spectral database.

Sample preparation

Cannabis oil was mixed by inversion prior to sample preparation. Then 10 µL of oil was diluted thousand-fold (1:1000 dilution) in 10 mL of diethyl ether and vortexed for 30 sec. Extracts were sonicated for 5 min and vortexed for 30 sec. The sonication and agitation cycles were performed twice more.

One hundred microliters (100 µL) of extract with 4 µL of ISTD (0.1 mg/mL) were evaporated to dryness under a gentle stream of nitrogen at 45 °C. For the derivatization procedure, 50 µL of MSTFA were added to the dried residue and vortexed for 10 sec. The tubes were heated on a thermo block at 60 °C for 20 min. A 2 µL aliquot of the resulting solution was injected into the GC–MS system.

▶ **Table 4** Optimized identification parameters for compounds

Compound	Retention Time (min)	Time window (min)	Quantification ion (m/z)	Qualifier ion 1 (m/z)	Qualifier ion 2 (m/z)	Dwell time (ms)	Peak width (m/z units)
CBD	20.944	20.50 – 21.30	390	337	458	80	0.5
Δ^9-THC	21.807	21.30 – 22.10	371	315	386	80	0.5
Δ^9-THC-d_3	21.787	21.30 – 22.10	374	–	–	80	0.5
CBN	22.564	22.10 – 22.90	367	238	310	80	0.5
CBD cannabidiol, Δ^9-THC Δ^9-tetrahydrocannabinol, Δ^9-THC-d_3 Δ^9-tetrahydrocannabinol-d_3 and CBN cannabinol.							

Validation procedure

The analytical method validation was performed in accordance with the guidelines of the FDA [30] and ICH [31]. The validation was performed following a 5-day validation protocol and included selectivity/specificity, linearity, limits, intra- and inter-day precision, and recovery.

Selectivity

Since it is not possible to obtain cannabis oil that is devoid of cannabinoids, blank samples were prepared using olive oil (n = 10). Samples were extracted and analyzed according to the previously described procedure.

Peaks at the retention time of interest were compared with those from olive oil samples spiked with analytes at the LLOQ.

The acceptance criteria for compounds identification were according to the WADA [34]. The method would be considered selective if no analyte could be identified in the blank samples by applying those criteria.

Calibration curves and limits

The linearity of the method was established on aliquots of ethanol (100 µL) spiked with the corresponding working solution to obtain calibrator samples. Replicates (n = 9) at each concentration were analyzed as described Fernandez et al. [35].

The lowest point of the calibration curve was the LLOQ of the method. The LLOQ was determined by analyzing 10 replicates of spiked blank olive oil samples (independent from those of the calibration curve). It was tested whether the signal-to-noise ratios (S/N) of all analytes was greater than 10. Furthermore, precision and accuracy data with a coefficient of variation (CV %) less than 20 % and a relative error (RE %) within ± 20 % of the nominal concentration were obtained.

Intra- and inter-day precision

In order to evaluate intra- and inter-day precision, different authentic samples containing different cannabinoid profiles were evaluated on 5 separate days as well as 5 times on the same day.

Precision, expressed as % RSD, was determined by calculating the percent ratio of the standard deviation divided by the calculated mean concentration times 100. Data were evaluated using a 1-way analysis of variance (ANOVA) with day as the grouping variable. RSD values below 15 % and at LLOQ below 20 % were acceptable for quantitative analysis.

Recovery

For the analysis of recovery, 2 sets of samples (n = 5) were prepared at LLOQ: sample set 1 representing the neat standard/ISTD and sample set 2 consisting of blank olive oil spiked before dilution. The ISTD were added to sample set 2 after dilution. The recovery results were obtained by comparison of peak areas ratio of sample set 1 to those of the corresponding peaks in sample set 2.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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