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Cannabinoids in *Cannabis sativa l.* and oil: Method validation and analysis by LC-MS/MS

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ABSTRACT

In recent years, *Cannabis sativa L.* has experienced cultural and legal acceptance in many countries, both for recreational and medicinal use, so plant material and cannabis products are frequently seized in a country by local and federal authorities to control traffic. Consequently, appropriate methods for quantifying the cannabinoids are essential to ensure regulatory compliance of these products. The main cannabinoids of regulatory and safety interest include Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN). In this study, an LC–MS/MS method was validated for the simultaneous analysis of THC, CBD, CBN and its acids CBDA, THCA, and CBNA in plant material and oil-based cannabis products, with LOQs ranging from 0.015 % to 0.18 %. The validated method was successfully applied to analyze 108 plant material (91 compressed, 11 inflorescences and six leaf) and four hashish samples provided by the Brazilian Federal Police, four cannabis roots provided by the Civil Police of the Federal District and 23 cannabis oils supplied by individuals or civil organizations. THC, THCA and CBN were found in all plant material samples, at the highest concentrations in compressed material (13.2, 8.69 and 2.77 %, respectively). None of the analyzed compounds were detected in the roots. All oil samples contained THC and CBD, with levels reaching 60.9 and 29.8 %, respectively; only four samples had THC levels within the Brazilian legal limit for medical use as phytopharmaceuticals or herbal medicine (up to 0.2 %). All hashish samples had THC (up to 58.5 %), THCA (up to 0.32 %) and CBN (up to 9.1 %). This study underscores the importance of quantifying these compounds amidst the diverse and rapidly changing regulatory landscape worldwide.

1. Introduction

Cannabis was one of the first domesticated plants in history, with its use dating back over 10,000 years. A member of the Cannabaceae family, along with the genus Humulus (hops), it is an annual herbaceous plant, mostly dioecious, wind-pollinated, with erect stems that can reach up to 5 m in height, depending on environmental conditions and genetic variability [1].

Cannabis contains more than 400 compounds, with over 120 cannabinoids already isolated, characterized by their typical C21 or C22 terpenophenolic structure [2,3]. Δ9–tetrahydrocannabinol (THC), which is the main psychoactive component of cannabis, cannabidiol (CBD) and cannabinol (CBN) are the major cannabinoids in the plant [4]. Fresh cannabis contains about 95 % of THC, CBD, and CBC present in their respective acidic forms (THCA, CBDA and CBCA), which are formed through enzymatic catalysis of cannabigerolic acid (Fig. 1).

The genus Cannabis was first classified as *C. sativa* by Linnaeus in 1753 as a single species, while Cannabis indica was identified by J.B. Lamarck in 1785, and Cannabis ruderalis by D.E. Janischewsky in 1924 [5], classifications derived from physical, morphological, chemical, and geographical data. However, Cannabis taxonomic classification is controversial, and the debate is whether the genus consists of a single species (C. sativa), multiple species (C. sativa, C. indica and C. ruderalis) or subspecies of C. sativa (sativa, indica and ruderalis) [6]. Fetterman et al. [7] proposed a classification of the Cannabis genus based on combined physical and chemical characteristics, allowing differentiation between drug-type (high THC content) and fiber-type (low THC content), while MacPartland and Small [8] proposed a classification of four subspecies of *C. sativa* based on the ratio of THC/CBD. However, more recently, genomic data have shown that the plant is most likely monotypic (*C. sativa*) with a wide range of phenotypes [6].

Cannabis is the most widely used psychoactive recreational drug in

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the world, with an estimated 244 million users in 2023, grown by one third over the past decade [9]. The plant can be found in various forms on the illicit market, including dried flowers, compressed blocks, or shredded material, which may contain other parts of the plant, and its composition varies widely from region to region [4]. The Brazilian Federal Police (BFP) is the national institution in charge of stifling drug trafficking at federal and international levels. Its role is crucial in dismantling large trafficking networks, fighting the transportation, production, financing, and money laundering associated with this criminal activity. BFP periodically carries out marijuana eradication operations both within Brazil and in cooperation with neighboring countries, such as Paraguay. The amount of marijuana eradicated in 2024 increased by 76 % compared to 2023. Similarly, drug seizures also increased by 15 %, totaling 479.1 tons [10].

The variety of cannabis cultivated for industrial use is referred to as hemp, which can be transformed into a range of commercial products, such as textiles, food, paper, animal feed, biofuel, and rope. These products typically have a THC content of up to 0.3 %, which is the threshold that differentiates therapeutic use and cannabis grown for recreational use [1,11]. The medicinal use of cannabis has gained global recognition over the years, primarily based on cannabidiol activity, including the treatment of epilepsy, Parkinson's, anxiety, autism, fibromyalgia, cancer, multiple sclerosis, and chemotherapy-induced nausea and vomiting [12,13]. Since 2019, medicinal cannabis is marketed in Brazil, with products containing up to 0.2 % THC, except for those indicated for palliative care in patients with no other therapeutic alternatives, which may contain higher THC levels [14]. As of May 2025, 39 products were registered in the country, 13 of which are herbal medicines (cannabis extract, up to 20 % CBD), 25 phytopharmaceuticals (CBD purified from the plant, up to 20 % CBD) and one medicament (2.5 and 2.7 % CBD and THC, respectively) [15]. The cultivation of industrial hemp by companies was approved in Brazil in 2024, exclusively for the manufacture of medicinal products [16]. Furthermore, it was defined that anyone who acquires, stores, deposits or transports up to 40 g of cannabis or six female plants will be presumed to be a user with no legal consequences [17].

Despite the availability of various cannabis- or CBD-based medical products on the market, the cost remains prohibitively high for most of the Brazilian population who need them. As a result, many patients and their families seek legal injunctions for home cultivation authorization or acquire products through civil associations.

When considering quantification methods, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is both sensitive and selective, making it ideal for monitoring structurally similar compounds, as is the case with phytocannabinoids. In this context, this study aims to validate a LC-MS/MS method to quantify THC, CBD, CBN and their acid forms and analyze plant and hashish seized material and oil provided by individuals or civil associations. The novelty and significance of the present study are related to its unique combination of scope, matrix complexity, and direct relevance to the evolving regulatory and forensic landscape in Brazil.

2. Materials and methods

2.1. Reference materials and reagents

Formic acid (85 %) was obtained from Labsynth Produtos para Laboratórios Ltda (São Paulo, Brazil), methanol (MeOH) HPLC grade from Tedia (Ohio, EUA) and acetonitrile (ACN) LC-MS grade from J.T. Baker (Pennsylvania, EUA).

Δ⁹-THC, CBD, CBN and CBNA (at 1 mg/mL), CBDA, THCA (0.500 mg/mL), and the deuterated compounds used as internal

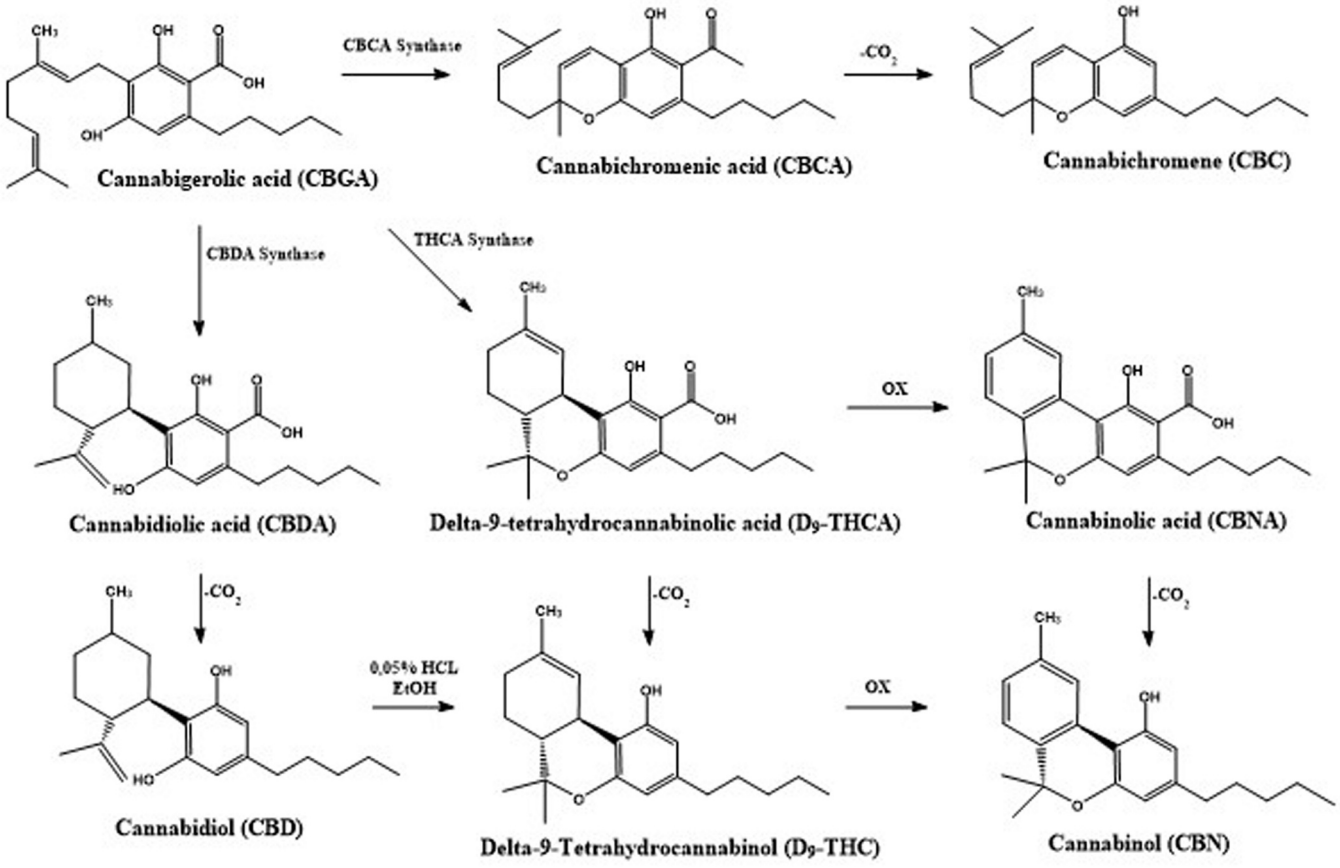

Fig. 1. Cannabinoid structures and biosynthesis, based on UNDOC [4].

standards (IS) (Δ^9-THC-d_3, CBD-d_3, CBN-d_3 and THCA-d_3 (0.1 mg/mL^{-1}) were obtained from Cerilliant (Texas, EUA). Mix standard working solutions were prepared in ACN containing the analytes at 5000, 500 and 50 ng/mL, which were used to prepare the analytical curve. A mixed working solution containing the IS was prepared in ACN at 60 ng/mL.

2.2. Instrumentation

The analysis was performed using an Ultra High Performance Liquid Chromatography (UHPLC) 1290 Infinity system (Agilent, MA, USA) coupled with a tandem mass spectrometer (MS/MS) equipped with a Turbo V ionization source. Chromatographic separation was performed in reverse phase using a UPLC ZORBAX Eclipse Plus C18 column (Agilent), with dimensions of (50 mm × 2.1 mm, 1.8 μm). The mobile phase consisted of ACN:water (85:15), acidified with 0.1 % formic acid, at a flow rate of 0.2 mL/min. The column oven temperature was set to 30 °C, and the injection volume of the autosampler to 10 μL. Total run time is 11 min.

Electrospray ionization parameters in MS/MS (ESI+) were determined for each analyte and optimized by flow infusion at 200 ng/mL: Declustering Potential (DP), Entrance Potential (EP), Collision Cell Entrance Potential (CEP), Collision Energy (CE), and Collision Cell Exit Potential (CXP) (Table S1). In multiple reaction monitoring (MRM), the precursor ion for each analyte was its protonated molecule ([M+H]+), and two transition reactions were monitored for each compound, with a detection window of 50 s and a target scan time of 0.2 s. Data acquisition was performed using Analyst 1.7 software, and data processing was carried out using Multiquant 3.0 software. The ESI source parameters were optimized for the analyte with the lowest intensity, which, according to the analyses performed, was identified as CBNA and its product ions. The optimized parameters of the ionization source were curtain gas at 50 psi, collision gas at 2 psi, ion spray voltage of 5000 V, temperature of 600 °C, gas 1 and gas 2 at 80 and 85 psi, respectively.

2.3. Samples

A total of 108 samples of cannabis plant material (91 compressed, 11 inflorescences and six leaf samples), and four hashish samples seized by the Brazilian Federal Police were provided to the study. Compressed materials were seized in 2021 and 2022 in the Federal District (12, all compressed materials), in the Brazilian states of Maranhão (5 leaves and one inflorescences), Mato Grosso (7 inflorescences and four compressed materials), and Mato Grosso do Sul (75, all compressed materials), and in Paraguay (4, all inflorescences); hashish of unknown origin was seized in 2018, 2021 and 2022. Additionally, six samples of plant root were provided by the Civil Police of the Federal District and 23 oil samples were provided by civil associations or individuals with legal authorization for oil production (Figure S1). All samples were kept at room temperature until analyzed, which occurred in the first semester of 2023.

2.4. Sample preparation

All plant material samples, except the roots, were ground using a mortar and pestle. The root samples were washed with running water, dried in an oven, and grated using a household device. Fifty milligrams of dried and ground plants were weighed in duplicate, 2.5 mL of an ACN: MeOH solution (80:20) was added to each sub-sample and homogenized/extracted for 2 min using a vortex mixer (Satra®). The mixture was centrifuged for 10 min; the supernatant was passed through a 0.45 μm PTFE filter and then diluted in the mobile phase (1:10^3–1:10^5) to fit within the analytical curve range. For oil and hashish samples, two sub-samples of 12 mg were prepared, like the plant material, except that the filtrate was diluted in proportions of 1:10, 1:10^3, or 1:10^5. The impact of using internal standards in the method was evaluated by adding 100 μL of an IS solution (60 ng/mL) to 500 μL of the final diluted solution. Fig. 2 shows an extracted ion chromatogram of all the analytes included in the study.

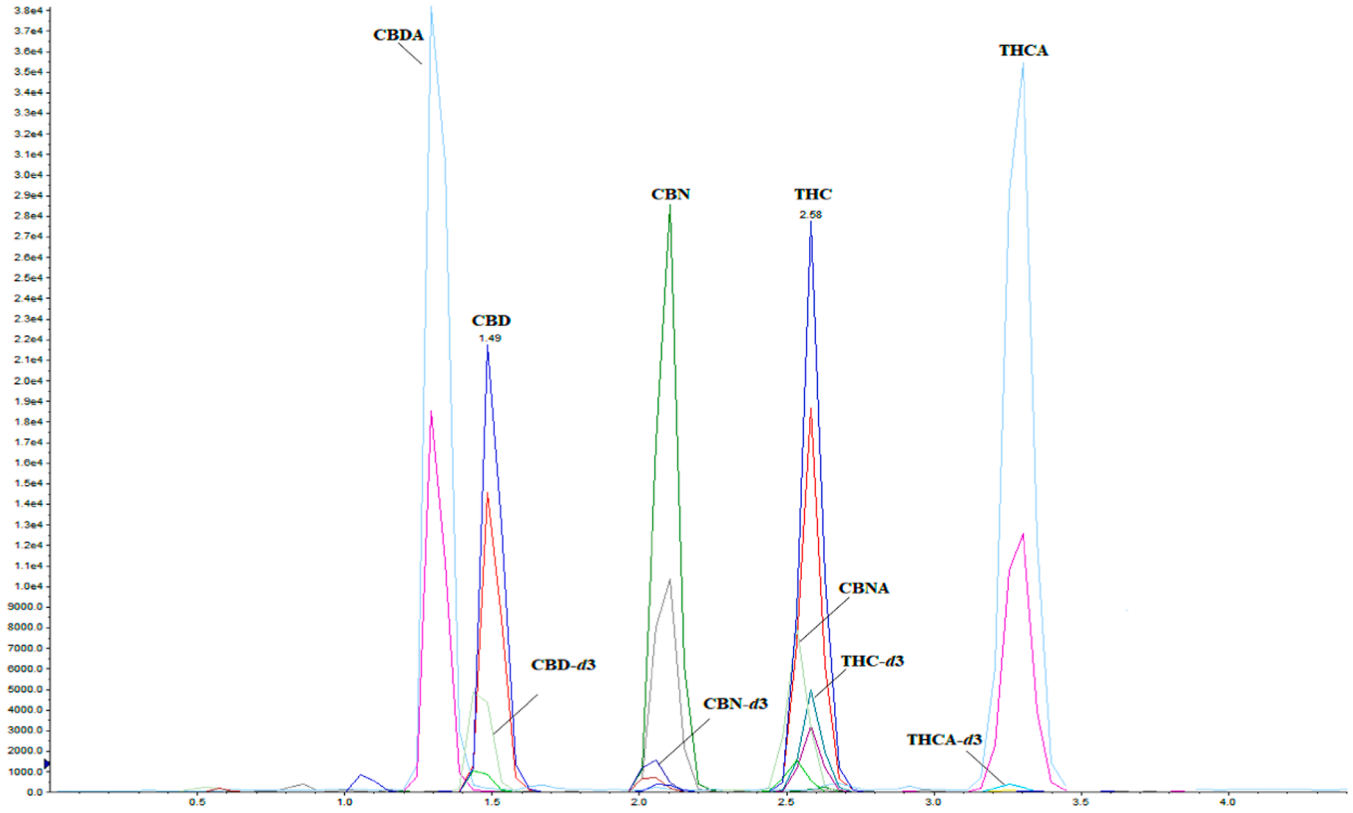

Fig. 2. Extracted ion chromatogram from LC-MS/MS API 3200 (Sciex) of neutral and acid cannabinoids at 200 ng/mL and the deuterated forms at 10 ng/mL.

2.5. Method validation

The method was validated for selectivity, linearity/working range, recovery, repeatability and intermediate precision. For plant material, a matrix control sample of hops, which bellongs to the same taxonomic family as cannabis but lacking cannabinoids [18], was used for validation. For oil and hashish, the matrix control sample was a mixture of sunflower oil, coconut oil, and extra virgin olive oil, which are commonly used in cannabis oil preparations. When the IS was used, the analyte response was normalized by dividing its area by the area of the respective IS. Isotopic labeled standards for CBDA and CBNA were not available, so THCA-d_3 was used as IS in these cases.

Selectivity was assessed by analyzing each matrix control samples for any interference at the same retention time and with the same transition ions as the analytes of interest.

Matrix effects (suppression or enhancement of the instrumental response) were evaluated by analyzing fortified control samples of plant extract diluted 1:10^2 and 1:10^4 and of oil matrices diluted 10 times, comparing the mean peak areas of the post-extraction fortified samples (matrix-matched) with the mean areas of the solvent-fortified samples, expressed as a percentage. The test was conducted with and without the addition of IS. Matrix effects were considered significant when they exceeded ±20 %.

The linearity of the calibration curve prepared in each matrix was evaluated at seven different concentration levels, with five authentic replicates (n) per level: 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 ng/mL for THC, CBD, and CBN; and 3.12, 6.25, 12.5, 25, 50, and 100 ng/mL for THCA, CBDA, and CBNA. The mean peak areas at each point were used to construct the calibration curve, and the Grubbs test was applied to detect outliers. The homoscedasticity of the calibration curve was evaluated for each analyte using linear least squares regression and the Levene test; the curve was considered homoscedastic when the variances did not differ significantly across the tested levels. The best linear regression fit for heteroscedastic calibration curves was tested for weighting factors of $1/x$, $1/x^2$, $1/x^{0.5}$, $1/y$, $1/y^2$, and $1/y^{0.5}$. Linearity of the calibration curve was considered acceptable when the coefficient of determination (r^2) was at least 0.99 [19].

Recovery, repeatability, and intermediate precision were assessed at levels of 1.56, 25, and 100 ng/mL for THC, CBD, and CBN; and 3.12, 25, and 100 ng/mL for THCA, CBDA, and CBNA. Recovery was expressed as a percentage (n = 4–5), and repeatability was defined as the spread of these results, expressed as relative standard deviation (RSD, %). The experiment was repeated on another day to determine intermediate precision (n = 9–10), also expressed as RSD. Acceptance criteria were recovery within the range of 80–110 %, and repeatability and intermediate precision up to 15 and 22 %, respectively [20].

To estimate the limit of detection (LOD) of the equipment, successive dilutions were performed until the lowest concentration with a signal-to-noise ratio of 3:1 was found. The method's limit of quantification (LOQ) for each analyte in each matrix was defined as the lowest level at which the method was validated within the acceptance criteria for recovery, repeatability and intermediate precision.

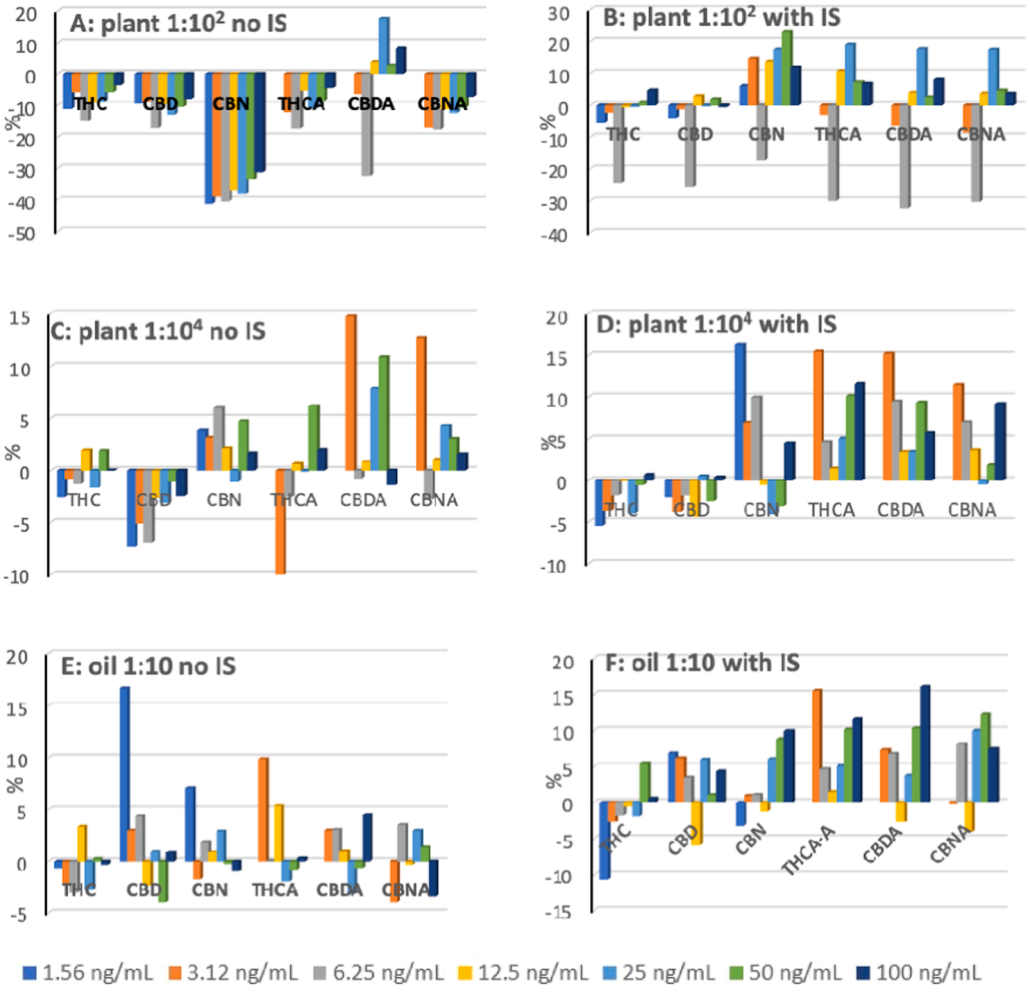

Fig. 3. Matrix effect of the plant material and of oil diluted at 1:10–1:10^4 with or without internal standard (IS).

3. Results and discussion

3.1. Method validation

No interference was observed at the retention times of the analytes in the control samples of plant material (hops) and the oil mixture (sunflower, coconut, and extra-virgin olive oils), indicating good method selectivity. The matrix effect evaluated in plants and oil at different dilution factors, with or without IS are shown in Fig. 3. Without IS and dilution at 1:10^2, signal suppression $>$ 20 % in plant matrix was observed for CBN at all fortification levels and for CBDA at the 6.25 ng/mL (Fig. 3A). With the addition of IS, signal suppression $>$ 20 % was observed for all analytes at the 6.25 ng/mL, except for CBN (17.3 %), which showed a slight signal enhancement at the 50 ng/mL fortification level (23 %; Fig. 3B). When the plant matrix was diluted at 1:10^4, no matrix effect was observed with or without IS at any fortification level ($<$ ±20 %; Figs. 2C and 2D). Oils diluted at 1:10 also did not show matrix effects $>$ 20 % for any of the analytes, regardless of the presence or absence of IS (Figs. 2E and 2F).

Considering that no matrix effect was observed in the plant material at 1:10^4 dilution, and in the oil matrix at 1:10 dilution, quantification of the analytes was performed against a calibration curve in solvent (mobile phase), at the concentration range of 1.56–100 ng/mL. Therefore, quantification of both plant material and oil samples can be performed in a single run using the same calibration curve. All analytes showed heteroscedastic behavior, and 1/x weighting gave the best fit, with $r^2 \geq 0.99$.

Recovery, repeatability and intermediate precision for the plant matrix are shown in Table 1. Recovery without IS was within the established criteria of 80–110 % at all three fortification levels, repeatability was $<$ 15 % (acceptance criterion), except for the lowest level of CBNA (31.1 %) and intermediate precision was higher than 22 % (acceptance criterion; [20]) for most compounds at least at one concentration level (Table 1). With IS, recovery for the acids and CBN at the highest concentration exceeded the accepted criteria (up to 110 %), but since repeatability and intermediate precision were within the acceptable limits at all concentrations and for all analytes, the results were considered satisfactory.

For the oil matrix (Table 2), recovery and repeatability were within the acceptable range, regardless of the presence or absence of IS, except for a slight increase in recovery for CBD (at the lowest level, 112 %) and CBDA (at the highest level, 111 %) with IS. Intermediate precision was satisfactory when IS was used, but higher than 22 % for the acid compounds at most concentrations without IS (Table 2).

Although no significant plant or oil matrix effects were observed when no IS was used, its inclusion improved the method's performance for both matrices and was used for the analysis of real samples. The LOQs are defined as the lowest level for which the method achieved the performance criteria (Tables S2 and S3). For plant matrices (1:10^4 dilution), LOQs were 0.09 % for the neutrals and 0.18 % for the acids, whereas for oils (1:10 ^{3} dilution), they were 0.015 and 0.03 %, respectively. LODs ranged from 0.0019 % to 0.023 % (Table S2).

The LOQs for oil achieved in this study were higher than the LOQ of 0.01 % reported by Galant et al. [21] for 13 cannabinoids (acids and neutrals), although it was not clear how the recovery experiment was conducted. McRae and Melanson [22] reported a very low LOQ for cannabinoids in hemp (0.0002 %), which was determined after sequential dilutions of the compound mixture in solvent to achieve a signal-to-noise ratio of 10 in the LC-MS/MS. This lower LOQ is probably because the authors did not consider the matrix effect of the sample, as they argue that there is no adequate matrix to perform method validation, which in the present study was done with hops, a member of the Cannabaceae family. In any case, the LOQs achieved in the present study are lower than the THC threshold levels of 0.2 or 0.3 % generally accepted to discriminate among cannabis grown for fiber production, therapeutic use or recreational purposes (i.e., non-drug-type vs. drug-type [7]).

3.2. Analysis of real samples

A total of 114 samples of plant material were analyzed by LC-MS/MS. A summary of the results is shown in Table 3 (except roots), and the results for each sample are shown in Table S3 (Supplementary Material).

CBDA and THCA are non-psychoactive precursors that undergo partial or complete decarboxylation to their neutral forms during drying, storage, and thermal processing [23,24] (Fig. 1). Additionally, the possibility of CBD converting into THC under acidic conditions during analysis cannot be discarded [25].

All the compressed material, inflorescence and leaf samples presented quantified levels of THC, THCA and CBN ($\geq$ LOQ). None of the cannabinoids investigated were detected in the six root samples analyzed ($<$ LOD). Indeed, not detected or very low levels of cannabinoids in cannabis roots was reported in other studies (THC $<$ 0.03 %; [26]).

THC showed the highest (13.2 %) and mean (5.15 %) values in compressed material (Table 3 and Table S3). Total THC ([=THC (%) + THCA (%) x (MW_{THC}/MW_{THCA})] [27]) ranged from 0.72 % to 18.1 % (Table 3). The levels of THC found in this study are higher than what was reported by de Oliveira et al. [28] in plants seized in the city of São Paulo

Table 1
Mean recovery (%), repeatability and intermediate precision (RSD, %) of the analytes in vegetal material[a].

	Recovery (n = 4–5)			Repeatability (n = 4–5)			Intermediate precision (n = 9–10)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
No internal standard									
THC	101	98.4	100	9.7	2.4	1.3	26.9	11.1	9.2
CBD	99.6	98.4	100	6.9	2.9	0.9	10.5	17.9	10.9
CBN	106	90.8	102	9.3	2.0	0.6	13.3	29.4	20.3
THCA	102	99.7	98.6	5.3	2.3	1.1	13.0	17.6	14.5
CBDA	96.7	97.9	105	8.0	3.0	1.3	16.8	25.7	25.9
CBNA	89.3	92.2	102	31.1	2.9	3.0	42.9	28.9	27.3
With internal standard									
THC	99.9	105.0	99.9	9.7	4.7	5.8	11.4	10.3	7.7
CBD	98.3	101.1	102	7.5	2.1	2.2	11.6	5.8	4.0
CBN	106	103.9	114	11.8	5.9	5.0	15.3	6.6	3.9
THCA	109	106.5	118	14.5	4.6	14.2	16.8	10.8	18.2
CBDA	101	104.6	116	14.2	5.3	12.3	14.9	4.5	9.4
CBNA	86.2	98.4	119	12.2	5.3	9.0	19.4	13.1	8.2

[a] Concentration levels: low, medium and high at 1.56. 25 e 100 ng/mL, respectively for THC, CBD and CBN, and of 3.12. 25 and 100 ng/mL for THCA. CBDA e CBNA.
RSD: relative standard deviation

Table 2
Mean recovery (%), repeatability and intermediate precision (RSD, %) of the analytes in oil[a].

	Recovery (n = 4–5)			Repeatability (n = 4–5)			Intermediate precision (n = 9–10)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
No internal standard									
THC	90.2	96.5	92.2	4.7	3.8	2.3	16.6	15.6	18.4
CBD	104	103	91.7	9.9	2.9	2.3	12.0	12.6	18.5
CBN	91.5	97.6	89.4	4.7	2.6	1.9	17.6	15.0	3.7
THCA	97.7	95.9	105	3.7	3.5	4.9	22.4	24.9	19.0
CBDA	103	93.2	107	3.4	7.6	2.8	21.1	32.7	31.2
CBNA	103	93.2	85.3	9.1	6.7	2.2	13.1	36.1	37.4
With internal standard									
THC	93.9	102	103	6.2	2.5	2.5	14.5	2.3	3.6
CBD	112	106	98.6	9.9	4.2	6.3	12.0	8.6	4.8
CBN	103	104	104	8.7	5.8	7.9	12.0	9.7	9.9
THCA	96.6	97.0	109	6.0	11.8	7.7	16.7	8.6	8.1
CBDA	102	93.9	111	6.7	11.9	10.3	13.5	17.3	9.9
CBNA	102	93.7	88.7	14.5	11.8	8.3	15.3	13.8	15.6

[a] Concentration levels: low, medium and high at 1.56. 25 e 100 ng/mL, respectively for THC, CBD and CBN, and of 3.12. 25 and 100 ng/mL for THCA, CBDA e CBNA. RSD: relative standard deviation

Table 3
Summary of cannabinoid levels found in the cannabis plant, oil, and hashish.

	THC	THCA	TTHC	THCATHC	CBD	CBDA	CBDA/CBD	CBN	CBNA	CBNA/CBN
	Pressed material (N = 91)[a]									
≥ LOQ, n	91	91	91	91	0	1	–	91	18	18
traces[c]/<LOD, n	0/0	0/0	–	–	81/10	10/80	–	0/0	60/13	–
Range[d], %	0.33–13.2	0.21–8.69	0.72–18.1	0.04–2.39	–	0.44	–	0.23–2.77	0.18–0.50	0.10–0.65
Mean[d] (sd), %	5.15 (2.24)	2.58 (1.78)	7.41 (3.04)	0.58 (0.45)	–	0.44	–	1.02 (0.52)	0.29 (0.12)	0.31 (0.15)
	Inflorescence (N = 11)[a]									
≥ LOQ, n	11	11	11	11	0	0	–	11	7	7
traces[c]/<LOD, n	0/0	0/0	–	–	9/2	2/9	–	0/0	0/4	–
Range[d], %	1.02–6.23	1.16–8.99	3.51–13.9	0.28–2.78	–	–	–	0.27–3.93	0.23–0.53	0.06–0.22
Mean[d] (sd), %	4.59 (1.43)	4.16 (2.19)	8.25 (2.97)	1.01 (0.68)	–	–	–	1.97 (1.33)	0.38 (0.11)	0.14 (0.05)
	Leaf N = 6)[a]									
≥ LOQ, n	6	6	6	6	0	0	–	6	0	–
traces[c]/<LOD, n	0/0	0/0	–	–	1/5	0/6	–	0/0	0/6	–
Range[d], %	0.24–2.26	0.72–2.36	0.87–4.09	0.92–3.00	–	–	–	0.34–1.10	–	–
Mean[d] (sd), %	1.16 (0.68)	1.52 (0.66)	2.50 (1.19)	1.59 (0.77)	–	–	–	0.50 (0.30)	–	–
	Oil (N = 23)[b]									
≥ LOQ, n	23	16	23	16	23	9	9	22	3	3
traces[c]/<LOD, n	0/0	1/6	–	–	0/0	0/14	–	0/1	0/20	–
Range[d], %	0.03–60.9	0.04–9.26	0.03–62.1	0.01–3.49	0.05–29.8	0.10–12.2	0.36–21.2	0.03–25.8	0.06–3.01	0.86–1.18
Mean[d] (sd), %	11.41 (17.6))	2.11 (2.52)	12.7 (17.9)	0.61 (1.09)	3.34 (6.95)	4.37 (3.70)	8.56 (7.72)	1.76 (5.47)	1.98 (1.66)	0.97 (0.19)
	Hashish (N = 4)[b]									
≥ LOQ, n	4	4		4	2	0	–	4	0	
Traces[c]/<LOD[c], n	0/0	0/0		–	0/2	0/4	–	0/0	0/4	–
Range[d], %	2.31–58.5	0.17–0.32	2.59–58.8	0.01–0.14	0.04–1.36	–	–	0.35–9.10	–	–
Mean[d] (sd), %	21.4 (25.5)	0.24 (0.08)	25.5	0.04 (0.06)	0.70 (0.94)	–	–	3.54 (4.0)	–	–

[a] Considering 1:10⁴ dilution, LOD/LOQ are 0.011/0.09 % for the neutrals (THC, CBD, and CBN), respectively, and 0.023/0.18 % for the acids (THCA, CBDA, and CBNA);
[b] Considering 1:10 ³ dilution, LOD/LOQ are 0.0019/0.015 % for the neutrals, and 0.0039/0.03 % for the acids.
[c] LOD ≤ trace < LOQ;
[d] only for quantified samples (≥ LOQ); sd: standard deviation. TTHC: Total THC: THC + THCA*0.88, where 0.88 is the ratio of the THC and THCA molecular mass (314.5/358.5 mol/g)

(Brazil) in 2007/2008 (up to 5.5 %). This is expected as cannabis varieties with higher THC content have been reported in the last decades [4]. Indeed, ElSohly et al. [29] have shown that the potency of illicit cannabis plant material seized in the United States increased from ~4 % THC in 1995 to ~12 % THC in 2014. The THCA/THC ratio in compressed material ranged from 0.04 to 2.39, with only 16.4 % of the samples having a ratio higher than 1 (Table 3). It's important to emphasize that most of the above studies analysed cannabinoids by gas chromatography, mainly coupled with mass spectrometer detector (GC-MS). In those studies, the reported THC levels include its acid form (THCA), as it undergoes decarboxylation and some degradation (~25 % loss; [27]) in the chromatographic injection system. Hence, unless derivatization of the acid form is conducted before the GC analysis, the result reflects approximately total THC [27].

THC levels in inflorescence samples (Table 3) reached 6.23 %, with total THC ranging from 3.51 % to 13.9 %. The ratio of THCA/THC reached 2.78, with approximately 27.2 % of the samples having a value higher than 1. The lower level of THCA compared to THC in most plant samples is expected, as decarboxylation to THC occurs during harvesting and drying, when the sample is exposed to heat and light, and/or during sample processing for chemical analyses [30]. However, decarboxylation in leaves was not so intense, with five of the six samples having a THCA/THC ratio higher than 1.

CBD was not quantified in any plant sample but was detected at trace levels (LOD ≤ trace < LOQ) in at least one sample of all plant matrices (in up to 91.2 % of the compressed material samples) (Table 3). Hence,

all the plant material can be classified as drug-type (> 0.3 % THC and low CBD levels). CBDA was quantified in only one compressed sample (at 0.44 %) and detected at trace levels in 10 and 2 samples of the compressed material and inflorescence samples, respectively. ElSohly et al. [29] also showed a decrease in CBD levels in illicit cannabis from 1995 (mean of 0.28 %) to 2014 (0.15 %), with an increase in the THC/CBD ratio during the period (from 14 to 80).

THC and THCA oxidize easily into CBN and CBNA, respectively, in the presence of oxygen and light during thermal decarboxylation and/or through aging (Fig. 1). CBNA was quantified in 19.9 % of the compressed material samples and 63.6 % of the inflorescence samples, at similar levels (0.18–0.53 %; Table 3), but the CBNA/CBN ratio was lower than 1 in all samples with both matrices (0.10–0.22). While CBN was quantified in all six leaf samples (up to 1.1 %), CBNA was not detected in any of them, indicating complete decarboxylation in this matrix (Table 3).

The stability of cannabinoids in plant material is an important consideration in determining total THC content, as both the rate of decarboxylation of THCA to THC, and degradation of THC to CBN exhibit nonlinear behavior [4,31,32]. In this study, all the inflorescence and leaf samples were seized in 2021, and about 71 % of the compressed samples were seized in 2022, although the age of the plants or the time of harvest is unknown. All samples were stored at room temperature before processing for analysis, which took place in 2023.

Some authors have used the Fetterman [7] approach to classify cannabis according to the phenotypic index ([(%THC+%CBN)/%CBD] [28,33]). If the index exceeds 1, the plant is classified as phenotype I (drug-cannabis); while if the phenotypic index is less than 1, it is classified as phenotype II (non-drug cannabis). Another index uses the ratio THC/CBD, and the same approach for classification [33,34]. In these studies, gas chromatography was used to determine THC, so the levels correspond approximately to total THC, as discussed previously. In the present study, all the seized plant samples with quantified CBD levels had both indices much higher than 1, reaching over 2000 for compressed plant and over 400 for inflorescence (data not shown), indicating that both materials can be classified as drug-cannabis destined for recreation. De Oliveira et al. [28] also found all the seized samples as drug-cannabis type, although the indexes were much lower (up to 101). Itś important to emphasize that seized material may have a mixture of different plant parts.

Cannabis is mainly trafficked as compressed herb and resin, with Paraguay being identified as one of the main source countries in South America. In 2019, it was the second country with the largest seizures worldwide and is described as a major supplier to Brazil and other countries in the region, underlining its central role in regional trafficking dynamics [35].

The flowering tops and their adjacent leaves contain the highest amounts of cannabinoids, as glandular trichomes are the primary sites of synthesis and accumulation. Female plants are preferred for cultivation because they produce higher amounts of cannabinoids [29,36], and are generally used to make oil for medical use or hashish (resin), which is commonly traded as such in illegal markets. In general, solvent-based extraction methods are used to prepare both matrices [37], and the resin can be dissolved/diluted in edible oil for medical purposes [34].

In this study, a total of 23 oil samples provided by civil associations or individuals who legally produce it for personal medical purposes were analyzed, in addition to four hashish samples seized by the Brazilian Federal Police (Table 3 and Table S4). All oil samples contained quantified levels of THC (up to 60.9 %), and 17 samples contained THCA, 16 at quantified levels (up to 9.26 %), with total THC reaching 62.1 %. Only three samples showed the THCA/THC ratio higher than 1 (up to 3.49). CBD was also found in all oil samples (up to 29.8 %), and CBDA in nine samples (up to 12.2 %), seven of which had a CBDA/CBD ratio higher than one (up to 21.2, Table 3). As expected, the acid forms were present at a lower frequency/concentration in the samples, as decarboxylation occurs during the extraction procedure. Decarboxylation was more extensive for CBNA, which was only detected in three of the 23 oil samples (up to 3.01 %) and a CBNA/CBN ratio from 0.86 to 1.18 (Table 3).

THC and THCA were quantified in all four hashish samples, with the highest THC concentration similar to that of the oil sample (around 60 %), but with a mean two times higher (Table 3). Decarboxylation occurred in all samples (THCA/THC ratio up to 0.14). CBD was present in two samples (up to 1.36 %) and CBN in all four samples (up to 9.10 %), but their acid forms were detected. Hanus et al. [38] analyzed 15 hashish samples from different origins (Lebanon, Morocco and/or India) seized in Israel and the Czech Republic, reporting THC levels ranging from 0.93 % to 16.4 % and CBD levels from 0.78 % to 13.1 %, much lower than the values found in the seized samples in the Federal District. In the United States, the mean THC levels of 814 samples seized from 1995 to 2014 were 21.8 (± 18.2), also lower than the values found here a decade later.

Fig. 4 shows the THC and CBD levels in the 23 oil samples analyzed. Most (61 % of the samples) had higher THC, with a THC/CBD ratio over 20 (up to 72) in about 30 % of the samples. Various studies report the benefit of the use of THC-rich products for neuropathic and chronic pain, cancer-related pain and chemotherapy-induced nausea, as well as THC and CBD balanced products for spasticity and multiple sclerosis, among other conditions, which was not the case of any of the samples analyzed [39].

Five oil samples had THC levels up to 0.3 %, with four of them in the range of 0.03–0.05 %, within the acceptable level according to the Brazilian legislation of up to 0.2 %, except for a registered medicament that contains 2.7 % THC [15]. Additionally, the levels of CBD in these four samples ranged from 1.6 % to 2.1 %, which are lower than the typical levels in cannabis extracts commercialized in the country (3.7–20 %). However, the therapeutic dose of these home-made extracts may be adjusted for each patient depending on the therapeutic response of the treatment.

In this study, the number of compressed material samples (91) analysed was much higher than the number of fluorescence, leaves and root samples, which is a limitation for comparison purposes.

4. Conclusion

Cannabis is the plant most involved in illegal traffic worldwide, an activity that leads to a high seizure rate. In this study, an LC-MS/MS method for detecting and quantifying six cannabinoids in plant and oil matrices within a single 11-minute chromatographic run was validated. The method can be adapted to various concentration ranges and other matrices, including cosmetics, herbal mixtures and edible products.

Furthermore, expanding it to include the analysis of terpenes and flavonoids may further strengthen its role, considering the importance of these compounds in the so-called entourage effect. Its applicability in routine laboratory workflows is particularly relevant in countries such as Brazil, where the distinction between recreational and medical cannabis is strictly regulated.

Although LC-MS/MS methods to analyze cannabinoids in other matrices is available, including biological matrices and cosmetics [40,41], the simultaneous, quantification of the major cannabinoids in plant parts and oil ensures a chemical profile that is essential for both forensic and regulatory compliance testing. The analysis of plant material samples seized by the Brazilian Federal Police provides a crucial, real-world snapshot of the illicit cannabis market in the country and the data is invaluable for law enforcement and public health authorities. The analysis of oil samples provided by civil associations and individuals directly addresses the rapidly evolving medicinal cannabis landscape in Brazil. The data highlights the need for quality control, as only a small fraction of the oils analyzed meets the country's legal limit for THC in phytopharmaceuticals (up to 0.2 %).

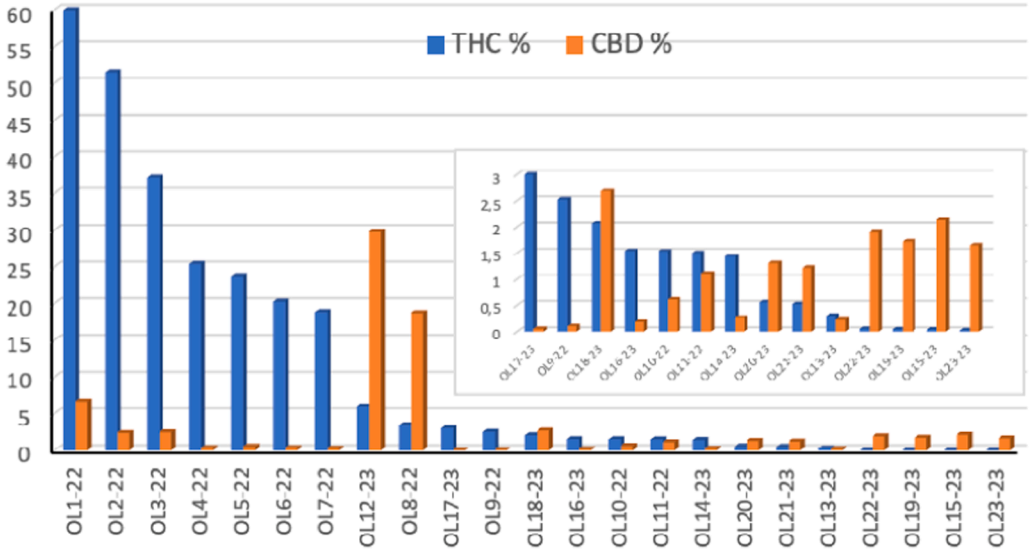

Fig. 4. THC and CBD levels found in oil samples provided by individual consumers or associations.

CRediT authorship contribution statement

Jorge Jardim Zacca: Writing – review & editing, Resources, Funding acquisition, Data curation, Conceptualization. **Patricia da Silva Montes Drobnjak:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Dutra Caldas Eloisa Dutra:** Writing – review & editing, Supervision, Project administration, Data curation, Conceptualization.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2025.112781.

References

[1] G. Micalizzi, F. Vento, F. Alibrando, D. Donnarumma, P. Dugo, L. Mondello, Cannabis sativa L.: a comprehensive review on the analytical methodologies for cannabinoids and terpenes characterization, J. Chromatogr. A 1637 (2021) 461864, https://doi.org/10.1016/j.chroma.2020.461864.

[2] M.A. ElSohly, M.M. Radwan, W. Gul, S. Chandra, A. Galal, Phytochemistry of Cannabis sativa L, in: A.D. Kinghorn, H. Falk, S. Gibbons, J. Kobayashi (Eds.), Phytocannabinoids, Springer International Publishing, Cham, 2017, pp. 1–36, https://doi.org/10.1007/978-3-319-45541-9_1.

[3] C.M. Andre, J.-F. Hausman, G. Guerriero, Cannabis sativa: the plant of the thousand and one molecules, Front. Plant Sci. 7 (2016), https://doi.org/10.3389/fpls.2016.00019.

[4] United Nations Office on Drugs and Crime (UNODC), World Drug Report 2022, United Nations publication, 2022 ⟨https://www.unodc.org/unodc/en/data-and-analysis/wdr-2022_booklet-3.html⟩ (Accessed July, 2025).

[5] J.M. McPartland, *Cannabis* Systematics at the levels of family, genus, and species, Cannabis Cannabinoid Res 3 (2018) 203–212, https://doi.org/10.1089/can.2018.0039.

[6] É. Lapierre, A.S. Monthony, D. Torkamaneh, Genomics-based taxonomy to clarify cannabis classification, Genome 66 (2023) 202–211, https://doi.org/10.1139/gen-2023-0005.

[7] P.S. Fetterman, E.S. Keith, C.W. Waller, O. Guerrero, N.J. Doorenbos, M. W. Quimby, Mississippi-Grown Cannabis sativa L.: Preliminary observation on chemical definition of phenotype and variations in tetrahydrocannabinol content versus age, sex, and plant part, J. Pharm. Sci. 60 (1971) 1246–1249, https://doi.org/10.1002/jps.2600600832.

[8] J.M. McPartland, E. Small, A classification of endangered high-THC cannabis (Cannabis sativa subsp. indica) domesticates and their wild relatives, PhytoKeys 144 (2020) 81–112, https://doi.org/10.3897/phytokeys.144.46700.

[9] United Nations Office on Drugs and Crime (UNODC), World Drug Report 2025, United Nations publication, 2025 ⟨https://www.unodc.org/documents/data-and-analysis/WDR_2025/WDR25_B1_Key_findings.pdf⟩ (Accessed August, 2025),

[10] Federal Police of Brazil, PF divulga balanço de resultados das ações de 2024 [Internet] (Accessed August), Federal Police of Brazil, *Brasilia*, 2025, ⟨https://www.gov.br/pf/pt-br/assuntos/noticias/2025/01/pf-divulga-balanco-de-resultados-das-acoes-de-2024⟩ (Accessed August).

[11] D. Fiorini, A. Molle, M. Nabissi, G. Santini, G. Benelli, F. Maggi, Valorizing industrial hemp (Cannabis sativa L.) by-products: Cannabidiol enrichment in the inflorescence essential oil optimizing sample pre-treatment prior to distillation, Ind. Crops Prod. 128 (2019) 581–589, https://doi.org/10.1016/j.indcrop.2018.10.045.

[12] H.J. Barrales-Cureño, L.G. López-Valdez, C. Reyes, V.M. Cetina-Alcalá, I. Vasquez-García, O.F. Diaz-Lira, B.E. Herrera-Cabrera, Chemical characteristics, therapeutic uses, and legal aspects of the cannabinoids of Cannabis sativa: A Review, Braz. Arch. Biol. Technol. 63 (2020) e20190222, https://doi.org/10.1590/1678-4324-2020190222.

[13] C.A. Legare, W.M. Raup-Konsavage, K.E. Vrana, Therapeutic potential of cannabis, cannabidiol, and cannabinoid-based pharmaceuticals, Pharmacology 107 (2022) 131–149, https://doi.org/10.1159/000521683.

[14] Brazil, Law n° 13.840, of June 5, 2019 [Internet]. Brasilia: Presidency of the Republic; 2019. ⟨https://www.planalto.gov.br/ccivil_03/_ato2019-2022/2019/lei/l13840.htm⟩ (Accessed August, 2025).

[15] National Health Surveillance Agency (ANVISA), Produtos de cannabis – ANVISA Consultas [Internet], ANVISA, Brasilia, 2025. ⟨https://consultas.anvisa.gov.br/#/cannabis/q/?nomeProduto=canabidiol⟩ (Accessed August, 2025).

[16] Superior Court of Justice (STJ). *STJ valida cultivo medicinal da cannabis por empresas e dá prazo para regulamentação* [Internet]. Brasília: STJ; 2024. ⟨https://www.stj.jus.br/sites/portalp/Paginas/Comunicacao/Noticias/2024/14112024-STJ-valida-cultivo-medicinal-da-cannabis-por-empresas-e-da-prazo-para-regulamentacao.aspx⟩ (Accessed August, 2025).

[17] Supreme Federal Court (STF), STF define 40 gramas de maconha como critério para diferenciar usuário de traficante [Internet]. Brasilia: STF; 2024. ⟨https://noticias.stf.jus.br/postsnoticias/stf-define-40-gramas-de-maconha-como-criterio-para-diferenciar-usuario-de-traficante/⟩ (Accessed August, 2025).

[18] E.C. Berthold, R. Yang, A. Sharma, S.H. Kamble, S.R. Kanumuri, T.I. King, R. Popa, J.H. Freeman, Z.T. Brym, B.A. Avery, C.R. McCurdy, Regulatory sampling of industrial hemp plant samples (Cannabis sativa L.) using UPLC-MS/MS method for detection and quantification of twelve cannabinoids, J. Cannabis Res. 2 (1) (2020) 42, https://doi.org/10.1186/s42238-020-00050-0.

[19] J.J. Zacca, C.P. Sandoval Jáuregui, F.M. Villafana Bardales, J. Remuzgo Yabar, J. E. Enciso Soria, V.D. Ayala Caro, Correlation coefficient distribution and its

application in the comparison of chemical data sets, Chemom. Intell. Lab. Syst. 248 (2024) 105091, https://doi.org/10.1016/j.chemolab.2024.105091.
[20] National Institute of Metrology, Quality and Technology (INMETRO). *Orientação sobre validação de métodos analíticos: documento de caráter orientativo* [Internet]. Rio de Janeiro INMETRO; DOQ-CGCRE-008 Revisão 09, 2025. ⟨https://www.gov.br/cdtn/pt-br/centrais-de-conteudo/documentos-cgcre-abnt-nbr-iso-iec-17025/doq-cgcre-008/view⟩ (Accessed August, 2025).
[21] N. Galant, J. Czarny, J. Powierska-Czarny, A. Piotrowska-Cyplik, Development and validation of the LC–MS/MS method for determination of 130 natural and synthetic cannabinoids in cannabis oil, Molecules 27 (2022) 8601, https://doi.org/10.3390/molecules27238601.
[22] G. McRae, J.E. Melanson, Quantitative determination and validation of 17 cannabinoids in cannabis and hemp using liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 412 (2020) 7381–7393, https://doi.org/10.1007/s00216-020-02862-8.
[23] C. Lanz, J. Mattsson, U. Soydaner, R. Brenneisen, Medicinal Cannabis: in vitro validation of vaporizers for the smoke-free inhalation of cannabis, PLOS ONE 11 (2016) e0147286, https://doi.org/10.1371/journal.pone.0147286.
[24] K. Żuk-Gołaszewska, K. Żuk-Gołaszewska, J. Gołaszewski, Cannabis sativa L. – cultivation and quality of raw material, J. Elem. (2018), https://doi.org/10.5601/jelem.2017.22.3.1500.
[25] M.N. Tahir, F.S. Raz, S. Rondeau-Gagné, J.F. Trant, The biosynthesis of the cannabinoids, J. Cannabis Res. 3 (2021) 7, https://doi.org/10.1186/s42238-021-00062-4.
[26] D. Jin, K. Dai, Z. Xie, J. Chen, Secondary Metabolites profiled in cannabis inflorescences, leaves, stem barks, and roots for medicinal purposes, Sci. Rep. 10 (2020) 3309, https://doi.org/10.1038/s41598-020-60172-6.
[27] R.B. Malabadi, M.R. Sadiya, K.P. Kolkar, L. Lakshminarayana, R.K. Chalannavar, Quantification of THC levels in different varieties of Cannabis sativa, Int. J. Sci. Res. Arch. 10 (2023) 860–873, https://doi.org/10.30574/ijsra.2023.10.2.1029.
[28] G.L. De Oliveira, M.H. Voloch, G.B. Sztulman, O.N. Neto, M. Yonamine, Cannabinoid contents in cannabis products seized in São Paulo, Brazil, 2006–2007, Forensic Toxicol. 26 (2008) 31–35, https://doi.org/10.1007/s11419-008-0046-x.
[29] M.A. ElSohly, Z. Mehmedic, S. Foster, C. Gon, S. Chandra, J.C. Church, Changes in Cannabis potency over the last 2 Decades (1995–2014): analysis of current data in the United States, Biol. Psychiatry 79 (2016) 613–619, https://doi.org/10.1016/j.biopsych.2016.01.004.
[30] M. Taschwer, M.G. Schmid, Determination of the relative percentage distribution of THCA and Δ9-THC in herbal cannabis seized in Austria – Impact of different storage temperatures on stability, Forensic Sci. Int. 254 (2015) 167–171, https://doi.org/10.1016/j.forsciint.2015.07.019.
[31] C. Citti, U.M. Battisti, D. Braghiroli, G. Ciccarella, M. Schmid, M.A. Vandelli, G. Cannazza, A metabolomic approach applied to a liquid chromatography coupled to High-Resolution Tandem Mass Spectrometry Method (HPLC-ESI-HRMS/MS): towards the comprehensive evaluation of the chemical composition of Cannabis medicinal extracts, Phytochem. Anal. 29 (2018) 144–155, https://doi.org/10.1002/pca.2722.
[32] V. Carvalho, A. Aguiar, L. Baratto, F. Souza, E. Rocha, Quantificação de canabinoides em extratos medicinais de cannabis por Cromatografia Líquida de Alta Eficiência, QuíM. Nova (2020), https://doi.org/10.21577/0100-4042.20170457.
[33] M. Stefanidou, A. Dona, S. Athanaselis, I. Papoutsis, A. Koutselinis, The cannabinoid content of marihuana samples seized in Greece and its forensic application, Forensic Sci. Int. 95 (1998) 153–162, https://doi.org/10.1016/S0379-0738(98)00083-8.
[34] E.P.M. De Meijer, H.J. Van Der Kamp, F.A. Van Eeuwijk, Characterisation of Cannabis accessions with regard to cannabinoid content in relation to other plant characters, Euphytica 62 (1992) 187–200, https://doi.org/10.1007/BF00041753.
[35] United Nations Office on Drugs and Crime (UNODC), World Drug Report 2021 (Accessed July,, United Nations publication, 2021–2025, ⟨https://www.unodc.org/res/wdr2021/field/WDR21_Booklet_3.pdf⟩ (Accessed July,.
[36] B.F. Thomas, M.A. ElSohly, The Botany of Cannabis sativa L. in: Anal. Chem. Cannabis, Elsevier, 2016, pp. 1–26, https://doi.org/10.1016/B978-0-12-804646-3.00001-1.
[37] M.P. Lazarjani, O. Young, L. Kebede, A. Seyfoddin, Processing and extraction methods of medicinal cannabis: a narrative review, J. Cannabis Res. 3 (2021) 32, https://doi.org/10.1186/s42238-021-00087-9.
[38] L.O. Hanuš, R. Levy, D. De La Vega, L. Katz, M. Roman, P. Tomíček, The main cannabinoids content in hashish samples seized in Israel and Czech Republic, Isr. J. Plant Sci. 63 (2016) 182–190, https://doi.org/10.1080/07929978.2016.1177983.
[39] G. Mick, P. Douek, Clinical benefits and safety of medical cannabis products: a narrative review on natural extracts, Pain. Ther. 13 (2024) 1063–1094, https://doi.org/10.1007/s40122-024-00643-0.
[40] M. Laloup, M.R. Fernandez, M. Wood, G. de Boeck, C. Henret, V. Maes, N. Samyn, Quantitative analysis of Δ9-tetrahydrocannabinol in preserved oral fluid by liquid chromatography–tandem mass spectrometry, J. Chromatogr. A 1082 (2005) 15–24, https://doi.org/10.1016/j.chroma.2005.03.031.
[41] Q. Meng, B. Buchanan, J. Zuccolo, M.M. Poulin, J. Gabriele, D.C. Baranowski, A reliable and validated LC-MS/MS method for the simultaneous quantification of 4 cannabinoids in 40 consumer products, PLoS One 13 (5) (2018) e0196396, https://doi.org/10.1371/journal.pone.0196396.