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DEVELOPMENT AND VALIDATION OF AN LC-MS/MS METHOD FOR THE QUANTIFICATION OF SIX CANNABINOIDS IN COMMERCIAL PRODUCTS

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ABSTRACT. Cannabidiol (CBD) oils and products have become extremely popular in the last decade. These products are marketed as having different effects and are recommended for many chronic diseases. Various food supplements with CBD are now available on the market, but due to legislative lacks the declared concentration of CBD and other cannabinoids in the products is often significantly different compared with the real concentration. Products that don't meet quality criteria result from a lack of control and standardization. The aim of this study was to develop and validate an LC-MS/MS method for the routine quantification of cannabinoids in herbal drugs and food supplements. An LC-MS/MS method was developed using an UHPLC system coupled with a QTOF mass spectrometer, and the chromatographic separations were performed on a C18 column with isocratic elution, electrospray ionization in negative mode with a run-time of 10

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minutes. According to the international guidelines, the method was validated concerning linearity, selectivity, stability, precision, and accuracy. Out of the ten tested products, in three of the supplements, significant differences were noticed in CBD concentration compared to the declared content. Other cannabinoids were also identified in some of the samples.

This study raises awareness regarding insufficient controlled food supplements.

Keywords: *cannabinoids, cannabidiol, LC-MS/MS, food supplements*

INTRODUCTION

Cannabinoids are lipophilic phenolic substances, benzopyran derivatives, whose concentration and distribution in commercial products are highly variable, depending on the *Cannabis* chemotype from which the extraction was made, as well as the technological process by which it was obtained [1].

In plants, cannabinoids are synthesized in the glandular trichomes. The biosynthetic pathway starts from hexanoyl-CoA, and the first cannabinoid precursor is cannabigerolic acid which is formed through the prenylation of olivetolic acid. THC and CBD are formed through the non-enzymatic decarboxylation of their acidic precursors, namely Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA) and cannabidiolic acid (CBDA). THCA is formed from cannabigerolic acid (CBGA) under the action of THCA synthase, CBDA is formed from CBGA under the action of CBDA synthase, and cannabichromenic acid is formed under the action of cannabichromenic acid synthase [2]. Until now, over 100 cannabinoids have been isolated in *Cannabis* species, but the most abundant ones are: Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), cannabichromene (CBC), Δ^9-THCA, CBDA, cannabigerol (CBG), cannabigerolic acid, cannabinol (CBN) [2,3]. CBDA and CBN are formed through the oxidation of Δ^9-THCA and Δ^9-THC, under different conditions (light, oxygen, long storage) [2,4,5].

Until now, there are only two approved (by the Food and Drug Administration and/or by European Medicines Agency) *Cannabis-based* drugs. Sativex® (nabiximols) is an oromucosal spray standardized in THC and CBD. Epidyolex® is an oral solution that contains CBD in a concentration of 100 mg/mL. It is approved by the European Medicines Agency for the treatment of epilepsy crisis associated with Lennox-Gastaut syndrome or with Dravet syndrome [6,7]. However, a plethora of food supplements with CBD, especially ‘CBD oils’, have appeared in pharmacies in the last years.

CBD products have rapidly gained popularity due to their marketed health benefits, ranging from anxiety relief to pain management. Due to the lack of definitions, it is unclear what type of oils are sold on the food supplements market (reconstituted from fatty oils with synthetic CBD or extracted from the plant product). However, the regulatory framework governing these products is unclear. The law in Romania regarding food supplements has numerous loopholes, which increases the risk of significant side effects. At the same time, the legislation in Romania is quite ambiguous regarding the products obtained from *Cannabis sp.* and/or products containing cannabidiol. *Cannabis*, *cannabis* resin, *cannabis* extracts, and tinctures belong to the category of narcotic substances of medical interest and are subjected to strict control [8]. Cannabidiol and other cannabinoids, except THC and THCA, are not included in this list, but there are also no clear regulations regarding their presence in different products (food supplements, cosmetics, oils, etc.). Regarding the effects of cannabidiol, numerous studies have highlighted multiple effects induced by a complex pharmacodynamic mechanism [9–11]. Also, other cannabinoids may act synergistically with CBD, enhancing its activity [12]. As noticed by Hayduc et al, these interactions are hard to predict, and are dose-dependent [13]. If extracted from the herbal drug, CBD oils contain other active lipophilic phytocompounds, different cannabinoids and non-cannabinoids such as volatile terpenes [14–17].

The toxicological studies have revealed that CBD is a compound with a good safety profile, with few and mild side effects even at high doses up to 1500 mg/day [18,19]. The most common side effects noticed in clinical trials were: decreased appetite, diarrhoea and dizziness [20,21].

The CBD market has quickly expanded, and the consumer has a very large portfolio of products from which he can choose. However, as how was previously noticed, there are many inconsistencies between the declared composition and the actual composition. Given the diverse nature of the routes of administration by which CBD has been studied, its pharmacokinetic profile is influenced by an important number of variables. All these variations are making the efficiency of CBD products to differ consistently between the products.

It is important to emphasize that more pharmacokinetic studies and more clinical trials are needed to validate the long-term efficacy and safety of using CBD as a food supplement.

CBD oils are standardized in CBD, but not in the other cannabinoids that can be extracted from hemp. A routine use of an LC-MS/MS analysis should be implemented for the evaluation of food supplements and other cannabinoid-based products. The analysis of cannabinoids using HPLC and LC-MS has evolved significantly over the past decade, offering a range of methods for precise and sensitive detection of these compounds. The

European Pharmacopoeia (11.5) method for determining cannabinoids in cannabis flower uses liquid chromatography with a polar-embedded octadecylsilyl silica gel column and a mobile phase of trifluoroacetic acid and acetonitrile, and a total run time of approximately 35 minutes. The process involves ethanol extraction, dilution, and filtration of the sample, with UV detection at 228 nm to identify and quantify cannabinoids like cannabidiol, THC, and their acidic forms based on retention times and reference standards [22].

The most practical detection method for analysing naturally occurring cannabinoids is mass spectrometric detection, which offers important structural details for differentiating different cannabinoids [23]. Table 1 summarizes relevant data from methods published between 2020 and 2025, detailing the types of methods, chromatographic conditions, the cannabinoids that were quantified, and the type of samples. The data highlight that the reversed-phase C18 columns commonly used for cannabinoid detection range in length from 50 mm to 150 mm, depending on the specific application and desired resolution. Particle sizes of columns used during this period predominantly fall below 3 µm, reflecting a trend toward improved separation efficiency and resolution. While advanced techniques such as nano-LC offer high sensitivity, they remain cost-prohibitive for many laboratories [24]. Therefore, while numerous validated methods exist, it is essential to revalidate protocols when instrumentation or columns are changed to ensure reliability.

Table 1. Analytical methods published between 2020 and 2025 regarding the analysis of cannabinoids in herbal products, food products and cosmetics

Method	Chromatografic conditions	Cannabinoids	Samples	Reference
LC-PDA Column: 2.1 × 50 mm, 1.8 µm (C18)	Mobile phase: 0.1% formic acid (A) and ACN containing 0.1% formic acid (B) Total run time: 7.5 min Detection: 210, 221 nm	CBD, CBDA, THC, THCA	CBD tea products	Mouton et al, 2024 [25]
LC-MS/MS Column: 4.6 × _50 mm, 2.7 µm (C18)	Mobile phase: water with 0.1% (v/v) formic acid (A) and MeOH (B) (gradient elution) Total run time: 10 min Detection: ESI in positive mode	CBN, 11-THC-OH, 11-THC-COOH, THC, CBD, CBC, CBG	Cannabis-based edibles	Christodoulou et al, 2023 [26]

Method	Chromatografic conditions	Cannabinoids	Samples	Reference
LC-MS/MS Column: 2.1 × 100 mm, 1.6 µm (C18)	Mobile phase: 0.1% formic acid in water (A) and acetonitrile (B) (isocratic elution) Total run time: 13 min Detection: HRMS/MS	CBDV, CBE, CBDVA, THCV, THCVA, CBD, CBDA, CBG, CBN, CBGA, Δ9-THC, Δ8-THC, CBL, CBC, CBT, CBNA, THCA	e-cigarette liquids	Barhdadi et al, 2023 [27]
LC-MS/MS Column: 3 x 100 mm, 2.6 µm (C18)	Mobile phase: water (A) and methanol (B); both contained 0.1% formic acid and 2 mM ammonium formate (gradient elution) Total run time: 13 min Detection: ESI/MS-MS in positive mode	THC, THCA-A, CBD, CBDA, CBG, CBGA, CBC, CBV, CBDVA, CBGVA, CBN, THCVA, THCV, + 117 synthetic cannabinoids	Food products and food supplements	Galant et al, 2022 [28]
LC-MS/MS Column: 3.0 mm x 100 mm, 25 µm (C18)	Mobile phase: 10 mM ammonia formate buffer (A) and 10 mM ammonia formate buffer in methanol (B) Total run time: 13 min Detection: ESI/MS-MS in positive mode	THC, CD, CBN	Hemp oil based cosmetic products	Hsu et al, 2021 [29]
LC-MS/MS Column: not specified	0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) (gradient elution) Total run time: 22 min	Δ9-THC	Food products and beverages	Pisciottano et al, 2021 [30]
LC-MS/MS Column: 2.1 x 100 × mm, 1.7 µm (C18)	Mobile phase: 0.1% formic acid in water (A) and acetonitrile (B) Total run time: 13 min Detection: ESI-MS/MS in positive and negative mode	CBD, CBDA Δ9-THC, THCA, CBN, CBC, CBCA, CBDV, CBDVA, CBG, CBGA, THCV, THCVA, Δ8-THC	Food samples	Christinat et al, 2020 [31]

Method	Chromatografic conditions	Cannabinoids	Samples	Reference
LC-MS/MS Column: 2.0 x 100 mm, 3 µm	Mobile phase: 0.1% formic acid in water (A) and acetonitrile (B) (gradient elution) Total run time: 18 min Detection: ESI-MS/MS in positive mode	CBD, THC	Food and dietary supplements	Lee et al, 2020 [32]
HPLC/UV Column: 4.6 x 150 mm, 2.7 µm	Mobile phase: water (A) and acetonitrile (B) both containing 0.085 % phosphoric acid (gradient elution) Total run time: 10 min Detection: 220 nm	CBDV, CBDA, CBGA, CBG, CBD, THCV, CBN, Δ9-THC, Δ8-THC, CBC, THCA	Cannabis light preparations (THC/CBD ratio <<1)	Dei Cas et al, 2020 [33]

Therefore, this study aimed to develop and validate a fast and reliable LC-MS/MS method for the routine quantification of cannabinoids in food supplements.

RESULTS AND DISCUSSION

Cannabinoid analysis is a popular topic with many challenges, and the interest in this domain has constantly increased in the last 20 years, worldwide. There are different proposed methods for the quantification of cannabinoids in different matrices, most of them based on HPLC-DAD [5,34–36]. Most HPLC-validated methods have long run times, making them not quite suitable for routine analysis. Tandem mass spectrometry has the advantage of offering high sensitivity and specificity, and it has been widely used in the last 5 years [28,30,32,33,36].

In the present study, an accurate, fast, and robust method was developed and validated for the quantification of 6 cannabinoids in food supplements. All standards showed a good linearity in the tested concentration range, with $R \geq 0.99$. The lower limit of quantification (LLOQ) was 5 ng/mL. Compared with the LC-MS/MS method proposed by Christodoulou et al, our method had a lower LOQ for CBD [26]. As anticipated, the sensitivity of our proposed LC-MS/MS method significantly surpasses that of the LC-PDA method developed by Mouton et al. [25].

The retention time of CBDA was 1.25 min, of CBGA was 1.41 min, of CBVA was 0.84 min, of CBD was 4.79 min, of CBG was 4.69 min, and of CBN was 8.34 min, with a total run time of 10 minutes. For the validation procedure the guidelines described by the USFDA and the EMA for bioanalytical method validation were used. Following those general guidelines and steps we validated the applicable and relevant parameters for our application [37,38]. In figure 1 and 2 MS spectrum and chromatograms of the six cannabinoids are presented.

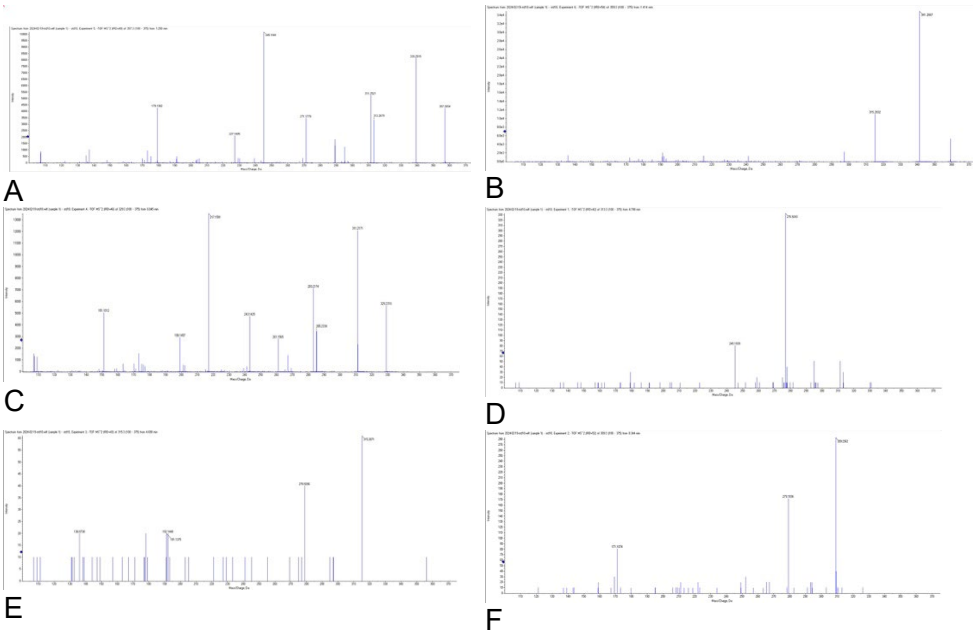

Figure 1. MS spectrum of compounds: A - CBDA, B - CBGA, C - CBVA, D - CBD, E - CBG, F – CBN

No carry-over effect was detected, with no peaks detected in the blank solution for any of the analytes.

No interfering peaks were observed at the retention times of the analytes in the blank solution. To provide the necessary sensitivity and selectivity, the areas of the analytes at the limit of quantification must be at least five times larger than the area of any peaks present in the blank samples.

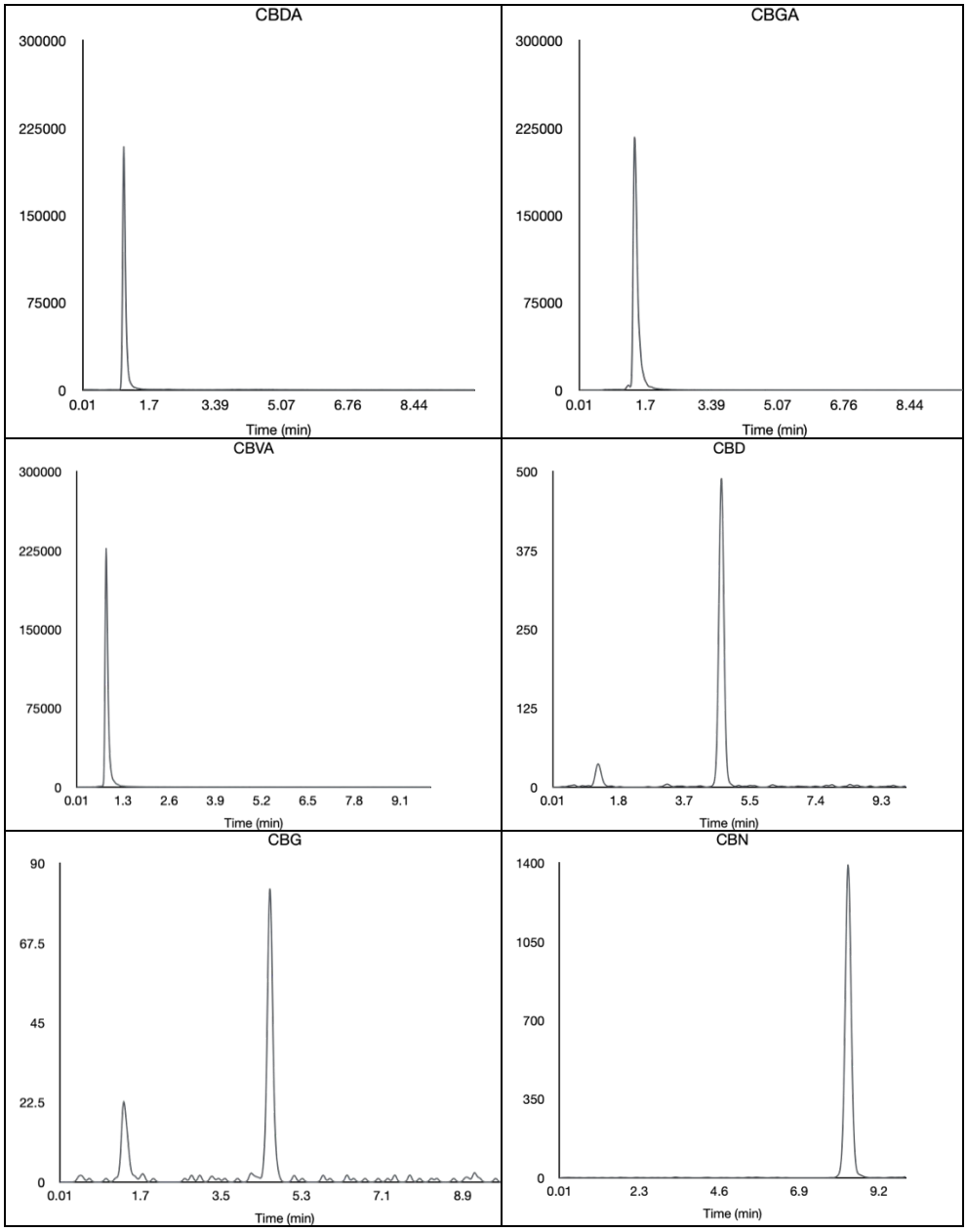

Figure 2. Representative chromatograms of the analyzed cannabinoids

Five samples of each control sample were analyzed in a single run to determine within-run accuracy and precision (table 2). The mean concentration, mean deviation from the nominal value (accuracy) and relative standard deviation (RSD% - precision) of the analyzed control standards were calculated. Average accuracy and precision were within ± 15% and ± 20% respectively in the case of LLOQ.

Table 2. Within-run accuracy and precision (n = 3)

Analyte	Nominal concentration (ng/ mL)	Measured concentration (ng/ mL)	Accuracy (%)	Precision (%)
CBDA	5	4.89 ± 0.15	97.8	3.07
	25	23.4 ± 2.31	93.4	9.90
	250	251 ± 8.68	100	3.46
	500	555 ± 38.6	111	6.97
	750	735 ± 68.1	98.0	9.27
CBGA	5	4.88 ± 0.23	97.6	4.83
	25	24.3 ± 2.55	97.1	10.5
	250	241 ± 5.59	96.5	2.32
	500	549 ± 31.7	110	5.77
	750	742 ± 43.1	98.9	5.81
CBVA	5	4.72 ± 0.18	94.4	3.84
	25	24.7 ± 1.85	98.8	7.50
	250	267 ± 14.7	107	5.51
	500	554 ± 26.4	111	4.76
	750	730 ± 31.3	97.4	4.29
CBD	5	5.33 ± 0.36	107	6.83
	25	27.1 ± 2.06	108	7.62
	250	233 ± 16.6	93.5	7.09
	500	551 ± 12.9	110	2.34
	750	702 ± 41.0	93.6	5.85
CBG	5	5.58 ± 0.48	112	8.63
	25	25.2 ± 2.15	101	8.52
	250	237 ± 21.4	94.9	9.03
	500	503 ± 35.1	101	6.98
	750	757 ± 45.7	101	6.04
CBN	5	5.43 ± 0.33	109	6.15
	25	25.3 ± 2.10	101	8.32
	250	272 ± 15.6	109	5.74
	500	538 ± 30.3	108	5.62
	750	746 ± 16.7	99.5	2.24

Five samples from each control sample, one from each sample in each sequence, were analyzed to determine between-run accuracy and precision (table 3). The mean concentration, mean deviation from the nominal value (accuracy), and relative standard deviation (RSD% - precision) of the analyzed control standards were calculated. Average accuracy and precision were within ±15% and ±20% respectively in the case of LLOQ.

Table 3. Between-run accuracy and precision (n = 3)

Analyte	Nominal concentration (ng/mL)	Measured concentration (ng/ mL)	Accuracy (%)	Precision (%)
CBDA	5	5.33 ± 0.32	107	5.95
	25	23.5 ± 1.86	94.1	7.90
	250	227 ± 19.6	90.8	8.63
	500	515 ± 41.5	103	8.07
	750	740 ± 51.8	98.7	7.00
CBGA	5	5.31 ± 0.53	106	9.93
	25	24.4 ± 1.99	97.8	8.16
	250	226 ± 18.9	90.3	8.39
	500	513± 17.7	102.6	3.44
	750	708 ± 66.5	94.4	9.39
CBVA	5	5.21 ± 0.64	104	12.2
	25	25.2 ± 1.94	101	7.68
	250	231. ± 29.8	92.5	12.9
	500	515 ± 26.7	103	5.18
	750	720 ± 61.7	96.0	8.56
CBD	5	5.23 ± 0.55	105	10.5
	25	26.6 ± 1.71	106	6.44
	250	224 ±11.6	89.49	5.17
	500	540 ± 32.3	108	5.99
	750	741 ± 68.6	98.8	9.27
CBG	5	5.28 ± 0.59	106	11.1
	25	25.3 ± 2.81	101	11.1
	250	244 ± 17.1	97.4	7.01
	500	521 ± 61.5	104	11.8
	750	745 ± 75.3	99.3	10.1
CBN	5	5.25 ± 0.59	105	11.4
	25	24.2 ± 1.89	96.7	7.80
	250	252 ± 19.4	101	7.69
	500	522 ± 27.4	104	5.25
	750	726 ± 30.4	96.8	4.18

Table 4. CBD concentration found in samples versus the declared concentration

Sample	Concentration (%)	Declared concentration (%)
1	11.5 ± 0.38	10
2	2.38 ± 0.20	2.5
3	0.15 ± 0.03	n.d
4	6.44 ± 0.04	10
5	2.01 ± 0.03	2.5 (CBD + terpenes)
6	2.10 ± 0.04	5
7	14.2 ± 0.08	10
8	0.02 ± 0.001	n.d.
9	0.53 ± 0.06	n.d.
10	0.21 ± 0.02	n.d.

The most abundant cannabinoids found in the tested samples were CBD, CBN, and CBG, as it can be seen in table 4 and 5. Within the tested products, in three products the CBD content was significantly different compared with the declared concentration. Samples 4 and 6 had lower concentrations of CBD compared to the declared concentration, while in sample 7 the CBD concentration was higher. Our results are in accordance with Johnson et al, who have concluded that only 8% of the tested products had CBD concentrations within 10% of the advertised values [39].

Table 5. Cannabinoid concentration found in samples (mg/100 g)

Sample	CBG	CBN	CBDA	CBGA	CBVA
1	447± 6.70	1386± 45.6	4.65 ± 0.21	0.07 ± 0.003	0.12 ± 0.01
2	42.2 ± 4.16	8.31 ± 1.28	110± 4.20	5.58 ± 0.22	13.0 ± 1.18
3	0.98 ± 0.24	1.86 ± 0.19	3.77 ± 0.11	4.20 ± 0.21	0.03 ± 0.002
4	904 ± 42.1	403± 9.55	79.2 ± 3.38	71.7 ± 1.51	11.46 ± 0.45
5	8.24 ± 0.09	3.99 ± 0.12	1.85 ± 0.19	0.58 ± 0.13	0.03 ± 0.003
6	9.26 ± 1.21	0.94 ± 0.09	6.43 ± 0.30	5.28 ± 0.42	0.08 ± 0.01
7	268 ± 11.9	21.1 ± 3.64	0.61 ± 0.03	0.51 ± 0.03	0.01 ± 0.0002
8	N.D.	0.31 ± 0.04	13.0 ± 1.04	0.59 ± 0.04	0.87 ± 0.05
9	35.4 ± 7.81	1.82 ± 0.10	55.9 ± 1.87	10.8 ± 1.26	6.04 ± 0.49
10	6.16 ± 0.39	2.40 ± 0.29	37.4 ± 0.70	5.24 ± 0.36	6.46 ± 0.22

N.D. – not detected

LC-MS/MS offers superior sensitivity, selectivity, and speed, which are essential for quantifying cannabinoids, particularly in complex food supplement matrices where multiple cannabinoids might co-exist at varying concentrations. Unlike GC, LC-MS/MS eliminates the need for derivatization and provides precise quantification of both neutral and acidic cannabinoids.

Additionally, the capability to operate in electrospray ionization mode further enhances detection specificity.

Most regulations regarding the cannabinoid levels in food supplements are only focused on THC because of its psychoactive effects. However, other non-psychoactive cannabinoids can have different actions and may act in synergy with CBD. Cannabigerol has been shown to have anti-inflammatory and neuromodulatory effects. These are primarily explained by the affinity of CBG to different receptors like α-2, 5-HT_{1A}, and PPARγ [40,41].

The present study showed the great diversity of commercial products that are found on the market. Our results are similar to those obtained by other researchers regarding the irregularities in the advertised concentration of CBD, with the mention that in our study, the problematic products were fewer compared with other studies [23].

Due to the wide variety of cannabinoid types and the products in which they can be found, there is no universal analytical method that allows for the accurate and precise quantification of these compounds with appropriate detection limits. Therefore, the analytical methodology must be adapted according to the sample treatment required by their matrix and the types and concentrations of substances expected or suspected to be present in products intended for consumption or medical treatment. Additionally, adapting a method published in the specialized literature often proves to be difficult or even impossible, either due to the inadequate technical performance of the equipment on which the method is intended to be implemented, the more laborious and costly sample preparation process proposed, or the discovery of limitations in methodologies that are not always clearly highlighted in the articles [42,43].

In this regard, the present study proposes an LC-MS/MS method that considers six cannabinoids as target analytes and two categories of products: oils and plant-based products. Based on the advanced type of mass spectrometry detection used, Q-TOF, the method ensures specificity/ selectivity and an appropriate quantification level for the intended purpose.

Through relatively simple sample preparation methods, such as dilution, solvent extraction from plants, and filtration, the developed and validated LC-MS/MS method allows for the identification and quantification of the selected analytes with accuracy and precision within a short analysis time of 10 minutes. It also enables the detection of some investigated substances in commercial products, even in cases where their presence is not declared.

Lastly, this study proposes an LC-MS/MS fingerprinting method for commercial dietary supplements and provides an overview of the situation in Romania. Based on a review of specialized literature, there are some recent data regarding the analysis of dietary supplement trends in Romania, but significantly fewer analytical studies on their quality control [44].

CONCLUSIONS

The validated method allows the simultaneous quantification of CBDA, CBGA, CBVA, CBD, CBG, and CBN in commercial products. The method showed good linearity across a wide concentration range for each cannabinoid. Regarding the quality control of the food supplements, significant differences in concentrations were observed for three tested products, between the advertised concentration versus the actual CBD concentration. Because of this unregulated industry is it difficult to assess the quality of these types of food supplements and to ensure the safety of cannabinoid-containing food supplements for consumers. Overall, our work contributes to the field by offering a robust alternative that can be readily adopted for routine and regulatory cannabinoid analysis.

EXPERIMENTAL SECTION

Chemicals and reagents

Cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), and cannabidivarinic acid (CBVA) were purchased from Cayman Chemical Company (Michigan, USA). LC-MS grade acetonitrile was purchased from Riedel-de Haen (Seelze, Germany), methanol was purchased from VWR International (Fontenays, France) and ammonium formiate was purchased from Fluka (Buchs, Switzerland). Ultrapure water was obtained from a Millipore Direct-Q water purification system (Millipore, Bedford, MA, USA).

Calibration curve

Stock solutions of 2.5 mg/ mL in methanol were prepared for CBDA, CBGA, CBVA, and CBG, while stock solutions of 5 mg/mL in methanol were prepared for CBD and CBN.

A solution of 1 µg/ mL of each CBD, CBN, CBG, CBDA, CBGA, and CBVA in a mixture of water:methanol (3:7, *v/v*) was obtained using these stock solutions and was further diluted with the same solvent to obtain calibration curve standards within the range of 5–1000 ng/mL. The calibration curve was composed of 10 levels with nominal concentrations between 5 – 1000 ng/mL for each analyte. The average calibration curve was plotted using a linear fit and $1/y^2$ weighting and the accuracy of each calibration standard was calculated for each calibration curve.

Sample preparation

Ten food supplements with CBD, from different manufacturers, were purchased from local pharmacies.

Table 6. The type of food supplements included in the present study

No. of sample	Type of product	Label mentions
1	10% CBD oil	
2	2.5 % CBD oil	
3	Mouthwash	
4	Hemp seed oil 1000 mg CBD	*5% CBDA, 5% CBGA*
5	2.5% CBD oil	
6	CBD	
7	CBD	
8	CBD tea	*tea mixture with 10% aerial parts from Cannabis sativa*
9	*Cannabis sativa* leaves tea	
10	*Cannabis sativa* CBD tea	

The CBD oils were diluted with a 1:1 (*v/v*) mixture acetonitrile: methanol, sonicated for 10 minutes at room temperature and centrifuged at 11000 rpm for 5 minutes with a digital angle centrifuge (Nahita 2615/1, Spain). From the herbal products, 1 g was extracted with 50 mL acetonitrile–methanol (1:1, *v/v*) mixture in an ultrasonic water bath (Nahita 626, Spain) for 30 minutes, at 40 °C [45,46]. All samples were filtered through a 0.45 µm Rotilabo mini-tip syringe filter, before the analysis.

LC-MS/MS instrumentation and conditions

The LC-MS/MS system consisted of an UHPLC Flexar FX-10 (Perkin Elmer, USA) system coupled with a triple quadrupole tandem mass spectrometer (QTOF 4600, AB Sciex). The chromatographic separations were carried out using a Kinetex XB-C18 column (3.0 x 100 mm, 2.5 µm), using a mixture with a ratio of 35% ammonium formate 20 mM (solvent A) and acetonitrile (solvent B) as a mobile phase, with isocratic elution and a flow rate set to 0.8 mL/min. The injection volume was 5 µL, the column temperature was set at 15 °C, the temperature of the samples was 20 °C, and the analysis time was 10 minutes. An electrospray ionization source with negative ion mode was used. Ionization parameters were as follows: Spray voltage: -4500V, vaporizer temperature: 450 ºC, Ion Gas Source 1: 50 bar, Ion Gas Source 2: 35 bar, Curtain Gas: 10 bar, Declustering Potential: -100V, Ion Release Delay: 42 ms, Ion Release Width: 18. Data files were processed by AB Sciex Analyst Mass Spectrometry Software.

The detections of analytes were performed by monitoring the sum of ion fragments generated from molecular ions at specific collision energy as follows:
- Cannabidiolic acid (CBDA) ions *m/z* 179.16, *m/z* 245.22, *m/z* 311.30 and *m/z* 339.29 from *m/z* 357.3 at a collision energy of -34V;
- Cannabigerolic acid (CBGA) ions *m/z* 191.16, *m/z* 241.23, *m/z* 297.30, *m/z* 315.32 and *m/z* 341.30 from *m/z* 359.32 at a collision energy of -20V;
- Cannabidivarinic acid (CBVA) ions *m/z* 217.18, *m/z* 243.17, *m/z* 283.25, *m/z* 311.25 and *m/z* 199.17 from *m/z* 329.2 at a collision energy of -32V;
- Cannabidiol (CBD) ions *m/z* 179.10, *m/z* 245.20 and *m/z* 311.25 made from *m/z* 313.3 at a collision energy of -25V;
- Cannabigerol (CBG) ions *m/z* 136.09 and *m/z* 191.15 made from *m/z* 315.3 at a collision energy of -31V;
- Cannabinol (CBN) ions *m/z* 171.13 and *m/z* 279.21 made of *m/z* 309.3 at a collision energy of -35V.

Method validation

Carry over

To check the carry-over effect, a blank solution (mobile phase) was injected immediately after the injection of the standard solution with the highest concentration.

Sensitivity and selectivity

To check the sensitivity and selectivity of the method, a blank solution was injected at the beginning of each sequence and the peaks (if any) at the retention times of the analytes were compared with those of the standard solution with the lowest concentration (LLOQ).

Linearity

Linearity was checked for each of the five validation sequences and for each sequence used for sample analysis. The fitter calibration curves were checked for correlation coefficient ($r > 0.99$) and accuracy of recalculated calibration standard concentrations compared to theoretical concentrations - a bias (%) not greater than ± 15%, except LLOQ for which a higher inaccuracy of ±20% was accepted.

Accuracy and precision

Accuracy and precision of the method were determined at 5 concentration levels (5, 25, 250, 500, and 750 ng/mL for each analyte) using quality control (QC samples). For accuracy a bias (%) not greater than ± 15% was considered acceptable, except LLOQ for which an inaccuracy of ± 20% was accepted. For precision, a coefficient of variation not greater than 15% was considered acceptable, except for LLOQ for which the acceptable value was 20%.

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