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Analysis of Cannabidiol, Δ^9-Tetrahydrocannabinol, and Their Acids in CBD Oil/Hemp Oil Products

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Hemp products · Gas chromatography coupled with mass spectrometry · Cannabidiol oil · Cannabidiol · Δ^9-Tetrahydrocannabinol

Abstract
Hemp products are readily available and are aggressively marketed for their health and medicinal benefits. Most consumers of these products are interested because of cannabidiol (CBD), which has taken the natural products industry by storm. The CBD and Δ^9-tetrahydrocannabinol (Δ^9-THC) concentrations in these products are often absent, and even where labeled, the accuracy of the label amounts is often questionable. In order to gain a better understanding of the CBD content, fifty hemp products were analyzed by gas chromatography coupled with mass spectrometry (GC-MS) for CBD, Δ^9-THC, tetrahydrocannabinolic acid (Δ^9-THCAA), and cannabidiolic acid (CBDA). Δ^9-THCAA and CBDA are the natural precursors of Δ^9-THC and CBD in the plant material. Decarboxylation to Δ^9-THC and CBD is essential to get the total benefit of the neutral cannabinoids. Therefore, analysis for the neutral and acid cannabinoids is important to get a complete picture of the chemical profile of the products. The GC-MS method used for the analysis of these products was developed and validated. A 10-m × 0.18-mm DB-1 (0.4 µ film) column was used for the analysis. The majority of the hemp products were oils, one of the products was hemp butter, one was a concentrated hemp powder capsule, and another was a hemp extract capsule. Most of the products contained less than 0.1% CBD and less than 0.01% Δ^9-THC. Three products contained 0.1–1% CBD, and 2 products contained 0.1–0.9% Δ^9-THC. All of the samples appeared to be decarboxylated since the CBDA and Δ^9-THCAA results were less than 0.001%. The developed method is simple, sensitive, and reproducible for the detection of Δ^9-THC, Δ^9-THCAA, CBD, and CBDA in CBD oil/hemp products.

Introduction

In the last 2 decades, there has been an escalation in Cannabis use in the USA, with growing public popularity and pressure, together with an inconsistent and confused regulatory picture. In the USA, the use and possession of

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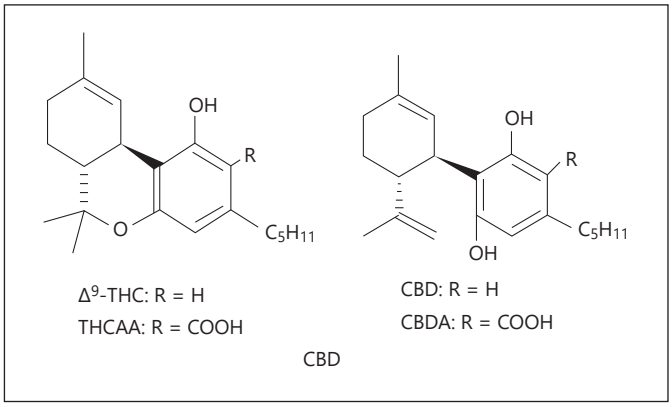

Fig. 1. Chemical structures of (–)-*trans*-Δ^9-THC, CBD, Δ^9-THCAA, and CBDA. Δ^9-THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; Δ^9-THCAA, Δ^9-tetrahydrocannabinol-acid-A; CBDA, cannabidiolic acid.

marijuana is a federal crime; however, medical marijuana legislation has been adopted in the District of Columbia and in 33 states, while recreational marijuana use has been legalized in 14 states and US territories [1]. In addition, 13 states have now passed legislation to allow certain CBD products, restricted in Δ^9-THC content, for specific disease indications [1]. In the states in which medical marijuana has been legalized, recent studies have shown that 16–26% of medical cannabis users also consume other hemp products [2, 3]. In December 2018, the passage of the "Farm Bill" (Agriculture Improvement Act of 2018) greatly accelerated the aggressive marketing of CBD products. That legislation redefined "hemp" as *Cannabis sativa* containing <0.3% dry wt. of the psychoactive cannabinoid Δ^9-THC, provided it is produced under regulations and guidelines stipulated in the statute [4]. It also removed "hemp," so defined, as a Schedule I substance. This increasing trend of legalization has led to the production of hundreds of kinds of hemp and hemp oil products, commercialized in various forms, including oils, balms, lotions, candies, and capsules. These products contain variable concentrations of Δ^9-THC and CBD.

Δ^9-THC exerts its actions through interactions with the CB_1 and CB_2 receptors [5]; the CB_1 agonist activity is, however, responsible for giving the user a feeling of being "high" when consumed in moderation. The pharmacological effects of CBD are much less well known. It has been reported that CBD may act as an inverse agonist or antagonist on CB_1 and CB_2 receptors [6]; however, this varies by cell type and the agonist ligand being studied. CBD also has activity on a number of other receptors: it antagonized the G-protein-coupled receptor GPR55 and the transient receptor potential channel TRPM8 [7]. When combined with Δ^9-THC, it may serve to counter some of the psychotropic effects of Δ^9-THC [8, 9].

Unfortunately, the concentration levels of these 2 cannabinoids are often unknown in these products which are widely sold on the Internet, and the labels are found to omit or inaccurately list these concentrations [10, 11]. It is important to identify these values as the concentration is determinant of the dosage required for medical use, as well as for the determination of the legality of the possession of hemp products.

In this article, we report the development and validation of a GC-MS method for the identification and quantitation of the 2 most principal cannabinoids, Δ^9-THC and CBD (Fig. 1), in CBD oil and hemp oil products. This GC-MS method is able to analyze these cannabinoids and their acid precursors down to low concentrations, with a limit of detection (LOD) and limit of quantitation (LOQ) of 0.1 μg (absolute) and 0.25 μg (absolute), respectively, in the products tested.

Table 1. GC temperature program

Time	Event, °C
0.00	180
1.00	180
7.66	280
11.00	280
13.00	180

GC, gas chromatography.

Materials and Methods

Instrumentation and GC Conditions

GC-MS analysis was performed on an Agilent Technologies 7890A gas chromatograph with an Agilent Technologies 5975C MSD and an Agilent Technologies 7693 autosampler. Separation was achieved on an Agilent Technologies 10-m × 0.18-mm DB-1 column (0.4 μ film). Helium was used as the carrier gas at a flow rate of 0.4 mL/min. The inlet was configured in splitless mode at a temperature of 250°C. The temperature program started at 180°C for 1 min and then ramped up at 15°C/min to 280°C for 5.33 min (Table 1). The total run time was ~13 min. Retention times of all analytes are shown in Table 2. Data acquisition was performed on ChemStation G1701EA E.02.01.117. Table 3 lists the ions acquired using the SIM mode.

Chemicals and Reagents

Hexane, chloroform, and hexane ethyl acetate (9:1) were all analytical grade. BSTFA+1% TMCS was purchased from Sigma

Aldrich; 1 n HCl was prepared by diluting 10 mL of concentrated HCl to 100 mL with deionized water, and 0.2 n methanolic NaOH was prepared by combining 450 mL of MeOH with 50 mL of 2 n NaOH.

Standard Solutions

Two 1.0 mg/mL cannabinoid standard solutions of Δ^9-THC and CBD were purchased from Cerilliant. Δ^9-THCAA (1.0 mg/mL) was purchased from Lipomed, and CBDA (1.0 mg/mL) was prepared at ElSohly Laboratories, Inc. All four 1.0 mg/mL standard solutions, Δ^9-THC, CBD, Δ^9-THCAA, and CBDA, as well as a 10 μg/mL dilution of each standard were used to prepare the calibration curves.

Internal Standard Solutions

Two 100 μg/mL internal standard solutions, d_3-Δ^9-tetrahydrocannabinol and d_3-cannabidiol, were purchased from Cerilliant. Both internal standards were added to all samples, calibrators, and controls at a concentration of 1 μg in each test sample.

Sample Preparation

An accurately weighed 50–100 mg of material was diluted with hexane to make 10 mg/mL samples. A volume of 1 mL (straight sample, 10 mg of oil) and 0.1 mL (dilute sample, 1 mg of oil) of the hexane solutions were spiked with 10 μL of d_3-Δ^9-tetrahydrocannabinol (100 μg/mL) and d_3-cannabidiol (100 μg/mL). The solutions were adjusted to 5 mL with hexane and vortexed. To this, 4 mL of 0.2 n sodium hydroxide was added and mixed. The solution was centrifuged, and the top layer (hexane) was discarded. A volume of 1.5 mL of 1 n HCl was added to the basic layer and mixed, with the pH checked to be between 1 and 2. A volume of 1 mL of hexane was added, and the sample was mixed and centrifuged. The top layer was transferred to a GC vial and evaporated. The sample was derivatized using *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide to make a trimethylsilyl derivative, followed by analysis using GC-MS.

Method Validation

The validation was executed using 100 mg of a hemp oil product certified to contain Δ^9-THC 5.5 μg/g, CBD 22.5 μg/g, Δ^9-THCAA 7.5 μg/g, and CBDA 100 μg/g. This control was used to validate the GC-MS method with 6 replicates over a period of 6 days with 4-point calibration curves (0.25, 0.5, 1.0, and 5.0 μg absolute). The accuracy was calculated using the standard addition method. The LOD, LOQ, and upper limit of linearity (ULOL) are listed in Table 2, and the accuracy, RSD, and precision for the 2 cannabinoids are listed in Tables 4 and 5.

Linearity

Linearity was calculated in 6 validation batches by using 4-point standard calibration curves (2.5, 5, 10, and 50 μg/g). The concentration-response relationship of the GC-MS method indicated a linear relationship between the concentration and response ratio with r^2 values of >0.99 for all the cannabinoids as follows: CBD (r^2 > 0.9999), Δ^9-THC (r^2 > 1.0000), CBDA (r^2 > 0.9999), and Δ^9-THCAA (r^2 > 0.9999).

Table 2. Retention times, LOD, LOQ, and ULOL of (−)-*trans*-Δ^9-THC, CBD, Δ^9-THCAA, and CBDA

	Δ^9-THC	CBD	Δ^9-THCAA	CBDA
Retention time, min	5.444	4.900	7.002	6.370
LOD, μg/g	1.00	1.00	1.00	1.00
LOQ, μg/g	2.50	2.50	2.50	2.50
ULOL, μg/g	250.00	100.00	250.0	250.0

ULOL, upper limit of linearity; LOD, limit of detection; LOQ, limit of quantitation; Δ^9-THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; Δ^9-THCAA, Δ^9-tetrahydrocannabinol-acid-A; CBDA, cannabidiolic acid.

Table 3. Ions monitored for the 4 cannabinoids and the internal standards

ID	Mass, amu	Q1, *m*/*z*	Q2, *m*/*z*
Δ^9-THC	314.00	371.00	386.00
THCAA	358.00	487.00	502.00
d_3-Δ^9-THC	317.00	374.00	389.00
CBD	314.00	393.00	304.00
CBDA	358.00	492.00	560.00
d_3-CBD	317.00	390.00	301.00

Δ^9-THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; Δ^9-THCAA, Δ^9-tetrahydrocannabinol-acid-A; CBDA, cannabidiolic acid.

Accuracy and RSD

The accuracy and RSD for the 4 cannabinoids were determined for within-batch and batch-to-batch (6 batches). For batch 1, the accuracy and RSD for the 5.5 μg/g control of Δ^9-THC were calculated to be 101.21% (RSD 0.03); the accuracy and RSD for the 22.5 μg/g control of CBD were determined to be 99.26% (RSD 0.02); the accuracy and RSD for the 7.5 μg/g control of Δ^9-THCAA were determined to be 92.67% (RSD 0.06); and the accuracy and RSD for the 100 μg/g control of CBDA were determined to be 90.78% (RSD 0.07). For batch 2, the accuracy and RSD for the 5.5 μg/g control of Δ^9-THC were calculated to be 104.55% (RSD 0.02); the accuracy and RSD for the 22.5 μg/g control of CBD were determined to be 105.78% (RSD 0.02); the accuracy and RSD

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Table 4. Within-batch mean, RSD, accuracy, and precision for (−)-*trans*-Δ^9-THC, CBD, Δ^9-THCAA, and CBDA

Concentration:	THC 5.50 5.50 μg/g				CBD 22.5 22.5 μg/g				THCAA 7.50 7.5 μg/g				CBDA 100 100 μg/g			
	conc.	accuracy, %	within batch		conc.	accuracy, %	within batch		conc.	accuracy, %	within batch		conc.	accuracy, %	within batch	
Batch 1	5.80	105	Mean	5.57	22.8	101	Mean	22.33	7.10	95	Mean	6.95	94.6	95	Mean	90.78
	5.50	100	SD	0.16	22.5	100	SD	0.47	7.70	103	SD	0.43	87.1	87	SD	6.60
	5.60	102	Accuracy	101.21%	22.5	100	Accuracy	99.26%	6.80	91	Accuracy	92.67%	85.4	85	Accuracy	90.78%
	5.60	102	Precision	97.07%	22.6	100	Precision	97.91%	6.60	88	Precision	93.78%	98.1	98	Precision	92.73%
	5.30	96	%CV	2.93%	21.5	96	%CV	2.09%	6.50	87	%CV	6.22%	82.5	83	%CV	7.27%
	5.60	102			22.1	98			7.00	93			97.0	97		
Batch 2	5.80	105	Mean	5.75	24.7	110	Mean	23.80	8.90	119	Mean	8.12	114.0	114	Mean	103.17
	5.70	104	SD	0.10	23.4	104	SD	0.52	8.90	119	SD	0.74	93.0	93	SD	12.01
	5.70	104	Accuracy	104.55%	23.6	105	Accuracy	105.78%	8.50	113	Accuracy	108.22%	96.0	96	Accuracy	103.17%
	5.90	107	Precision	98.18%	24.1	107	Precision	97.81%	7.40	99	Precision	90.90%	105.5	106	Precision	88.36%
	5.80	105	%CV	1.82%	23.7	105	%CV	2.19%	7.70	103	%CV	9.10%	90.5	91	%CV	11.64%
	5.60	102			23.3	104			7.30	97			120.0	120		
Batch 3	5.30	96	Mean	5.27	22.5	100	Mean	22.68	7.60	101	Mean	8.43	97.0	97	Mean	90.00
	5.10	93	SD	0.16	22.8	101	SD	0.44	7.90	105	SD	0.53	83.0	83	SD	13.10
	5.40	98	Accuracy	95.76%	23.0	102	Accuracy	100.81%	8.40	112	Accuracy	112.44%	86.0	86	Accuracy	90.00%
	5.10	93	Precision	96.90%	22.1	98	Precision	98.08%	8.30	111	Precision	93.74%	94.0	94	Precision	85.45%
	5.20	95	%CV	3.10%	22.4	100	%CV	1.92%	8.70	116	%CV	6.26%	93.0	93	%CV	14.55%
	5.50	100			23.3	104			9.70	129			87.0	87		
Batch 4	5.50	100	Mean	5.27	23.5	104	Mean	22.57	7.60	101	Mean	7.78	102.0	102	Mean	100.20
	5.50	100	SD	0.40	23.8	106	SD	1.56	7.20	96	SD	0.49	88.8	89	SD	9.96
	5.20	95	Accuracy	95.76%	22.1	98	Accuracy	100.30%	7.30	97	Accuracy	103.78%	93.0	93	Accuracy	100.20%
	5.60	102	Precision	92.34%	23.7	105	Precision	93.10%	8.40	112	Precision	93.68%	117.0	117	Precision	90.06%
	5.30	96	%CV	7.66%	22.6	100	%CV	6.90%	8.00	107	%CV	6.32%	96.4	96	%CV	9.94%
	4.50	82			19.7	88			8.20	109			104.0	104		
Batch 5	5.30	90	Mean	5.13	23.0	102	Mean	22.42	6.50	87	Mean	7.28	103.0	103	Mean	105.00
	5.30	90	SD	0.15	23.8	101	SD	1.22	8.00	107	SD	0.76	117.0	117	SD	7.60
	5.10	86	Accuracy	87.01%	22.5	100	Accuracy	99.63%	7.50	100	Accuracy	97.11%	98.6	99	Accuracy	105.00%
	5.10	86	Precision	97.074%	22.4	100	Precision	94.55%	7.90	105	Precision	89.59%	108.0	108	Precision	92.77%
	5.10	86	%CV	2.93%	22.5	100	%CV	5.45%	6.50	87	%CV	10.41%	95.4	95	%CV	7.23%
	4.90	83			21.3	95			7.30	97			108.0	108		
Batch 6	5.30	90	Mean	5.20	23.9	106	Mean	22.50	7.40	99	Mean	6.98	107.0	107	Mean	108.67
	5.00	85	SD	0.23	21.7	96	SD	0.91	6.50	87	SD	0.38	113.0	113	SD	3.83
	5.00	85	Accuracy	88.14%	21.9	97	Accuracy	100.00%	6.60	88	Accuracy	93.11%	106.0	106	Accuracy	108.67%
	5.10	86	Precision	95.61%	21.8	97	Precision	95.98%	6.90	92	Precision	94.61%	107.0	107	Precision	96.48%
	5.20	88	%CV	4.39%	22.4	100	%CV	4.02%	7.20	96	%CV	5.39%	114.0	114	%CV	3.52%
	5.60	95			23.3	104			7.30	97			105.0	105		

Δ^9-THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; Δ^9-THCAA, Δ^9-tetrahydrocannabinol-acid-A; CBDA, cannabidiolic acid.

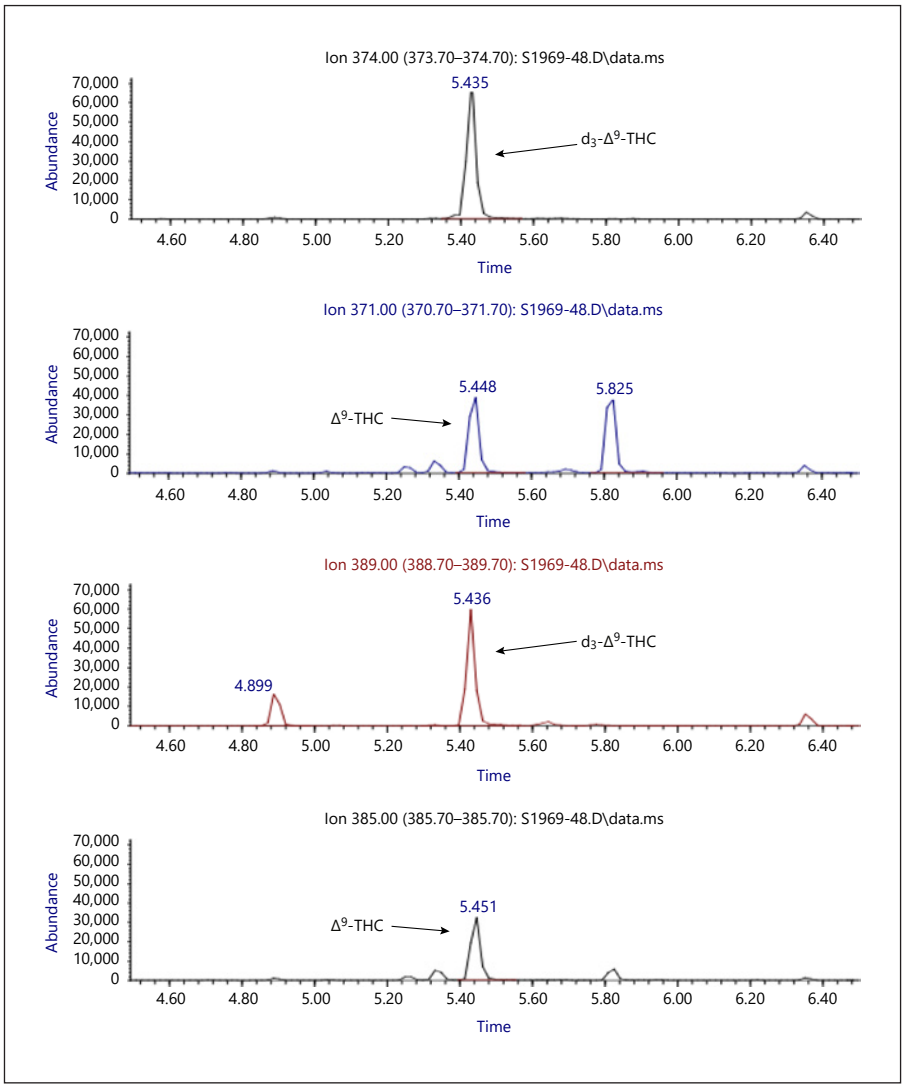


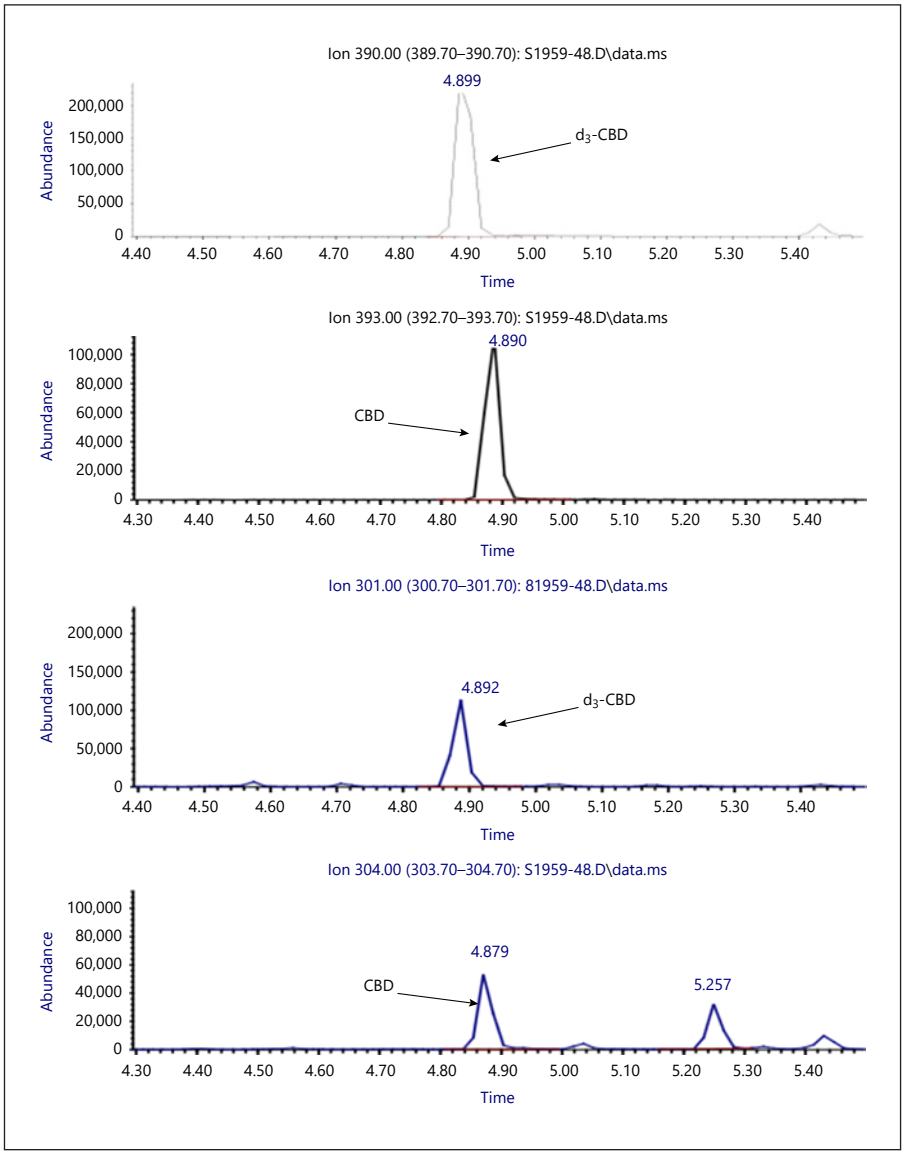
Fig. 2. Representative chromatograms showing the ions monitored for (–)-*trans*-Δ^9-THC and the deuterated internal standard (d_3-Δ^9-THC) in the control (5.5 µg/g for Δ^9-THC). Δ^9-THC, Δ^9-tetrahydrocannabinol.

Table 5. Overall mean, RSD, accuracy, and precision for (–)-*trans*-Δ^9-THC, CBD, Δ^9-THCAA, and CBDA

Overall	THC 5.50	CBD 22.50	THCAA 7.5	CBDA 100
Mean	5.36	22.72	7.59	99.64
Accuracy, %	97.53	100.96	101.22	99.64
n = 6 batches				
SD	0.11	0.46	0.16	3.50
Precision, %	98.02	97.97	97.90	96.49
%CV, %	1.98	2.03	2.10	3.51
n = 36 samples				
SD	0.30	0.93	0.79	10.31
Precision, %	94.33	95.91	89.60	89.65
%CV, %	5.67	4.09	10.40	10.35

Δ^9-THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; Δ^9-THCAA, Δ^9-tetrahydrocannabinol-acid-A; CBDA, cannabidiolic acid.

for the 7.5 µg/g control of Δ^9-THCAA were determined to be 108.22% (RSD 0.09); and the accuracy and RSD for the 100 µg/g control of CBDA were determined to be 103.17% (RSD 0.12). For batch 3, the accuracy and RSD for the 5.5 µg/g control of Δ^9-THC were calculated to be 95.76% (RSD 0.03); the accuracy and RSD for the 22.5 µg/g control of CBD were determined to be 100.81% (RSD 0.02); the accuracy and RSD for the 7.5 µg/g control of Δ^9-THCAA were determined to be 112.44% (RSD 0.06); and the accuracy and RSD for the 100 µg/g control of CBDA were determined to be 90.00% (RSD 0.15). For batch 4, the accuracy and RSD for the 5.5 µg/g control of Δ^9-THC were calculated to be 95.76% (RSD 0.08); the accuracy and RSD for the 22.5 µg/g control of CBD were determined to be 100.30% (RSD 0.07); the accuracy and RSD for the 7.5 µg/g control of Δ^9-THCAA were deter-



Color version available online

Fig. 3. Representative chromatograms showing the ions monitored for CBD and the deuterated internal standard (d_3-CBD) in the control (22.5 μg/g for CBD). CBD, cannabidiol.

mined to be 103.78% (RSD 0.06); and the accuracy and RSD for the 100 μg/g control of CBDA were determined to be 100.20% (RSD 0.10). For batch 5, the accuracy and RSD for the 5.5 μg/g control of Δ^9-THC were calculated to be 87.01% (RSD 0.03); the accuracy and RSD for the 22.5 μg/g control of CBD were determined to be 99.63% (RSD 0.06); the accuracy and RSD for the 7.5 μg/g control of Δ^9-THCAA were determined to be 97.11% (RSD 0.10); and the accuracy and RSD for the 100 μg/g control of CBDA were determined to be 105.00% (RSD 0.07). For batch 6, the accuracy and RSD for the 5.5 μg/g control of Δ^9-THC were calculated to be 88.14% (RSD 0.04); the accuracy and RSD for the 22.5 μg/g control of CBD were determined to be 100% (RSD 0.04); the accuracy and RSD for the 7.5 μg/g control of Δ^9-THCAA were determined to be 93.11% (RSD 0.05); and the accuracy and RSD for the 100 μg/g control of CBDA were determined to be 108.67% (RSD 0.04).

For the overall calculations, the accuracy for the 5.5 μg/g control of Δ^9-THC was determined to be 97.53%; the accuracy for the 22.5 μg/g control of CBD was determined to be 100.96%; the accuracy for the 7.5 μg/g control of Δ^9-THCAA was determined to be 101.22%; and the accuracy for the 100 μg/g control of CBDA was determined to be 99.64%. For the overall n = 36 samples, the RSD for the 5.5 μg/g control of Δ^9-THC was calculated to be 0.06; the

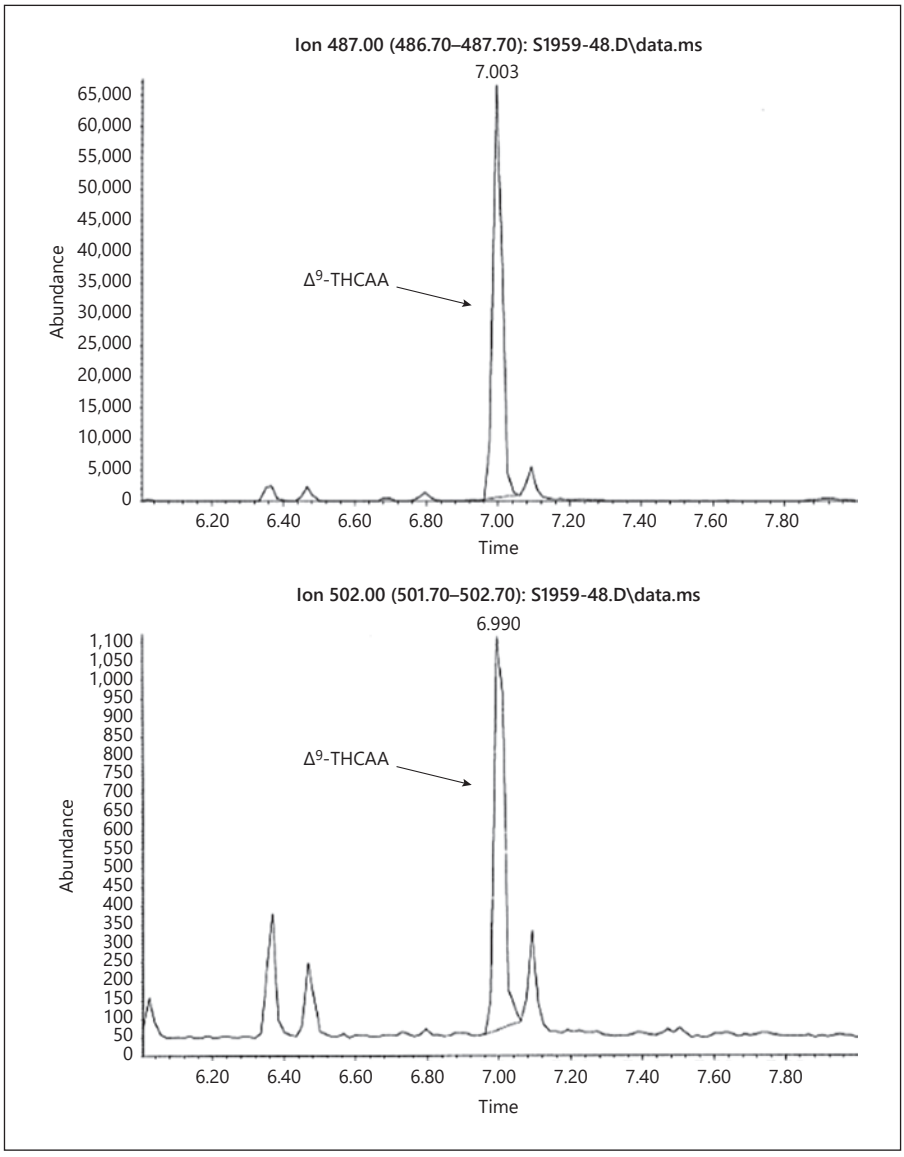


Fig. 4. Representative chromatograms showing the ions monitored for Δ^9-THCAA in the control (7.5 μg/g for Δ^9-THCAA). Δ^9-THCAA, Δ^9-tetrahydrocannabinol-acid-A.

RSD for the 22.5 μg/g control of CBD was determined to be 0.04; the RSD for the 7.5 μg/g control of Δ^9-THCAA was calculated to be 0.10; and the RSD for the 100 μg/g control of CBDA was determined to be 0.10. For the overall $n = 6$ batches, the RSD for the 5.5 μg/g control of Δ^9-THC was calculated to be 0.02; the RSD for the 22.5 μg/g control of CBD was determined to be 0.02; the RSD for the 7.5 μg/g control of Δ^9-THCAA was calculated to be 0.02; and the RSD for the 100 μg/g control of CBDA was determined to be 0.04.

Precision

The precision for the 4 cannabinoids was calculated for within-batch (6 batches) and overall ($n = 36$ samples and $n = 6$ batches). For batch 1, the precision for the 5.5 μg/g control of Δ^9-THC was calculated to be 97.07%; the precision for the 22.5 μg/g control of CBD was determined to be 97.91%; the precision for the 7.5 μg/g control of Δ^9-THCAA was calculated to be 93.78%; and the precision for the 100 μg/g control of CBDA was determined to be 92.73%. For batch 2, the precision for the 5.5 μg/g control of Δ^9-THC was calculated to be 98.18%; the precision for the 22.5 μg/g control of CBD was determined to be 97.81%; the precision for the 7.5 μg/g control of Δ^9-THCAA was

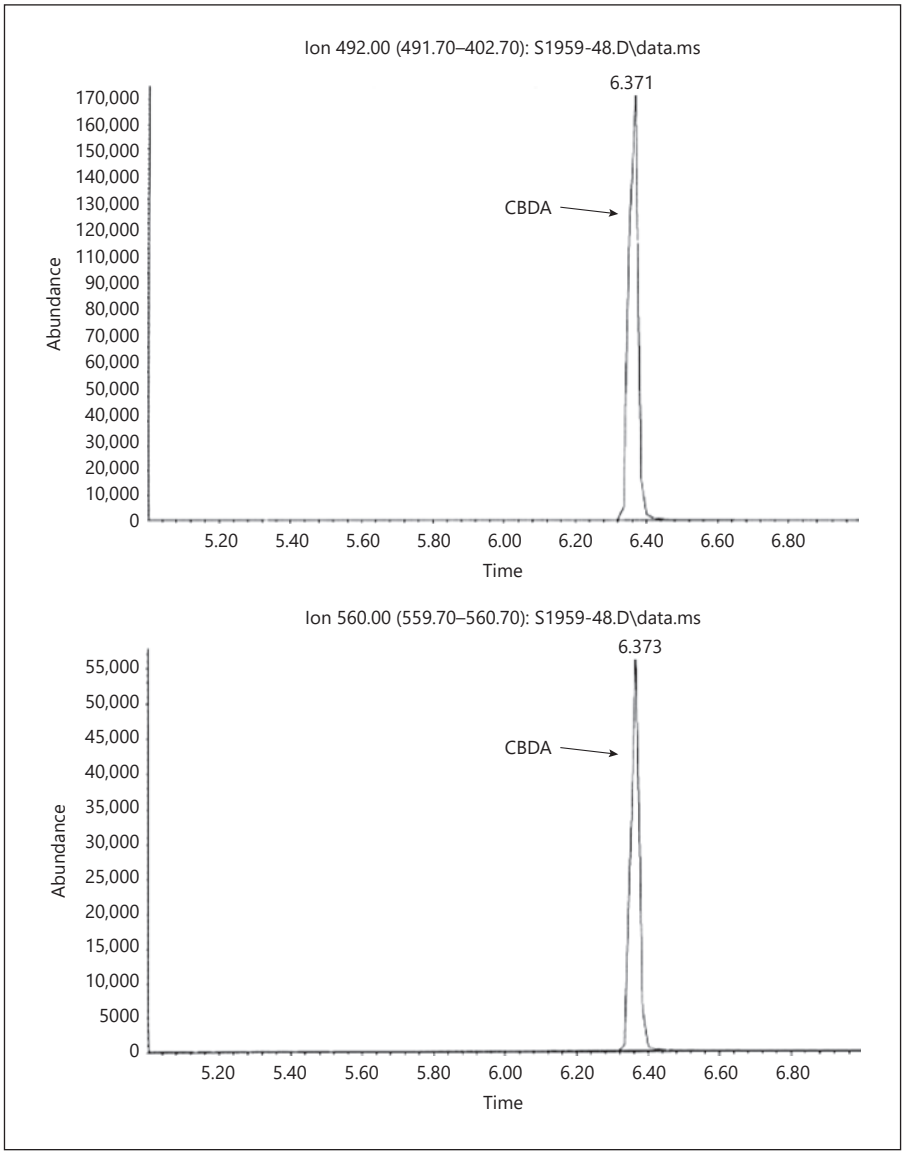


Fig. 5. Representative chromatograms showing the ions monitored for CBDA in the control (100 μg/g for CBDA). CBDA, cannabidiolic acid A.

calculated to be 90.90%; and the precision for the 100 μg/g control of CBDA was determined to be 88.36%. For batch 3, the precision for the 5.5 μg/g control of Δ^9-THC was calculated to be 96.90%; the precision for the 22.5 μg/g control of CBD was determined to be 98.08%; the precision for the 7.5 μg/g control of Δ^9-THCAA was calculated to be 93.74%; and the precision for the 100 μg/g control of CBDA was determined to be 85.45%. For batch 4, the precision for the 5.5 μg/g control of Δ^9-THC was calculated to be 92.34%; the precision for the 22.5 μg/g control of CBD was determined to be 93.10%; the precision for the 7.5 μg/g control of Δ^9-THCAA was calculated to be 93.68%; and the precision for the 100 μg/g control of CBDA was determined to be 90.06%. For batch 5, the precision for the 5.5 μg/g control of Δ^9-THC was calculated to be 97.07%; the precision for the 22.5 μg/g control of CBD was determined to be 94.55%; the precision for the 7.5 μg/g control of Δ^9-THCAA was calculated to be 89.59%; and the precision for the 100 μg/g control of CBDA was determined to be 92.77%. For batch 6, the precision for the 5.5 μg/g control of Δ^9-THC was calculated to be 95.61%; the precision for the 22.5 μg/g control of CBD was determined to be 95.98%; the precision for the 7.5 μg/g control of Δ^9-THCAA was calculated to be 94.61%; and the precision for the 100 μg/g control of CBDA was determined to be 96.48%.

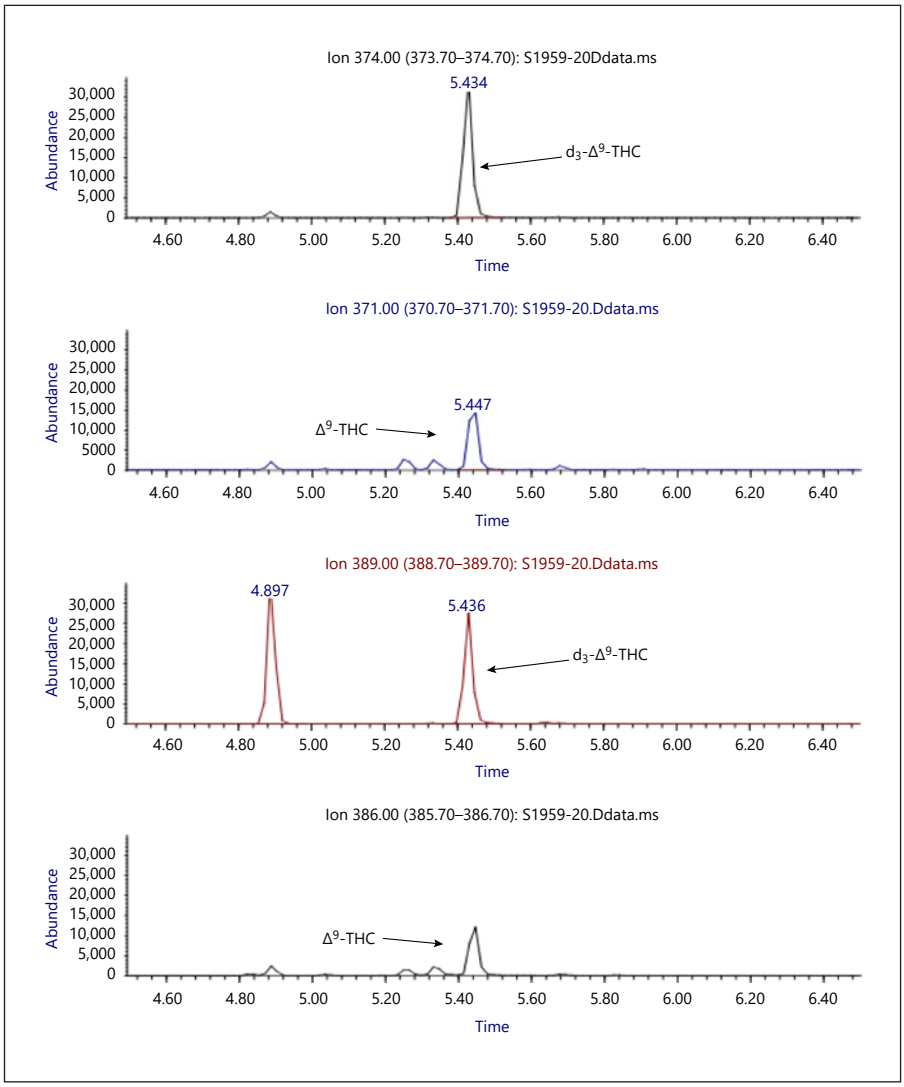


Fig. 6. Representative chromatograms showing the ions monitored for (−)-*trans*-Δ^9-THC and the deuterated internal standard (d_3-Δ^9-THC) in a real sample (CY346). Δ^9-THC, Δ^9-tetrahydrocannabinol.

For the overall 36 samples, the precision for the 5.5 μg/g control of Δ^9-THC was calculated to be 94.33%; the precision for the 22.5 μg/g control of CBD was determined to be 95.91%; the precision for the 7.5 μg/g control of Δ^9-THCAA was calculated to be 89.60%; and the precision for the 100 μg/g control of CBDA was determined to be 89.65%. For the overall $n = 6$ batches, the precision for the 5.5 μg/g control of Δ^9-THC was calculated to be 98.02; the precision for the 22.5 μg/g control of CBD was determined to be 97.97%; the precision for the 7.5 μg/g control of Δ^9-THCAA was calculated to be 97.90%; and the precision for the 100 μg/g control of CBDA was determined to be 96.49%.

Results

A GC-MS method was developed and validated for the quantification of Δ^9-THC, CBD, Δ^9-THCAA, and CBDA in CBD oil/hemp oil products. The structures of the cannabinoids are shown in Figure 1. The GC conditions, including the temperature program (Table 1), were optimized in order to achieve the highest sensitivity of the cannabinoids' peaks (Table 2). Chromatograms of the control (Δ^9-THC at 5.5 μg/g, CBD at 22.5 μg/g, Δ^9-THCAA at 7.5 μg/g, and CBDA at 100 μg/g) are shown in Figures 2–5, and a chromatogram showing these ions in a real sample (CY346) is shown in Figures 6, 7. The ions monitored for the 2 cannabinoids and internal standards are presented in Table 3.

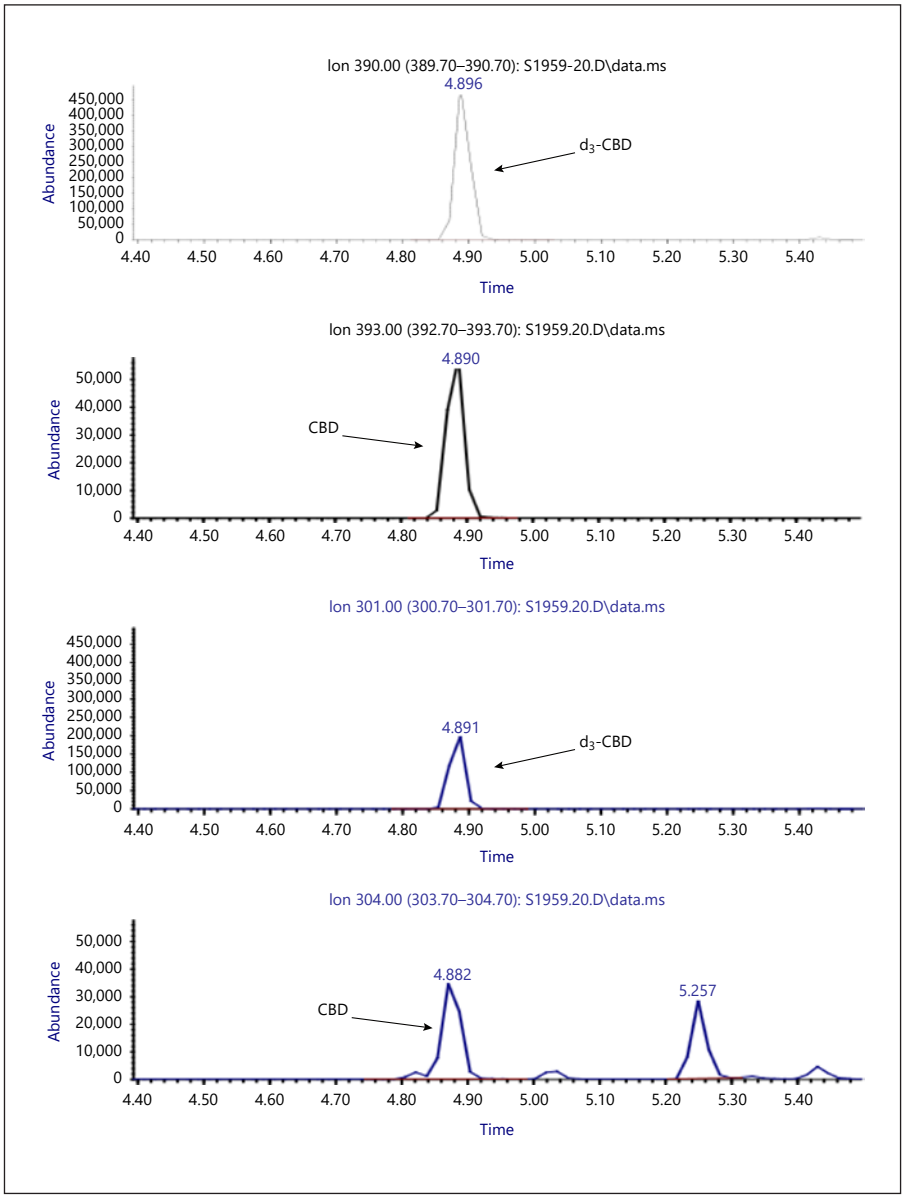


Fig. 7. Representative chromatograms showing the ions monitored for CBD and the deuterated internal standard (d_3-CBD) in a real sample (CY346). CBD, cannabidiol.

The standard curves for Δ^9-THC, CBD, Δ^9-THCAA, and CBDA were linear and had correlation coefficient (r^2) values of 0.9999, 1.0000, 0.999, and 0.9996, respectively. The standard calibration curves for all cannabinoids are shown in Figure 8.

The LOD for Δ^9-THC, CBD, Δ^9-THCAA, and CBDA was calculated to be 1.0 μg/g for each, and the LOQ for the cannabinoids was determined to be 2.5 μg/g for each. The ULOL for Δ^9-THC, Δ^9-THCAA, and CBDA was determined to be 250 μg and 100 μg/g for CBD. The LOD, LOQ, and ULOL of the cannabinoids are shown in Table 2.

The method was validated using 6 replicates of 100 mg hemp oil containing 5.5 μg/g Δ^9-THC, 22.5 μg/g CBD, 7.5 μg/g Δ^9-THCAA, and 100.0 μg/g CBDA in 6 GC-MS batches. The individual and overall accuracies, precisions, and RSD values are shown in Tables 4 and 5.

The developed and validated GC-MS method was applied for the analysis of 50 different CBD oil/hemp products, shown in Table 6. The cannabinoids were identified in each product sample based on their mass spectra and specific retention times.

It can be seen (Table 6) that other than 8 samples, all products had Δ^9-THC and CBD ranging from 0.00002 to

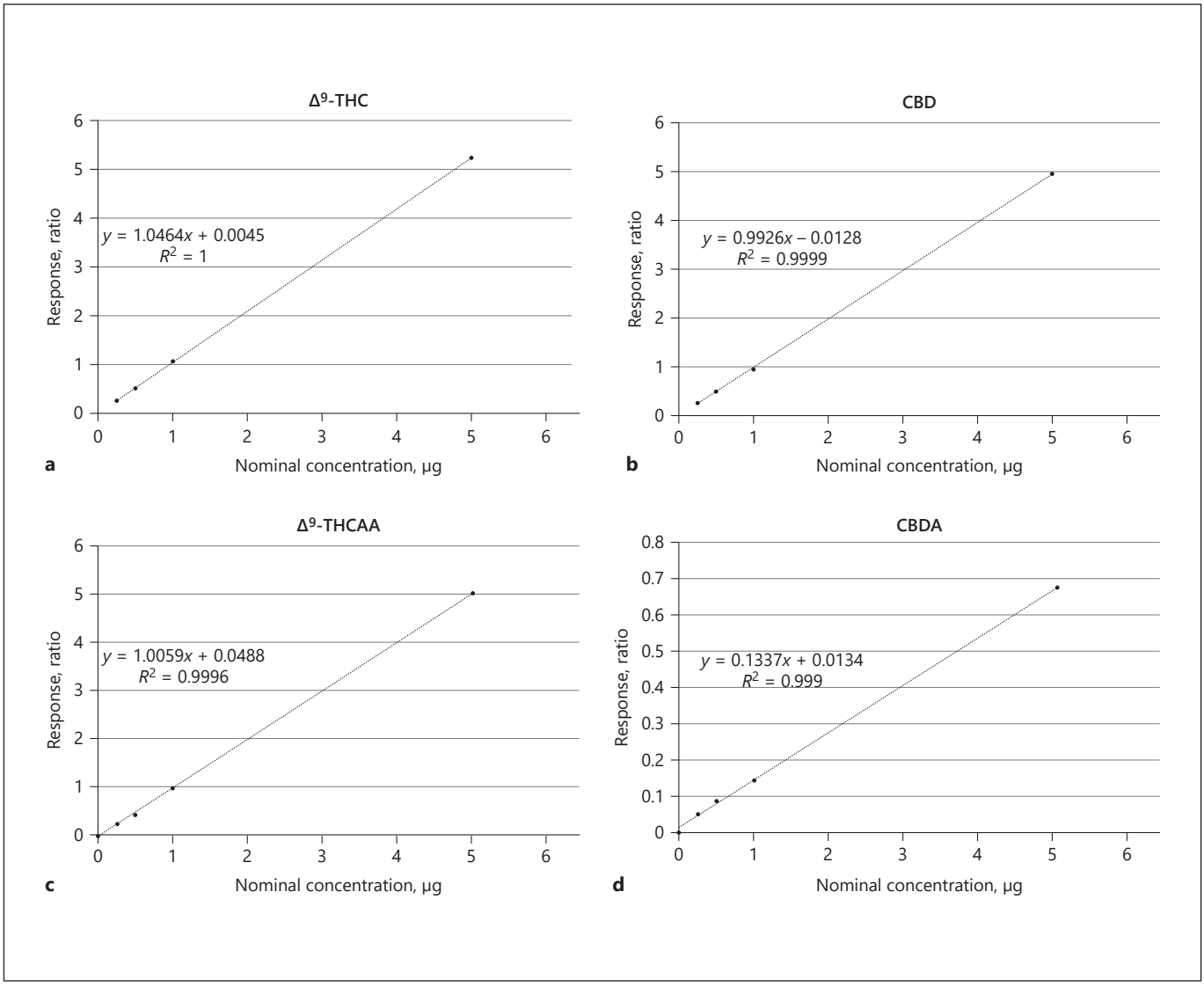


Fig. 8. a Average calibration curve for (–)-*trans*-Δ^9-THC. **b** Average calibration curve for CBD. **c** Average calibration curve Δ^9-THCAA. **d** Average calibration curve for CBDA. Δ^9-THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; Δ^9-THCAA, Δ^9-tetrahydrocannabinol-acid-A; CBDA, cannabidiolic acid.

0.04% in the products tested. The 8 samples were determined to have significant concentrations of Δ^9-THC (0.006–0.797%) and CBD (0.116–17.73%) (Fig. 9; Table 6). These samples were analyzed for concentrations of both Δ^9-THCAA and CBDA. The concentration of Δ^9-THCAA ranged from <0.001 to 0.01%, and the CBDA concentration ranged from <0.001 to 0.44%. This indicated that most products were substantially decarboxylated.

Discussion/Conclusion

A GC-MS method was successfully developed and validated for the analysis of Δ^9-THC, CBD, Δ^9-THCAA, and CBDA in CBD oil/hemp oil products. The method was reproducible for all cannabinoids and was used for the analysis of 50 commercial products. The majority of the products analyzed were oils. One of the products was butter, one was a concentrated powder capsule, and another was a hemp extract capsule. The majority of the products contained less than 0.1% CBD and less than 0.01% THC.

Table 6. Average concentration of Δ^9-THC, CBD, Δ^9-THCAA, and CBDA in CBD oil/hemp oil products

Product name	Accessioning#	% THC	% CBD	% THCA	% CBDA
Cibdex hemp oil nutritional supplement	CY304	0.00125	0.0396	–	–
Hemp oil hemp honey 10 mL bottle 150 mg CBD new bubble gum flavor	CY311	0.00107	0.0587	–	–
Cibdex CBD original flavor hemp oil supplement, 1 oz – 100 mg	CY336	0.00131	0.0576	–	–
Cibdex 1 oz bottle of vanilla hemp oil drops/spray – 100 mg CBD – nutritional supplement	CY337	0.00061	0.0193	–	–
Cibdex 1 oz bottle of unflavored CBD-rich hemp oil drops/spray – CBD – nutritional supplement	CY347	0.00103	0.0357	–	–
Cannabis oil organic extract virgin CBD 1 glass bottle with dropper with 2 oz	CY352	0.00058	0.00292	–	–
Dixie Botanicals Dew Drops natural flavor hemp oil supplement, 1 oz 500 mg + hemp salvation balm, 1.3 oz	CY353	0.00085	0.0363	–	–
Nutiva organic hemp seed oil, 8 oz liquid, cold pressed	CY269	0.00044	0.0022	–	–
Nutiva organic hemp seed oil, 8 oz liquid, cold pressed	CY270	0.00046	0.00233	–	–
Hemp seed 100% pure carrier/base oil, 3.4 oz, 100 mL	CY272	0.0168	0.00033	–	–
Manitoba Harvest hemp seed oil, 12 oz liquid, unrefined cold pressed	CY276	0.00033	0.00283	–	–
Hansi Organics natural hemp seed oil, 4 oz	CY278	0.00023[a]	0.00063	–	–
Organic hemp seed oil, 8 fL oz, unrefined cold pressed	CY280	0.00025	0.00064	–	–
Hemp seed oil, 2 oz – 100% pure, undiluted, cold pressed, unrefined, virgin – high in linoleic and linolenic acids, omega 3 and 6 fatty acids, antioxidants, and vitamins	CY282	0.00026[a]	0.00179	–	–
Hemp seed oil –100% natural, 16 oz	CY283	0.00002	0.00009[a]	–	–
Raw organic hemp seed oil – freshly pressed, 2 oz	CY286	0.00024[a]	0.00015[a]	–	–
Hemp seed oil organic virgin carrier, cold pressed unrefined pure, 4 oz	CY289	<0.0001	0.00018[a]	–	–
Hemp seed oil organic virgin carrier, cold pressed unrefined pure, 16 oz	CY290	<0.0001	0.00028[a]	–	–
Seitenbacher organic hemp oil, 8.4 oz, cold pressed	CY291	0.00022[a]	<0.0001	–	–
Hemp seed oil (cold pressed), 16 fL oz	CY292	0.00024[a]	0.00069	–	–
100% pure certified organic virgin/unrefined hemp seed oil (also edible), 8 oz – imported from Canada	CY294	0.00027	0.0017	–	–
Dr. Adorable Inc. hemp seed butter organic 100% pure raw, 8 oz	CY295	<0.0001	0.00033[a]	–	–
Braham and Murray Good Hemp seed oil, 500 mL	CY298	0.00017	0.00021[a]	–	–
Madina – 100% pure hemp seed oil, 16 oz	CY301	0.00026[a]	0.00037	–	–
NHR organic oils – organic hemp base oil	CY303	0.00040[a]	<0.0001	–	–
Dr. Adorable Inc. – hemp seed oil pure organic cold pressed, 4 oz	CY306	<0.0001	<0.0001	–	–
Dr. Adorable Inc. – hemp seed oil pure organic, 4 oz	CY307	<0.0001	<0.0001	–	–
Dr. Adorable Inc. – hemp seed oil pure organic, 8 oz	CY308	<0.0001	<0.0001	–	–
Dr. Adorable Inc. – hemp seed oil pure organic, 16 oz/1 pint	CY309	<0.0001	<0.0001	–	–
Ultra oil skin and coat supplement with hemp seed oil, 32 oz	CY315	<0.0001	<0.0001	–	–
Hemp oil supplement – concentrated powdered formula capsules	CY325	0.1530	5.5900	<0.0001	0.0040
Concentrated refined CBD oil (pure)	CY329	0.7970	17.7300	<0.0001	0.0090
Life-Flo pure hemp seed body oil, 16 oz	CY339	0.00031[a]	0.00087	–	–
Foods alive organic artisan cold-pressed 100% hemp oil, 8 oz	CY341	0.0001	0.00152	–	–
Tasty drops CBD hemp oil dietary supplement (cinnamon)	CY346	0.0471	0.7300	<0.0001	0.0200
Cibdex CBD peppermint flavor hemp oil supplement	CY348	0.0126	0.1690	<0.0001	<0.0001
Hemp CBD Miracle Drops organic extract virgin CBD	CY351	0.0062	0.1160	<0.0001	0.0040
Canada Hemp Foods, organic hemp oil, 17 fL oz, cold pressed	CY354	0.00005	0.0012	–	–
Golden Kings of Ukraine hemp seed oil	CY288	<0.0001	<0.0001	–	–
Earthly Body Miracle oil, 1 oz	CY356	<0.0001	<0.0001	–	–
Herbal Choice Mari organic hemp seed oil, 100 mL/3.4 oz	CY370	0.00032[a]	0.00093	–	–
Nature's Alchemy hemp seed oil, 100% pure, 4 fL oz, 118 mL	CY371	<0.0001	<0.0001	–	–
Manitoba Harvest hemp oil softgels, 60/1,000 mg	CY434	<0.0001	0.00065	–	–
Dr. Adorable Inc. hemp seed oil, 4 fL oz (organic unrefined pure cold pressed)	CY435	<0.0001	<0.0001	–	–
Manitoba Harvest organic hemp oil cold pressed, 8.4 fL oz	CY436	0.00027[a]	0.00071	–	–
Podor premium oil, hemp seed oil, 3.4 fL oz, 100 mL, cold pressed	CY438	<0.0001	0.00036[a]	–	–
Plant Therapy refined hemp seed oil (Cannabis sativa) 100% pure carrier oil, 4 fL oz	CY439	0.0155	0.0001[a]	–	–
Charlotte's Web hemp extract in mint chocolate, 200 mg, batch # A00045	DB137	0.0278	3.7800	<0.0001	0.0009
Charlotte's Web hemp extract in capsules, dietary supplement, batch # A00022	DB169	0.0762	3.7000	<0.0001	0.0008
RA CBD oil, 50 mg, 5 mL	DD336	0.0850	1.5000	0.0110	0.4400

THCAA, tetrahydrocannabinol-acid-A; CBDA, cannabidiolic acid; CBD, cannabidiol. [a] The concentrations of THCAA and CBDA are not included for 42 products, as they were not detected/not analyzed in these samples.

Of the products analyzed, 3 products contained 0.1–1.0% CBD, 2 products contained 0.1–0.9% THC, and 5 products contained amounts of CBD greater than 1%. Almost all the samples appeared to be partially or totally decarboxylated, as most of the CBDA and Δ^9-THCAA results were below 0.001%.

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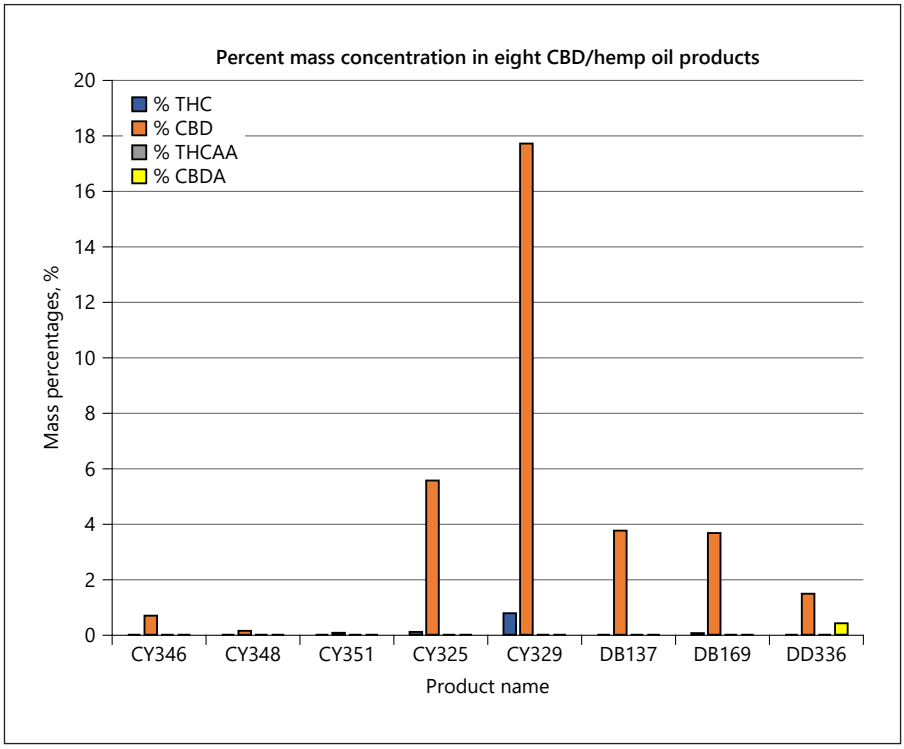
Statement of Ethics

No animal or human subjects were used in this study.



Color version available online

Fig. 9. Percent mass concentrations of Δ^9-THC, CBD, Δ^9-THCAA, and CBDA in 8 CBD/hemp oil products. Δ^9-THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; Δ^9-THCAA, Δ^9-tetrahydrocannabinol-acid-A; CBDA, cannabidiolic acid.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.A.E. was involved in study design. I.K. contributed in the acquisition of the samples. T.P.M. contributed to the analytical method development and execution of sample analyses. Both T.P.M. and W.G. contributed to reviewing the validation data. L.A.W., W.G., M.A.E., and T.P.M. organized the manuscript draft. All authors contributed to writing and finalizing the text of the manuscript.

References

1 National conference of state legislatures. Available from: https://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx. Posted 2020 Mar 10.
2 Grella CE, Rodriguez L, Kim T. Patterns of medical marijuana use among individuals sampled from medical marijuana dispensaries in Los Angeles. J Psychoactive Drugs. 2014;46(4):267–75.
3 Walsh Z, Callaway R, Belle-Isle L, Capler R, Kay R, Lucas P, et al. Cannabis for therapeutic purposes: patient characteristics, access, and reasons for use. Int J Drug Policy. 2013;24(6):511–6.
4 U.S. Government Publishing Office. Public law 115–334; 2018. https://www.govinfo.gov/link/plaw/115/public/334?link-type=pdf.
5 Pertwee RG. Pharmacological actions of cannabinoids. Handb Exp Pharmacol. 2005;168:1–51.
6 Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol, and Δ9-tetrahydrocannabivarin. Br J Pharmacol. 2008;153(2):199–215.
7 Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, et al. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB_1 and CB_2. Pharmacol Rev. 2010;62(4):588–631.
8 Colizzi M, Bhattacharyya S. Does cannabis composition matter? differential effects of delta-9-tetrahydrocannabinol and cannabidiol on human cognition. Curr Addict Rep. 2017;4(2):62–74.
9 Schubart CD, Sommer IE, van Gastel WA, Goetgebuer RL, Kahn RS, Boks MP. Cannabis with high cannabidiol content is associated with fewer psychotic experiences. Schizophr Res. 2011;130(1–3):216–21.
10 Vandrey R, Raber JC, Raber ME, Douglass B, Miller C, Bonn-Miller MO. Cannabinoid dose and label accuracy in edible medical cannabis products. JAMA. 2015;313(24):2491–3.
11 Bonn-Miller MO, Loflin MJE, Thomas BF, Marcu JP, Hyke T, Vandrey R. Labeling accuracy of cannabidiol extracts sold online. JAMA. 2017;318(17):1708–9.