Quantitative Determination of Δ^9 -THC, CBG, CBD, Their Acid Precursors and Five Other Neutral Cannabinoids by UHPLC-UV-MS

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ABSTRACT

Cannabinoids are a group of terpenophenolic compounds in the medicinal plant *Cannabis sativa* (Cannabaceae family). Cannabigerolic acid, Δ^9 -tetrahydrocannabinolic acid A, cannabidiolic acid, Δ^9 -tetrahydrocannabinol, cannabigerol, cannabidiol, cannabichromene, and tetrahydrocannabivarin are major metabolites in the classification of different strains of *C. sativa*. Degradation or artifact cannabinoids cannabinol, cannabicyclol, and Δ^8 -tetrahydrocannabinol are formed under the influence of heat and light during processing and storage of the plant sample. An ultrahigh-performance liquid chromatographic method coupled with photodiode array and single quadrupole mass spectrometry detectors was developed and validated for quantitative determination of 11 cannabinoids in different *C. sativa* samples. Compounds 1–11 were baseline separated with an acetonitrile (with 0.05% formic acid) and water (with 0.05% formic acid) gradient at a flow rate of 0.25 mL/min on a Waters Cortec UPLC C18 column (100 mm $\times$ 2.1 mm I.D., 1.6 μ m). The limits of detection and limits of quantitation of the 11 cannabinoids were below 0.2 and 0.5 μ g/mL, respectively. The relative standard deviation for the precision test was below 2.4%. A mixture of acetonitrile and methanol (80:20, v/v) was proven to be the best solvent system for the sample preparation. The recovery of all analytes was in the range of 97–105%. A total of 32 *Cannabis* samples including hashish, leaves, and flower buds were analyzed.

Introduction

Cannabis sativa L. is an annual herbaceous plant belonging to the family Cannabaceae. The cannabis plant is the most commonly used illicit plant in the form of marijuana or hashish, but is also a highly promising medicinal plant for treating various medical conditions [1–4]. The cannabinoids, a group of terpenophenolic compounds, are the most interesting and specific constituents of the cannabis plant. In addition, terpenoids and noncannabinoid phenols have been identified from this plant [5, 6].

In the biosynthetic pathway of cannabinoids in plant tissues, cannabinoids are biosynthesized in an acidic (carboxylated) form. CBGA (► **Fig. 1**) is the first cannabinoid product in the cannabis plant. THCAA, CBDA, and CBCA are biosynthesized from CBGA following different pathways, each by a particular synthase [7, 8]. Almost no neutral cannabinoid can be found in significant quantities in fresh plant material [9]. However, the carboxyl group is readily lost under the influence of heat or light, resulting in the corresponding neutral cannabinoids such as cannabigerol, cannabidiol, Δ^9 -THC, and CBC. Δ^9 -THC and CBD are two key marker cannabi-

ABBREVIATIONS

Δ^8 -THC	Δ^8 -tetrahydrocannabinol
Δ^9 -THC	Δ^9 -tetrahydrocannabinol
CBC	cannabichromene
CBCA	cannabichromenic acid
CBD	cannabidiol
CBDA	cannabidiolic acid
CBG	cannabigerol
CBGA	cannabigerolic acid
CBL	cannabicyclol
CBN	cannabinol
LOD	limits of detection
LOQ	limits of quantitation
RSD	relative standard deviation
THCV	tetrahydrocannabivarin
THCAA	Δ^9 -tetrahydrocannabinolic acid A
UHSFC	ultrahigh-performance supercritical fluid chromatography

noids in the cannabis plant. Three main chemotypes (chemical phenotypes) of the cannabis plant can be recognized on the basis of total Δ^9 -THC and total CBD contents, namely, drug-type plants (chemotype I, total Δ^9 -THC/total CBD ratio $\gg 1.0$), intermediate-type plants (chemotype II, total Δ^9 -THC/total CBD ratio close to 1.0), and fiber-type plants (chemotype III, total Δ^9 -THC/total CBD ratio $\ll 1.0$) [5]. During all stages of growing, harvesting, processing, and storage, degradation product CBN is produced in aged cannabis. Δ^8 -Tetrahydrocannabinol (Δ^8 -THC) is an artifact transformed from Δ^9 -THC [7]. The core skeleton of CBL is similar to CBC and considered an artifact resulting from a 2 + 2 cyclization of the double bonds when CBC is exposed to sun light.

The most common approaches for the characterization of cannabinoids are GC and HPLC coupled with various detection techniques, for example, GC-flame ionization detector, GC-MS, HPLC-UV, and HPLC-MS [5, 7, 10–17]. However, GC methods are difficult to directly determine acidic cannabinoids such as CBGA, CBDA, and THCAA because acidic compounds are decarboxylated into their corresponding neutral forms during analysis [7]. HPLC is a suitable tool to analyze the native composition of the cannabis plant, but the total run time of one analysis on HPLC including the time of separation and column re-equilibration is usually more than 25 min. In recent years, UHPLC and UHSFC have been introduced to improve the separation of cannabinoids and reduce the run time of the analysis [18–20].

In addition to the development of more efficient methods to extract major and trace components from complex plant matrices, various extraction solvents including ethanol, methanol, chloroform, hexane, petroleum ether, and mixtures of these solvents were used in previous studies [3, 5, 21, 22], but the recovery rates were not reported. A mixture of methanol and chloroform (9:1, v/v) has been used for sample preparation and given recovery rates above 90% to major components [7, 14]. However, recovery rates of constituents CBGA, CBG, and Δ^8 -THC are lower than 85% [14].

The primary purpose of this work was to find extraction solvents and determine major and minor cannabinoids from cultivated cannabis plants and various confiscated samples. An ultra-high-performance liquid chromatographic method coupled with photodiode array and single quadrupole mass spectrometry detectors (UHPLC-UV-SQD) was developed. In order to achieve satisfied recoveries for 11 cannabinoids (► Fig. 1) in cannabis samples, extraction solvents were optimized. The validated UHPLC-UV-MS method was applied for the analysis of 32 cannabis samples including hashish, leaves, and flower buds.

Results and Discussion

Extraction solvents including methanol, ethanol, and acetonitrile were optimized. Fifty milligrams of S-28 were extracted with each solvent. Recoveries of cannabinoids CBDA, THCAA, and Δ^9 -THC, etc., in methanol and acetonitrile were better than that in ethanol. Therefore, mixtures of acetonitrile and methanol in different ratios, such as acetonitrile/methanol = 9:1, 8:2, 7:3, and 5:5 (v/v), were further evaluated. 80% Acetonitrile mixed with 20% methanol (v/v) was proven to be the best extraction solvent system.

In a preliminary test for the separation of cannabinoids, analytes were applied on different UHPLC columns. The columns tested were Cortecs UPLC C18 (100 mm $\times$ 2.1 mm I.D., 1.6 μ m), Acquity UPLC HSS T3 (100 mm $\times$ 2.1 mm I.D., 1.8 μ m), UPLC BEH C18 (100 mm $\times$ 2.1 mm I.D., 1.7 μ m), and UPLC BEH Shield RP18 (100 mm $\times$ 2.1 mm I.D., 1.7 μ m). The best separation and peak shape were achieved on a 100 mm $\times$ 2.1 mm Cortecs UPLC C18 column. Optimal chromatographic separation was observed with a solvent composition of acetonitrile with 0.05% formic acid (v/v) and 0.05% formic acid in water as the mobile phase.

The calibration curves of reference compounds 1–11 showed a linear correlation between the analytes concentration and peak area. As shown in ► Table 1, calibration data indicated that the linearity ($r^2 > 0.99$) of the detector response for all standard compounds were from LOQs up to 100 μ g/mL. For UV detection at 220 nm, the LODs were 0.2 μ g/mL for CBDA, CBGA, CBG, CBD, THCV, and CBN, respectively, and 0.4 μ g/mL for Δ^9 -THC, Δ^8 -THC, CBL, CBC, and THCAA, respectively. The LOQs of cannabinoids 1–11 are listed in ► Table 1.

The accuracy was evaluated for the recovery of standard compounds from spiked samples. Plant sample S-28 was exhaustively extracted, spiked with standard compounds, and extracted under the optimized conditions. The recovery of cannabinoids (1–8, 10 and 11) in this study is listed in ► Table 2 in the range of 97–105%. CBC (9) was not tested in the recovery study of higher concentrations because the amount of the CBC standard was not sufficient.

The precision was determined by intra- and inter-day assays on 3 consecutive days with 3 repetitions each day. All samples were injected in triplicate. The RSDs obtained in the intra- and inter-day studies were within 2.5%; the maximum RSD was 2.4% for CBG of the inter-day assays (► Table 3). Intra- and inter-day assays as well as multiple injections showed that the results were highly reproducible and had a low standard error. This method was suitable for routine analysis of cannabinoids from the cannabis plant.

► **Fig. 1** Structures of standard cannabinoids (1, CBDA; 2, CBGA; 3, CBG; 4, CBD; 5, THCV; 6, CBN; 7, Δ^9 -THC; 8, Δ^8 -THC; 9, CBL; 10, CBC and 11, THCAA).

► **Table 1** Regression equation, correlation coefficient (r^2), LOD, and LOQ of cannabinoids 1–11 (1, CBDA; 2, CBGA; 3, CBG; 4, CBD; 5, THCV; 6, CBN; 7, Δ^9 -THC; 8, Δ^8 -THC; 9, CBL; 10, CBC; 11, THCAA).

Compound name	Calibration curve	r^2	Linearity range ($\mu\text{g/mL}$)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
CBDA	$Y = 1.94\text{e}+004 X + 2.01\text{e}+004$	0.998	0.5–100	0.2	0.5
CBGA	$Y = 1.55\text{e}+004 X + 1.78\text{e}+004$	0.998	0.5–100	0.2	0.5
CBG	$Y = 1.72\text{e}+004 X + 1.15\text{e}+004$	0.998	0.5–100	0.2	0.5
CBD	$Y = 2.07\text{e}+004 X + 6.34\text{e}+003$	0.999	0.5–100	0.2	0.5
THCV	$Y = 1.78\text{e}+004 X + 1.55\text{e}+004$	0.997	0.5–100	0.2	0.5
CBN	$Y = 3.36\text{e}+004 X + 8.85\text{e}+003$	0.999	0.5–100	0.2	0.5
Δ^9 -THC	$Y = 1.23\text{e}+004 X + 5.55\text{e}+003$	0.999	1.0–100	0.4	1.0
Δ^8 -THC	$Y = 1.08\text{e}+004 X + 3.27\text{e}+002$	0.999	1.0–100	0.4	1.0
CBL	$Y = 1.35\text{e}+004 X + 2.78\text{e}+004$	0.994	1.0–50	0.4	1.0
CBC	$Y = 1.49\text{e}+004 X + 3.43\text{e}+003$	0.999	1.0–100	0.4	1.0
THCAA	$Y = 1.53\text{e}+004 X + 1.36\text{e}+004$	0.999	1.0–100	0.4	1.0

In testing samples, high-concentrated cannabinoids were up to 1000-fold higher than low-concentrated cannabinoids in the same sample. To determine all 11 cannabinoids in one sample, further dilutions and several analytical runs are necessary. This allows that all analytes are within the linear range of the calibration curve. Typical chromatograms of cannabinoids by UV detection at 220 nm are shown in ► **Fig. 2**. Contents of 11 cannabinoids in *C. sativa* samples and the ratio of Δ^9 -THC/CBD are summarized in ► **Table 4**.

Out of the 32 *C. sativa* samples, there are 22, 4, and 6, respectively, of flower bud, leaf, and hashish samples. The six hashish samples (S-1, S-12, S-15, S-18, S-29, and S-30) are shown in ► **Table 4**. Δ^9 -THC and THCAA are in the range of 1.8–9.6 and 0.08–82% (mg in 100 mg sample weight), respectively. For hashish samples, sample S-1 contains the highest amount of Δ^9 -THC and THCAA, and samples S-12 and S-29 have the lowest content of Δ^9 -THC and THCAA, respectively. CBL (9) was identified in samples S-

12, S-15, S-18, and S-29. The chemical structures of CBC and CBL are similar. CBL is considered an artifact resulting from a 2 + 2 cyclization of the double bonds during processing. Not all hashish samples are drug-type according to ratios of Δ^9 -THC/CBD in ► **Table 4**. Samples S-29 and S-30 are two intermediate-type plants.

The content of THCAA, Δ^9 -THC, and CBN in four leaf samples (S-6, S-13, S-21, and S-28) were in the range of 0.056–12, 0.33–2.5, and 0.018–0.22%, respectively. CBDA and CBGA varied from a trace amount to 1.7 and 0.38%, respectively. Δ^8 -THC and CBL were not detected in these samples; other cannabinoids, namely, CBD, CBG, and THCV were identified in trace amounts in most of the samples. Considering the ratios of Δ^9 -THC/CBD in ► **Table 4**, three out of four leaf samples are drug-type plants, but sample S-28 is an intermediate-type plant.

Among 22 flower bud samples in ► **Table 4**, THCAA, Δ^9 -THC, CBGA, and CBDA are the major constituents with a range of

► Table 2 Recovery of cannabinoids 1–11 (1, CBDA; 2, CBGA; 3, CBG; 4, CBD; 5, THCV; 6, CBN; 7, Δ^9 -THC; 8, Δ^8 -THC; 9, CBL; 10, CBC; 11, THCAA).

Compound name	Recovery sample 1 (n = 2)			Recovery sample 2 (n = 2)		
	Amount found (μg)	Std. added (μg)	Recovery (%)	Amount found (μg)	Std. added (μg)	Recovery (%)
CBDA	19.68	20.04	98.2	8.25	8.02	102.9
CBGA	33.87	34.98	96.8	14.05	13.99	100.4
CBG	26.80	26.74	100.3	11.01	10.69	103.0
CBD	22.80	22.36	102.0	9.40	8.94	105.1
THCV	25.15	25.75	97.7	10.44	10.30	101.3
CBN	25.56	25.15	101.6	10.53	10.06	104.6
Δ^9 -THC	29.21	29.21	100.0	11.87	11.68	101.6
Δ^8 -THC	22.96	22.39	102.6	9.30	8.96	103.8
CBC	–	–	–	9.01	9.05	99.5
THCAA	27.17	27.51	98.8	10.92	11.00	99.2

Recovery tests used the samples of defatted cannabinoids. The recovery rate is averaged from duplicate assays.

► Table 3 Intra- and inter-day assays of sample S-28.

Compound name	Day 1 (n = 3)			Day 2 (n = 3)			Day 3 (n = 3)			Inter-day (n = 9)		
	Content (%)	SD	RSD	Content (%)	SD	RSD	Content (%)	SD	RSD	Content (%)	SD	RSD
CBDA	1.7	0.0047	0.27	1.7	0.024	1.4	1.7	0.0162	0.96	1.7	0.025	1.5
CBGA	3.5×10^{-2}	0.00030	0.87	3.5×10^{-2}	0.00057	1.6	3.5×10^{-2}	0.00011	0.32	3.5×10^{-2}	0.00034	0.99
CBG	2.1×10^{-2}	0.00028	1.3	2.1×10^{-2}	0.00004	0.19	2.0×10^{-2}	0.00018	0.88	2.1×10^{-2}	0.00050	2.4
CBD	3.3×10^{-1}	0.00036	0.11	3.3×10^{-1}	0.0044	1.3	3.3×10^{-1}	0.00255	0.78	3.3×10^{-1}	0.0034	1.0
THCV	2.1×10^{-2}	0.00015	0.73	2.1×10^{-2}	0.00003	0.14	2.1×10^{-2}	0.00012	0.57	2.1×10^{-2}	0.0001	0.61
CBN	1.8×10^{-2}	0.00018	1.0	1.8×10^{-2}	0.00027	1.6	1.8×10^{-2}	0.00019	1.1	1.8×10^{-2}	0.00021	1.2
Δ^9 -THC	6.3×10^{-1}	0.0024	0.38	6.3×10^{-1}	0.00044	0.070	6.3×10^{-1}	0.0028	0.44	6.3×10^{-1}	0.0033	0.53
Δ^8 -THC	ND			ND			ND			ND		
CBL	ND			ND			ND			ND		
CBC	2.1×10^{-2}	0.00043	2.1	2.1×10^{-2}	0.00032	1.5	2.1×10^{-2}	0.00011	0.51	2.1×10^{-2}	0.00046	2.2
THCAA	8.6×10^{-1}	0.0016	0.19	8.4×10^{-1}	0.012	1.5	8.4×10^{-1}	0.0072	0.86	8.5×10^{-1}	0.010	1.2

Content %: mg/100 mg testing sample; SD: standard derivation; RSD: relative standard derivation; ND: not detected.

0.053–19.5, 0.034–10.6, 0.018–0.81, and 0.015–11.2%, respectively. Δ^8 -THC and CBL were not detected from these samples. The amounts of the other cannabinoids, including CBD, CBG, CBN, CBC and THCV, were varied. Out of the 22 flower bud samples, plants belonging to drug-type and intermediate-type were 13 and 9, respectively. Some intermediate-type plants have low Δ^9 -THC/CBD ratios, such as samples S-24, S-31, and S-32 with 0.5, 0.2, and 0.2, respectively.

In summary, a sensitive UHPLC-UV-MS method was established for the quantitative analysis of 11 cannabinoids from *C. sativa*. The developed method was validated in terms of extraction solvents, precision, accuracy, LOD, LOQ, and linearity range. All analytes were baseline separated in a 10-min run time. Different extraction solvents were evaluated and a mixture of acetonitrile and methanol (80:20, v/v) showed the best extraction efficiency. The validated method was applied for the quantitative analysis of 32 dif-

ferent cannabis samples. THCAA + Δ^9 -THC, CBDA + CBD, and CBGA + CBG were major components in hashish, leaves, and flower buds. CBL was only identified from hashish samples, which suggests that CBL could be an artifact formed under the influence of light or heat during processing.

Material and Methods

Instrumentation and chromatographic conditions for UHPLC-UV-MS analysis

All analyses were performed on a Waters Acquity UPLC system (Waters) that included a binary solvent manager, sample manager, heated column compartment, photodiode array (PDA) detector, and a single quadrupole detector (SQD) of a mass spectrometer (Waters). The instrument was controlled by Waters Empower 2

software. For analysis of 11 cannabinoids, a Cortec UPLC C18 column (100 mm × 2.1 mm I.D., 1.6 μm) from Waters was used. The column and sample temperature were maintained at 35 °C and 15 °C, respectively. The mobile phase consisted of water containing 0.05% formic acid (A) and acetonitrile with 0.05% formic acid (B). Analysis was performed using the following gradient elution at a flow rate of 0.25 mL/min: 0–8 min, 70 to 80% B; 8–10 min, 80 to 100% B. The analysis was followed by a 4-min washing procedure with 100% B and a re-equilibration period of 3.5 min with initial conditions. A strong needle wash solution (90/10; acetonitrile/water, v/v) and weak needle wash solution (10/90; acetonitrile/water) were used. The injection volume was 2 μL. The PDA detection wavelength was 220 nm. Peaks were assigned by spiking the samples with standard compounds and comparison of UV spectra, mass spectra, and retention times.

The ESI-MS experiments were carried on a Waters SQD Mass Spectrometer (Waters) that was connected to the UHPLC system via an ESI interface. The ESI source was operated in the positive ionization mode with the capillary voltage at 3.0 kV. The temperature of the source and desolvation were set at 150 and 350 °C, respectively. The cone and desolvation gas flows were 25 and 650 L/h, respectively. The cone voltage was set at 30 V. All data collected in centroid mode were acquired using Empower 2 software (Waters).

Chemicals and reagents

Eleven reference compounds, namely, CBDA (1), CBGA (2), CBG (3), CBD (4), THCV (5), CBN (6), Δ⁹-THC (7), Δ⁸-THC (8), CBL (9), CBC (10), and THCAA (11) were isolated at the National Center for Natural Products Research (NCNPR), University of Mississippi, Mississippi, USA. The identity of standard compounds 1–11 were certified on the basis of the spectral data (¹H- and ¹³C-NMR and HR-ESIMS). The purity was confirmed that all were above 95% by chromatographic methods (data not shown). The stability of cannabinoids 1–11 was found to be stable in extraction solvents for up to 96 h. All tested samples were kept in a –20 °C freezer for up to 3 weeks with no changes observed.

Methanol, acetonitrile, and formic acid were HPLC grade and purchased from Fisher Scientific. Water for the HPLC mobile phase was purified using a Millipore Synergy UV Water Purification System (Millipore SAS).

Plant material and confiscated products

C. sativa L. samples including flower buds, leaves, and hashish were received from the Drug Enforcement Administration (DEA) of the United States or obtained from materials produced for research of different varieties at the Waller Labs, University of Mississippi (UM). The information for these samples is listed in ► **Table 3**. Specimens of all samples are deposited at Waller Labs, University of Mississippi, University, Mississippi, USA.

Sample preparation

Fine powder of plant material (50 mg) was accurately weighed into a 15-mL centrifuge tube and extracted with 2.5 mL of extraction solvents (acetonitrile/methanol = 80:20, v/v) in an ultrasonic water bath for 30 min, followed by centrifugation at 959 g for 15 min. The supernatant was transferred to a 10-mL volumetric flask. The procedure was repeated three more times and the re-

► **Fig. 2** a Chromatograms of a mixture of cannabinoid standards 1–11 (1, CBDA; 2, CBGA; 3, CBG; 4, CBD; 5, THCV; 6, CBN; 7, Δ⁹-THC; 8, Δ⁸-THC; 9, CBL; 10, CBC and 11, THCAA) and b–f different varieties of *C. sativa* at UV 220 nm.

spective supernatants were combined. The final volume was adjusted to 10 mL with extraction solvents. Prior to LC analysis, the prepared sample was mixed thoroughly and an adequate volume of extracts was passed through a 0.45-μm PTFE filter and collected in an LC sample vial. The final solutions of some samples were further diluted up to 20-fold in order that all concentrated analytes were within the linear range of calibration curve.

Standard solutions of cannabinoids

An individual stock solution of standard compounds 1–11 was prepared at a concentration of 2.0 mg/mL in the extraction solvent. The calibration curves were prepared at five different concentration levels. The ranges of calibration curves were from LOQs up to 100 μg/mL as shown in ► **Table 2** for cannabinoids 1–11.

Validation procedure

In order to achieve a better extraction solvent system with satisfied recovery for 11 cannabinoids, solvents including methanol, ethanol, and acetonitrile were tested. CBDA, THCAA, and Δ⁹-THC were the top three most abundant components in sample S-28. Fifty milligrams of S-28 were extracted with ultra-sonication. Recoveries in methanol, acetonitrile, and ethanol were 68, 93, and 63%, respectively, for CBDA; 87, 62, and 82% for THCAA; and 81, 100, and 77% for Δ⁹-THC. Further on, mixtures of acetonitrile and

► **Table 4** Sample type, code, source, content of cannabinoids 1–11 in *C. sativa* (%), mg/100 mg testing sample), and ratio of Δ^9 -THC/CBD.

Sample type	Sample code	Sample source	Compound name and content (%), mg in 100 mg samples)											Ratio (Δ^9 -THC/CBD)
			CBDa (1)	CBGA (2)	CBG (3)	CBD (4)	THCV (5)	CBN (6)	Δ^9 -THC (7)	Δ^8 -THC (8)	CBL (9)	CBC (10)	THCAA (11)	
Hashish	S-1	DEA	7.3×10^{-2}	1.4	6.0×10^{-1}	4.2×10^{-2}	7.7×10^{-2}	2.9×10^{-1}	9.6	ND	ND	2.1×10^{-1}	82	231
	S-15	DEA	1.4×10^{-1}	1.2	1.9×10^{-1}	1.1×10^{-2}	8.1×10^{-2}	2.0	5.8	ND	DUL	3.3×10^{-2}	32	542
	S-18	DEA	3.2×10^{-1}	2.6×10^{-1}	5.8×10^{-2}	2.5×10^{-2}	1.0×10^{-1}	7.3	6.3	1.1×10^{-1}	2.1×10^{-2}	2.4×10^{-1}	12	254
	S-12	DEA	1.5×10^{-2}	1.6×10^{-1}	3.1×10^{-2}	DUL	6.9×10^{-2}	3.1×10^{-1}	1.8	ND	DUL	5.3×10^{-2}	4.3	226
	S-30	DEA	8.5	2.6×10^{-1}	1.2×10^{-1}	4.0	1.7×10^{-1}	7.2×10^{-1}	4.0	ND	ND	1.9×10^{-1}	2.5	1
	S-29	DEA	2.1×10^{-1}	8.4×10^{-2}	2.5×10^{-1}	1.8	1.2×10^{-1}	2.9	6.9	ND	3.2×10^{-2}	3.1×10^{-1}	8.2×10^{-2}	4
Leaf	S-6	DEA	3.1×10^{-2}	3.8×10^{-1}	4.9×10^{-2}	DUL	8.8×10^{-2}	1.6×10^{-1}	2.5	ND	ND	3.1×10^{-2}	12	312
	S-13	DEA	1.5×10^{-2}	1.0×10^{-1}	2.4×10^{-2}	DUL	8.1×10^{-2}	2.2×10^{-1}	1.3	ND	ND	5.6×10^{-2}	4.2	165
	S-21	DEA	DUL	DUL	DUL	DUL	DUL	1.6×10^{-1}	3.3×10^{-1}	ND	ND	5.2×10^{-1}	5.6×10^{-2}	41
	S-28	DEA	1.7	3.5×10^{-2}	2.1×10^{-2}	3.3×10^{-1}	2.1×10^{-2}	1.8×10^{-2}	6.3×10^{-1}	ND	ND	2.1×10^{-2}	8.5×10^{-1}	2
	S-4	DEA	4.3×10^{-2}	3.7×10^{-1}	1.0×10^{-1}	DUL	1.1×10^{-1}	4.8×10^{-1}	4.6	ND	ND	9.3×10^{-2}	20	581
	S-2	DEA	2.5×10^{-2}	3.7×10^{-1}	9.7×10^{-2}	DUL	1.2×10^{-1}	8.1×10^{-1}	6.8	ND	ND	2.1×10^{-2}	16	851
Flower bud	S-3	DEA	2.9×10^{-2}	8.1×10^{-1}	1.4×10^{-1}	DUL	2.3×10^{-1}	2.6×10^{-1}	5.5	ND	ND	2.2×10^{-2}	16	681
	S-7	DEA	4.2×10^{-2}	5.0×10^{-1}	1.5×10^{-1}	DUL	1.1×10^{-1}	7.3×10^{-1}	4.7	ND	ND	DUL	15	585
	S-8	UM	4.2×10^{-2}	7.9×10^{-1}	5.3×10^{-2}	DUL	4.5×10^{-2}	5.2×10^{-1}	2.5	ND	ND	DUL	14	313
	S-5	DEA	2.9×10^{-2}	2.2×10^{-1}	7.0×10^{-2}	DUL	1.2×10^{-1}	5.2×10^{-1}	2.9	ND	ND	2.0×10^{-2}	12	359
	S-10	DEA	3.1×10^{-2}	2.9×10^{-1}	1.6×10^{-1}	1.3×10^{-2}	1.1×10^{-1}	9.7×10^{-1}	11	ND	ND	8.1×10^{-2}	9.5	799
	S-14	DEA	2.7×10^{-2}	2.5×10^{-1}	7.8×10^{-2}	DUL	1.2×10^{-1}	1.0	4.7	ND	ND	2.8×10^{-2}	7.2	583
	S-26	UM	11	7.6×10^{-1}	6.7×10^{-2}	9.8×10^{-1}	8.0×10^{-2}	5.7×10^{-2}	2.4	ND	ND	7.1×10^{-2}	7.1	2
	S-9	UM	2.0×10^{-2}	3.4×10^{-1}	1.7×10^{-2}	DUL	8.4×10^{-2}	2.3×10^{-2}	1.2	ND	ND	4.1×10^{-2}	6.8	155
	S-19	DEA	3.0×10^{-1}	5.7×10^{-1}	1.0×10^{-1}	7.8×10^{-2}	8.5×10^{-2}	4.3×10^{-1}	2.5	ND	ND	3.3×10^{-2}	6.4	32
	S-11	UM	1.5×10^{-2}	2.9×10^{-1}	2.7×10^{-2}	DUL	7.8×10^{-2}	2.4×10^{-2}	1.4	ND	ND	8.7×10^{-2}	4.3	172
	S-25	UM	5.8	5.1×10^{-1}	6.6×10^{-2}	1.0	7.1×10^{-2}	7.3×10^{-2}	2.5	ND	ND	8.0×10^{-2}	3.8	2
	S-27	UM	6.0	6.2×10^{-1}	7.6×10^{-2}	9.4×10^{-1}	7.1×10^{-2}	7.2×10^{-2}	2.5	ND	ND	7.2×10^{-2}	3.7	3
	S-16	DEA	1.8×10^{-2}	1.6×10^{-1}	2.7×10^{-2}	DUL	7.5×10^{-2}	1.7×10^{-1}	1.9	ND	ND	5.3×10^{-2}	3.6	234
	S-20	DEA	4.2×10^{-1}	2.7×10^{-1}	1.4×10^{-1}	4.2×10^{-1}	1.0×10^{-1}	1.2	2.5	ND	ND	5.6×10^{-2}	3.5	6
	S-17	UM	1.9×10^{-2}	1.6×10^{-1}	DUL	DUL	2.4×10^{-2}	3.5×10^{-1}	1.2	ND	ND	DUL	2.2	156
	S-23	DEA	9.0×10^{-1}	8.1×10^{-2}	3.3×10^{-2}	4.5×10^{-1}	5.6×10^{-2}	3.7×10^{-1}	1.0	ND	ND	5.2×10^{-2}	1.7	2
Content %: mg/100 mg testing sample. UM: University of Mississippi; DEA: Drug Enforcement Administration; ND: not detected; DUL: detected under limits of quantitation	S-22	DEA	8.0×10^{-1}	1.3×10^{-1}	7.7×10^{-2}	6.3×10^{-1}	5.2×10^{-2}	1.1	1.2	ND	ND	7.6×10^{-2}	1.5	2
	S-31	UM	2.7	7.0×10^{-2}	DUL	1.7×10^{-1}	DUL	DUL	3.4×10^{-2}	ND	ND	2.1×10^{-2}	3.6×10^{-1}	0.2
	S-32	UM	4.3	1.4×10^{-1}	1.3×10^{-2}	2.1×10^{-1}	DUL	DUL	3.5×10^{-2}	ND	ND	2.2×10^{-2}	1.1×10^{-1}	0.2
	S-24	DEA	5.8×10^{-2}	1.8×10^{-2}	3.4×10^{-2}	4.9×10^{-1}	2.4×10^{-2}	1.8	2.7×10^{-1}	ND	ND	1.0×10^{-1}	5.3×10^{-2}	0.5

methanol in different ratios, such as acetonitrile/methanol = 9:1, 8:2, 7:3, and 5:5 (v/v), were evaluated. The recoveries in an order of acetonitrile/methanol = 5:5, 7:3, 8:2, and 9:1 (v/v) were 88, 96, 100, and 100% for CBDA; 92, 96, 99, and 96% for THCA; and 92, 97, 100, and 100% for Δ^9 -THC. Therefore, the best extraction solvent system was a mixture of 80% acetonitrile and 20% methanol (v/v).

The UHPLC method was validated in terms of precision, accuracy, LOD, LOQ, and linearity range according to ICH guidelines [23]. The LODs and LOQs were determined by injecting a series of dilute solutions with known concentrations. LOD and LOQ were defined as the signal-to-noise ratio equal to 3:1 and 10:1, respectively.

The accuracy analysis of cannabinoids in *C. sativa* samples was determined by spiking known amounts of standard compounds 1–8, 10, and 11 in a plant sample. After 50 mg plant sample (S-28) was exhaustively extracted six times and dried completely, it was spiked with 0.1 and 0.25 mg, respectively, of each standard compound. The spiked samples were extracted and analyzed under the same optimized conditions. The recovery of cannabinoids (1–8, 10, and 11) was calculated and is listed in ► Table 2.

The precision was evaluated by carrying out three independent analyses each day, and the assays were performed on three different days for S-28 *C. sativa* plant samples. All samples were injected in triplicate.

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Conflict of Interest

The authors declare no conflict of interest.

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