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High-throughput LC-PDA method for determination of Δ^9-THC and related cannabinoids in *Cannabis sativa*

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ABSTRACT

Before the passage of the *Agriculture Improvement Act of 2018*, more commonly referred to as the 2018 Farm Bill, forensic laboratories were only required to perform qualitative measurements to confirm the identity of seized plant samples as *Cannabis sativa* (hemp or marijuana). The new law defines hemp at a federal level as *Cannabis sativa* containing 0.3 % or less Δ^9-THC. Because forensic laboratories were not adequately equipped with the proper methods or training to meet these requirements, significant backlogs in casework resulted. The National Institute of Standards and Technology (NIST) responded by providing analytical tools to the forensic community. An accurate and precise method was previously developed to determine Δ^9-THC, Δ^9-THCA, and total Δ^9-THC in botanical samples based on liquid chromatography with photodiode array detection (LC-PDA). *Cannabis* plant samples were ground and extracted with methanol using routine laboratory equipment. The original sample preparation procedure was time-consuming, taking over 70 min. The method described here has been optimized with the time required for sample preparation and LC-PDA analysis has been reduced to less than 30 min.

Introduction

Hemp was removed from the United States Drug Enforcement Administration (US DEA) controlled substances list with the passage of the *Agriculture Improvement Act of 2018* (2018 Farm Bill) that defined hemp as *Cannabis sativa* containing 0.3 % or less Δ^9-tetrahydrocannabinol (Δ^9-THC) [1]. Before this legislation, forensic laboratories were only required to perform qualitative analysis to confirm the presence of Δ^9-THC. An example of this analysis includes the combination of colorimetric tests [2] and gas chromatography with mass spectrometry (GC–MS) [2,3]. Forensic laboratories are now required to perform quantitative analysis of seized *Cannabis* plant samples to distinguish hemp from marijuana. As a result, the Chemical Science Division (CSD) at the National Institute of Standards and Technology (NIST) created analytical tools for forensic laboratories, including the Cannabis Laboratory Quality Assurance Program (CannaQAP), hemp Reference Materials (RMs), robust sample preparation procedures, and analytical methods [4].

The accurate determination of native levels of Δ^9-THC in *Cannabis* by GC-based methods requires derivatization during sample preparation to prevent the conversion of Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA) to Δ^9-THC in the GC inlet [5–11]. Derivatization also makes the GC determination of Δ^9-THCA possible, which is not feasible for the underivatized compound. Forensic labs have been reluctant to incorporate a derivatization step due to the increased length of the sample preparation process. As a result, many forensic laboratories prefer using liquid chromatography (LC) combined with ultraviolet (UV) or photodiode array (PDA) based analytical methods [12–19] to measure Δ^9-THC and Δ^9-THCA separately and then calculate the total Δ^9-THC (Eq. (1)).

$$\%\ \text{Total}\ \Delta^9\text{-THC} = (0.877 \times \%\ \Delta^9\text{-THCA}) + \%\ \Delta^9\text{-THC} \quad (1)$$

Similarly, total CBD can be calculated from knowledge of cannabidiolic acid (CBDA) and cannabidiol (CBD) (Eq. (2)).

$$\%\ \text{Total CBD} = (0.877 \times \%\ \text{CBDA}) + \%\ \text{CBD} \quad (2)$$

NIST CSD previously investigated an integrated commercial *Cannabis Analyzer* instrument and a high-sensitivity method (Shimadzu) for identifying 11 cannabinoids in *Cannabis* plants [19,20,22] and *Cannabis* oils [19,21]. Δ^9-THC, Δ^9-THCA, and total Δ^9-THC were accurately quantified in four hemp reference plant samples from the University of Kentucky Proficiency Testing (UK-PT) program using this LC-PDA method [19]. NIST has recently expanded these efforts to include 53 commercially available samples of smokable hemp plants from online

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sources [20]. The level of total Δ^9-THC exceeded the 0.3 % threshold set in the 2018 Farm Bill in approximately 93 % of the hemp samples examined. Additionally, levels of Δ^9-THC reported by the manufacturers did not agree with measurements performed at NIST. These differences can arise from inaccurate measurements, sample inconsistency, batch variation, improper storage, and misleading product information.

The sample preparation procedure previously implemented at NIST was adapted from an approved Association of Official Analytical Collaboration (AOAC) INTERNATIONAL Official First Action Method for quantifying cannabinoids in dried *Cannabis* plant samples [14]. Samples were ground using a consumer-grade blender and extracted with two 30 min stepwise methanol (MeOH) extractions. The extracts were combined, filtered, and diluted before LC-PDA analysis. Although the procedure was developed using standard laboratory equipment to facilitate the adoption by forensic laboratories, the time required was deemed unacceptable for laboratories with high-volume workloads. The current study describes a new sample preparation procedure consisting of grinding, extraction, and clean-up steps that can be completed in a maximum time of 15 min. When combined with the previously reported LC-PDA method, the total sample analysis time has been reduced to less than 30 min. Previously characterized hemp samples from the NIST Cannabis Quality Assurance Program (CannaQAP) were used to compare the performance of each procedure [23].

Experimental

Chemicals and materials

A calibration solution containing 11 cannabinoids in acetonitrile (ACN) was obtained from Shimadzu Instruments, LLC (Columbia, MD). The solution has a nominal concentration of 250 mg/L for each cannabinoid, including cannabidivarin (CBDV), CBDA, cannabigerolic acid (CBGA), cannabigerol (CBG), CBD, Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabinol (CBN), Δ^9-THC, Δ^8-tetrahydrocannabinol (Δ^8-THC), cannabichrome (CBC), and Δ^9-THCA. HPLC grade ACN and water (H_2O) with 0.085 % phosphoric acid (PA) concentration were purchased from Shimadzu Instruments, LLC. MeOH was purchased from Fisher Scientific (St. Louis, MI). *Cannabis* plant samples were stored in the dark at −20 °C until analysis.

Calibration standards

Four calibration solutions were gravimetrically prepared to have final mass concentrations of approximately 2.5 mg/L, 10 mg/L, 25 mg/L, and 50 mg/L for the individual cannabinoids.

Grinding procedure

Commercial hemp bud samples (10 g–20 g) were ground at room temperature without drying using a small portable Magic Bullet grinder (Capital Brands, Los Angeles, CA). At a minimum, four separate 5 s–10 s pulses were employed to prevent the generation of heat that could cause the decarboxylation of Δ^9-THCA into Δ^9-THC. Similar procedures are used in forensic laboratories to measure Δ^9-THC, Δ^9-THCA, and other cannabinoids of interest in seized *Cannabis* samples. The grinding vessel cups and blades were cleaned with one water wash and four MeOH washes (Fig. S1).

Sieving procedure

The physical characteristics of the ground plant samples were evaluated by, approximately sieving 10 g–20 g portions using a small sieve shaker and test sieves (Humboldt H-4326, Elgin, IL) with five 3″ sieves for 20 min. All sieves were U.S.A. Standard Test Sieves meeting ASTM E11 specifications with the following screen sizes: 1 mm (ASTM #18), 0.500 mm (ASTM #35), 0.425 mm (ASTM #40), 0.250 mm (ASTM #60), and 0.125 mm (ASTM #120).

Sample extraction and clean-up

Ground plant samples were mixed thoroughly to promote homogeneity. Three replicate subsamples (0.50 g ± 0.05 g) of each plant sample were each weighed into 50 mL centrifuge tubes. Samples were extracted with 5 mL portions of MeOH. Samples were vortex-mixed for 10 s to ensure initial mixing and mechanically shaken for 5 min at 5.24 rad/s (50 rpm) using a large-capacity mixer (Glas-Col: Tools for Scientists, Terre Haute, IN). Samples were centrifuged for 1 min at 157.2 rad/s (1500 rpm) using an Allegra X-14R Centrifuge (Beckman Coulter, Brea, CA). MeOH extracts were decanted from the sample and an additional 5 mL aliquot of MeOH was added to the plant sample for a second extraction. The extracts were combined and filtered with a 0.45 µm polytetrafluoroethylene (PTFE) membrane syringe filter. Sample extracts (0.1 mL) were diluted volumetrically with MeOH to prepare 10-fold (0.9 mL) and 100-fold sample dilutions (9.9 mL) for measurements.

LC-PDA

The LC-PDA method was performed on a Shimadzu *Cannabis Analyzer* using a NexLeaf CBX for Potency C_{18} column (Shimadzu) with the following characteristics: 15.0 cm length, 4.6 mm i. d., and 2.7 µm average particle diameter. Separations were carried out at a flow rate of 1.6 mL/min, column temperature of 40 °C, and the following mobile phase gradient: hold at 70/30 ACN/H_2O for 3 min, increase to 85 % ACN over 4 min, increase to 95 % ACN over 0.1 min, hold for 0.9 min, and re-equilibrate for 2 min under the initial chromatographic conditions. The injection volume was 5 µL. The PDA detector collected data from 190 nm to 700 nm. An external standard calibration approach was implemented using a linear regression model with detection at 220 nm. Sample extracts were diluted 10-fold and 100-fold to ensure the chromatographic response falls within the calibration curve. Δ^9-THC and other cannabinoids were identified in the *Cannabis* samples by LC-PDA based on matching retention times and absorbance spectra to reference standards.

Results and discussion

Previous NIST studies for the analysis of *Cannabis* have focused on developing a rapid and accurate LC-PDA separation method in <15 min [19,20]. A significant portion of the time required to analyze botanical samples is spent on sample preparation. This process entails grinding, extraction, filtration, and the cleaning of grinding vessels utilized in sample processing. Optimal sample preparation is paramount for ensuring accurate quantitative analysis. An inherent challenge lies in balancing the quantitative extraction of solid matrix samples and the associated time investment.

Proper grinding of the sample is essential for quantitative solvent extraction. The characteristics of the ground material significantly impact the effectiveness of subsequent sample preparation steps. Decreasing the particle size reduces extraction time by shortening the diffusion distances for the solvent into and out of the particles, thereby increasing analyte recovery and more accurate results. Poorly ground samples that contain a broad particle size distribution require more extensive and lengthy extraction protocols to achieve accurate results. Ideally, samples should be ground to particle sizes less than 1 mm [24,25]; however, grinding protocols typically produce some particles that exceed 1 mm. Extensive grinding may result in sample heating causing Δ^9-THCA to be decarboxylated to Δ^9-THC, which should be avoided, and a balance exists between grinding time and grinding efficiency.

Several variables influence extraction efficiency that provide the basis of extraction recovery studies for analytical methods involved in analyzing complex matrix samples, such as *Cannabis*. The variables include the selection of the extraction solvent, sample mass, extraction

Table 1
Sieved percentages of ground *Cannabis* plant materials.

Sieve #	Particle Size (mm)	Percentage of Sieved Plant Materials		
		Hemp Samples		Marijuana Sample
18	1.000	17.0	13.6	3.5
25	0.710	9.5	11.8	8.0
35	0.500	30.0	30.0	10.6
60	0.250	40.3	43.6	30.9
120	0.125	3.2	1.0	14.2
Pan		0.0	0.0	32.8

volumes, temperature, number of cycles, and methodology (e.g., shaking, sonication, pressurized fluid extraction, etc.). These parameters can be optimized by comparisons with reference materials or other well-characterized samples or through exhaustive extractions with large solvent volumes, long extraction times, and multiple extraction cycles. The ultimate goal is to develop an extraction protocol that is fit for purpose, reducing the time required for an analysis and ensuring accurate results [19,20].

The original extraction procedure employed by NIST CSD was a slightly modified method previously approved as an AOAC Official First Action Method, with MeOH substituted for ethanol (EtOH) [14]. Approximately 0.5 g ± 0.05 g portions of plant samples and 20 mL aliquots of MeOH were weighed into 50 mL centrifuge tubes. The samples were vortex mixed for 10 s, mechanically shaken for 30 min, and centrifuged for 5 min at 157 rad/s (1500 rpm). The MeOH layer was decanted and 20 mL of fresh MeOH was added to the plant samples to repeat the extractions. The extracts were combined, filtered, and diluted with MeOH to make 10-fold and 100-fold sample dilutions for analysis by LC-PDA. While this method provides quantitative extraction, the total time required (approximately 70 min) is deemed unacceptable in forensic laboratories due to the high number of *Cannabis* samples seized annually. As a result, NIST has optimized the sample preparation procedure, reducing the processing time required before analysis.

Sample homogenization

Cannabis plant materials are naturally heterogeneous because the highest levels of Δ^9-THC, Δ^9-THCA, and other cannabinoids exist primarily in the trichomes of the *Cannabis* bud [26]. Sample homogeneity is crucial in preparing seized *Cannabis* plant material. Homogenizing the plant samples generally involves grinding the sample to a consistent particle size distribution ≤ 1 mm [24,25]. As part of this research, NIST developed a grinding procedure depicted through the photographs in Fig. S1. *Cannabis* samples were not dried prior to grinding because all results are reported on an as-received basis. Approximately 10 g–20 g of *Cannabis* buds were placed in a grinding vessel (cup and blades) and hand tightened. The grinding vessel was locked onto the blender and tilted to one side as the motor was activated in short 5 s–10 s pulses. Pulses were used to minimize heating and prevent decarboxylation of the acidic cannabinoids. The blender was shaken while grinding to ensure even grinding. Afterward, the walls of the grinding cup were scraped to recover any remaining plant residue. The ground material was manually mixed before extraction. For long-term storage, unused plant materials were stored at −80 °C in vacuum-sealed plastic bags.

A comparison of the *Cannabis* plant materials before and after grinding is depicted in Fig. S2, showing a wide range of particle sizes in the ground materials, including small stem pieces. After grinding, three ground samples were subjected to sieving to evaluate the particle size distribution. The particle size fractions are detailed in Table 1 and illustrated in Fig. S3. Two pre-packaged commercial hemp plant materials (≈ 15 g) and a marijuana plant sample (≈ 20 g) were evaluated. Ground hemp's largest fractions were collected using sieves #35 (≈ 30 %) and #60 (≈ 42 %). For ground marijuana, primary fractions were collected using sieve #60 (≈ 31 %), sieve #120 (≈ 14 %), and the collection pan (≈ 33 %). A more finely ground plant material resulted from marijuana, possibly due to the material's age and reduced moisture content. The portions of the hemp materials collected with sieve #18 (13.6 % and 17.0 %) were attributed primarily to stems [25]. When obtaining sub-samples to characterize the physical composition, it is essential not to use the sieved samples for chemical analysis. Sieving can introduce bias into the analytical process because cannabinoid levels differ in different plant parts. Removing specific particle size fractions, such as larger particles consisting mainly of stems or analyzing only fine particles consisting primarily of buds, can lead to higher cannabinoid levels that are not representative of the original sample.

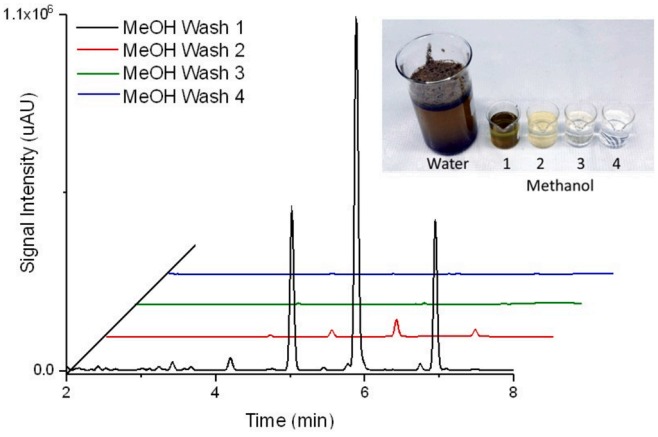

Fig. 1. LC-PDA chromatograms at 220 nm of the four MeOH washes of the grinding vessel after grinding a confiscated marijuana sample (454 g).

The blender was cleaned after grinding to ensure that cannabinoid residues were removed. Approximately 300 mL of H_2O was added to the cup, and the grinding vessel was reassembled and hand-tightened. The blender was pulsed in a few short bursts to dislodge plant residue from the vessel's walls. The vessel was emptied and the process was repeated with 50 mL aliquots of MeOH. An illustration showing the successive washes and corresponding LC-UV chromatograms at 220 nm is presented in Fig. 1, demonstrating the progressive reduction of detected cannabinoids following each MeOH wash. As a result, four washes with MeOH were used to clean the grinding vessel, which was then allowed to air dry for 2 h.

Sample extraction procedure

Solvent extractions are required for most quantitative analytical methods used for botanicals and have recently been summarized in several *Cannabis* review articles [8,27,28]. The NIST CannaQAP conducted an interlaboratory study including over 200 participants with the option to measure up to 17 cannabinoids in ground *Cannabis* plant samples [23]. Approximately 90 % of the laboratories reported results using analytical methods that required liquid extraction with at least one organic solvent. Some critical extraction conditions have been identified from CannaQAP Exercise 2 data and are summarized in Fig. S4. Sample size can impact the quantity of solvent or length of time required to extract Δ^9-THC and other cannabinoids completely. For example, 1 g samples require larger extraction solvent volumes or extraction times than 0.1 g samples. NIST used 0.1 g samples to refine our method based on the number of laboratories using small sample sizes in CannaQAP and discussions with local police department crime laboratories. The choice of sample size must also consider the homogeneity of the sample and the experimental design for sample replicates. The current study extracted a minimum of three subsamples for each ground sample to minimize heterogeneity effects.

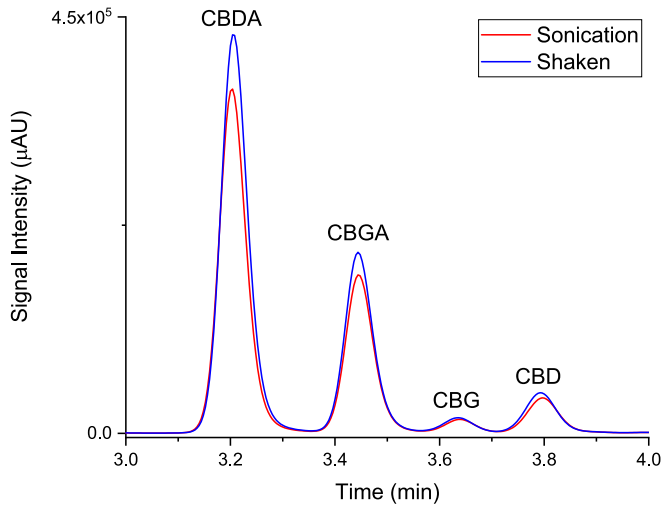

Fig. 2. LC-PDA chromatogram at 220 nm of CBDA, CBGA, CBG, and CBD in an extracted hemp plant sample comparing sonication (red) and mechanical shaking (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

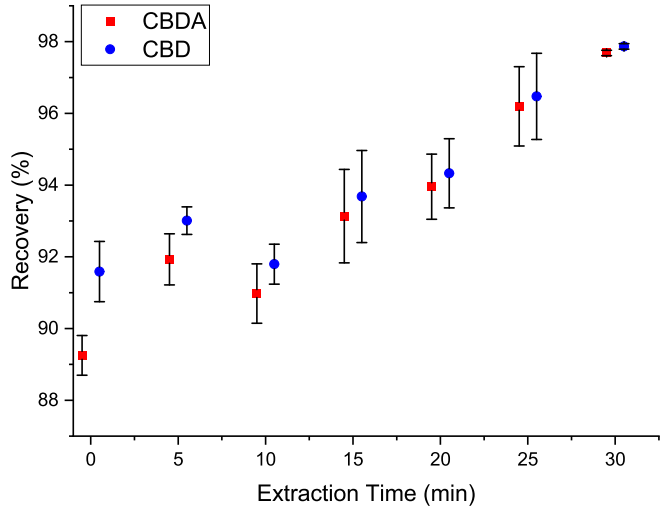

Fig. 3. Percentage of CBDA and CBD recovered over the first 30 min of the MeOH extraction. Error bars correspond to one standard deviation of the mass fraction mean (N = 3).

Extraction equipment

Solvent extractions of *Cannabis* plant samples are typically performed by initial suspension with vortex mixing, followed by sonication or mechanical shaking. Variations in these approaches were compared for ground plant samples (0.1 g), MeOH aliquots (20 mL), and extraction times (30 min). Extracts were centrifuged, decanted, filtered, and analyzed by LC-PDA. Very similar results were obtained for shaking and sonication extraction approaches as summarized in Fig. 2. Mechanical shaking was chosen over sonication extraction to avoid heating the ultrasonic water bath (and sample), which could occur with prolonged use.

Extraction solvent

Selectivity, solubility, cost, and chemical safety must be considered when selecting the extraction solvent for cannabinoids. The use of various extraction solvents has been documented in the literature, spanning polar solvents such as alcohols or ACN to non-polar solvents such as hexane. Polar solvents are advantageous for neutral and acidic cannabinoid measurements. In Exercise 2 of CannaQAP (Fig. S4), participants favored using MeOH or EtOH for extractions. The current study chose MeOH due to its lower cost.

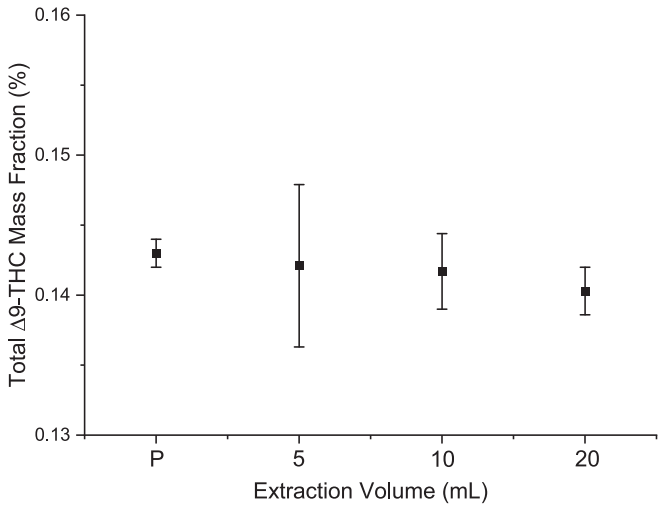

Fig. 4. Mass fractions (%) of total Δ^9-THC in a hemp sample using the previous method (P) and various extraction volumes. Error bars correspond to one standard deviation of the mass fraction mean (N = 3).

Extraction time and cycles

Extraction time usually refers to the time the sample is in contact with the solvent. Studies by Vaclavik et al. [14] demonstrated the importance of additional extraction cycles for extracting 0.5 g ground hemp samples with 20 mL aliquots of EtOH. Approximately 90 % recovery was obtained in the initial 30 min extraction and > 99 % recovery was obtained with a second extraction cycle. A similar approach using 20 mL aliquots of MeOH, 0.1 g samples, and vortex-mixing extraction was evaluated for a ground hemp reference sample (HM19SEP-1). Extract samples of 100 μL were collected at 10 s, 5 min, 10 min, 15 min, 20 min, 25 min, and 30 min, then diluted with 900 μL of MeOH and analyzed by LC-PDA. Most cannabinoids are detected in samples extracted after 10 s of vortex mixing, with a recovery ranging from 87 % to 93 %, as shown in Fig. 3 for CBDA and CBD. The percent recovery slowly increased to ≈ 97 % after 30 min of shaking, and the remaining ≈ 3 % was extracted with a second extraction cycle. It can be concluded that the extraction time has less effect on extraction recovery than the use of additional extraction cycles. The optimized extraction procedure employed 5 min of shaking with two MeOH extraction cycles to limit processing time to <15 min.

Extraction volume

The volume of solvent used for the extraction of cannabinoids from *Cannabis* plant samples should be proportional to the size of the sample. In Exercise 2 of CannaQAP, laboratories normally used larger extraction volumes as the sample size increased. The original extraction procedure implemented at NIST involved the extraction of cannabinoids from 0.5 g of *Cannabis* plant with 20 mL of MeOH. The sample size was reduced to 0.1 g for the optimized procedure. 0.1 g hemp plant samples were extracted with different volumes of MeOH (5 mL, 10 mL, and 20 mL) to evaluate the effect of extraction volume. Levels of Δ^9-THC are plotted as a function of extraction volume in Fig. 4. Within the measurement error, the levels of Δ^9-THC recovered are consistent for the different extraction volumes. The data indicates that 5 mL extraction volumes of MeOH are sufficient for 0.1 g samples.

Centrifugation time

After extraction, sample extracts were decanted aided by centrifugation. The centrifugation time was evaluated between 1 min to 5 min

Table 2
Summary of cannabinoid mass fraction (mean ± 1 SD) comparisons for three hemp plant samples using two MeOH extraction procedures on an as-received basis.

Cannabinoid	Original procedure		New procedure	
	Mass fraction (%)	%RSD	Mass fraction (%)	%RSD
Hemp Sample 1				
CBDV	0.208 ± 0.017	8.06	0.203 ± 0.000	0.23
CBDA	2.241 ± 0.115	5.13	1.817 ± 0.095	5.21
CBGA	2.480 ± 0.172	6.93	2.524 ± 0.015	0.61
CBG	0.366 ± 0.020	5.35	0.345 ± 0.006	1.70
CBD	0.452 ± 0.026	5.78	0.398 ± 0.007	1.80
Δ^9-THC	0.065 ± 0.001	1.92	0.063 ± 0.001	1.72
Δ^9-THCA	0.060 ± 0.001	2.22	0.062 ± 0.003	4.48
Total CBD	2.417 ± 0.127	5.25	1.992 ± 0.090	4.53
Total Δ^9-THC	0.118 ± 0.002	2.06	0.117 ± 0.003	2.98
Hemp Sample 2				
CBDA	8.110 ± 0.211	2.59	8.266 ± 0.404	2.59
CBG	0.030 ± 0.001	3.01	0.026 ± 0.001	5.38
CBD	0.587 ± 0.011	1.87	0.573 ± 0.006	1.03
Δ^9-THC	0.068 ± 0.002	2.37	0.069 ± 0.001	1.67
Δ^9-THCA	0.240 ± 0.005	2.00	0.237 ± 0.017	7.12
Total CBD	7.720 ± 0.180	2.33	7.823 ± 0.347	4.44
Total Δ^9-THC	0.279 ± 0.006	2.08	0.277 ± 0.014	5.10
Hemp Sample 3				
CBDA	4.210 ± 0.230	5.46	4.294 ± 0.194	4.51
CBD	0.241 ± 0.023	9.54	0.255 ± 0.013	5.18
Δ^9-THC	0.028 ± 0.003	9.29	0.032 ± 0.001	2.91
Δ^9-THCA	0.136 ± 0.011	8.09	0.148 ± 0.004	2.46
Total CBD	3.930 ± 0.200	5.09	4.021 ± 0.182	4.53
Total Δ^9-THC	0.147 ± 0.012	8.16	0.161 ± 0.004	2.55

Table 3
Cannabinoid mass fraction (mean ± 1 SD) comparisons for three marijuana plant samples using two MeOH extraction procedures on an as-received basis.

Cannabinoid	Original procedure		New procedure	
	Mass fraction (%)	%RSD	Mass fraction (%)	%RSD
Marijuana Sample 1				
CBDA	15.13 ± 0.170	1.12	14.55 ± 0.672	4.62
CBG	0.054 ± 0.002	4.26	0.057 ± 0.001	1.81
CBD	1.050 ± 0.025	2.38	1.207 ± 0.044	3.61
Δ^9-THC	0.127 ± 0.008	5.92	0.133 ± 0.005	3.55
Δ^9-THCA	0.424 ± 0.026	1.79	0.366 ± 0.014	3.77
Total CBD	14.32 ± 0.140	0.98	13.95 ± 0.622	4.46
Total Δ^9-THC	0.498 ± 0.015	3.01	0.454 ± 0.017	3.70
Marijuana Sample 2				
CBDA	13.82 ± 0.160	1.16	13.91 ± 2.200	15.8
CBG	0.066 ± 0.004	5.30	0.068 ± 0.003	4.81
CBD	1.134 ± 0.018	1.59	1.223 ± 0.034	2.78
CBN	0.030 ± 0.001	3.34	0.030 ± 0.001	4.23
Δ^9-THC	0.292 ± 0.007	2.33	0.275 ± 0.008	2.92
Δ^9-THCA	1.289 ± 0.024	1.86	1.120 ± 0.021	1.91
Total CBD	13.25 ± 0.140	1.12	13.42 ± 1.877	14.0
Total Δ^9-THC	1.422 ± 0.028	1.91	1.256 ± 0.027	2.12
Marijuana Sample 3				
CBDA	12.99 ± 0.110	0.85	12.69 ± 0.930	7.33
CBG	0.086 ± 0.001	0.58	0.076 ± 0.000	0.47
CBD	1.392 ± 0.048	3.45	1.265 ± 0.113	8.97
CBN	0.065 ± 0.001	1.55	0.063 ± 0.000	0.43
Δ^9-THC	0.442 ± 0.016	3.62	0.393 ± 0.034	8.64
Δ^9-THCA	1.996 ± 0.077	3.86	1.767 ± 0.176	9.98
Total CBD	12.79 ± 0.100	0.78	12.39 ± 0.820	6.62
Total Δ^9-THC	2.193 ± 0.083	3.78	1.942 ± 0.188	9.70

based on visual assessment with 0.1 g samples and 5 mL aliquots of MeOH. The appropriate centrifugation time was 157 rad/s (1500 rpm) for 2 min.

Sample clean-up procedure

After extraction, samples must be filtered and diluted to maintain instrument performance and prevent overloading effects that may affect measurement accuracy. Filtration limits the introduction of particulate matter into chromatographic instrumentation and it helps minimize maintenance and repair costs for the equipment. Approximately 2 mL sample extracts were processed through a 0.45 μm PTFE syringe filter for the original and optimized procedure. The filtered extracts are diluted with MeOH to create 10-fold and 100-fold sample dilutions to ensure the chromatographic response falls within the LC-UV calibration curve. Sample dilutions also help prevent instrumental downtime because of additional routine maintenance and costly repairs. Better accuracy will routinely be achieved by gravimetric dilutions rather than volumetric dilutions. NIST performs sample dilutions gravimetrically when assigning mass fractions to reference materials [22]; however, NIST used calibrated pipettes to simulate the more common volumetric practice used by forensic and *Cannabis* testing laboratories for the more rapid sample preparation procedure.

Comparison of MeOH extraction procedures

Six ground *Cannabis* plant samples (Hemp Sample 1–3 and Marijuana Sample 1–3) from the NIST CannaQAP Program were utilized in this study. NIST made LC-PDA measurements with the original extraction protocol to help assign target values for performance assessment [23]. The same *Cannabis* samples were analyzed by LC-PDA using the optimized MeOH extraction procedure. Three subsamples (0.1 g ± 0.01 g) of each C*annabis* sample were weighed into 50 mL centrifuge tubes with 5 mL of MeOH. The subsamples were vortex mixed for 10 s, mechanically shaken for 5 min, and centrifuged for 2 min at 157 rad/s (1500 rpm).

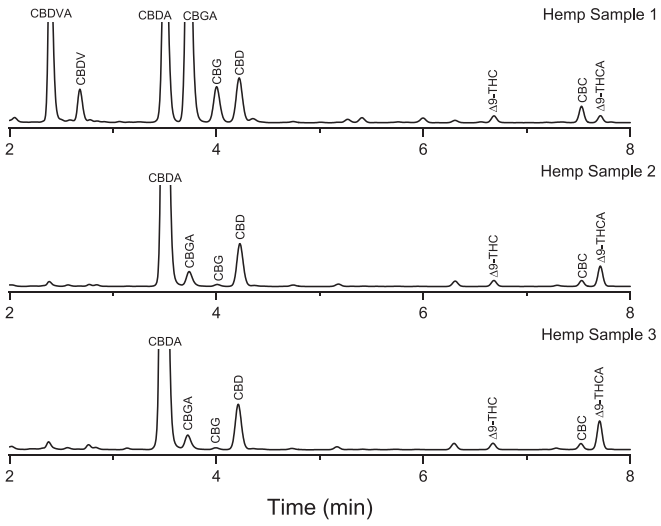

Fig. 5. LC-UV chromatogram at 220 nm for Hemp Sample 1 to Hemp Sample 3 in the non-diluted sample extracts.

The MeOH layer was decanted and a 5 mL aliquot of MeOH was added to the samples to repeat the extraction process. The extracts were combined, filtered, and diluted with MeOH to make 10-fold and 100-fold sample dilutions for LC-PDA analysis. NIST used three criteria to identify cannabinoids to increase confidence in the analysis. Retention times for individual cannabinoids in plant samples must match retention times to reference standards (± 0.10 min). Normalized absorbance spectra collected at the apex of cannabinoid peaks in *Cannabis* plants must provide similar profiles to absorbance spectra collected for individual cannabinoids in reference standards. Lastly, peak purity is assessed by examining the UV absorbance spectra collected across the entire

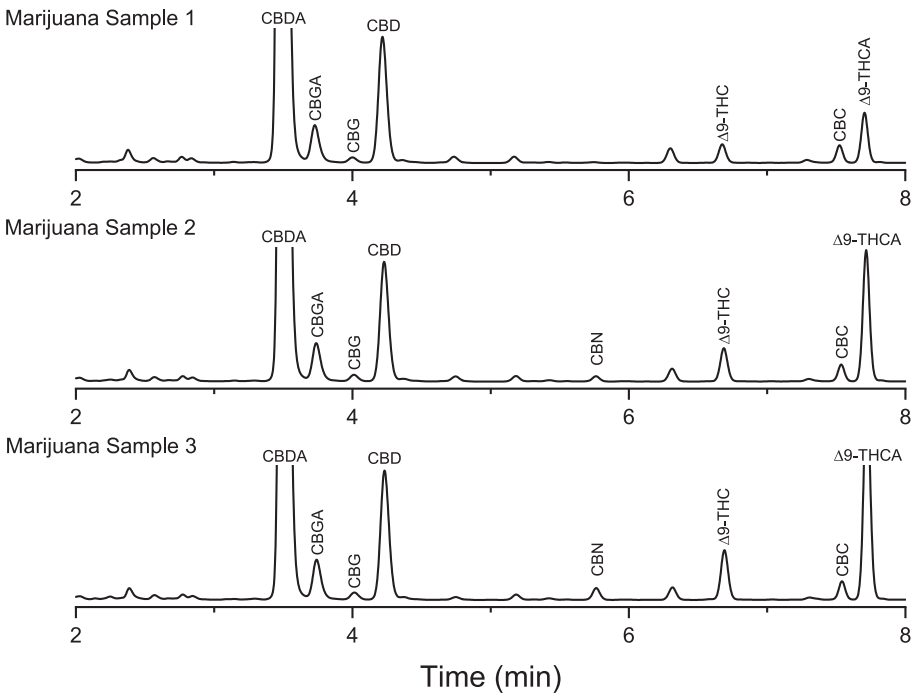

Fig. 6. LC-UV chromatogram at 220 nm for Marijuana Sample 1 to Marijuana Sample 3 in the non-diluted sample extracts.

chromatographic peak to identify significant changes resulting from chromatographic interferences.

Cannabinoid levels (%, mass fraction) are summarized in Tables 2 and 3 for the three hemp and three marijuana plant samples, using the original and new MeOH extraction methods and LC-PDA analysis. Example LC-UV chromatograms at 220 nm of the *Cannabis* samples are shown in Figs. 5 and 6 for undiluted sample extracts for hemp and marijuana samples, respectively. The cannabinoid levels agree well at the 95 % confidence level [29]. Except for the results of CBDA and total CBD in Marijuana Sample 2, the percent relative standard deviation (RSD) was <10 % with the new extraction procedure, comparable to the original method with only the requirement of 15 min of sample preparation.

Conclusions

A new sample preparation procedure and LC-PDA method have been developed for the determination of CBDV, CBDA, CBGA, CBG, CBD, Δ^9-THCV, CBN, Δ^9-THC, Δ^8-THC, CBC, and Δ^9-THCA in *Cannabis* plant samples. The optimized method offers advantages that promote its use in high-throughput environments. Advantages include reduced processing time (15 min), smaller sample size (0.1 g), reduced solvent volumes (10 mL), the use of only standard laboratory equipment (vortex mixer, shaker, and centrifuge), and rapid instrumental analysis by LC-PDA (10 min). Using the new extraction method, levels of Δ^9-THC and Δ^9-THCA were determined in three marijuana and three hemp samples, with each analysis taking less than 30 min to complete. Cannabinoid levels agree well at the 95 % confidence level with measurements obtained using a previously established method that utilized a lengthy extraction procedure.

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Disclaimer

Certain equipment, instruments, software, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement of any product or service by NIST, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

CRediT authorship contribution statement

Walter B. Wilson: Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition. **Aaron A. Urbas:** Methodology. **Haley Jensen:** Methodology. **Lane C. Sander:** Writing – original draft, Methodology.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.forc.2024.100610.

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