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ORIGINAL

Determination of 11 Cannabinoids in Hemp Plant and Oils by Liquid Chromatography and Photodiode Array Detection

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Abstract

Drug scheduling has directed the testing approaches for forensic laboratories since the 1970s when *Cannabis* (marijuana and hemp) and its psychoactive constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC), were classified as Schedule 1 controlled substances. Seized evidence is tested by federal, state, and local crime laboratories following a qualitative test scheme. However, the 2018 Farm Bill defined hemp as *Cannabis* containing 0.3% or less of decarboxylated-Δ^9-THC (total THC) and removed hemp from the controlled substance list. As a result of this change, forensic laboratories are required to quantify the level of total THC to distinguish *Cannabis* as marijuana (an illegal controlled substance) or as hemp (a legal commodity). The National Institute of Standards and Technology (NIST) has recently established an integrated *Cannabis* measurement services program for forensic and cannabis testing laboratories to help ensure the quality of analytical measurements through the development of a Cannabis Quality Assurance Program (CannaQAP) and Reference Materials (RMs). To support these efforts, NIST is developing, implementing, and validating analytical methods for screening bulk hemp and marijuana samples; these methods may potentially be implemented in forensic laboratories. In this article, an LC-PDA method is evaluated for the determination of 11 cannabinoids in 4 hemp plant reference samples from the University of Kentucky Proficiency Testing Program (UK-PT) for cannabinoids, and 15 commercially available hemp oils. Samples were extracted following a previously approved methanol (MeOH) extraction method by an AOAC Expert Review Panel. The results summarized here demonstrate the accuracy and precision of the LC-PDA method for the screening of future RMs and/or CannaQAP samples.

Keywords Hemp · *Cannabis* · Marijuana · Cannabinoids · Delta-9-tetrahydrocannabinol · Liquid chromatography

Introduction

In 2016, the legal cannabis market in the US was worth an estimated $7.2B [1] and has grown to an estimated worth of $61B in 2020 with gross sales of $18.3B [2]. Much of this growth can be attributed to passage of the 2018 Farm Bill that defined hemp as cannabis plant or finished products containing 0.3% or less of decarboxylated-Δ^9-tetrahydrocannabinol (total THC) and removed hemp from the United States Drug Enforcement Agency controlled substances list [3]. Additionally, to date, medical marijuana has been legalized in 36 states and 17 states as well as the District of Columbia permit recreational marijuana usage [4]. As a result, the legal cannabis market has exploded with a wide range of finished products. By the end of 2020 [5], the cannabis plant represented 32% of sales, whereas edibles/ingestibles and vape cartridges each represented 26% of sales. Hemp oils are the most popular type of ingestible on the market and are often used as the primary component in vape cartridges. As the industry grows, so does the need for cannabis testing laboratories and forensic laboratories to have reliable methods for the differentiation between legal and illegal hemp oils and the hemp plant materials used in their production. The National Institute of Standards and Technology (NIST) has started an integrated measurement services program to help ensure the quality of analytical

Disclaimer Certain commercial equipment or materials are identified in this paper to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified is necessarily the best available for the purpose.

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methods though a Cannabis Quality Assurance Program (CannaQAP), Reference Materials (RM) production, and development of robust analytical methods.

Forensic laboratories generally utilize a qualitative test scheme with minimal sample homogenization, extraction, and/or clean-up for seized cannabis [6], which includes macro- and microscopic identification of plant features [7], colorimetric testing for presence of Δ^9-THC [8], and gas chromatography with mass spectrometry (GC–MS) for confirmation of Δ^9-THC [9]. GC and liquid chromatography coupled to a photodiode array detector (LC-PDA) are the primary separation techniques used for quantitative determination of individual cannabinoids and total THC in cannabis products. Leghissa et al. [10] recently summarized the published GC and LC methods for the chemical characterization of cannabis natural products. Of these two approaches, GC is generally favored in forensic laboratories because of shorter separation times, reduced solvent consumption, familiarity, and simplicity [8, 9]. GC may be coupled with flame ionization detection (FID) or MS, but MS provides the distinct advantage of enabling a positive identification of Δ^9-THC based on its mass spectrum in seized samples.

The quantitative determination of Δ^9-THC, THCA, and total THC in cannabis products is primarily achieved in the cannabis industry through LC coupled with PDA or Ultraviolet–visible absorbance detection [10–18]. LC-PDA facilitates rapid confirmation of Δ^9-THC and THCA by collection of absorbance spectra. Methods are linear over 4–5 orders of magnitude, and the use of external standard calibration and reduced sample preparation is often feasible. In addition, LC-PDA permits the quantitation of Δ^9-THC and THCA separately to enable calculation of total THC mass fraction (%) values (Eq. 1). As a result, federal, state, and local crime laboratories have started implementing these techniques for the analysis of seized cannabis plant and finished products.

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

Ciolino et al. [11] demonstrated the accuracy and precision of LC-PDA for the quantitative analysis of 11 cannabinoids in a range of commercial cannabis products, such as plant and hemp oils. Samples were extracted via sonication with 95% ethanol and 5% water for 30 min and diluted prior to analysis. The samples were separated on a MacMod ACE 5 C18-AR analytical column with an isocratic mobile phase of 66% acetonitrile and 34% water (0.5% acetic acid). An extensive method validation was conducted demonstrating the accuracy and precision for the determination of 5 primary cannabinoids; however, the LC run time of 50 min limits its potential usage in forensic laboratories. LC-PDA has been shown to provide shorter run times through the use of a gradient mobile phase [14–18].

In the current study, 11 cannabinoids (Table S1) are determined in 4 hemp plant reference samples and 15 commercial hemp oils using the *Cannabis Analyzer* LC-PDA method with a run time of 10 min. The commercial LC-PDA Cannabis Analyzer method was designed specifically for cannabinoid measurements and marketed to forensic laboratories as an all-in-one instrument. This method was combined with a published methanol (MeOH) extraction method previously approved as an AOAC Official Method [18]. RMs are the best samples to use for evaluating the accuracy and precision of analytical methods; however, only a few hemp reference materials are available with measurable amounts of Δ^9-THC, THCA, CBD, and CBDA. The four hemp samples analyzed here are from the University of Kentucky Proficiency Testing (UK-PT) program accompanied with a *Certificate of Analysis* (COA) summarizing assigned mass fraction (%) values for five cannabinoids, total THC, and total CBD [19]. A hemp oil RM is available for purchase from Emerald Scientific; however, the assigned mass fraction (%) values for Δ^9-THC and THCA are less than or equal to 0.01% and are lower than the typical commercial hemp oil. As a result, the 15 hemp oils analyzed here were obtained from 4 commercial sources consisting of various types of carrier oils (coconut, olive, and hemp seed) and flavorings (peppermint, lemon, orange, etc.). The results indicate the analytical method to be accurate and precise for the screening of future RMs and/or CannaQAP samples.

Experimental

Chemicals and Materials

The certified reference material (CRM) mixture solution of 11 cannabinoids in acetonitrile was obtained from Shimadzu (part # 220-91239-21) with a concentration of 250 mg/L. HPLC-grade acetonitrile (ACN) and water (H_2O) with 0.085% phosphoric acid (PA) concentration were purchased from Shimadzu Instruments, LLC (Columbia, MD). Methanol (MeOH) was purchased from Fisher Scientific (St. Louis, MI). Four reference hemp plant samples were obtained from the UK-PT program that are accompanied with a COA and a summary of their PT results [19]. Fifteen commercial hemp oil samples were purchased from four commercial sources. All plant and oil samples were stored in the dark at − 80 °C.

Sample Preparation

Calibration Standards

Four calibration solutions (calibrants) were individually prepared volumetrically from the Shimadzu cannabinoid RM

mixture (250 mg/L) to have final mass concentrations of 2.5 mg/L, 10 mg/L, and 25 mg/L.

University of Kentucky Hemp Reference Samples

The plant samples were extracted following the guidelines described in an official AOAC method with a modification of using methanol instead of ethanol [14]. The plant samples were equilibrated at room temperature for 1 h and mixed thoroughly by hand to ensure homogeneity. Four subsamples (≈ 0.50 g ± 0.05 g) of each cannabis sample were weighed into 50 mL centrifuge tubes. MeOH (20 mL) was added to each sample and vortexed for 10 s to ensure initial suspension. Samples were then shaken for 30 min and centrifuged for 5 min at 1000 rpm. The MeOH extract was removed and a second 20 mL aliquot of MeOH was added to the cannabis sample. After shaking and centrifugation, the extract was decanted and combined with the initial MeOH extract. Extracts were filtered with a 0.45 µm nylon membrane filter and tenfold and 100-fold methanol dilutions were prepared for LC-PDA measurements.

Commercial Hemp Oils

Hemp oil samples were prepared following the guidelines described in an official AOAC method [14]. The oil samples were equilibrated at room temperature for 3 h and mixed by hand to ensure homogeneity. Three subsamples of each of the hemp oil samples were weighed (≈ 0.50 g ± 0.05 g) into 50 mL centrifuge tubes. MeOH (25 mL) was added to each sample and the mixture was vortexed for 10 s to ensure initial mixing. Samples were then shaken for 15 min and centrifuged for 1 min. The hemp oil sample extract was filtered with a 0.45 µm nylon membrane filter and MeOH was added to prepare tenfold and 100-fold sample dilutions for LC-PDA measurements.

LC-PDA

The LC-PDA measurements were performed on a Shimadzu *Cannabis Analyzer* equipped with a binary pump, degasser, auto-sampler, column compartment, and a photodiode array detector (Shimadzu, Columbia, Maryland). The instrument was computer controlled using commercial software (Lab Solutions, Shimadzu). Separations were carried out on a NexLeaf CBX for Potency C_{18} column purchased from Shimadzu with the following characteristics: 15.0 cm length, 4.6 mm i.d. and 2.7 µm particle diameter. The LC column was protected with the installation of a NexLeaf CBX guard column purchased from Shimadzu. The separation conditions were previously optimized by Shimadzu as a "high-sensitivity method" and are summarized in Table 1.

Table 1 LC-PDA operating parameters

LC conditions			
Injection volume	5 µL		
Column temperature	40 °C		
Flow rate	1.6 mL/min		
Mobile phase program			
	Time (min)	0.085% PA in H_2O	0.085% PA in ACN
	0.0	30	70
	3.0	30	70
	7.0	15	85
	7.1	5	95
	8.0	5	95
	8.1	30	70
	10.0	30	70
PDA conditions			
Wavelength range	190–700 nm		
Quantitative wavelength	220 nm		

Results and Discussion

Liquid Chromatography—Photodiode Array Detection of 11 Cannabinoids

The work summarized here focuses on the evaluation of a LC-PDA method for the initial screening of cannabis plant and oil samples to be used in CannaQAP. The 3D chromatogram obtained with the LC-PDA analysis of a 250 mg/L calibration solution is shown in Fig. 1A. The extracted chromatogram at 208 nm and the absorbance spectra of CBDV are shown in Fig. 1B, C, respectively. The absorbance spectra for all 11 cannabinoids are shown in Figures S1–S11. The chromatographic retention times and absorbance peak wavelengths for this LC-PDA method are summarized in Table S1. Baseline separation was obtained for 7 of the 11 cannabinoids resulting in two co-eluting pairs: (1) CBD/THCV and (2) Δ^9-THC/Δ^8-THC.

Screening measurements by the LC-PDA method are based on the external standard calibration method, which is typically performed in cannabis (hemp or marijuana) testing laboratories and has become more prevalent in forensic laboratories. In this study, each calibration solution was measured three times and the individual chromatograms are shown in Figure S12–S17. The common wavelength of 220 nm was selected to provide the highest response for all cannabinoids. The individual, mean, standard deviation, and relative standard deviations (RSDs) for the chromatographic peak areas are summarized in Table S2. Method repeatability at the various concentrations was less than 3.0% except for CBC and THCA at 2.5 mg/L calibration solutions.

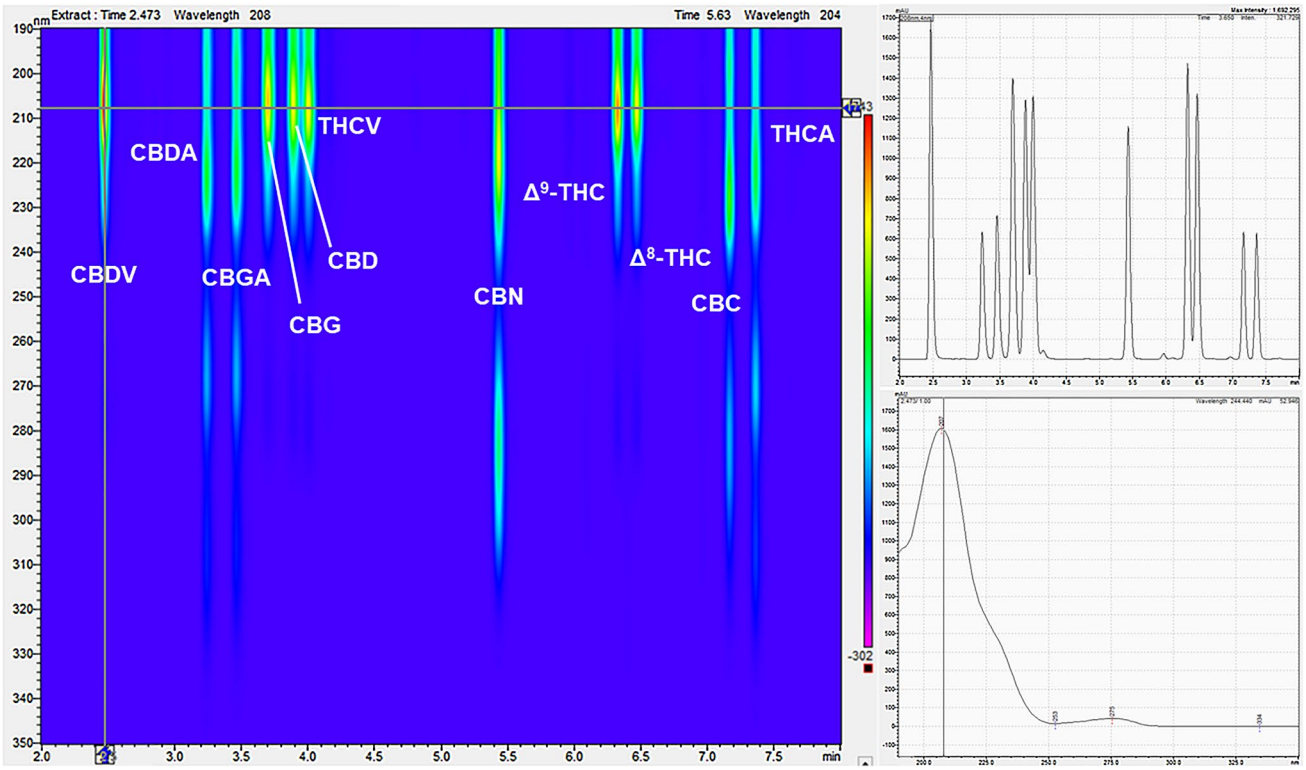

Fig. 1 LC-PDA analysis of the 250 mg/L calibration solution: **A** 3D chromatogram; **B** extracted wavelength chromatogram at 208 nm; and **C** UV absorbance spectra of CBDV at 2.473 min. The 11 cannabinoids included in this analysis are cannabidivarin (CBDV), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), cannabigerol (CBG), cannabidiol (CBD), tetrahydrocannabivarin (THCV), cannabinol (CBN), Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-tetrahydrocannabinol (Δ^8-THC), cannabichromeme (CBC), and Δ^9-tetrahydrocannabinolic acid (THCA)

In the case of Δ^9-THC, the calibration curve obtained from 2.5 to 250 mg/L (corresponding to mass fractions of Δ^9-THC in solution of 0.32–32.0%; respectively) is shown in Fig. 2A with a correlation coefficient (r^2) of 0.9999. This value is normally considered excellent; however, low Δ^9-THC levels are poorly fit to the regression model, which exhibits a significant y-intercept of 0.2917. Similar observations were made for the additional cannabinoids except for CBDV and THCV. A calibration curve for Δ^9-THC constructed over a narrower concentration interval (2.5 to 25 mg/L; 0.32–3.2% mass fractions Δ^9-THC in solution) is shown in Fig. 2B with $r^2 = 0.9997$ a y-intercept of 0.0658. As a result, the narrower calibration range was selected for all future studies to ensure the most accurate measurements summarized in Table S3 for the hemp plant and oil samples in further sections.

LC-PDA Analysis of 4 University of Kentucky Hemp Reference Samples

Four hemp reference samples (HM19SEP-1, HM19SEP-2, HM19NOV-1, and HM19NOV-2) were obtained from the UK-PT program for cannabinoids. This program was initiated to evaluate laboratory performance for determination of Δ^9-THC, CBD, and other cannabinoids in hemp, after legalization in the 2018 Farm Bill. Each participating laboratory receives dried ground hemp samples with total THC concentrations less than or equal to 0.3% to ensure samples meet the current legal definition of hemp. The PT results are summarized in reports based on ISO 13528:2015 statistical methods for inter-laboratory comparisons. At completion of the PT studies, UK makes the remaining hemp samples available for purchase as reference samples. The assigned mass fraction (%) values for these samples are based on the results of from 18 to 41 laboratories that participated in the UK-PT exercise, using multiple LC-UV, LC–MS, and GC-FID methods [19].

Four replicate extractions of each of the four reference samples were performed with the methanol extraction protocol, sample dilutions (10-fold and 100-fold), and LC-PDA method summarized above. The LC-PDA measurements using extracted wavelength chromatograms (220 nm) are compared to the PT results in Table 2. Mass fraction (%) values were assigned to the reference samples by the UK-PT program for CBDA, CBD, CBN, Δ^9-THC, THCA, total THC, and total CBD; however, the NIST method was able to

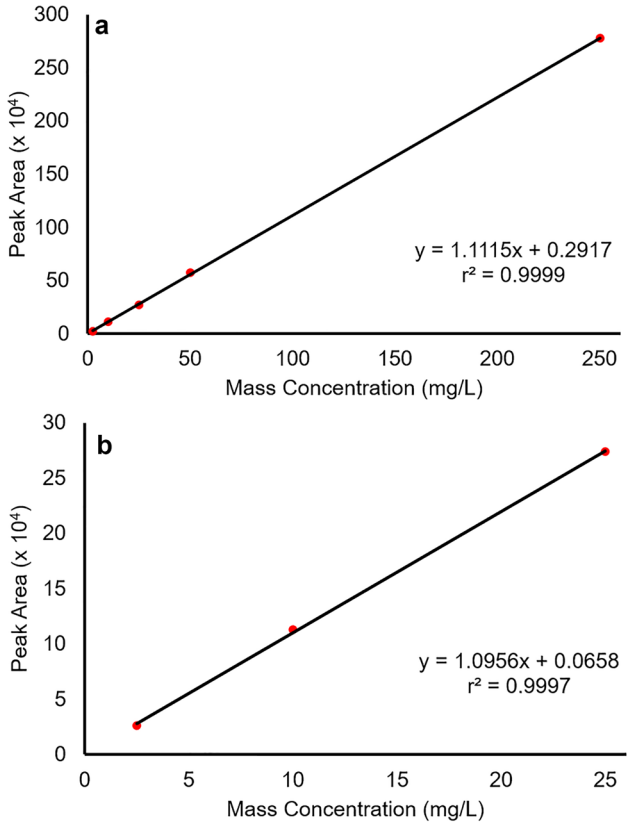

Fig. 2 Δ^9-THC calibration curves by LC-PDA analysis at wavelength 220 nm for a two orders of magnitude calibration range (**A**) and one order of magnitude calibration range (**B**)

quantify four additional cannabinoids: CBDV, CBGA, CBG, and CBC. All nine cannabinoids were identified based on the chromatographic retention times and UV absorbance spectra summarized in Table S1. A typical 3D chromatogram and extracted wavelength chromatograms for HM19SEP-1 are shown in Figs. 3 and 4, respectively.

In general, the UK-PT and NIST mass fraction values for most of the cannabinoids are in good agreement; however, NIST values for CBD and total CBD were slightly higher than the range of the PT study measurements. CBD and CBDA mass fractions values were only determined using the 100-fold dilution sample ($N=4$) to ensure accuracy because the higher mass fraction samples were outside the calibration range used in this study. The mass fraction values for the other seven cannabinoids were determined using undiluted and tenfold dilution samples ($N=8$). The mass fraction values for total THC are in good agreement for all four samples; however, the NIST results are notably higher for Δ^9-THC and lower in THCA in sample HM19NOV-2. This result indicates a potential conversion of THCA to Δ^9-THC in the plant material over time.

LC-PDA Analysis of 15 Commercial Hemp Oil Samples

Due to the limited availability of reliable RMs, 15 hemp oils were obtained from 4 commercial sources that were formulated with various carrier oils including coconut oil (9), olive oil (1), and hemp seed oil (5). The carrier oils differ in density, viscosity, and types of fatty acids. Carrier oils play a role in promoting the absorption of CBD in the body as well as providing potential health benefits. Coconut oil, specifically MCT oil, is predominantly used by manufacturers due to its low cost and lack of flavor [20]. Added matrix complexity is introduced by flavorings; these additives potentially complicate the analysis of commercial CBD formulations. The influence of flavorings on method specificity and accuracy was examined for mint chocolate, olive oil, lemon, and orange flavors utilized by a single manufacturer.

None of the 15 hemp oils were accompanied with COAs; however, the product labels did provide mass concentrations of CBD that ranged from 1.6 (Hemp Oil 13) to 83.3 mg/mL (Hemp Oil 12) corresponding to mass fraction (%) values of 0.170 and 9.22%. Most state regulators require the reported values on the label to be accurate within 15% [21], and this uncertainty was uniformly applied to the determined mass fraction (%) values. Our analyses quantified additional cannabinoids not reported by the manufacturers. These results are summarized in Tables 3, 4, 5 and 6 and are discussed in detail below. The mass fractions (%) reported here are based on three replicate extractions of each of the hemp oils, carried out with the MeOH extraction protocol, 10-fold and 100-fold sample dilutions, and the LC-PDA method summarized above.

Manufacturer 1 Hemp Oils

The NIST-determined mass fraction (%) values for Manufacturer 1 samples consisting of five flavored Hemp Oils (1–5) are summarized in Table 3. The extracted wavelength chromatograms at 220 nm obtained for the undiluted, tenfold dilution, and 100-fold dilution sample preparations of the five hemp oil samples are shown in Figures S18–S22. The flavored hemp oil samples were advertised as full-spectrum CBD oils, which contain a full range of cannabinoids, terpenes, and other compounds. Nevertheless, the complexity of the samples did not negatively impact the separation of the targeted cannabinoids. Levels of CBN were lower in these flavored hemp oils than in all other samples. The mass fraction levels of Δ^9-THC and CBG were consistently similar across all samples. Among the cannabinoids determined, CBD was present at the highest levels across all samples. The CBD mass fraction values provided on the bottles of the five hemp oils were listed as either $5.54 \pm 0.83\%$ or $6.64 \pm 1.00\%$. CBD levels determined by NIST were

Table 2 Mass fraction (%) value comparisons for the UK-PT hemp samples

	UK PT Results			NIST Results[b]		
	Mean ± SD	Labs	RSD (%)	Mean ± SD[a]	*N*	RSD (%)
HM19SEP-1						
CBDV				0.0310 ± 0.0040	8	13.5
CBDA	3.59 ± 0.45	35	3.29	4.167 ± 0.038	4	0.90
CBGA				0.0950 ± 0.0080	8	8.75
CBG				0.0970 ± 0.008	8	8.31
CBD	5.21 ± 0.43	38	8.23	6.620 ± 0.075	4	1.11
CBN	0.037 ± 0.011	29	29.7	0.0370 ± 0.0030	8	6.94
Δ^9-THC	0.248 ± 0.029	41	11.5	0.262 ± 0.014	8	5.38
CBC				0.298 ± 0.016	8	5.53
THCA	0.039 ± 0.016	35	42.6	0.0330 ± 0.0040	8	11.6
Total THC	0.287 ± 0.044	52	15.3	0.291 ± 0.015	8	5.14
Total CBD	8.42 ± 0.81	39	9.60	10.3 ± 0.11	4	1.03
HM19SEP-2						
CBDV				0.0200 ± 0.0010	8	6.96
CBDA	1.72 ± 0.25	35	14.6	1.870 ± 0.095	8	4.97
CBGA				0.0380 ± 0.0050	8	12.1
CBG				0.0320 ± 0.0050	8	15.3
CBD	1.48 ± 0.13	38	9.01	1.95 ± 0.10	8	5.08
CBN	0.0138 ± 0.0096	18	69.1	0.0090 ± 0.0010	8	9.09
Δ^9-THC	0.110 ± 0.016	40	14.5	0.1210 ± 0.0020	8	1.28
CBC				0.1390 ± 0.0040	8	2.68
THCA	0.039 ± 0.015	35	38.9	0.0340 ± 0.0040	8	13.0
Total THC	0.144 ± 0.027	52	19.0	0.1500 ± 0.0040	8	2.68
Total CBD	3.02 ± 0.34	40	11.3	3.52 ± 0.13	8	7.28
HM19NOV-1						
CBDV				0.0140 ± 0.0040	8	10.5
CBDA	5.20 ± 0.54	41	10.4	5.83 ± 0.28	4	4.79
CBGA				0.1550 ± 0.0080	8	2.79
CBG				0.0610 ± 0.0020	8	3.14
CBD	1.59 ± 0.15	42	9.22	2.23 ± 0.13	8	5.63
CBN	0.023 ± 0.023	13	98.4	0.0060 ± 0.0010	8	9.86
Δ^9-THC	0.161 ± 0.021	47	13.0	0.2000 ± 0.0040	8	2.15
CBC				0.1570 ± 0.0040	8	2.38
THCA	0.159 ± 0.028	48	17.6	0.1400 ± 0.0050	8	3.34
Total THC	0.300 ± 0.038	60	12.6	0.3230 ± 0.0070	8	2.25
Total CBD	6.18 ± 0.50	46	8.12	7.43 ± 0.18	4	4.66
HM19NOV-2						
CBDV				0.0060 ± 0.0010	7	19.7
CBDA	1.407 ± 0.1467	41	10.4	1.500 ± 0.090	8	5.93
CBGA				0.0570 ± 0.0030	8	5.73
CBG				0.0230 ± 0.0040	8	16.4
CBD	0.4412 ± 0.0411	42	9.31	0.59 ± 0.070	8	11.7
CBN	0.0148 ± 0.0139	6	93.9	0.0040 ± 0.0010	7	25.7
Δ^9-THC	0.0351 ± 0.0056	42	15.7	0.0350 ± 0.0030	8	8.74
CBC				0.0410 ± 0.0020	8	4.01
THCA	0.0351 ± 0.0104	43	29.1	0.0290 ± 0.0020	8	7.93
Total THC	0.0686 ± 0.0138	56	20.1	0.0610 ± 0.0040	8	6.79
Total CBD	1.6881 ± 0.1669	46	9.89	1.910 ± 0.075	8	7.68

[a]CBD and CBDA measurements were determined with the 100-fold dilution sample preparation; other cannabinoids were determined with undiluted and tenfold dilution sample preparations

[b]The NIST-determined mass fractions include two significant figures for the measurement SD and the mean values match the number of decimal places to the SD

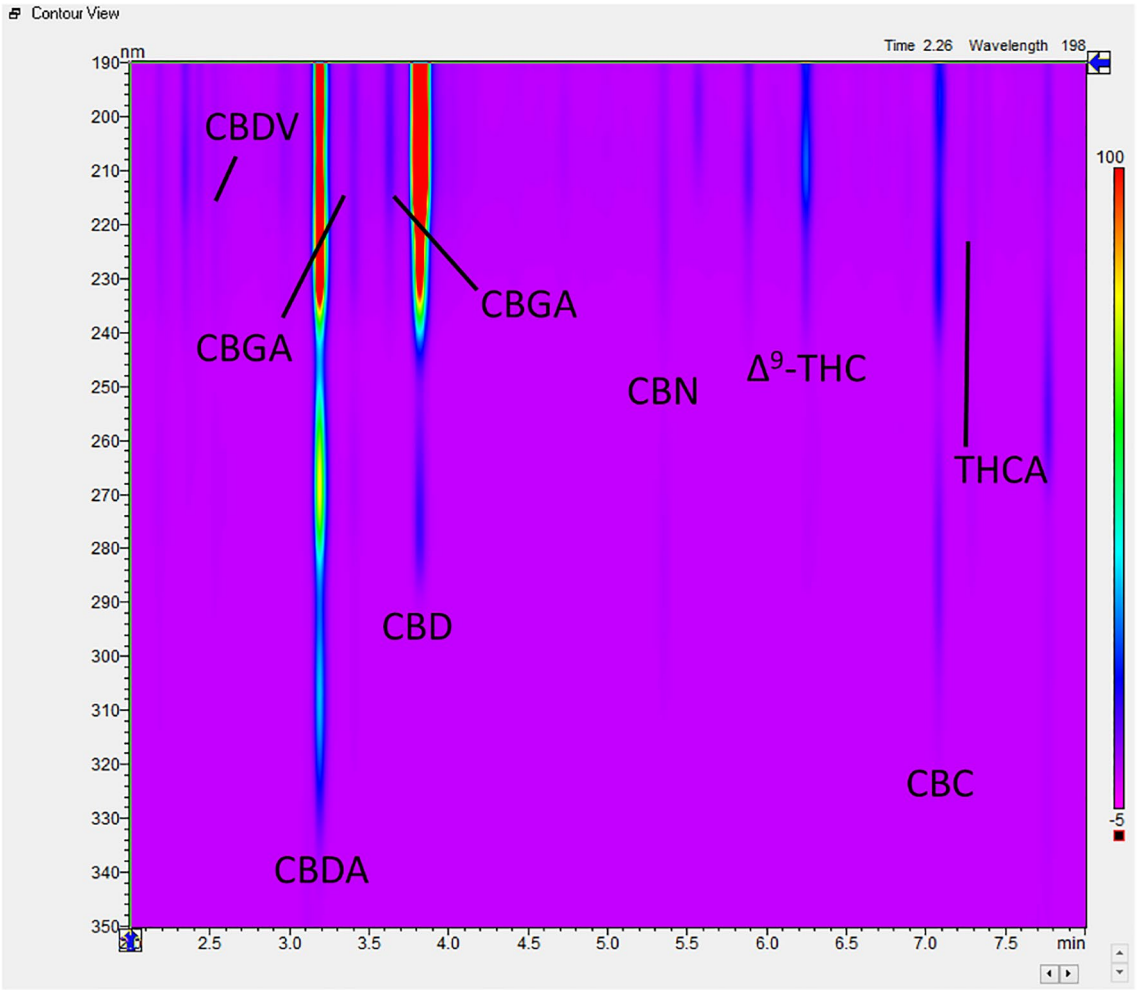

Fig. 3 Example 3D chromatogram obtained from the LC-PDA analysis of HM19SEP-1

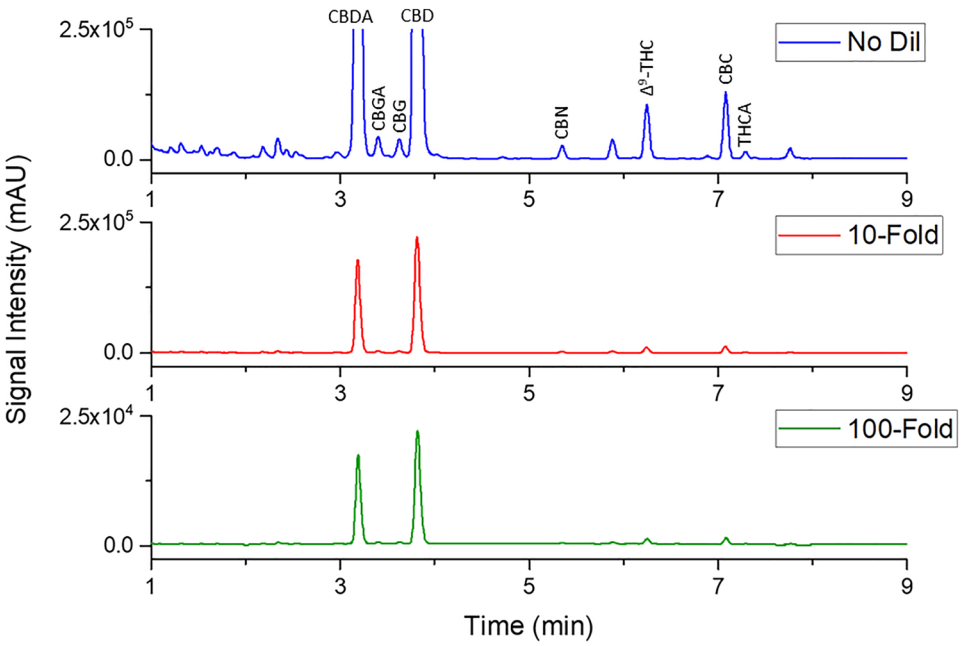

Fig. 4 Extracted wavelength chromatogram at 220 nm for HM19SEP-1

Table 3 Mass fraction (%) values for cannabinoids determined in Manufacturer 1 hemp oils by LC-PDA

Samples	Cannabinoids	Label (%)[f]	Mean ± SD (%)[g]	*N*
Hemp Oil 1[a]	CBDV	NA	0.0200 ± 0.0044	6
	CBG	NA	0.095 ± 0.014	6
	CBD	5.54 ± 0.83	7.10 ± 0.50	3
	CBN	NA	0.0180 ± 0.0035	6
	Δ^9-THC	NA	0.158 ± 0.010	6
	CBC	NA	0.399 ± 0.034	6
Hemp Oil 2[b,c]	CBDV	NA	0.0220 ± 0.0051	6
	CBG	NA	0.114 ± 0.012	6
	CBD	5.54 ± 0.83	6.66 ± 0.18	3
	CBN	NA	0.0160 ± 0.0012	6
	Δ^9-THC	NA	0.1600 ± 0.0063	6
	CBC	NA	0.383 ± 0.010	6
Hemp Oil 3[b,c]	CBDV	NA	0.0260 ± 0.0034	6
	CBDA	NA	0.362 ± 0.032	6
	CBG	NA	0.141 ± 0.013	6
	CBD	6.64 ± 1.00	8.242 ± 0.064	3
	CBN	NA	0.0560 ± 0.0063	6
	Δ^9-THC	NA	0.209 ± 0.012	6
	CBC	NA	0.515 ± 0.030	6
Hemp Oil 4[d]	CBDV	NA	0.0390 ± 0.0044	6
	CBDA	NA	0.281 ± 0.026	6
	CBG	NA	0.119 ± 0.012	6
	CBD	6.64 ± 1.00	7.68 ± 0.18	3
	CBN	NA	0.0430 ± 0.0059	6
	Δ^9-THC	NA	0.1800 ± 0.0068	6
	CBC	NA	0.412 ± 0.019	6
Hemp Oil 5[e]	CBDV	NA	0.0300 ± 0.0040	6
	CBDA	NA	0.206 ± 0.027	6
	CBG	NA	0.118 ± 0.015	6
	CBD	6.64 ± 1.00	7.36 ± 0.21	3
	Δ^9-THC	NA	0.1990 ± 0.0075	6
	CBC	NA	0.276 ± 0.014	6

[a]Olive oil flavored and carrier oil

[b]Mint chocolate flavored

[c]Coconut carrier oil

[d]Orange flavored

[e]Lemon flavored

[f]A – Not Available

[g]The NIST-determined mass fractions include two significant figures for the measurement SD and the mean values match the number of decimal places in the SD value

Table 4 Mass fraction (%) values for cannabinoids determined in Manufacturer 2 hemp oils by LC-PDA

Samples[a]	Cannabinoids	Label (%)[d]	Mean ± SD (%)[e]	*N*
Hemp Oil 6[b]	CBDV	NA	0.1843 ± 0.0013	6
	CBDA	NA	0.1165 ± 0.0017	6
	CBD	0.92 ± 0.14	0.915 ± 0.070	6
Hemp Oil 7[c]	CBDV	NA	0.0209 ± 0.0018	6
	CBD	3.71 ± 0.56	3.51 ± 0.26	3
Hemp Oil 8[b]	CBDV	NA	0.00880 ± 0.00020	2
	CBDA	NA	0.01300 ± 0.00040	2
	CBGA	NA	0.00310 ± 0.00010	2
	CBG	NA	0.0132 ± 0.0010	2
	CBD	3.71 ± 0.56	3.48 ± 0.24	3
	CBN	NA	0.0288 ± 0.0033	5
	CBC	NA	0.0360 ± 0.0060	5
Hemp Oil 9[b]	CBDV	NA	1.6683 ± 0.0087	6
	CBDA	NA	0.01350 ± 0.00060	3
	CBD	9.22 ± 1.38	9.27 ± 0.62	3

[a]Coconut carrier oil

[b]Cool mint flavored

[c]Unflavored

[d]NA – Not Available

[e]The NIST-determined mass fractions include two significant figures for the measurement SD and the mean values match the number of decimal places in the SD value

slightly higher than the values provided by Manufacturer 1, with Hemp Oil 3 showing the greatest difference. The CBD mass fraction determined by NIST in Hemp Oil 3 (8.24 ± 0.064%) is outside of the estimated 15% uncertainty interval (5.64–7.64%). The CBD mass fractions determined for the other four Hemp Oils also vary from the product labels; however, the NIST measurement standard deviations (SD) overlap the product label 15% uncertainty ranges.

Sample dilutions of the oil extracts were also included in these studies to accurately measure the cannabinoids within a narrow calibration range (2.5 to 25 mg/L; see Table S2). Hemp Oil 4 with the orange flavoring was selected for this study (see Fig. 5 and Table S4). The mass fraction values determined for CBD are higher for the 100-fold dilution. The chromatographic response for undiluted samples and tenfold dilution samples are ≈ 2 and ≈ 1 orders of magnitude outside the calibration range, respectively. It is important to note that CBD was the only cannabinoid that required the 100-fold dilution to be within the range of the calibration curve. Similar observations were made for CBD in all the hemp oils from Manufacturer 1. The mass fraction (%) values of CBDA, Δ^9-THC, and CBC for undiluted samples were outside the calibration range and required a tenfold dilution. CBG mass fraction values for undiluted and tenfold dilutions were at the high and low end of the calibration range. The 100-fold dilution was significantly lower than the calibration range. This may account for discrepancies compared with undiluted samples and samples with tenfold dilutions. In general, the precision of the mass fraction values was best when sample dilutions were within the specified calibration range. For these reasons, the results reported for the mean

Table 5 Mass fraction (%) values for cannabinoids determined in Manufacturer 3 hemp oils by LC-PDA

Samples	Cannabinoids	Label (%)[d]	Mean ± SD (%)[e]	*N*
Hemp Oil 10[a,b]	CBDV	NA	0.0230 ± 0.0033	6
	CBDA	NA	0.0820 ± 0.0041	6
	CBG	NA	0.0330 ± 0.0022	6
	CBD	2.72 ± 0.41	3.225 ± 0.064	3
	CBN	NA	0.0130 ± 0.0023	6
	Δ^9-THC	NA	0.0740 ± 0.0041	6
	CBC	NA	0.0880 ± 0.0040	6
Hemp Oil11[a]	CBDV	NA	0.076 ± 0.010	6
	CBDA	NA	0.0250 ± 0.0045	6
	CBG	NA	0.082 ± 0.013	6
	CBD	8.70 ± 1.31	10.1 ± 0.13	3
	CBN	NA	0.0440 ± 0.0075	6
	Δ^9-THC	NA	0.1940 ± 0.0050	6
	CBC	NA	0.2650 ± 0.0075	6
Hemp Oil 12[c]	CBDV	NA	0.072 ± 0.010	6
	CBG	NA	0.081 ± 0.014	6
	CBD	8.86 ± 1.33	9.25 ± 0.66	3
	CBN	NA	0.0130 ± 0.0029	6
	Δ^9-THC	NA	0.157 ± 0.012	6
	CBC	NA	0.160 ± 0.013	6

[a]Hemp Seed Carrier Oil

[b]Peppermint Flavored

[c]Coconut Carrier Oil

[d]NA – Not Available

[e]The NIST-determined mass fractions include two significant figures for the measurement SD and the mean values match the number of decimal places in the SD value

Table 6 Mass fraction (%) values for cannabinoids determined in Manufacturer 4 hemp oils by LC-PDA

Samples [a]	Cannabinoids	Label (%) [b]	Mean ± SD (%) [c]	*N*
Hemp Oil 13	CBD	0.170 ± 0.026	0.176 ± 0.019	6
	Δ^9-THC	NA	0.00200 ± 0.00020	6
	CBC	NA	0.0130 ± 0.0019	6
Hemp Oil 14	CBD	0.540 ± 0.081	0.528 ± 0.051	6
	Δ^9-THC	NA	0.0040 ± 0.0011	6
	CBC	NA	0.0040 ± 0.0010	6
Hemp Oil 15	CBD	1.09 ± 0.16	1.11 ± 0.13	6
	Δ^9-THC	NA	0.0090 ± 0.0012	6
	CBC	NA	0.0080 ± 0.0010	6

[a]Hemp seed carrier oil and unflavored

[b]NA – Not Available

[c]The NIST-determined mass fractions include two significant figures for the measurement SD and the mean values match the number of decimal places in the SD value

mass fractions in Tables 3, 4, 5 and 6 for hemp oils are based on measurements within the one order of magnitude calibration range.

Manufacturer 2 Hemp Oils

The NIST-determined mass fraction values for broad-spectrum hemp oils produced by Manufacturer 2 are summarized in Table 4. The extracted wavelength chromatograms at 220 nm obtained for undiluted, tenfold dilution, and 100-fold dilution sample preparations are shown in Figures S23–S26. Broad-spectrum oils contain a range of cannabinoids in addition to CBD but lack Δ^9-THC. NIST measurements confirm the absence of Δ^9-THC in the four samples. These hemp oils were flavorless and used coconut oil as the carrier oils, which is immiscible with MeOH used in sample extraction. The NIST-determined CBD values were consistent with the values provided on the product labels of the four hemp oils. The greatest number of cannabinoids was detected in Hemp Oil 8, which contained CBDV, CBDA, CBG, CBG, CBN, and CBC. All other samples only contained CBDV, CBDA, and CBD, excluding Hemp Oil 7, which only contained CBDV and CBD. CBDA levels were significantly higher in Hemp Oil 6 compared with Hemp Oil 9. CBD values were the highest in the Hemp Oil 9, consistent with the manufacturer's claims.

Manufacturer 3 Hemp Oils

The NIST-determined mass fraction values are summarized in Table 5 for full-spectrum Hemp Oil 10, Hemp Oil 11, and Hemp Oil 12 from Manufacturer 3. The extracted wavelength chromatograms at 220 nm obtained for the undiluted, tenfold dilution, and 100-fold dilution sample preparations of the three hemp oil samples are shown in Figures S27–S29. Samples were distinguished on the product labels by CBD concentration using the term "extra strength"; however, this designation is not universally employed by all manufacturers. Hemp Oil 11 and Hemp Oil 12 were classified as "extra strength" with significantly higher levels of CBD than Hemp Oil 10. The CBD levels determined by NIST were higher than CBD values listed on the product labels. Hemp Oil 10 and Hemp Oil 11 used hemp seed carrier oils and showed the greatest difference in CBD values compared with NIST values; however, Hemp Oil 12 used a coconut carrier oil and the NIST results agree well with the product labels. The use of a hemp seed carrier oil could potentially contribute small additional quantities of CBD to the mixture, leading to higher levels of CBD and other cannabinoids in the finished hemp oil. This aspect is relevant if the label values are based on the results before addition of the carrier. Manufacturer 1 and Manufacturer 3 hemp oils are both classified as full-spectrum hemp oils; notably, the materials from

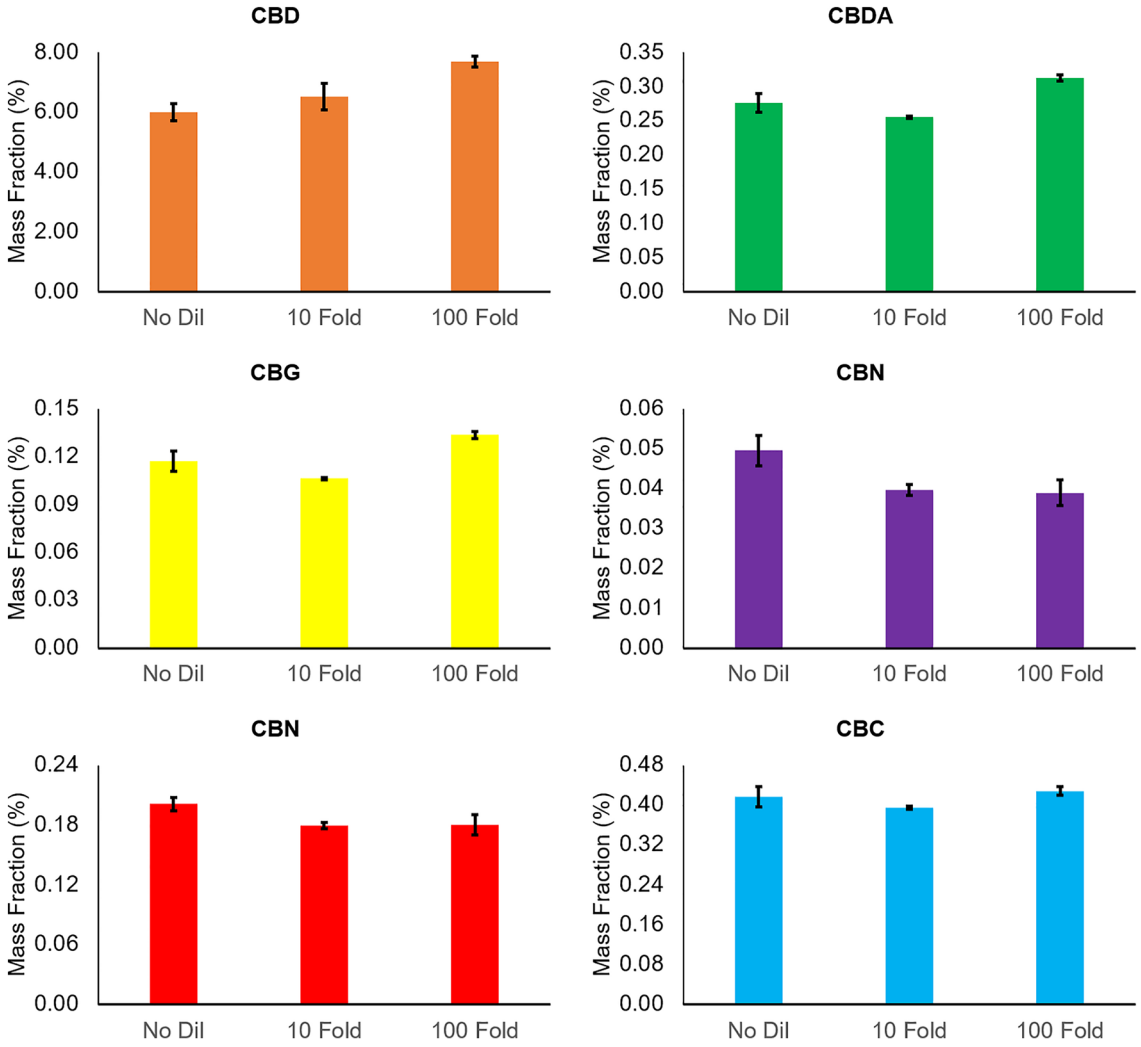

Fig. 5 Mass fractions of cannabinoids determined in Hemp Oil 4 from Manufacturer 1 with the various sample dilutions. The error bars represent one standard deviation of the mean mass fraction value

Manufacturer 3 have detectable levels of Δ^9-THC ranging from 0.074 to 0.194%.

Manufacturer 4 Hemp Oils

The NIST-determined mass fraction values are summarized in Table 6 for full-spectrum Hemp Oil 13, Hemp Oil 14, and Hemp Oil 15 from Manufacturer 4. The extracted wavelength chromatograms at 220 nm obtained for the undiluted, tenfold dilution, and 100-fold dilution sample preparations of the six hemp oil samples are shown in Figures S30–S32. The CBD mass fraction values for Hemp Oil 13 (0.17%) and Hemp Oil 15 (1.09%) were almost identical to NIST values (0.176 ± 0.019%) and (1.11 ± 0.13%), respectively. CBDA levels were consistent across all samples, ranging from 0.003 to 0.004%. Among all samples, the concentration of Δ^9-THC was highest in Hemp Oil 15, while CBC had the lowest concentration.

Conclusion

An LC-PDA method combined with a MeOH extraction method was evaluated for the determination of 11 cannabinoids in 4 UK-PT hemp plant reference samples and 15 commercial hemp oils. NIST quantified 9 of 11 target cannabinoids in hemp plant samples; levels were in good agreement for CBDA, CBD, CBN, Δ^9-THC, THCA, total THC, and total CBD. None of the 15 hemp oils were accompanied with COAs, but the product labels did permit the calculation of CBD mass fractions ranging from 0.170 ± 0.026% (Hemp Oil 13) to 9.22 ± 1.38% (Hemp Oil 12). For these studies, NIST assigned an uncertainty of 15% to product

label values based on current state regulations to permit comparisons with the determined NIST values. The NIST values were determined using a narrow calibration range and 100-fold sample dilutions for the determination of CBD to permit measurements with improved accuracy. NIST values for most samples were near or above the upper limit calculated based on the uncertainty estimate. The analytical methods summarized here were found to be sufficient for the determination of 11 cannabinoids in plant and oil samples as recently summarized in the final report for Exercise 1 of CannaQAP [12]. The LC-PDA provides accurate and precise measurements in less than 10 min; however, the lengthy extraction times (70 min) are to long for forensic laboratories and optimization of sample extraction is in progress to increase efficiency.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Research Involving Human Participants and/or Animals This article does not contain any studies with human participant or animals performed by any of the authors.

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