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Determination of Δ^9-THC, THCA, Δ^8-THC, and total Δ^9-THC in 53 smokable hemp plant products by liquid chromatography and photodiode array detection

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ABSTRACT

The passage of the 2018 Farm Bill defined hemp as cannabis plant containing 0.3 % or less of Δ^9-THC, which led to a large increase in hemp production in the United States in 2021. Approximately 76 % of it focused on floral hemp that is used to produce hemp-derived finished products such as smokable hemp (*e.g.*, manicured, roll your own, or cigarettes). As a result, forensic laboratories have seen a significant increase in confiscated cannabis samples, but few reliable analytical methods exist for differentiation between hemp and marijuana. In response to the need for reliable quantitative methods, the National Institute of Standards and Technology (NIST) has developed and evaluated analytical methods to provide forensic scientists the tools necessary. In this manuscript, 53 smokable hemp plant products were analyzed for Δ^8-THC, Δ^9-THC, THCA, and total Δ^9-THC by a previously established liquid chromatography with photodiode array detection (LC-PDA) method using a methanol extraction procedure. Over 90 % of the samples analyzed by NIST were determined to have a total Δ^9-THC mass fraction above 0.3 % even though samples were being marketed as hemp. Surprisingly, often the associated online documentation reported total Δ^9-THC mass fractions of $\geq$ 0.3 %. Mass fractions determined by NIST were compared with manufacturer's online documentation for 22 samples. Measurements differed by $\approx$ 55 % for total Δ^9-THC, $\approx$ 68 % for THCA, and $\approx$ 18 % for Δ^9-THC. Poor agreement may result from method difference, sample inhomogeneity, batch to batch variability, changes due to storage conditions, and/or product labels or online documentation that are not representative of actual products.

1. Introduction

The United States (US) Department of Agriculture National Agricultural Statistics Services recently released the results of a 2021 Hemp Acreage and Production Survey that estimated the value of hemp production in the United States to be $824 M [1]. Floral hemp represented $\approx$ 76 % of all hemp production used in the manufacture of hemp finished products. One of these products is smokable hemp, which refers to the hemp flower cultivated for smoking purposes similar to traditional marijuana except with lower levels of Δ^9-THC. Smokable hemp can be hand ground and rolled similar to marijuana or it can be purchased pre-rolled as cigarettes.

The 2018 Farm Bill defined hemp as cannabis plant containing 0.3 % or less of decarboxylated-Δ-9-tetrahydrocannabinol (Δ^9-THC) and removed hemp from the US Drug Enforcement Agency controlled substances list [2]. Because enforcement of this distinction is based on quantitative measurements, reliable analytical methods are needed by forensic laboratories to distinguish hemp and marijuana in seized cannabis samples. In response to this need, the National Institute of Standards and Technology (NIST) established a Cannabis Research Program to provide forensic laboratories the necessary tools to quantitatively measure Δ^9-THC, its isomeric compounds (*e.g.* Δ^8-THC, etc.), and its acidic precursor tetrahydrocannabinolic acid (THCA).

Prior to the legal definition of hemp, forensic laboratories performed qualitative characterizations including, macro- and microscopy [3], colorimetric tests [4], and gas chromatography with mass spectrometry (GC–MS) [5] to identify and confirm marijuana. Now, with a quantitative limit, method accuracy is of paramount importance since legal

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actions are based on the resulting measurements [2]. Many forensic laboratories have switched from GC–MS to liquid chromatography due to incomplete conversion of THCA to Δ^9-THC in the GC inlet resulting in measurement bias. Methods that utilize LC with absorbance detection (ultraviolet [UV] or photodiode array [PDA]), mass spectrometry (MS), or tandem mass spectrometry (MS/MS) determine THCA and Δ^9-THC separately to provide the analysts the ability to calculate the total Δ^9-THC mass fraction (%) present in the seized sample. The ability to determine THCA is important in cannabis samples as it is often present as the primary form of Δ^9-THC in cannabis samples. As with GC–MS, PDA enables the collection of complementary data (*i.e.*, absorbance spectra) to permit confirmation of Δ^9-THC, Δ^8-THC, and/or THCA. Recent studies in the literature have demonstrated the accuracy and precision of LC-PDA or LC-UV [6–15] and LC-MS/MS [16–18]; however, LC-PDA and LC-UV are the primary analytical techniques used in cannabis testing laboratories due to their simplicity and lower cost.

Previous studies at NIST [15] have evaluated an LC-PDA method that utilized methanol (MeOH) for sample extraction and clean-up. Δ^9-THC, THCA, and nine other cannabinoids were determine in hemp plant and oil samples using the all-in-one *Cannabis Analyzer* high sensitivity LC-PDA method marketed to forensic laboratories. The analytical method was found to be accurate and precise through the analysis of four hemp reference samples obtained from the University of Kentucky Proficiency Testing (UK-PT) program [19]. The current study will expand on the initial study to include 53 smokable hemp samples from five different commercial sources. Smokable hemp samples were selected because they are typically found to have total Δ^9-THC mass fraction levels at ≈ 0.3 %. Several samples were selected to study potential measurement challenges that might arise from high levels of Δ^8-THC, which has become popular in the cannabis industry for smokable hemp and other finished products. The current study will expand on the initial work through the investigation of the effects and interferences when nine additional cannabinoids (*e.g.,* Δ^{10}-THC stereoisomers and *exo*-THC) are added to LC-PDA method. The results published are not limited to smokable hemp plant products, but also relevant to other types of hemp plant samples (*e.g.*, hemp bio-mass) or hemp matrix (*e.g.*, oils, edibles, etc.).

2. Experimental

2.1. Chemicals and materials

A calibration standard mixture of 11 cannabinoids in acetonitrile (ACN) was obtained from Shimadzu Instruments, LLC (Columbia, MD) with a nominal concentration of 250 mg/L for each individual cannabinoid including cannabidivarin (CBDV), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), cannabigerol (CBG), cannabidiol (CBD), Δ^9-tetrahydrocannabivarin (THCV), cannabinol (CBN), Δ^9-THC, Δ^8-THC, cannabichromene (CBC), and THCA. Individual calibration standards of cannabidivarinic acid (CBDVA), Tetrahydrocannabivarinic acid (THCVA), *exo*-THC, cannabinolic acid (CBNA), 9S-Δ^{10}-THC, 9R-Δ^{10}-THC, cannabichromenic acid (CBCA), cannabicyclic (CBL), and cannabicyclolic acid (CBLA) in ACN or MeOH were obtained from Cayman Chemical (Ann Arbor, MI). HPLC grade ACN and water (H_2O) with 0.085 % phosphoric acid (PA) concentration was purchased from Shimadzu Instruments, LLC. MeOH was purchased from Fisher Scientific (St. Louis, MI). A total of 53 smokable hemp plant product samples were purchased from five commercial sources and stored in the dark at room temperature prior to analysis.

2.2. Calibration standards

For quantitative purposes, 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. For qualitative purposes, working solutions were individually prepared volumetrically from individual standards of CBDVA, THCVA, *exo*-THC, CBNA, 9S-Δ^{10}-THC, 9R-Δ^{10}-THC, CBCA, CBL, and CBLA (1000 mg/L) to have final mass concentrations of ≈ 10 mg/L to investigate potential chromatographic interferences.

2.3. Sample extraction

The plant samples (10 g to 20 g) were ground at room temperature using a small portable Magic Bullet grinder in four or five separate 5 s pulses to represent similar procedures forensic laboratories have been using to measure total Δ^9-THC in seized cannabis plant samples. Ground plant samples were mixed thoroughly by hand to ensure homogeneity. Ground plant samples were extracted following the procedure published previously using reference samples from UK-PT program [15], which included a modification of using MeOH instead of ethanol in the original method [8]. Three replicate subsamples (0.50 g ± 0.05 g) of each of the hemp plant samples were weighed into 50 mL centrifuge tubes. Samples were extracted with the addition of 20 mL of MeOH. The samples were vortexed for 10 s to ensure initial mixing. Next, samples were mechanically shaken for 30 min at 50 rpm using a large capacity mixer from Glas-Col®: Tools for Scientists® (Terre Haute, IN). Samples were then centrifuged for 5 min at 1000 rpm using an Allegra X-14R Centrifuge from Beckman Coulter (Brea, CA). MeOH extracts were removed from the sample and 20 mL of fresh MeOH was added to the plant sample for a second extraction. Both extracts were combined and filtered with a 0.45 μm PTFE membrane filter. Lastly, sample extracts (0.1 mL) were diluted gravimetrically with MeOH to prepare 10-fold (0.9 mL) and 100-fold sample dilutions (9.9 mL) for measurements.

2.4. LC-PDA

The LC-PDA method was performed on a Shimadzu *Cannabis Analyzer* equipped with a binary pump, degasser, autosampler, column compartment, and a photodiode array detector. Separations were carried out on 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, and hold for 0.9 min. The column was re-equilibrated between each run for 2 min under the initial chromatographic conditions. A 5 μL injection size was used and the PDA detector collected data from 190 nm to 700 nm. An external calibration approach was used in the linear regression model with detection at 220 nm. Internal standards were not used for the LC-PDA method as it was demonstrated previously to provide accurate results [15] and NIST routinely monitors the methods performance through the use of control samples such as UK PT hemp samples [19].

3. Results and discussion

3.1. Investigation of potential cannabinoid interferences for the LC separation of Δ^8-THC, Δ^9-THC, and THCA

Previous work reported by Wilson and Abdur-Rahman [15] evaluated the "high sensitivity" method from Shimadzu Instruments for the determination of 11 cannabinoids in four hemp plant and 15 oil samples. This method used a C_{18} column, ACN:H_2O mobile phase with 0.085 % PA, flow rate of 1.6 mL/min, and a column temperature of 40 °C. The detector was set to a 10 Hz sampling rate collecting absorbance spectra from 190 nm to 700 nm. Example spectra for all cannabinoids studied in this manuscript are reported in Fig. S1 –Fig. S20 and an extracted wavelength chromatogram (220 nm) of the calibrant 11 cannabinoid mixture (black) is shown in Fig. 1. Potential interferences of 9 commercially available cannabinoids were investigated relative to the separation of Δ^8-THC, Δ^9-THC, and THCA. Chromatograms for each

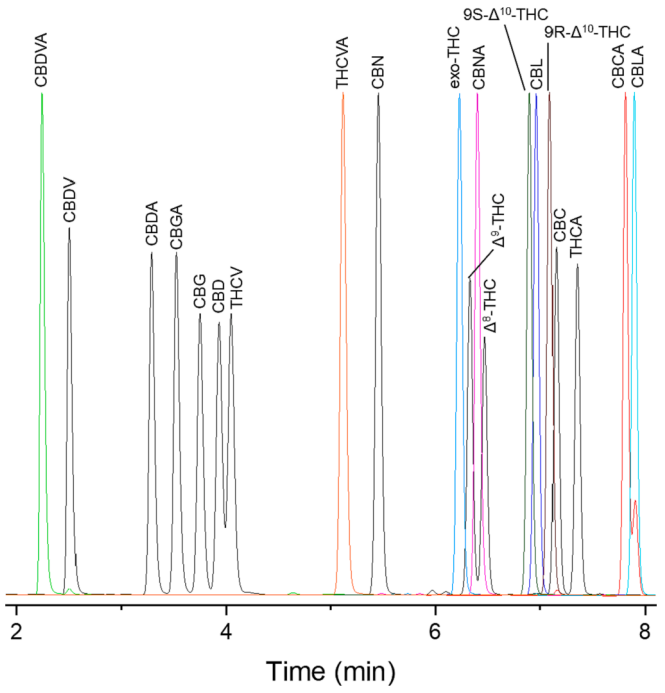

Fig. 1. LC-UV chromatogram at 220 nm for the 20 cannabinoid standards.

cannabinoid are shown in Fig. 1. The retention times of these cannabonoids are summarized in Table S1 using this LC-PDA method for these analysis. CBDVA, THCVA, CBCA, and CBLA were separated from the initial 11 cannabinoids; however, two sets of closely eluting cannabinoids remained with partial co-elution: (1) *exo*-THC, Δ^9-THC, CBNA, and Δ^8-THC and (2) 9S-Δ^{10}-THC, CBL, 9R-Δ^{10}-THC, CBC, and THCA.

The separations from Fig. 1 are enlarged in Fig. 2A to focus on the first set of cannabinoids. *exo*-THC elutes prior to Δ^9-THC while CBNA elutes between Δ^9-THC and Δ^8-THC. Adequate separation of *exo*-THC from Δ^9-THC is important since selective detection is not possible due to the similarity of absorbance spectra as shown in Fig. 2B. CBNA is the acidic precursor of CBN and is most often not detected or detected at low levels in cannabis plant samples. The interference from CBNA on Δ^9-THC could not be eliminated through selective detection due to the broad overlap in the absorbance spectra for CBNA and Δ^9-THC (Fig. 1C). For quantitative measurements, the co-eluting peaks are integrated using a valley-baseline approach; positive or negative biases could potentially result for the determination of Δ^9-THC, depending on relative levels of CBNA.

The chromatographic separation of the second set of cannabinoids is shown in Fig. 3A. THCA is baseline separated from the all cannabinoids; however, partial co-elution exists between 9S-Δ^{10}-THC/CBL and 9R-Δ^{10}-THC/CBC. The baseline separation of the two Δ^{10}-THC stereoisomers may become more important with the introduction of different commercial products since the compounds exhibit identical absorbance spectra (Fig. 3B and Fig. 3C), which are significantly different from the absorbance spectra of their co-eluting cannabinoids. Although Δ^8-THC,

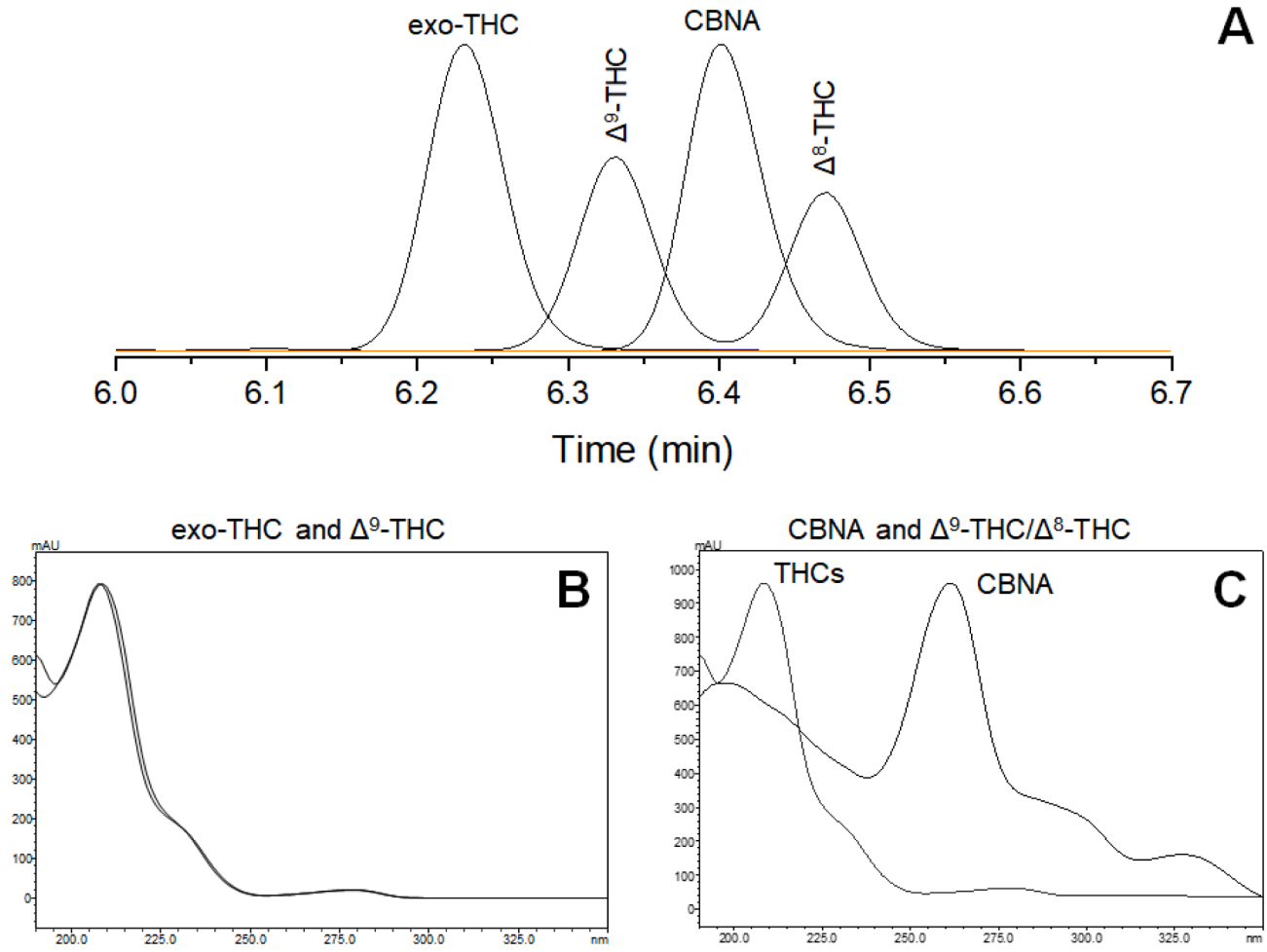

Fig. 2. LC-PDA analysis of 20 cannabinoid solutions: extracted chromatogram at 220 nm (A); absorbance spectra of *exo*-THC/Δ^9-THC (B) and CBNA/Δ^9-THC/Δ^8-THC (C).

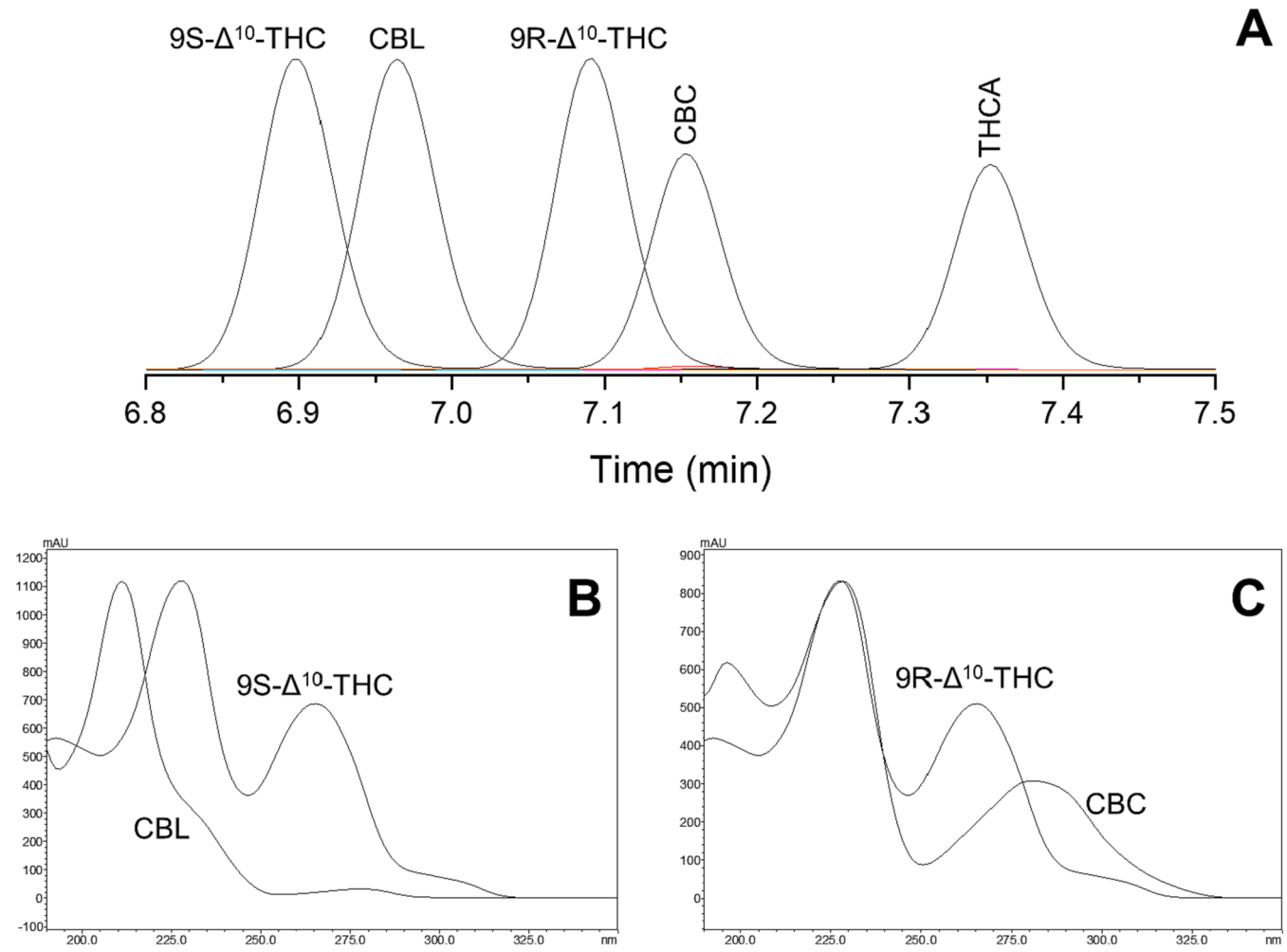

Fig. 3. LC-PDA analysis of 20 cannabinoid solutions: enhanced extracted chromatogram at 220 nm (A); absorbance spectra of CBL/9S-Δ^{10}-THC (B) and CBC/9R-Δ^{10}-THC (C).

Δ^9-THC, and THCA can be measured accurately using the current method, the chromatographic retention times and absorbance spectra of the 9 cannabinoids will be utilized for potential identification in future studies of smokable hemp samples.

3.2. LC-PDA analysis of commercial smokable hemp samples

The LC-PDA method was further evaluated for applications to hemp plant samples and smokable hemp products (buds only). Five commercial sources were identified (Manufacturer 1 – 5) as representative samples available in commerce as examples that potentially could be classified as marijuana if the threshold limit for hemp was exceeded. Although all samples were sold as hemp with Δ^9-THC mass fractions less than or equal to the federal limit of 0.3 %, LC-PDA analysis completed at NIST indicates that ≈ 93 % of the samples were above the 0.3 % threshold for total Δ^9-THC (see Fig. 4). Online documentation provided by the cannabis testing laboratory selected by the manufacturer for 45 of 53 hemp samples analyzed by NIST are summarized in Table 1. Although Δ^9-THC mass fractions are ≤ 0.3 %, THCA mass fractions are > 0.3 % for most samples.

Total Δ^9-THC mass fractions on an as-received basis for these samples are summarized in Table 2 and plotted in Fig. 4. Corresponding Δ^9-THC, Δ^8-THC, and THCA mass fractions are based on three replicate extractions of each of the smokable hemp samples extracted with MeOH and diluted (10-fold and 100-fold) prior to analysis by LC-PDA (see Fig. 5 for Manufacturer 1 and Fig. 6 for Manufacturer 2, 3, and 5). Δ^9-

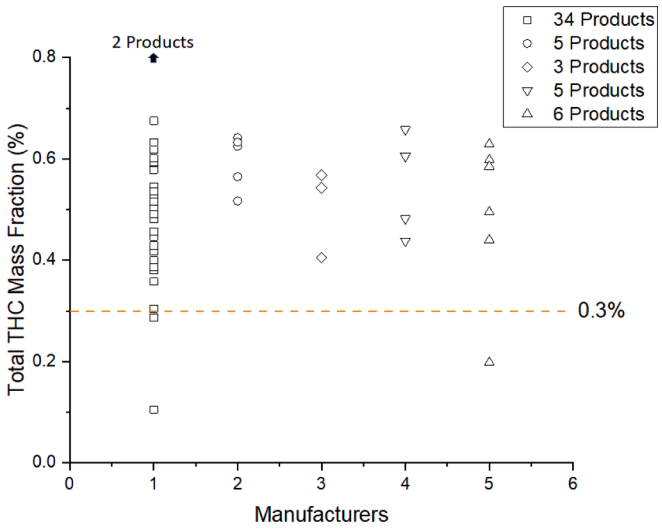

Fig. 4. Mass fractions of total Δ^9-THC in 53 commercial smokable hemp samples obtained from five commercial sources.

THC mass fractions were not included in Fig. 6 due to limited data provided by the manufacturers. Uncertainty estimates for the manufacturer data were arbitrarily set at 15 % because uncertainties were not

Table 1
Mass fraction (%) reported by the manufacturers of 53 commercial smokable hemp products.

Samples	Δ^9-THC	Δ^8-THC	THCA	Total Δ^9-THC
Manufacturer 1				
Sample 1	0.06	ND	0.43	0.44
Sample 2*	<0.04	<0.04	0.58	0.509
Sample 3*	ND	ND	ND	ND
Sample 4	NA	NA	NA	NA
Sample 5	0.0472	<0.04	0.919	0.853
Sample 6	0.06	<0.05	0.16	0.20
Sample 7	NA	NA	NA	NA
Sample 8*	0.06	ND	0.53	0.52
Sample 9	<0.024	<0.024	0.703	0.617
Sample 10	<0.04	<0.04	0.447	0.392
Sample 11*	<0.05	<0.05	0.758	0.691
Sample 12	0.11	NA	1.69	1.59
Sample 13	0.07	<0.04	0.92	0.88
Sample 14	ND	NA	0.49	ND
Sample 15	0.0471	<0.04	0.770	0.772
Sample 16	0.053	NA	0.466	0.462
Sample 17*	<0.1	NA	0.61	0.53
Sample 18*	ND	ND	0.87	0.76
Sample 19	<0.100	<0.100	0.615	0.539
Sample 20*	<0.04	<0.04	0.447	0.392
Sample 21*	0.106	<0.05	0.181	0.265
Sample 22	<0.04	NA	0.302	0.265
Sample 23	0.055	<0.04	0.658	0.632
Sample 24	<0.1	NA	0.865	0.759
Sample 25*	<0.024	<0.024	0.719	0.631
Sample 26	<0.04	NA	0.650	0.570
Sample 27	0.094	<0.023	0.794	0.790
Sample 28	<0.04	<0.04	0.563	0.494
Sample 29*	ND	ND	0.330	0.289
Sample 30	NA	NA	NA	NA
Sample 31	<0.1	<0.1	0.649	0.659
Sample 32*	0.30	<0.02	0.25	0.52
Sample 33	0.10	<0.04	0.71	0.72
Sample 34	0.063	ND	0.790	0.756
Manufacturer 2				
Sample 35	0.096	<0.087	0.807	0.804
Sample 36	<0.009	24.12	0.396	0.347
Sample 37	NA	NA	NA	NA
Sample 38	<0.1	ND	0.684	0.600
Sample 39	<0.009	31.11	0.354	0.311
Manufacturer 3				
Sample 40	ND	ND	0.356	0.312
Sample 41	NA	NA	NA	NA
Sample 42	ND	ND	0.489	0.429
Manufacturer 4				
Sample 43	0.06	NA	NA	NA
Sample 44	0.06	NA	NA	NA
Sample 45	NA	11.53	NA	NA
Sample 46	NA	16.49	NA	NA
Sample 47	0.17	NA	NA	NA
Manufacturer 5				
Sample 48	<0.1	<0.1	0.555	0.487
Sample 49	<0.1	<0.1	0.529	0.464
Sample 50	0.136	<0.1	0.623	0.682
Sample 51	<0.1	<0.1	0.673	0.590
Sample 52	<0.1	<0.1	0.605	0.531
Sample 53	NA	NA	NA	NA

*Indicates samples that had different mass fractions on the product packaging than listed in the online documentation.

provided and most state regulators require product labels to be accurate within this range [21]. The uncertainty estimates for the NIST-determined values correspond to four times the standard deviation from triplicate measurements. Δ^8-THC was not included in these graphs due to limited sample data.

Thirty-four samples from Manufacturer 1 were analyzed with data from 11 of the samples included in Fig. 5. Samples with inconsistency in the online documentation and product labels and samples where the manufacturer reported none detected, not available, or below the limits of quantitation (LOQ) for the cannabinoids were not included in the

Table 2
Mass fraction (%) determined at NIST (N = 3) for the target cannabinoids in 53 commercial smokable hemp products.

Samples	Δ^9-THC	Δ^8-THC	THCA	Total Δ^9-THC
Manufacturer 1				
Sample 1	0.209 ± 0.013	ND	0.323 ± 0.031	0.502 ± 0.026
Sample 2	0.160 ± 0.016	ND	0.434 ± 0.064	0.545 ± 0.068
Sample 3	0.199 ± 0.029	ND	0.328 ± 0.060	0.490 ± 0.080
Sample 4	0.137 ± 0.008	ND	0.445 ± 0.028	0.532 ± 0.030
Sample 5	0.278 ± 0.014	ND	0.232 ± 0.026	0.483 ± 0.036
Sample 6	0.084 ± 0.006	ND	0.023 ± 0.011	0.105 ± 0.015
Sample 7	0.696 ± 0.144	ND	1.226 ± 0.066	1.783 ± 0.360
Sample 8	0.206 ± 0.016	ND	0.212 ± 0.016	0.394 ± 0.030
Sample 9	0.099 ± 0.001	ND	0.650 ± 0.033	0.675 ± 0.030
Sample 10	0.060 ± 0.006	ND	0.361 ± 0.060	0.381 ± 0.056
Sample 11	0.146 ± 0.014	ND	0.434 ± 0.048	0.531 ± 0.060
Sample 12	0.143 ± 0.031	ND	0.243 ± 0.048	0.358 ± 0.072
Sample 13	0.215 ± 0.032	ND	0.426 ± 0.080	0.593 ± 0.100
Sample 14	0.514 ± 0.022	ND	0.098 ± 0.002	0.601 ± 0.023
Sample 15	0.201 ± 0.015	ND	0.315 ± 0.038	0.481 ± 0.048
Sample 16	0.248 ± 0.034	ND	0.173 ± 0.018	0.400 ± 0.049
Sample 17	0.188 ± 0.013	ND	1.254 ± 0.033	1.300 ± 0.128
Sample 18	0.182 ± 0.014	ND	0.265 ± 0.014	0.417 ± 0.020
Sample 19	0.391 ± 0.040	ND	0.102 ± 0.010	0.482 ± 0.052
Sample 20	0.125 ± 0.002	ND	0.363 ± 0.018	0.446 ± 0.018
Sample 21	0.216 ± 0.006	ND	0.355 ± 0.024	0.531 ± 0.025
Sample 22	0.114 ± 0.006	ND	0.195 ± 0.017	0.287 ± 0.010
Sample 23	0.214 ± 0.046	ND	0.190 ± 0.033	0.380 ± 0.075
Sample 24	0.239 ± 0.006	ND	0.075 ± 0.008	0.304 ± 0.014
Sample 25	0.276 ± 0.015	ND	0.277 ± 0.017	0.522 ± 0.030
Sample 26	0.292 ± 0.038	ND	0.224 ± 0.013	0.491 ± 0.048
Sample 27	0.321 ± 0.056	ND	0.074 ± 0.006	0.386 ± 0.067
Sample 28	0.159 ± 0.012	ND	0.535 ± 0.017	0.633 ± 0.023
Sample 29	0.206 ± 0.006	ND	0.419 ± 0.014	0.578 ± 0.016
Sample 30	0.381 ± 0.025	ND	0.150 ± 0.010	0.515 ± 0.034
Sample 31	0.099 ± 0.004	ND	0.404 ± 0.011	0.457 ± 0.044
Sample 32	0.250 ± 0.015	ND	0.202 ± 0.064	0.429 ± 0.116
Sample 33	0.208 ± 0.006	ND	0.370 ± 0.032	0.536 ± 0.033
Sample 34	0.197 ± 0.005	ND	0.474 ± 0.015	0.618 ± 0.016
Manufacturer 2				
Sample 35	0.354 ± 0.084	ND	0.308 ± 0.050	0.625 ± 0.125

(*continued on next page*)

Table 2 (*continued*)

Samples	Δ^9-THC	Δ^8-THC	THCA	Total Δ^9-THC
Sample 36	0.220 ± 0.012	3.015 ± 0.368	0.339 ± 0.031	0.517 ± 0.040
Sample 37	0.322 ± 0.092	ND	0.365 ± 0.067	0.642 ± 0.151
Sample 38	0.398 ± 0.026	ND	0.268 ± 0.026	0.633 ± 0.049
Sample 39	0.193 ± 0.035	2.423 ± 0.123	0.424 ± 0.010	0.565 ± 0.044
Manufacturer 3				
Sample 40	0.207 ± 0.012	ND	0.383 ± 0.021	0.543 ± 0.030
Sample 41	0.221 ± 0.040	ND	0.396 ± 0.105	0.568 ± 0.134
Sample 42	0.113 ± 0.008	ND	0.333 ± 0.019	0.405 ± 0.025
Manufacturer 4				
Sample 43	0.296 ± 0.052	ND	0.212 ± 0.012	0.482 ± 0.063
Sample 44	0.369 ± 0.011	ND	0.270 ± 0.020	0.606 ± 0.028
Sample 45	0.222 ± 0.073	4.487 ± 1.290	0.246 ± 0.095	0.438 ± 0.156
Sample 46	0.385 ± 0.092	1.266 ± 0.276	0.312 ± 0.084	0.658 ± 0.101
Sample 47	0.290 ± 0.026	ND	0.359 ± 0.074	0.605 ± 0.090
Manufacturer 5				
Sample 48	0.126 ± 0.020	ND	0.422 ± 0.052	0.495 ± 0.065
Sample 49	0.069 ± 0.007	ND	0.423 ± 0.170	0.440 ± 0.157
Sample 50	0.287 ± 0.064	ND	0.355 ± 0.013	0.598 ± 0.076
Sample 51	0.126 ± 0.015	ND	0.573 ± 0.064	0.629 ± 0.065
Sample 52	0.164 ± 0.076	ND	0.479 ± 0.019	0.585 ± 0.024
Sample 53	0.067 ± 0.019	ND	0.150 ± 0.011	0.198 ± 0.028

graph. In the cases where the online documentation did not match the product label it is assumed that the online documentation is for different batches or lots of a similar material. These samples are denoted in Table 1 with an asterisk (*) and are not included in the graphs. Manufacturer 4 samples were not included in the Fig. 6 comparisons because no online documentation was available for the samples purchased.

The NIST-determined and manufacturer mass fractions were significantly different for most samples. Several sources of these differences can be proposed. Manufacturers typically employ cannabis testing laboratories to assess cannabinoid levels; measurement error could originate with methods used by these laboratories. Discrepancies could also originate from sample inhomogeneity, improper sampling, sample stability from storage conditions, or variations in production lots. The following sections will discuss the results from each manufacturer samples in detail and provide examples of these issues.

3.2.1. *Manufacturer 1*

CBDA, CBGA, CBG, CBD, Δ^9-THC, CBC, and THCA were detected in all 34 samples as shown in the chromatograms in Fig. S21 to Fig. S26. CBDVA was the only cannabinoid to be detected in a few samples from the nine cannabinoids studied in Section 3.1. Manufacturer mass fractions for total Δ^9-THC are statistically equivalent with NIST measurements for only 5 of 11 samples within a 95 % confidence interval (Fig. 5A) [20]. Poorer agreement was observed for THCA (2) and Δ^9-THC (1) in Fig. 5B and Fig. 5C, respectively. Discrepancies in THCA and Δ^9-THC values could potentially result from conversion of THCA to Δ^9-THC during storage. This is evident for samples obtained by NIST since mass fractions for Δ^9-THC are higher and levels for THCA are lower than mass fractions reported in online documentation for all 11 samples. It

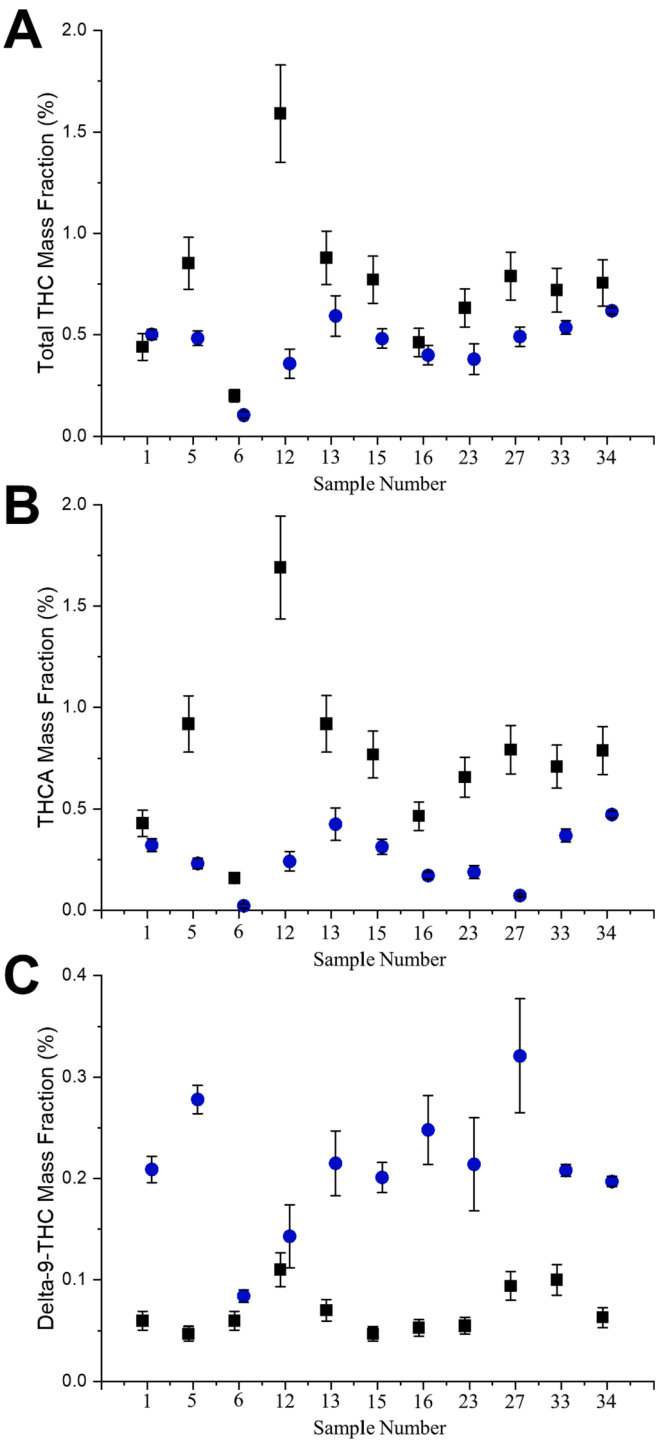

Fig. 5. Levels of total Δ^9-THC (A), THCA (B), and Δ^9-THC (C) in commercial samples from Manufacturer 1 (black squares) and NIST (blue circles). The error bars for the manufacturer results represents an arbitrary error estimate of 15%. The error bars for the NIST results represent an expanded uncertainty of four standard deviations of the mean mass fraction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

can be noted that mass fractions reported for Δ^9-THC are below the LOQs for 8 samples and are not included in Fig. 5 (Table 1), whereas the NIST results were well above LOQ and LOD values for the LC-PDA method at NIST (≈ 0.009 % and ≈ 0.003 %, respectively). NIST measurements performed in early 2021 support the theory of decarboxylation relative to product measurements performed in 2019 and 2020 as

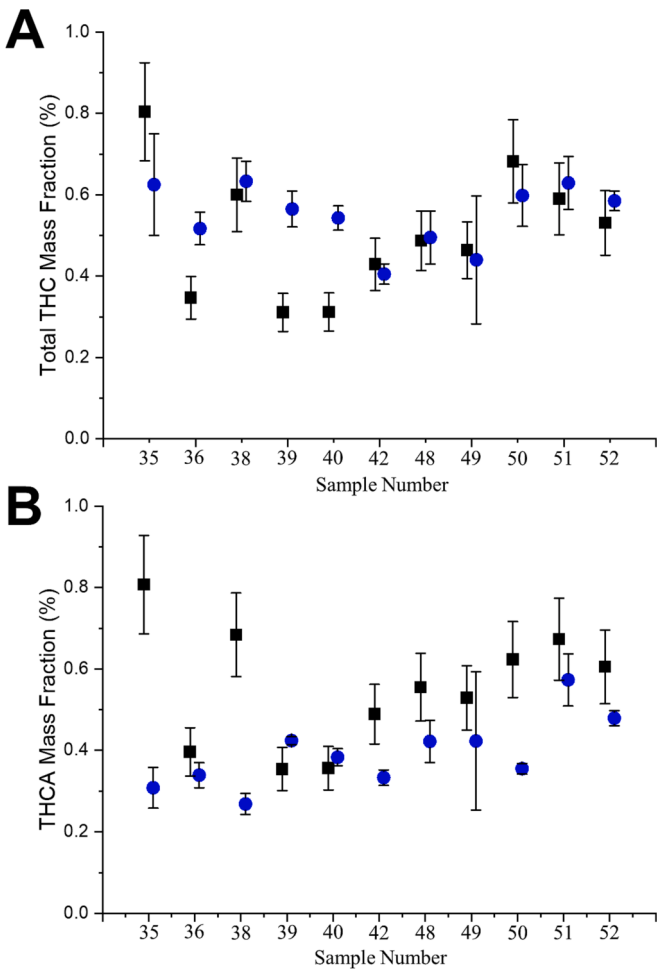

Fig. 6. Levels of total Δ^9-THC (A) and THCA (B) in commercial samples from Manufacturer 2, 3, or 5 (black squares) and NIST (blue circles). The error bars for the manufacturer results represents an arbitrary error estimate of 15%. The error bars for the NIST results represent an expanded uncertainty of four standard deviations of the mean mass fraction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

a consequence of poor storage conditions.

3.3.2.2. Manufacturer 2. CBDA, CBGA, CBG, CBD, Δ^9-THC, CBC, and THCA were detected in all five samples, while Δ^8-THC was only detected in Sample 36 and Sample 39 (Fig. S27). Δ^9-THC, THCA, and total Δ^9-THC mass fractions provided by Manufacturer 2 (Table 1) are compared in Fig. 6 to the NIST-determined mass fractions (Table 2). The total Δ^9-THC mass fraction determined by NIST in Samples 35 and 38 agree with manufacturer mass fractions within a 95 % confidence interval, while NIST mass fractions were significantly higher than manufacturer mass fractions for Samples 36 and 39 [20]. Mass fractions by the manufacture was not available for Sample 37. The LOQ for Δ^9-THC reported by the manufacturer was greater than the mass fractions determined (*i.e.*, <0.1 %). The NIST-determined mass fractions for Δ^9-THC are well above the corresponding LOQs (0.193 % to 0.398 %). The THCA mean mass fraction for Samples 35 (0.807 %) and 38 (0.684 %) were significantly higher than the NIST-determined mass fractions of 0.308 % and 0.268 %, respectively [20]. The online documentation provided for these samples are dated October 2019 and November 2020. Samples obtained by NIST in 2021 may have changed through decarboxylation of THCA to Δ^9-THC as a consequence of storage conditions. Similar results were observed for CBDA and CBD in these two samples (data not shown). THCA mass fractions listed in the online documentation for Samples 36 and 39 were measured in February 2021 and agree within a 95 % confidence interval of the NIST mass fractions determined during the same time [20]. Regarding Δ^8-THC, the NIST-determined mass fractions are approximately one order of magnitude lower in Samples 36 and 39 than reported in their respective online documentation. Δ^8-THC does not exist naturally in cannabis plant materials at the levels reported by the manufacturer and suggests application to the plant after harvesting. This procedure could easily lead to inhomogeneous materials that result in package-to-package variability and/or poor manufacture sampling plans for these measurements that may not be representative of the entire materials prior to packaging.

3.3.2.3. Manufacturer 3. CBDA, CBGA, CBG, CBD, Δ^9-THC, CBC, and THCA were detected in Samples 40 to 42 as shown in Fig. S28. The manufacturer provided online documentation for Samples 40 and 42 with mass fractions for THCA and total Δ^9-THC. Δ^9-THC and Δ^8-THC are reported as not detected; however, mass fractions determined by NIST for Δ^9-THC ranged from 0.113 % to 0.221 %. These values are well above the limits of detection (LOD) and LOQs reported in the online documentation for Δ^9-THC in both samples (0.047 % and 0.069 %, respectively). The NIST-determined mass fractions for THCA are in good agreement with the online documentation for Sample 40 [20]. THCA mass fractions determined at NIST for Sample 42 is approximately 32 % lower than manufacturer mass fractions potentially due to decarboxylation into Δ^9-THC. Similar results were observed for CBDA and CBD in Sample 42 (data not shown).

3.3.2.4. Manufacturer 4. Online documentation was not available for the five samples obtained from Manufacturer 4 and a direct comparison to the NIST-determined mass fraction values summarized in Table 2 was not possible. However, limited data are provided in Table 1 for Δ^9-THC and/or Δ^8-THC based on product packaging. Separations for the 10-fold dilution of Samples 43 to 47 are shown in Fig. S29. CBDA, CBGA, CBG, CBD, CBC, and THCA were detected in each of the five samples. CBDVA and CBDV were detected only in Sample 45, while Δ^8-THC was detected in Samples 45 and 46. Samples 43 and 44 were labeled to contain Δ^9-THC at a mass fraction of 0.06 %, but NIST measurements determined Δ^9-THC mass fractions as 0.296 % and 0.369 %, respectively. NIST also determined levels of THCA and total Δ^9-THC, which ranged from 0.212 % to 0.359 % and 0.438 % to 0.658 %, respectively. NIST determined the mass fraction of Δ^8-THC to be 4.487 % and 1.266 % in Samples 45 and 46, respectively, that are significantly lower than the quantities reported on the packaging (11.53 % and 16.49 %, respectively). This is similar to results reported above for Δ^8-THC in Samples 36 and 39.

3.3.2.5. Manufacturer 5. Separations for the 10-fold dilution of Samples 48 to 53 are shown in Figure S30. CBDA, CBGA, CBG, CBD, Δ^9-THC, CBC, and THCA were detected in all six samples. With the exception of Sample 53, mass fractions of THCA and total Δ^9-THC were reported in online online documentation for these samples. The NIST-determined mass fractions agreed within a 95 % confidence interval of the manufacturer values for total Δ^9-THC; however, the NIST mass fractions for THCA were significantly lower in Sample 50 then the manufacturer mass fractions [20]. Manufacturer mass fractions for Δ^9-THC were below the reported LOQ (<0.1 %) with the exception of Sample 50 (0.136 %), which is significantly lower than the NIST value of 0.287 % ± 0.064 %. These results are clear indication of decarboxylation of THCA into Δ^9-THC.

4. Conclusions

Δ^9-THC, Δ^8-THC, THCA, and total Δ^9-THC were determined in 53 smokable hemp samples purchased from five commercial sources using a previously established high sensitivity LC-PDA method with a MeOH

extraction using routine analytical laboratory equipment. The LC-PDA method separates 11 cannabinoids in less than 10 min. Additional cannabinoids identified in cannabis samples including *exo*-THC and Δ^{10}-THC stereoisomers were investigated with CBNA being found to interfere with Δ^{9}-THC and Δ^{8}-THC, but THCA was baseline resolved from all cannabinoids.

All samples were sold as smokable hemp with Δ^{9}-THC levels less than or equal to the federal limit of 0.3 %; however, the LC-PDA measurements at NIST indicated that ≈ 93 % of the samples were above the 0.3 % federal limit. Nearly half of the online documents provided by the manufacturers differed from corresponding product labels, and direct comparisons to NIST values were limited to 22 samples. For products with consistent labeling, NIST assigned an uncertainty of 15 % to mass fractions based on current state regulations to permit comparison with NIST values. NIST measurements agreed with mass fractions for ≈ 55 % for total Δ^{9}-THC, ≈ 68 % for THCA, and ≈ 18 % for Δ^{9}-THC. NIST values were generally higher for Δ^{9}-THC and lower for THCA indicating decarboxylation of THCA and conversion to Δ^{9}-THC with aging. These studies demonstrate the need for accurate analytical measurements, batch homogeneity measurements, appropriate long-term storage conditions, and updated product information. These results also highlight the need for reference materials in the cannabis industry to establish measurement accuracy.

CRediT authorship contribution statement

Walter B. Wilson: Supervision, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. **Aaron A. Urbas:** Methodology. **Maryam Abdur-Rahman:** Methodology. **Arianna Romares:** Methodology. **Ewelina Mistek-Morabito:** .

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

I do have permission to share the data.

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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 are necessarily the best available for the purpose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.forc.2024.100550.

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