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Abstract

The National Institute of Standards and Technology (NIST) Reference Material (RM) 8210 Hemp Plant delivers non-certified values for cannabinoids and toxic elements in a dried ground hemp plant material to help cannabis and forensic laboratories for use as a control and research material. A unit of RM 8210 contains three sample packets (approximately 1.5 g each), each sealed with a desiccant pouch in an aluminized polyester bag. This publication documents the production, analytical methods, and computations involved in characterizing this product.

Keywords

arsenic; cadmium; cannabichromene (CBC); cannabidivarin (CBDV); cannabidiolic acid (CBDA); cannabigerol (CBG); cannabigerolic acid (CBGA); cannabidiol (CBD); cannabinol (CBN); *Cannabis sativa*; hemp; lead; marijuana; mercury; Δ^9 tetrahydrocannabinol (Δ^9-THC); tetrahydrocannabinolic acid (THCA); toxic elements

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1. Introduction

The need to accurately measure cannabinoids and moisture in hemp plant became more important following passage of the 2018 Farm Bill [1]. The legislation legalized hemp in the United States (US) by removing hemp from the DEA Scheduled 1 controlled substance list [2] and defined it as *Cannabis sativa* with a Δ^9-tetrahydrocannabinol (Δ^9-THC) concentration of less than or equal to 0.3 % on a dry-weight basis. In 2021, the United States Department of Agriculture published a final rule providing regulation on hemp production in the US clarifying these measurements must be based on total Δ^9-THC (including its acidic precursor Δ^9-tetrahydrocannabinolic acid [THCA]) [3]. Toxic elements have been identified as high priority measurands due to the public health concerns associated with exposure.

The Chemical Sciences Division at the National Institute of Standards and Technology (NIST) has developed a Cannabis Research Program [4] that includes development of analytical methods, Cannabis Quality Assurance Program (CannaQAP), and the development of the reference material RM 8210 Hemp Plant. This RM was characterized at NIST for cannabinoids, toxic elements, and moisture; it has a total Δ^9-THC concentration of ≤ 0.3 %. RM 8210 was developed for use by cannabis and forensic laboratories as a quality control and research material; it is not intended for establishing metrological traceability to the International System of Units (SI).

2. Production

2.1. Materials

RM 8210 is dried hemp plant, *Cannabis sativa L.*, containing materials obtained from two sources. The first source was freshly harvested hemp plant bio-mass including plant buds, leaves, and stems purchased by NIST from Klersun, LLC (Portland, Oregon). After delivery to NIST, the material was stored at room temperature for a few weeks while being packaged into ≈ 3 kg polypropylene bags for storage in a -80 °C freezer. The second source was only hemp stems obtained from James Madison University (JMU) through a Material Transfer Agreement and stored at room temperature.

2.2. Preparation

Prior to grinding, the Klersun hemp bio-mass material was brought to room temperature for 1 h and stems were manually separated from buds and leaves. The stems were stored at –80 °C until further processing. The buds and leaves were ground using a high-power Vitamix blender (Cleveland, OH). The ground material was sieved to ensure a particle size between 250 µm to 710 µm and stored in tightly sealed plastic buckets. The individual bucket materials were mixed using a paint mixer drill bit for 30 min and stored in the dark at –80 °C until the material could be analyzed for cannabinoids.

The ground buds and leaves from the Klersun material was determined to have a total Δ^9-THC mass fraction slightly above the federal legal threshold of 0.3 % [1,2]. In order to reduce the total Δ^9-THC mass fraction, the Klersun hemp stems and JMU hemp stems were ground and sieved following the same procedures described above. The ground bud and leaf material was mixed with the ground stem material in a ≈ 76 L (20-gallon) high density polyethylene (HDPE) container (Vittles Vault®) using a paint mixer drill bit for 30 min to result in a final material having a total Δ^9-THC mass fraction of slightly less than 0.3 %.

Approximately 400 portions (≈ 3 g each) of the mixture were packaged in plastic packets, sealed into individualized aluminized polyester bags along with one desiccant silica pouch (1 g), and stored at –20 °C for use as Plant Sample 4 in Exercise 2 of CannaQAP [5]. The remaining bulk material was sealed in a ≈ 19 L (5-gallon) HDPE airtight container (United States Plastic Corp.) and stored at –80 °C until it could be packaged as RM 8210 by the Office of Reference Materials (ORM) at NIST.

2.3. Packaging

ORM produced a total of 2160 sample packets of RM 8210, each packet containing approximately 1.5 g of the material. The packets used for holding the RM were pink polyethylene sleeves from Uline (5.1 cm x 7.6 cm x 0.01 cm, Pleasant Prairie, WI). Approximately 160 sample packets were packaged per day. The sample packets and unpackaged material were stored at – 80 °C to prevent moisture addition or decarboxylation of acidic cannabinoids into their neutral cannabinoids. The total material was packaged in the polyethylene packets across 20 days. The packets were stored in 13 boxes containing 160 packets each and one box containing 80 packets. These boxes were

labeled in accordance with the packaging order. Nested random sampling was used to select packets for use in characterizing the material and stored in –20 °C freezer. ORM then packaged the polypropylene sample packets into aluminized polyester bags (7 cm x 14 cm) with desiccant pouches (1 g each) and stored them at –80 °C. A unit of RM 8210 consists of three sample packets of hemp plant each sealed inside an aluminized polyester bag with a desiccant pouch. Each sample packets contains approximately 1.5 g of material.

3. Cannabinoids

3.1. Initial Assessment

Measurements of cannabinoids in RM 8210 were made using NIST's published liquid chromatographic with a photodiode array detector (LC-PDA) method [6,7]. This method was used to determine the mass fractions of cannabichromene (CBC), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabidivarin (CBDV), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabinol (CBN), Δ^9-THC, and THCA. Chemical structures for these cannabinoids are provided in Table 1, along with their CAS registry numbers, molecular formulas, and molar masses. Additionally, the measured mass fractions were used to compute the mass fractions of total CBD and total Δ^9-THC.

3.1.1. Materials

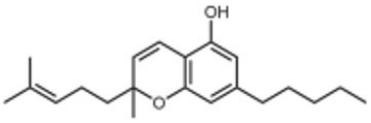
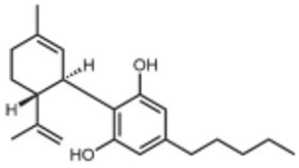
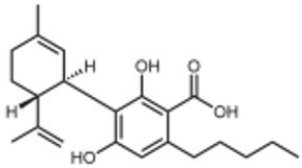
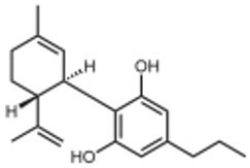
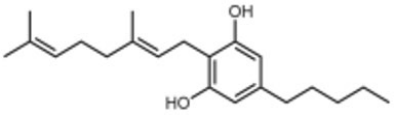
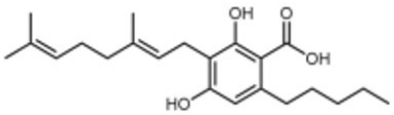
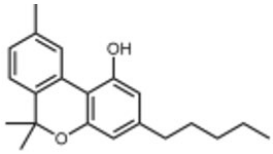
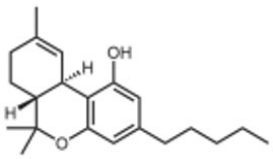
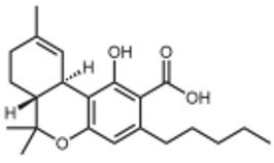
High-Performance Liquid Chromatography (HPLC) grade water with 0.085 % phosphoric acid (PA, part #220-91394-90) and HPLC grade acetonitrile (ACN) with 0.085 % PA (part #220-91394-91) were purchased from Shimadzu Corp. (Kyoto, Japan). HPLC grade methanol (MeOH) was purchased from Fisher Scientific.

Cayman Chemical (Ann Arbor, MI USA) Phytocannabinoid Mixture 11 [8] calibration solution was obtained from Shimadzu. This solution contains 11 cannabinoids in ACN, with mass concentrations of 249.6 mg/L to 251.3 mg/L of each cannabinoid and 95 % level of confidence expanded uncertainties ranging from 3.5 mg/L to 4.2 mg/L. In the following sections of this report, this solution is referred to as the "Cayman" calibrant.

Single component Supelco calibration standard solutions of Δ^9-THC [9] and THCA [10] were purchased from Cerilliant (Round Rock, TX, USA). Both cannabinoids are supplied as MeOH solutions with mass concentration values of 1.000 mg/mL with 95 % level of confidence expanded uncertainties of 0.018 mg/mL for Δ^9-THC and 0.006 mg/mL for THCA. In the following Sections of this report, these materials are referred to as the "Supelco" calibrants.

One bottle of National Research Council of Canada (NRC) HEMP-1 Certified Reference Material of dried, ground hemp [11] was used as a control material; this CRM was donated to NIST by NRC. HEMP-1 delivers certified mass fractions for 16 cannabinoids, total CBD, and total Δ^9-THC. These measurands include all of the cannabinoids of interest in RM 8210.

Table 1. Cannabinoids of Interest to RM 8210.

Chemical Information	Chemical Structure [a]
Code: CBC Name: cannabichromene CAS registry number: 20675-51-8 Molecular formula: $C_{21}H_{30}O_2$ Molar mass: 314.461 ± 0.012	
Code: CBD Name: cannabidiol CAS registry number: 13956-29-1 Molecular formula: $C_{21}H_{30}O_2$ Molar mass: 314.461 ± 0.012	
Code: CBDA Name: cannabidiolic acid CAS registry number: 1244-58-2 Molecular formula:$C_{22}H_{30}O_4$ Molar mass: 358.470 ± 0.013	
Code: CBDV Name: cannabidivarin CAS registry number: 24274-48-4 Molecular formula: $C_{19}H_{26}O_2$ Molar mass: 286.407 ± 0.11	
Code: CBG Name: cannabigerol CAS registry number: 25654-31-3 Molecular formula: $C_{21}H_{32}O_2$ Molar mass: 316.477 ± 0.012	
Code: CBGA Name: cannabigerolic acid CAS registry number: 25555-57-1 Molecular formula: $C_{22}H_{32}O_4$ Molar mass: 360.464 ± 0.013	
Code: CBN Name: cannabinol CAS registry number: 521-35-7 Molecular formula: $C_{21}H_{26}O_2$ Molar mass: 310.429 ± 0.012	
Code: Δ^9-THC Name: Δ^9-tetrahydrocannabinol CAS registry number: 1972-08-3 Molecular formula: $C_{21}H_{30}O_2$ Molar mass: 314.461 ± 0.012	
Code: THCA Name: Δ^9-tetrahydrocannabinolic acid CAS registry number: 23978-85-0 Molecular formula: $C_{22}H_{30}O_4$ Molar mass: 358.470 ± 0.013	

[a] Oriented chemical structures from HEMP-1 Certificate of Analysis [11]

3.1.2. Calibration Standards Preparation

One vial of the Cayman calibration solution was removed from the –80 °C freezer and allowed to equilibrate for one hour prior to preparation of working solution and calibrants. One ≈ 25 mg/L working solution was gravimetrically prepared in MeOH. Nine calibrants with target mass concentrations of 0.25 mg/L, 1.5 mg/L, 2.75 mg/L, 4.0 mg/L, 5.25 mg/L, 6.5 mg/L, 7.75 mg/L, 9 mg/L, and 10.25 mg/L were gravimetrically prepared in MeOH using either the Cayman calibration solutions as received or the 25 mg/L working solution.

Δ^9-THC and THCA mixed working solutions at 250 mg/L and 25 mg/L were gravimetrically prepared in MeOH from the Supelco calibration solutions. Nine calibrants with the same target mass concentrations as above were then gravimetrically prepared in MeOH using either the 250 mg/L or 25 mg/L working solution.

3.1.3. Sample Preparation

A set of 10 packets of RM 8210 were selected from the –20 °C freezer following a stratified random sampling based on packaging order. The packets were labelled sequentially based on their packaging order.

Samples were extracted and cleaned up using published procedures [7,6]. The ten packets of RM 8210 and one bottle of HEMP-1 were equilibrated at room temperature for 1 h and mixed thoroughly by hand prior to sampling. Two aliquots (numbered 1 and 2) were prepared from each packet of RM 8210. Three aliquots (numbered 1 to 3) were prepared from HEMP-1.

For each aliquot, approximately 0.5 g of hemp sample was weighed into a 50 mL polypropylene centrifuge tube using a Mettler Toledo XPR205 balance. A 20 mL aliquot of MeOH was added to the hemp sample and the mixture was shaken for 30 min at 4.2 rad/s (40 rpm) with a pulse setting of 100 on a Glas-Col Tools for Scientists Large Capacity Mixer (Model #099A LC1012, Terre Haute, IN USA). The mixture was then centrifuged at 105 rad/s (1000 rpm) for 5 min on a Beckman Coulter Allegra X-14R centrifuge. The MeOH was decanted into a new 50 mL polypropylene centrifuge tube and the hemp sample was extracted once more following the same procedure. The two MeOH extracts were combined and stored in a –80 °C freezer.

Samples were removed from –80 °C and equilibrated at room temperature for 1 h. Approximately 3 mL of the extract was filtered through a 0.45 µm polytetrafluoroethylene (PTFE) membrane filter (Phenomenex, AF0-1102-52) into a 15 mL polypropylene centrifuge tube. Samples were gravimetrically diluted 10-fold and 100-fold using MeOH and stored at –80 °C.

Samples were removed the next day from –80 °C, vortexed briefly, then transferred into amber autosampler vials and kept at 4 °C in a Shimadzu temperature controlled autosampler tray prior to and during analysis. Sample preparation measurements are summarized in Table 2.

One MeOH blank was carried through the extraction and dilution processes to be used as a check that there were no cannabinoids in the extraction or dilution MeOH and to be used as a blank in the processing method for background subtraction.

Table 2. Mass and Volume Measurements for the RM 8210 Samples.

	All Samples		10-Fold Dilution		100-Fold Dilution	
Sample [a]	m_{sam} [b]	V_{MeOH} [c]	m_{ext} [d]	$M_{ext} + m_{dil}$ [e]	m_{ext} [d]	$M_{ext} + m_{dil}$ [e]
34-1	0.51433	39.935	0.06973	0.86831	0.07130	7.86454
34-2	0.50622	40.100	0.07150	0.84833	0.07240	7.91248
250-1	0.49690	40.042	0.07123	0.78895	0.07076	7.89676
250-2	0.52334	40.041	0.07022	0.85394	0.07179	7.90428
466-1	0.50321	39.948	0.07108	0.79109	0.07160	7.88247
466-2	0.50802	39.921	0.07168	0.85550	0.07195	7.87664
682-1	0.48966	39.941	0.07318	0.75738	0.07179	7.88969
682-2	0.50532	39.958	0.07169	0.86043	0.07163	7.86937
898-1	0.49950	39.915	0.07095	0.84953	0.07125	7.86989
898-2	0.50714	39.963	0.07091	0.85010	0.07045	7.86691
1114-1	0.50595	39.937	0.06980	0.85712	0.07226	7.86675
1114-2	0.50913	39.956	0.07094	0.84699	0.06991	7.89966
1330-1	0.52010	39.996	0.07129	0.86051	0.07210	7.89250
1330-2	0.51454	39.957	0.07101	0.86755	0.07135	7.87120
1546-1	0.50274	39.980	0.07104	0.85264	0.07204	7.88243
1546-2	0.51709	39.966	0.07109	0.84796	0.07132	7.85915
1762-1	0.54872	39.935	0.07058	0.84937	0.07238	7.88376
1762-2	0.50307	39.939	0.07157	0.79409	0.07123	7.86840
2146-1	0.50323	39.909	0.07111	0.86432	0.07194	7.88347
2146-2	0.51032	39.971	0.06980	0.86126	0.07130	7.86970

a Packet-Aliquot
b Mass of plant aliquot extracted, in grams (g)
c Total volume of methanol used to extract the sample aliquot, converted from measured mass of methanol using the 0.789 g/mL density of MeOH at 21.1 °C, in milliliters (mL)
d Mass of non-diluted extract used in the given dilution, in grams (g)
e Total mass of diluted extract, the sum of the masses of non-diluted extract and added methanol, in grams (g)

3.1.4. Instrumental Method

LC-PDA measurements were performed on a Shimadzu *Cannabis Analyzer* equipped with a binary pump, degasser, autosampler, column compartment, and a photodiode array detector. The instrument was computer controlled using commercial software (Lab Solutions, Version 5.99, Shimadzu). Chromatographic separation was achieved on a NexLeaf CBX for Potency C_{18} column (Shimadzu part #220-91525-70, 15.0 cm × 4.6 mm, 2.7 µm) with a NexLeaf CBX guard column (Shimadzu parts #220-91525-72 and #220-91525-73). The operating parameters were optimized by Shimadzu as a “high sensitivity method” and are summarized in Table 3. Nine cannabinoids were tentatively identified based on their retention times. The LC-PDA chromatograms at 220 nm for RM 8210 non-diluted extracts, 10-fold dilutions, and 100-fold dilutions are shown with overlapping calibrant chromatograms in Fig. 1. Confirmation of the cannabinoids was made visually by comparing the absorbance spectra collected at the maximum of the chromatographic peaks for the hemp plant samples to the calibration reference spectra, shown in Fig. 2.

Table 3. Shimadzu Optimized LC-PDA Operating Conditions.

Parameter	Setting		
PDA: Wavelength Range PDA: Single Wavelength	(190 to 700) nm 220 nm		
LC: Injection Volume LC: Column Temperature LC: Flow rate	5 µL 40 ˚C 1.6 mL/min		
LC: Gradient Program	Time (min)	HPLC Water (%)	HPLC 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

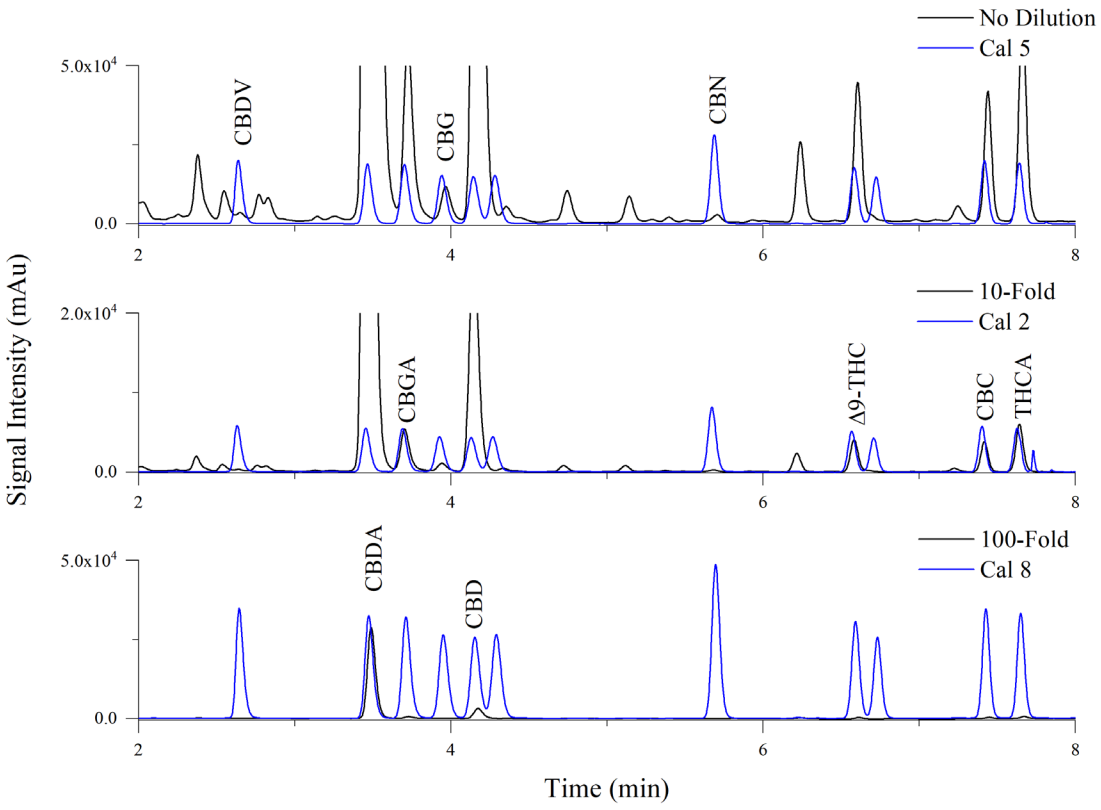


Fig. 1. LC-UV Chromatogram of RM 8210 and Selected Calibrants.

Black traces depict exemplar LC-PDA chromatograms at 220 nm for RM 8210 non-diluted, 10-fold diluted, and 100-fold diluted extracts. The superimposed blue traces depict chromatograms of calibrants derived from the Cayman CRM that most closely match the signal intensity of one or more of the RM 8210 peaks.

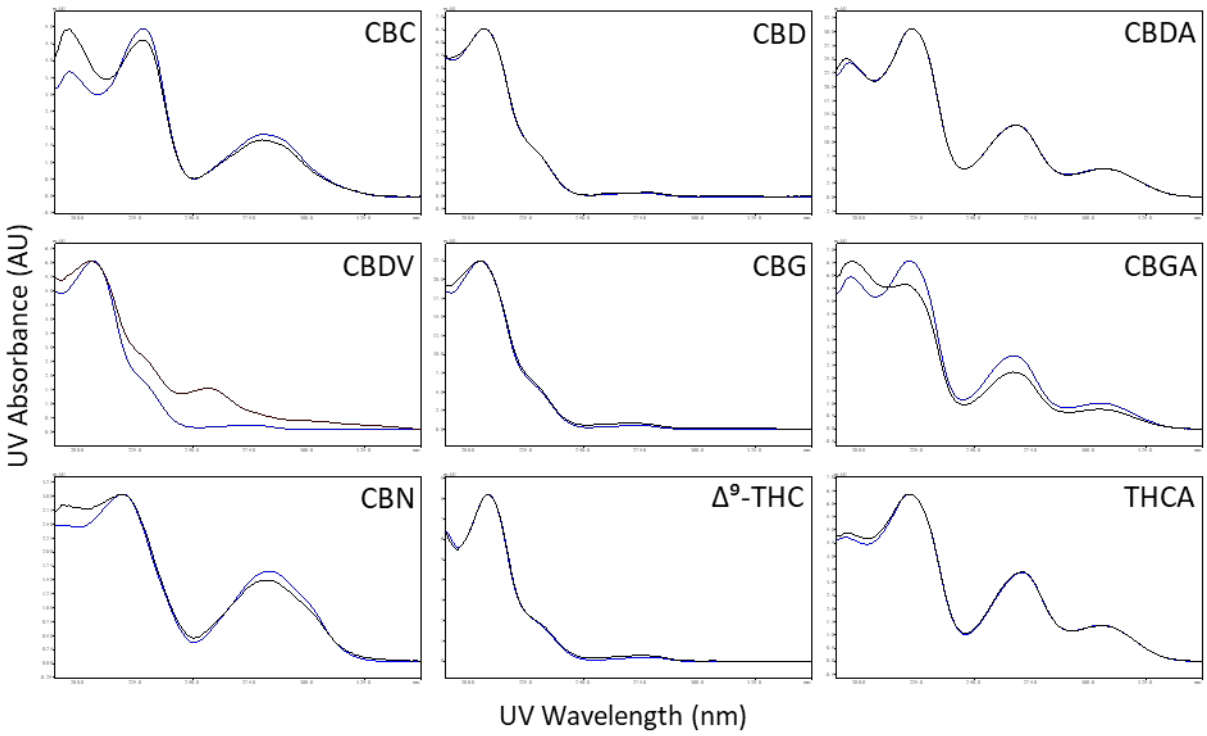


Fig. 2. LC-UV Absorbance Spectra for Cannabinoids in RM 8210.

Black traces depict exemplar UV absorbance spectra from 190 nm to 350 nm at the chromatographic peak maximum. The blue traces depict the spectra at the peak maximum of a best-signal-intensity-matched Cayman CRM-derived calibrant.

The absorbance spectra were visually well correlated between the samples and the standards indicating no co-elution with interfering species except for CBDV, CBGA, and CBC. The differences observed in the absorbance spectra for CBGA and CBC was minimal and not expected to affect the LC-PDA method accuracy. The differences observed in the absorbance spectra for CBDV were more significant as would be expected based on the co-elution shown in Fig. 1 and will not be included in the RMIS of RM 8210.

3.1.5. Calibration Functions

An external standard calibration method was used for all quantitative cannabinoid measurements. Calibration standards, samples, and blanks were injected in a random order throughout the chromatographic sequence and each calibrant was injected twice. Mass concentration (mg/L) values for the cannabinoids in the extracts are calculated using linear functions without forcing through zero defined by regression of the measured peak areas of the cannabinoids in the calibration solutions, A_{cal}, on their measured mass concentrations, γ_{cal}:

$$A_{\mathrm{cal}} = a + b \times \gamma_{\mathrm{cal}} \quad (1)$$

where the intercept, a, has units of area and the slope, b, has units of area per milligram of cannabinoid per liter of solution.

The signal response of each analyte was plotted as a function of concentration. For the preliminary analysis, calibration functions were parameterized using classical linear regression with the concentrations regarded as exact. Calibration curves were constructed using two injections of four to nine calibrants depending on the response range of the analyte in the samples. Calibration linearity was evaluated by the magnitude of the linear regression correlation coefficient (r^2). The functions for all nine cannabinoids are adequately linear, with r^2 values ranging from 0.9978 to 0.9999. The measured peak areas and mass concentrations for the calibrants prepared from the Cayman calibration solutions are listed in Table 4; the calibration data and preliminary calibration functions are visualized in Fig. 3. The measured peak areas and mass concentrations for the calibrants prepared from the Supelco calibration solutions are listed in Table 5; the calibration data and calibration functions are visualized in Fig. 4.

Table 4. Calibration Measurements for Calibrants Derived from the Cayman CRM.

	CBC		CBD		CBDA		CBDV		CBG		CBGA		CBN		Δ⁹-THC		THCA	
Analysis [a]	A_{cal} [b]	γ_{cal} [c]	A_{cal} [b]	γ_{cal} [c]	A_{cal} [b]	γ_{cal} [c]	A_{cal} [b]	γ_{cal} [c]	A_{cal} [b]	γ_{cal} [c]	A_{cal} [b]	γ_{cal} [c]	A_{cal} [b]	γ_{cal} [c]	A_{cal} [b]	γ_{cal} [c]	A_{cal} [b]	γ_{cal} [c]
Cay-1-1	3025	0.2275	3070	0.2285	3805	0.2291	3208	0.2286	3275	0.2275	3729	0.2277	4757	0.2280	3167	0.2275	2971	0.2278
Cay-1-2	3357	0.2275	3408	0.2285	3489	0.2291	3239	0.2286	4145	0.2275	4476	0.2277	4923	0.2280	3133	0.2275	3481	0.2278
Cay-2-1	19197	1.3828	17782	1.3889	20833	1.3922	19326	1.3894	18057	1.3828	21257	1.3839	29798	1.3856	18496	1.3828	19181	1.3844
Cay-2-2	19148	1.3828	18812	1.3889	20721	1.3922	19482	1.3894	19574	1.3828	22134	1.3839	31772	1.3856	18198	1.3828	20042	1.3844
Cay-3-2	35685	2.6147	33498	2.6262	38653	2.6325	36074	2.6273	33715	2.6147	39487	2.6168	56045	2.6199	33851	2.6147	34927	2.6179
Cay-3-3	36380	2.6147	33683	2.6262	38942	2.6325	36577	2.6273	33428	2.6147	39822	2.6168	55944	2.6199	34573	2.6147	35615	2.6179
Cay-4-1	52416	3.6508	47616	3.6669	55335	3.6756	51824	3.6683	48166	3.6508	56409	3.6537	80267	3.6581	49022	3.6508	50000	3.6552
Cay-4-2	51669	3.6508	48914	3.6669	55843	3.6756	52653	3.6683	50542	3.6508	58710	3.6537	80994	3.6581	49612	3.6508	51831	3.6552
Cay-5-1	66077	4.8316	61432	4.8529	71473	4.8645			62308	4.8316			103276	4.8413	63321	4.8316		
Cay-5-2	66644	4.8316	63799	4.8529	72349	4.8645			65261	4.8316			104405	4.8413	63741	4.8316		
Cay-6-1	82207	5.9523	78400	5.9785	88380	5.9928			80123	5.9523			127630	5.9642	77489	5.9523		
Cay-6-2	81862	5.9523	77398	5.9785	89426	5.9928			79893	5.9523			128569	5.9642	78558	5.9523		
Cay-7-1			92074	7.2171	105825	7.2344							153618	7.1999				
Cay-7-2			93277	7.2171	106836	7.2344							155294	7.1999				
Cay-8-1			107881	8.4144	123098	8.4346												
Cay-8-2			110443	8.4144	124207	8.4346												
Cay-9-1					139379	9.4473												
Cay-9-2					140006	9.4473												

a Calibration solution index from least concentrated (1) to most concentrated (9), concatenated with injection order (1 or 2).

b Peak area in arbitrary units

c Mass concentration of cannabinoid in the calibration solution, in milligrams per liter, mg/L.

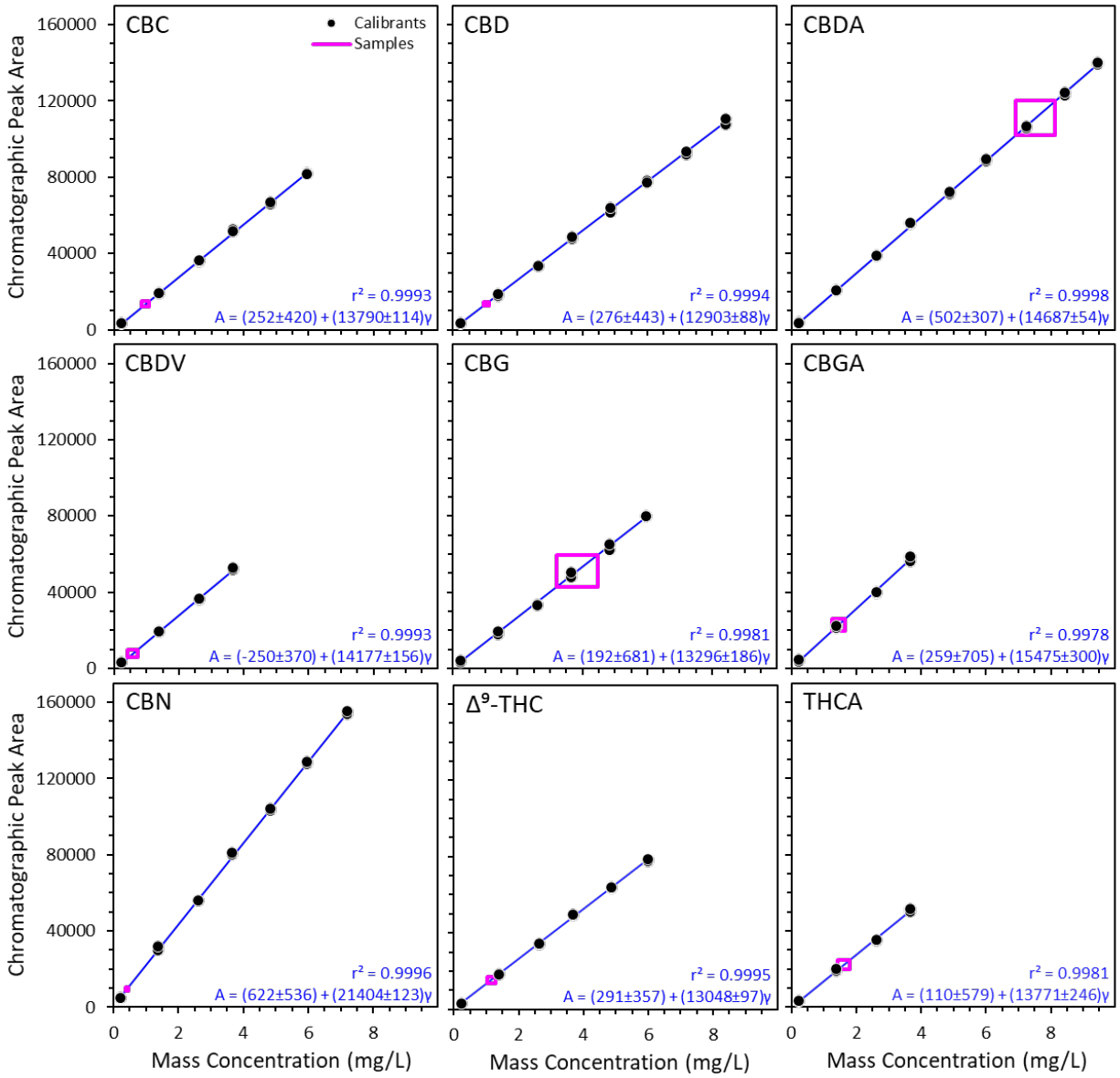


Fig. 3. Calibration Data and Functions Derived from the Cayman CRM.

Each panel presents the calibration model developed for the cannabinoid identified in the upper left corner. Each solid circle represents the measured calibrant peak area for the given cannabinoid as a function of its measured mass concentration using the calibrants derived from the Cayman calibration solution. The diagonal line represents an equally weighted least-squares regression linear fit to the {mass concentration, peak area} measurements; the value of the correlation coefficient and the regression intercept and slope are listed along the bottom right of the panel. The magenta "box" along the line marks the region relevant to the RM 8210 sample peak area measurements.

Table 5. Calibration Measurements for Calibrants Derived from the Supelco CRMs.

	Δ⁹-THC		THCA	
Analysis [a]	A_{cal} [b]	γ_{cal} [c]	A_{cal} [b]	γ_{cal} [c]
Sup-1-1	2469	0.1930	2602	0.2064
Sup-1-2	2405	0.1930	2517	0.2064
Sup-2-1	15484	1.1832	16644	1.2654
Sup-2-2	15112	1.1832	16489	1.2654
Sup-3-2	30765	2.3865	33659	2.5523
Sup-3-3	30688	2.3865	33558	2.5523
Sup-4-1	45383	3.4959	49477	3.7388
Sup-4-2	45579	3.4959	49429	3.7388
Sup-5-1	56095	4.3584		
Sup-5-2	56482	4.3584		
Sup-6-1	69858	5.3598		
Sup-6-2	69800	5.3598		

a Calibration solution index from least concentrated (1) to most concentrated (6), concatenated with injection order (1 or 2).
b Peak area in arbitrary units
c Mass concentration of cannabinoid in the calibration solution, in milligrams per liter, mg/L.

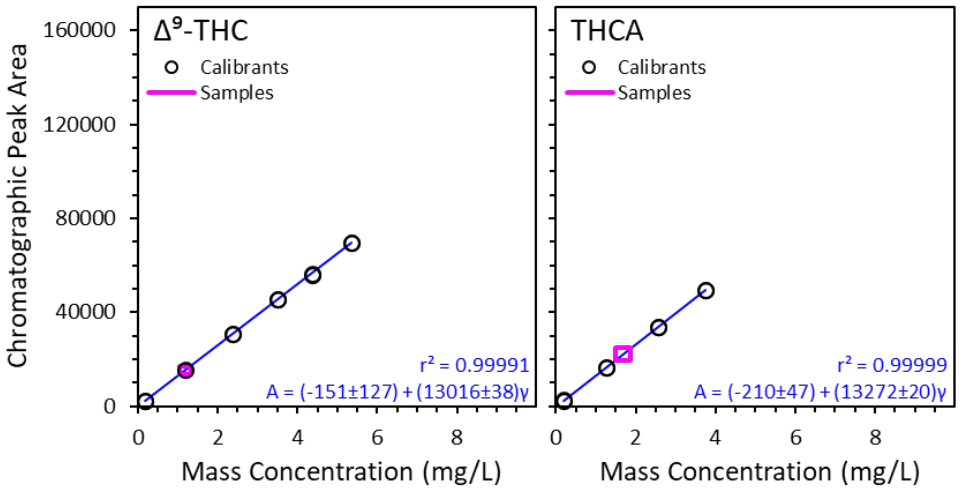


Fig. 4. Calibration Data and Functions Derived from the Supelco CRMs.

Each panel presents the calibration model developed for the cannabinoid identified in the upper left corner. Each solid circle represents the measured calibrant peak area for the given cannabinoid as a function of its measured mass concentration using the calibrants derived from the Supelco calibration solutions. The diagonal line represents an equally weighted least-squares regression linear fit to the {mass concentration, peak area} measurements; the value of the correlation coefficient and the regression intercept and slope are listed along the bottom right of the panel. The magenta "box" along the line marks the region relevant to the RM 8210 sample peak area measurements.

3.1.6. Calibrant Comparison for Δ⁹-THC and THCA

The Cayman and Supelco calibration functions for Δ⁹-THC and THCA are compared in Fig. 5. While the Δ⁹-THC curves overlap, the THCA curves do not have overlapping 95 % confidence intervals. An F-test conducted using the OriginPro (OriginLab Corp, Northampton, MA USA) dataset comparison tool at a *p*-value of 0.05 indicated that the functions for both Δ⁹-THC and THCA are statistically different. Δ⁹-THC and THCA are quantified using the calibrant solutions derived from the Supelco calibration solutions.

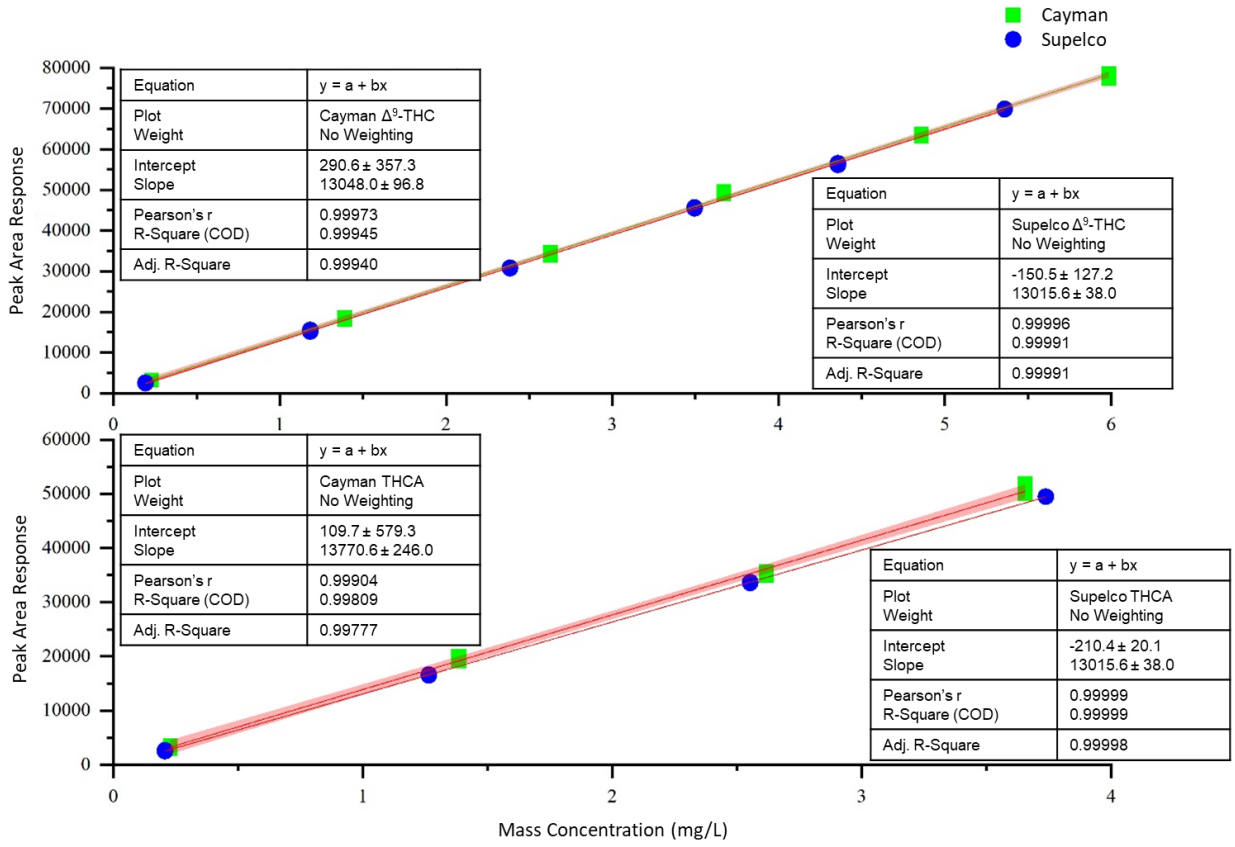


Fig. 5. Comparison of Cayman and Supelco Calibration Functions.

The top panel displays the nearly overlapping Δ⁹-THC functions. The bottom panel displays the slightly divergent THCA functions. Various linear regression fit statistics for the Cayman functions are displayed in the top left of the panels. The statistics for the Supelco functions are displayed at the bottom right corners. The shaded regions represent 95 % confidence intervals for each function.

3.1.7. Mass Concentrations of Sample Extracts

Mass concentrations in the sample extracts, γ_{ext}, are estimated using the measured peak areas, A_{ext}, and the model parameter values for each cannabinoid of interest:

$$\gamma_{\mathrm{ext}} = (A_{\mathrm{ext}} - a)/b \; . \qquad (2)$$

Samples were randomly injected and analyzed in duplicate. Prior to integration, the blank that was extracted with the samples and carried through each dilution step was used as the spectral background file for background subtraction. For the non-diluted extract samples, the non-diluted MeOH blank was used, and the 10-fold and 100-fold diluted MeOH blanks were used as background subtraction files for the 10-fold and 100-fold diluted samples, respectively. Specificity was determined by matching the acquired spectra and elution order/retention times of cannabinoids from the samples with those from the Cayman calibration solution. Peak areas for the RM 8210 sample aliquots injections are listed in Table 6, along with the extraction, dilution, and analysis sequences. Preliminary analyses of the RM 8210 and HEMP-1 materials utilized slope and intercept coefficients estimated using unweighted linear regression.

3.1.8. Mass Fractions of Samples

Mass fractions of the cannabinoids in the samples, w_{sam}, are calculated from γ_{ext}, the sample mass, m_{sam}, the mass of MeOH, m_{MeOH}, the 0.789 g/mL density of MeOH at 21.1 °C, and (for diluted extracts) the mass of undiluted extract in a diluted extract, m_{extr}, and the mass of the MeOH used to dilute the extract, m_{dil}:

$$w_{\mathrm{sam}} = \frac{\gamma_{\mathrm{ext}}}{10000} \times \left(\frac{m_{\mathrm{MeOH}}}{0.789}/m_{\mathrm{sam}}\right) \times \frac{m_{\mathrm{est}}+m_{\mathrm{dil}}}{m_{\mathrm{ext}}} \; . \qquad (3)$$

The factor 1×10^4 is required for the conversion of mass concentration in milligrams per liter to mass fraction in grams per one hundred grams (percent, %). The mass and volume measurements for undiluted, 10-fold diluted, and 100-fold diluted RM 8210 extracts are listed in Table 2.

Table 6. Cannabinoid Peak Areas and Extraction, Dilution, and Analysis Orders.

		Non-Diluted				10-Fold Dilution						100-Fold Dilution			
Sample [a]	Ext [b]	Run [c]	CBDV	CBG	CBN	Dil [d]	Run [c]	CBC	CBGA	Δ⁹-THC	THCA	Dil [d]	Run [c]	CBD	CBDA
34-1-a	10	27	8799	57992	9466	8	39	13192	22205	15078	21696	3	19	14026	114907
34-1-b	10	171	7305	50287	10577	8	128	13571	22170	15433	23759	3	145	14110	116436
34-2-a	20	59	8096	54997	9446	21	84	12834	22762	15018	21646	10	48	13784	115004
34-2-b	20	137	7308	48249	9954	21	163	13564	22740	15505	22167	10	188	13996	114737
250-1-a	9	30	7452	55507	9249	5	83	13864	23787	16112	22820	23	69	13759	110787
250-1-b	9	104	7178	54507	8851	5	130	13998	23702	16059	23793	23	143	13469	110640
250-2-a	19	11	9335	59663	9666	15	7	13771	24568	15515	22537	11	8	14212	116516
250-2-b	19	195	9231	52329	10308	15	168	13934	24593	16131	22307	11	125	14070	116998
466-1-a	4	51	7257	56329	8949	2	58	13806	24285	16107	23121	6	18	13445	111146
466-1-b	4	146	6744	46534	9262	2	157	14517	25308	16816	24197	6	179	13363	112637
466-2-a	16	14	7576	55888	9537	14	91	12890	22519	16361	21322	13	20	13801	113756
466-2-b	16	120	9770	58438	9815	14	164	12950	22841	15333	21360	13	193	14136	114177
682-1-a	11	75	6779	51157	8551	1	46	14860	25841	16884	24402	18	93	13429	111966
682-1-b	11	175	6507	43847	9359	1	142	14881	25215	17137	24676	18	180	e	e
682-2-a	22	62	7136	54589	9311	11	23	12756	20984	14479	20640	16	79	13392	109094
682-2-b	22	169	6772	46794	10010	11	152	13257	22346	14708	21668	16	112	13267	109221
898-1-a	8	26	7775	48198	9868	17	86	12968	22000	15059	21289	19	44	13570	111493
898-1-b	8	106	5677	59090	9296	17	129	13271	23502	15481	22148	19	159	14607	112932
898-2-a	17	67	6069	59022	9756	19	56	14830	24180	16180	24721	14	43	13838	114080
898-2-b	17	134	9101	52091	10384	19	197	13538	22815	15381	22490	14	133	13925	115076
1114-1-a	6	68	7453	56173	9214	13	52	12345	19677	13572	20050	12	97	13216	109952
1114-1-b	6	156	6824	46885	9630	13	192	12692	21891	14415	21221	12	108	13186	110280
1114-2-a	15	50	7819	55030	9648	22	12	12886	25282	14586	20459	22	31	13171	109501
1114-2-b	15	132	6604	47438	9870	22	141	12901	21681	14733	21445	22	121	13299	108328
1330-1-a	7	102	7255	55011	9286	12	76	13185	22376	15027	22754	5	42	14189	115511
1330-1-b	7	154	10050	45202	9576	12	122	12848	22695	14708	21939	5	187	14014	116276
1330-2-a	13	47	7516	58430	9700	9	63	12532	21814	14379	20726	21	55	13502	109990
1330-2-b	13	148	8371	49960	9999	9	118	12281	22138	13961	20795	21	109	13422	110855
1546-1-a	1	15	8869	53596	9910	16	15	13411	23457	15040	21291	8	38	13952	112222
1546-1-b	1	181	7873	46838	10578	16	191	13642	23417	16013	22439	8	160	14543	113492
1546-2-a	21	80	7580	53060	9101	20	10	12847	21853	14518	21251	2	65	13332	108775
1546-2-b	21	105	7160	48405	9148	20	185	13057	22502	15178	21651	2	151	13224	109553
1762-1-a	3	60	8655	59083	10364	18	94	13502	23666	15591	22482	9	98	14694	119983
1762-1-b	3	124	8246	58829	10258	18	113	13296	23692	15366	22426	9	114	14731	119844
1762-2-a	14	34	7859	51192	8873	6	77	12719	22149	14956	21361	4	22	12682	102202
1762-2-b	14	196	5721	42757	9539	6	173	13060	23692	15466	22328	4	150	12555	103163
2146-1-a	2	24	7271	54565	9460	7	72	13106	20131	13612	20958	7	32	12789	103418
2146-1-b	2	167	6850	46425	9692	7	189	12384	20718	14465	19717	7	176	13002	105258
2146-2-a	18	81	6665	48948	9177	10	6	12068	20921	13909	19804	15	28	12551	104715
2146-2-b	18	126	6436	45624	9941	10	184	12056	21292	13709	20158	15	161	13236	105778

a Packet-Aliquot-Injection.

b Extraction sequence of the RM 8210 sample aliquots. A total of 23 sample aliquots were extracted, two each for the ten RM 8210 packets and three for the one bottle of HEMP-1.

c Run (chromatographic analysis) sequence of the RM 8210 sample aliquot injections of the given dilution.

d Dilution sequence of the RM 8210 sample aliquots of the given dilution.

e Compromised injection, no usable results.

3.1.9. Agreement with Control Material.

Measurement results for the cannabinoids in the NRC HEMP-1 control material were compared to their certified values in Fig. 6. With a 95 % level of confidence, there were no statistically significant differences between the values. While the UV absorbance spectra for CBDV, CBGA, and CBC in RM 8210 indicated a potential for measurement bias, the measured values for these analytes were well within the certified values.

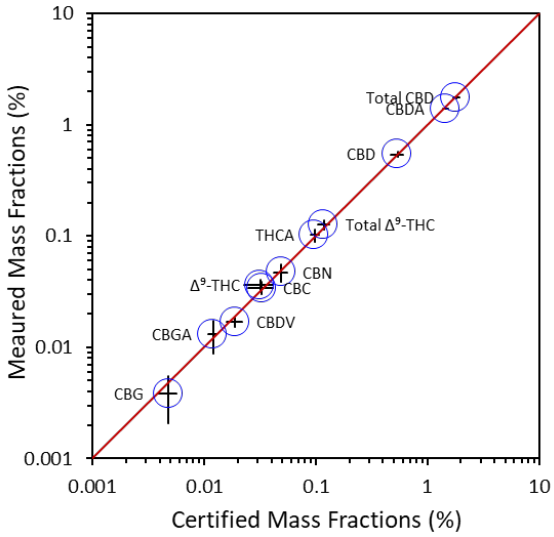


Fig. 6. Comparison of Certified and Measured Cannabinoid Values for the HEMP-1 Control Material.

Horizontal error bars span the certified 95 % level of confidence expanded uncertainties. Vertical error bars span approximate 95 % level of confidence expanded uncertainties estimated from the measured standard deviations and the Student's *t* coverage value of 4.3 for two degrees of freedom. The diagonal line represents equality of the values. Open circles are for graphical emphasis.

3.1.10. Trend Evaluations.

Mass fractions and linear trend lines for all nine of the cannabinoids are shown in Fig. 7 as functions of sample LC run order, extraction order, and packet order. Mass Fractions and trend lines for the six cannabinoids evaluated in 10-fold (CBC, Δ^9-THC, CBGA, THCA) and 100-fold (CBD, and CBDA) diluted extracts are shown in Fig. 8 as functions of dilution order. There are no obvious non-linear trends.

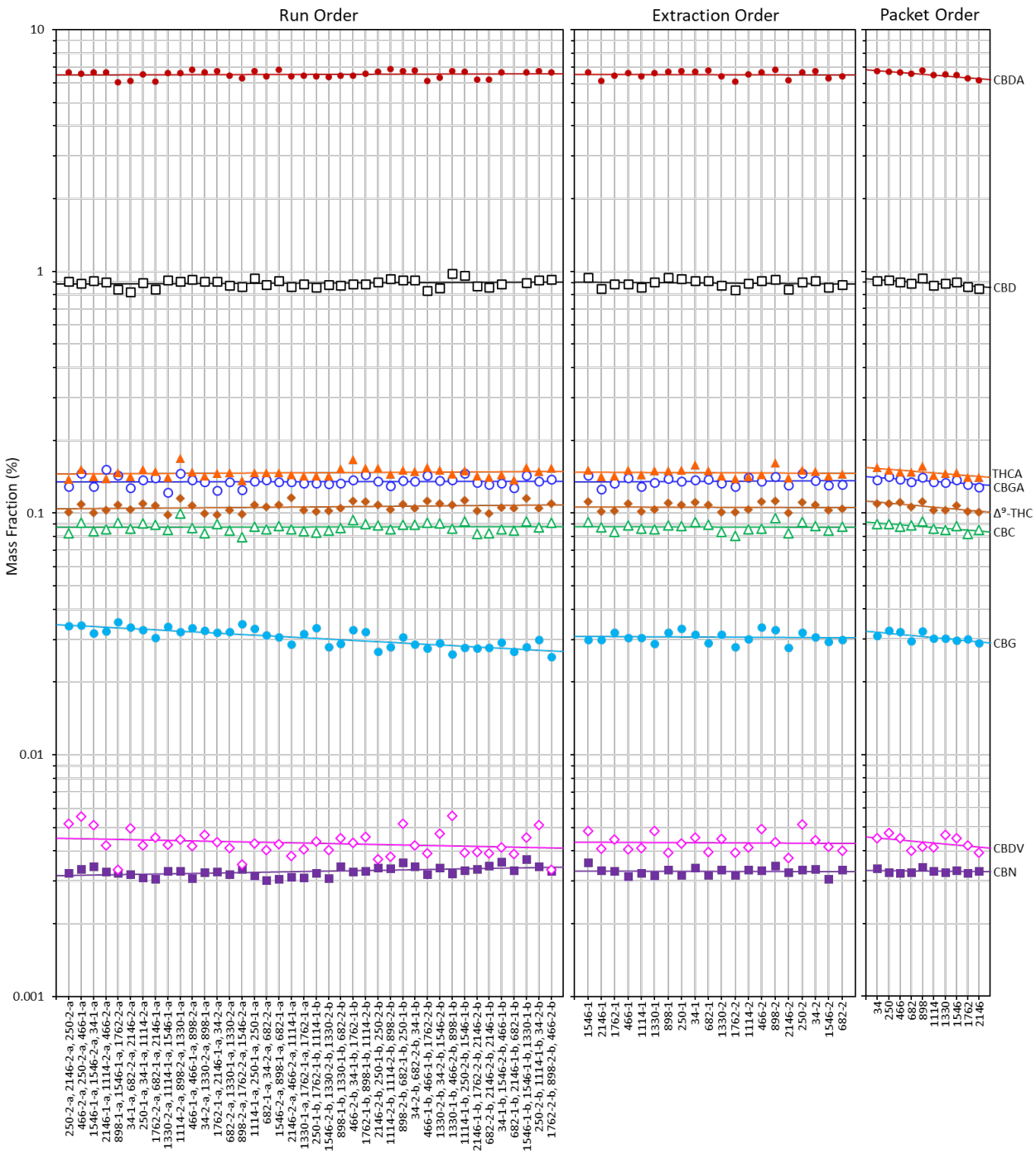


Fig. 7. Run, Extraction, and Packet Order Trends for RM 8210 Measurements.

Symbols in the Run Order panel (left) represent results for individual injections of each aliquot arranged in the order of the chromatographic analysis; labels are arranged in order: non-diluted, 10-fold diluted, and 100-fold diluted extracts. Symbols in the Extraction Order panel (middle) represent the average of two injections for each aliquot, arranged in order of aliquot extraction. Symbols in the Packet Order panel (right) represent the average of four injections (two each for each aliquot) arranged by packet index. Lines represent least-squares fits of the estimated mass fractions to the ordering. Cannabinoids are identified along the right edge of the Packet Order panel.

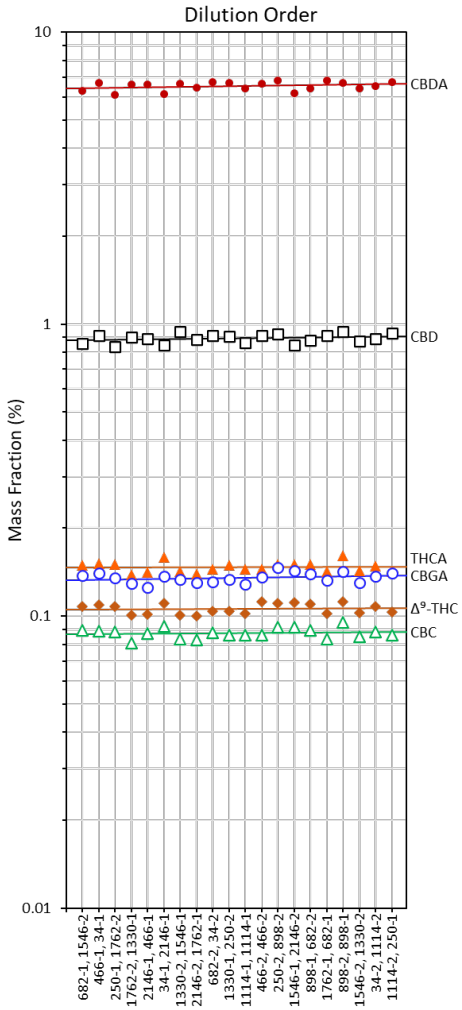


Fig. 8. Dilution Order Trends for 10-Fold and 100-Fold Diluted Extracts.

Symbols represent the average of two injections for each aliquot, arranged in order of extract dilution; labels are arranged in order: 10-fold diluted and 100-fold diluted extracts. Lines represent least-squares fits of the estimated mass fractions to the ordering. Cannabinoids are identified along the right edge of the panel.

Table 7 summarizes the estimated linear trend line slopes, sorted in order of increasing statistical significance.

$$|t| = |b|/u(b) \tag{4}$$

where t is the "t-score", b is the slope of the linear trend and $u(b)$ is the standard uncertainty of the estimated slope. Absolute t-scores greater than about two suggest statistical significance at about a 95 % level of confidence. There are no statistically significant linear trends with extraction order or dilution order. The CBN mass fraction increases with run order and the CBG mass fraction decreases, suggesting slight thermal degradation over the course of the LC analysis. The change in CBG mass fraction is too large to account for the increase in CBN but is too small for its degradation product(s) to have significantly increased the mass fractions of the other

cannabinoids. The increase in CBN is compatible with a slight degradation of Δ^9-THC [12]. With the exception of CBN and (perhaps) CBDV, there is a decreasing trend with packet order. More detailed analysis suggests that there is also a difference between samples from boxes 1 to 9 and those from boxes 10 to 14. However, the needs of the user community combined with the level of heterogeneity observed in the material suggest that these trends do not prevent the cannabinoid content of the material from being fit for its intended purpose.

Table 7. Summary of Linear Trends with Run, Extraction, Dilution, and Packet Order.

Order	Cannabinoid	Slope	u(Slope)	*t*-score [a]
Run	CBDV	0.000	0.045	0.0
Dilution	THCA	0.00000	0.00023	0.0
Extraction	Δ^9-THC	-0.00001	0.00015	-0.1
Run	CBGA	0.000008	0.000090	0.1
Extraction	CBDA	-0.0010	0.0079	-0.1
Extraction	CBDV	-0.000002	0.000014	-0.2
Extraction	CBG	-0.000012	0.000059	-0.2
Extraction	CBGA	0.00004	0.00019	0.2
Extraction	CBN	-0.0000011	0.0000043	-0.3
Extraction	CBC	-0.00004	0.00013	-0.3
Dilution	Δ^9-THC	0.00005	0.00016	0.3
Extraction	THCA	-0.00007	0.00021	-0.3
Dilution	CBC	0.00005	0.00014	0.3
Run	CBC	0.000021	0.000054	0.4
Packet	CBN	-0.0000039	0.0000070	-0.6
Run	CBDA	0.0021	0.0031	0.7
Extraction	CBD	-0.0007	0.0011	-0.7
Run	THCA	0.000091	0.000094	1.0
Run	CBD	0.00047	0.00048	1.0
Dilution	CBGA	0.00021	0.00020	1.1
Dilution	CBDA	0.0095	0.0079	1.2
Dilution	CBD	0.0014	0.0011	1.3
Packet	CBDV	-0.000041	0.000030	-1.4
Run	Δ^9-THC	0.000102	0.000064	1.6
Packet	CBC	-0.00070	0.00026	-2.8 [b]
Packet	CBG	-0.00031	0.00011	-2.8 [b]
Packet	CBD	-0.0064	0.0021	-3.0 [b]
Packet	CBGA	-0.00101	0.00031	-3.2 [b]
Run	CBN	0.0000063	0.0000019	3.3 [c]
Packet	THCA	-0.00128	0.00039	-3.3 [b]
Packet	Δ^9-THC	-0.00103	0.00029	-3.5 [b]
Packet	CBDA	-0.057	0.011	-5.0 [b]
Run	CBG	-0.000189	0.000023	-8.4 [b]

a Slope divided by the estimated standard uncertainty of the slope. Absolute *t*-scores greater than two suggest that the slope has statistical significance at about the 95 % level of confidence.

b Statistically significant decrease in mass fraction with increasing run order or packet index.

c Statistically significant increase in mass fraction with increasing run order.

3.1.11. Bayesian Mass Fraction Assessment

To rigorously estimate cannabinoid mass fractions and uncertainties, the calibration functions were evaluated using a Bayesian errors-in-variables model. These coefficients were used to estimate mass concentrations on an as-received basis from the measured peak areas listed in Table 6 (Eq. 2). The mass concentrations were converted to mass fraction using the volume and mass measurement listed in Table 2 (Eq. 3). Quantification of uncertainty in the mass fraction was performed with a Monte Carlo method using OpenBUGS [13] software as is described in Toman et al., 2016 [14]. The individual sample mass fractions were combined to obtain a consensus value using the Linear Pool model [15]. The uncertainty analysis accounts for the effects of calibration, within-aliquot, between-aliquot, and between-packet variability for the samples. Any heterogeneity between samples (boxes) is included in the standard and expanded uncertainties of the mass fractions.

3.1.11.1. Mass Fraction Estimates

Table 8 summarizes the mass fraction results, stated as percent of sample, for the nine quantified cannabinoids in RM 8210. The results for each sample aliquot are presented in Fig. 9 as function of the sample packet number.

Table 8. Values and Uncertainties for As-Received Cannabinoid Mass Fractions, %.

	Mass Fractions (%)				
Cannabinoid	$\hat{\mu}$ [a]	$u(\hat{\mu})$ [b]	U_{95}(Low) [c]	U_{95}(High) [c]	%CV [e]
CBN	0.00330	0.00012	0.00307	0.00358	3.6
CBDV	0.00433	0.00038	0.00376	0.00515	8.8
CBG	0.0307	0.0016	0.0277	0.0337	5.2
CBC	0.0875	0.0036	0.0807	0.0954	4.1
Δ^9-THC	0.1095	0.0043	0.1033	0.1159	3.9
CBGA	0.1354	0.0053	0.1256	0.1462	3.9
THCA	0.1550	0.0061	0.1461	0.1695	3.9
CBD	0.894	0.032	0.838	0.947	3.6
CBDA	6.54	0.22	6.10	6.83	3.4

a Linear pool consensus value.
b Standard uncertainty associated with the consensus value
c 2.5^{th} percentile of the posterior distribution.
d 97.5^{th} percentile of the posterior distribution.
e Coefficient of variation (aka relative standard deviation) expressed as a percentage: %CV = 100 × $u(\hat{\mu})/\hat{\mu}$.

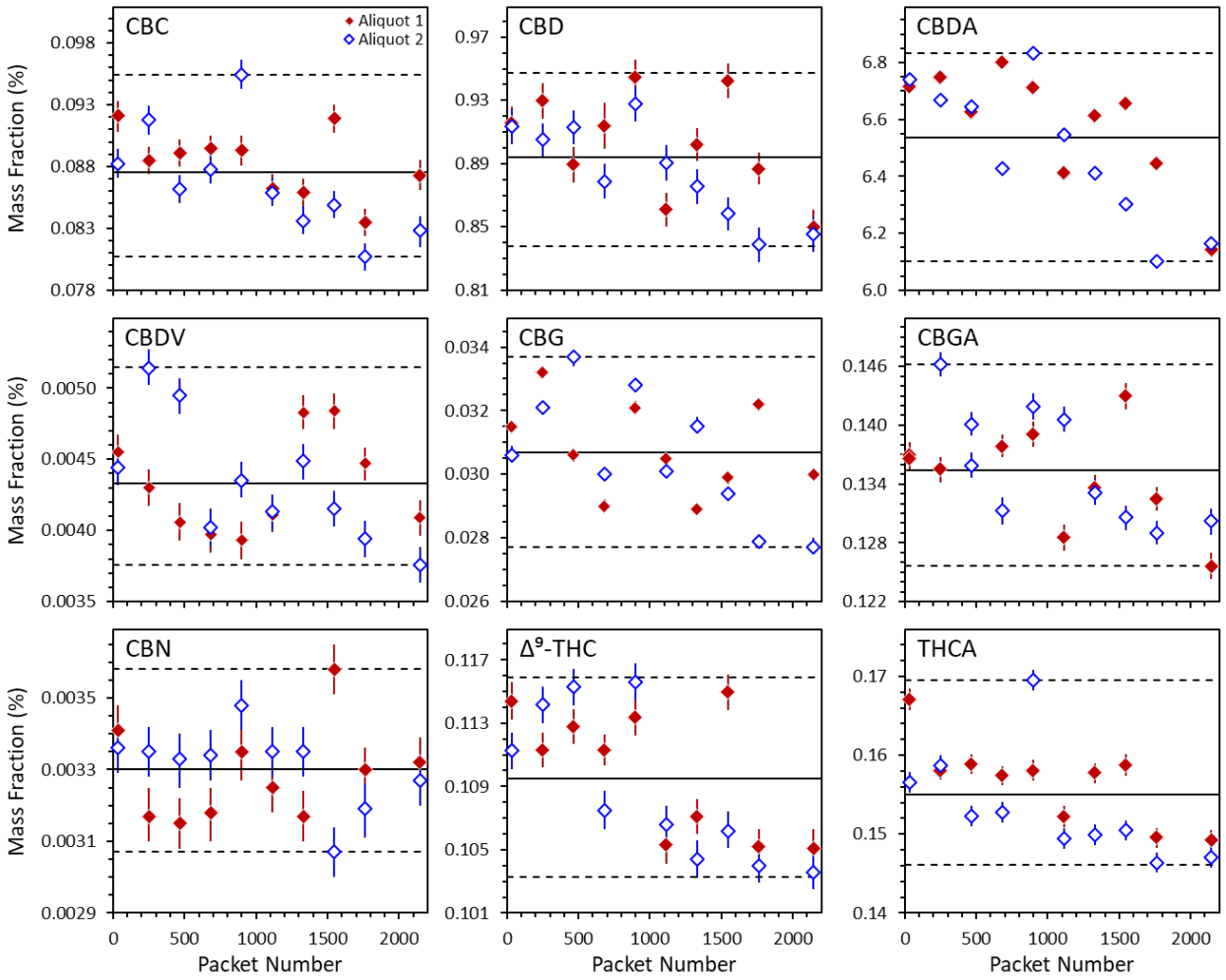


Fig. 9. Estimated Mass Fractions (%) as Functions of Packet Number.

Each panel presents the OpenBUGS results for one cannabinoid. Solid diamonds represent results for the first aliquot of each sample, open diamonds for the second aliquot. Error bars represent approximate 95 % confidence uncertainty intervals. The solid horizontal line represents the mean of the posterior distribution of the mass fraction (as percent) of the cannabinoid in RM 8210 on an as-received basis (see Section 3.1.8 for conversion of mass concentration to mass fraction). The dashed horizontal lines bound the central 95 % of the posterior distribution.

3.1.11.2. Total THC and Total CBD

The conventional total Δ^9-THC mass fraction, $w_{\text{Total THC}}$, is estimated as the sum of the calculated Δ^9-THC mass fraction, w_{THC}, and the THCA mass fraction, w_{THCA}, corrected to its equivalent Δ^9-THC content by the molar mass ratio,

[(314.461 ± 0.012) g/(mol Δ^9-THC)]/[(358.470 ± 0.013) g/(mol THCA)] = 0.87723 ± 0.00005, rounded to three significant digits:

$$w_{\text{Total THC}} = w_{\text{THC}} + 0.877 \times w_{\text{THCA}} \,. \tag{5}$$

Likewise, the conventional total CBD mass fractions, $w_{\text{Total CBD}}$, are estimated as the sum of the calculated CBD mass fraction, w_{CBD}, and the CBDA mass fraction, w_{CBDA}, corrected to its equivalent CBD content by the same molar mass ratio:

$$w_{\text{Total CBD}} = w_{\text{CBD}} + 0.877 \times w_{\text{CBDA}} \,. \tag{6}$$

The NIST Uncertainty Machine [16] provides a convenient mechanism to combining the mass fractions. Example Uncertainty Machine Input and output screens are displayed in Fig. 10. Table 9 summarizes the mass fraction results, stated as percent of sample, for total Δ^9-THC and total CBD in RM 8210.

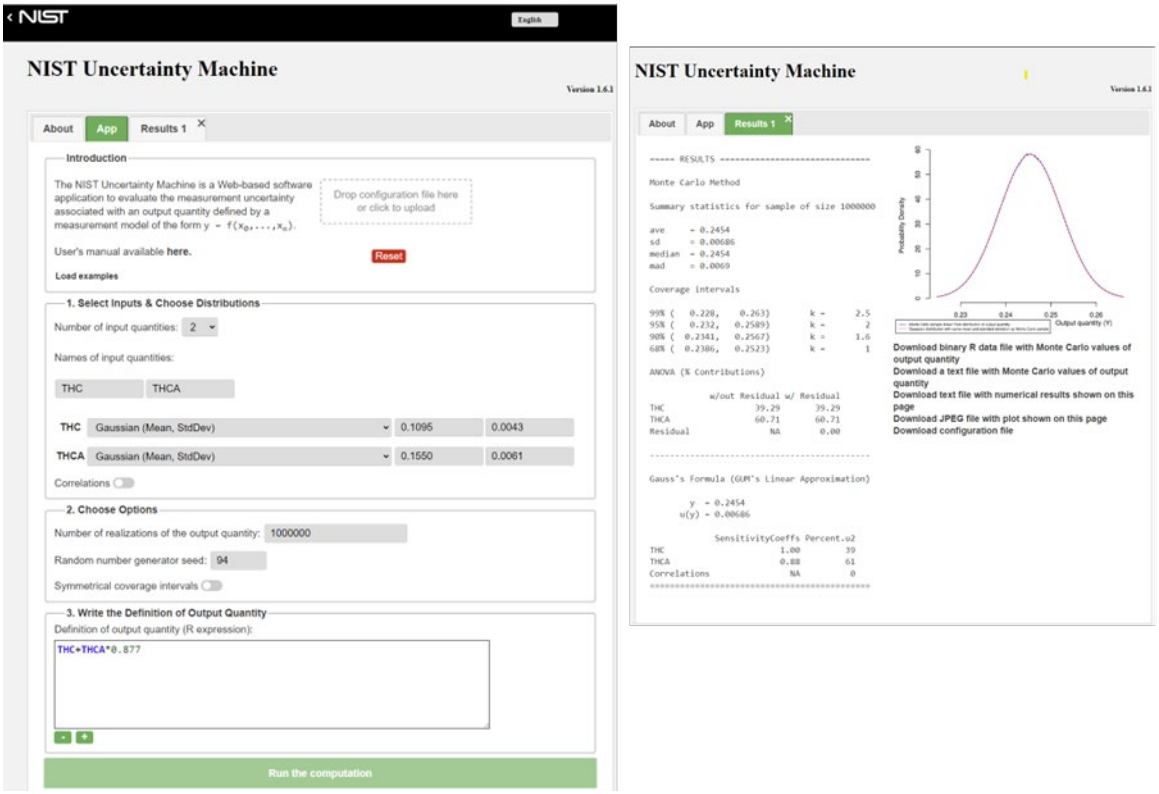


Fig. 10. Screen Shots of the NIST Uncertainty Machine Input and Output Tabs for Total THC.

Table 9. Values and Uncertainties for As-Received Total THC and Total CBD Mass Fractions, %.

	Mass Fractions (%)				
Cannabinoid	$\hat{\mu}$ [a]	$u(\hat{\mu})$ [b]	U_{95}(Low) [c]	U_{95}(High) [c]	%CV [e]
Total THC	0.2454	0.0069	0.2320	0.2589	2.8
Total CBD	6.63	0.20	6.24	7.01	3.0

a Linear pool consensus value.
b Standard uncertainty associated with the consensus value
c 2.5$^{\text{th}}$ percentile of the posterior distribution.
d 97.5$^{\text{th}}$ percentile of the posterior distribution.
e Coefficient of variation (aka relative standard deviation) expressed as a percentage: %CV = 100 × $u(\hat{\mu})/\hat{\mu}$.

3.2. Five Month Measurements

Cannabinoid mass fractions were evaluated five months after completion of the initial RM 8210 assessment. The measurement materials and processes used were similar to those described in Section 3.1; the few differences are detailed in this section.

3.2.1. Materials

HPLC grade ACN, water, MeOH, and 85 % PA were purchased from Fisher Scientific. Supelco Calibration Mixture (Neutrals) – 8 Component [17] was purchased from Cerilliant. This calibration solution delivers mass concentrations of ≈ 500 mg/L ± 3 mg/L of CBC, CBD, CBDV, CBG, CBN, Δ^9-THC, Δ^8-tetrahydrocannabinol (Δ^8-THC), and tetrahydrocannabivarin (THCV) in MeOH. Supelco calibration standard solutions for Cannabidiolic acid (CBDA) [18], Cannabigerolic acid (CBGA) [19], and THCA [20] were purchased from Cerilliant. These one component calibration solutions deliver mass concentrations of ≈ 1000 mg/L ± 6 mg/L in ACN. One aliquot was taken from each of six packets of ASTM Cycle 2209 Hemp material for use as a control material. This material had been previously value-assigned for use in an ASTM-sponsored proficiency testing program [21].

3.2.2. Calibration Standards Preparation

One calibration stock solution was prepared gravimetrically from the Supelco cannabinoid standards. The eleven cannabinoids in this mixture were each at a mass concentration of ≈ 200 mg/L. Four calibration solutions were individually prepared gravimetrically from the stock solution mixture to have final mass concentrations of approximately 2 mg/L, 4 mg/L, 8 mg/L, and 16 mg/L of each cannabinoids.

3.2.3. Sample Preparation

Six of the same packets of RM 8210 previously opened and used in the initial assessment and six packets of the ASTM Cycle 2209 materials were removed from the –20 °C freezer, equilibrated at room temperature for 1 h, and mixed thoroughly by hand to ensure homogeneity. One 0.50 g ± 0.05 g aliquot of each packet was weighed into individual 50 mL polypropylene centrifuge tubes using a Mettler Toledo XPR205 balance. MeOH (20 mL) was added to each sample and vortexed for 10 s to ensure initial suspension. Samples were then mechanically shaken at room temperature using a large capacity Glas-Col Tools Model # 099A LC1012 mixer at room temperature for 30 min at 5.2 rad/s (50 rpm). Samples were then centrifuged at room temperature using an Allegra X-14R Centrifuge from Beckman Coulter for 5 min at 2285 m/s^2 (233 g force). The supernatant was removed and a second 20 mL aliquot of MeOH was added. After shaking and centrifugation, the supernatant was decanted and combined with the initial MeOH extract. Extracts were filtered through a 0.45 µm PTFE polymer membrane filter (Phenomenex, AF0-1102-52) and MeOH dilutions (10-fold and 100-fold) were gravimetrically prepared for LC-PDA measurements.

3.2.4. Mass Fractions

All samples were identified, analyzed, and quantified as described in Section 3.1. The seven cannabinoids present at mass concentrations sufficiently high to be quantified with the prepared calibrants are identified in Fig. 11. Section 3.1.8 details conversion of mass concentration to mass fraction and Table 10 lists the cannabinoid mass fractions determined for each of the six packets of RM 8210 five months after the initial analysis. The mean mass fractions in RM 8210 and the ASTM Cycle 2209 control materials are plotted as function of their initial assessments in Fig. 12. The values are in good visual agreement.

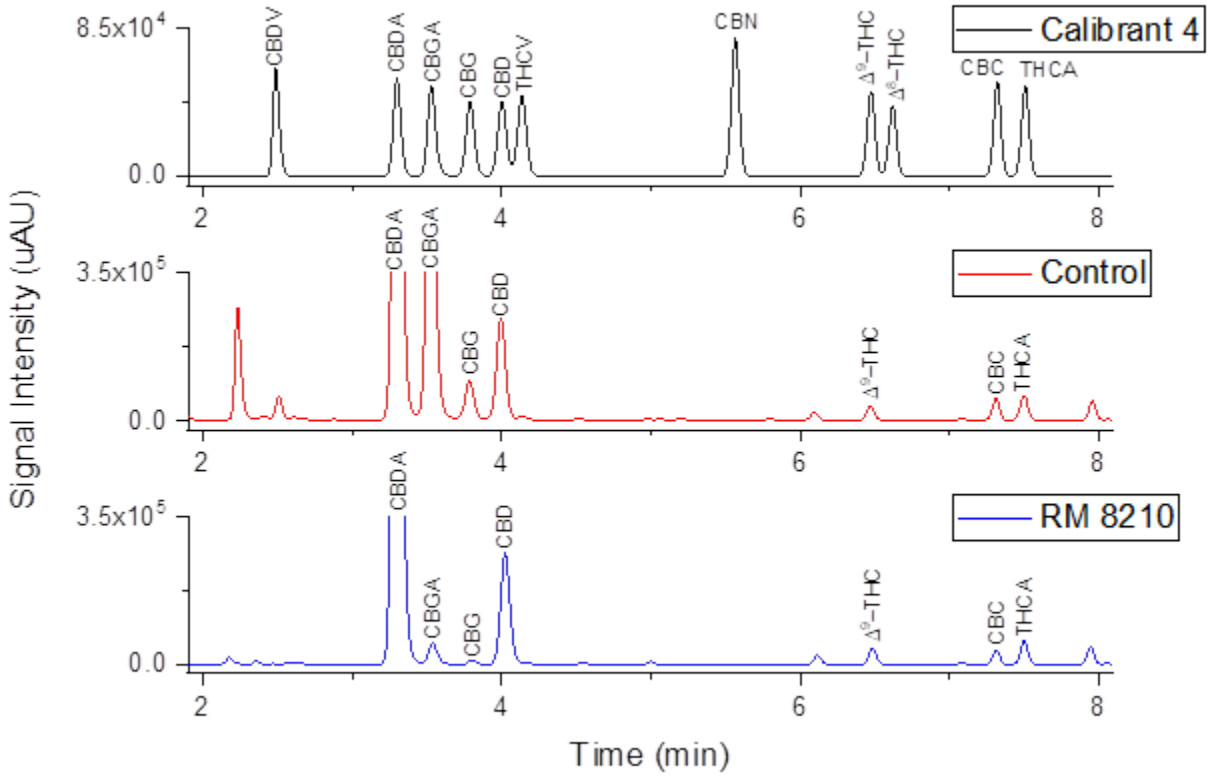


Fig. 11. LC-UV Chromatograms of Calibrant and Undiluted Control and RM 8210 Samples.

Table 10. Cannabinoid Mass Fractions Determined at Five Months, %

Packet	CBGA	CBG	CBC	Δ^9-THC	THCA	Total THC	CBD	CBDA	Total CBD
34	0.1222	0.0341	0.0911	0.1116	0.1531	0.2458	0.931	7.46	7.48
250	0.1454	0.0339	0.0901	0.1087	0.1495	0.2398	0.990	7.36	7.45
466	0.1203	0.0338	0.0897	0.1085	0.1518	0.2416	0.902	7.17	7.19
682	0.1383	0.0358	0.0947	0.1144	0.1591	0.2540	0.925	7.15	7.20
898	0.1347	0.0340	0.0896	0.1089	0.1517	0.2420	0.938	7.38	7.41
1546	0.1291	0.0325	0.0872	0.1063	0.1486	0.2366	0.907	6.82	6.89
Mean:	0.1317	0.0340	0.0904	0.1097	0.1523	0.2433	0.932	7.22	7.27
Standard Deviation:	0.0097	0.0011	0.0025	0.0028	0.0037	0.0060	0.032	0.23	0.22

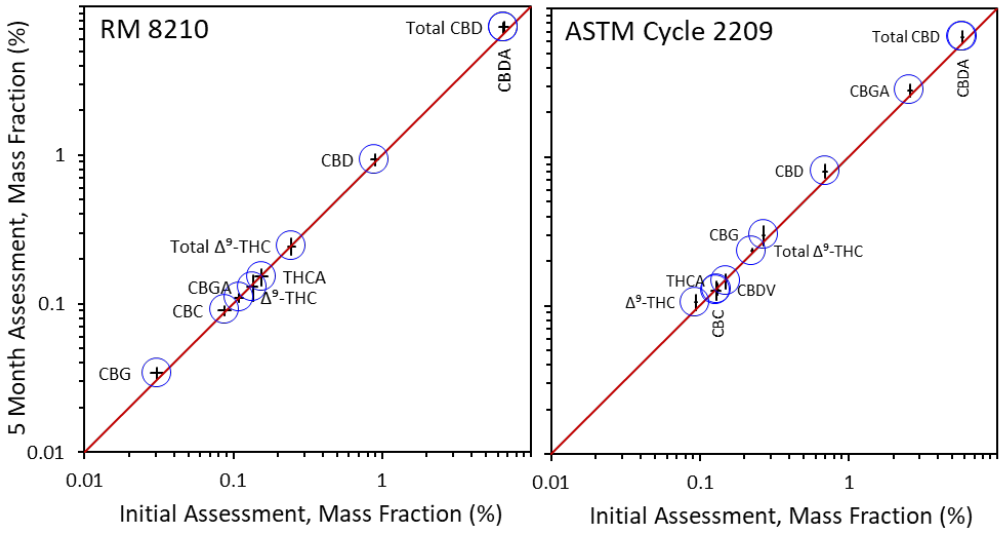


Fig. 12. Five Month Measurements of RM 8210 and Control Materials.

The panel to the left displays the five-month assessment of cannabinoid mass fractions of RM 8210 as functions of the assessments presented in Section 3.1.11. The panel to the right displays the mass fraction assessments of the ASTM Cycle 2209 control material as functions of target values assigned approximately three months earlier. Horizontal error bars span the estimated 95 % level of confidence expanded uncertainties of the initial assessments of the two materials. Vertical error bars span approximate 95 % level of confidence expanded uncertainties of the five month assessments. The diagonal lines represent equality of the values. Open circles are for graphical emphasis.

By the criterion outlined in Linsinger 2010 [22], CBGA, CBG, CBD, Δ^9-THC, CBC, THCA, and total Δ^9-THC mass fractions are not significantly different ($\alpha = 0.05$). The CBDA and total CBD values differ with marginal statistical significance for both RM 8210 and ASTM Cycle 2209 materials, indicating potential biases in the CBDA mass concentration in the calibration solutions. The variance in total CBD mass fractions is a result of the difference among CBDA mass fractions. In addition to the increase in CBDA mass fraction, the additional measurements are on average slightly higher than the original assessments in both materials. For RM 8210, the ratio between the 5-month and 15-month measurements and the initial assessments is 1.04 ± 0.06; for the ASTM Cycle 2209 material the ratio is 1.07 ± 0.07. This average increase suggests that the materials have not degraded over time but that different calibration solutions from different sources are not necessarily equivalent. When stored in the dark at –20 °C, RM 8210 hemp materials in previously opened packets appear to be stable for at least 5 months.

3.3. Fifteen Month Measurements

Cannabinoid concentrations in RM 8210 were evaluated fifteen months after completion of the initial assessment. The measurement materials and processes used were nearly identical to those described in Section 3.2 except for: 1) thirteen sample packets were analyzed instead of six sample packets, 2) two packets of ASTM Cycle 2205 Hemp were used as a control material rather than six packets of ASTM Cycle 2209 Hemp, and 3) five calibration solutions were used instead of four. The final mass concentrations in the calibrants were approximately 1.8 mg/L, 3.7 mg/L, 6.5 mg/L, 9.8 mg/L, and 13.3 mg/L for each of the cannabinoids.

3.3.1. Mass Fractions

All samples were identified, analyzed, and quantified as described in Section 3.1. The seven cannabinoids present at mass concentrations sufficiently high to be quantified with the prepared calibrants are identified in Fig. 13. The chromatographic separations are very similar to those at five months. Section 3.1.8 details conversion of mass concentration to mass fraction and Table 11 lists the cannabinoid mass fractions determined for each of the thirteen packets of RM 8210 fifteen months after the initial analysis.

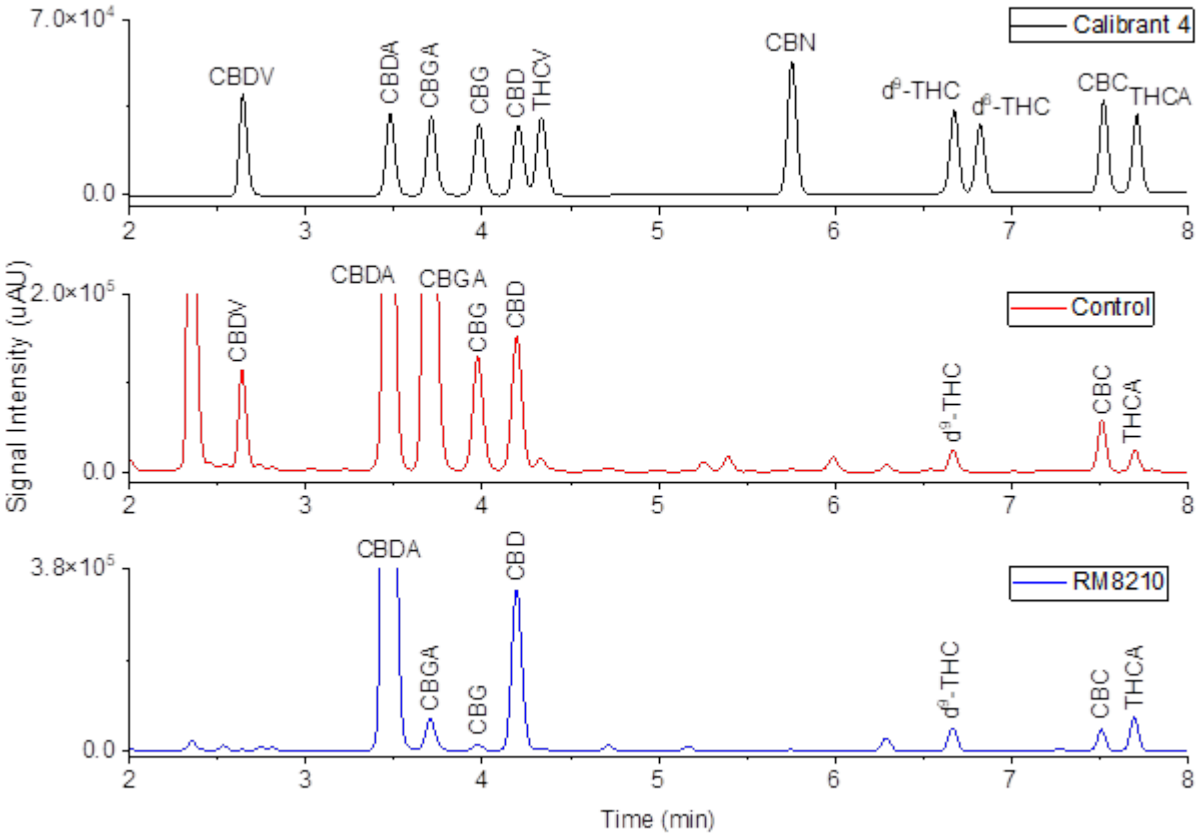


Fig. 13. LC-UV Chromatograms of Calibrant and Undiluted Control and RM 8210 Samples.

Table 11. Cannabinoid Mass Fractions Determined at Fifteen Months, %

Box	CBGA	CBG	CBC	Δ⁹-THC	THCA	Total THC	CBD	CBDA	Total CBD
1	0.1561	0.0348	0.0936	0.1127	0.1530	0.2472	0.982	7.47	7.52
2	0.1419	0.0351	0.0890	0.1079	0.1413	0.2287	0.926	6.96	6.99
3	0.1408	0.0350	0.0900	0.1066	0.1407	0.2278	0.926	6.97	7.00
4	0.1545	0.0343	0.0923	0.1120	0.1521	0.2472	0.994	7.46	7.49
5	0.1415	0.0347	0.0889	0.1078	0.1433	0.2310	0.916	7.05	7.09
6	0.1613	0.0336	0.0905	0.1096	0.1499	0.2431	0.971	6.67	6.71
7	0.1502	0.0342	0.0931	0.1112	0.1477	0.2405	0.969	7.30	7.34
8	0.1482	0.0358	0.0902	0.1081	0.1460	0.2364	0.950	7.10	7.15
9	0.1519	0.0347	0.0914	0.1100	0.1484	0.2397	0.964	7.27	7.31
10	0.1381	0.0345	0.0905	0.1082	0.1355	0.2201	0.885	7.09	7.09
11	0.1414	0.0353	0.0895	0.1077	0.1444	0.2323	0.921	7.02	7.06
12	0.1416	0.0324	0.0890	0.1049	0.1366	0.2229	0.905	6.80	6.83
13	0.1392	0.0346	0.0862	0.1032	0.1354	0.2207	0.893	6.78	6.81
Mean:	0.1467	0.0345	0.0903	0.1085	0.1442	0.2337	0.939	7.07	7.11
Standard Deviation:	0.0075	0.0008	0.0020	0.0027	0.0060	0.0095	0.035	0.25	0.25

The mean mass fractions in RM 8210 and the ASTM Cycle 2205 control materials are plotted as function of their initial assessments in Fig. 14. The values for RM 8210 are in good visual agreement. Given that only two samples of the control material were evaluated, the values are in adequate agreement. The mass fraction values determined in the initial, 5 month, and 15 month assessments are displayed in Fig. 15. While the CBG and CBDA values have slightly increased, there is no evidence of the time-related cannabinoid decreases expected had degradation had occurred.

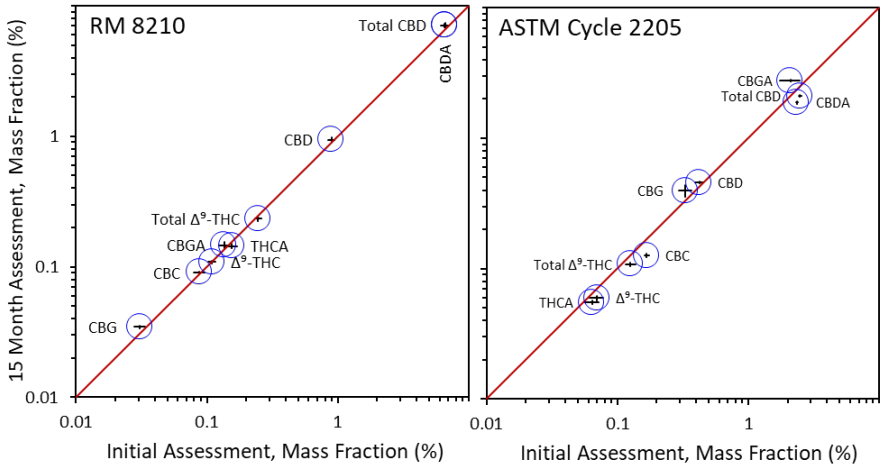


Fig. 14. Fifteen Month Measurements of RM 8210 and Control Materials.

The panel to the left displays the fifteen-month assessment of cannabinoid mass fractions of RM 8210 as functions of the assessments presented in Section 3.1.11. The panel to the right displays the mass fraction assessments of the ASTM Cycle 2205 control material as functions of target values assigned approximately fifteen months earlier. Horizontal error bars span the estimated 95 % level of confidence expanded uncertainties of the initial assessments of the two materials. Vertical error bars span approximate 95 % level of confidence expanded uncertainties of the fifteen month assessments. The diagonal lines represent equality of the values. Open circles are for graphical emphasis.

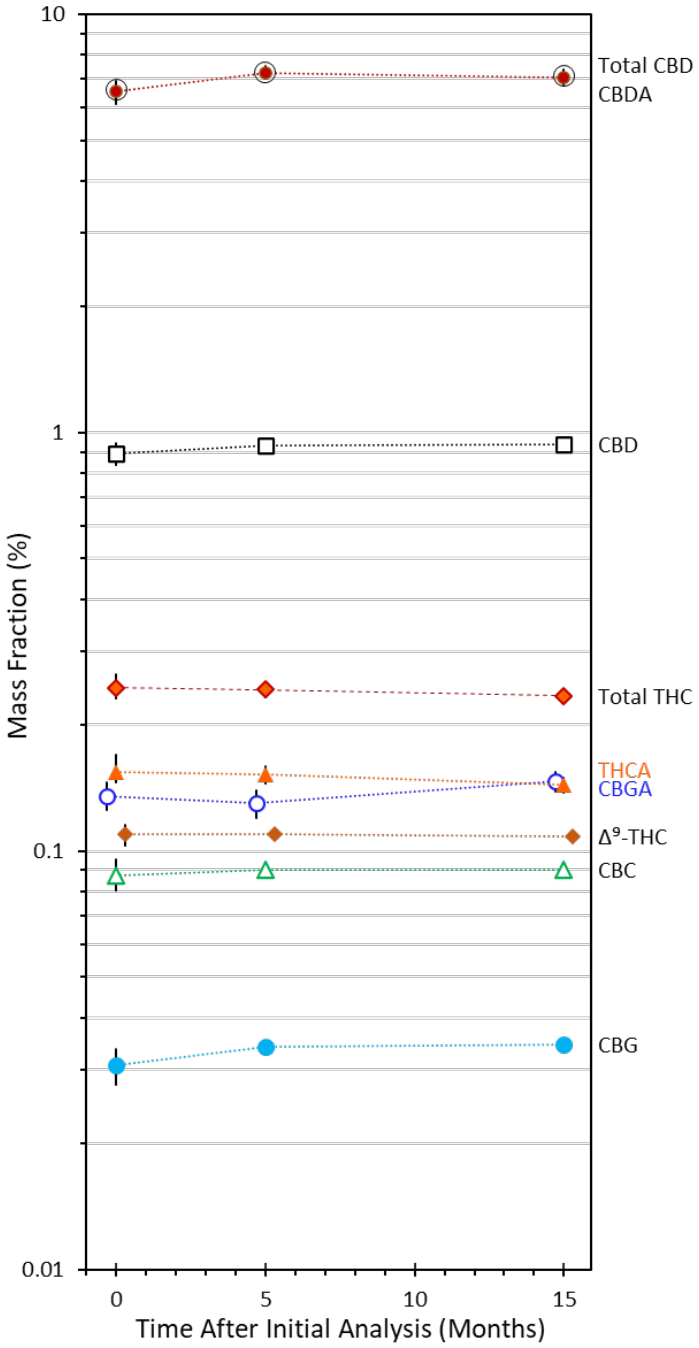


Fig. 15. Cannabinoid Mass Fraction Assessments over Time.

Symbols represent mean mass fraction results on an as-received basis as initially determined, evaluated at five months, and again at fifteen months. Error bars represent approximate 95 % confidence uncertainty intervals. Dotted lines connect the time-points for graphical emphasis. Cannabinoids are identified along the right edge of the panel.

3.4. Consensus Values

The measurements performed represent independent realizations of cannabinoid mass fractions as determined using the analytical method described in Wilson WB and Abdur-Rahman M, 2022 [7]. The results are derived from different calibration solutions, used different subsamples of mostly different RM 8210 packets, and were made by different analysts at well-separated times. Combining the results from the three studies therefore should provide more representative estimates of cannabinoid mass fractions. Table 12 lists the value and uncertainties for the three studies as estimated using the Bayesian analyses described in Section 3.1.11 and **Error! Reference source not found.**. Table 12 also provides consensus values obtained using the Linear Pool procedure of the NIST Consensus Builder [14,15]. This method was chosen because it does not use the stated uncertainties of the individual location estimates to derive the consensus.

Table 12. Summary and Consensus Values and Uncertainties for As-Received Cannabinoid Mass Fractions, %.

	Initial [a]		5 Month [b]		15 Month [c]		Consensus [d]	
Cannabinoid	$\hat{\mu}$ [e]	$u(\hat{\mu})$ [f]	$\hat{\mu}$ [e]	$u(\hat{\mu})$ [f]	$\hat{\mu}$ [e]	$u(\hat{\mu})$ [f]	$\hat{\mu}$ [e]	$u(\hat{\mu})$ [f]
CBGA	0.1354	0.0053	0.1315	0.0082	0.1467	0.0026	0.1378	0.0080
CBG	0.0307	0.0016	0.0340	0.0009	0.0345	0.0003	0.0331	0.0017
CBC	0.0875	0.0036	0.0904	0.0021	0.0903	0.0007	0.0894	0.0030
Δ^9-THC	0.1095	0.0043	0.1097	0.0024	0.1084	0.0009	0.1092	0.0032
THCA	0.1550	0.0061	0.1523	0.0031	0.1442	0.0021	0.1505	0.0057
Total THC	0.2454	0.0069	0.2434	0.0052	0.2337	0.0032	0.2408	0.0068
CBD	0.894	0.032	0.932	0.025	0.939	0.012	0.921	0.030
CBDA	6.54	0.22	7.223	0.107	7.073	0.072	6.95	0.29
Total CBD	6.63	0.20	7.270	0.103	7.105	0.072	7.00	0.27

a Location value and its standard uncertainty from Table 8.
b Location value and its standard uncertainty Bayesian analysis (Section **Error! Reference source not found.**) of results in Table 10.
c Location value and its standard uncertainty Bayesian analysis (Section **Error! Reference source not found.**) of results in Table 11.
d Results of Linear Pool consensus evaluation [14,15].
e Location estimate.
f Standard uncertainty associated with the location estimate.

4. Toxic Elements

As detailed in Section **Error! Reference source not found.**, some of the bulk dried ground hemp plant material was packaged for use in CannaQAP Exercise 2, and NIST target value measurements were done for toxic elements [5]. The CannaQAP Exercise 2 toxic element NIST target value measurements did not reveal heterogeneity concerns. Additionally, examining RM 8210 homogeneity was factored into toxic element value assignment measurements, which are detailed in the following sections.

4.1. Manganese (Mn)

RM 8210 was analyzed for manganese (Mn) content using inductively coupled plasma optical emission spectrometry (ICP-OES).

4.1.1. Materials

Ten packets of RM 8210 (packets 7, 233, 439, 655, 871, 1087, 1303, 1519, 1735, and 2155) were analyzed to assign an as-received Mn mass fraction. Two bottles of SRM 1573a Tomato Leaves [23] and two bottles of SRM 1575a Trace Elements in Pine Needles (*Pinus taeda*) [24] were analyzed as control materials. SRM 3132 Manganese (Mn) Standard Solution [25] was used to prepare calibration solutions. SRM 3102a Antimony (Sb) Standard Solution [26] and SRM 3144 Rhodium (Rh) Standard Solution [27] were used as internal standards (IS). OPTIMA grade nitric acid (HNO_3) and OPTIMA grade hydrofluoric acid (HF) were purchased from Fisher Scientific (Suwanee, GA).

4.1.2. Sample Preparation

A Mettler AT261 Delta Range analytical balance was used for weighing in the preparation of samples and standards. Duplicate 0.50 g aliquots were taken from each packet of RM 8210, each bottle of SRM 1573a and SRM 1575a and placed in Teflon microwave vessels. The packets and bottles were designated A or B to distinguish between the two aliquots taken from each packet or bottle. Aliquots labeled “A” were run on the first day of measurement and those labeled “B” run on the second day.

Nine procedural reagent blanks were a prepared along with the samples. To each vessel, 8 mL of HNO_3 and 2 mL of HF were added, along with 0.5 mL of a 1000 ng/g solution of Sb and 0.7 mL of a 100 ng/g solution of Rh as internal standards. Samples were digested using CEM MARSXpress vessels in a CEM Mars 5 Microwave Digestion system using the method in

Table 13. After microwave digestion, solutions were transferred to polyethylene bottles and diluted to 60 g using 18 MΩ cm water. All samples were analyzed in as-received condition. The results for SRM 1573a and SRM 1575a were corrected for moisture content to enable comparison with the certified values.

Table 13. Microwave Method for Sample Digestion.

Step	Power, W	Power Setting, %	Ramp Time, min	T, °C	Hold Time (min)
1	800	100	15:00	195	20:00
2	1600	85	20:00	210	15:00

4.1.3. ICP-OES Analysis and Quantification

A Perkin-Elmer Optima 5300 Dual View inductively coupled plasma optical emission spectrometer (ICP-OES) was used for the analysis. Manganese was measured in two one-day runs at a wavelength of 260.568 nm, axial plasma view, integration time of 0.5 s, and a read time of 1.0 s. Quantification was achieved using the linear calibration model

$$Y = a + (b \times w_{\text{calibrant}}) \tag{7}$$

where: Y is the instrument response,

a is the value of Y when $w_{\text{calibrant}}$ = 0 µg/g,

b is the sensitivity (slope), and

$w_{\text{calibrant}}$ is the mass fraction of analyte in calibration solutions (µg/g).

The parameterized model is then used to obtain the mass of the analyte, w_{sample}, from a sample which produces an observed response, Y:

$$w_{\text{sample}} = (Y - a)/b\ . \tag{8}$$

The a and b are assigned by equal-weighted least squares regression on a set of n pairs of values (R_i, $w_{cal,i}$). Results are corrected for the mean blank values from their corresponding runs by subtracting the mean total mass of a given analyte found in the blanks from the mass found of that analyte in each individual sample before calculating the as-received mass fraction (µg/g).

Four calibration solutions were prepared from SRM 3132 for each one-day run. Four instrumental measurements were averaged for each sample aliquot. After exporting raw data to a spreadsheet wherein final mass fractions were calculated using the calibration curve determined for each day.

4.1.4. Results for Mn

The measurement results for the SRM 1573a and 1575a controls completely overlay the values listed in their respective COAs, indicating that the measurement process was in adequate control and calibration. The results for RM 8210 are listed in

Table 14 along with standard (mean, standard deviation) and robust (median, Q_n) location and dispersion summary estimates. The Q_n is an efficient high-breakdown estimator of standard deviation [28, 29]. The results are displayed as a function of packet number in Fig. 16.

Table 14. Mn Mass Fraction Values, µg/g, and Summary Statistics.

	Mass Fraction, µg/g	
Packet	A	B
7	137.4	131.5
233	133.6	130.0
439	130.1	128.1
655	143.6	129.5
871	136.2	137.6
1087	139.8	122.5
1303	124.4	139.4
1519	130.9	131.5
1735	120.0	123.3
2155	122.8	124.0

Statistic	Value
Number of Results	20
Mean:	130.8
Standard Deviation:	6.7
Median:	130.5
Q_n:	7.5
$1.24 \times Q_n$	9.3

4.2. Arsenic (As), Cadmium (Cd), and Lead (Pb)

RM 8210 was analyzed for arsenic (As), cadmium (Cd), and lead (Pb) using inductively coupled plasma mass spectrometry (ICP-MS).

4.2.1. Materials

The RM 8210 samples described in Section 4.1 that were digested with internal standards were analyzed for As, Cd, and Pb to assign as-received mass fractions. The SRM 1573a and 1575a samples described in Section 4.1 were analyzed as control materials. SRM 3103a Arsenic (As) Standard Solution [30], SRM 3108 Cadmium (Cd) Standard Solution [31], and SRM 3128 Lead (Pb) Standard Solution [32] were sources for the spikes.

4.2.2. ICP-MS Analysis and Quantification

An Agilent 7500cs ICP-MS equipped with a Peltier-cooled, inert sample introduction system was used. The analytes in the solutions were measured according to the parameters in Table 15 using H_2 as a collision gas to minimize polyatomic interferences.

Table 15. ICP-MS Parameter Values.

Element	Mass, amu	Integration Time, s	Read Time, s
As	75	0.1	3
Cd	114	0.1	3
Pb	207	0.1	3
Sb	123	0.1	3

Analyte mass fractions were quantified by the method of standard additions. Samples were diluted so that analytes were present at appropriate mass fractions. From each dilution, two aliquots were taken, with a spike added to one. Ten instrumental measurements were taken and averaged for each sample aliquot and each spiked aliquot. The raw data were exported to a spreadsheet wherein final mass fractions were calculated. The method of standard additions refers to the calibration of an analytical instrument by measuring the increase in the analytical signal that occurs when a known amount of the analyte is added to the sample. It avoids

multiplicative types of matrix interferences (enhancements or suppressions) since the calibrant is present with the same matrix as the sample.

The mass fraction of the analyte in the sample, w_{sample}, is calculated as:

$$w_{\text{sample}} = R_{\text{u}} \left(\frac{\left({}^{m_{\text{sp}} w_{\text{sp}}}/_{m_{\text{spsolu}}} \right)}{R_{\text{sp}} - R_{\text{u}}} \right) \left(\frac{m_{\text{solu}}}{m_{\text{sample}}} \right) \quad (9)$$

where: w_{sp} mass fraction of the analyte in the spiking solution,
m_{sample} mass of sample that is present in the solution to be analyzed,
m_{solu} total mass of the sample solution after addition of the IS spike,
m_{sp} mass of the analyte spiking solution delivered to the solution,
m_{spsolu} mass of the solution that will be spiked,
R_{sp} analyte/IS signal ratios for the spiked solution,
R_{u} analyte/IS signal ratios for the unspiked solution.

All samples were analyzed in as-received condition. The results for SRM 1573a and SRM 1575a were corrected for moisture content to enable comparison with the certified values.

4.2.3. Results for As, Cd, and Pb

The As, Cd, and Pb measurement results for the SRM 1573a and 1575a controls overlap the values listed in their respective COAs, indicating that the measurement process was in adequate control and calibration. The As, Cd, and Pb results for RM 8210 are listed in

Table 16 along with standard (mean, standard deviation) and robust (median, Q_n) location and dispersion summary estimates. The Q_n is an efficient high-breakdown estimator of standard deviation [28,29]. The results are displayed as a function of packet number in Fig. 16.

Table 16. As, Cd, and Pb Mass Fraction Values, ng/g, and Summary Statistics.

		Mass Fraction, ng/g		
Packet	Aliquot	As	Cd	Pb
7	A	40.03	81.60	205.6
	B	38.94	82.79	193.9
233	A	48.83	80.95	249.7
	B	45.33	75.48	223.6
439	A	42.20	79.60	208.8
	B	32.57	77.73	199.4
655	A	43.44	83.54	185.8
	B	39.07	74.84	218.0
871	A	29.37	86.88	172.2
	B	36.19	75.80	185.7
1087	A	43.13	91.23	203.0
	B	42.69	73.40	181.2
1303	A	43.28	79.15	177.0
	B	42.40	80.33	176.4
1519	A	38.09	77.30	317.3
	B	41.17	86.35	292.4
1735	A	39.14	73.51	189.0
	B	35.19	77.38	200.5
2155	A	37.57	77.47	198.2
	B	44.14	76.17	207.2
	n:	20	20	20
	Mean:	40.14	79.58	209.2
	Standard Deviation:	4.55	4.73	37.6
	Median:	40.60	78.44	200.0
	Q_n:	4.44	4.59	23.3
	1.24 × Q_n:	5.51	5.70	28.9

4.3. Mercury (Hg)

RM 8210 was analyzed for mercury (Hg) content using direct combustion atomic absorption spectrometry.

4.3.1. Materials

Ten packets of RM 8210 (packets 77, 293, 509, 725, 941, 1157, 1373, 1589, 1805, and 2091) were analyzed to assign an as-received Hg mass fraction. One bottle of SRM 1547 Peach Leaves [33] was analyzed as a control material and results for were corrected for moisture content to enable comparison with the certified values. SRM 3133 Mercury Standard Solution [34] was used to make calibration solutions.

4.3.2. Analysis

The mass fraction of total Hg was determined with a direct Hg analyzer DMA-80 (Milestone Scientific, Shelton, CT) by external calibration. The external calibration curves were prepared by gravimetrically aliquoting different masses (between 0.0211 g and 1.0257 g) of aqueous dilutions of SRM 3133 into quartz sample boats following an established protocol.

Mercury was measured in RM 8210 and control samples by weighing approximately 120 mg of material into pre-cleaned nickel weigh boats and placing them into the instrument auto-sampler rotor. Duplicate samples were measured from each packet of candidate RM 8210. Control material samples and procedural blanks (six empty nickel weigh boats, each of nominal mass 1 g) were bracketed between blocks of RM 8210 samples to verify instrument calibration and monitor instrumental drift. Table 17 lists the method parameters used.

Table 17. Mercury Analyzer Method Parameters.

	Ramp			
Sample Type	From	To	Duration	Hold
Calibration Solutions	Ambient	200 °C	90 s	30 s
	200 °C	650 °C	90 s	180 s
Plant Materials	Ambient	200 °C	90 s	30 s
	200 °C	300 °C	60 s	60 s
	300 °C	450 °C	60 s	30 s
	450 °C	650 °C	60 s	240 s

4.3.3. Quantification

An external calibration curve (peak area versus Hg concentration) was established using calibration solutions derived from SRM 3133. The relationship between the measured Hg peak areas, *A*, and the mass of Hg deliver by the calibrants, $m_{\text{calibrant}}$, was determined using a second-order polynomial calibration model

$$A = a \times m_{\text{calibrant}}^2 + b \times m_{\text{calibrant}} + c \quad (10)$$

This non-linear model was used to account for an asymptotic or slight rollover effect due to non-ideal Beer-Lambert Law behavior.

Values for the calibration model were estimated using classical least squares regression. The coefficients, measured peak areas reported by the Hg analyzer, and measured sample masses were used to calculate the mass fraction of Hg in the samples, w_{sample}:

$$w_{\text{sample}} = \left(\frac{-b \pm \sqrt{b^2 - 4a(c-A)}}{2\text{a}}\right) / m_{\text{sample}} - w_{\text{blank}} \quad (11)$$

where: *a, b, c* coefficients of the second-order polynomial,
m_{sample} mass of the sample (g), and
w_{blank} mean of the procedural blank corrections (ng/g).

The w_{blank} values were estimated in the same manner, replacing m_{sample} with m_{blank} and ignoring the blank subtraction. The measured w_{blank} values ranged from (0.0045 to 0.1135) ng/g for an average of 0.05 ng/g.

4.3.4. Results for Hg

The measurement result for the SRM 1547 control was completely contained within the certified uncertainty interval, indicating that the measurement process was in adequate control and calibration. The results for RM 8210 are listed in Table 18, along with standard (mean, standard deviation) and robust (median, Q_n) location and dispersion summary estimates. The Q_n is an efficient high-breakdown estimator of standard deviation [28,29]. The results are displayed as a function of packet number in Fig. 16.

Table 18. Hg Mass Fraction Values, ng/g, and Summary Statistics.

	Mass Fraction, ng/g	
Packet	A	B
77	7.87	7.67
293	7.13	6.15
509	7.73	6.59
725	7.44	6.71
941	6.58	7.44
1157	6.14	6.43
1373	6.34	6.51
1589	6.88	7.83
1805	7.04	7.53
2091	7.34	7.35

Statistic	Value
Number of Results	20
Mean:	7.04
Standard Deviation:	0.57
Median:	7.09
Q_n:	0.60
$1.24 \times Q_n$	0.74

4.4. Cobalt (Co), Molybdenum (Mo), Nickel (Ni), Selenium (Se), and Uranium (U)

RM 8210 was analyzed for cobalt (Co), molybdenum (Mo), nickel (Ni), selenium (Se), and uranium (U) using inductively coupled plasma mass spectrometry (ICP-MS).

4.4.1. Materials

Ten packets of RM 8210 (packets 139, 355, 571, 787, 1003, 1219, 1435, 1651, 1867, and 2082) were analyzed to assign as-received Co, Mo, Ni, Se, and U mass fractions. Two bottles of SRM 1573a Tomato Leaves [23] and two bottles of SRM 1575a Trace Elements in Pine Needles (*Pinus taeda*) [24] were analyzed as control materials. SRMs 3113 Cobalt Standard Solution [35], 3134 Molybdenum Standard Solution [36], 3136 Nickel Standard Solution [37], and 3149 Selenium Standard Solution [38] were sources for spike and calibration solutions. A commercial CRM from High Purity Standards (North Charleston, SC USA), Uranium at 1,000 µg/mL in 2% HNO_3 from a Natural Uranium Source, was used in the spike. SRM 3102a Antimony (Sb) Standard Solution [26] and SRM 3144 Rhodium (Rh) Standard Solution [27] were used as internal standards (IS). OPTIMA grade nitric acid (HNO_3) and OPTIMA grade hydrofluoric acid (HF) were purchased from Fisher Scientific (Suwanee, GA).

4.4.2. Sample Preparation

Duplicate 0.5 g aliquots from 10 individual packets of RM 8210 and five replicate 0.5 g aliquots each from SRM 1573a and SRM 1575a were weighed into Teflon microwave vessels using a calibrated Mettler AT261 Delta Range analytical balance. Ten procedural blanks were prepared along with the samples. For digestion, 8 mL HNO_3 and 2 mL HF were added to each vessel, along with 0.5 mL of a 1000 ng/g solution of Sb and 0.7 mL of 100 ng/g solution of Rh as internal standards.

4.4.3. Sample Digestion

All samples and procedural blanks were digested according to the microwave method in

Table 13 (Section 4.1.2) using a CEM Mars 5 microwave system with CEM MARSXpress digestion vessels (CEM, Matthews, NC). Following microwave digestions, sample vessels were cooled to room temperature, slowly vented, and digests diluted to 60 g in pre-weighed polyethylene bottles using 18 MΩ cm water.

4.4.4. ICP-MS Analysis and Quantification

An Agilent ICP-MS 7500cs ICP-MS, equipped with a Peltier-cooled, inert sample introduction system, was used for analysis of all samples. The analytes in digested sample solutions were measured according to the parameters displayed in Table 19 using collision cell mode to minimize polyatomic interferences. He was used as the collision gas for Co, whereas H_2 gas was used as the collision gas for Mo, Ni, Se, and U. Sb was used as the IS for the determination of Co, while Rh was employed as IS for the determination of Mo, Se, and U.

Table 19. ICP-MS Parameter Values for Co, Mo, Ni, Se, and U.

Element	Mass, amu	Integration Time, s	Read Time, s	# Runs	Gas
Co	59	0.1	3	2	He
Mo	95	0.1	3	2	H_2
Ni	60	0.1	3	2	H_2
Se	78	0.1	3	2	H_2
U	238	0.1	3	2	H_2
Rh	103	0.1	3	2	
Sb	121	0.1	3	2	
Sb	123	0.1	3	2	

For each sample dilution, two aliquots were taken, one of which had a spike added to it and the other was left unspiked. Ten instrumental replicates were measured and averaged for each unspiked sample aliquot and each spiked aliquot. After exporting raw data into a spreadsheet, final mass fractions were calculated for Co, Mo, Se, and U using the using the one-point standard addition method described in Section 4.2.2.

For Ni, four-point external linear calibration curves were constructed for each day of analysis as described in Section 4.1.3. Nickel mass fractions were then computed using the following:

$$w_{\text{sample}} = \frac{\frac{\frac{I_{\text{unspk}}-a}{b}}{\left(\frac{I_{\text{spk}}-a}{b}\right)-\left(\frac{I_{\text{unspk}}-a}{b}\right)} \times w_{\text{sp}} \times f_{\text{dil}} - w_{\text{blank}}}{m_{\text{sample}}} \qquad (12)$$

where: I_{unsp} measured Ni^{60} intensity of the unspiked sample
I_{sp} measured Ni^{60} intensity of the spiked sample
a intercept of the calibration curve
b slope of the calibration curve
w_{sp} mass-fraction concentration of the Ni spike solution added to the spiked sample,
f_{dil} dilution factor for the sample
w_{blank} mean mass fraction of the procedural blanks
m_{sample} mass of the sample.

All samples were analyzed in as-received condition. The results for SRMs 1573a and 1575a were corrected for moisture content to enable comparison with the certified values.

4.4.5. Results for Co, Mo, Ni, Se, and U

The measured Co value for SRMs 1573a and 1575a overlapped with the certified values. The Mo and U values overlapped with the SRM 1573a non-certified values; SRM 1575 does not deliver values for Mo or U. Although the Ni and Se values did not overlap well with the SRM 1573a certified values, they were in excellent agreement with the SRM 1575a certified values. The measurement processes were in adequate control and calibration.

The Co, Mo, Ni, Se, and U results for RM 8210 are listed in

Table 20, along with standard (mean, standard deviation) and robust (median, Q_n) location and dispersion summary estimates. The Q_n is an efficient high-breakdown estimator of standard deviation [28,29]. The results are displayed as a function of packet number in Fig. 16.

Table 20. Co, Mo, Ni, Se, and U Mass Fraction Values, ng/g, and Summary Statistics.

		Mass Fraction, ng/g				
Packet	Aliquot	Co	Mo	Ni	Se	U
94	A	199.0	304.8	3130	75.17	4.82
	B	212.3	305.6	3650	87.57	4.42
310	A	171.0	302.0	3364	73.73	3.97
	B	199.0	296.2	3678	79.11	4.98
526	A	189.5	296.0	3925	85.38	4.63
	B	188.4	318.4	3724	75.11	4.40
742	A	194.6	314.3	3801	78.57	4.33
	B	196.8	305.5	3724	83.30	4.75
958	A	185.2	284.3	5388	86.83	4.19
	B	186.6	301.4	3861	66.54	3.93
1174	A	185.3	299.1	3704	76.69	3.72
	B	172.0	303.9	4090	77.98	4.48
1390	A	179.8	286.2	3838	72.08	4.19
	B	175.5	309.0	3949	75.80	3.87
1606	A	181.4	280.3	4226	79.20	3.84
	B	194.8	292.4	3739	76.77	4.21
1822	A	170.0	292.2	3629	68.29	3.62
	B	325.8	282.3	4587	68.47	5.04
2101	A	176.6	310.7	3862	75.13	3.68
	B	183.8	308.7	3479	82.73	3.66
	n:	20	20	20	20	20
	Mean:	193.4	299.7	3867	77.22	4.24
	Standard Deviation:	33.0	10.8	470	5.93	0.45
	Median:	186.0	301.7	3770	76.73	4.20
	Q_n:	13.6	11.2	280	6.35	0.49
	1.24 × Q_n:	16.9	13.9	347	7.87	0.60

4.5. Beryllium, Chromium, and Vanadium

RM 8210 was analyzed for beryllium (Be), chromium (Cr), and vanadium (V) content using inductively coupled plasma - tandem mass spectrometry (ICP-MS/MS). An analytical quantification and validation scheme using the method of single-point standard additions was employed for each analyte. Single-point standard additions methods mitigate matrix effects by splitting a single sample and spiking one of the sample splits, to maintain matrix matching.

4.5.1. Materials

Ten packets of RM 8210 (packets 94, 310, 526, 742, 958, 1174, 1390, 1606, 1822, and 2101) were analyzed to assign as-received Be, Cr, and V mass fractions. One bottle each of SRMs 1547 Peach Leaves [33] and 1573a Tomato Leaves [23] were analyzed as control materials. SRM 3105a Beryllium Standard Solution [39], SRM 3112a Chromium Standard Solution [40], and SRM 3165 Vanadium Standard Solution [41] was used to make spike solutions. SRM 3148a Scandium Standard Solution [42] and SRM 3167a Yttrium Standard Solution [43] were used as internal standards. OPTIMA grade nitric acid (HNO_3) and OPTIMA grade hydrochloric acid (HCl) were purchased from Fisher Scientific (Suwanee, GA).

4.5.2. Sample Preparation

All RM 8210 material, control material, and procedural blank preparations were weighed by difference using a four-place balance that had been internally calibrated and checked using external weights prior to use. A mixed internal standard (IS) stock solution was made with Sc (1.1467 mg/kg) and Y (0.9274 mg/kg). Samples (approximately 0.5 g) along with approximately 0.25 g IS stock solution were digested in acid-cleaned quartz microwave vessels with 5 mL HNO_3 and 1 mL HCl. Only one aliquot from each packet was prepared and analyzed. Microwave digestions were carried out in an Anton Paar (Ashland, VA) Multiwave 5000 microwave using the method in Table 21.

Table 21. Microwave Method for Sample Digestion.

Step	Power, W	Ramp Time, min	Hold Time (min)
1	600	10:00	15:00
2	1400	10:00	20:00
3	0	0:00	30:00

After microwave digestion and cooling, the digests were quantitatively and gravimetrically transferred. Samples were transferred to 50 mL acid-cleaned polypropylene centrifuge tubes, diluted to approximately 50 g using high-purity deionized water (18 MΩ cm), and weighed. Half of each sample solution was then transferred into another acid-cleaned 50 mL polypropylene centrifuge tube and weighed; spiked (approximately 0.1 g for procedural blanks and 0.25 g for control materials and RM 8210) with multi-element custom spike solutions and weighed; and each tube was diluted back to approximately 50 g with high-purity deionized water and weighed.

The custom multi-element spike solutions described in Table 22 were prepared from SRM 3100 series single-element standard solutions to spike samples at approximately 3 to 4 times that of the native mass fraction of the trace element in the unspiked sample.

Table 22. Nominal Mass Fractions of Custom Multi-Element Spikes, ng/g.

	Sample Type			
Element	SRM 1547	SRM 1573a	RM 8210	Blank
Beryllium	25.3	--	8.0	8.0
Chromium	--	6053	1004	1004
Vanadium	1210	2551	793	793

4.5.3. ICP-MS/MS Measurements with Single-Point Standard Additions

An Agilent 8800 triple quadrupole (QQQ-ICP-MS) ICP-MS/MS system (Agilent, Santa Clara, CA) was used for measuring the analytical samples and blanks. The instrument working conditions were optimized prior to data collection by running a performance test to optimize general plasma conditions followed by tuning with 1 µg/kg Be, V, and Cr and 2 µg/kg Sc and Y solutions. The signals were monitored in no gas mode and helium reaction gas mode for the isotopes of interest listed in Table 23.

Table 23. Tandem Chemical Transitions for Measured Isotope.

Element	Mode	Q1 (*m/z*)	Q2 (*m/z*)
Be	No Gas	9	9
	He Gas	9	9
Sc	No Gas	45	45
	He Gas	45	45
Cr	No Gas	50	50
	He Gas	52	52
V	No Gas	51	51
	He Gas	51	51
Y	No Gas	89	89
	He Gas	89	89

The metals of interest were run in multiple QQQ-ICP-MS gas modes as an internal quality check on the data generated for each mode of gas operation. Reported results for each metal were selected by which isotope and instrument gas mode optimized reducing interferences and offered the greatest sensitivity. The “No gas” mode was utilized for Be. Additional forward argon gas was added to move the Be more quickly through the plasma to help prevent loss of this light atomic mass element. Helium collision gas mode was utilized for measuring Cr and V.

Elements were measured in all samples (unspiked and spiked) by the QQQ-ICP-MS in pulse counting mode, which was suitable for the dynamic range of the mass fractions in the diluted samples. Scandium was chosen as the IS for Be, and Y was chosen as the IS for Cr and V calculations due to proximity in atomic mass and ionization potential. The mass fractions of trace metals in RM 8210 samples, control materials, and procedural blanks were calculated using Eq. 9.

Four procedural blanks were processed and measured concurrently with the samples. The mass fractions of the analytes in candidate RM 8210 and control material samples were procedural blank corrected by subtracting the mean of the procedural blank measurements.

4.5.4. Results for B, Cr, and V

While neither of the control materials delivered results for Be, the mass fraction results for Be in SRM 1547 agreed with values reported in previous studies. The mass fractions for V and Cr overlapped with certified values in SRM 1573a. The mass fraction result for V in SRM 1547 were in good agreement with the certified value. The measurement processes were in adequate control and calibration. The mean values of the procedural blanks for B, Cr, and V were 0.0 ng/g, 8.5 ng/g, and 5.4 ng/g, respectively.

The B, Cr, and V results for RM 8210 are listed in Table 24, along with standard (mean, standard deviation) and robust (median, Q_n) location and dispersion summary estimates. The Q_n is an efficient high-breakdown estimator of standard deviation [28,29]. The results are displayed as a function of packet number in Fig. 16.

Table 24. Be, Cr, and V Mass Fraction Values, ng/g, and Summary Statistics.

	Mass Fraction, ng/g		
Packet	Be	Cr	V
94	2.36	443.9	195.7
310	2.21	546.2	266.7
526	1.53	2397.4	202.3
742	1.57	532.7	225.4
958	2.65	526.1	226.9
1174	2.08	752.3	214.2
1390	1.35	505.2	249.2
1606	2.52	460.5	269.8
1822	1.33	519.4	202.3
2101	3.03	493.4	254.3
n:	10	10	10
Mean:	2.06	717.7	230.7
Standard Deviation:	0.59	596.2	27.6
Median:	2.15	522.7	226.2
Q_n:	0.71	63.2	33.2
$1.24 \times Q_n$:	0.88	78.4	41.2

4.6. Value Assignments

The mass fraction values for As, Be, Cd, Co, Cr, Hg, Mn, Mo, Ni, Pb, Se, U, and V are displayed in Fig. 16 as functions of packet number. These displays identify issues that complicate assigning representative mass fraction values:

- Two values are displayed per packet for metals characterized using two independent aliquots per packet. The within-packet differences for As, Cd, Co, Hg, Mn, Mo, Ni, Pb, Se, and U are of the same magnitude as the between-packet differences. Given that RM 8210 units contain about 1.5 g hemp and that the typical sample mass per aliquot was 0.5 g, value assignment must address within- and between packet heterogeneity to be fit for customer purpose.
- At least four metals (Co, Cr, Ni, and Pb) have at least one atypically high aliquot result. There are no known technical issues associated with these values and the values are too divergent to reflect normally distributed measurement variability. They likely represent either single 0.5 g samples that contain a highly concentrated “nugget” of the metal (Co, Cr, and Ni, plausibly from flakes of metal from tools used in harvesting or preparing the hemp materials) or a packet containing (for some unknown reason) consistently atypical material (Pb).

A variant of the Bayesian analysis approach described in Section 3.1.11 can be used to estimate credible consensus values and their standard and expanded uncertainties in the presence of within- and between-packet heterogeneity. However, this approach is not robust towards extremely atypical results, nor can it be used for the elements characterized using only one sample per packet (Be, Cr, and V).

Therefore, as-received mass fraction values for the toxic elements are assigned through a three-stage approach:

- Bayesian analysis of the two-aliquot results for As, Cd, Co, Hg, Mn, Mo, Ni, Pb, Se, and U.
- Establishing a relationship between the Bayesian results and the usual summary means and standard deviations for metals that do not have strongly atypical aliquots, as evaluated by differences between the usual and robust summary location and/or dispersion statistics.
- Establishing a relationship between the usual and robust estimates of dispersion.

4.6.1. Measurement Uncertainty Budgets

The measurement uncertainty budgets for As, Be, Cd, Co, Cr, Hg, Mn, Mo, Ni, Pb, Se, U, and V include some or all components related to gravimetric preparation of samples, calibrants and/or spikes, and blanks; uncertainty of the primary CRM standard(s) used to prepare calibrants and/or spikes; calibration model lack-of-fit; and sample and blank replicability. The contributions of these measurement process components may contribute to the observed within- and between-packet variability but are not explicitly addressed in the value assignment analyses.

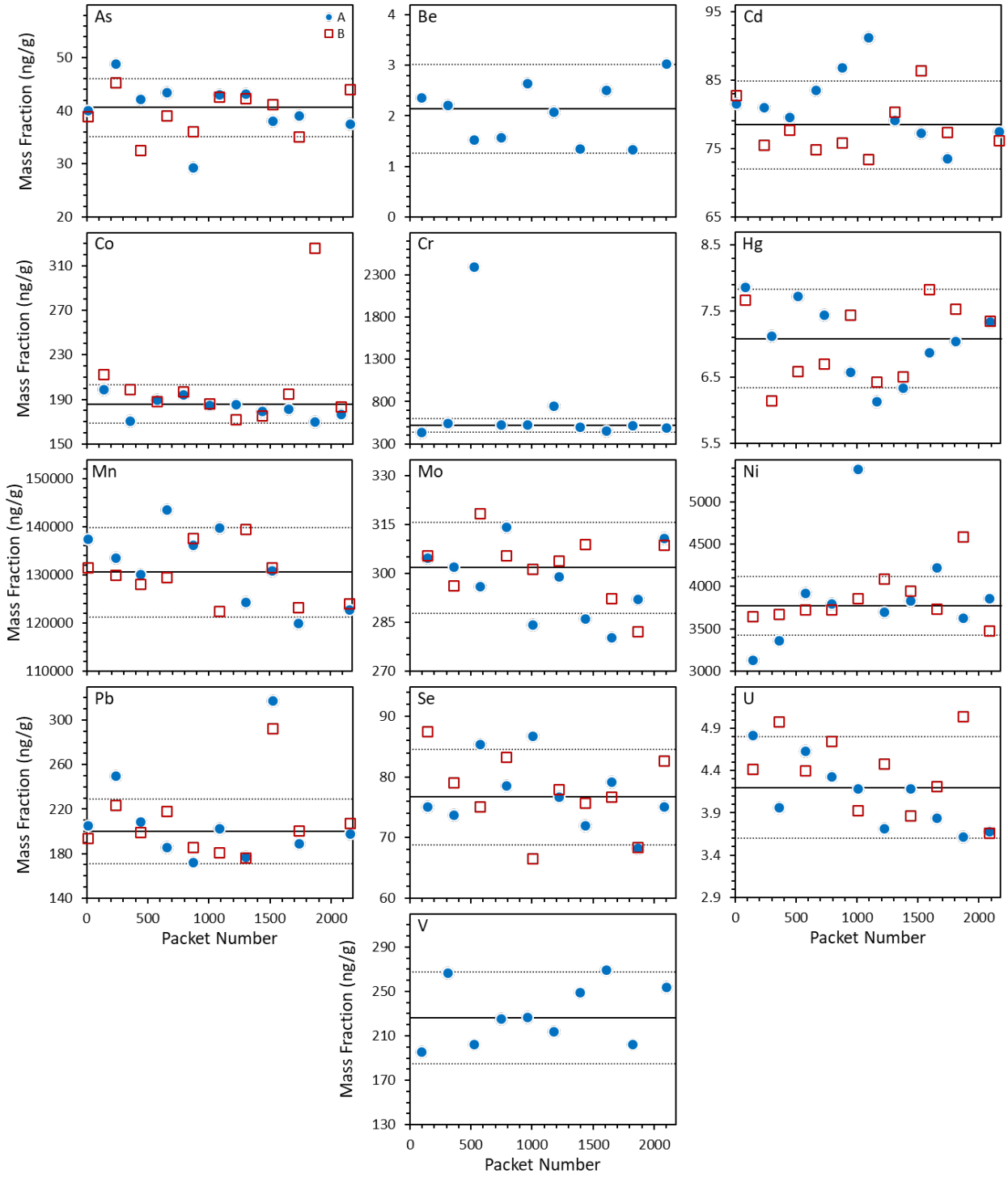


Fig. 16. Measurement Results for the Toxic Elements as Functions of Packet Number.

Each panel displays the measurement results for one element as a function of packet number. Solid blue circles represent the measurement results for the A aliquots; open red squares represent results for the B aliquots. Solid horizontal lines represent medians, horizontal dotted lines bound the intervals [median – 1.24 × Q_n, median + 1.24 × Q_n].

4.6.2. Bayesian Uncertainty Analysis

Unlike the cannabinoids, the differing analytical approaches used in the determination of the toxic elements and lack of within-aliquot replication limits the Bayesian analysis to combining mass fraction estimates as determined for each element. The single per-aliquot mass fractions are combined to obtain a consensus value using the Linear Pool model [15] with OpenBUGS [**Error! Bookmark not defined.**] software.

4.6.3. Bayesian Vs Sample Population Estimates

The ratios between the Bayesian Linear Pool location estimates, $\hat{\mu}$, and the measurement means, Mean, for both the cannabinoids and the toxic elements are displayed in Fig. 17. All of the ratios are between 0.98 and 1.01. The expected values for "typical" 0.5 g samples are well predicted by the sample means.

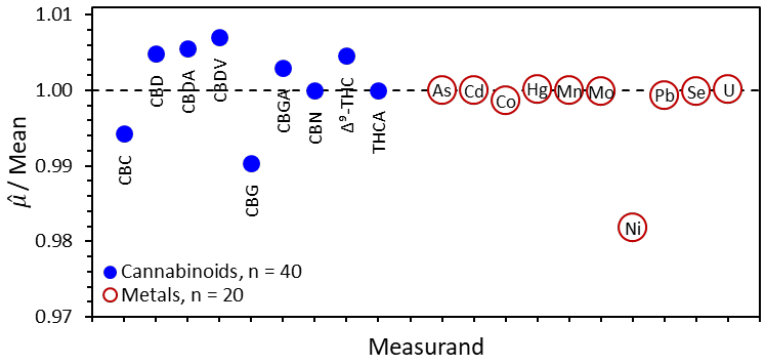


Fig. 17. Ratio of Bayesian Location Estimates and Sample Means.

Solid blue circles represent $\hat{\mu}$/Mean ratios for the cannabinoid measurands discussed in Section 3. Open red circles represent the $\hat{\mu}$/Mean ratios for the toxic elements. The dashed horizontal line represents the unit ratio.

The ratios between the Bayesian standard uncertainty of the Linear Pool location estimates, and the measurement standard deviations (SD(Sample)) for both the cannabinoids and the toxic elements are displayed in Fig. 18. None of the ratios for any of the measurands are less than 0.5. All of the ratios for elements free of extremely atypical aliquot results are greater than 0.85. The uncertainty in the linear pool estimates do not become smaller in proportion to the inverse of the square root of the number of independent measurements. The expected uncertainty in the location estimates of the elements for "typical" 0.5 g samples well if slightly over-estimated by the sample standard deviations. The near unit $u(\hat{\mu})$/SD(Sample) ratio for Pb, for which both aliquots from one packet are equally atypical, suggests that the Bayesian model used for the elements is most sensitive to within-packet differences.

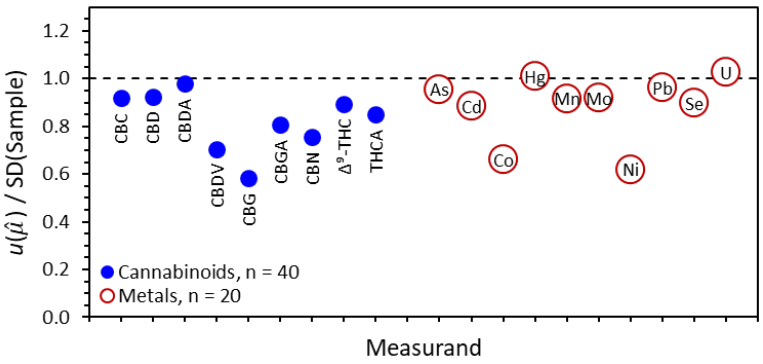


Fig. 18. Ratio of Bayesian Location Uncertainties and Sample Standard Deviations.

Solid blue circles represent $u(\hat{\mu})$/SD ratios for the cannabinoid measurands discussed in Section 3. Open red circles represent the $u(\hat{\mu})$/SD ratios for the toxic elements. The dashed horizontal line represents the unit ratio.

4.6.4. Standard Vs Robust Sample Population Estimates

The ratios between the robust median and the usual mean location estimates for both the cannabinoids and the toxic elements are displayed in Fig. 19. Except for Cr, for which one aliquot has an extremely atypical value, all of the ratios are between 0.95 and 1.05. The values expected for "typical" 0.5 g samples are well predicted by the robust medians.

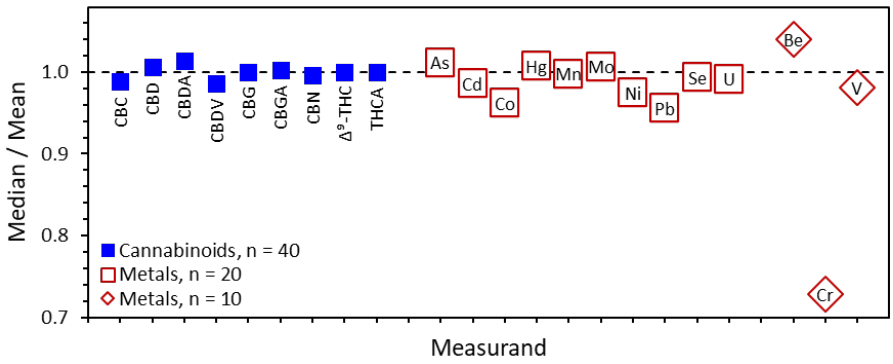


Fig. 19. Ratio of Robust and Standard Location Estimates: Median/Mean.

Solid blue squares represent median/mean ratios for the cannabinoid measurands discussed in Section 3. Open red squares represent the median/mean ratios for toxic elements characterized with two aliquots per packet. Open red diamonds represent the median/mean ratios for toxic elements characterized with just one aliquot per packet. The dashed horizontal line represents the unit ratio.

The ratios between the robust Q_n [28,29] estimate of standard deviation and the usual standard deviation estimates of dispersion for both the cannabinoids and the toxic elements are displayed in Fig. 20. Except for the elements with at least one extremely atypical measurement result, the ratios are between 0.95 and 1.20. While the ratios for fully "typical" sample populations on average more from unity as the number of measurements in the population decreases, the values expected for "typical" 0.5 g samples are well predicted by the robust medians.

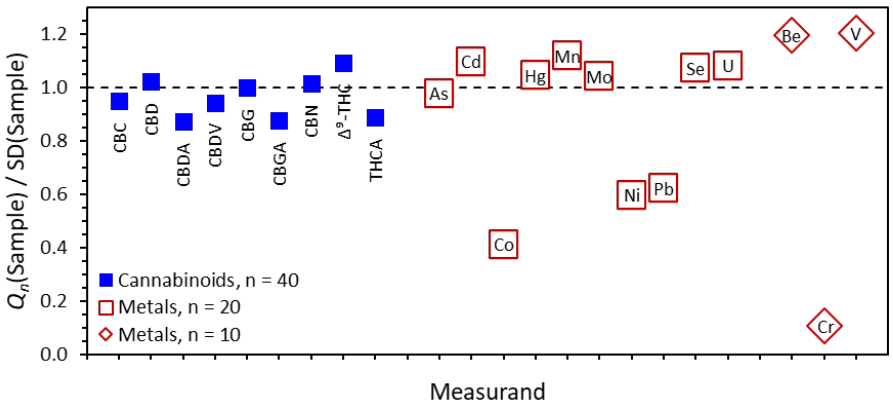


Fig. 20. Ratio of Robust Standard Dispersion Estimates: Q_n and Standard Deviation.

Solid blue squares represent Q_n/SD ratios for the cannabinoid measurands discussed in Section 3. Open red squares represent the Q_n/SD ratios for toxic elements characterized with two aliquots per packet. Open red diamonds represent the Q_n/SD ratios for toxic elements characterized with just one aliquot per packet. The dashed horizontal line represents the unit ratio.

The standard deviation and *Qn* estimates of dispersion as functions of the mean and median estimates of location are displayed in Fig. 21. The robust estimates for the four "atypical" elements (Co, Cr, Ni, and Pb) are more consistent with the (median, Q_n) relationship for the seven elements fully "typical" elements (As, Cd, Hg, Mn, Mo, Se, and U) than they with the (mean, standard deviation) relationship. Both (location, dispersion) relationships are equally descriptive for the two "typical" metals (Be and V) characterized with one aliquot per packet.

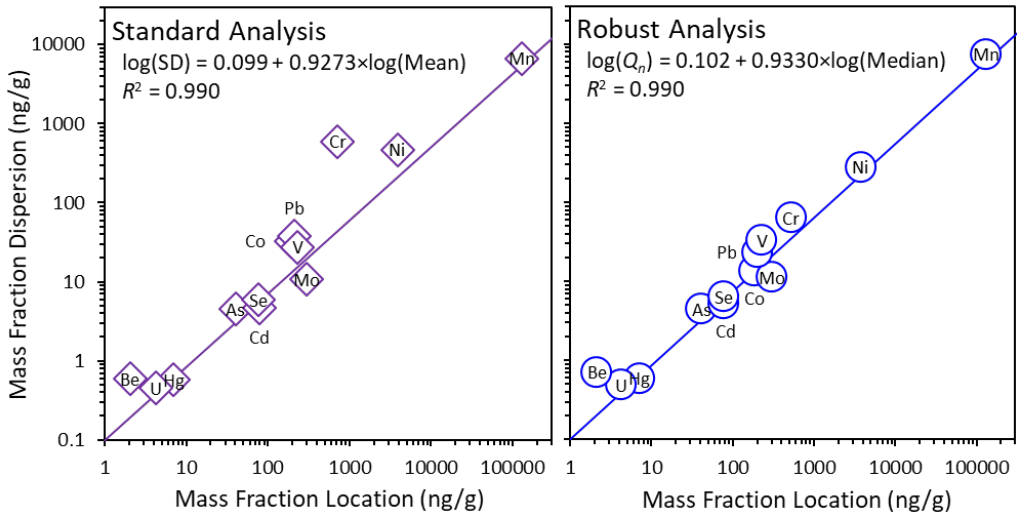


Fig. 21. Dispersion Estimates as Functions of Location Estimates.

The panel to the left presents standard deviations as a function of mean values; the panel to the right presents the robust Qn estimates of standard deviation as a function of the robust median values. The solid diagonal lines represent linear trend lines calculated using just (dispersion, location) estimates for As, Cd, Hg, Mn, Mo, Se, and U.

4.6.5. Summary Estimates for Toxic Element Measurements and Uncertainties

Given the relatively large number of atypical 0.5 g sample measurements, it is prudent to use robust estimates of as-received mass fraction for all the toxic elements described in the above Sections. The Q_n estimates the standard deviation of a set of values. The expected uncertainty for locations estimated as medians is a factor of approximately 1.25 times the uncertainty of locations estimated as means [44].

$$u(\text{Median}) = 1.25 \times Q_n \quad (13)$$

Approximate 95 % level of confidence expanded uncertainties can be estimated by multiplying estimated standard uncertainties by an appropriate expansion factor, k_{95}, based on the Student's t distribution $t_{(0.05,n-1)}$.

$$U_{95}(\text{Median}) = k_{95} \times u(\text{Median}) = t_{(0.05,n-1)} \times u(\text{Median}) \quad (14)$$

The resulting locations and uncertainties are listed in Table 25.

Table 25. Values and Uncertainties for As-Received Mass Fractions, ng/g.

			Mass Fraction, ng/g				
Element	n	K_{95}	Median	Qn	u(Median)	U_{95}(Median)	%CV [a]
As	20	2.09	40.6	4.4	5.6	12	14
Be	10	2.26	2.15	0.71	0.89	2.0	41
Cd	20	2.09	78.4	5.2	6.5	14	8
Co	20	2.09	186	14	17	36	9
Cr	10	2.26	523	63	79	180	15
Hg	20	2.09	7.09	0.60	0.75	1.6	11
Mn	20	2.09	130500	7500	9400	20000	7
Mo	20	2.09	302	11	14	29	5
Ni	20	2.09	3770	280	350	730	9
Pb	20	2.09	200	23	29	61	15
Se	20	2.09	76.7	6.3	7.9	17	10
U	20	2.09	4.20	0.49	0.61	1.3	14
V	10	2.26	226	33	42	94	18

a Coefficient of variation (aka relative standard deviation) expressed as a percentage: %CV = 100×u(Median)/Median.

4.7. CannaQAP Exercise 2

NIST launched a Cannabis Laboratory Quality Assurance Program (CannaQAP) in 2020 to improve the comparability of the analytical measurements of cannabis and cannabis-derived products in forensic and cannabis (hemp and marijuana) testing laboratories. CannaQAP is an interlaboratory study mechanism that is similar to a proficiency testing scheme; however, the focus is on education without assigning pass/fail grades to the anonymized participants. CannaQAP helps inform NIST about the current measurement capabilities of, and challenges faced by the analytical cannabis community. This in turn assists NIST in the design and characterization of cannabis reference materials (RMs).

CannaQAP Exercise 2 [5] focused on the determination of toxic elements in two hemp materials and a control material provided by NIST. As, Be, Cd, Co, Cr, Pb, Mn, Hg, Mo, Ni, Se, U, and V were the toxic elements chosen based on interest expressed by the cannabis community for safety and regulations. The samples distributed as "Plant Sample 4" in CannaQAP Exercise 2 contained the RM 8210 material but were packaged at an earlier time, in different quantity, and in different packaging. The results from Exercise 2 are therefore not necessarily representative of RM 8210 and are not eligible for use in value-assigning RM 8210 [45]. However, as can be seen in Fig. 22 the Exercise 2 consensus mean results of the participants agree quite well with the NIST RM 8210 values.

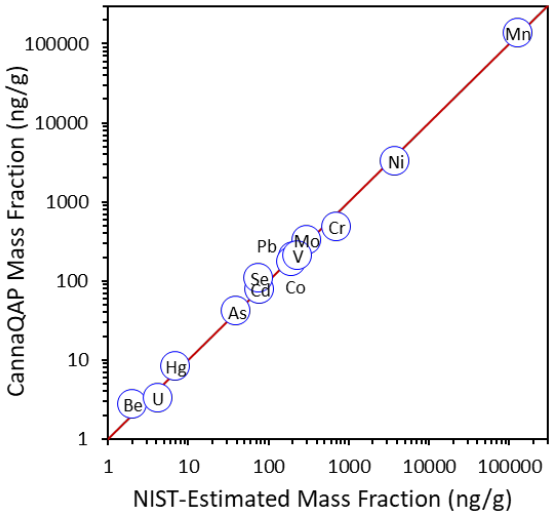


Fig. 22. Comparison of NIST and CannaQAP Exercise 2 Results.

Open circles represent the CannaQAP Exercise 2 consensus mean mass fractions of the participants as functions of the NIST location estimates reported in Table 25. Standard uncertainty error crosses are smaller than the circles. The diagonal line represents equality of the values.

5. Moisture

To avoid loss of volatile organic compounds from oven heating, moisture content was determined by drying the hemp material in a desiccator over magnesium perchlorate ($Mg(ClO_4)_2$) at ambient temperature. Two studies were conducted. The initial study evaluated packets before they had been placed in aluminized polyester bags with a desiccant packet and that were stored at -20 °C. The second study, conducted a year after the first, addressed potential differences in moisture content due to packaging and storage conditions.

5.1. Study 1

Samples were taken from each of twelve packets (152, 332, 512, 692, 872, 1052, 1172, 1232, 1414, 1592, 1952, 2132). The packets were mixed by rotation prior to sampling. For each packet, an empty glass weighing vessel and lid were weighed and the mass recorded. Next, approximately 1 g of material were scooped into the vessel and the combined mass of the vessel, lid, and material were recorded. Then the vessels and lids (removed from vessel while in the desiccator) were placed into a desiccator over fresh anhydrous magnesium perchlorate ($Mg(ClO_4)_2$). On day (5, 7, 14, 21, 28, and 35) after the start of the study, the lids were placed on the vessels and the material, vessel, and lid were weighed again and weights were recorded. A Mettler AT261 Delta Range analytical balance was used for all weighing's.

Assuming all mass loss was due to loss of moisture, the percent mass fraction of moisture, w_{moisture}, in the RM 8210 samples was calculated as

$$w_{\text{moisture}} = 100 \times \frac{m_{\text{w}} - m_{\text{d}}}{m_{\text{w}} - m_{\text{b}}} \quad (15)$$

where: m_{b} represents the weight of the empty weighing vessel and lid,
m_{w} was the initial weight of the weighing vessel, lid, and hemp sample, and
m_{d} was the weight of the weighing vessel, lid, and hemp on day "d".

The resulting mean mass fractions are displayed as a function of days in the desiccator are displayed in Fig. 23

5.2. Study 2

The same technical protocol as used in Study 1 was followed. The desiccator was charged with 500 g of fresh anhydrous $Mg(ClO_4)_2$ obtained from GFS Chemicals (Powell, OH, USA). Mettler Toledo Model XS105 Dual Range analytical balance was used for all weighing's. Two empty glass vessels with lids were carried through the moisture measurement to make sure mass variability was within the expanded uncertainty of the balance. The vessels were weighed on days (5, 7, 9, 16, 23, 28, and 36) after the start of the study. The resulting mean mass fractions are displayed as a function of days in the desiccator are displayed in Fig. 23.

5.2.1. Materials

Fifteen samples of RM 8210 were evaluated in the following manner:

- Packets from Box 1 marked (1, 30, 61, 90, and 120) packaged in aluminized polyester bags with desiccant pouches and stored at -80 °C,
- Polyethylene packets marked (box-unit: 2-161, 4-521, 7-1120, 10-1521, and 14-2160) and stored at -80 °C,
- Polyethylene packets marked (box-unit: 1-3, 5 651, 7-1083, 10-1515, and 14-2113) and stored at -20 °C.

Samples from the three different storage conditions were randomized prior to the initial wet mass measurement and also prior to each subsequent mass measurement.

5.2.2. Comparisons Between Storage Conditions

Moisture content from the three storage conditions were compared to each other and to the moisture measurements made in Study 1 using a one-way analysis of variance (ANOVA) with a Tukey-Kramer post hoc test [46]. The assumption of equal variances was checked using the Levene test for equality of variances, which is less sensitive to departures from normality than similar tests for equality of variance [47].

On Day 36, the mass fraction moisture content was (5.82 ± 0.09) % for the samples packaged in: aluminized polyester bags with desiccant pouches stored at -80 °C, (5.93 ± 0.59) % for polypropylene packets stored at -80 °C, and (5.86 ± 0.30) % for polypropylene packets stored at -20 °C. There was no significant difference in moisture content of RM 8210 due to storage conditions based on the results from the ANOVA (p-value = 0.905). However, the hemp packaged in aluminized polyester bags with desiccant pouches had a much smaller variability than the samples not packaged in aluminized polyester bags with desiccant pouches. The variability differences are either a function of the aluminized polyester bags with desiccant pouches or that the aluminized polyester bags were all from Box 1. Results from the five packets stored in aluminized polyester bags with desiccant pouches are not used in further analysis to avoid skewing results.

5.3. Comparisons Between Studies

The moisture loss as a function of days in the desiccator is displayed in Fig. 23. Mass loss plateaus at about 28 days in both. However, the apparent moisture content at 28 days in the Study 2 materials was approximately 11 % greater than in Study 1. The increase is unexplained beyond speculation of analyst, environmental, operational, and/or and equipment differences between the studies.

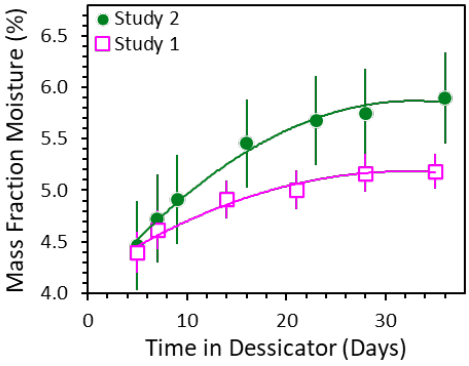


Fig. 23. Moisture Mass Fraction As a Function of Days in Desiccator.

Open pink squares represent the mean results from Study 1, solid green circles represent mean results from the ten samples stored as polyethylene bags in study 2. Error bars represent standard deviations. The solid curved lines represent empirical quadratic trend lines.

5.4. Value assignment

Combining the 28-day results of the two studies captures the likely limiting variability of the desiccator method for the RM 8210 material. Table 26 lists the pertinent results.

Table 26. Mass Fraction Moisture Content at 28 Days, %.

Study 1		Study 2	
Packet	Moisture	Packet	Moisture
152	5.14	3	5.69
332	5.28	161	6.63
512	5.12	521	5.69
692	5.34	651	5.70
872	5.19	1083	6.12
1052	5.47	1120	5.93
1172	5.13	1515	5.72
1232	5.33	1521	5.61
1412	5.20	2113	5.34
1592	5.01	2160	5.01
1952	5.06		
2132	4.74		

Because of the strong difference in the 28-day limiting moisture content results between the two studies, the results are combined using the same Bayesian Linear Pool model used in Section 4.6.2. with OpenBUGS software [13].

This analysis estimates the percent mass fraction moisture in the RM 8210 material as 5.46 %, a standard uncertainty of 0.32 %, and an approximate 95 % level of confidence interval of 0.64 %. The data and summary estimates are displayed in Fig. 24. The downward moisture content trend with packet number in both studies is similar to the trends for many of the cannabinoid (Fig. 9) and some of the toxic elements (Fig. 16). Since a loss of moisture would tend to increase the mass fraction of non-volatile components, moisture loss is not responsible for the observed decreases

in measurand mass fractions. The decreases are suggestive of small but systematic changes in material composition towards the end of the packaging operations.

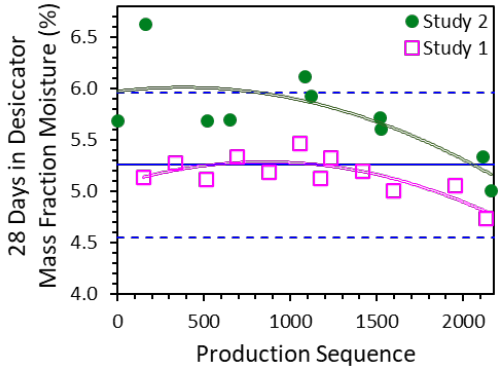


Fig. 24. Moisture Mass Fraction at 28 Days in Desiccator.

Open pink squares represent the mean results from Study 1, solid green circles represent mean results from the ten samples stored as polyethylene packets in study 2. The solid curved lines represent empirical quadratic trend lines. The solid horizontal line denotes the consensus mass fraction moisture; the dashed horizontal lines bound approximate 95 % level of confidence interval on the consensus value.

6. Mass Fractions on a Dry Mass Basis

The individual cannabinoid results reported in Table 8, the total THC and total CBD results in Table 9, and the toxic elements in Table 25 are on an as-received basis, w_{ar}. The mass fractions can be converted to a dry mass basis, w_{dry}, using the formula

$$w_{\mathrm{dry}} = w_{\mathrm{ar}} \times \left(1 + \frac{w_{\mathrm{moisture}}}{100}\right) \quad (16)$$

where $w_{moisture}$ is the moisture content determined in the previous section: 5.46 % ± 0.32 %. The moisture variability component of uncertainty contributed minimally to the overall dry mass basis standard uncertainty, therefore not impacting the resulting values. Table 27 summarizes the assigned values for cannabinoids and toxic elements in RM 8210 after the conversion from the as-received to the dry mass basis. These are not the final assigned values, which are only available in the current RMIS available on the NIST SRM website. The NIST Uncertainty Machine [16] was used to propagate the uncertainties.

Table 27. Measurand Values and Uncertainties on a Dry Mass Basis.

		As-Received Basis		Dry Mass Basis			
Measurand	Units	w_{ar} [a]	$u(w_{ar})$ [b]	W_{dry} [c]	$u(w_{dry})$ [d]	$U_{95}(w_{dry})$ [e]	%CV [f]
CBN [g]	%	0.00330	0.00012	0.00348	0.00013	0.00025	4
CBDV [g]	%	0.00433	0.00038	0.00457	0.00040	0.00079	9
CBG [h]	%	0.0331	0.0017	0.0349	0.0021	0.0042	6
CBC [h]	%	0.0894	0.0030	0.0943	0.0031	0.0062	3
Δ^9-THC [h]	%	0.1092	0.0032	0.1152	0.0034	0.0068	3
CBGA [h]	%	0.1378	0.0080	0.145	0.0092	0.018	6
THCA [h]	%	0.1505	0.0057	0.159	0.0067	0.014	4
CBD [h]	%	0.921	0.030	0.972	0.033	0.067	3
CBDA [h]	%	6.95	0.29	7.33	0.35	0.70	5
Total THC [h]	%	0.2408	0.0068	0.254	0.0078	0.016	3
Total CBD [h]	%	7.00	0.27	7.38	0.32	0.64	4
As [i]	ng/g	40.6	5.6	42.8	5.9	12	14
Be [i]	ng/g	2.15	0.89	2.27	0.94	1.9	42
Cd [i]	ng/g	78.4	6.5	82.7	6.9	14	9
Co [i]	ng/g	186	17	196	18	35	10
Cr [i]	ng/g	523	79	552	83	170	16
Hg [i]	ng/g	7.09	0.75	7.48	0.79	1.6	11
Mn [i]	ng/g	130500	9400	137600	9900	20000	8
Mo [i]	ng/g	302	14	319	15	29	5
Ni [i]	ng/g	3770	350	3980	370	730	10
Pb [i]	ng/g	200	29	211	31	60	15
Se [i]	ng/g	76.7	7.9	80.9	8.3	17	11
U [i]	ng/g	4.20	0.61	4.43	0.64	1.3	15
V [i]	ng/g	226	42	238	44	87	19

a Measurand mass fraction on the as-received basis.
b Standard uncertainty of the as-received measurand mass fraction.
c Measurand mass fraction converted to a day mass basis.
d Standard uncertainty of the dry mass measurand mass fraction.
e Approximate 95 % level of confidence expanded uncertainty of the dry mass measurand mass fraction.
f Coefficient of variation (aka relative standard deviation) expressed as a percentage: %CV = 100×$u(w_{dry})/w_{dry}$.
g As-received result from the initial assessment, Table 8.

h As-received result from the three-study consensus analysis, Table 12.
i As-received result from robust analysis, Table 25.

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Appendix A. List of Acronyms

%CV	coefficient of variation as a percentage (aka relative standard deviation)
CannaQAP	Cannabis Quality Assurance Program
CBC	cannabichromene
CBDV	cannabidivarin
CBDA	cannabidiolic acid
CBG	cannabigerol
CBGA	cannabigerolic acid
CBD	cannabidiol
CBN	cannabinol
COA	Certificate of Analysis
CRM	certified reference material
HDPE	high density polyethylene
HPLC	high-performance liquid chromatography
ICP-MS	inductively coupled plasma mass spectrometry
ICP-MS/MS	inductively coupled plasma - tandem mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
IS	internal standard
LC	liquid chromatography
LC-PDA	liquid chromatography with photodiode array detection
LC-UV	liquid chromatography with UV absorbance detection
MeOH	methanol
NIST	National Institute of Standards and Technology
ORM	Office of Reference Materials
PTFE	polytetrafluoroethylene
QQQ-ICP-MS	triple quadrupole inductively coupled plasma mass spectrometry
SI	International System of Units
SRM	Standard Reference Material
Δ^9-THC	Δ^9-Δtetrahydrocannabinol
THCA	tetrahydrocannabinolic acid