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Commercial cannabis consumer products part 2: HPLC-DAD quantitative analysis of cannabis cannabinoids

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ABSTRACT

Quantitative analysis for the cannabis cannabinoids such as cannabidiol and Δ^9-tetrahydrocannabinol in commercial products is necessary for evaluating label information, and assessing dosages and exposures when the products are consumed. Herein is presented a broadly applicable HPLC-DAD method for the determination of cannabis cannabinoids in commercial consumer products and traditional plant-related substances. The current method provides chromatographic resolution of 11 cannabinoids using a commercial, mixed C18-aromatic functionality stationary phase. The method uses 95% or pure ethanol for extraction, and certain modifications which address specific matrix types are detailed herein. Extensive method validation including precision and accuracy was conducted for five cannabinoids of primary interest (CBD, Δ^9-THC, CBDA, THCA, and CBN). UV detection provided excellent sensitivity with limits of quantitation (LOQs) of 10 μg/g across cannabinoids. The method was applied to about 60 commercial products representing diverse product types and a broad range of cannabinoids amounts (0.01–350 mg/g).

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1. Introduction

In addition to traditional cannabis plants and plant extracts, the recent surge in the sale of cannabis-based consumer products has added a host of sample types for analysis including foods, candies, beverages, topicals, vapes/eliquids, and oral supplements in various forms. A GC–MS method [1] for the qualitative analysis of cannabis cannabinoids which addresses the current diversity in cannabis-based samples was presented in Part 1. In Part 2, the quantitative analysis of the cannabinoids is addressed.

The expansion of the cannabis market has coincided with continued development of quantitative analytical methods for the cannabinoids. For quantitation which addresses both acidic and neutral cannabinoids, methods based on HPLC–MS [2,3], SFC-DAD-MS [4], HPLC-DAD [5–13], HPLC-DAD-MS [14,15], and GC-FID [16] have been reported. Most of the reported methods [3–10,12–16] were developed for plant materials or plant extracts/oils, including illicit, medicinal, and/or hemp varieties. One of the HPLC–MS methods was used for the determination of cannabidiol (CBD) only in eliquids [2]. One of the HPLC-DAD methods was used for the determination of Δ^9-tetrahydrocannabinol (hereafter "d9THC") only in baked goods, beverages, candies, and chocolates [11]. This latter report provided no details of the extraction conditions or HPLC parameters [11].

Each of these methods is applicable to specific sample types, and does not address the broad range of cannabis-based sample types which currently exist. As for the qualitative testing [1], there is a need to develop alternative extraction procedures which extract the target cannabinoids while minimizing interferences. Extraction requirements for quantitative analysis are more stringent than for qualitative analysis, demanding high recoveries of the target analytes to provide accurate results. The objective of this part was to develop and validate a broadly applicable HPLC-DAD method for the quantitative analysis of cannabis cannabinoids. As for Part 1, the method scope includes both acidic and neutral cannabinoids, and all varieties of cannabis plants (recreational, medical, and hemp), plant extracts or preparations, and consumer, medical, or illicit products. The HPLC-DAD method will likewise provide chromatographic resolution of the 11 commercially available cannabis cannabinoids: cannabidiol (CBD), cannabidiolic acid (CBDA), Δ^9-tetrahydrocannabinol (d9THC), tetrahydrocannabinolic acid-A (hereafter "THCA"), cannabinol (CBN), Δ^8-tetrahydrocannabinol (d8THC), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabidivarin (CBDV), tetrahydrocannabivarin (THCV), and cannabichromene (CBC). Extensive method validation is conducted for five cannabinoids of primary interest (CBD, d9THC, CBDA, THCA, and CBN).

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2. Materials and methods

2.1. Standards, chemicals, and reagents

Cannabis cannabinoids were obtained as pure substances (≥98%) or concentrated stock solutions (10 mg/ml) in methanol or methylacetate from Cayman, Sigma, or Fluka. For the pure substances, stock solutions in the range 0.5–2.0 mg/ml were prepared using methanol or acetonitrile. Certified 1.0 mg/ml cannabinoid stock solutions from Cerilliant Corporation were also used. For analysis, stock solutions were diluted in 95% ethanol to yield concentrations within the calibration ranges.

Ethanol (200 proof, USP/ACS grade) was obtained from Sigma-Aldrich. Acetonitrile (HPLC grade), methanol (HPLC grade), glacial acetic acid (HPLC grade), and hexanes (Optima grade) were obtained from Fisher Scientific (Fair Lawn, NJ). Deionized water was obtained from a Millipore 18 ohm filtration system fed by a service deionized water source. 95% ethanol(aq) (hereafter "95% ethanol") was prepared in our laboratory from the 200 proof ethanol and deionized water.

2.2. Cannabis plant materials, commercial products, hempseed oils, hash oils, kief, and dronabinol capsules

Five THCA-rich varieties of cannabis plant materials were obtained from retail markets in Colorado in 2017. The plant materials consisted of dried buds which were ground in a mortar prior to extraction. Commercial cannabis consumer products including oral supplements, foods, candies, beverages, vapes/eliquids, and topicals were obtained between 2015 and 2017 via purchases from internet web stores. Two different Canada-sourced hempseed oils were obtained in 2014 or 2017. Generic dronabinol capsules (2.5 mg and 5.0 mg strengths) were obtained from a local pharmacy.

Two crude hash oils and one fine kief material were prepared in the authors' laboratory from a commercial THCA-rich bud material obtained previously in 2016. 3.1 g of ground material were extracted with 30 ml ethanol (hash oil 1) or isopropyl alcohol (hash oil 2). The entire extract was filtered through a 0.45 μm nylon membrane filter, after which the solvent was evaporated in a heated water bath (45–50 °C) under a nitrogen purge. Both hash oils were dark red amber, with yields of 0.71 g (hash oil 1) or 0.68 g (hash oil 2). For the fine kief, 3.1 g of ground bud material, 50 ml water, and 117 g ice were stirred in a beaker for 45 min. The preparation was successively filtered through a series of five metal sieves, with mesh sizes of 10, 20, 60, 80, and 100. The materials collected on the sieves were allowed to air dry, with a yield of 0.13 g fine kief material obtained from the 60 mesh sieve.

2.3. Concentrated cannabis plant extracts for spiking

Three different types of concentrated plant extract filtrates were used for the negative product matrices spike/recovery experiments (see Section 2.7). THCA-rich concentrated plant extracts were prepared from the commercial bud materials (Section 2.2) by extracting the ground bud with 100% ethanol (2.8 g bud material in 20 ml ethanol) according to the general procedure (Section 2.4). Depending on the batch, nominal THCA and d9THC concentrations for the finished filtrates were in the range 21–23 mg/ml and 2.3–5.9 mg/ml, respectively. A d9THC-rich concentrated plant extract was prepared by first heating the THCA-rich ground bud material in an oven for 2.5 h at 250 °C, followed by three days ambient, dark storage with the dried material exposed to air. After extraction with 100% ethanol (4.4 g plant material in 35 ml ethanol), nominal d9THC and CBN concentrations for the finished filtrate were 21 mg/ml and 0.69 mg/ml, respectively. A concentrated CBD-rich extract was prepared from a commercial oral supplement product which was labeled as a hemp oil. Assay results for the CBD and CBDA contents of the product were 159 and 10 mg/g, respectively. 5.4 g of the oral supplement was extracted with 30 ml of 100% ethanol yielding finished CBD and CBDA filtrate concentrations of 21 mg/ml and 1.4 mg/ml, respectively.

2.4. Sample preparation

Sample weights and extraction volumes were adjusted according to cannabinoid contents, with sample weights in the range 0.03–2.5 g and extraction volumes in the range 0.5–30 ml. For tablets and capsules, composites of 3–10 units were prepared if possible. For some capsules (e.g. dronabinol capsules), it was not possible to prepare a quantitative composite since the capsule contents were viscous liquids. For these capsules, analysis was conducted using several individual whole capsules. All extractions were performed using 95% ethanol or 100% ethanol with variations in the general procedure according to the sample matrix (see below). Extractions for sample matrices which completely dissolved were generally carried out in volumetric flasks, and all other extractions were performed in scintillation vials or Erlenmeyer flasks.

Product contents were mixed or shaken as needed prior to sampling. A portion of sample was weighed into the extraction vessel. The extracting solvent was added, and the samples were capped, then vortexed thoroughly to dissolve or disperse the matrix. Samples which did not fully dissolve were sonicated for 30 min. A portion of the extract was filtered through a 0.45 μm nylon membrane filter. Depending on the cannabinoid(s) content, HPLC-DAD analysis was conducted on the filtrate, or the filtrate was further diluted using 95% ethanol to reach a target final concentration within the standard calibration ranges. In many cases, it was necessary to analyze two different extract dilutions (i.e., analyze the filtrate and a dilution of the filtrate, or analyze two separate dilutions of the filtrate) so that the sample concentrations of the targeted cannabinoids fell within the standard calibration ranges.

Variations were applied to specific matrix types as follows. For semisolid fat/oil matrices such as butter, margarine, virgin coconut oil, and various nonpolar topical ointments, creams, or balms, the sample vessel was warmed on a hot plate after addition of the extraction solvent to melt the matrix. The sample was then vortexed to disperse the matrix, continuing with the general procedure. For specific high sugar and carbohydrate matrices including hard candies, fruit preserves, and honey, the aqueous portion of the 95% ethanol extractant was added first, followed by warming of the sample vessel on a hot plate to cause the sugars and carbohydrates to fully dissolve. The ethanol portion of the extractant was then added as 100% ethanol to give a final proportion of approximately 95% ethanol. The sample was then vortexed to disperse the sample, continuing with the general procedure. For any highly aqueous matrices such as prepared coffee or tea beverages, and certain oral supplements/pharmaceuticals, the extractant was 100% ethanol with the general procedure followed thereafter.

2.5. HPLC-DAD analysis

All HPLC analysis was conducted using Agilent 1100, 1200, or 1260 HPLC-DAD systems. Separations were carried out using MacMod ACE 5 C18-AR analytical columns (5 μm, 4.6 mm ID × 250 mm length). The mobile phase comprised 66:34 acetonitrile: 0.5% acetic acid (no pH adjustment, nominal pH 2.9). The injection volume was 25 μl, flow rate 1.0 ml/min., and run time 50 min. Several detection wavelengths (220 nm, 240 nm, 270 nm,

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307 nm) were used according to the specific compound, compound levels, and sample matrices (see also Table 1 and Section 3): Chromatographic peak spectra were obtained over the range 190–400 nm.

2.6. Spike recovery for hempseed oils and cannabis based consumer products

Spike recovery experiments for two hempseed oils and 28 commercial cannabis consumer products were conducted as follows. The hempseed oils were spiked with all five target cannabinoids at two different spiking levels (ca. 20 μg/g and in range 120–140 μg/g). The consumer products were spiked only with the cannabinoids which were detected and quantitated in the assay work for the given product, and at spiking levels close to the assayed levels. Spike/recovery experiments were conducted in duplicate for each hempseed oil or consumer product. The cannabinoid contents present in the matrix prior to spiking was accounted for when calculating spike recovery.

For spiking, a portion of oil or product was weighed into a 20 ml glass scintillation vial or volumetric flask, after which an aliquot(s) (10–300 μl) of a standard solution(s) or a standard mix was deposited onto the sample. The vessel was capped and thoroughly vortexed to distribute the spiking solution as completely as possible into the matrix. For semisolid products, the vial was warmed and further vortexed as needed to melt the product and mix in the spike. For solid product matrices, the solvent was allowed to completely evaporate from the product matrix under a bench top vacuum snorkel prior to extraction. For liquid and semisolid matrices, the extraction was performed without evaporating the spiking solvent. After spiking, all extractions were carried out according to the general procedure (see Section 2.4).

2.7. Negative product matrices and concentrated cannabis spikes

38 cannabinoid-negative products including edible oils, foods and beverages, topicals, and oral OTC medicines were obtained from local stores for use in spike/recovery experiments with concentrated cannabis plant extracts (see Section 2.3). In addition, one Canada-sourced hempseed oil (Section 2.2), one cannabinoid-negative commercial cannabis herbal cream (Section 2.2), and one pharmaceutical cream base containing no active ingredients (obtained from a local pharmacy) were included in the study. Spike/recovery experiments were conducted in duplicate for each product matrix and plant extract. Spiking was conducted as follows: a portion of product (200–300 mg) was weighed into a 20 ml glass scintillation vial or volumetric flask, after which an aliquot (150–200 μl) of the concentrated cannabis extract spiking solution was deposited onto the product. The vessel was capped and thoroughly vortexed to distribute the spiking solution as completely as possible into the product matrix. For semisolid products, the vessel was warmed and further vortexed as needed to melt the product and mix in the spike. For solid products, the solvent was allowed to completely evaporate from the product matrix under a bench top vacuum snorkel. For liquid and semisolid products, the extraction was performed without evaporating the spiking solvent. After spiking, all extractions were carried out according to the general procedure (Section 2.4). The extraction volume was typically 5.0 or 10.0 ml.

3. Results and discussion

3.1. HPLC analysis

This method provides chromatographic resolution of the following 11 cannabis cannabinoids for which standards were commercially available: CBD, CBDA, d9THC, THCA, CBN, d8THC, CBG, CBGA, CBDV, THCV, and CBC. Chromatographic resolution was made more difficult by two critical compound pairs with very similar structures: CBD/CBG (closed vs. open ring), and d9THC/d8THC (double bond position isomers), see structures in Part 1 [1]. A key finding from the development work was the improved separation of these compound pairs by use of a mixed C18/aromatic functionality stationary phase (MacMod ACE 5 C18-AR). Highlights of the development work follow.

Four derivatized silica stationary phases were evaluated for the resolution of nine cannabinoids available at the time (CBD, CBDA, d9THC, THCA, CBN, d8THC, CBG, THCV, and CBC). The stationary phases (all 25 cm, 4.6 mm ID, 5 μm particle diameter) were a conventional C18 phase (Luna C18-2), a phenylhexyl phase (Luna), and two phases with mixed C18/aromatic functionality (MacMod ACE 5 C18-AR and Siliachrom C18/phenyl). Mobile phase variables included buffer type (acetic acid, formic acid, or citric acid), buffer pH (tested pH range 2.5–5.1), and the use of acetonitrile vs. methanol. Overall retention for the cannabinoids was highest with the C18 column and lowest with the phenylhexyl column. Due to

Table 1
Target compounds retention, spectral detection and UV response.

Compound	Nominal ret time min	Observed λ maxes nm	Detection λ nm	Linear range[a] μg/ml	Slope
CBDA	12	308, 269, 224[b]	307	0.50–1000	18.3
			270	0.50–1000	44.3
			240	0.50–1000	29.9
			220	0.50–500	97.2
CBD	15	276, 235(s), rise[c]	240	0.50–750	18.1
			220	0.50–500	94.1
CBN	25	301(s), 285, 221[b]	240	0.50–750	54.2
			220	0.50–500	130
Δ^9-THC	29	279, 235(s), rise[c]	240	0.50–1000	20.3
			220	0.50–750	87.4
THCA	39	306, 272, 223[b]	307	0.50–1000	21.3
			270	0.50–1000	50.7
			240	0.50–1000	32.6
			220	0.50–750	96.8

[a] Listed concentration ranges represent tested ranges for which linearity was established.
[b] Apparent maximum observed at listed wavelength, observed maximum may be artefact of acetic acid buffer background subtraction.
[c] Low end of spectrum characterized by continual steep rise in absorbance with no defined maximum.

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their lower pH relative to acetic acid, formic acid buffers resulted in unnecessarily long retention for THCA. Resolution of all nine cannabinoids was only achieved using the ACE 5 C18 column, and identified two alternate sets of mobile phase conditions: (1) 66:34 acetonitrile:0.5% acetic acid (no pH adjustment, nominal pH 2.9); and (2) 83:17 methanol: 50 mM citrate (pH adjusted to 4.2). Because retention of THCA is extremely pH dependent, the first set of mobile phase conditions was chosen as consistent buffer pH's are obtained without the need for adjustment.

Fig. 1 shows the separation of 11 cannabinoids using the ACE 5 C18-AR column (Fig. 1A) vs. the conventional C18 column (Fig. 1B) with mixed 0.5% acetic acid/acetonitrile mobile phases for both columns. Resolution of all 11 cannabinoids with excellent peak shape was obtained using the ACE 5 C18-AR column. Next to the ACE 5 C18-AR column, the C18 column provided the next best resolution but iterative mobile phase adjustments always left two or more cannabinoids unresolved. Fig. 2 shows HPLC-DAD chromatograms for two commercial cannabis products: an oral liquid supplement for which CBD was the predominant cannabinoid (Fig. 2A), and a topical balm for which CBDA was the predominant cannabinoid (Fig. 2B). These chromatograms demonstrate the typical case that the cannabinoids are resolved from other compounds in the chromatogram. The series of compounds which elute prior to CBDA for both the oral liquid and topical balm are completely excluded from detection by using 307 nm as the detection wavelength (see next paragraph).

Table 1 summarizes retention time, and UV spectral and response data, for the five target compounds (CBD, d9THC, CBDA, THCA, and CBN). Multiple absorbance maxima are observed in each compound's spectrum. Multiple detection wavelengths were evaluated and used for the five compounds based on their inherent UV maxima, relative UV responses, and in order to avoid interferences from matrix components in practice. While none of the compounds showed a maximum at 240 nm, this wavelength tended to equalize the response (slope) across the five compounds, except for CBN which has the highest response. 220 nm provides the highest response for all five compounds and is useful for low level quantitation. 270 and 307 nm provide high selectivity for CBDA and THCA, which was used to minimize or eliminate detection of matrix interferences as needed. Detection wavelengths in the range 270–280 nm were problematic for CBD, CBN, and d9THC due to interferences encountered with many matrix types in the retention windows for these compounds. Linear ranges were established at two or more wavelengths as listed in the table for the five target compounds. The listed ranges are considered strict ranges established by visual inspection of the concentration vs. response data graphs, calculation of correlation coefficients (r), consideration of absorbance magnitudes, and assessment for spectral distortions. All correlation coefficients (r) were >0.999. The low end of the calibration ranges was taken as 0.50 μg/ml (see also Section 3.4).

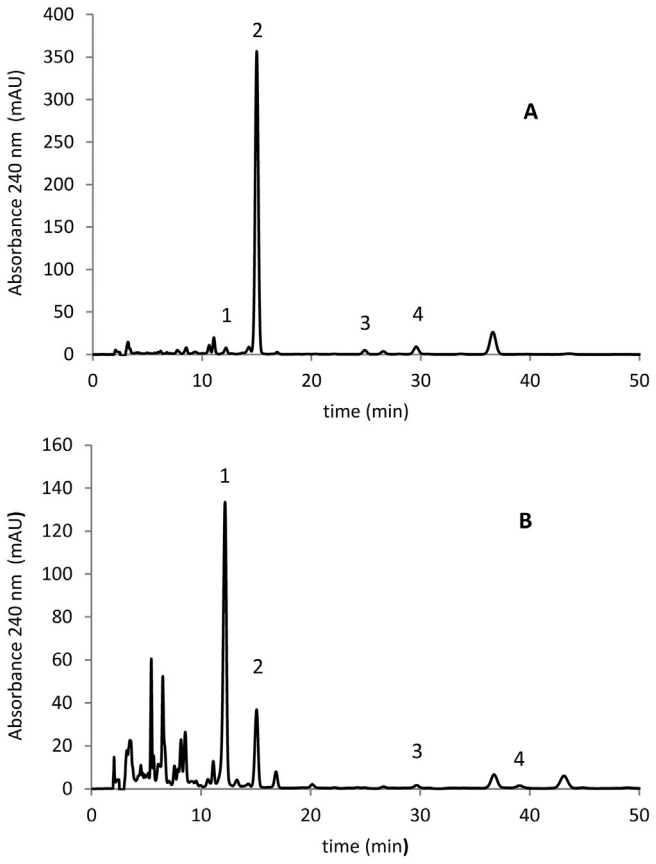

Fig. 2. HPLC-DAD chromatograms for two commercial Cannabis products: an oral liquid (A) and a topical balm (B). Peak labels and cannabinoid eluate concentrations (μg/ml): (A) 1-CBDA (3.7); 2- CBD (420); 3-CBN (2.3); 4-d^{9}THC (15). (B) 1-CBDA (90); 2-CBD (42); 3-d^{9}THC (2.8); 4-THCA (1.8).

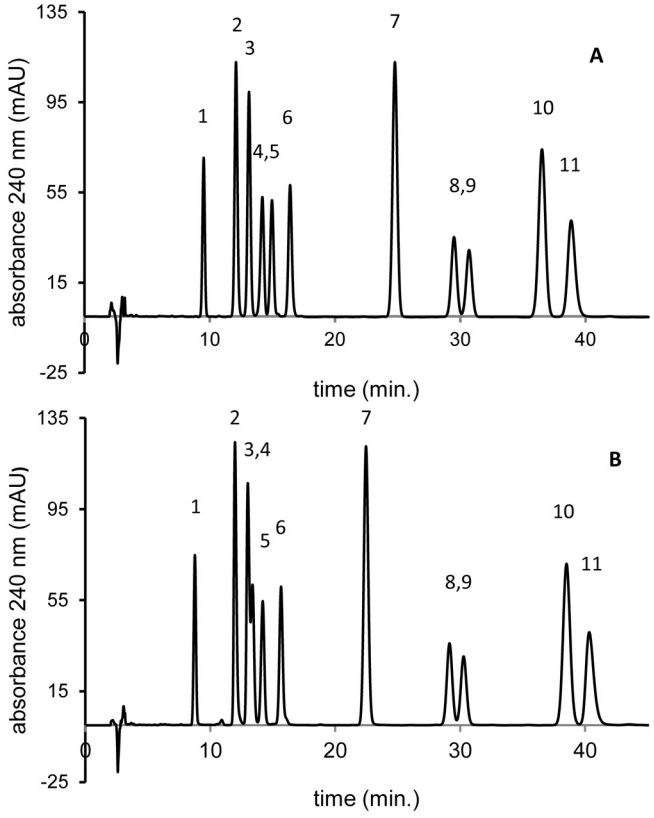

Fig. 1. HPLC-DAD separation of 11 cannabinoids (nominally 50 μg/ml each) using ACE 5 C18 AR column and 66:34 acetonitrile 0.5% HAc mobile phase (A) vs. Luna C18 column and 74:26 acetonitrile 0.5%HAc mobile phase (B) Elution order 1-CBDV, 2-CBDA, 3-CBGA, 4-CBG, 5-CBD, 6-THCV, 7-CBN, 8-d9THC, 9-d8THC, 10-CBC, 11-THCA.

3.2. Extraction for quantitative analysis

Extraction for quantitative analysis requires dissolution or fine dispersion of the sample matrix in the extracting solvent, as well as sufficient solubility of the target compounds. Both of these requirements are dictated by the chemical and physical natures of both the target analytes and the sample matrices. As a whole, the cannabis cannabinoids are medium to low polarity compounds.

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CBD, d9THC, and CBN possess weakly acidic phenolic hydroxyl groups, while CBDA and THCA possess both carboxylic acid and phenolic hydroxy groups. The target sample matrices are diverse including cannabis plants, plant extracts, plant oils, and a host of commercial products made using cannabis plants, extracts, or oils. These products range from highly polar (sugary foods or beverages) to nonpolar (certain ointments/balms, butter) matrices, with many product types of intermediate polarity (many foods and topicals, oral supplements). Physical forms included solids, semi-solids, aqueous and nonaqueous solutions, dispersions, emulsions, and viscous or free flowing liquids.

Method development with extractants focused on comparing ethanol and acetonitrile, either as pure solvents or with the addition of up to 15% water. The goal was to investigate whether small amounts of water could boost extractability for the acidic cannabinoids (CBDA, THCA, etc.) which are capable of ionizing and/or help diminish coextraction of unwanted matrix components such as oils. Any solvents containing acids or bases were ruled out due to the instability of several cannabinoids [17–19] in acidic or basic solutions. Extractants containing chloroform were also ruled out due to CBD instability [20,21]. This included the extractant 9:1 methanol:chloroform, for which major CBD degradation (15–40%) was observed in extracts of several concentrated hemp oils/pastes within one day's storage under ambient conditions.

In development work, experiments were conducted comparing the following four extractants for quantitation of THCA, d9THC, CBGA, and CBDA from five different commercial THCA-rich plant bud products: (1) ethanol; (2) acetonitrile; (3) 95% ethanol, and (4) 90% acetonitrile(aq). 95% ethanol provided the highest average extractability across the five plant materials for all four of the cannabinoids. The average relative amounts extracted with 95% ethanol were 1.4% higher vs. pure ethanol, and 3–5% higher vs. the acetonitrile-based solvents. The same four extractants were compared for the quantitation of CBD, d9THC, CBDA, THCA, and CBN in a concentrated hemp oil, and two commercial oral supplements containing oils such as hempseed, coconut, grapeseed, and peppermint (note that THCA was not detected in the oral supplements). 95% ethanol and pure ethanol provided similar extractability (within 1% relative), and both ethanol-based extractants provided much higher extractability (10–15% higher) relative to the acetonitrile-based extractants. Hence, acetonitrile-based solvents were ruled out for use in quantitative analysis. Unlike for the GC–MS analysis [1], the higher coextraction of sugars, glycerin, or propylene glycol by the ethanol-based solvents did not cause any chromatographic interferences in the HPLC-DAD analysis since these coextracted compounds are not detected by UV.

Table 3
Assay results and precision for concentrated, CBD-rich Cannabis oral supplement syringe products.

Product No.	*n*	CBD		d9-THC		CBN		CBDA	
		mg/g	%RSD	mg/g	%RSD	mg/g	%RSD	mg/g	%RSD
1	3	144	0.95	8.5	0.58	1.9	2.1	ND	–
2	3	145	1.4	6.4	1.6	1.2	0.94	ND	–
3	4	151	11	6.6	7.0	1.2	8.8	ND	–
4	3	245	1.2	8.6	1.5	0.80	5.6	0.24	2.8
5	3	350	0.74	1.6	4.2	8.2	2.8	ND	–
6	3	257	1.4	8.4	1.6	0.70	3.2	0.24	8.3
7	3	144	0.83	5.0	0.80	0.39	2.6	ND	–
Average %RSD		2.5		2.5		3.7		5.5	

Products 1–3 were extracted with 100% ethanol; products 4–7 were extracted with 95% ethanol.
n, number of trials.
All reported values (mg/g) are averages of 3– 4 trials.
ND, not detected.

As discussed above, it was difficult to measure differences in extractability for pure ethanol vs. 95% ethanol. Extractability for ethanol-water mixtures fell off with higher water amounts, especially above 10%. Overall, the data indicated that 95% ethanol may provide marginally higher extractability vs. pure ethanol for CBDA, which is the most polar of the target compounds and capable of ionizing. Excellent peak shapes were obtained for the cannabinoids across the entire range of product types and matrices (Tables 2–6) with 95% ethanol extracts. Good peak shapes were observed for pure ethanol extracts except for matrices which contained high levels of oils, or solvents such as propylene glycol or glycerin. The additional "solvency load" from the oils or solvents caused peak broadening in the chromatograms.

Most product matrices containing sugars and carbohydrates dispersed well and yielded quantitative recoveries with the 95% ethanol extractant. However, as in the GC–MS work, specific high sugar, high carbohydrate products such as hard candies, strawberry preserves, and honey required a modification of the extraction procedure. These matrices did not disperse well in either 95% or pure ethanol, and tended to form glassy, impervious precipitates during extraction. The net result was a physical trapping of the cannabis cannabinoids, and low recoveries (17–66%) of the cannabinoids, whether from cannabis-containing products or when conducting spiking experiments in negative product matrices. This problem was resolved by adding the aqueous portion of the 95% ethanol extractant first, followed by warming of the extraction vessel on a hot plate to cause the carbohydrates and sugars to fully dissolve. The ethanol portion of the extractant was then added as 100% ethanol to give a final proportion of approximately 95% ethanol. The ethanol addition

Table 2
Assay results and precision for commercial THCA-rich Cannabis plant buds and plant preparations ($n = 3$).

Matrix	THCA		d9THC		CBGA		CBDA		CBN	
	mg/g	%RSD	mg/g	%RSD	mg/g	%RSD	mg/g	%RSD	mg/g	%RSD
Plant buds 1	138	6.4	4.4	7.4	3.4	3.7	0.26	6.4	0.016	13
Plant buds 2	212	1.6	7.6	5.9	8.8	0.46	0.51	4.9	0.020	12
Plant buds 3	241	1.8	7.9	4.3	6.9	2.0	0.54	1.7	0.034	4.9
Plant buds 4	154	3.6	18	2.0	15	2.1	0.33	1.7	0.24	4.3
Plant buds 5	239	6.8	11	6.0	2.5	0.72	0.58	3.5	0.026	6.6
Hash oil 1	379	3.2	149	3.7	14	2.9	0.76	3.5	4.3	2.3
Hash oil 2	399	1.8	172	1.4	15	0.55	0.86	3.8	4.9	0.14
Fine kief	214	6.8	46	4.8	4.0	7.7	0.43	10	9.4	5.6
Average %RSD	4.0		4.4		2.5		4.4		6.1	

n, number of trials.
All reported values (mg/g) are averages of the 3 trials.

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Table 4
Assay results, precision, and spike recovery for target cannabinoids in imported Canadian hempseed oils.

Hempseed oil product	Compound	Determined amount (n) μg/g	%RSD	Low spiking level μg/g	%Recovery	High spiking level μg/g	%Recovery
1	CBDA	14 (3)	3.0	18	87	125	90
	CBD	12 (8)	8.9	19	105	142	96
	THCA	1.5 (6)	5.8	19	83	142	88
	d9THC	trace	NA	19	97	142	98
	CBN	ND	NA	19	99	142	94
	Averages		5.9		94		93
2	CBDA	56 (3)	0.89	18	91	119	91
	CBD	26 (8)	3.0	20	101	137	101
	THCA	3.1 (6)	3.3	21	84	137	87
	d9THC	trace	NA	20	104	137	103
	CBN	ND	NA	20	102	137	93
	Averages		2.4		96		95

All determined amounts (μg/g) are averages of 3–8 trials.
n, number of trials for assay given in parentheses; $n = 2$ for all spiking experiments.
ND, not detected.
NA, not applicable.
Note that reported THCA levels are less than the specified LOQ (10 μg/g).

Table 5
Assay results, precision, and spike recovery for commercial Cannabis CBD products.

Product description	n	CBD				d9THC				CBDA				CBN			
		Assay mg/g	% RSD	Spike mg/g	% Rec	Assay mg/g	% RSD	Spike mg/g	% Rec	Assay mg/g	% RSD	Spike mg/g	% Rec	Assay mg/g	% RSD	Spike mg/g	% Rec
Oral nonaqueous liquid	6	145	3.2	137	96	5.3	3.8	4.9	99	0.54	4.3	0.53	100	0.21	7.8	0.21	93
Oral nonaqueous liquid	3	50	0.78	37	103	1.8	1.3	1.8	95	0.046	9.1	0.37	96	0.057	3.5	0.37	98
Oral nonaqueous liquid	3	28	0.59	21	114	trace	–	–	–	–	–	–	–	–	–	–	–
Oral nonaqueous liquid	3	15	0.18	13	93	0.85	0.65	0.77	92	–	–	–	–	0.097	1.3	0.086	89
Oral nonaqueous liquid	3	15	0.70	15	104	0.53	0.81	0.56	100	0.13	1.3	0.14	105	0.082	3.8	–	–
Oral nonaqueous liquid	3	9.5	0.93	7.7	100	0.29	2.4	0.25	104	0.012	1.9	0.011	98	0.012	5.3	0.011	109
Oral nonaqueous liquid	3	8.1	2.8	8.0	99	0.29	0.50	0.31	95	0.046	1.5	0.041	100	0.010	4.4	0.010	99
Oral nonaqueous liquid	2	6.9	0.51	6.6	97	0.21	0.19	0.30	98	--	--	–	–	0.018	5.8	0.025	94
Oral semisolid	2	6.8	0.22	5.6	92	0.40	1.2	0.31	87	0.30	2.6	0.22	98	0.12	0.83	0.11	87
Oral nonaqueous liquid	2	6.6	0.87	6.6	100	0.21	0.28	0.22	92	–	–	–	–	0.018	0.92	0.018	98
Tablets	2	1.5	2.5	1.9	99	0.021	10	0.067	98	–	–	–	–	0.028	3.1	0.038	98
Oral nonaqueous liquid	2	1.2	2.4	0.74	97	0.035	7.4	0.026	100	–	–	–	–	–	–	–	–
Capsules powder	6	0.56	1.1	0.51	90	0.014	5.9	0.014	96	0.0096	3.6	0.010	100	–	–	–	–
Aqueous supplement	2	0.30	0.53	0.30	100	0.012	2.4	0.011	101	–	–	–	–	–	–	–	–
Oral liquid dispersion	3	0.23	1.6	0.22	106	trace	–	–	–	–	–	–	–	–	–	–	–
Oral nonaqueous liquid	3	0.011	2.5	0.013	112	–	–	–	–	0.055	0.89	0.070	99	–	–	–	–
Vape/eliquid	2	9.0	0.54	6.4	99	–	–	–	–	–	–	–	–	–	–	–	--
Vape/eliquid	2	7.4	4.8	7.4	100	0.069	6.0	0.10	111	–	–	–	–	0.015	5.27	0.012	102
Vape/eliquid	2	0.28	0.40	0.22	98	–	–	–	–	–	–	–	–	–	–	–	–
Vape/eliquid	3	0.27	1.2	0.30	100	–	–	–	–	–	–	–	–	–	–	–	–
Vape/eliquid	3	0.26	0.82	0.27	100	–	–	–	–	–	–	–	–	–	–	–	–
Topical gel	3	16	0.94	12	107	0.41	3.1	0.40	97	0.018	3.8	0.017	100	0.014	2.44	0.017	92
Topical balm	2	0.45	1.7	0.43	91	0.032	6.0	0.020	102	0.94	0.18	0.86	101	–	–	–	–
Whole coffee bean	3	0.82	9.0	0.54	97	0.034	8.8	0.022	95	–	–	–	–	trace	–	–	–
Strawberry jam	3	0.62	5.1	0.70	113	0.023	9.4	0.026	108	–	–	–	–	–	–	–	–
Hard candies	12	0.31	1.5	0.33	98	0.018	6.7	0.018	90	–	–	–	–	–	–	–	–
Flavored liquid	3	0.28	0.66	0.29	94	–	–	–	–	–	–	–	–	–	–	–	–
Coffee beverage	3	0.042	0.63	0.036	96	–	–	–	–	–	–	–	–	–	–	–	–
Averages		%RSD	1.7	%Rec	99.8	%RSD	4.0	%Rec	97.9	%RSD	2.9	%Rec	99.7	%RSD	3.7	%Rec	96.3

extracted the cannabinoids, while also causing formation of a finely dispersed precipitate. This latter approach resulted in quantitative recovery (85–113%) of the cannabinoids, see also Tables 5 and 6. To assess other high sugar matrices for the potential to trap the cannabinoids and the need to use the modified extraction, it is a matter of evaluating the physical form of the precipitates formed during extraction. Glassy or amorphous precipitates may trap the cannabinoids, while finely dispersed precipitates tend not to interfere with extraction.

Semisolid fat and oil matrices such as butter, margarine, virgin coconut oil, and various nonpolar topical ointments, creams, or balms did not disperse well in either 95% or pure ethanol. Two alternative extraction conditions were evaluated to address this problem: (1) the use of 95% ethanol combined with hexane (10–30% v/v) for extraction; (2) a simple warming of the 95% ethanol extract on a hot plate. The hexane-containing extractants did provide good dispersion of the sample matrices during extraction, and resulted in quantitative cannabinoid recoveries (91–105%). The warming of the 95% ethanol extract caused the semisolid matrices to melt and remain dispersed throughout the extraction, and also provided quantitative recoveries (86–103%). The latter approach which avoided the need for hexane in the extractant was preferred, and minimized coextraction of fatty matrix components. Finally, pure ethanol was used for the extraction of Cannabinoids from highly

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Table 6
Cannabinoids %spike recovery from consumer products.

Spiking extract source	Fresh bud		Decarboxylated bud		Decarboxylated hemp oil	
Cannabinoid	THCA	d9THC	d9THC	CBN	CBD	CBDA
Spiking level range (mg/g)	13–19	1.7–5.8	13–20	0.41–0.69	16–19	0.96–1.2
Edible oils						
Liquid coconut oil	97.2	103	96.2	100	91.6	91.5
Virgin coconut oil	91.7	100	98.9	103	93.3	93.0
Grapeseed oil	90.2	94.9	95.7	99.0	93.4	94.3
Safflower seed oil	92.5	97.5	94.7	101	93.8	95.8
Sunflower seed oil	92.8	99.8	95.9	98.9	93.3	95.1
Olive oil	94.2	100	97.4	100	92.2	93.6
Hempseed oil 1	92.4	100	95.9	101	94.9	96.4
Hempseed oil 2	93.0	101	95.8	101	94.4	96.0
Sesame oil	93.0	100	97.2	100	93.8	95.0
Corn oil	91.9	100	98.8	102	93.2	94.9
Foods/beverages						
Frosted brownie	102	100	98.8	98.6	92.3	95.3
Milk chocolate bar	107	110	101	99.7	96.3	99.6
Dark chocolate bar	101	102	98.3	96.2	94.3	95.7
Hard candy	100	105	95.5	103	99.3	102
Honey	99.5	106	94.3	99.3	95.7	96.8
Strawberry preserves	92.6	101	102	109	101	102
Whipped butter	89.8	95.1	95.5	97.7	96.5	99.2
Vegetable oil spread	94.8	102	92.2	96.9	96.8	96.9
Cream cheese spread	94.8	99.8	91.8	93.8	96.7	97.2
Powdered cocoa mix	101	105	99.8	101	96.8	97.9
Ground coffee	93.8	97.8	96.0	101	97.0	93.2
Flavored coffee beverage	90.2	100	94.8	97.1	97.7	97.1
Flavored green tea beverage	91.7	102	96.3	98.7	98.6	102
Creamy peanut butter	91.0	93.5	98.3	99.7	95.2	96.4
Loose English tea	93.5	99.4	97.5	100	99.7	100
Greek yogurt	93.0	99.5	99.3	102	95.3	94.3
Pancake dry mix	96.4	98.7	95.6	98.4	98.4	93.2
Tapioca pudding dry mix	102	107	102	105	94.1	94.9
Flavored gelatin dry mix	105	108	103	106	95.7	96.3
Topicals						
Hand/body lotion	96.3	99.5	92.3	97.7	89.3	84.7
Pharmaceutical cream base	94.5	100	94.2	95.7	89.8	87.1
Trolamine salicylate cream	98.4	101	95.9	98.8	97.8	94.4
Menthol pain gel	97.3	100	93.9	95.7	90.3	87.7
Menthol/methylsalicylate balm	95.2	100	98.9	102	90.7	85.8
Herbal pain cream	87.8	93.5	95.8	100	92.5	90.5
Camphor/menthol ointment	96.4	102	94.6	101	94.9	88.2
Oral OTC pharmaceuticals						
Acetaminophen tablets	95.4	101	98.3	99.7	90.8	90.5
Ibuprofen tablets	93.8	99.4	96.2	99.8	91.5	91.5
Acetaminophen suspension	97.7	98.9	98.3	99.4	92.2	90.8
Ibuprofen soft gels	98.6	98.7	96.3	96.6	91.5	91.7
Acetaminophen/caffeine liquid	98.9	103	99.0	99.0	91.6	90.6
Overall average % recovery	95.6	101	96.9	100	94.5	94.4

aqueous sample matrices such as beverages. Depending on the sample weights and extraction volumes, the water in these matrices mixed with the ethanol extractant to produce a finished extract with >90% ethanol. Quantitative recoveries (90–102%) were obtained.

While no formal stability studies were conducted, sample extracts using 95% ethanol or pure ethanol were found to be stable for all product types. Sample extracts were placed in amber HPLC vials and queued in an autosampler under ambient conditions. No evidence of conversion of the carboxylated cannabinoids (CBDA, THCA) to their decarboxylated forms (CBD, d9THC), or of conversion of d9THC to CBN, was observed in the analytical time frames (extracts were analyzed up to three days after preparation when in a sample queue).

3.3. Method validation

Method validation consisted of evaluating precision and accuracy. Precision was assessed by calculating relative standard deviation (%RSD) for replicate analyses of commercial cannabis products, or other cannabis matrices. Accuracy was primarily determined by measuring the recoveries of cannabinoid spikes from numerous commercial cannabis products, and from a series of consumer products which we spiked to mimic the commercial products. No certified cannabis reference materials were available for testing, but both 2.5 and 5.0 mg prescription dronabinol capsules were analyzed to further test method accuracy. The 2.5 and 5.0 mg dronabinol capsules were from different generic drug manufacturers, with both formulas including glycerin and sesame oil as excipients. Excellent accuracy was obtained, with the d9THC assay results equivalent to 95% and 101% of the labeled amounts for the 2.5 and 5.0 mg capsules, respectively. The results of extensive precision and accuracy testing are detailed below. The authors' inhouse laboratory targets for precision were %RSDs of 20% or less, and accuracy were 80–120% recovery of the spiked or labeled amounts.

Table 2 provides the analysis results for five THCA-rich cannabis plant bud products obtained from Colorado retail markets in 2017,

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as well as crude hash oils and fine kief which were prepared in the authors' laboratory (Section 2.2). No CBDA-rich varieties were available in the retail markets visited. The commercial plant buds were packaged in capped plastic product jars containing from 1.75 to 3.5 g total contents weight. The plant buds were thoroughly ground in a mortar prior to analysis with no further attempts to homogenize the materials, and extracted with pure ethanol. The crude hash oils and kief were extracted with 95% ethanol. The table lists assay and precision results for the cannabinoids THCA, d9THC, CBGA, CBDA, and CBN. THCA was by far the predominant cannabinoid in the commercial buds (ranging from 138 to 241 mg/g) with very small amounts of CBN (all less than 0.24 mg/g), consistent with fairly fresh plant material. Good assay precision was obtained over several orders of magnitude for the cannabinoid determined amounts (0.016–399 mg/g) with average %RSDs ranging from 2.5 to 6.1% across the five cannabinoids. As is typical for low level quantitation, the highest individual %RSDs (12 or 13%) were observed for the CBN assays which were near the LOQ (0.01 mg/g).

Table 3 provides the analysis results for seven concentrated, CBD-rich oral supplement syringe products obtained from internet web stores between 2014 and 2016. Six of the syringe products contained semisolid paste or gel like substances, and one syringe product contained a low viscosity liquid. Most of the products were labeled as "hemp oil" with additional label text indicating "mature stalk", "seed and stalk", "stem and stalk", or "aerial plant parts". The product contents were dispensed directly from the product syringes for analysis, and extracted with pure ethanol or 95% ethanol (see table). The table lists assay and precision results for the four cannabinoids CBD, d9THC, CBN, and CBDA. CBD amounts were high ranging from 144 to 350 mg/g. CBDA was either not detected or found in low amounts in the products indicating that the product contents were most likely subjected to a heated decarboxylation process during manufacture. Again, good assay precision was obtained over several orders of magnitude for the cannabinoid determined amounts (0.24–350 mg/g) with average % RSDs ranging from 2.5 to 5.5% across the cannabinoids. No THCA was detected in the products.

Table 4 provides assay, precision (%RSD), and accuracy (%spike/recovery) data for the five target cannabinoids in two Canadian sourced hempseed oils (one obtained in 2014 and the other in 2017). All extractions were conducted using 95% ethanol. Note that cannabinoid levels are typically low in hempseed oils, and all results are given in μg/g. Determined amounts of CBDA and CBD in the first hempseed oil were close to the specified LOQ (0.01 mg/g, equivalent to 10 μg/g) for this method, and determined amounts of THCA were below the LOQ in both hempseed oils. Two spiking levels (nominally 20 μg/g or 120–140 μg/g) were tested for each cannabinoid, which fall within the ranges of cannabinoid contents reported for hempseed oils [5,22]. Recoveries for both spiking levels averaged across the five target cannabinoids ranged from 93–96%. However, recoveries tended to be lower (83–88%) for THCA. Through all of the work with other edible oils and product matrices, hempseed oil was the most difficult matrix from which to extract low levels of THCA (less than 200 μg/g).

Table 5 provides assay results, precision (%RSD), and accuracy (%spike/recovery) data for CBD, d9THC, CBDA, and CBN in 28 cannabis based consumer products obtained between 2015 and 2017 from internet web stores. The products included oral supplements (oral/sublingual liquids/semisolid, tablets, capsules), vapes/eliquids, topicals, and foods or beverages. Many of the orals liquids were sold in dropper bottles, and were formulated with various edible oils (grapeseed, sunflower seed, aerial hemp, hempseed, liquid or virgin coconut, sesame), and may have also contained ethanol, glycerin, propylene glycol, and flavors. All products were extracted with 95% ethanol. CBD was declared as an ingredient on many of the product labels, and was frequently present in highest amount relative to the other cannabinoids. Interferences were not encountered in most cases, except for CBDA for which sometimes there was partial coelution with unknown interferents. These CBDA interferences were effectively eliminated by using the most selective wavelength for this compound (307 nm). For all cannabinoids, expert examination of the peak UV spectra in the product chromatograms was made, and cannabinoid identification was only confirmed if the spectra were consistent with the respective cannabinoid standard.

In the table, the products are listed in descending order of CBD content within each product category. CBD product levels spanned a broad range from 0.011 to 145 mg/g. If detected, the d9THC content ranged from 0.012 to 5.3 mg/g across the products. Good precision was obtained, with average %RSDs across products ranging from 1.7 to 4.0%. Spiking experiments were conducted for each of the quantified cannabinoids using a spiking level which approximated the amounts determined in the assay. Excellent recoveries were obtained, with the averages across products ranging from 96 to 100% for each cannabinoid. THCA was only detected in one of the products listed in Table 5, the topical balm with a determined content of 0.019 mg/g (3.3%RSD). Another consumer product which is not listed in the table consisted of an oral liquid tincture which contained only THCA and d9THC with determined amounts of 0.14 mg/g (0.35% RSD) and 0.026 mg/g (4.7% RSD), respectively. Spike recovery for THCA in the topical balm was 100% (0.020 mg/g spiking level) and in the oral tincture was 102% (0.15 mg/g spiking level).

In order to further challenge the method's broad applicability, additional spiking experiments were conducted with still more products/sample matrices. Table 6 provides accuracy (%spike/recovery) data for all five target cannabinoids in 39 negative product matrices, and two Canadian hempseed oils which contained low cannabinoid levels (see Table 4) prior to spiking. Most of the sample matrices were consumer products obtained from local grocery stores or pharmacies, and included edible oils, foods and beverages, topicals, and oral OTC pharmaceuticals. As a whole, these matrices are physically and chemically diverse, and may contain fats, oils, sugars, starches, food proteins, flavors, water, or various topical/pharmaceutical excipients. The edible oils include several which were encountered by the authors in commercial cannabis containing oral supplements.

The products/matrices were spiked with three different types of concentrated cannabis extracts (Section 2.3): (1) an ethanolic THCA-rich extract which was prepared in the authors' laboratory from fresh THCA-rich cannabis bud material; (2) an ethanolic d9THC-rich extract which was prepared in the authors' laboratory from decarboxylated cannabis bud material; (3) an ethanolic CBD-rich extract which was prepared in the authors' laboratory from a commercial decarboxylated hemp oil (the authors' analysis of the CBD content of the product was ca. 160 mg/g). By using whole cannabis extracts for spiking, this set of experiments more closely mimics manufactured cannabis consumer products because the extracts contain additional cannabinoids and a host of other cannabis plant components which extract into ethanol. Another goal was to achieve somewhat higher spiking levels, especially for THCA and d9THC. To this end, spike recoveries were only measured for the two cannabinoids which were most prominent in the spiking extracts, i.e. THCA and d9THC for the first spiking set, d9THC and CBN for the second spiking set, and CBD and CBDA for the third spiking set. For brevity, spiking levels for each individual matrix are not listed in the table. Rather, the ranges of spiking levels across the 41 matrices are listed, and were in the range 10–20 mg/g for the predominant cannabinoid and 0.4–5.8 mg/g for the second cannabinoid in each set. All individual spike recoveries fell within the range 85–110%. The recoveries for the five target

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compounds averaged across all 41 matrices were excellent, ranging from 94 to 101%. The low levels of cannabinoids already present in the two hempseed oils prior to spiking were accounted for in the spike recovery calculations. None of the additional product actives (trolamine salicylate, acetaminophen, ibuprofen, etc.) from the topical or oral pharmaceuticals caused interferences for the cannabinoids.

3.4. Method LODs and LOQs

The method limit of quantitation (LOQ) was taken as 10 μg/g (0.010 mg/g) in all cannabis-related samples including finished products. For UV detection, limits of detection (LODs) are specific to the response slope at a given wavelength. For the five target cannabinoids, the slopes ranged from a low of 18 for CBD at 240 nm up to 130 for CBN at 220 nm (Table 1). The concentration based limit of detection (LOD) for the injected standard solution was established as 0.20 μg/ml, and was based on the lowest response compound (CBD) yielding the most conservative value. The concentration based LOD was confirmed by injection of low level standards and in practice with real sample extracts. The concentration based LOQ was taken as 2.5 times this value, or 0.50 μg/ml.

For LOQ calculations in finished products, a 1 g sample weight and 5.0 ml extraction volume were taken to represent a fairly concentrated sample extract. While this calculation would yield a finished products LOQ value of 2.5 μg/g, the LOQ was specified at the more conservative 10 μg/g value based on other considerations. These include the intended use of the method for a series of cannabinoids in a tremendous variety of product types and matrices, and the tendency for poorer precision at low analyte levels in complex matrices. The specified LOQ does not preclude the possibility of achieving lower limits for specific cannabinoids in specific product or matrix types by various means, such as the use of preconcentration of product extracts.

As demonstrated in this work, the method sensitivity was well suited for analysis of commercial cannabis products for which target cannabinoid levels were at or well above the current method LOQ. Furthermore, certain legal limits or definitions based on the cannabinoid content(s) in cannabis materials are currently in use throughout the world. Maximum d9THC contents in the range 0.1–0.3%w/w [23–26] are currently specified in the EU, Australia, US, and Canada for plant materials to meet a legal definition of "hemp". By contrast, maximum allowable levels of d9THC in hemp seeds/hempseed oils for consumption are much lower, ranging from zero to 20 μg/g [24,26,27] depending on the governing country. The current method LOQ falls well below the sensitivity needed to classify hemp vs. marijuana plants, but may not be suitable for the stricter d9THC limits in hemp seeds/hempseed oils. As discussed for the GC–MS procedure [1], this method has not been evaluated for the determination of cannabinoids in washed or unwashed hemp seeds, or hemp seed kernels.

4. Conclusion

The HPLC-DAD method presented herein addresses the need for a reliable quantitative procedure for the determination of the cannabis cannabinoids in a wide range of product and sample types. The method is applicable to foods, candies, beverages, topicals, vapes/eliquids, oral liquid supplements, pastes, capsules, tablets, cannabis plants, and plant extracts or preparations. In earlier work (data not presented), spiking experiments for CBC in 10 products and CBG in three products were conducted, and quantitative recoveries were obtained. Although the extensive validation studies were limited to the five cited cannabinoids, this method may be suitable for the quantitation of other cannabis cannabinoids, with additional validation conducted for the specific compounds of interest.

Declaration of interests

None.

References

[1] L.A. Ciolino, T.L. Ranieri, A.M. Taylor, Commercial cannabis consumer products Part 1: GC–MS qualitative analysis of cannabis cannabinoids, Forensic Sci. Int. xxxxxx.
[2] M.R. Peace, K.E. Butler, C.E. Wolf, J.L. Poklis, A. Poklis, Evaluation of two commercially available cannabidiol formulations for use in electronic cigarettes, Front. Pharmacol. 29 (2016) 1–6.
[3] O. Aizpurua-Olaizola, J. Omar, P. Navarro, M. Olivares, N. Etxebarria, A. Usobiaga, Identification and quantitation of cannabinoids in cannabis sativa L. plants by high performance liquid chromatography–mass spectrometry, Anal. Bioanal. Chem. 406 (2014) 7549–7560.
[4] M. Wang, Y.H. Wang, B. Avula, M.M. Radwan, A.S. Wanas, Z. Mehmedic, J. van Antwerp, M.A. ElSohly, I.A. Khan, Quantitative determination of cannabinoids in cannabis and cannabis products using ultra-high performance supercritical fluid chromatrography and diode array/mass spectrometric detection, J. Forensic Sci. 62 (2017) 602–611.
[5] C. Citti, B. Pacchetti, M.A. Vandelli, F. Forni, G. Cannazza, Analysis of cannabinoids in commercial hemp seed oil and decarboxylation kinetics studies of cannabidiolic acid (CBDA), J. Pharm. Biomed Anal. 149 (2018) 532–540.
[6] B. Patel, D. Wene, Z. Fan, Qualitative and quantitative measurement of cannabinoids in cannabis using modified HPLC/DAD method, J. Pharm. Biomed. Anal. 146 (2017) 15–23.
[7] V. Brighenti, F. Pellati, M. Steinbach, D. Maran, S. Benvenuti, Development of a new extaction technique and HPLC method for the analysis of non-psychoactive cannabinoids in fibre-type cannabis sativa L. (hemp), J. Pharm. Biomed. Anal. 143 (2017) 228–236, doi:http://dx.doi.org/10.1016/j.jpba.2017.05.049 [Epub ahead of print].
[8] W. Peschel, Quality control of traditional cannabis tinctures, Sci. Pharm. 84 (2016) 567–584.
[9] W. Gul, S.W. Gul, M.M. Radwan, A.S. Wanas, Z. Mehmedic, I.I. Khan, M.H. Sharaf, M.A. ElSohly, Determination of 11 cannabinoids in biomass and extracts of different varieties of cannabis using high-performance liquid chromatography, J. AOAC Int. 98 (2015) 1523–1528.
[10] W. Peschel, M. Politi, ^{1}HNMR and HPLC/DAD for cannabis sativa L. chemotype distinction, extract profiling, and specification, Talanta 140 (2015) 150–165.
[11] R. Vandrey, J.C. Raber, M.E. Raber, B. Douglass, C. Miller, M.O. Bonn-Miller, Cannabinoid dose and label accuracy in edible medical cannabis products, JAMA 313 (2015) 2491–2493.
[12] L. Ambach, F. Penitschka, A. Broillet, S. Konig, W. Weinmann, W. Bernhard, Simultaneous quantification of delta-9-THC, THC-acid A, CBN and CBD in seized drugs using HPLC-DAD, Forensic Sci. Int. 243 (2014) 107–111.
[13] B. De Backer, B. Debrus, P. Lebrun, L. Theunis, N. Dubois, L. Decock, A. Verstraete, P. Hubert, C. Charlier, Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material, J. Chromatogr. B 877 (2009) 4115–4224.
[14] Y.H. Wang, B. Avula, M.A. ElSohly, M.M. Radwan, M. Wang, A.S. Wanas, Z. Mehmedic, I.A. Khan, Quantitative determination of d9-THC, CBG, CBD, their acid precursors and five other neutral cannabinoids by UHPLC-UV-MS, Planta Med. 84 (2018) 260–266.
[15] C. Citti, G. Ciccarella, D. Braghiroli, C. Parenti, M.A. Vandelli, G. Cannazza, Medicinal cannabis: principal cannabinoids concentration and their stability evaluated by a high performance liquid chromatography coupled to diode array and quadrupole time of flight mass spectrometry method, J. Pharm. Biomed. Anal. 128 (2016) 201–209.
[16] E.A. Ibrahim, W. Gul, S.W. Gul, B.J. Stamper, G.M. Hadad, R.A.A. Salam, A.K. Ibrahim, S.A. Ahmed, S. Chandra, H. Lata, M.A. Radwan, M.A. EhSohly, Determination of acid and neutral cannabinoids in extracts of different strains of cannabis sativa using GC-FID, Planta Med. 84 (2018) 250–259.
[17] R. Mechoulam, L. Hanus, Cannabidiol: an overview of some chemical and pharmacological aspects. Part 1: chemical aspects, Chem. Phys. Lipids 121 (2002) 35–43.
[18] R. Mechoulam, Marihuana chemistry, Science 168 (1970) 1159–1165.
[19] O. Zoller, P. Rhyn, B. Zimmerli, High-performance liquid chromatographic determination of d9-tetrahydrocannabinol and the corresponding acid in hemp containing foods with special regard to the fluorescence properties of d9-tetrahydrocannabinol, J. Chromatogr. A 872 (2000) 101–110.
[20] J.M. Parker, M.L. Borke, L.H. Block, T.G. Cochran, Decomposition of cannabidiol in chloroform solution, J. Pharm. Sci. 63 (1974) 970–971.
[21] J.W. Fairbairn, J.A. Liebmann, M.G. Rowan, Stability of cannabis and its preparations, J. Pharm. Pharmacol. 28 (1976) 1–7.
[22] M. Petrović, Ž. Debeljak, N. Kezić, P. Džidara, Relationship between cannabinoids content and composition of fatty acids in hempseed oils, Food Chem. 170 (2015) 218–225.
[23] European Monitoring Centre for Drugs and Drug Addiction, Cannabis legislation in Europe: an overview, Publication Office of the European Union, Luxembourg, 2017.

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[24] Therapeutic Goods Administration, Department of Health, Australia, Scheduling delegate's final decisions: Cannabis, (2017) . May https://www.tga.gov.au/book-page/11-cannabis.
[25] US. Department of Agriculture, Drug Enforcement Administration, Food and Drug Administration, Statement on Principles of Industrial Hemp. Federal Register Vol. 81 No. 156 (August 12, 2016) 53395–53396.
[26] Minister of Justice, Government of Canada, Industrial Hemp Regulations (SOR/98-156), current to 2018-01-18.
[27] Drug Enforcement Administration, Exemption from control of certain industrial products and materials from the cannabis plant. Federal Register Vol. 68 No. 55 (March 21, 2003) 14119–14126.

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