APPLICATION NOTE

Liquid Chromatography

Author:

Wilhad M. Reuter

PerkinElmer, Inc.
Shelton, CT

Cannabinoid Monitoring in Dried Cannabis Flowers by HPLC-PDA

Introduction

Cannabis sativa, from which marijuana is derived, has been a source of medicinal, commercial and recreational commodities for centuries. Currently, 29 states and the District of Columbia (DC) permit the medicinal use of marijuana. Eight of these states, Alaska, California, Colorado, Massachusetts, Nevada, Oregon and Washington, as well as DC, also allow for some form of limited recreational use. Although the U.S. Department of Justice and the Drug Enforcement Administration (DEA) do not officially condone either medicinal or recreational use of marijuana,[1] the indicated states are setting a precedent that could pose a significant challenge for the federal government to reverse without considerable difficulty.

Among the supporting states, there is a rapid expansion in the cultivation of marijuana and in the labs that are focused on processing and analyzing marijuana. To assure the quality, safety and potency of marijuana products, reliable analytical procedures are pivotal for the quantitative analysis of the cannabinoids and terpenes, as well as any pesticides that may be absorbed during cultivation.

Naturally occurring cannabinoids, the main biologically active component of the cannabis plant, form a complex group of closely related compounds, of which 113 are known and 70 are well described. Of these, the primary focus has been on Δ^9-tetrahydrocannabinol (THC), as the primary active ingredient due to its pharmacological and toxicological characteristics, upon which strict legal limits have been enforced.[2] However, processing labs must also focus on Δ^9-tetrahydrocannabinolic acid (THC-A), as it is the naturally occurring precursor to THC and is readily decarboxylated to THC via the drying and/or heating of cannabis.

This application describes an analytical method for the chromatographic separation and quantitative monitoring of seven primary cannabinoids (structures shown in Figure 1), including THC and THC-A, in cannabis extracts by HPLC with PDA detection.

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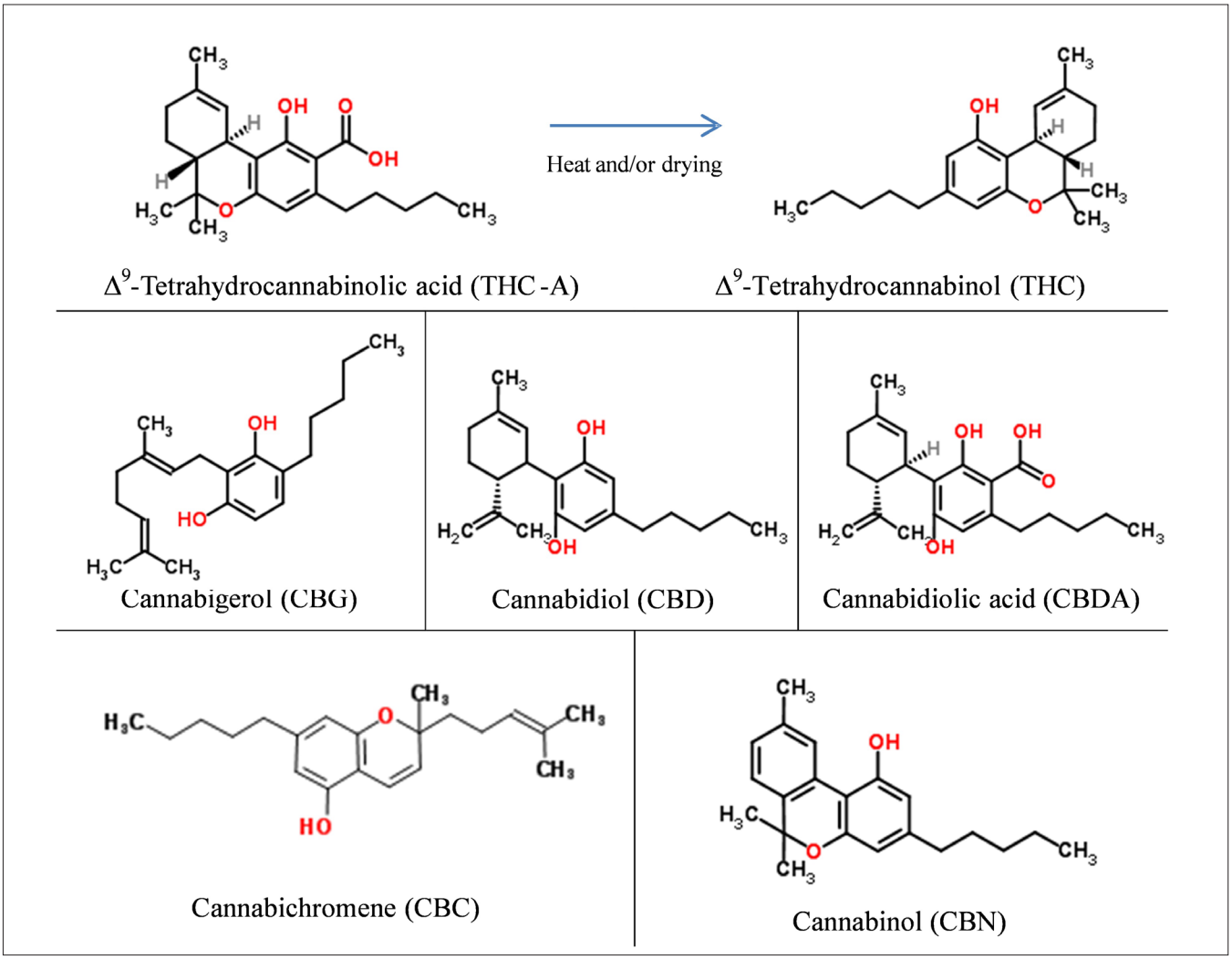

Figure 1. Chemical structure of the seven cannabinoids analyzed in this study.

Experimental

Hardware/Software

A PerkinElmer Flexar™ HPLC system was used, including a quaternary pump, autosampler with Peltier cooling, column heater and PDA (photodiode array) detector. A PerkinElmer Brownlee™ SPP C18, 2.7 µm, 3.0 x 150 mm column was used for all analyses (PerkinElmer, Shelton, CT, USA). All instrument control and data analysis/processing was performed via the PerkinElmer Chromera® CDS software.

Method Parameters

The LC method parameters are shown in Table 1.

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade and filtered via 0.45-µm filters. All diluents were 80:20 methanol/water.

1 mg/mL (in 1 mL of methanol) standards of Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinolic acid (THC-A), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabigerol (CBG), cannabinol (CBN) and cannabichromene (CBC) were obtained from Sigma-Aldrich®, Inc (Allentown, PA) and the Restek® Corporation (Bellefonte, PA).

Table 1. HPLC Method Parameters.

Column	PerkinElmer Brownlee SPP C18, 2.7 µm, 3.0 x 150 mm (Part# N9308411)
Mobile Phase	Solvent A: Water with 0.1% formic acid Solvent B: Acetonitrile with 0.1% formic acid Solvent Program:

Step	Step Time (min.)	Flow Rate (mL/min.)	%A	%B
0 (Equil)	4.5	1.0	30.0	70.0
1	4.0	1.0	5.0	95.0
2	2.0	1.0	5.0	95.0

Analysis Time	6.0 min.; equilibration time: 4.5 min.
Flow Rate	1.0 mL/min.
Pressure	4600 psi/317 bar maximum
Oven Temp.	40 °C
PDA Detection	Wavelength: 228 nm
Injection Volume	10 µL
Sampling (Data) Rate	10 pts./sec
Diluent	80:20 methanol/water

A 100-µg/mL working standard of the six standards was prepared by adding the entire 1 mL of each standard to a 10-mL volumetric flask and filling to mark with the 80:20 methanol/water diluent. This also served as the level-6 calibration standard. 50, 20, 5, 1 and 0.5-µg/mL calibration standards were then prepared via serial dilution of the working standard.

Two 5-mL previously-prepared cannabis extract samples were obtained from a cannabis lab in Oregon. These were prepared by first adding 10 mL of methanol to 1 gram of ground-up dried cannabis flowers, vortexing for three minutes, filtering 2 mL of the supernatant through a 0.45-µm filter and then diluting the filtered supernatant 3-fold with methanol. This resulted in an overall 30-fold concentration dilution with respect to the initial product. The extract samples were individually labeled Samples 1 and 2. Upon receipt, each extract was further diluted 50-fold with diluent and refrigerated until further use. The considerable dilution was required to stay within the concentration range of the calibrants (0.5-100 µg/mL). As cannabinoid standards are only commercially (and legally) obtainable at 1 mg/mL, once prepared as part of the calibration mix, the individual analyte concentration at the highest level was 100 µg/mL. This level is considerably lower than that expected for some cannabinoids in undiluted cannabis extract, particularly for THC-A; hence, the significant dilution requirement of the samples. Note: depending on individual cannabinoid content, some cannabis extract samples, particularly those with higher THC-A content, may require further dilution.

All calibrants and prepared samples were subsequently filtered through 0.45-µm filters and then injected on column. The results reflect the averaged triplicate injections for all calibrants and samples.

Results and Discussion

Figure 2 shows the chromatogram of a 100-µg/mL standard mixture containing the seven cannabinoids, all well resolved in under five minutes.

As shown in Figure 3, chromatographic repeatability was shown via ten replicate injections of the 100-µg/mL standard mixture. The retention time %RSD for all peaks was less than 0.05%. This confirms the reliable performance of this chromatographic method, which is essential for ensuring the integrity of the results for the medicinal cannabis industry, as confident product composition is pivotal in helping to assure the safety of released products.

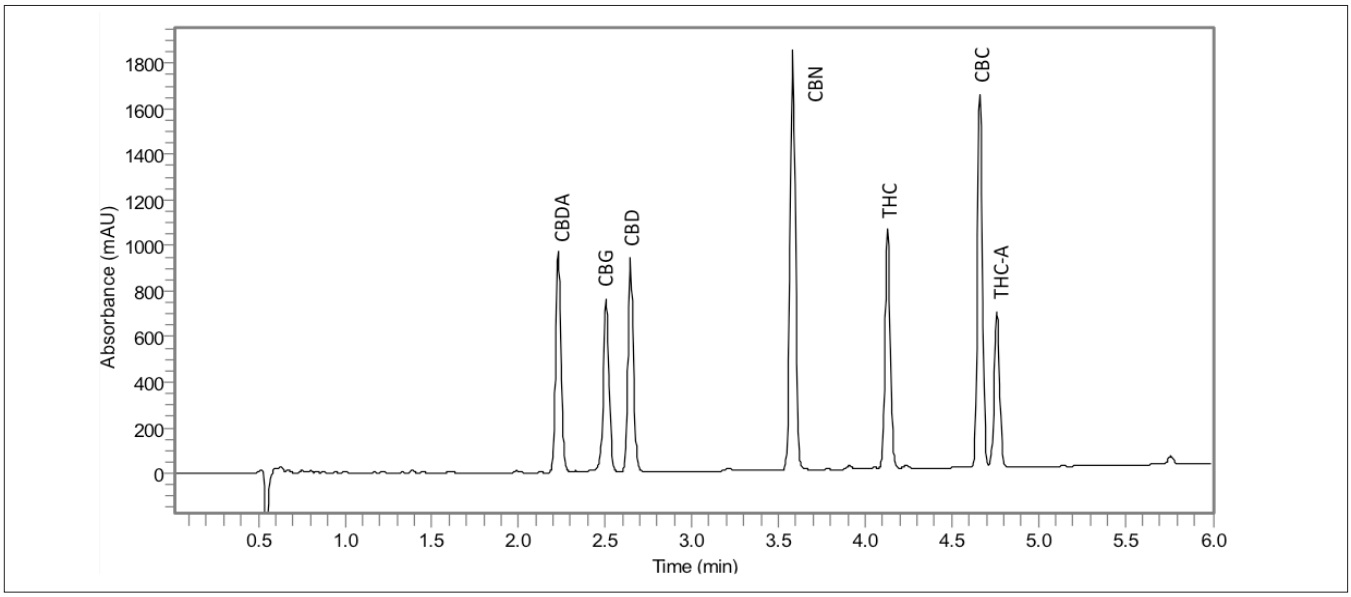

Figure 2. LC chromatogram showing separation of the seven cannabinoids in the Level-6 standard; λ = 228 nm.

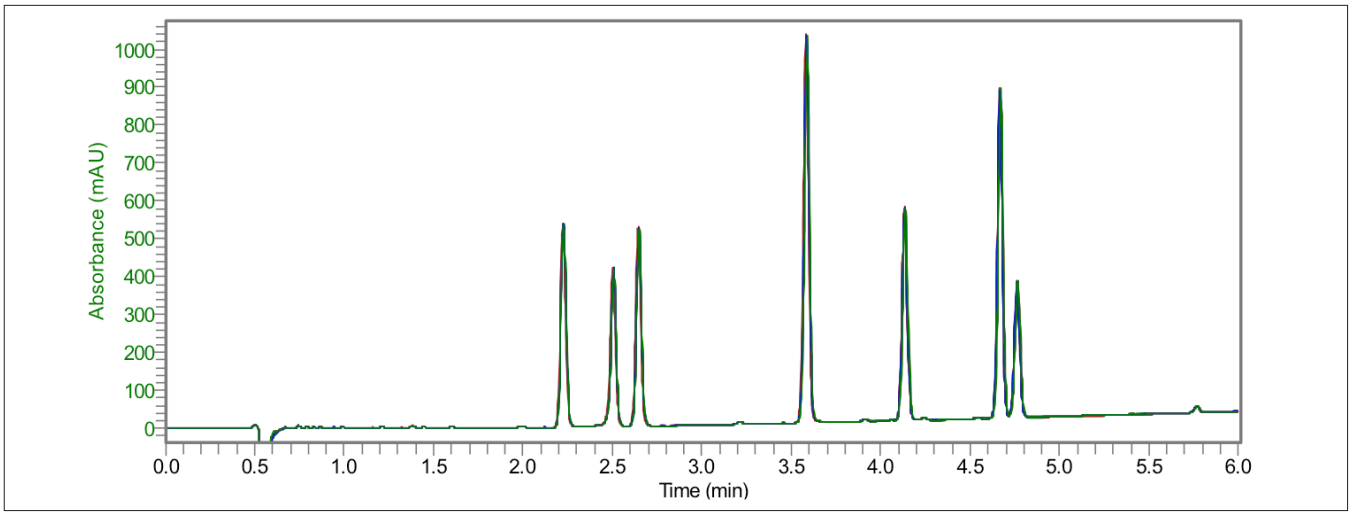

Figure 3. Overlay of ten replicates of the Level-6 (100-µg/mL) standard.

Linearity was determined for all seven cannabinoids. Representative 6-level linearity plots for THC and THC-A, are shown in Figure 4a and 4b, respectively. The R^2 values for all seven cannabinoids were above 0.998.

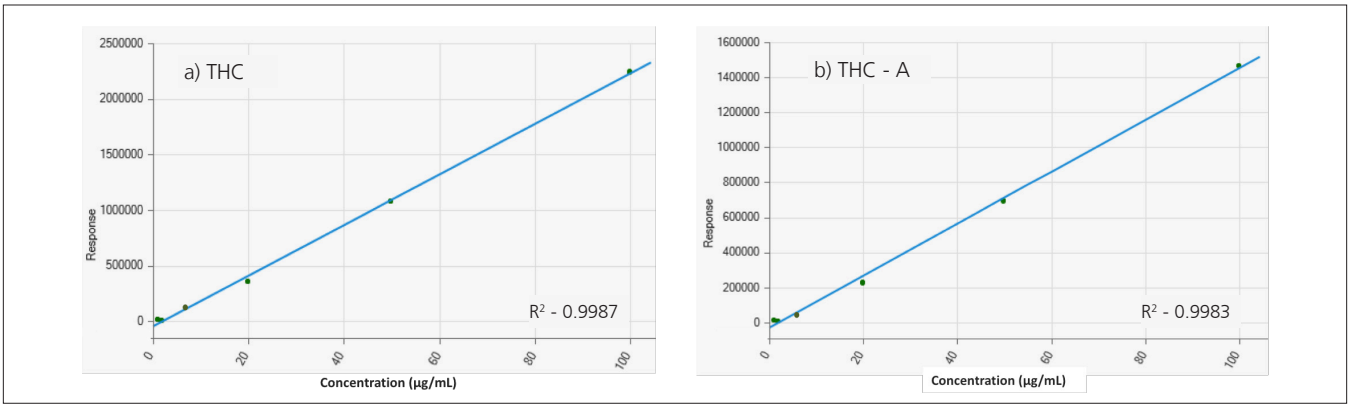

Figure 4. Linearity plots for THC (a) and THC-A (b); concentration range: 0.5 - 100 µg/mL in 80:20 methanol/water.

As listed in Table 2, LOQ (limit of quantitation) levels were established for each cannabinoid, based upon their averaged Level-1 calibration standard response. The LOQs (≥10 S/N) were <0.1 µg/mL for all analyzed cannabinoids. As cannabinoids are typically tested for high-end potency, these levels are well below the current concentrations of interest for the primary cannabinoids being analyzed.

Figures 5 and 6 show the chromatographic results for Samples 1 and 2, respectively. Comparing the two chromatographic results, though none of the samples showed any detectable levels of CBN, their analyte profiles were otherwise quite different from one another, Sample 1 was observed to contain considerably more CBDA, while Sample 2 showed appreciably more THC and THC-A. Though both samples showed a few unknown matrix components, these were all well resolved from the target analytes and considered not to interfere with analyte quantitation. Therefore, these were not further investigated in this study.

Table 2. LOQs for all seven cannabinoids; PDA at 228 nm.

Cannabinoid	LOQ (µg/mL)
Cannabidiolic acid (CBDA)	0.06
Cannabigerol (CBG)	0.08
Cannabidiol (CBD)	0.06
Cannabinol (CBN)	0.03
Δ^9-Tetrahydrocannabinol (THC)	0.06
Cannabichromene (CBC)	0.04
Δ^9-Tetrahydrocannabinolic acid (THC-A)	0.09

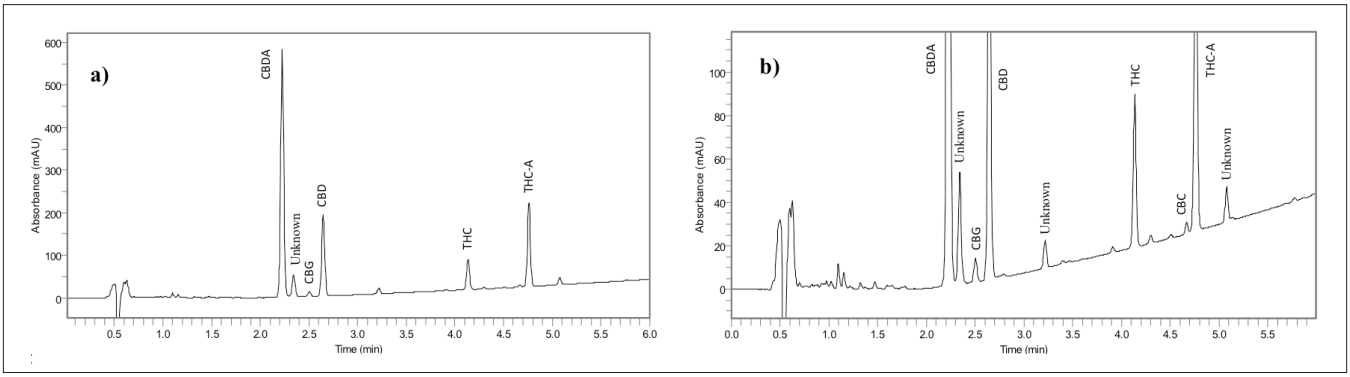

Figure 5. Chromatographic results for Sample 1; a) full view; b) expanded view.

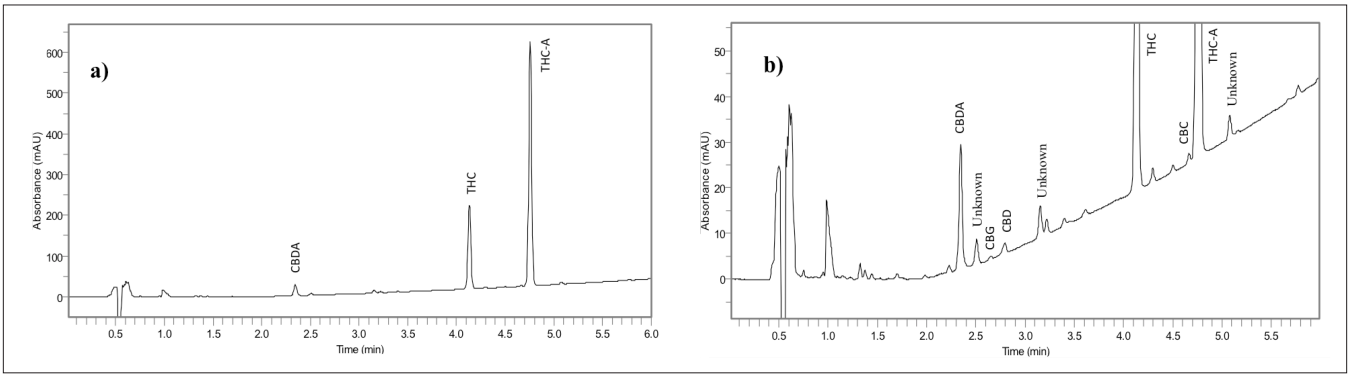

Figure 6. Chromatographic results for Sample 2; a) full view; b) expanded view.

Table 3 shows the calculated concentrations (µg/mL) for the seven cannabinoids found in each of the two sample extracts. Sample 1 was found to contain a significantly higher CBDA concentration and, thus, points toward an outlier-type cannabis strain, one that may peak considerable interest for possible medicinal purposes. Sample 2 showed the highest concentration (13.08%) of THC-A, setting it apart from the other sample and making it a better candidate for possible recreational purposes.

Conclusion

This work has demonstrated the effective chromatographic separation and quantitation of seven cannabinoids, including THC and THC-A, in cannabis extracts using the PerkinElmer Flexar™ HPLC system with a photodiode array detector. The method provided exceptional chromatographic repeatability and provided LOQs well below the current concentrations of interest for the primary cannabinoids being analyzed.

Table 3. Cannabinoid concentrations found in each of the two cannabis extracts.

	Analytes	Extract Concentration (µg/mL)*	Extract Concentration (Wgt./Vol. %)*	Concentration in Actual Dried Cannabis Sample (Wgt./Wgt. %)**
Sample 1	CBDA	3150	0.315	9.45
	CBG	175	0.018	0.54
	CBD	1100	0.110	3.30
	CBN	ND	ND	ND
	THC	435	0.044	1.32
	CBC	120	0.012	0.36
	THC-A	1500	0.150	4.5
Sample 2	CBDA	116	0.012	0.36
	CBG	142	0.014	0.42
	CBD	Trace	Trace	Trace
	CBN	ND	ND	ND
	THC	1059	0.106	3.18
	CBC	Trace	Trace	Trace
	THC-A	4359	0.436	13.08

* Accounting for 1/50 extract dilution; average of three replicates.
** Accounting for 30-fold dilution during initial sample extraction in lab of origin.
ND - Not detected

References

1. *DEA Position on Marijuana,* U.S. Department of Justice and the DEA, April 2013; dea.gov/docs/marijuana_position_2011.pdf.
2. "Cannabis oil vs hemp seed oil; Cannabis oil, CDB Oil, Medical Marijuana". http://cbd.org/cannabis -oil-vs-hemp-oil.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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