APPLICATION NOTE

Liquid Chromatography

Author:

Wilhad M. Reuter

PerkinElmer, Inc.
Shelton, CT

Analysis of Cannabinoids in Cannabis by UHPLC Using PDA Detection

Introduction

Cannabis sativa, from which marijuana is derived, has been a source of medicinal, industrial, and recreational commodities for centuries. Currently, 26 states (soon to become 29) and the District of Columbia (DC) permit the medicinal use of marijuana. Five of these states, Alaska, Colorado, Oregon, Washington and DC, allow for some form of limited recreational use and California, Massachusetts and Nevada will have joined by end of 2016. 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 which will likely be followed by others and will be a 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 number of labs that are focused on processing and analyzing marijuana. To assure both the quality and safety 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.

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Naturally occurring cannabinoids, the main biologically active components of the cannabis plant, form a complex group of closely related compounds, of which 70 are known and 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 a method for the chromatographic separation and quantitative monitoring of seven primary cannabinoids, including THC and THC-A, in cannabis extracts by UHPLC combined with PDA detection. Figure 1 shows the chemical structures of the analyzed cannabinoids.

Experimental

Hardware/Software

A PerkinElmer UHPLC system was used, including a 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, analysis and data processing was performed via associated 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).

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.

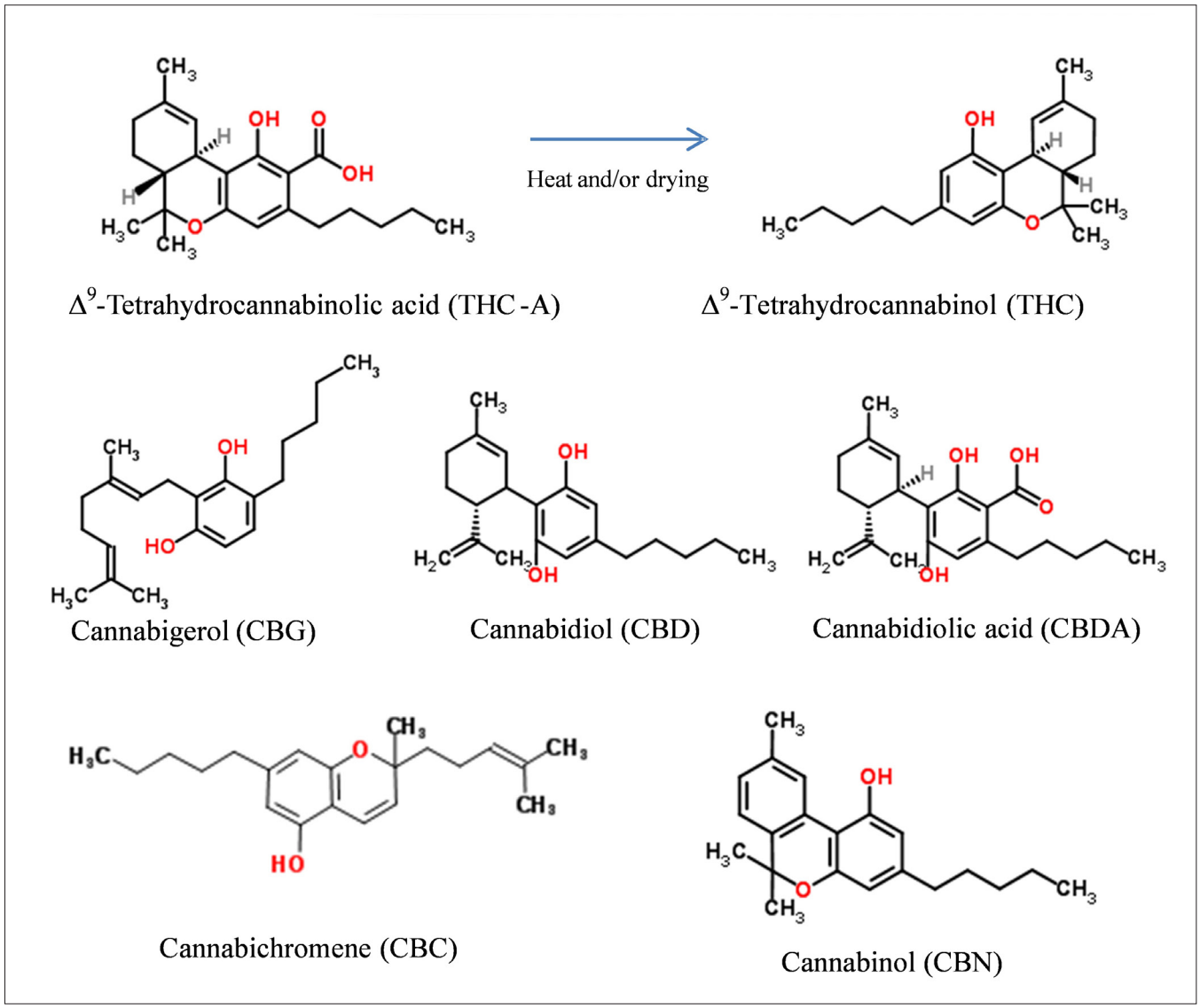

Figure 1. Chemical structure of the seven cannabinoids analyzed in this study.

Table 1. LC Method Parameters.

Column	PerkinElmer Brownlee SPP C18, 2.7 µm, 3.0 x 150 mm (Part# N9308411)
Mobile Phase	Solvent A: 0.1% formic acid in water Solvent B: 0.1% formic acid in acetonitrile Solvent program:

	Time (min)	Flow Rate (mL/min)	%A	%B
1	Initial	1.0	30.0	70.0
2	4.0	1.0	5.0	95.0
3	6.0	1.0	5.0	95.0
4	6.1	1.0	30.0	70.0

Analysis Time	6.2 min; equilibration time: 4.0 min
Flow Rate	1.0 mL/min
Pressure	~5900 psi/407 bar maximum
Oven Temperature	40 °C
PDA Detection	Wavelength: 228 nm
Injection Volume	4 µL
Sampling (Data) Rate	10 pts/sec
Diluent	80:20 methanol/water

Four 5-mL prepared cannabis extract samples, all in methanol, were obtained from the 3B Analytical lab in Portland Oregon. These were prepared by first adding 10 mL of methanol to one gram of ground-up dried cannabis flowers, vortexing for three minutes, filtering 2 mL of the supernatant through 0.45-µm filters 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 A, B, C and D. Upon receipt, each was further diluted 100-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 commercially (and legally) only 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.

All calibrants and samples were subsequently filtered through 0.45-µm filters and then injected (4 µL).

Results and Discussion

Figure 2 shows the chromatogram of a standard mixture containing the seven cannabinoids, all separated in under four minutes. The gradient ramp was due to the relatively low concentrations of the high-end calibrants; again, limited by the obtainable concentrations of the standards.

As shown in Figure 3, chromatographic repeatability was shown via ten replicate injections of the 100-ppm 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 medicinal cannabis analysis. In this industry, confident product composition is pivotal in helping to assure the safety of released products.

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 0.999.

As listed in Table 2, LOQ (limit of quantitation) levels were established for each cannabinoid, based upon calibration standard responses. The LOQs (≥10 S/N) were <0.26 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.

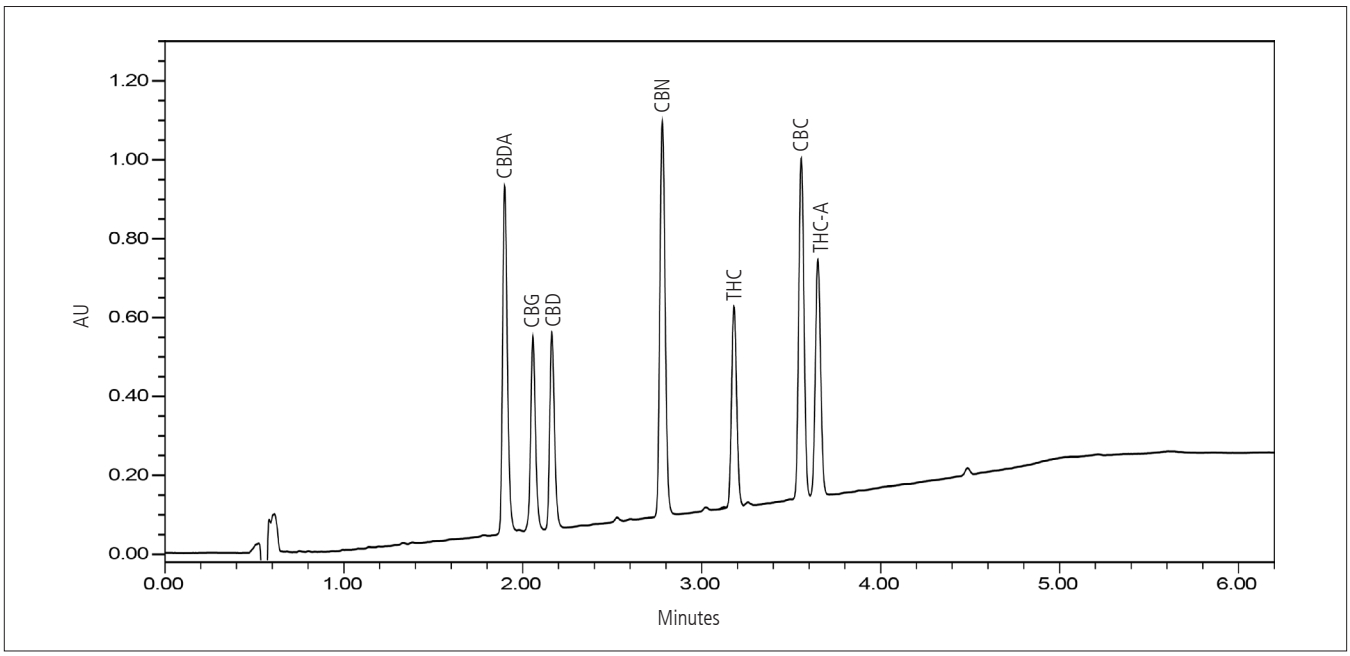

Figure 2. LC chromatogram showing separation of the seven cannabinoids in the 100-ppm standard; λ = 228 nm.

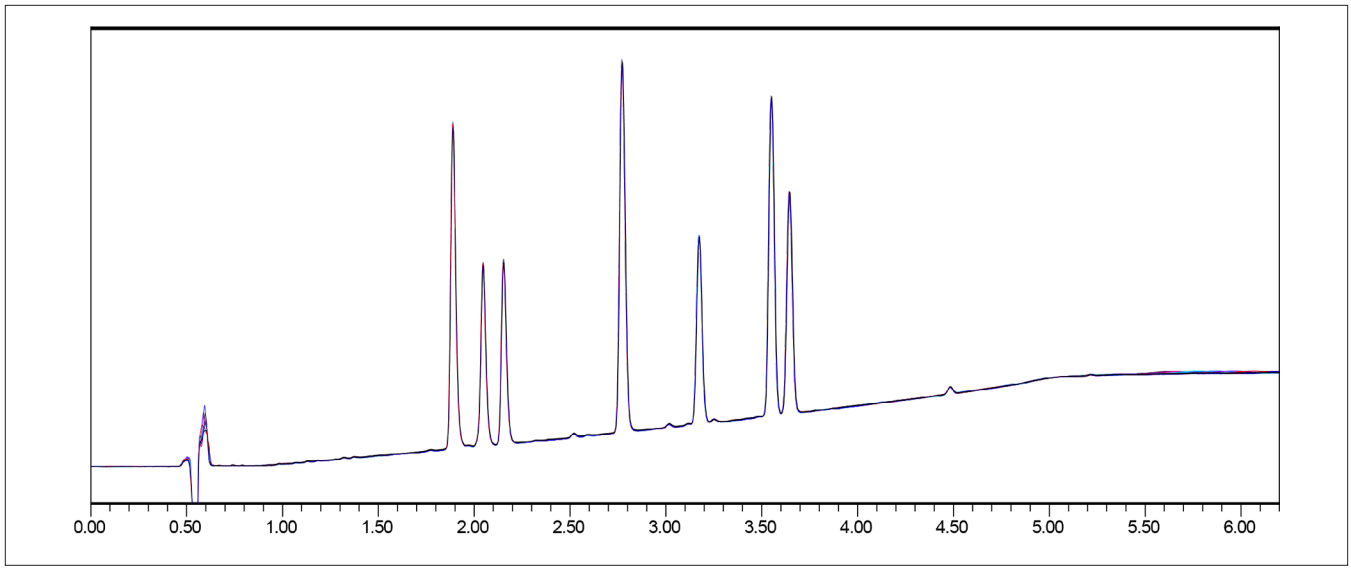

Figure 3. Overlay of ten replicates of the Level-6 (100-ppm) standard.

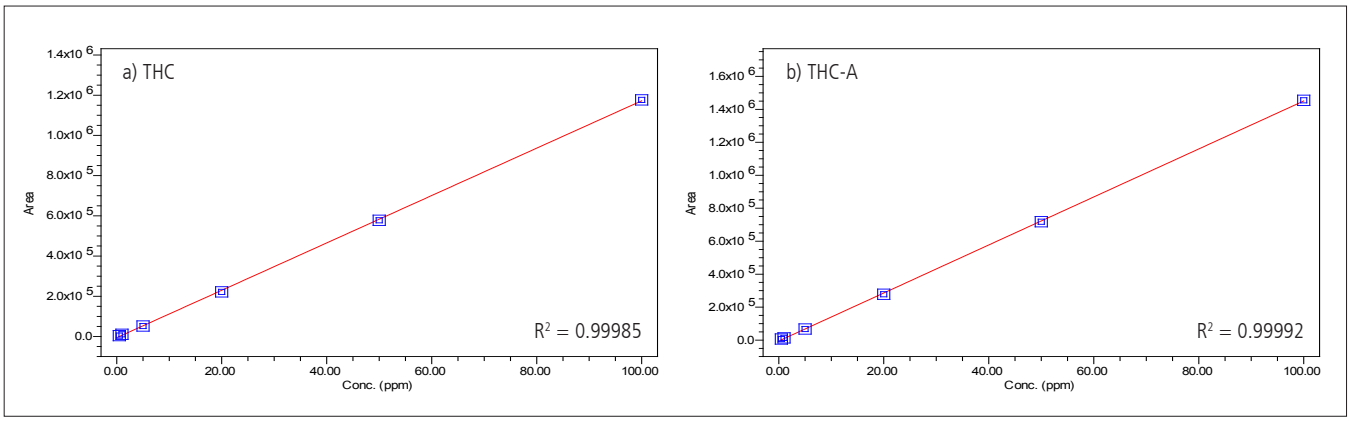

Figure 4. Linearity plots for THC (a) and THC-A (b); concentration range: 0.5-100 µg/mL in 80:20 methanol/water diluent.

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

Cannabinoid	LOQ (µg/mL)
Cannabigerol (CBG)	0.13
Cannabidiol (CBD)	0.26
Cannabidiolic acid (CBDA)	0.24
Cannabinol (CBN)	0.11
Δ^9-Tetrahydrocannabinol (THC)	0.23
Cannabichromene (CBC)	0.13
Δ^9-Tetrahydrocannabinolic acid (THC-A)	0.18

Figures 5-9 show the chromatographic results for Samples A through D, respectively. Comparing chromatograms, none of the samples showed any detectable levels of CBN and only Sample A showed any detectable amount of CBC.

Sample A appeared quite different from the rest, in that it contained a significantly greater proportion of CBDA than all the others, while containing significantly less THC and THC-A. It was also the only sample found to contain quantitatable amounts of CDA, as well as an unknown matrix component eluting on the backside of CBDA.

Otherwise, Samples B through D appeared quite similar to one another.

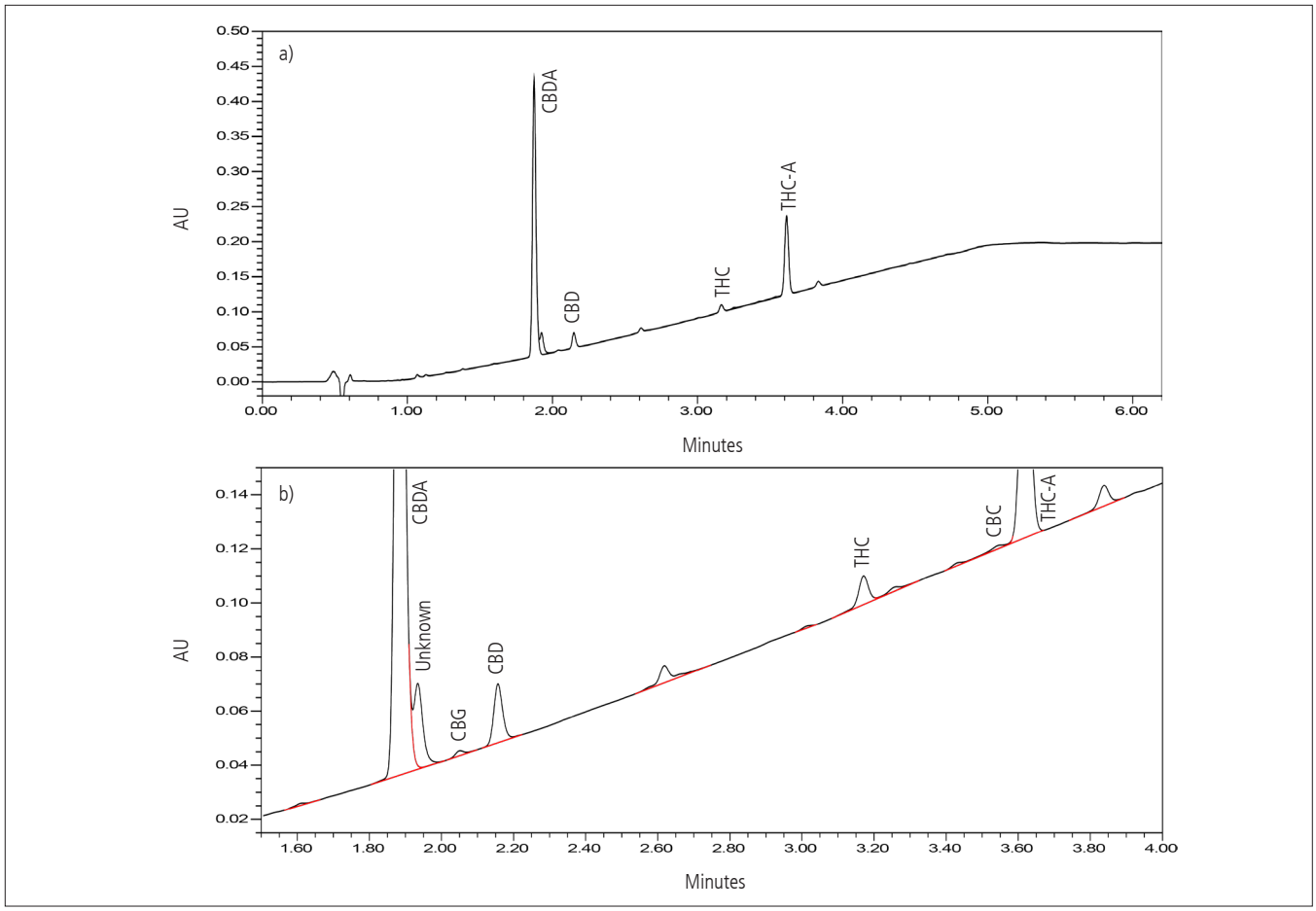

Figure 5. Chromatographic results for Sample A; a) full view; b) expanded view.

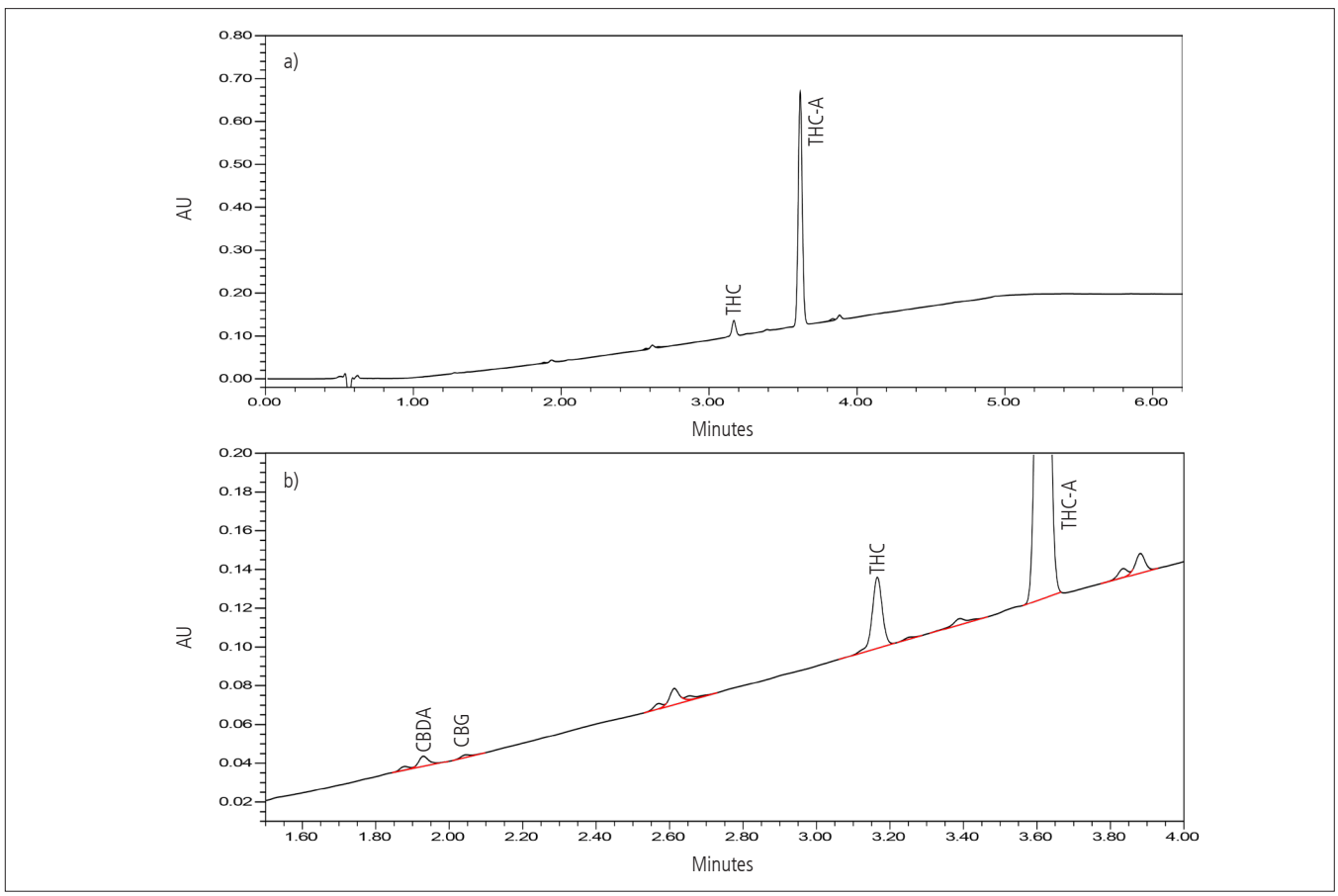

Figure 6. Chromatographic results for Sample B; a) full view; b) expanded view.

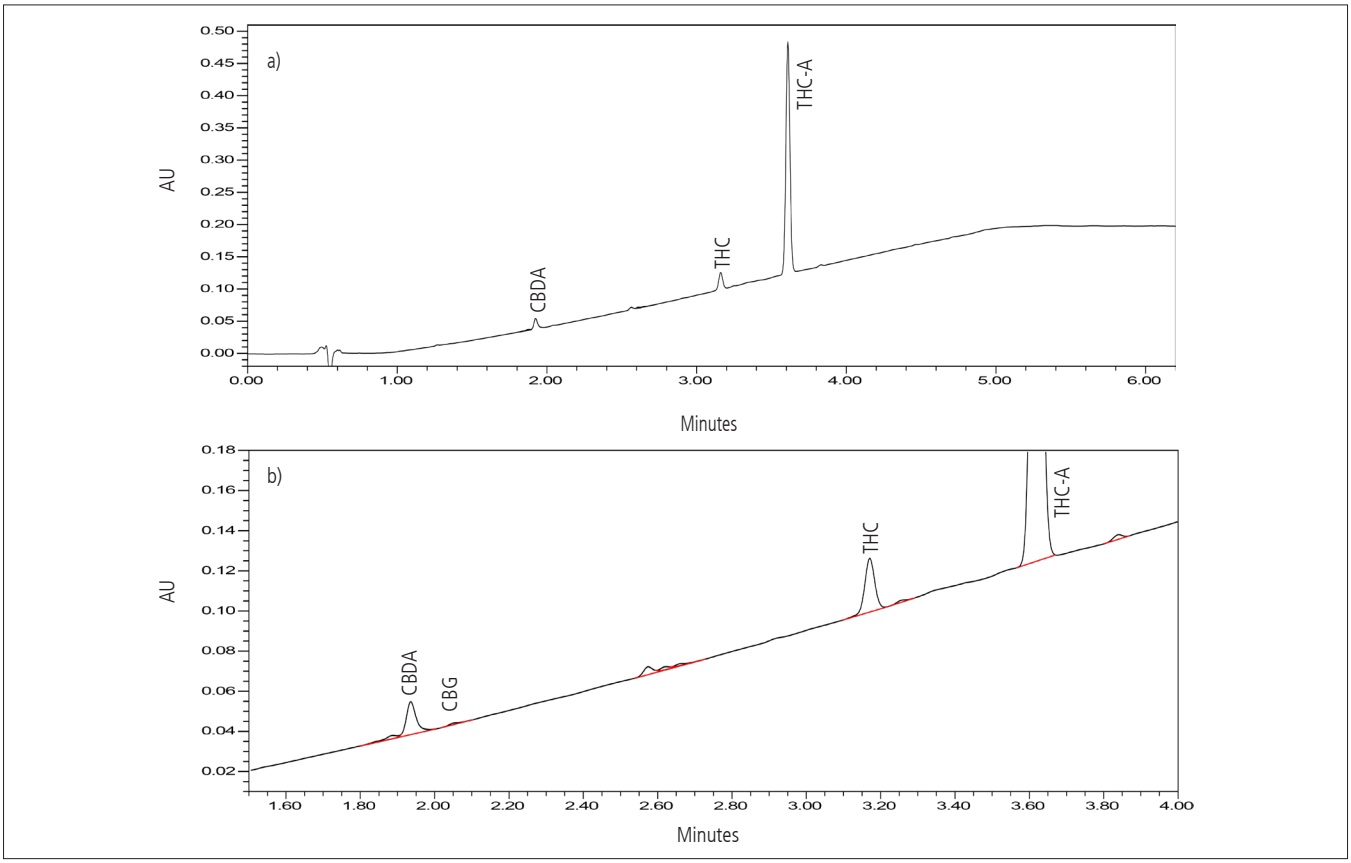

Figure 7. Chromatographic results for Sample C; a) full view; b) expanded view.

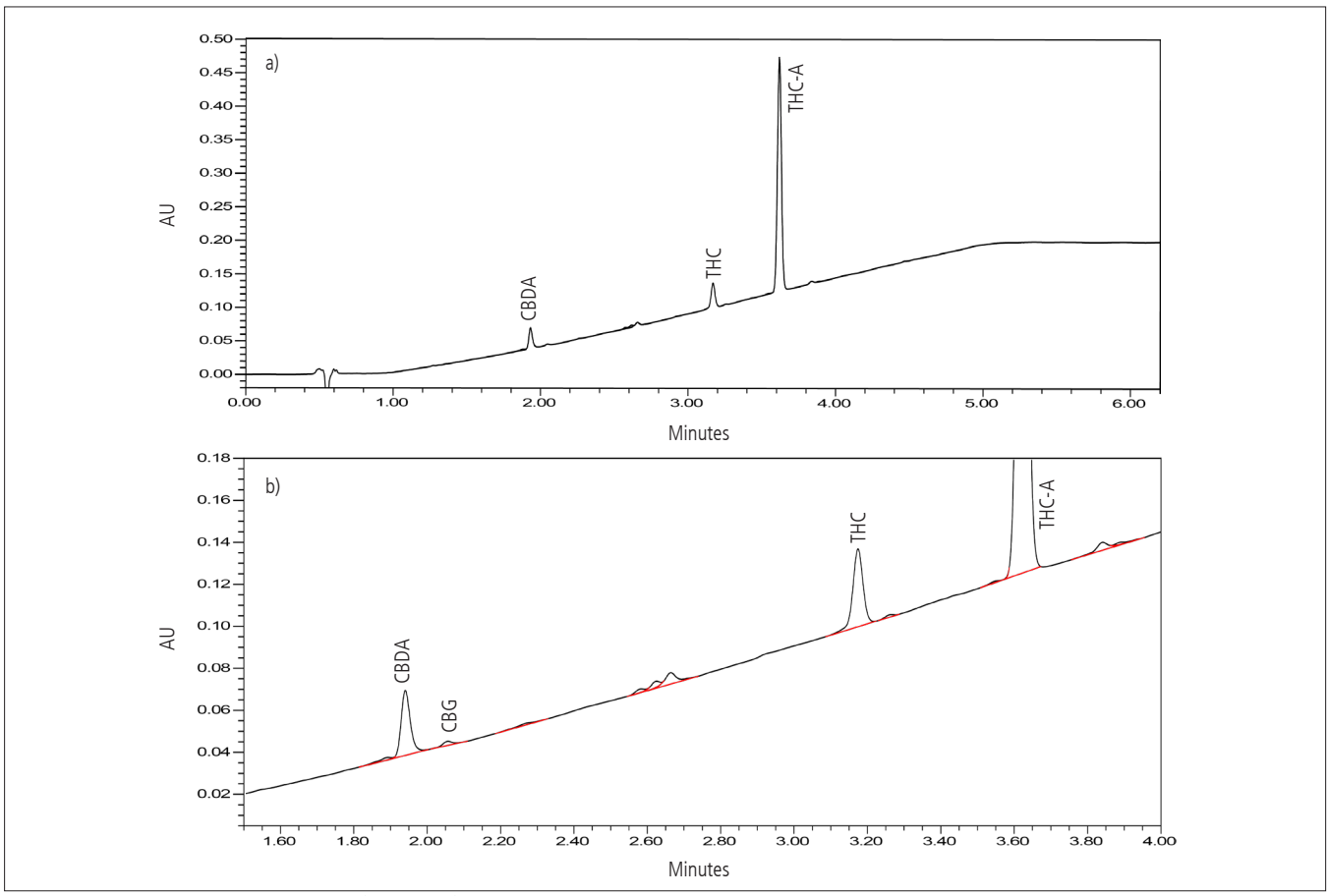

Figure 8. Chromatographic results for Sample D; a) full view; b) expanded view.

Table 3 shows the calculated concentrations (µg/mL) for the seven cannabinoids found in each of the samples. Per 3B Analytical labs, all values were verified as agreeing with the expected values, obtained from an independent GC-based analysis.

Sample A exhibits 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 B showed the highest concentration (21.4%) of THC-A, setting it apart from all other samples, making it a relative front-runner for recreational purposes. Samples C and D were quite similar, both chromatographically and quantitatively, suggesting rather similar cannabis strains.

Table 3. Cannabinoid concentrations found in each of the four samples.

	Analytes	Extract Concentration (µg/mL) *	Extract Concentration (Wgt./Vol. %) *	Concentration in Actual Dried Cannabis Sample (Wgt./Wgt. %) **
Sample A	CBDA	3430	0.343	10.29
	CBG	84	0.008	0.24
	CBD	408	0.041	1.23
	CBN	ND	ND	ND
	THC	221	0.022	0.66
	CBC	62	0.006	0.18
	THC-A	1500	0.150	4.5
Sample B	CBDA	Trace	Trace	Trace
	CBG	77	0.008	0.24
	CBD	Trace	Trace	Trace
	CBN	ND	ND	ND
	THC	650	0.065	1.95
	CBC	ND	ND	ND
	THC-A	7118	0.712	21.36
Sample C	CBDA	Trace	Trace	Trace
	CBG	69	0.007	0.21
	CBD	ND	ND	ND
	CBN	ND	ND	ND
	THC	473	0.047	1.41
	CBC	ND	ND	ND
	THC-A	4665	0.467	14.01
Sample D	CBDA	85	0.009	0.27
	CBG	85	0.009	0.27
	CBD	ND	ND	ND
	CBN	ND	ND	ND
	THC	640	0.064	1.92
	CBC	Trace	Trace	Trace
	THC-A	4533	0.453	13.59

*Accounting for 1/100 extract dilution; average of three replicates.

** Accounting for 30-fold dilution during initial sample extraction in lab of origin.

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 UHPLC system with photodiode array detector. The method provided exceptional linearity for each of the seven cannabinoids over the tested concentration range and the sample results were verified to be consistent with those obtained by independent GC-analysis.

Acknowledgements

The author would like to thank Meghan Brock of 3B Analytical for kindly offering to prepare and provide the sample extracts used for this study, as well as for providing product details to help analyze and verify the sample results.

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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