APPLICATION BRIEF

Liquid Chromatography

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Analysis of 16 Cannabinoids Using the PerkinElmer LC 300 HPLC System with Photodiode Array Detection

Introduction

Current trends for the analysis of the cannabinoid content in commercially available cannabis flower and fortified products, such as foods, depend on liquid chromatography for ensuring label-claim accuracy in product content descriptions. As the use of cannabis products increases with state-level recreational legalization, complete and robust quantification of a range of cannabinoid compounds becomes increasingly important, as failure to do so can result in negative health impacts, as well as a loss in consumer confidence. Recent studies of edible cannabis products purchased from licensed dispensaries in the state of California revealed that only 17% of the 75 edible products purchased were labelled accurately, with the remaining either under-labeled (23%) or over-labeled (60%) with respect to THC content[1].

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Thereupon, this work describes a simple and fast chromatographic method for the analysis of sixteen commonly analyzed cannabinoids. The cannabinoid structures are shown in Figure 1.

Experimental

Hardware/Software

Chromatographic separation was achieved using a PerkinElmer LC 300 HPLC system, consisting of an LC 300 10k psi pump and an LC 300 autosampler equipped with an integrated column oven. Detection was achieved using an LC 300 Photodiode Array (PDA) detector. Instrument control, analysis and data processing were performed using the Simplicity™Chrom CDS software platform.

Method parameters

The LC parameters are shown in Table 1.

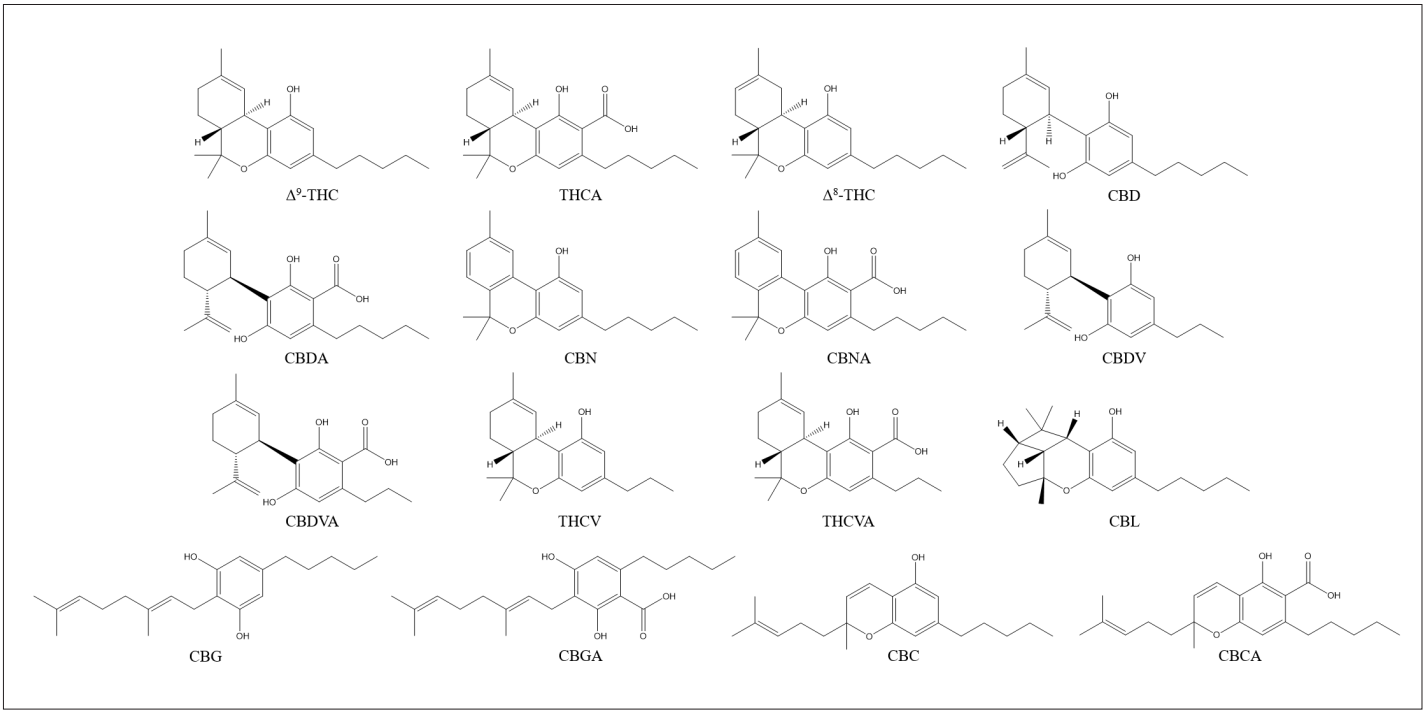

Figure 1. Chemical structures of the sixteen cannabinoids analyzed in this study.

Table 1. LC Parameters.

Column	PerkinElmer Quasar™ SPP C18, 2.6 µm, 150x3.0 mm (Part# N9308913)
Mobile Phase	**Solvent A:** Water with 0.1% formic acid and 8 mM ammonium formate **Solvent B:** Acetonitrile with 0.1% formic acid **Solvent Program:** Isocratic

Step	Time (min)	Flow Rate (mL/min)	%A	%B
1	0.0	1.0	29	71
2	8.0	1.0	29	71

Analysis Time	8 min; Equilibration Time: 0.5 min
Pressure	4300 psi/300 bar maximum
Oven Temperature	40 °C
Sample Temperature	Ambient
Injection Volume	10 µL (Partial Loop)
Analytical Wavelength	228 nm Bandwidth: 5 nm Reference Wavelength: 380 nm Bandwidth: 5 nm
Data Collection Rate	5 pts/sec (Hz)
PDA Flowcell	10 mm (standard)

Solvents and Standards

All solvents used in this method were HPLC grade. Unless otherwise specified, standard dilutions were prepared using 80:20 methanol/water.

The sixteen 1-mg/mL cannabinoid standards were obtained from Cerilliant® (Round Rock, TX). These included: Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^9-tetrahydrocannabinolic acid (THCA), Δ^8-tetrahydrocannabinol (Δ^8-THC), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabinol (CBN), cannbinolic acid (CBNA), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabichromene (CBC), cannabichromenic acid (CBCA), cannabidivarin (CBDV), cannabidivarinic acid (CBDVA), tetrahydrocannabivarin (THCV), tetrahydrocannabivarinic acid (THCVA) and cannabicyclol (CBL).

A 50-µg/mL stock standard mix solution was prepared by pipetting 1 mL of each standard into a 20 mL volumetric flask, and filling to the mark with water. This stock standard mix also served as the Cal-L6 calibration standard.

Additional calibrants were prepared by serially diluting the standard mix to concentration levels of 25, 10, 5, 1, and 0.4 µg/mL, providing a 6-level calibration set.

Results and Discussion

The chromatogram of the 50 µg/mL standard is shown in Figure 2, with all sixteen cannabinoids eluting in under seven minutes.

Figure 3 shows the overlay of 10 replicate 50 µg/mL cannabinoid standard injections, demonstrating exceptional reproducibility. All analyzed cannabinoids had a peak retention time precision (%RSD) of less than 0.16%, and a peak area precision of less than 0.7% (with most analytes below 0.5%).

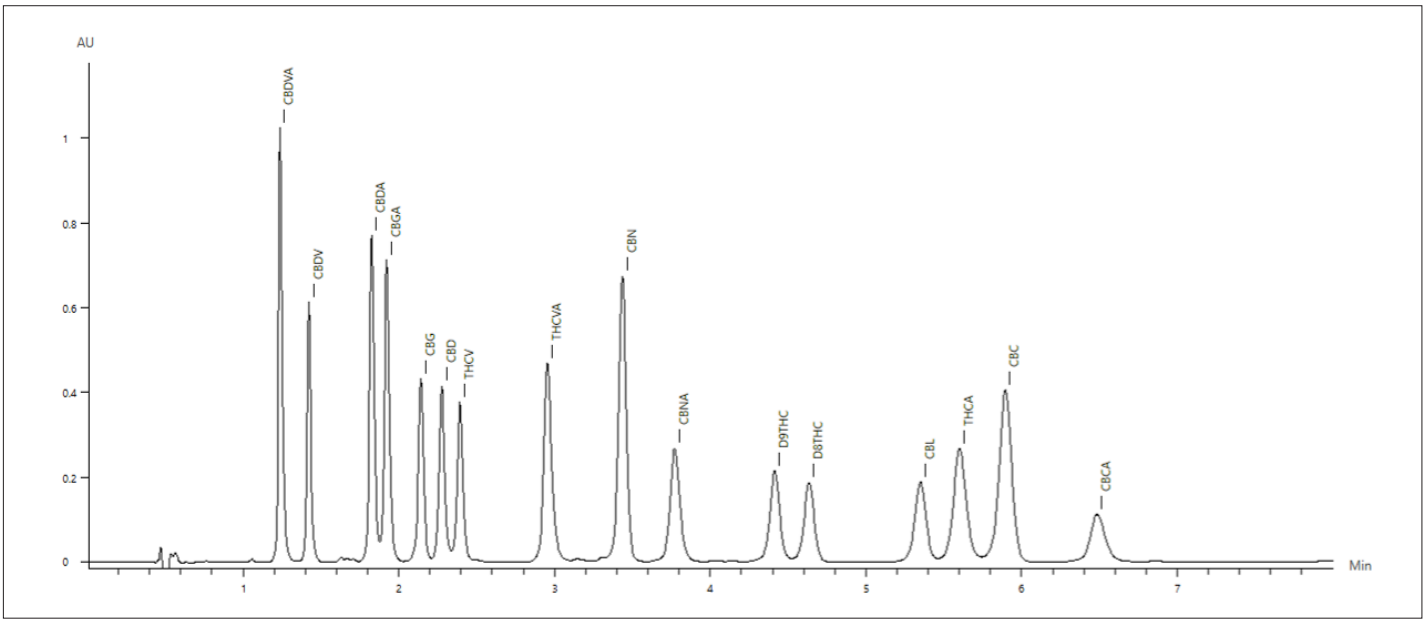

Figure 2. Chromatogram of the the 50 µg/mL cannabinoid standard.

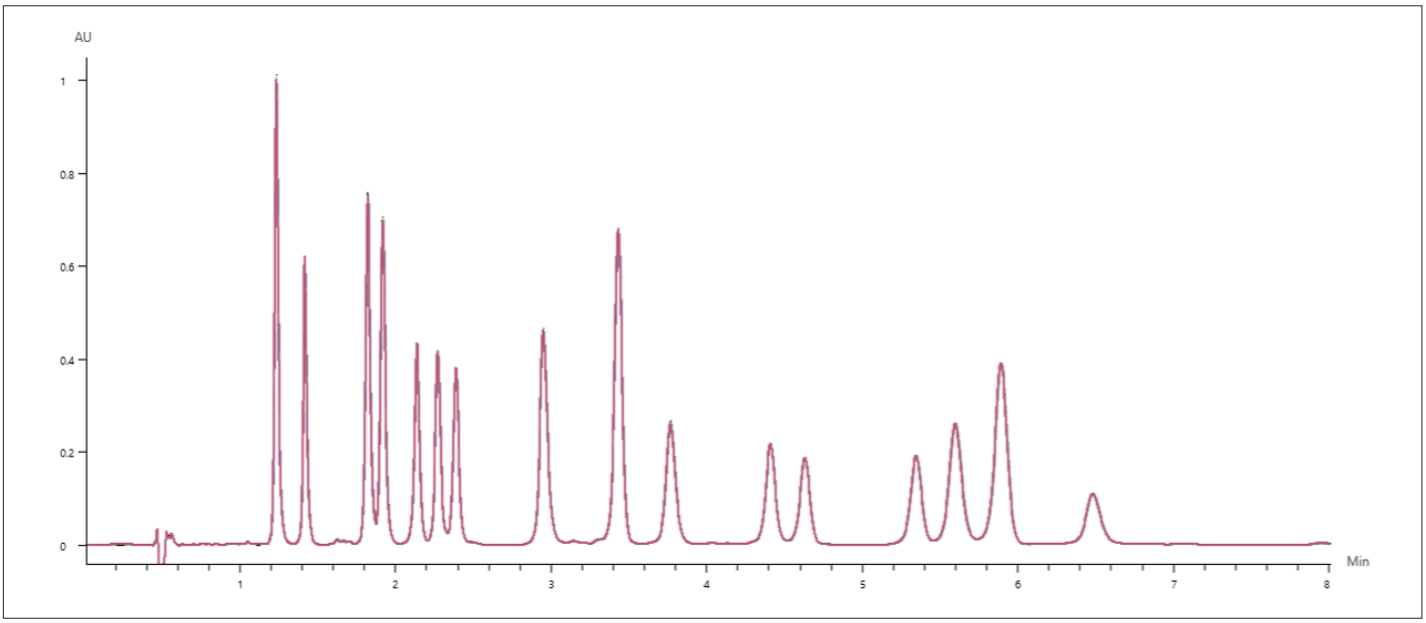

Figure 3. Chromatographic overlay of 10 replicates of the 50 µg/mL cannabinoid standard.

Figure 4 shows the calibration results for four representative cannabinoids over the concentration range of 0.4 to 50 µg/mL. All sixteen cannabinoids followed a linear (1st order) fit, and had R^2 coefficients greater than 0.999 (n=3 at each level).

The chromatogram of the low-level 0.4 µg/mL cannabinoid standard is shown in Figure 5.

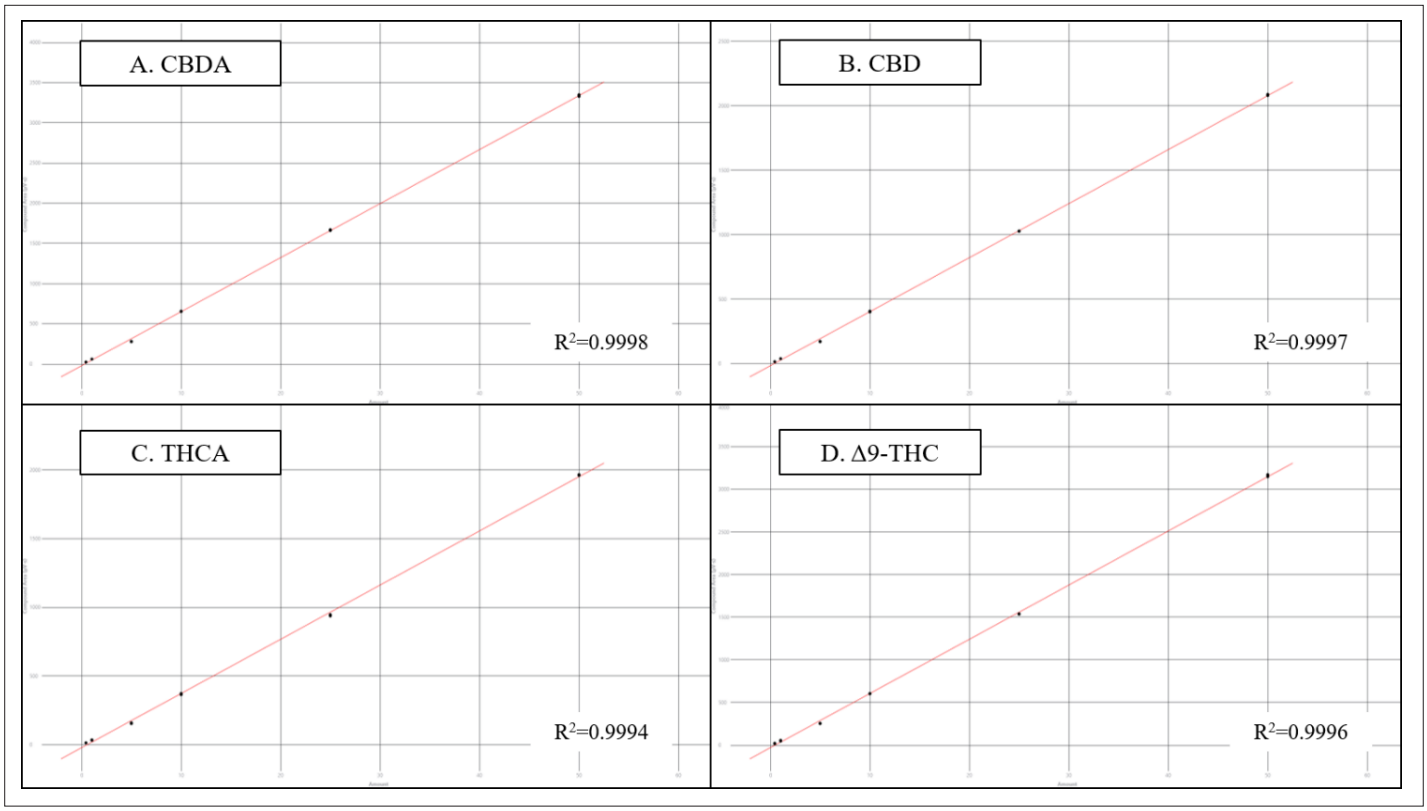

Figure 4. Results for the 6-level calibration sets for four example cannabinoids.

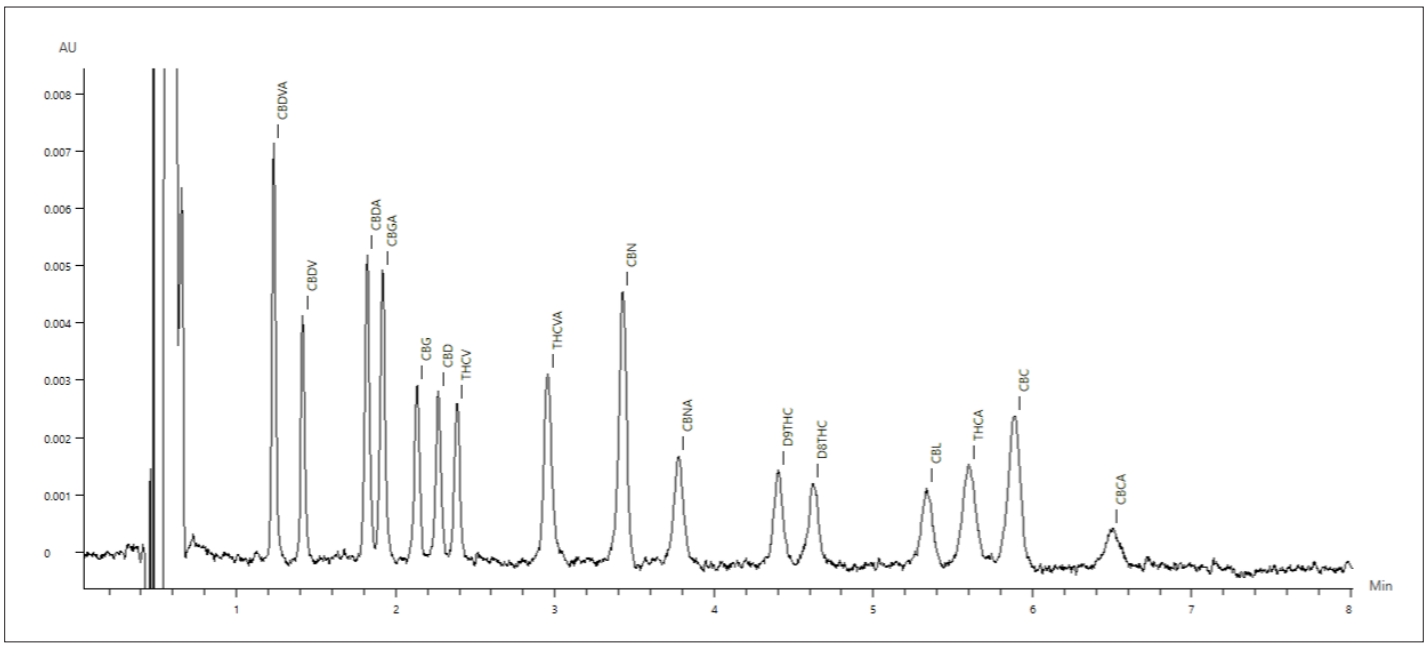

Figure 5. Chromatogram of the the 0.4 µg/mL cannabinoid standard.

An 80:20 methanol/water solvent blank was injected following triplicate injections of the Cal-L6 (50 µg/mL) standard, and no carryover was observed for any of the sixteen cannabinoids.

As listed in Table 2, LOQ (limit of quantitation) levels were established for each analyte based upon the averaged response and signal-to-noise ratio for the level 1 (0.4 µg/mL) calibration standard, run in triplicate.

Table 2. LOQs for the sixteen analytes, in order of elution.

Analyte	Calculated LOD (µg/mL; S/N ≥ 3)	Calculated LOQ (µg/mL; S/N ≥ 10)
CBDVA	0.009	0.032
CBDV	0.016	0.053
CBDA	0.013	0.043
CBGA	0.014	0.045
CBG	0.022	0.074
CBD	0.023	0.078
THCV	0.025	0.082
THCVA	0.021	0.071
CBN	0.014	0.048
CBNA	0.037	0.124
Δ^9-THC	0.042	0.141
Δ^8-THC	0.048	0.161
CBL	0.051	0.170
THCA	0.041	0.138
CBC	0.025	0.083
CBCA	0.090	0.301

Conclusion

This work has demonstrated the fast and robust chromatographic separation and quantitation of sixteen commonly analyzed cannabinoids using a PerkinElmer LC 300 HPLC system with PDA detection. The results exhibited very good retention time repeatability, as well as excellent linearity over the tested concentration ranges. The method also affords LOQs of ≤ 0.15 µg/mL for most analytes.

References

1. Vandrey R, Raber JC, Raber ME, Douglass B, Miller C, Bonn-miller MO. Cannabinoid Dose and Label Accuracy in Edible Medical Cannabis Products. JAMA. 2015;313(24):2491-3.
2. Cannabis oil vs hemp seed oil; Cannabis oil, CDB Oil, Medical Marijuana. http://cbd.org/cannabis -oil-vs-hemp-oil

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