APPLICATION BRIEF

Liquid Chromatography

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High Speed Chromatographic Analysis of 16 Cannabinoids by HPLC-PDA

To further validate the performance of this method for the industry, The Emerald Test Proficiency Test (PT) for Potency was conducted. The Emerald Test™ is an Inter-Laboratory Comparison and Proficiency Test (ILC/PT) program for cannabis testing labs. The results from the PT inter-laboratory samples passed; therefore, the method meets inter-laboratory reproducibility and accuracy. The method was awarded the Emerald Test Badge seen on the right.
https://pt.emeraldscientific.com/

Introduction

Current trends for the analysis of the cannabinoid content in cannabis flower extracts/concentrates and commercially available fortified foods depend on liquid chromatography for potency testing and/or ensuring the label-claim accuracy in product content descriptions.

Thereupon, this work describes a fast chromatographic method for the analysis of 16 cannabinoids commonly monitored in cannabis-centric labs. Their structures are shown in Figure 1.

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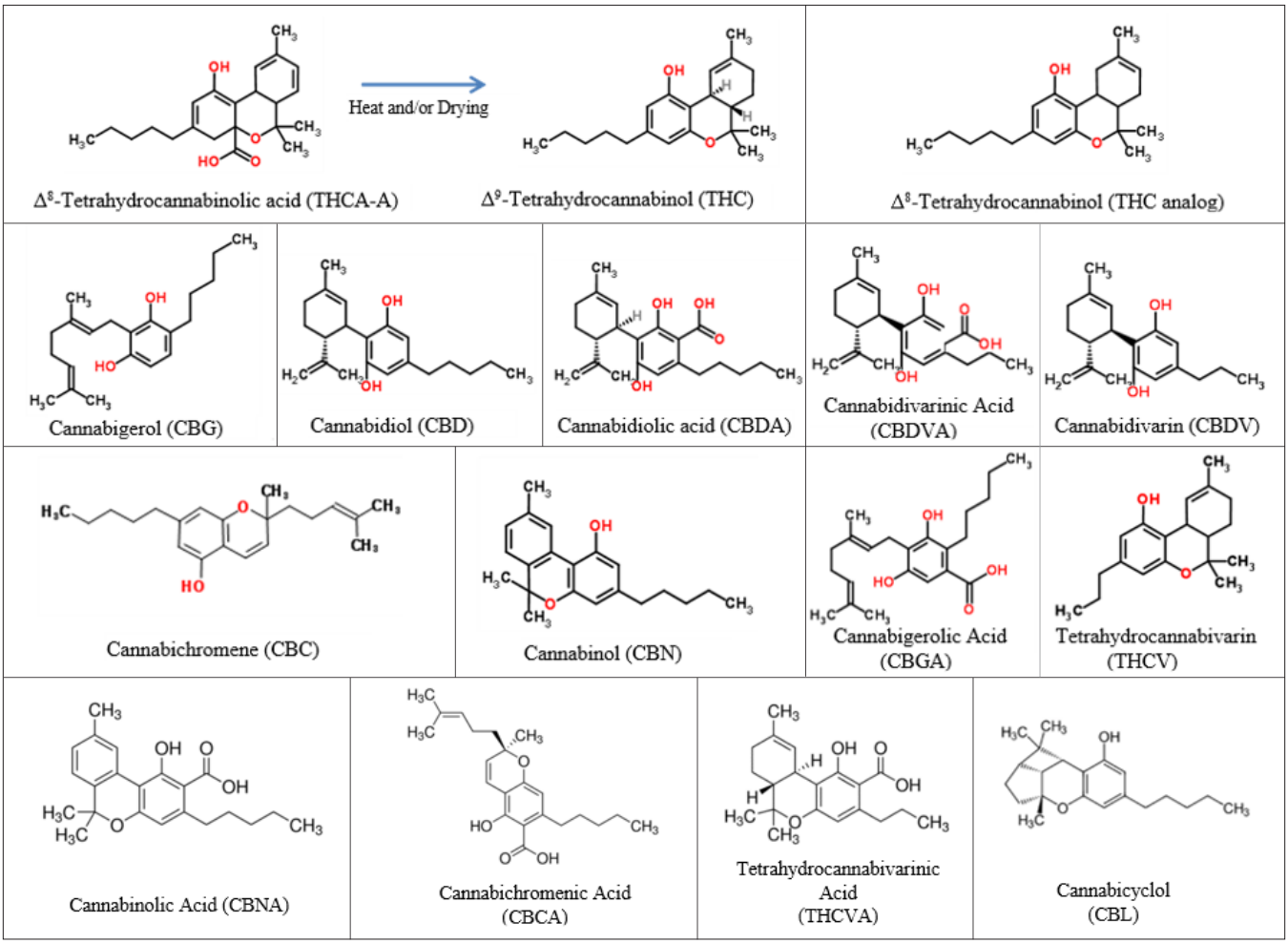

Figure 1. Chemical structures of the sixteen cannabinoids analyzed in this study.

Experimental

Hardware/Software

For the chromatographic separation, a PerkinElmer Flexar™ HPLC system was used with a PDA (photodiode array) Plus detector. Instrument control, analysis and data processing were performed using the Chromera® software platform.

Method Parameters

The LC parameters are shown in Table 1.

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade. Unless otherwise specified, standard and sample extract dilutions were prepared using 80:20 methanol/water.

The sixteen 1-mg/mL cannabinoid standards were obtained from Sigma-Aldrich®, Inc (Allentown, PA). These included: Δ^9-tetrahydrocannabinol (Δ9-THC), Δ^9-tetrahydrocannabinolic acid (THCA-A), Δ^8-tetrahydrocannabinol (Δ8-THC), tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), cannabidivarinic acid (CBDVA), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabinol (CBN), cannabichromene (CBC), cannabicyclol (CBL), cannabichromenic acid (CBCA), cannabinolic acid (CBNA) and tetrahydrocannabivarinic acid (THCVA).

Table 1. LC Parameters.

Column:	PerkinElmer SPP C18, 2.7 µm, 150 x 3.0 mm (Part # N9308411)	
Mobile Phase:	**Solvent A:** Water with 0.1% formic and 5 mM ammonium formate **Solvent B:** Acetonitrile with 0.1% formic **Solvent Program:**	
Analysis Time:	6 min.	**Data Collection Rate:** 5 pts/sec (Hz)
Pressure:	4400 psi/300 bar maximum	
PDA Wavelengths:	228 nm	**PDA Flowcell:** 10 mm (standard)
Oven Temp.:	40 °C	
Injection Vol.:	10 µL	

Step	Time (min)	Flow Rate (mL/min)	%A	%B
Equil.	4.5	1.0	33	67
1	6	1.0	5	95
2	2	1.0	5	95

A 50-ppm stock standard mix solution was prepared by adding the entire contents of each standard into a 20-mL volumetric flask and filling to mark with water. This also served as the L10 calibration standard.

Additional calibrants were prepared by serially diluting the standard mix to concentration levels of 25, 10, 5, 2.5, 1.0, 0.5, 0.25, 0.10 and 0.05 µg/mL (ppm), providing a 10-level calibration set. Depending on the response, the lowest level (0.05 µg/mL) was not used for some analytes.

Results and Discussion

The chromatogram of the 50-µg/mL standard is shown in Figure 2, with all 16 cannabinoids eluting in under five minutes.

A 10-replicate chromatographic overlay of the 10-µg/mL cannabinoid standard is shown in Figure 3, highlighting very good repeatability.

The linearity plots for three representative cannabinoids are shown in Figure 4. The R^2 values for all 16 cannabinoids were above 0.999.

The chromatogram of the low-level 0.1-µg/mL cannabinoid standard is shown in Figure 5.

The chromatogram of an 80:20 methanol/water blank injection is shown in Figure 6, run right after the high-level calibrant set, showing no carryover or interferences.

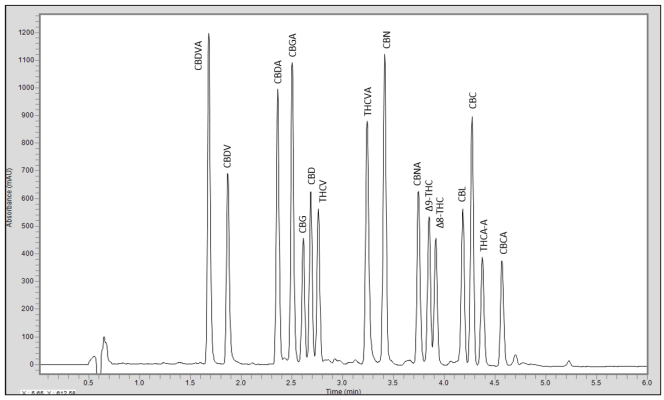

Figure 2. Chromatogram of the the 50-µg/mL cannabinoid standard.

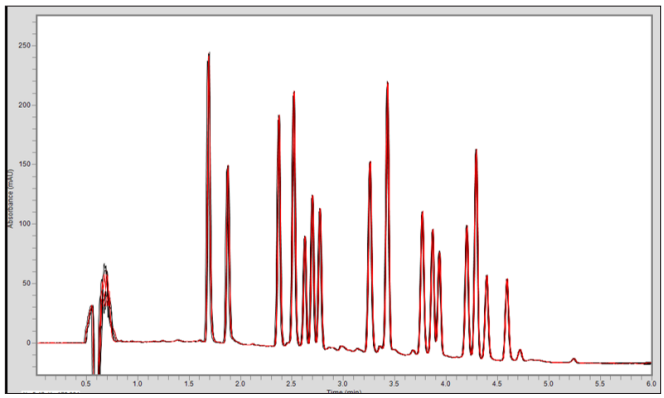

Figure 3. Chromatographic overlay of the 10-replicate injections of the 10-µg/mL cannabinoid standard.

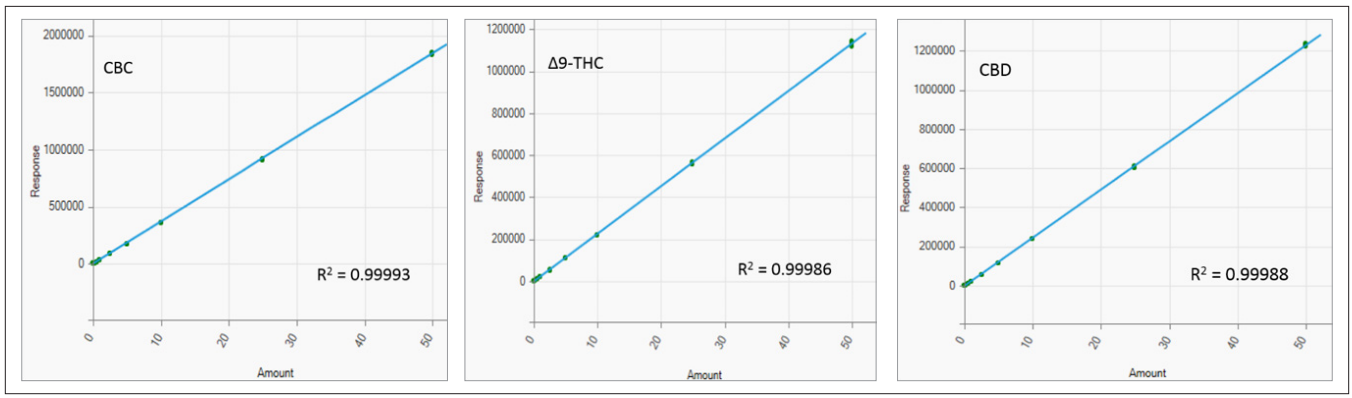

Figure 4. Linearity plots for three example cannabinoids.

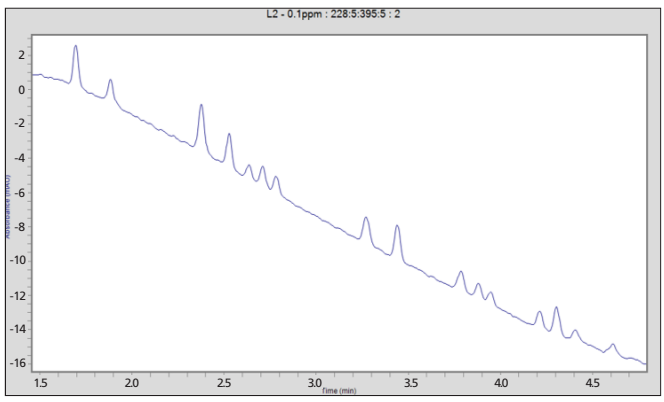

Figure 5. Chromatogram of the the 0.1-µg/mL cannabinoid standard.

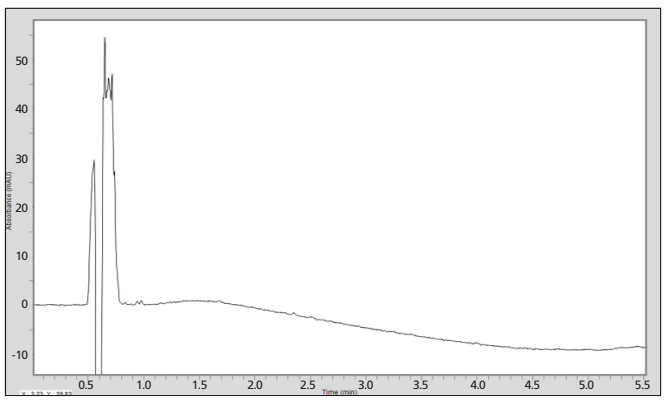

Figure 6. Chromatogram of a diluent "blank" injection.

As listed in Table 2, LOQ (limit of quantitation) levels were established for each analyte, based upon the averaged response for the 0.1 µg/mL calibration standard, run in triplicate.

Conclusions

- This work has demonstrated the fast and robust chromatographic separation and quantitation of 16 cannabinoids commonly analyzed in cannabis-centric labs, using the PerkinElmer Flexar HPLC system with a PDA detector.
- The method provides very good chromatographic repeatability and affords LOQs of ≤ 0.1 µg/mL for most analytes.
- If needed, additional sensitivity can be gained using the PDA's optional 50-mm flow cell.

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

Analyte	Calculated LOQ (µg/mL; S/N = 10)	Analyte	Calculated LOQ (µg/mL; S/N = 10)
CBDV	0.05	CBC	0.05
CBDVA	0.03	CBCA	0.11
CBG	0.10	CBL	0.08
CBGA	0.04	Δ9-THC	0.09
CBD	0.07	Δ8-THC	0.11
CBDA	0.03	THCA-A	0.12
CBN	0.04	THCV	0.08
CBNA	0.07	THCVA	0.05

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