The LC-UV Analysis of 16 Cannabinoids of Interest in Commercially Available CBD Oils

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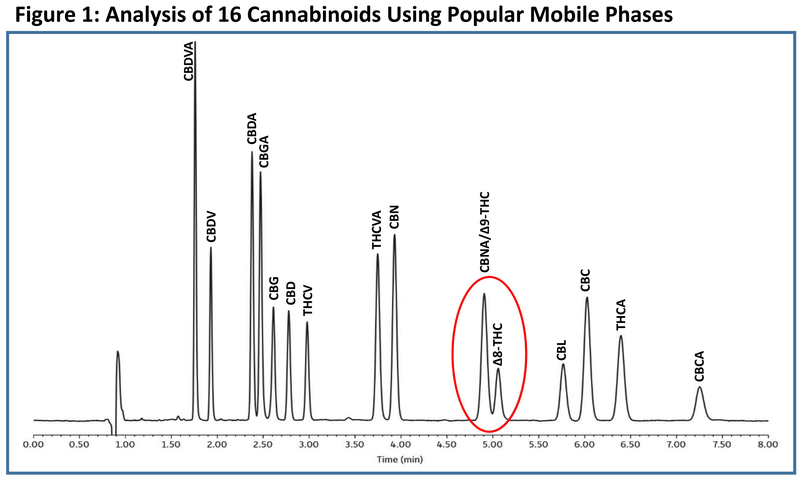
Introduction

More than 100 cannabinoids have been isolated from cannabis in addition to the five most commonly tested: THC, THCA, CBD, CBDA, and CBN. While methods have been published that show the separation of these major cannabinoids, many do not take into account the possibility of interference from other cannabinoids that may be present. This is most problematic in concentrates where minor cannabinoids can be enriched to detectable levels that were not observed in the flower. Additionally, some terpenes have been shown to absorb UV light at 228 nm, the wavelength cannabinoids are typically detected, which can result in an additional source of interference. In this study, the LC-UV separation of 16 cannabinoids of interest was performed while monitoring for the potential impact from minor cannabinoids and terpenes on reported potency values. The method is applied to commercially available CBD oils that have recently become suspect due to inaccurate label claims.

Method Development

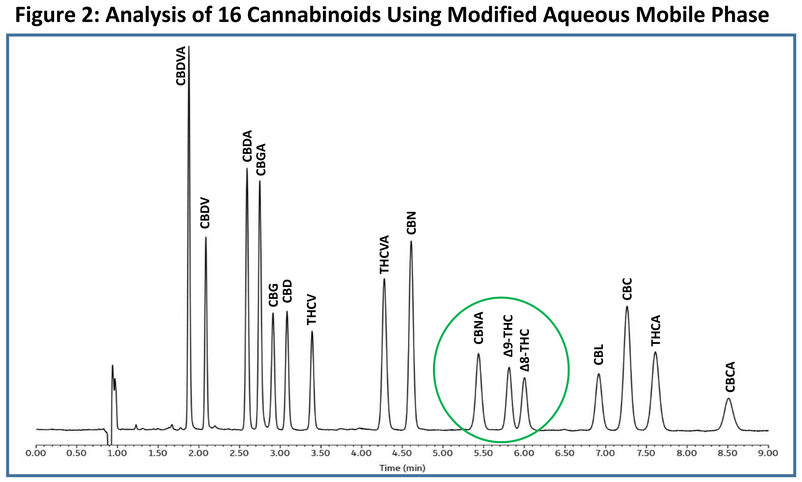
Method development began by using popular mobile phases for the analysis of cannabinoids (0.1% formic acid in water and 0.1% formic acid in acetonitrile) under isocratic conditions with a Raptor ARC-18 2.7 µm, 150 x 4.6 mm analytical column (Restek cat# 9314A65). Using these mobile phases, it was found that cannabinolic acid (CBNA) co-eluted with Δ9-tetrahydrocannabinol (Δ9-THC) regardless of flow rate, column temperature, or mobile phase composition (Figure 1).

Figure 1: Analysis of 16 Cannabinoids Using Popular Mobile Phases



In order to resolve CBNA from Δ9-THC without compromising established separations, ammonium formate was added to the aqueous mobile phase to a concentration of 5 mM in solution. The increase in ionic strength, combined with a decreased column temperature, resulted in reduced retention for carboxylated cannabinoids relative to neutral cannabinoids which enabled the baseline separation of CBNA from Δ9-THC without impacting the separation of the remaining cannabinoids (Figure 2).

Figure 2: Analysis of 16 Cannabinoids Using Modified Aqueous Mobile Phase



Sample Preparation

Calibration standards and QC samples were prepared in 25:75 water: methanol across a linear range of 5 – 500 µg/mL. 50 µL of CBD product was extracted using 950 µL of methanol followed by vortexing for 30 seconds at 3000 rpm. 750 µL of the sample was then mixed with 250 µL of water followed by vortexing for 10 seconds at 3000 rpm. 400 µL of the sample was then filtered using a Thomson SINGLE StEP Standard Filter Vial (0.2 µm PVDF membrane, cat# 25895) prior to analysis.

Analytical Method

Table 1: Analytical Conditions

Column:	Raptor ARC-18 2.7 µm, 150 mm x 4.6 mm (cat# 9314A65)		
Guard Column:	Raptor ARC-18 EXP Guard Column Cartridge 2.7 µm, 5 x 4.6 mm (cat# 9314A0250)		
Mobile Phase A:	Water, 5 mM Ammonium Formate, 0.1% Formic Acid		
Mobile Phase B:	Acetonitrile, 0.1% Formic Acid		
Time Program:	Time (min.)	Flow (mL/min.)	%B
	0.00	1.5	75
	9.00	1.5	75
Oven Temp.:	30 °C		
Sample Temp.:	10 °C		
Inj. Volume:	5 µL		
Wavelength:	228 nm		

Chromatograms

Figure 3: Terpene Interferences Observed Using Final Method Conditions

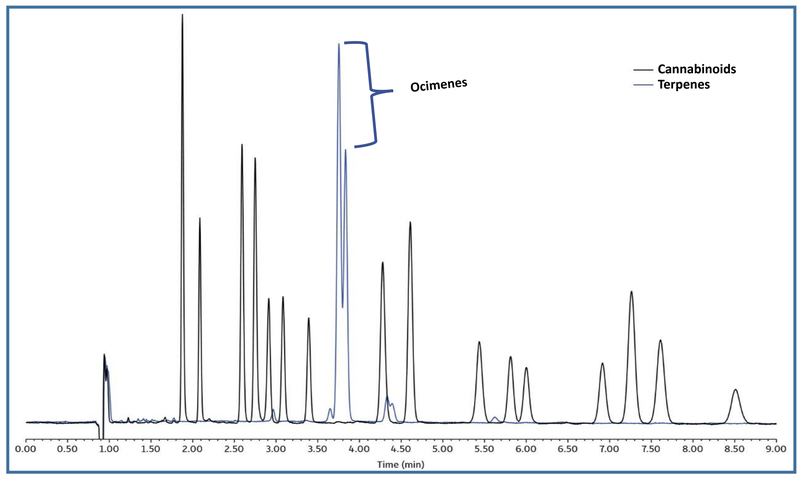


Figure 4: Example Chromatogram of a Commercially Available CBD Product

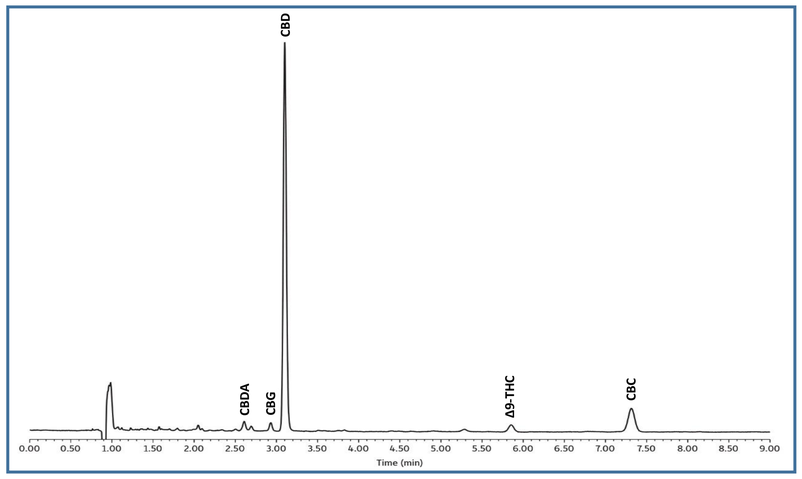
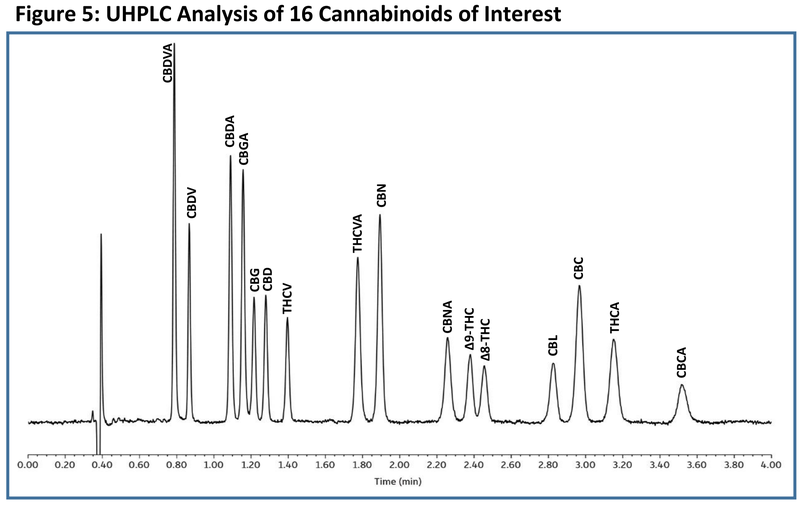


Figure 5: UHPLC Analysis of 16 Cannabinoids of Interest



Results and Discussion

Linearity: Using linear 1/x weighted regression, the method showed good linearity for CBD with an r^2 value of 0.999 across a range from 5 – 500 µg/mL.

Accuracy & Precision: The method accuracy was demonstrated to be within 3.67% of the nominal concentration for all QC levels. The %RSD was within 3.14% for all QC levels indicating good method precision (Table 2).

Table 2: Inter-run Accuracy and Precision

Analyte	QC LLOQ 5.00 µg/mL			QC Low 30.0 µg/mL			QC Mid 150 µg/mL			QC High 400 µg/mL			QC Dilution (20-fold) 1,000 µg/mL		
	Avg. Conc. (µg/mL)	Avg. Accuracy (%)	%RSD	Avg. Conc. (µg/mL)	Avg. Accuracy (%)	%RSD	Avg. Conc. (µg/mL)	Avg. Accuracy (%)	%RSD	Avg. Conc. (µg/mL)	Avg. Accuracy (%)	%RSD	Avg. Conc. (µg/mL)	Avg. Accuracy (%)	%RSD
CBD	4.96	99.2	3.14	30.8	103	1.84	156	104	0.455	396	99.0	0.455	1,036	104	0.341

Terpene Interference: A mix of 21 common terpenes was prepared at a concentration of 50 µg/mL. The mix was injected using the conditions listed in table 1 and overlayed with a separate injection of the cannabinoids at 50 µg/mL utilizing the same conditions (Figure 3). The terpene that best absorbs UV light at 228 nm, ocimenes, does not impact the quantitation of any monitored cannabinoids. Minor terpene interferences were found that could potentially impact the quantitation of CBGA and THCVA if present in high enough concentrations, but the quantitative analysis of these cannabinoids is currently not required.

Sample Analysis: Six commercially available CBD products (three vape oils, two sublingual oils, and one raw hemp oil) were quantitatively evaluated for CBD. All products were diluted into the linear range as necessary. According to California regulations, a cannabis product must not exceed the labeled concentration of CBD by ± 15%. Applying this labeling guideline to the samples evaluated in this study results in the possibility of 5 out of 6 products not being in compliance with regulations (Table 3). Vape oils did not contain a significant amount of CBDA to account for the discrepancy in the labeling. The large discrepancy in the result for Product 3 is most likely due to photodegradation from improper packaging. In addition to CBD, additional cannabinoids were found in most samples (Figure 4). Additional cannabinoids that were found included: CBDVA, CBDV, CBDA, CBG, Δ9-THC, Δ8-THC, CBN, CBNA, and CBC. The concentrations of these cannabinoids were not quantitatively evaluated, but the concentration of Δ9-THC in all samples appeared to be well below 0.3% w/w based upon the peak height of a 50 µg/mL standard.

Table 3: Concentration of CBD in Commercially Available CBD Oils

Product Name	Product Type	CBD - Label Claim (mg/mL)	±15% Range (mg/mL)	CBD - Actual Concentration (mg/mL)
Product 1	Vape Oil	3.33	2.83 – 3.83	2.05
Product 2	Vape Oil	6.67	5.67 – 7.67	5.59
Product 3	Vape Oil	500	425 – 575	0.242
Product 4	Sublingual Oil	11.0 (Hemp Extract)	NA	6.24
Product 5	Sublingual Oil	6.11	5.19 – 7.03	5.21
Product 6	Raw Hemp Oil	30.0	25.5 – 34.5	18.4

Recovery: Recovery was evaluated by spiking each product type with an additional 2.00 mg/mL of CBD. Recovery results for vape oil, sublingual oil, and raw hemp oil were 102%, 98.5%, and 105%, respectively.

UHPLC Analysis: In order to improve the speed of analysis, the method was translated to a Raptor ARC-18 1.8 µm, 100 x 3.0 mm analytical column (cat# 931421E). The conditions listed in Table 1 were adjusted to a flow rate of 1.0 mL/min with an injection volume of 1 µL. The analysis was reduced to less than four minutes while maintaining the separation of all 16 cannabinoids (Figure 5).

Conclusions

It was demonstrated that 16 cannabinoids of interest can be separated in under nine minutes using a Raptor ARC-18 2.7 µm, 150 x 4.6 mm analytical column on a traditional HPLC system. CBNA was identified as a source of interference for Δ9-THC using popular mobile phases for the analysis of cannabinoids (0.1% formic acid in water and 0.1% formic acid in acetonitrile). No major sources of terpene interference were identified that would impact the quantitation of THC, THCA, CBD, CBDA, or CBN. The method was successfully applied to commercially available CBD oils. For high-throughput analysis, the method was successfully transferred to UHPLC-UV which enabled the analysis of 16 cannabinoids in less than four minutes.