

# Application News

## High Performance Liquid Chromatography

### The Potency Determination of 16 Cannabinoids by UHPLC with Diode-Array Detection

No. HPLC-020

#### ■ Introduction

Since the legalization of cannabis in several US states and, recently, Canada, the quantitative determination of cannabinoids in cannabis products has been of great interest. There are more than 100 cannabinoids that can be found in the plant or extracts<sup>(1)</sup>. Tetrahydrocannabinol (THC) and cannabidiol (CBD) are two of the highest priority in potency testing along with their acidic forms. The acidic forms, Tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), are primarily found in the plant, subsequently converting to THC and CBD through decarboxylation from exposure to heat and light<sup>(2)</sup>.

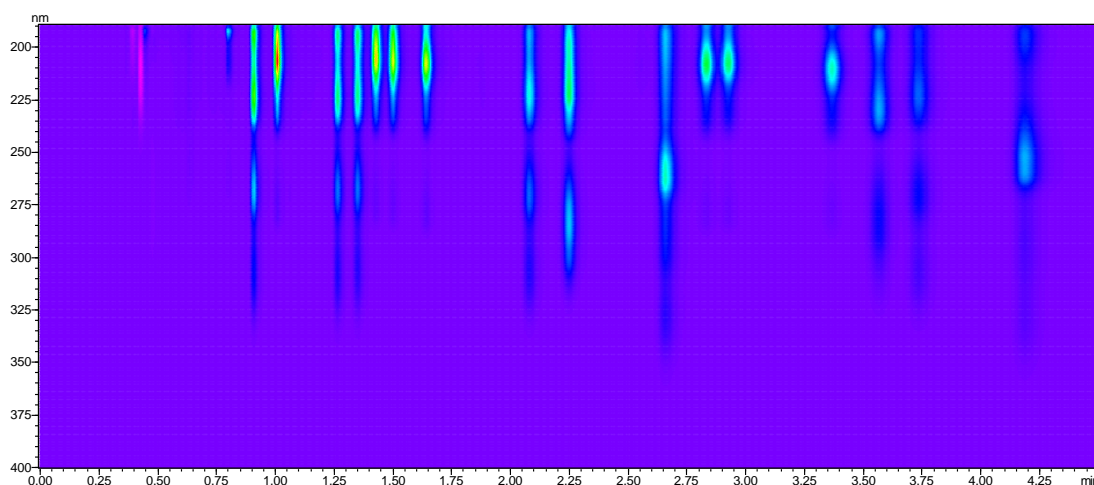
Traditional HPLC is the gold standard for cannabinoids analysis, including the acidic forms, providing nearly complete separation of the cannabinoids, and robust quantitation. Several methods have been developed for optimal results of resolution, sensitivity, and throughput. To assist in optimizing for high throughput while maintaining sensitivity and resolution, this application note proves a 4.5-minute isocratic method using a UHPLC system.

#### ■ Experimental

Potency analysis was performed using a Shimadzu Nexera-i (LC-2040C 3D) UHPLC with a photodiode array detector. The method conditions are shown in Table 1. Historically, 276 nm is ideal for acidic cannabinoids, but non-acidic cannabinoids give weak responses. Consistent with previous literature, a wavelength of 228 nm was chosen as an acceptable compromise<sup>(3)</sup>. Experimentation with the PDA supported this finding (Figure 1).

**Table 1:** Instrument Method Parameters

Liquid Chromatography	Nexera-i (LC-2040C 3D)
Mobile Phase A	Water, 5 mM Ammonium Formate, 0.1% Formic Acid
Mobile Phase B	Acetonitrile, 0.1% Formic Acid
MP Composition	Isocratic, 25/75
Column	Shimadzu NexLeaf CBX II, 1.8 µm, 3.0 x 100mm (220-91525-75) Shimadzu NexLeaf CBX II Guard, 1.8 µm (220-91525-76)
Oven temperature	30°C
Flow rate	1.0 mL/min
Wavelength Monitored	228 nm



**Figure 1:** PDA contour plot showing wavelengths 190 to 400 nm

■ Standards Preparation

An 11 component cannabinoids mixture (Shimadzu 220-91239-21) was supplemented with five additional cannabinoids (Cerilliant) to create a 100.0 ppm comprehensive mix of 16 cannabinoids. Table 2 shows a complete list of cannabinoids with initial standard concentration.

Table 2: Cannabinoid Standards

Standard	Compounds	Conc. (mg/L)
Shimadzu	CBDV	250
Shimadzu	CBDA	250
Shimadzu	CBGA	250
Shimadzu	CBG	250
Shimadzu	CBD	250
Shimadzu	THCV	250
Shimadzu	CBN	250
Shimadzu	Δ9-THC	250
Shimadzu	Δ8-THC	250
Shimadzu	CBC	250
Shimadzu	Δ9-THCA	250
Cerilliant	CBDVA	1000
Cerilliant	THCVA	1000
Cerilliant	CBNA	1000
Cerilliant	CBL	1000
Cerilliant	CBCA	1000

■ Results and Discussion

Chromatography

Figure 2 shows the chromatographic separation of the cannabinoids at 100 ppm. USP Resolution greater than 1.0 was indicated for all analytes.

A six-point calibration curve was prepared ranging from 0.5 to 100.0 ppm (n=6). Due to the wide linear range (0.5 – 100.0 ppm) a best-fit weighting method was used (1/c). Selected standard curves are shown in figure 3. A series of low, middle, and high calibration checks were also run in replicates of six injections (Figure 4). Full statistical results can be seen in Table 3.

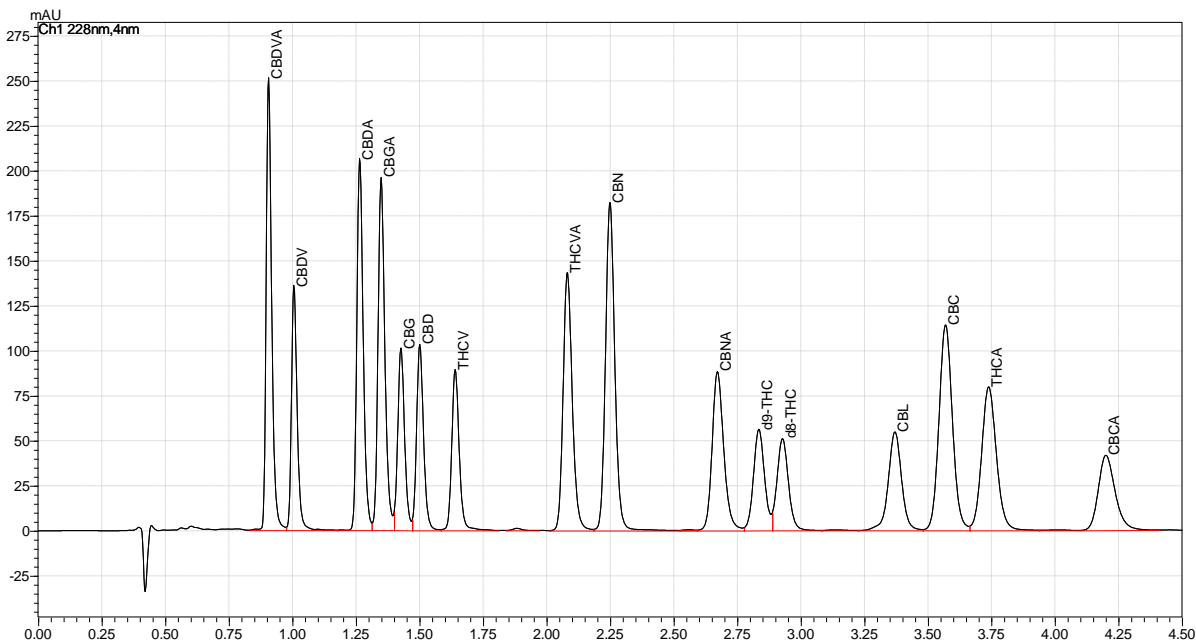


Figure 2: UHPLC-PDA Chromatogram - 16 Cannabinoids

Figure 3: Selected Calibration Curves

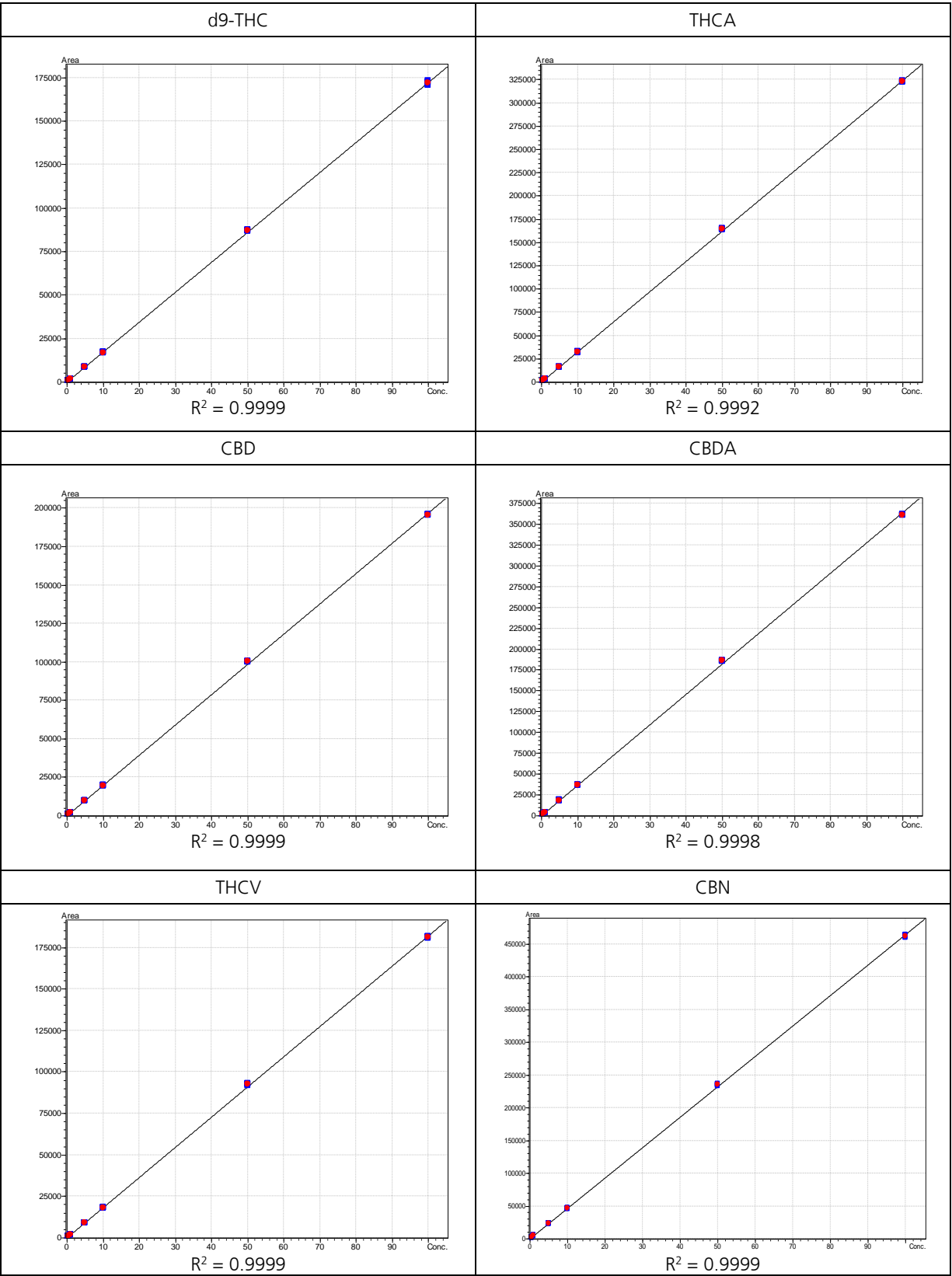


Table 3: Statistical Results of 6-Point Calibration Results for 16 Cannabinoid Comprehensive Mix

Analyte	Calibration Results		2.5 ppm			25.0 ppm			75.0 ppm		
	1/C		(n=6)			(n=6)			(n=6)		
	RF RSD (%)	R2	Mean Conc	RSD (%)	Accuracy (%)	Mean Conc	RSD (%)	Accuracy (%)	Mean Conc	RSD (%)	Accuracy (%)
CBDVA	1.758	0.9998	2.435	0.804	97.4	25.18	0.496	100.7	73.44	0.288	97.9
CBDV	2.622	0.9999	2.428	3.08	97.1	24.95	0.396	99.8	73.25	0.195	97.7
CBDA	1.962	0.9998	2.473	1.72	98.9	25.06	0.8	100.3	73.45	0.175	97.9
CBGA	2.198	0.9999	2.459	1.843	98.4	24.98	0.225	99.9	73.08	0.209	97.4
CBG	3.065	0.9999	2.469	1.94	98.8	25.16	0.265	100.6	73.79	0.281	98.4
CBD	1.814	0.9999	2.468	1.121	98.7	25.2	0.884	100.8	73.93	0.348	98.6
THCV	2.919	0.9999	2.413	1.945	96.5	25.09	0.332	100.3	73.78	0.469	98.4
THCVA	1.934	0.9999	2.457	1.971	98.3	25.03	0.288	100.1	73.59	0.168	98.1
CBN	1.949	0.9999	2.447	1.919	97.9	24.99	0.559	100	73.76	0.238	98.3
CBNA	2.688	0.9999	2.441	2.646	97.7	24.79	0.365	99.2	73.6	0.232	98.1
d9-THC	5.303	0.9999	2.411	2.524	96.4	24.56	0.454	98.3	72.85	0.387	97.1
d8-THC	3.32	0.9999	2.487	2.119	99.5	25.18	0.532	100.7	74.06	0.675	98.8
CBL	3.224	0.9999	2.469	3.447	98.7	24.84	0.231	99.4	73.67	0.302	98.2
CBC	2.172	0.9999	2.43	1.988	97.2	24.89	0.25	99.6	73.52	0.183	98
THCA	2.741	0.9992	2.449	2.764	97.9	24.89	0.316	99.6	73.61	0.219	98.1
CBCA	5.258	0.9999	2.424	2.843	96.9	24.76	0.382	99	73.92	0.266	98.6

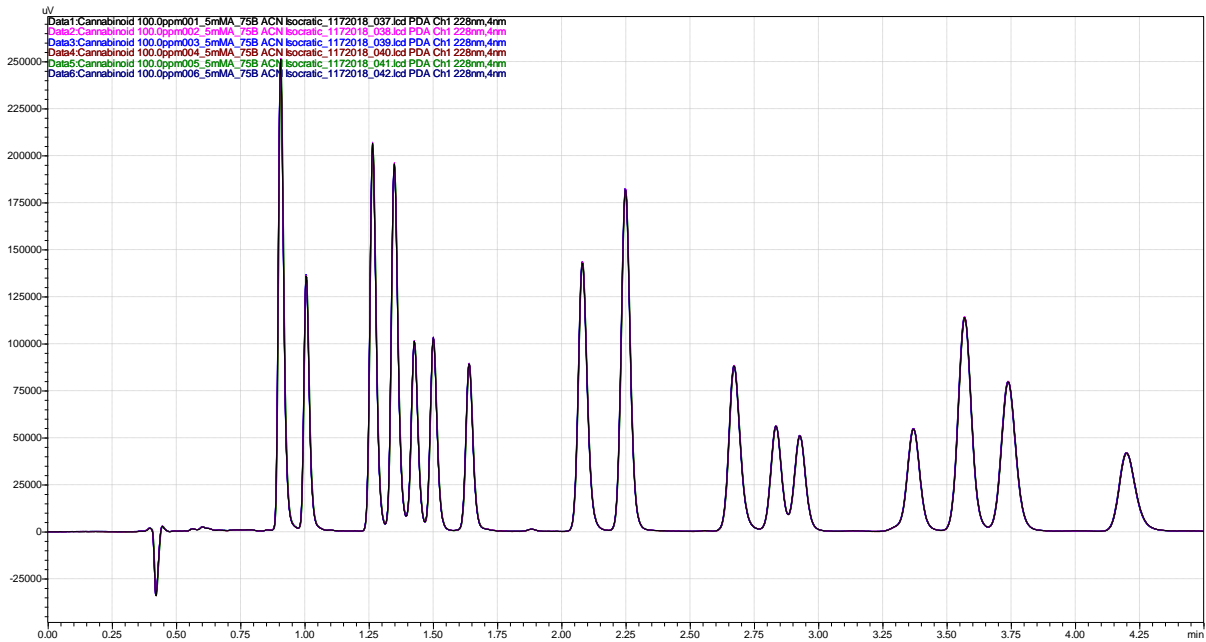


Figure 4: 16 Cannabinoid Mixture - Overlay of 6 injections (1 µL; 100 ppm)

#### *Carryover Assessment and Samples in Matrix*

Following 36 injections of neat standards, a series of solvent blank injections was performed. The first blank injection showed a minor peak attributed to CBDVA, with no discernable quantities of other cannabinoids. Subsequent blank injections showed no detectable carryover from any of the target components. When running samples in matrix, a column wash is recommended to minimize the chance of carryover affecting column performance over time.

Two approaches are common: First, a pump program column wash or flush could be added as a program modification of approximately 2 minutes to the end of the run (gradient control mode); Second, a wash method could be applied with a null injection or solvent injection run at regular intervals in the batch, perhaps after each 10<sup>th</sup> injection. Further testing is required to determine optimal results.

#### ■ References

1. Wang, M., Wang, Y. H., Avula, B., Radwan, M. M., Wanas, A. S., van Antwerp, J., ... & Khan, I. A. (2016). Decarboxylation study of acidic cannabinoids: a novel approach using ultra-high-performance supercritical fluid chromatography/photodiode array-mass spectrometry. *Cannabis and cannabinoid research*, 1(1), 262-271.
2. ElSohly, M., & Gul, W. (2014). Constituents of cannabis sativa. *Handbook of cannabis*, 3.
3. Restek. (2017). 16 Cannabinoids on Raptor ARC-18 1.8 µm by LC-UV.



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