



# Expanding the Cannabinoid Target List for a Rugged HPLC Potency Method

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## Introduction

Non-psychoactive cannabinoids do not directly alter perception or consciousness, yet still show medicinal effects. These include pain relief, inflammation reduction, appetite stimulation, nausea reduction, anxiety relief, psychosis relief, seizure reduction, muscle spasm suppression, blood sugar management, nervous system degeneration prevention, psoriasis treatment, reduces risk of artery blockage, anti-bacterial, cancerous cell growth inhibition, and bone growth promotion effects.

Prior work in support of the turn-key package, Shimadzu Cannabis Analyzer for Potency™ includes a method for the separation of 11 cannabinoids in a reasonable time frame of 30 minutes. This study expands the chromatographic target list to 18 cannabinoids using High Resolution method.

## Equipment and Method

A Shimadzu Cannabis Analyzer for Potency™ – an integrated HPLC system with built-in UV detector – was used for this study. Table 1 shows a summary of the instrument and method parameters.

Table 1: Instrument and Method Parameters

Item	Description
Standard (Shimadzu)	11 components (CRM) in acetonitrile (1mL x 250ug/mL), 220-91239-21
HPLC System	Cannabis Analyzer for Potency™, 220-94420-00
Detector	UV-Vis
Wavelength Monitored (nm)	220
Mobile Phase A	0.085% Phosphoric Acid in Water
Mobile Phase B	0.085% Phosphoric Acid in methanol
Gradient Program	60% B for 5 min; 60%-72% B over 11 min; 72%-95% B over 6 min; 95% B for 2 min; 95%-60% B over 1 min; 60% B for 5 min
Column	Shim Pack XR-ODS II, 75 mm x 3.0 mm, 2.2 um, 228-41624-91
Guard column	NexLeaf CBX Guard Column Cartridge, 2.7 um, 220-91525-72; and NexLeaf Guard Holder 220-91525-73
Flowrate (mL/min)	1
Run time per injection (min)	30
Oven Temperature (°C)	50
Injection Volume (µL)	5

## Results and Discussion

Six levels of calibration standards were prepared ranging from 0.5 to 85 mg/L, in addition three Quality Control (QC) standards at 2.5 mg/L, 25 mg/L and 75 mg/L, were prepared. Calibration curves and QC standards were evaluated using seven replicate injections and evaluating the correlation coefficient (R<sup>2</sup>) of the linear regression. All calibration curves passed the high-resolution method criteria (R<sup>2</sup>≥0.999). The statistical results were processed in LabSolutions, version 5.99. Results are shown in table 2. Figure 1 shows the 18-cannabinoid mixture resolution.

Table 2: Statistical analysis of 6-point calibration curve with seven replicates for calibration standards and quality control (QC) standards for the 18-cannabinoid mixture

No.	Compound	Calibration (n=7) R <sup>2</sup>	2.5 ppm (QC Low) (n=7)			25.0 ppm (QC Medium) (n=7)			75.0 ppm (QC High) (n=7)		
			Mean Conc.	RSD (%)	Accuracy (%)	Mean Conc.	RSD (%)	Accuracy (%)	Mean Conc.	RSD (%)	Accuracy (%)
1	CBDV	0.9998	2.52	0.273	101.1	25.38	0.285	101.5	74.30	0.103	99.1
2	CBDVA	0.9998	2.49	0.360	99.9	25.03	0.340	100.1	74.31	0.077	99.1
3	CBCO	0.9998	2.51	0.230	100.4	24.87	0.259	99.5	74.47	0.067	99.3
4	THCV	0.9998	2.53	0.310	101.4	25.45	0.290	101.8	74.10	0.093	98.8
5	CBD	0.9998	2.52	0.357	100.8	25.34	0.302	101.3	74.28	0.093	99.0
6	CBG	0.9998	2.54	0.272	101.6	25.30	0.310	101.2	74.40	0.095	99.2
7	CBDA	0.9998	2.50	0.302	100.1	24.98	0.343	99.9	74.41	0.048	99.2
8	CBCV	0.9998	2.53	0.354	101.5	25.16	0.386	100.6	74.26	0.191	99.0
9	CBGA	0.9998	2.56	0.350	102.7	24.71	0.357	98.9	75.01	0.212	100.0
10	CBN	0.9998	2.53	0.228	101.2	25.05	0.282	100.2	74.51	0.073	99.4
11	d9-THC	0.9996	2.55	0.148	102.3	25.72	0.292	102.9	74.20	0.060	98.9
12	d8-THC	0.9997	2.51	0.274	100.7	25.45	0.275	101.7	74.27	0.073	99.0
13	CBC	0.9998	2.55	0.392	102.0	25.25	0.291	101.0	74.33	0.049	99.1
14	CBNA	0.9998	2.51	0.426	100.4	24.74	0.286	98.9	74.25	0.052	99.0
15	d9-THCP	0.9997	2.51	0.960	100.4	25.75	0.702	103.0	74.33	0.417	99.1
16	THCA	0.9998	2.56	0.489	102.6	25.01	0.729	100.0	74.20	0.305	98.9
17	CBLA	0.9998	2.59	0.409	103.7	25.10	0.601	100.4	74.46	0.073	99.3
18	CBCA	0.9996	2.50	0.639	100.2	24.95	1.862	99.8	74.60	0.221	99.5

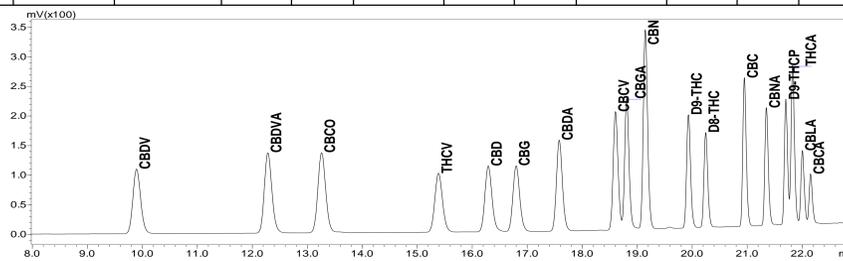


Fig. 1 18-cannabinoid mixture resolution (5 µL injection at 50 ppm)

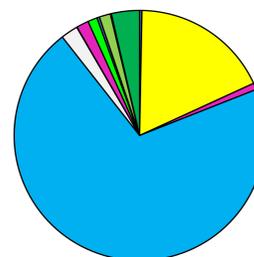
The measured potency for the dry sample (flower) is represented in Tables 3. The results were in consistency with those from the packaging label, as a total-CBD level of more than 90% (wt.%) was expected.

Table 3: Measured potency for flower

Compound	Conc. (wt.%)
CBDVA	0.36
CBD	21.16
CBG	1.01
CBDA	83.51
CBGA	2.62
CBN	0.05
D9-THC	1.87
CBC	1.45
CBNA	0.38
THCA	1.71
CBLA	0.15
CBCA	4.30

<b>Total CBD (%)</b>	<b>94.39</b>
<b>Total CBD (mg/g)</b>	<b>943.98</b>
<b>Total THC (%)</b>	<b>3.363</b>
<b>Total THC (mg/g)</b>	<b>33.637</b>



Figures 2 and 3 illustrate the results for a commercially available concentrated CBD tincture hemp. Table 4 shows the summary of cannabinoids quantitation. For the tinctures, or in general for any form of oil structure, we define the potency in mg/mL to be consistent with the manufacturer's label. Using our method, we obtained a total CBD of 637.8 mg CBD (label claimed 500 mg CBD), for a commercially available tinctures, respectively.

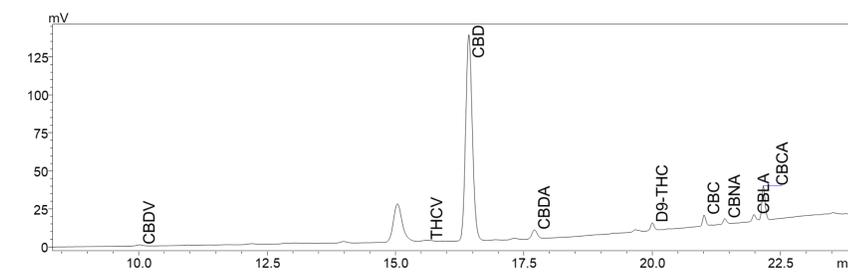


Fig 2. commercially available tincture oil (1000x diluted) profiles (5 µL injection volume)

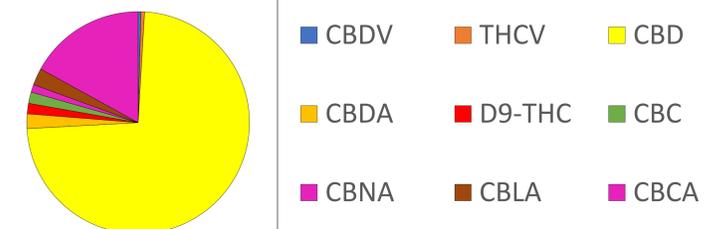


Fig 3: Summary of cannabinoids content for a 10 mL (or 0.33 oz) commercially available tincture

Table 4: Summary of CBD and THC quantitative determination for samples

ID #	Sample Name	Tincture Volume (mL)	Dry Weight (mg)	Extraction Volume (mL)	Dilution	Measured Mean Conc.	
						Total CBD (mg/mL)	Total THC (mg/mL)
1	Dry flower	-	100	10	100	9.63	0.17
3	Tincture	10	-	-	1000	63.78	1.30

## Conclusion

Given that there is already one FDA-approved drug derived from CBD, there is a significant interest in the development of therapies from CBD and/or THC. A simple and rugged HPLC method for differentiating cannabis and hemp is presented, building on the existing High Sensitivity Method using the Shimadzu Cannabis Analyzer for Potency™. Potency method was optimized for the quantitative determination of four major cannabinoids.