A Fast and Comprehensive Potency Determination of 21-Cannabinoids in Hemp Material and Finished Tinctures using the Cannabis Analyzer for Potency™

Niloufar Pezeshk and Eberhardt Kuhn
Shimadzu Scientific Instruments, Inc.

Introduction

Cannabis is a plant of the Cannabaceae family and contains more than one hundred biologically active chemical compounds. The most commonly known compounds are delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is the component that produces the "high" associated with marijuana use. Much interest has been seen around CBD and its potential related to health benefits. Since 2018, the FDA has approved one CBD-containing drug Epidiolex, to treat two rare and severe forms of epilepsy. The same year, the 2018 Farm Bill was signed into law. Hemp is defined as product with no more than 0.3 percent THC on a dry weight basis.
Currently the potency for hemp oil is reported in concentration units of mg/mL. For effective results, individuals' prescriptions are different. Doses can be as low as 0.5-10 mg CBD per day, in other cases 100-200 mg CBD daily, or as high as 300-800 mg CBD daily in clinical trials. Tinctures are concentrated liquids commonly in quantities of 0.5, 1, or 2 ounces, with a dropper for measuring a specific number of drops (25-30 drops = 1 mL). The label shows the potency or mg of CBD per mL of solution.
The research shows that the "entourage" effect of hemp makes it an effective therapeutic. There are conditions that respond to pure CBD, but many cases in which CBD is more effective in a broad-spectrum oil. In this application note, an HPLC method is presented which builds on the well-established potency method using the Shimadzu Cannabis Analyzer for Potency™, a comprehensive and fast determination of 21-cannabinoids in only 15 minutes (including the wash-step). Cannabinoid profiles for commercially available dry hemp and finished tinctures are presented.

Equipment and Method

For this study a Shimadzu Cannabis Analyzer for Potency™ – an integrated HPLC system with built-in UV detector – was used. Table 1 shows the instrument and method parameters summary. Table 2 shows a list of initial concentrations for each standard. Quality Control (QC) standards were prepared using the same method as the calibration standards.

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 Acetonitrile
Gradient Program	70% B for 3 min; 70%-85% B over 7 min; 85%-95% B over 0.01 min; 95% B for 1.99 min; 95%-70% B over 0.01 min; 70% B for 2.99 min
Column	NexLeaf CBX for Potency 150 mm x 4.6 mm, 2.7 um, 220-91525-70
Guard column	NexLeaf CBX Guard Column Cartridge, 2.7 um, 220-91525-72; and NexLeaf Guard Holder, 220-91525-73
Flowrate (mL/min)	1.6
Run time per injection (min)	15
Oven Temperature (°C)	35
Injection Volume (µL)	5

Results and Discussion

A six-point calibration curve ranging from 0.5 to 85 mg/L and three Quality Control (QC) standards, 2.5 mg/L 30 mg/L and 70 mg/L, were prepared. Calibration curves and QC standards were evaluated using seven replicate injections and evaluating the correlation coefficient (R^2) of the linear regression. All calibration curves passed the high-sensitivity method criteria (R2≥0.999). A best-fit weighting method was selected for the linear regression for calibration curve quantitation. The statistical results were processed via Browser in LabSolutions, version 5.99. Results are shown in Table 2. Figures 1 and 2 show the 21-cannabinoid mixture resolution and repeatability, respectively.

Table 2: Statistical analysis of 6-point calibration curve with seven replicates for calibration standards and quality control (QC) standards for the 21-cannabinoid mixture

No.	Compound	Calibration (n=7)	2.5 ppm (QC Low) (n=7)			30.0 ppm (QC Medium) (n=7)			70.0 ppm (QC High) (n=7)		
		R^2	Mean Conc.	RSD (%)	Accuracy (%)	Mean Conc.	RSD (%)	Accuracy (%)	Mean Conc.	RSD (%)	Accuracy (%)
1	CBDVA	0.99994	2.50	1.424	100.2	29.48	1.911	98.2	73.37	0.109	104.8
2	CBDV	0.99993	2.58	1.234	103.3	29.92	0.498	99.7	71.60	0.124	102.3
3	CBCO	0.99998	2.54	1.299	101.9	29.66	0.387	99.0	75.00	0.123	107.1
4	CBDA	0.99994	2.51	1.539	100.5	28.91	0.281	96.4	72.11	0.181	103.0
5	CBGA	0.99994	2.51	1.448	100.4	29.46	1.667	98.2	72.29	0.136	103.3
6	CBG	0.99994	2.54	2.329	101.6	29.56	0.246	98.5	72.15	0.151	103.1
7	CBD	0.99990	2.50	2.147	100.1	29.67	0.146	99.0	72.05	0.166	102.9
8	THCV	0.99996	2.50	1.633	100.3	29.85	0.364	99.5	72.09	0.241	103.0
9	CBCV	0.99997	2.62	2.260	105.0	29.80	0.397	99.2	71.66	0.143	102.4
10	THCVA	0.99995	2.49	2.352	99.9	29.44	0.191	98.1	80.27	0.163	114.7
11	CBN	0.99997	2.55	1.703	102.1	29.69	0.135	99.0	72.15	0.153	103.1
12	CBDP	0.99997	2.60	2.580	104.3	29.89	0.218	99.6	69.97	0.220	100.0
13	D9-THC	0.99990	2.58	1.665	103.1	29.88	0.137	99.6	71.89	0.234	102.7
14	D8-THC	0.99994	2.54	1.376	101.8	29.77	0.183	99.3	71.85	0.308	102.7
15	CBL	0.99991	2.51	2.453	100.5	30.00	1.416	100.0	74.58	0.145	106.5
16	CBC	0.99994	2.53	1.897	101.2	29.74	0.316	99.1	72.01	0.119	102.9
17	THCA	0.99989	2.53	2.356	101.2	29.47	0.385	98.2	71.93	0.195	102.8
18	D8-THCA	0.99980	2.54	2.432	101.6	29.67	0.167	98.9	76.28	0.164	109.0
19	CBCA	0.99958	2.66	5.398	106.5	29.33	1.274	97.8	72.87	0.575	104.1
20	D9-THCP	0.99937	2.51	2.954	100.4	29.88	0.692	99.6	71.76	0.465	102.5
21	CBT	0.99976	2.59	2.210	103.7	29.91	0.890	99.7	72.33	0.234	103.3

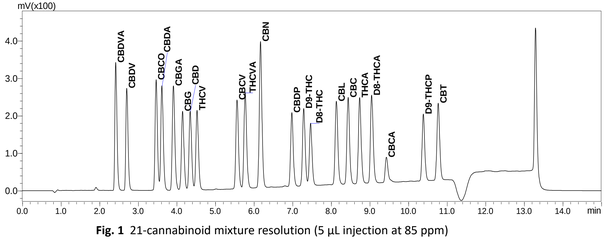


Fig. 1 21-cannabinoid mixture resolution (5 µL injection at 85 ppm)

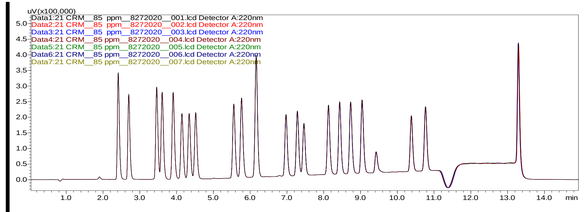


Fig 2. 21-cannabinoid mixture repeatability and overlay of seven-injections (5 µL injection at 85 ppm)

Figure 3 shows an example of a commercial purified CBD hemp (dry sample). No d9-THC or THCA were detected.

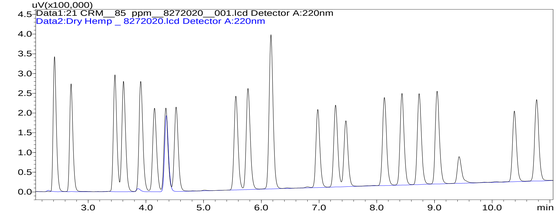


Fig 3. CBD Hemp dry-sample (diluted 100x) vs. 85 ppm 21-Component Standards Mixture (5 µL injection volume)

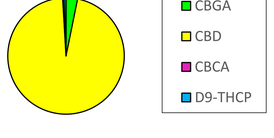
Equations (1) and (2) were used for the total amount of THC (contributions from d9-THC and THCA) on dry weight basis. Similar equations can be used to calculate the total CBD (contributions from CBD and CBDA). These equations can be edited by the user when reporting the potency for dry samples.

Total THC (wt.%) = Conc. d9-THC (wt.%) + (Conc. THCA (wt.%) x 0.877) ... [Eq.1]
Total THC (mg/g) = [Conc. d9-THC (wt.%) + (Conc. THCA (wt.%) x 0.877)] x10 ... [Eq.2]

The measured potency for the dry sample is represented in Table 3. The results were consistent with those from the manufacturer, as a CBD level of more than 75% (wt.%) and no level of THC was expected.

Table 3: Measured potency for dry hemp flower

Compound	Conc. (wt.%)
CBGA	2.534
CBD	76.201
CBCA	0.309
D9-THCP	0.428



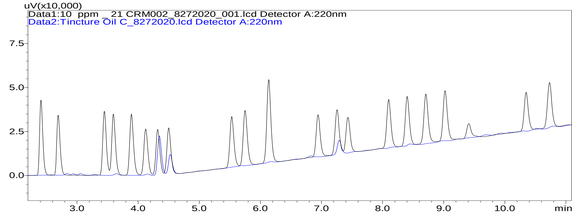
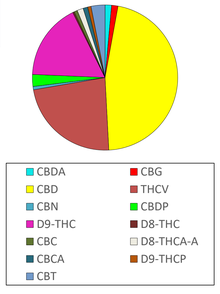


Fig 4. Tincture oil (1000x diluted) vs. 10 ppm 21-Component Standard (5 µL injection volume)

Table 4: Measured potency for commercial tincture oil (30 mL)

Compound	Measured Conc. (mg/mL)	Amt. per 30 mL (mg)	% of Total
CBDA	0.27	8.28	1.44
CBG	0.27	8.22	1.43
CBD	8.87	266.31	46.31
THCV	4.43	132.99	23.13
CBN	0.14	4.20	0.73
CBDP	0.51	15.30	2.66
D9-THC	3.25	97.71	16.99
D8-THC	0.06	1.83	0.32
CBC	0.14	4.35	0.76
D8-THCA-A	0.26	7.80	1.36
CBCA	0.19	5.97	1.04
D9-THCP	0.14	4.29	0.75
CBT	0.59	17.82	3.10
Total	19.16	575.07	100.00



For the commercial tincture (representative of oils in general) we found the potency to be consistent with the manufacturer's label. Using our method, we obtained a total CBD of 273.6 mg (label claimed 300 mg CBD)

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 more effective broad-spectrum CBD oil over isolates. In response to the demand for a comprehensive development of chromatography techniques in potency testing of cannabis and hemp, we developed a method that fully resolved 21-cannabinoids in only 15 minutes (wash-step was included) using the Shimadzu Cannabis Analyzer for Potency™. The statistical results show retention time and peak area repeatability, quantitative accuracy and sensitivity, provided a robust potency results for cannabinoid profiles for commercially available dry hemp and tincture oil.