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Application News

No. HPLC-039

High Performance Liquid Chromatography

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

■ 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. There is much interest in CBD and its potential related 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 total THC on a dry weight basis.

Hemp oil potency is commonly reported in concentration units of mg/mL. Effective doses vary with the individual and makeup of the oil, ranging from 0.5-10 mg CBD per day up to 100-200 mg CBD daily. Doses have been 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.

Research shows that the "entourage" effect of hemp makes it an effective therapeutic. There are conditions that respond to pure CBD but it may be 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

A Shimadzu Cannabis Analyzer for Potency™ – an integrated HPLC system with built-in UV detector – was used for this study. 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: Summary of method and instrument 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 (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

Table 2: Initial concentrations for the 21 cannabinoids prior to mixture preparation

Reference Standard	Abbreviation	Stock Conc. (mg/L)	Standard
cannabidivarin	CBDV	250	Shimadzu
tetrahydrocannabivarin	THCV	250	Shimadzu
cannabidiol	CBD	250	Shimadzu
cannabigerol	CBG	250	Shimadzu
cannabidiolic acid	CBDA	250	Shimadzu
cannabigerolic acid	CBGA	250	Shimadzu
cannabinol	CBN	250	Shimadzu
delta-9-tetrahydrocannabinol	D9-THC	250	Shimadzu
delta-8-tetrahydrocannabinol	D8-THC	250	Shimadzu
cannabichromene	CBC	250	Shimadzu
tetrahydrocannabinolic acid	THCA	250	Shimadzu
delta-9-tetrahydrocannabiphorol	D9-THCP	5,000	Cayman
delta-8-trans-tetrahydrocannabinolic acid	D8-THCA	10,000	Cayman
cannabidiphorol	CBDP	10,000	Cayman
(±)-cannabichromeorcin	CBCO	1,000	Cayman
cannabichromevarin	CBCV	1,000	Cayman
cannabicitran	CBT	1,000	Cayman
cannabidivarinic acid	CBDVA	1,000	Cerilliant
tetrahydrocannabivarinic acid	THCVA	1,000	Cerilliant
(±)-cannabicyclol	CBL	1,000	Cerilliant
cannabichromenic acid	CBCA	1,000	Cerilliant

■ Hemp Sample Preparation (Dry Flower and Tincture Oil)

Samples were comprised of dry flower material or tincture. The preparation is dependent on the nature/form of the sample. The initial amounts for the sample and extraction volume depend on the availability of the sample and solvent. A Geno/Grinder is a necessary step for the dry sample form, if the dry sample is not already a homogenized powder. It is necessary to use a 0.45 um filtration step to avoid guard column clogging.

Step-by-step preparation of flower hemp (dry sample) to reach a dilution factor of 100x:
- Weigh 100 mg dry sample into a 50 mL centrifuge tube.
- Transfer two 9.5 mm O.D. steel balls into the tube.
- Shake at 1000 rpm for 5 minutes using a 2010 Geno/Grinder.
- Add 10 mL of methanol to the tube.
- Shake at 1000 rpm for 1 minute using a vortex mixer.
- Wait 15 minutes.
- Transfer 10 µL of extraction supernatant to a 1.5 mL microtube.
- Add 990 µL of methanol to the microtube.
- Mix using a vortex mixer for 1 minute.
- Agitate for 30 seconds.
- Filter using a 0.45 µm PTFE or Nylon syringe filter into an HPLC vial.
- Secure the vial with a septum and cap.

Step-by-step preparation of the tincture sample (oily sample) to achieve a dilution factor of 1000x:
- Obtain a glass scintillation vial.
- Pipette 10 µL tincture or oil to the vial.
- Add 2 mL isopropanol and completely dissolve.
- Agitate the mixture for 30 seconds.
- Add 8 mL methanol.
- Filter the mixture through a 0.45 µm PTFE or Nylon syringe filter into an HPLC vial.
- Secure the vial with a septum and cap.

■ 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 (low), 30 mg/L (mid) and 70 mg/L (high), 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 ($R^2 \geq 0.999$).

Figure 1 shows the calibration curves for the 21 target cannabinoids. A best-fit weighting method (1/C) 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 3. Figures 2 and 3 shows the 21-cannabinoid mixture resolution and repeatability.
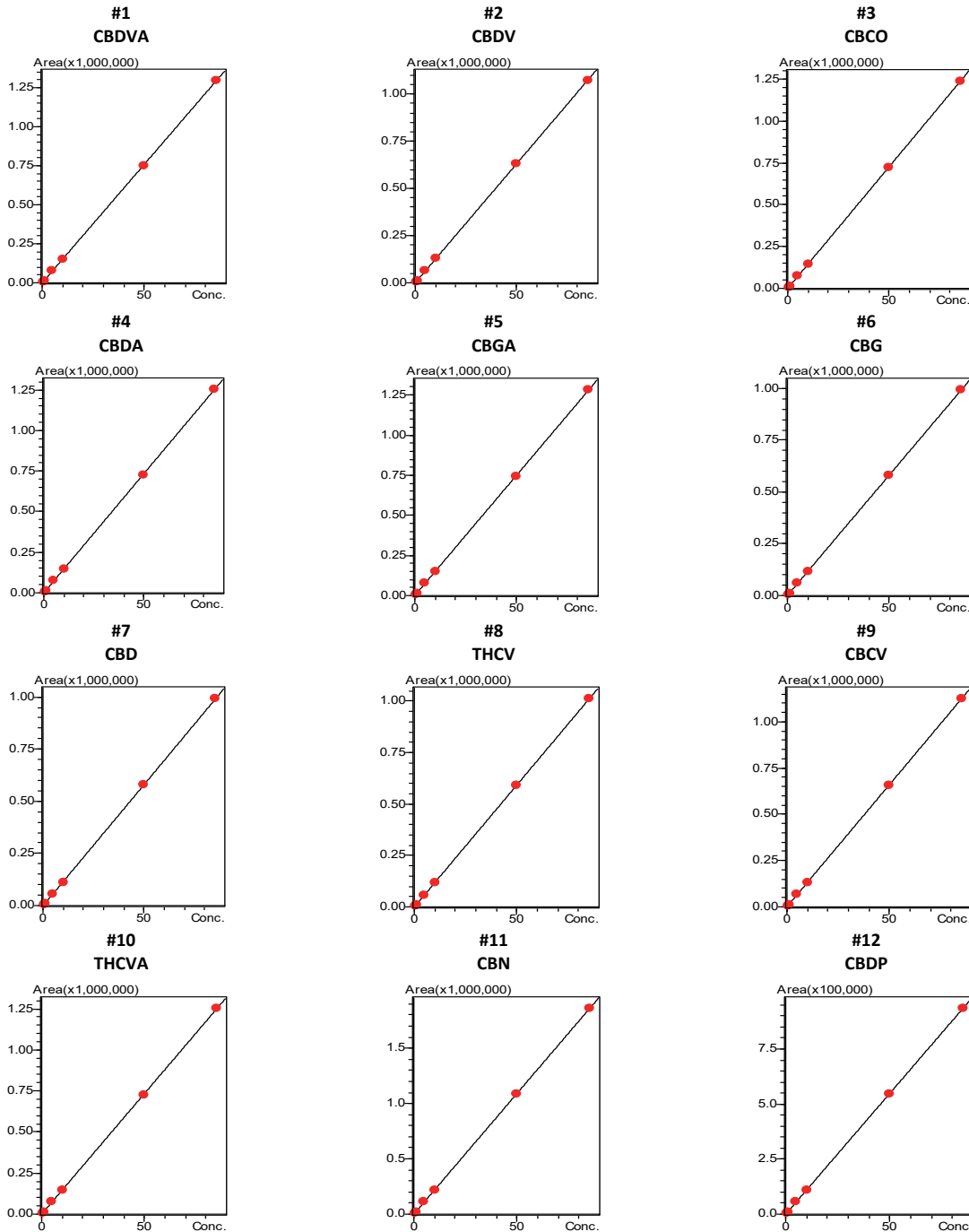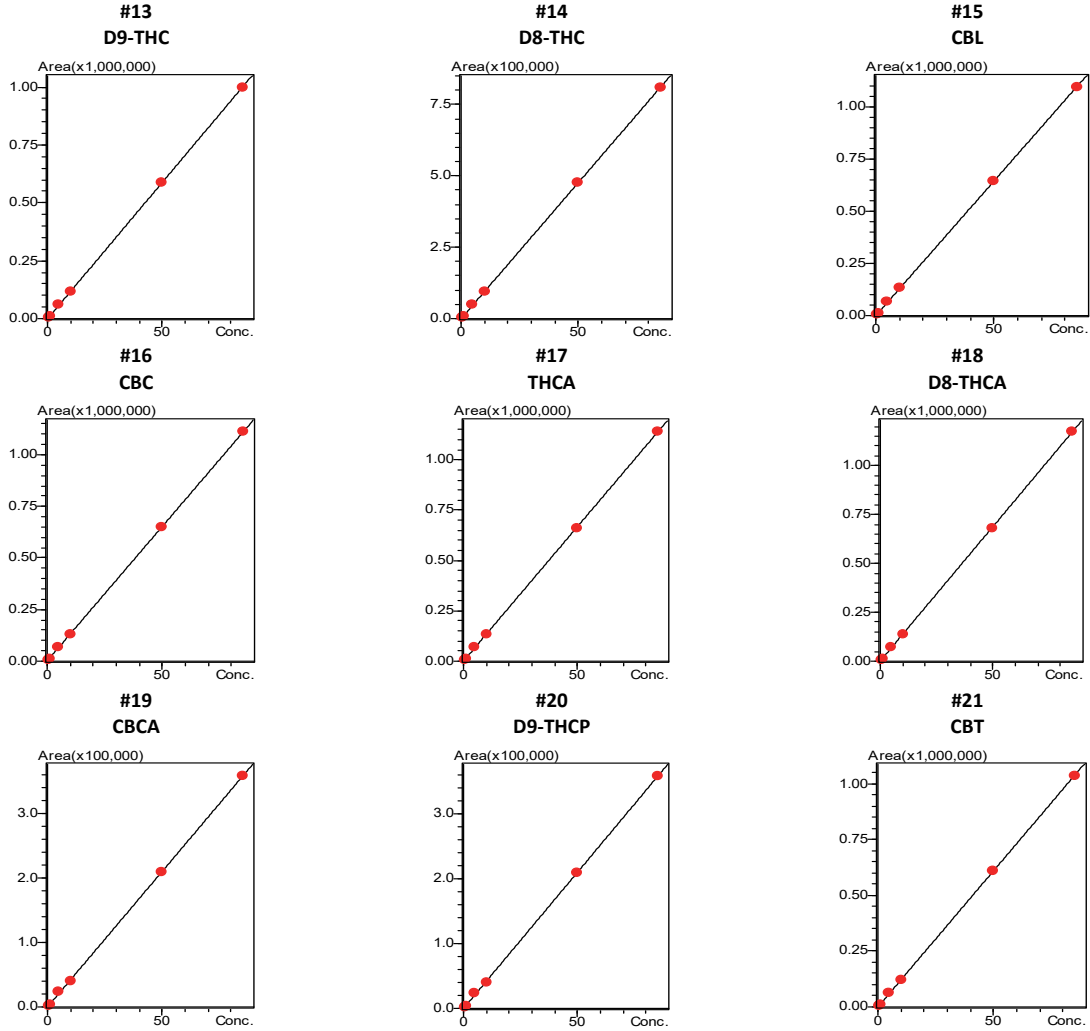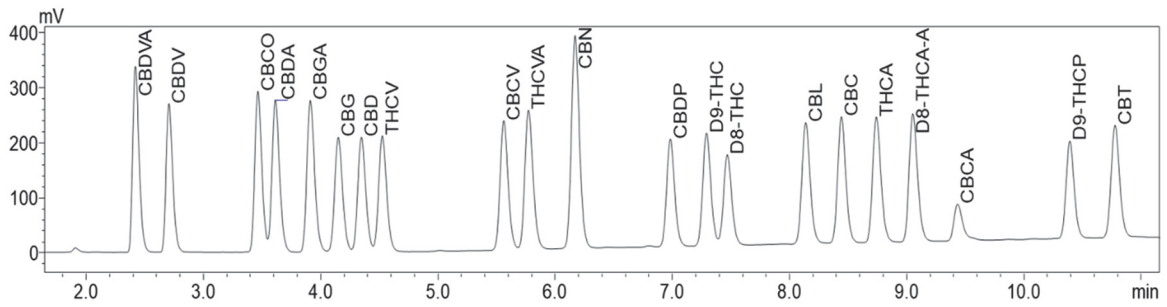
Figure 1: Standard curves for 21-cannabinoids

Figure 2: 21-cannabinoid mixture resolution (5 µL injection at 85 ppm)

Table 3: 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
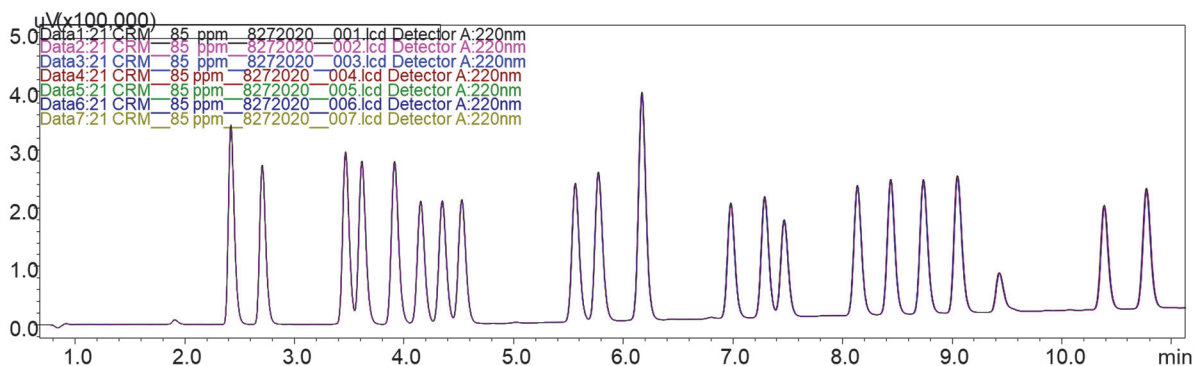
Figure 3: Repeatability and overlay of seven-injections (5 μL injection at 85 ppm)

Figure 4 shows an example of a commercial purified CBD hemp (dry sample). Neither D9-THC nor THCA was detected.
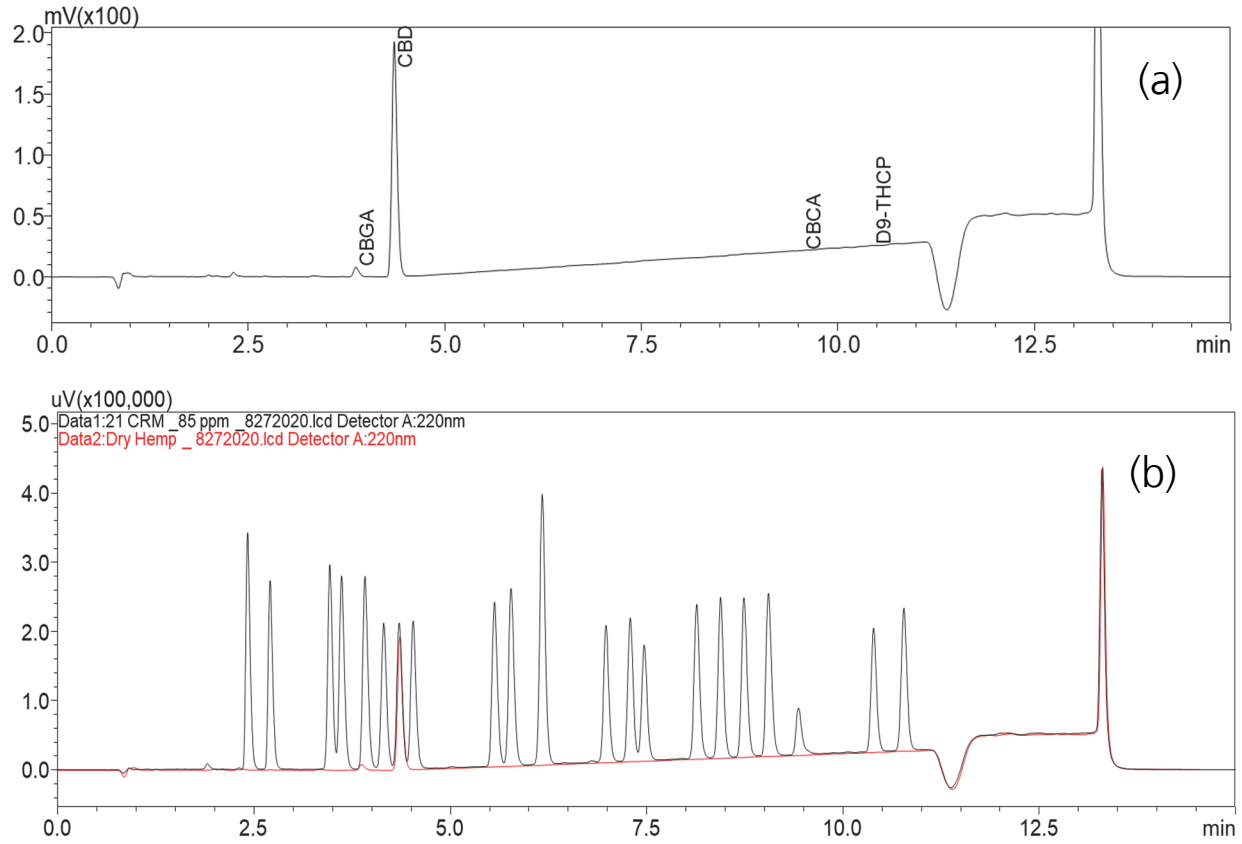

Figure 4: CBD Hemp profile (A); and CBD Hemp 100x diluted (dry sample) (B) 85 ppm 21-Component Standards Mixture. Results obtained at 5 µL injection volume. Peaks listed in Table 4 in order of retention time.

Reports can be generated based on custom calculations. For example, using equations (1) and (2) below, the total amount of THC (contributions from d9-THC and THCA) can be determined 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.

Individual cannabinoid (wt. %) percentage or potency for dry basis can be calculated using equation (3). These calculations can be performed in LabSolutions by adding the Dilution Factor of the sample. Dilution Factor was calculated using equation (4).

$$\text{Total THC (wt.\%)} = \text{Conc. d9-THC (wt.\%)} + (\text{Conc. THCA (wt.\%)} \times 0.877) \qquad \dots [Eq.1]$$

$$\text{Total THC (mg/g)} = [\text{Conc. d9-THC (wt.\%)} + (\text{Conc. THCA (wt.\%)} \times 0.877)] \times 10 \qquad \dots [Eq.2]$$

$$Cannabinoid\ (wt.\%) = \begin{pmatrix} Concentration \\ of \\ Component,\ ppm \end{pmatrix} \left(\frac{Extraction\ Vol,\ mL}{Sample\ Aliquot,\ mg}\right) \begin{pmatrix} Additional \\ Dilution \\ Factor \end{pmatrix} \begin{pmatrix} Conversion \\ mL\ to\ L \end{pmatrix} . 100 \quad \dots [Eq.3]$$

$$Dil.\ Factor = (Extraction\ Vol,\ mL) \begin{pmatrix} Additional \\ Dilution \\ Factor \end{pmatrix} (1/1000). 100 \dots [Eq.4]$$

The measured potency for the dry sample is represented in Table 4. 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.

Figure 5 illustrates the reports generated for the dry sample. Figures 6 and 8 illustrate the chromatograms for two commercially available CBD-rich tinctures. Tables 5 and 6 show the measured potency (mg/mL). Figures 7 and 9 show the tincture report generated in LabSolutions.

Table 4: Measured potency for dry hemp flower

Compound	Conc. (mg/L)	Conc. (wt.%)	Total CBD (%)	Total CBD (mg/g)
CBGA	258.4	2.534	-	-
CBD	7772.4	76.201	76.20	762.00
CBCA	31.4	0.309	-	-
D9-THCP	43.6	0.428	-	-

CANNABIS ANALYZER
FOR POTENCY

<Sample Information>

System Administrator
Sample Name   : Dry Hemp-8272020
Sample ID   : 001
Data Filename   : Dry Hemp _ 8272020.lcd
Method Filename : HighSensitivityMethod_21CRM.lcm
Vial #   : 1-17
Sample Type   : Unknown
Injection Volume : 5 uL
Sample Amount   : 102 mg
dilution Facor   :100
Date Acquired   : 8/28/2020 1:25:42 PM   Acquired by   : System Administrator
Date Processed   : 10/20/2020 3:04:27 PM   Processed by   : System Administrator

<Chromatogram>

<Quantitative Results>

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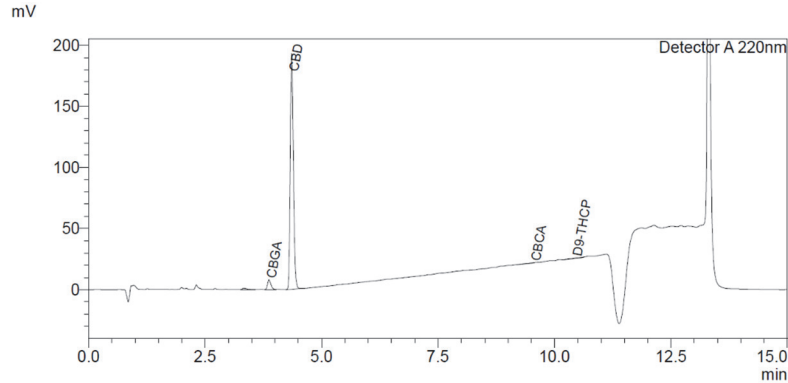
ID#	Name	Ret. Time	Conc.	Unit
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2	CBDV	--	--	%
3	CBCO	--	--	%
4	CBDA	--	--	%
5	CBGA	3.869	2.534	%
6	CBG	--	--	%
7	CBD	4.358	76.201	%
8	THCV	--	--	%
9	CBCV	--	--	%
10	THCVA	--	--	%
11	CBN	--	--	%
12	CBDP	--	--	%
13	D9-THC	--	--	%
14	D8-THC	--	--	%
15	CBL	--	--	%
16	CBC	--	--	%
17	THCA	--	--	%
18	D8-THCA-A	--	--	%
19	CBCA	9.548	0.309	%
20	D9-THCP	10.440	0.428	%
21	CBT	--	--	%

Total THC	0.00	%
Total THC	0.00	mg/g
Total CBD	76.20	%
Total CBD	762.01	mg/g

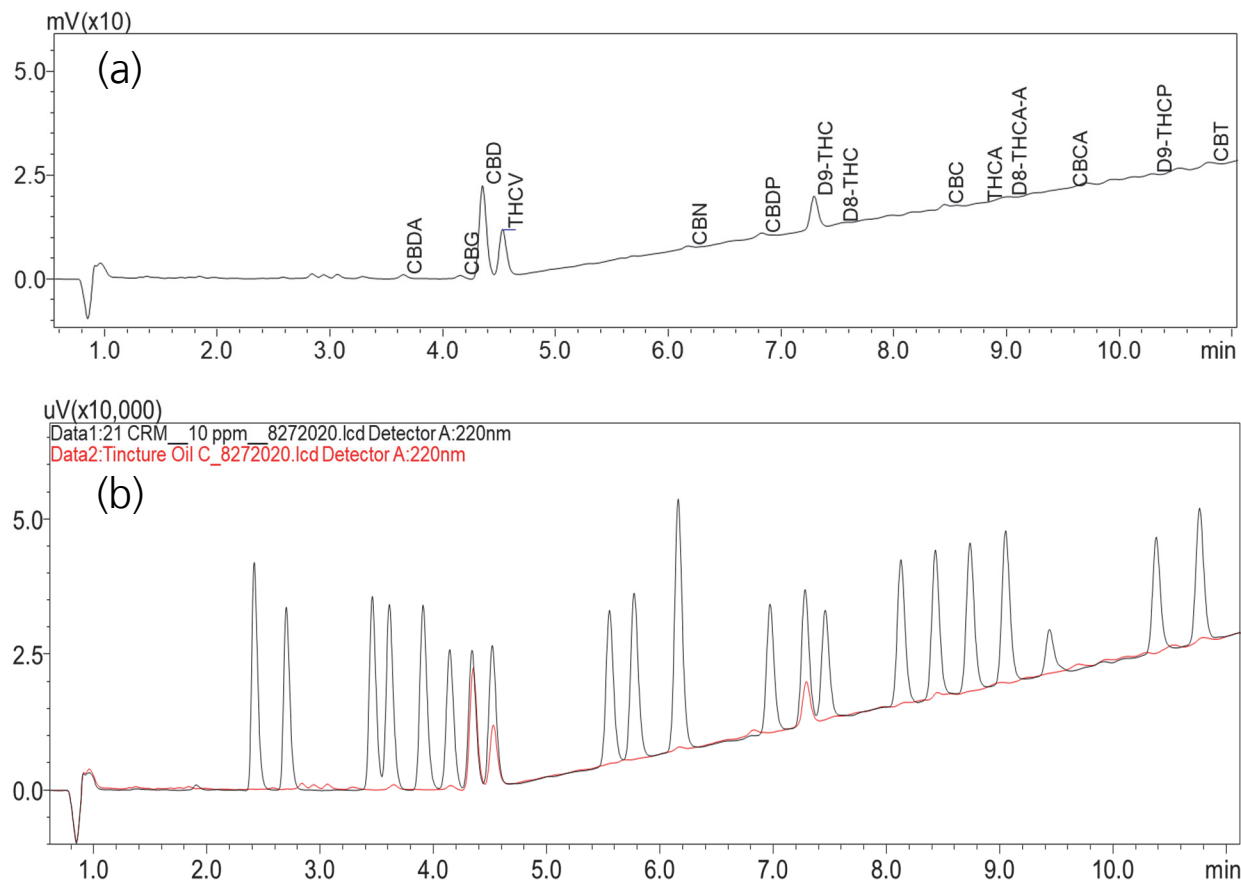**Figure 5**: Dry sample report from Cannabis Analyzer Overlay Software

Figure 6: Tincture oil profile (A); Tincture 1000x diluted (B) 10 ppm 21-Component Standard. Results obtained at 5 µL injection volume. Peaks listed in Table 5 in order of retention time.

**Table 5** (: Measured potency for commercial tincture oil (30 mL)

Compound	Measured Conc. (mg/mL)	Amt. per 30 mL (mg)	% of Total
CBDA	0.276	8.28	1.44
CBG	0.274	8.22	1.43
CBD	8.877	266.31	46.31
THCV	4.433	132.99	23.13
CBN	0.14	4.2	0.73
CBDP	0.51	15.3	2.66
D9-THC	3.257	97.71	16.99
D8-THC	0.061	1.83	0.32
CBC	0.145	4.35	0.76
D8-THCA-A	0.26	7.8	1.36
CBCA	0.199	5.97	1.04
D9-THCP	0.143	4.29	0.75
CBT	0.594	17.82	3.10
Total	19.169	575.07	100.00

<Sample Information>

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Sample ID   : 001
Data Filename   : Tincture Oil C_8272020 - Copy (2).lcd
Method Filename : HighSensitivityMethod_21CRM.lcm
Vial #   : 1-10   Sample Type   : Unknown
Injection Volume : 5 uL
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Date Processed   : 10/6/2020 3:59:03 PM   Processed by   : System Administrator

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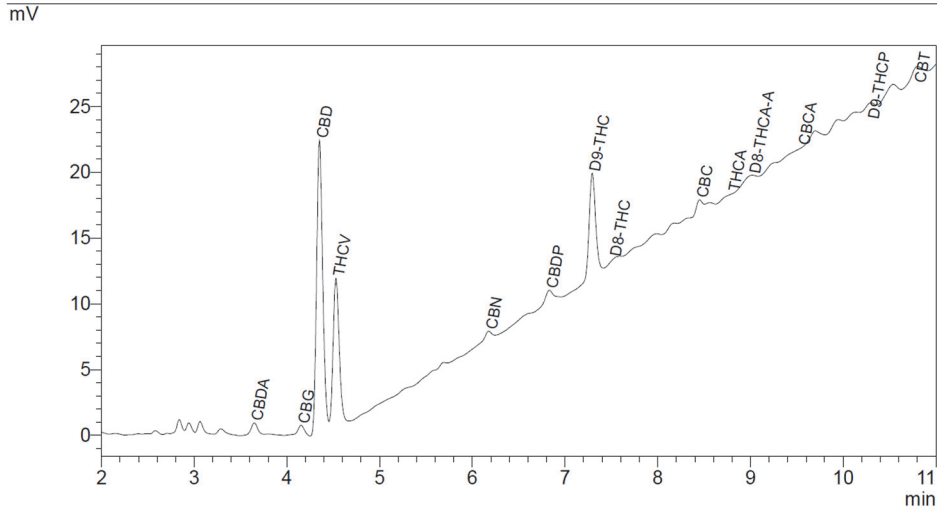
Detector A

ID#	Name	Ret. Time	mg/mL
1	CBDVA	--	0.00000
2	CBDV	--	0.00000
3	CBCO	--	0.00000
4	CBDA	3.649	0.27618
5	CBGA	--	0.00000
6	CBG	4.153	0.27427
7	CBD	4.351	8.87675
8	THCV	4.529	4.43270
9	CBCV	--	0.00000
10	THCVA	--	0.00000
11	CBN	6.176	0.13989
12	CBDP	6.830	0.50989
13	D9-THC	7.293	3.25670
14	D8-THC	7.515	0.06054
15	CBL	--	0.00000
16	CBC	8.451	0.14499
17	THCA	8.795	0.00000
18	D8-THCA-A	9.018	0.25950
19	CBCA	9.548	0.19895
20	D9-THCP	10.298	0.14348
21	CBT	10.799	0.59416

Total THC	3.26	mg/mL
Total CBD	9.12	mg/mL

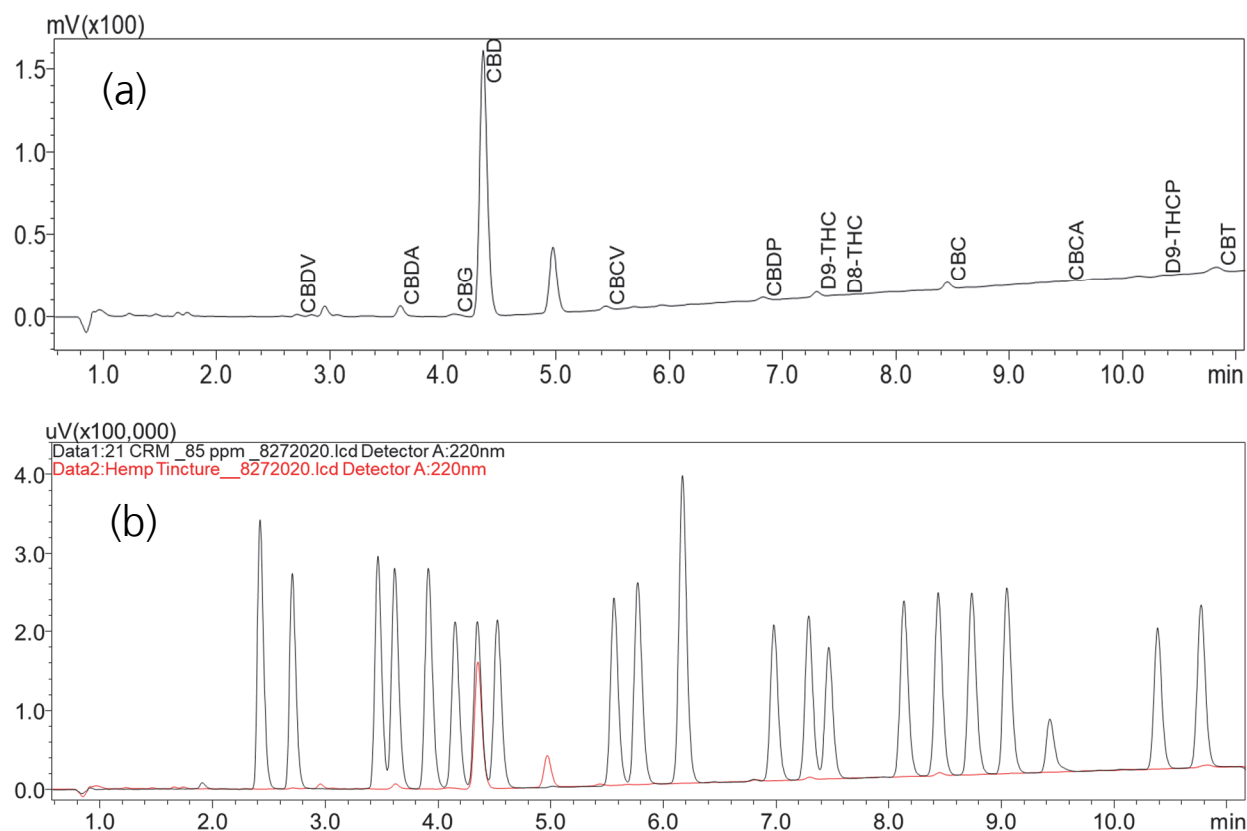**Figure 7**: Tincture report from Cannabis Analyzer Overlay Software

Figure 8: Hemp Tincture profile (A); Tincture 1000x diluted (B) 85 ppm 21-Component Standards Mixture. Results obtained at 5 µL injection volume. Peaks listed in Table 6 in order of retention time.

Table 6: Measured potency for Hemp tincture (10 mL)

| Compound	Measured Conc. (mg/mL)	Amt. per 10 mL (mg)	% of Total
CBDV	0.414	4.14	0.545
CBDA	2.016	20.16	2.655
CBG	0.819	8.19	1.079
CBD	65.391	653.91	86.120
CBCV	0.783	7.83	1.031
CBDP	1.008	10.08	1.328
D9-THC	1.194	11.94	1.573
D8-THC	0.135	1.35	0.178
CBC	1.354	13.54	1.783
CBCA	0.140	1.40	0.184
D9-THCP	0.659	6.59	0.868
CBT	2.017	20.17	2.656
Total	75.930	759.30	100.00

<Sample Information>

System Administrator
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Sample ID   : 002
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Method Filename : HighSensitivityMethod_21CRM.lcm
Vial #   : 1-12   Sample Type   : Unknown
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Date Acquired   : 8/28/2020 9:50:42 AM   Acquired by   : System Administrator
Date Processed   : 10/6/2020 3:53:09 PM   Processed by   : System Administrator

<Chromatogram>

<Quantitative Results>
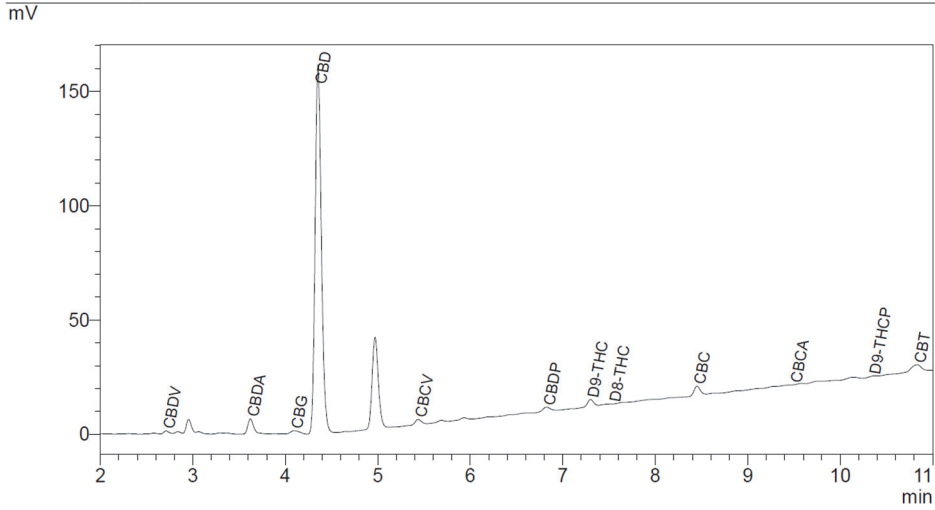
Detector A

ID#	Name	Ret. Time	mg/mL
1	CBDVA	--	0.00000
2	CBDV	2.710	0.41406
3	CBCO	--	0.00000
4	CBDA	3.622	2.01609
5	CBGA	--	0.00000
6	CBG	4.097	0.81935
7	CBD	4.353	65.39084
8	THCV	--	0.00000
9	CBCV	5.436	0.78296
10	THCVA	--	0.00000
11	CBN	--	0.00000
12	CBDP	6.825	1.00760
13	D9-THC	7.300	1.19363
14	D8-THC	7.535	0.13458
15	CBL	--	0.00000
16	CBC	8.452	1.35379
17	THCA	--	0.00000
18	D8-THCA-A	--	0.00000
19	CBCA	9.495	0.13964
20	D9-THCP	10.347	0.65868
21	CBT	10.832	2.01697

Total THC	1.19	mg/mL
Total CBD	67.16	mg/mL

Figure 9: Tincture sample report from Cannabis Analyzer Overlay Software

Table 7 shows the summary of cannabinoids quantitation. For dry-hemp samples, a Dilution Factor of 100 yielded appropriate detector sensitivity to the array of cannabinoids (selected so that the response was within the established quantitative dynamic range established for that sample).

For the tinctures (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), and a total 671.6 mg CBD (label claimed 500 mg CBD), for two commercially available tinctures, respectively.

Table 7: Summary of CBD and THC quantitative determination for three samples.

Sample ID #	Sample Name (Dilution factor)	Dry Weight (mg)	Extraction Volume (mL)	Dilution	Dil. Factor	Measured Mean Conc. (mg/mL)	
						Total CBD	Total THC
1	CBD Hemp, dry sample	102	10	100	100	7.77	0
2	Tincture Oil	-	-	1000	-	9.12	3.257
3	Tincture Hemp	-	-	1000	-	67.16	1.194

■ 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.

SHIMADZU

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