SHIMADZU
Excellence in Science

Application News

No. HPLC-040

High Performance Liquid Chromatography

A High-Throughput Potency Determination of 19 Cannabinoids in Hemp Plant Material and Finished Tinctures using LabSolutions and the Cannabis Analyzer for Potency™

■ Introduction

CBD is growing in popularity as a medicinal treatment for inflammation, pain and anxiety. Since the passage of Agricultural Improvement Act of 2018, which legalized cannabis and hemp products under the FDA's authority, the cannabis market has dramatically expanded. 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. 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, which builds on the well-established potency method using the Shimadzu Cannabis Analyzer for Potency™, for fast and high-throughput determination of 19 cannabinoids in only 8 minutes (including the wash-step) is presented. 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 a summary of the instrument and method parameters, while 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)	10 components (CRM) in acetonitrile (1mL x 250ug/mL), 220-91239-20
HPLC System	Cannabis Analyzer for Potency™, 220-94420-00
Detector	UV-Vis
Wavelength Monitored (nm)	220
Mobile Phase A	0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Acetonitrile
Gradient Program (Original HT Method)	70%-80% B over 5 min; 80%-85% B over 1 min; 85%-70% B over 0.1 min; 70% B for 1.99 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)	1.5
Oven Temperature (°C)	50
Injection Volume (µL)	5

Table 2: Initial concentrations for the 19 cannabinoids prior to mixture preparation

Reference Standard	Abbreviation	Stock Conc. (mg/L)	Standard
cannabidivarin	CBDV	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-8-trans-tetrahydrocannabinolic acid	d8-THCA	10,000	Cayman
(±)-cannabichromeorcin	CBCO	1,000	Cayman
cannabidiphorol	CBDP	10,000	Cayman
(±)-cannabichromevarin	CBCV	1,000	Cayman
(±)-cannabicyclol	CBL	1,000	Cerilliant
tetrahydrocannabivarinic acid	THCVA	1,000	Cerilliant
cannabidivarinic acid	CBDVA	1,000	Cerilliant
cannabicyclolic acid	CBLA	500	Cerilliant
cannabichromenic acid	CBCA	1,000	Cerilliant

■ Hemp Sample Preparation (Dry Flower and Tincture Oil)

Samples come as either dry flower or tincture oil. 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 necessary for the dry sample form if it is not already a homogenized powder. After the extraction step, it is necessary to filter prior to injection to prevent clogging of the column.

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 preparing the tincture sample (oily sample) to achieve a dilution factor of 1000x:

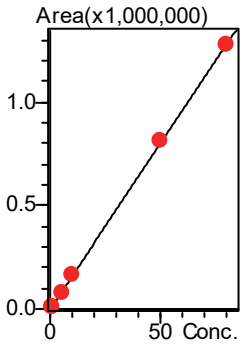
- Obtain a glass scintillation vial.
- Pipette 10 µL tincture or oil to the vial.
- Add 2 mL isopropanol and completely dissolve.
- Agitate the mixture for 30 seconds.
- Add 8 mL methanol.
- Filter the mixture through a 0.45 µm PTFE or Nylon syringe filter into an HPLC vial.
- Secure the vial with a septum and cap.

■ Results and Discussion

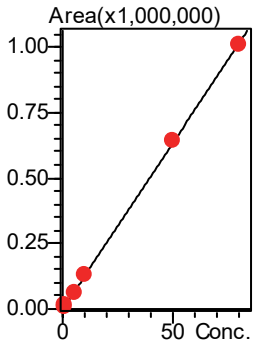
Six levels of calibration standards, ranging from 0.5 to 80 mg/L, and three Quality Control (QC) standards at 2.5 mg/L, 25 mg/L and 60 mg/L were prepared. Calibration curves and QC standards were evaluated using seven replicate injections and evaluating the correlation coefficient (R^2) of the linear regression.

Figure 1 shows the calibration curves for the 19 target cannabinoids. 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 3. Figures 2 and 3 show the 19-cannabinoid mixture resolution and repeatability, respectively.

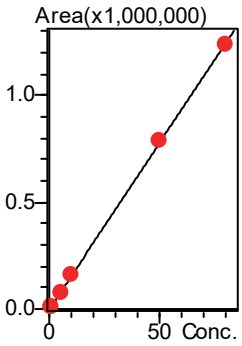
#1
CBDVA



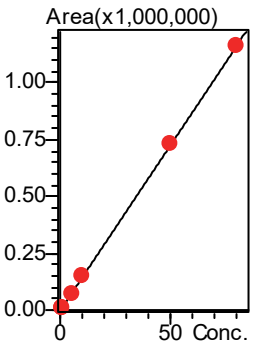
#2
CBDV



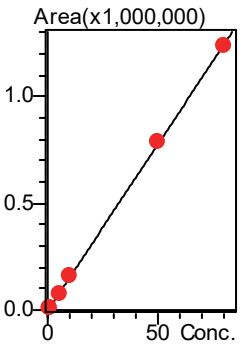
#3
CBCO



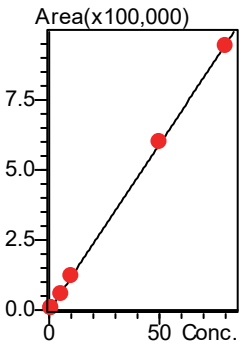
#4
CBDA



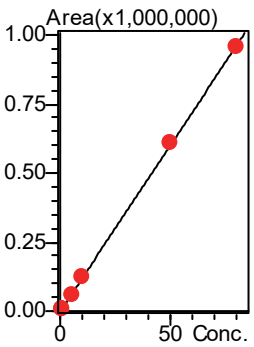
#5
CBGA



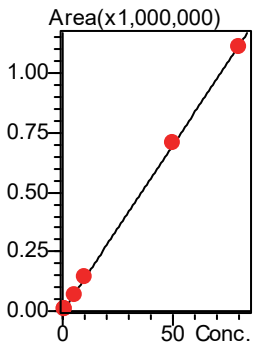
#6
CBG



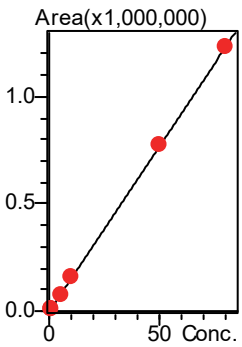
#7
CBD



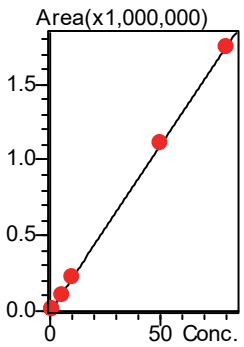
#8
CBCV



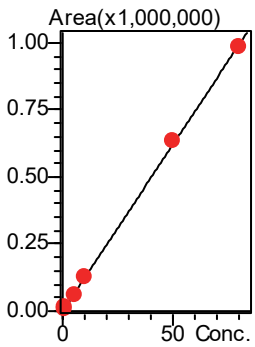
#9
THCVA



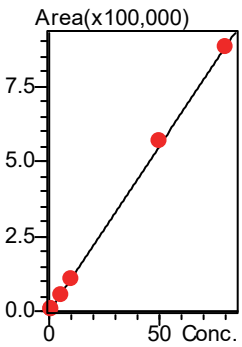
#10
CBN



#11
CBDP



#12
d9-THC



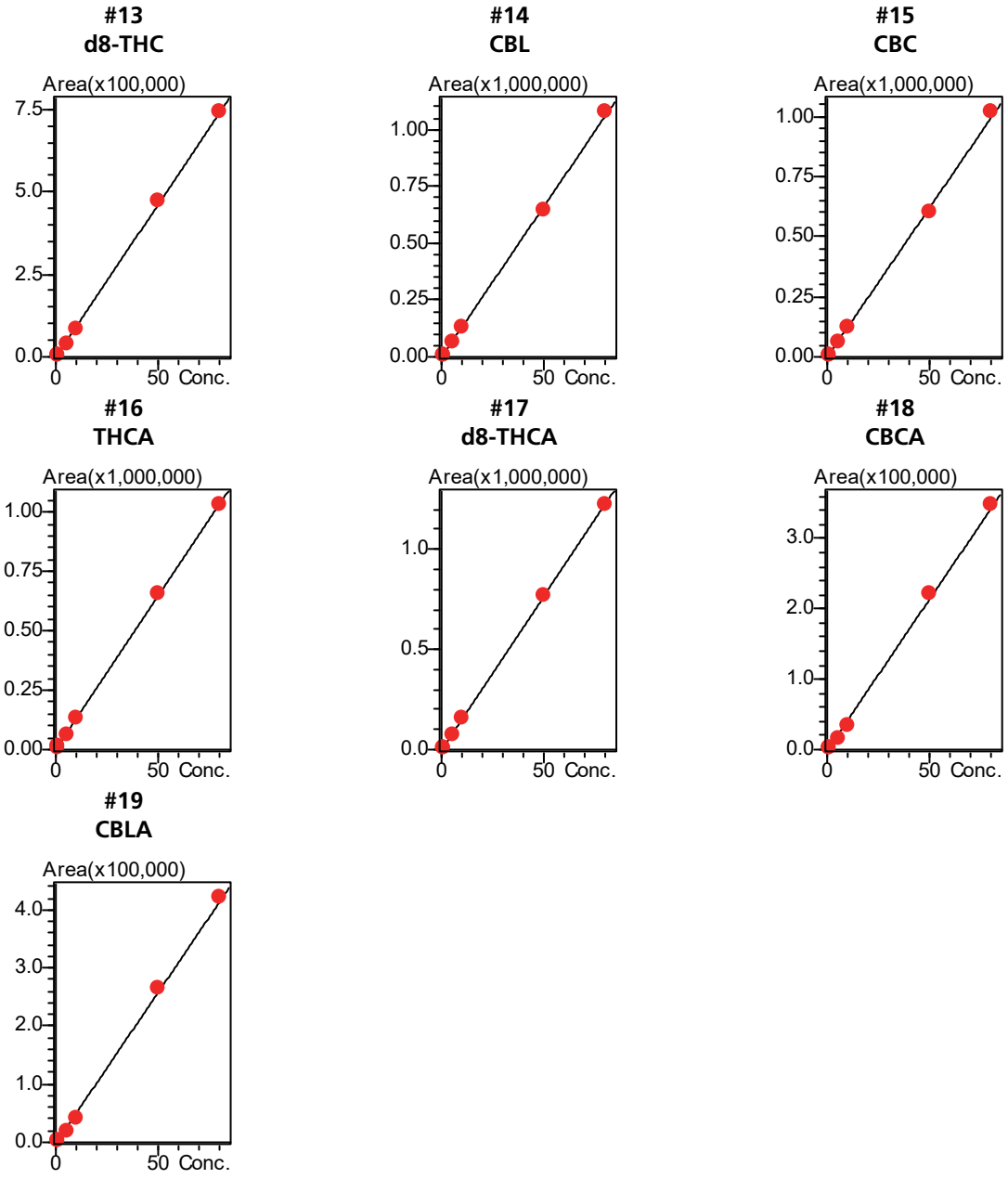


Figure 1: Standard curves for 19 cannabinoids

Table 3: Statistical analysis of a 6-point calibration curve from 0.5 ppm to 80 ppm, with seven replicates for calibration standards and quality control (QC) standards for the 19-cannabinoid mixture

No.	Compound	Calibration (n=7)	2.5 ppm (QC Low) (n=7)			25.0 ppm (QC Medium) (n=7)			60.0 ppm (QC High) (n=7)		
		R^2	Mean Conc.	RSD (%)	Accuracy (%)	Mean Conc.	RSD (%)	Accuracy (%)	Mean Conc.	RSD (%)	Accuracy (%)
1	CBDVA	0.99990	2.52	0.333	100.9	25.48	0.168	102	59.84	0.502	99.7
2	CBDV	0.99985	2.48	1.030	99.2	25.73	0.159	102.9	60.09	0.490	100.2
3	CBCO	0.99977	2.59	0.489	104.0	25.75	0.394	103.0	60.43	0.721	100.7
4	CBDA	0.99977	2.68	1.011	107.4	25.32	0.617	101.3	59.30	0.826	98.8
5	CBGA	0.99976	2.61	1.138	104.7	25.54	0.430	102.2	59.68	0.557	99.5
6	CBG	0.99985	2.49	1.546	99.7	25.68	0.207	102.7	60.01	0.489	100.0
7	CBD	0.99985	2.45	0.698	98.3	25.73	0.164	102.9	60.17	0.501	100.3
8	CBCV	0.99979	2.59	1.186	103.8	25.74	0.418	103.0	59.94	0.807	99.9
9	THCVA	0.99989	2.59	2.270	103.7	25.41	0.411	101.7	59.86	0.857	99.8
10	CBN	0.99989	2.50	0.884	100.1	25.59	0.223	102.4	60.03	0.523	100.1
11	CBDP	0.99975	2.56	1.222	102.5	25.77	0.176	103.1	59.71	0.548	99.5
12	d9-THC	0.99952	2.44	1.524	97.8	23.81	0.247	95.2	60.55	0.681	100.9
13	d8-THC	0.99875	2.40	2.578	96.2	22.61	0.378	90.5	60.68	0.543	101.1
14	CBL	0.99960	2.55	2.515	102.3	25.09	0.224	100.4	58.06	0.514	96.8
15	CBC	0.99922	2.49	2.613	99.9	24.70	0.292	98.8	57.87	0.516	96.5
16	THCA	0.99989	2.47	3.054	99.0	25.42	0.198	101.7	59.76	0.550	99.6
17	d8-THCA	0.99992	2.46	2.177	98.6	25.50	0.162	102.0	59.86	0.479	99.8
18	CBCA	0.99489	2.24	3.702	89.8	24.19	12.994	96.8	60.59	1.023	101.0
19	CBLA	0.99523	2.11	6.259	84.8	24.22	13.370	96.9	61.35	1.033	102.3
	Average (n=19)	**0.99921**	**2.49**	**1.907**	**99.6**	**25.12**	**1.644**	**100.5**	**59.88**	**0.640**	**99.8**

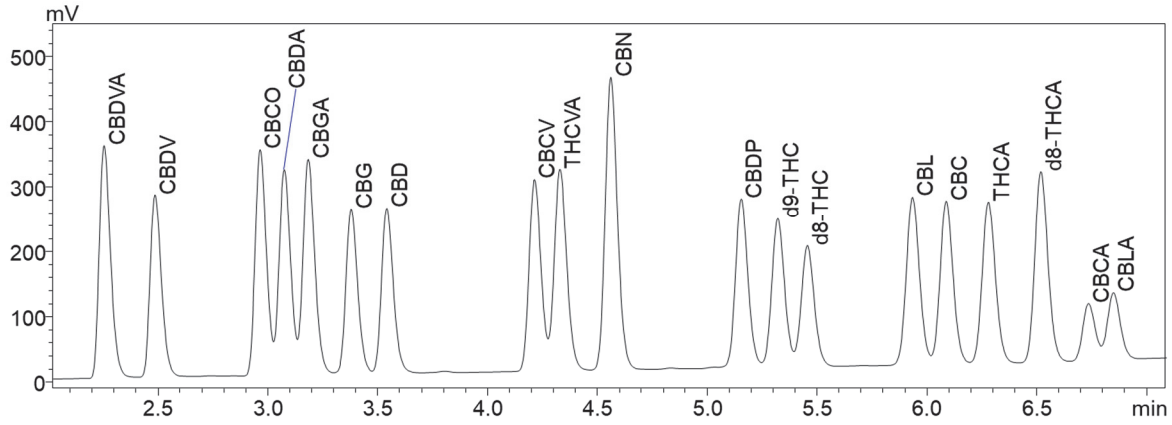


Figure 2: 19-cannabinoid mixture resolution (5 µL injection at 80 ppm)

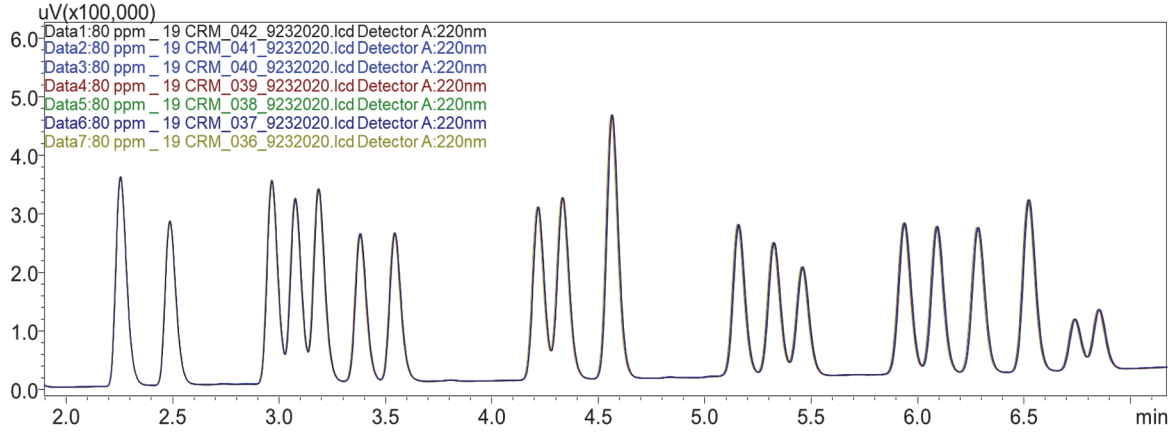


Figure 3: 19-cannabinoid mixture repeatability and overlay of seven injections (5 µL injection at 80 ppm)

Figures 4 and 5 show examples of two commercially available dry samples.

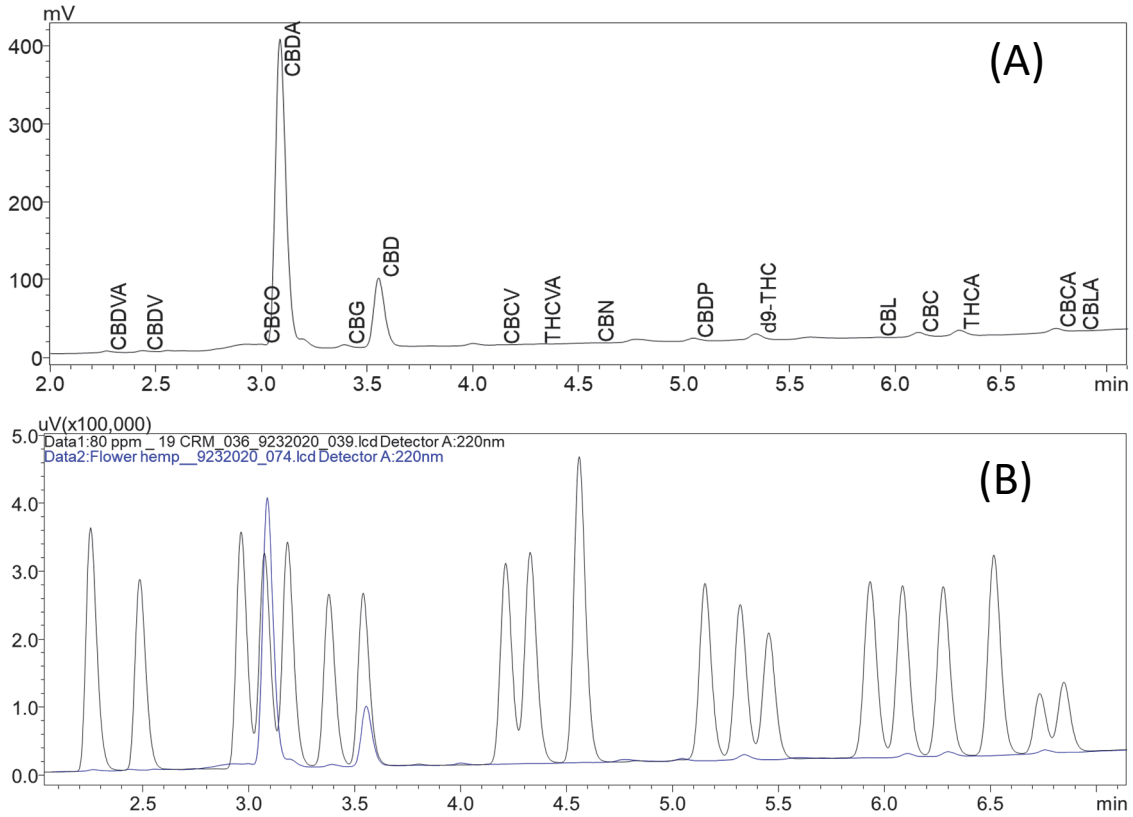


Figure 4: Flower profile (A); and flower 20x diluted (blue trace) vs 80 ppm 19-standards (black trace) (B). Results obtained at 5 µL injection volume. Peaks listed in Table 4 in order of retention time.

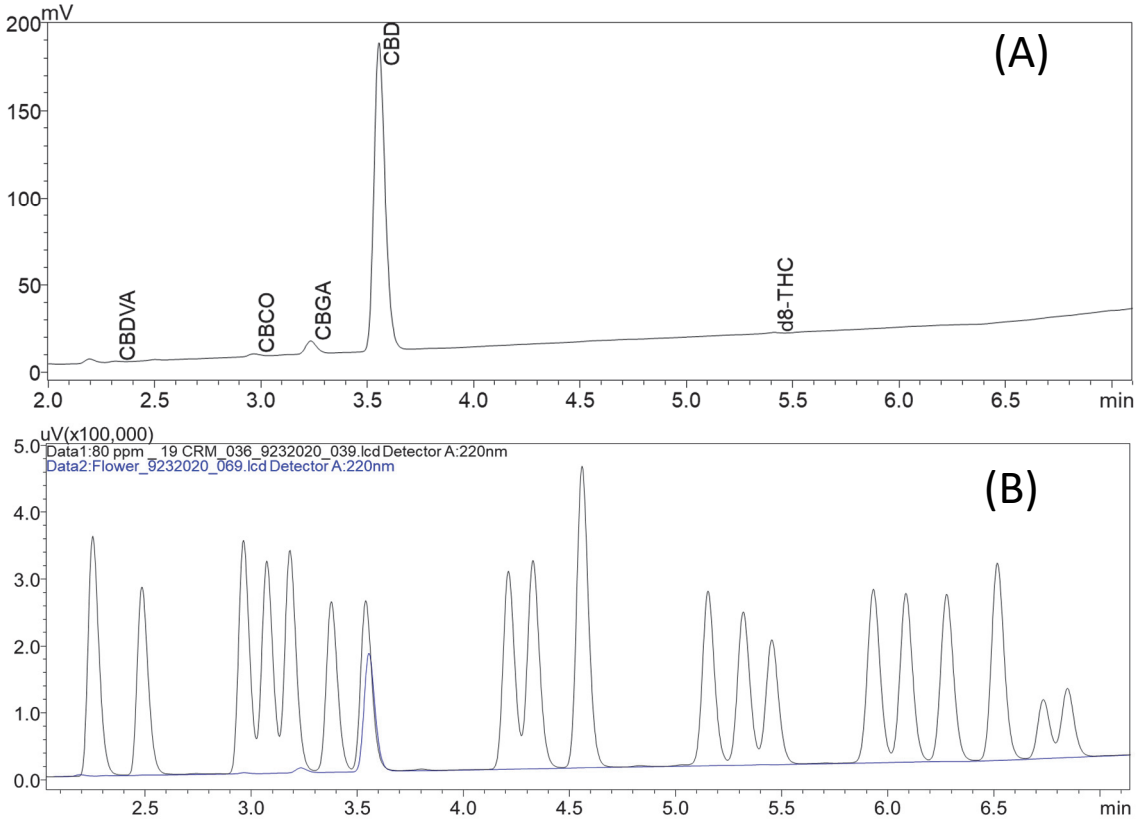


Figure 5: Flower profile (A); and flower 150x diluted (blue trace) vs 80 ppm 19-standards (black trace) (B). Results obtained at 5 µL injection volume. Peaks listed in Table 6 in order of retention time

Figure 6 illustrates potency equations for this app note in LabSolutions, where equations (1) and (2) explain the formula for the total amount of THC on dry basis. Similar equations can be used to calculate the total CBD (%) and the total CBD (mg/g). 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). To perform this calculation in LabSolutions, the "Dil.Factor" and "Sample Amount" will need to be added to the batch-file in the PostRun analysis and the data needs to be reprocessed. The Dil. Factors were calculated based on equation (4).

	Title	Formula	Const A	Const B	Const C
1	Total CBD (%)	(Conc[7]+(Conc[4]*0.877))	1	1	1
2	Total THC (%)	(Conc[12]+(Conc[16]*0.877))	1	1	1
3	Total CBD (mg/g)	(Conc[7]+(Conc[4]*0.877))*10	1	1	1
4	Total THC (mg/g)	(Conc[12]+(Conc[16]*0.877))*10	1	1	1
5	Dry weight %	Conc*100	1	1	1

Figure 6: Custom Calculations using PostRun LabSolutions

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]*

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

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

The measured potency for the dry sample (flower hemp_074) is represented in Tables 4 and 5. This sample does not fall within the definition for "hemp," based on criteria in the Farm Bill of 2018, as the total THC (1.024%) exceeds 0.3% (wt. %).

Table 4: Measured potency for flower hemp_074

Compound	Conc. (mg/L)	Conc. (wt. %)
CBDVA	14.58	0.146
CBDV	14.12	0.141
CBCO	29.94	0.299
CBDA	2007.4	20.074
CBG	31.74	0.317
CBD	543.54	5.435
CBCV	3.62	0.036
THCVA	5.74	0.057
CBN	5.38	0.054
CBDP	23.34	0.233
d9-THC	61.96	0.620
CBL	6.38	0.064
CBC	33.04	0.330
THCA	46.14	0.461
CBCA	107.04	1.070
CBLA	9.34	0.093

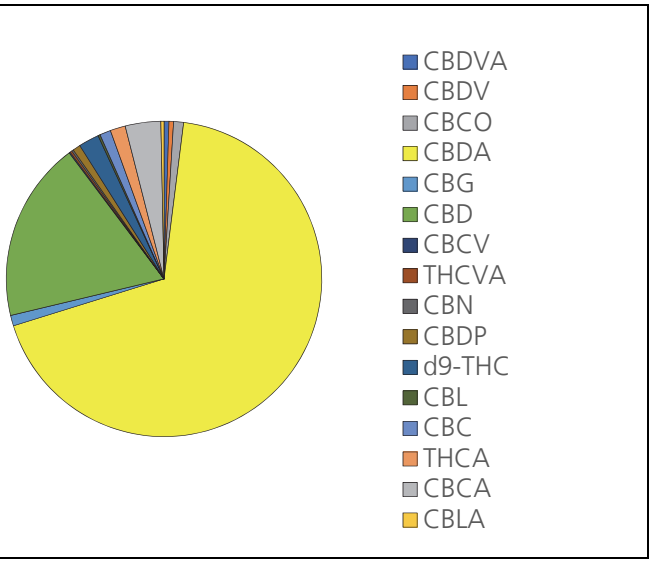
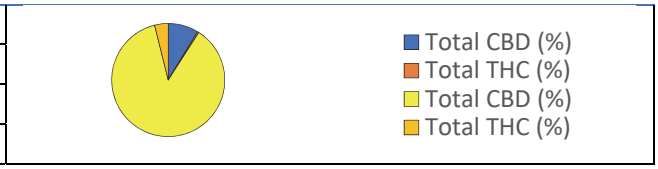


Table 5: Measured CBD:THC potency for flower hemp_074

Total CBD (%)	23.040
Total CBD (mg/g)	230.403
Total THC (%)	1.024
Total THC (mg/g)	10.242



The measured potency for the dry flower_069 is represented in Tables 6 and 7. A total-CBD level of 77.85% (wt. %) or a total 778.56 (mg/g) was measured. No d9-THC or THCA was detected. And the summation of the rest of the cannabinoids accounted for less than 1% (wt. %). This sample is considered a hemp.

Table 6: Measured potency for flower_069

Compound	Conc. (mg/L)	Conc. (wt.%)
CBDVA	26.40	0.258
CBCO	61.05	0.599
CBGA	266.10	2.609
CBD	7941.30	77.856
d8-THC	44.93	0.441

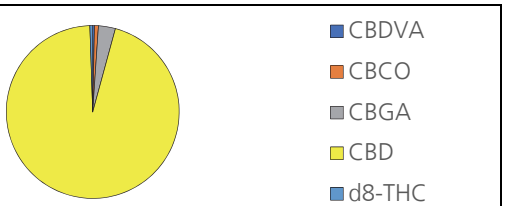


Table 7: Measured total CBD for flower_069

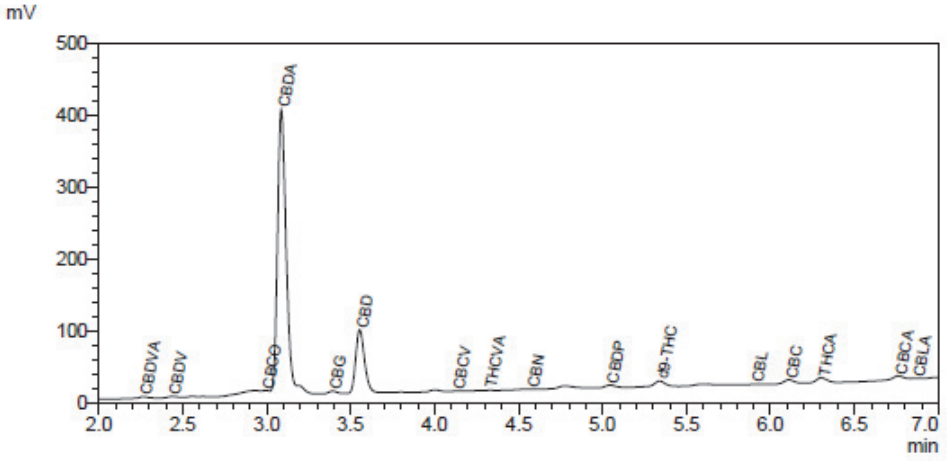
Total CBD (%)	77.856
Total CBD (mg/g)	778.561

<Sample Information>

System Administrator

Sample Name	: Flower Hemp_9232020		
Sample ID	: 074		
Method Filename	: HighThroughput.lcm		
Vial #	: 1-17	Sample Type	: Unknown
Injection Volume	: 5 uL		
Sample Amount	: 200 mg		
Dilution Factor	: 40		
Date Acquired	: 9/24/2020 1:10:54 AM	Acquired by	: System Administrator
Date Processed	: 11/3/2020 2:31:33 PM	Processed by	: System Administrator

<Chromatogram>



<Quantitative Results>

Detector A

ID#	Name	Ret. Time	Conc.	Unit
1	CBDVA	2.266	0.146	%
2	CBDV	2.437	0.141	%
3	CBCO	2.993	0.299	%
4	CBDA	3.087	20.074	%
5	CBGA	–	–	%
6	CBG	3.393	0.317	%
7	CBD	3.554	5.435	%
8	CBCV	4.127	0.036	%
9	THCVA	4.320	0.057	%
10	CBN	4.573	0.054	%
11	CBDP	5.045	0.233	%
12	d9-THC	5.340	0.620	%
13	d8-THC	–	–	%
14	CBL	5.907	0.064	%
15	CBC	6.110	0.330	%
16	THCA	6.303	0.461	%
17	d8-THCA	–	–	%
18	CBCA	6.761	1.070	%
19	CBLA	6.867	0.093	%

Total THC	1.02	%
Total THC	10.24	mg/g
Total CBD	23.04	%
Total CBD	230.40	mg/g

Figure 7: Dry sample report from Cannabis Analyzer Overlay Software

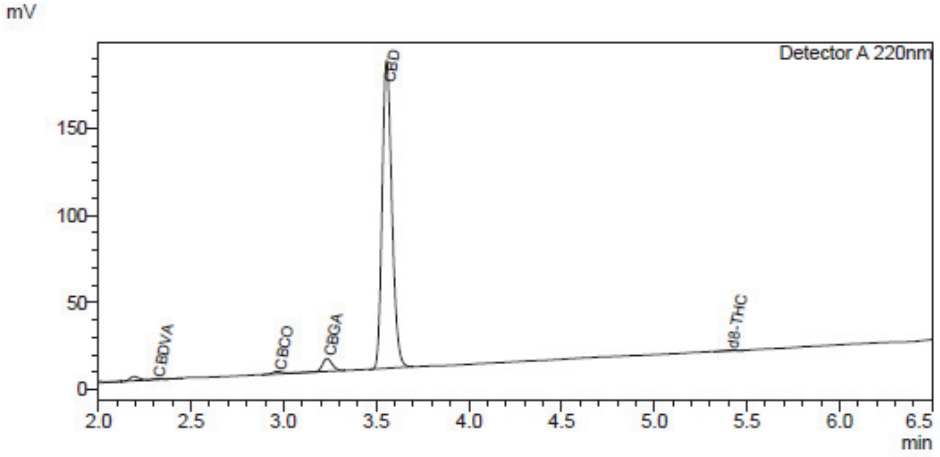
CANNABIS ANALYZER

<Sample Information>

System Administrator

Sample Name	: Flower_9232020		
Sample ID	: 069		
Method Filename	: HighThroughput.lcm		
Vial #	: 1-12	Sample Type	: Unknown
Injection Volume	: 5 uL		
Sample Amount	: 102 mg		
Dilution Factor	: 150		
Date Acquired	: 9/24/2020 12:29:10 AM	Acquired by	: System Administrator
Date Processed	: 11/3/2020 2:41:39 PM	Processed by	: System Administrator

<Chromatogram>



<Quantitative Results>

Detector A

ID#	Name	Ret. Time	Conc.	Unit
1	CBDVA	2.313	0.258	%
2	CBDV	–	–	%
3	CBCO	2.967	0.599	%
4	CBDA	–	–	%
5	CBGA	3.234	2.609	%
6	CBG	–	–	%
7	CBD	3.555	77.856	%
8	CBCV	–	–	%
9	THCVA	–	–	%
10	CBN	–	–	%
11	CBDP	–	–	%
12	d9-THC	–	–	%
13	d8-THC	5.407	0.441	%
14	CBL	–	–	%
15	CBC	–	–	%
16	THCA	–	–	%
17	d8-THCA	–	–	%
18	CBCA	–	–	%
19	CBLA	–	–	%

Total THC	0.00	%
Total THC	0.00	mg/g
Total CBD	77.86	%
Total CBD	778.56	mg/g

Figure 8: Dry sample report from Cannabis Analyzer Overlay Software

Figure 9 illustrates the chromatograms for a commercially available concentrated CBD tincture hemp. And table 8 shows the measured potency in (mg/mL) for the quantified cannabinoids in the tincture. Figure 10 shows the tincture report.

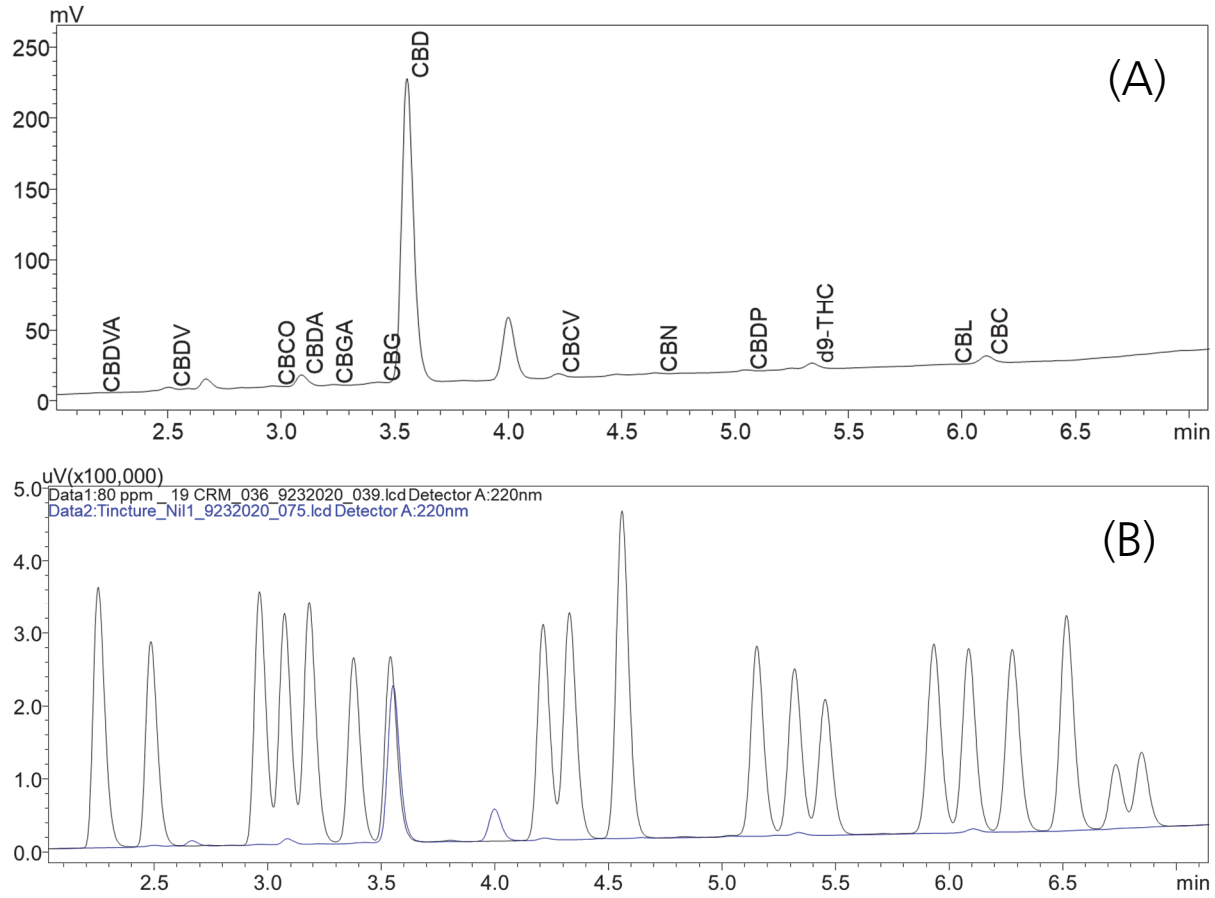
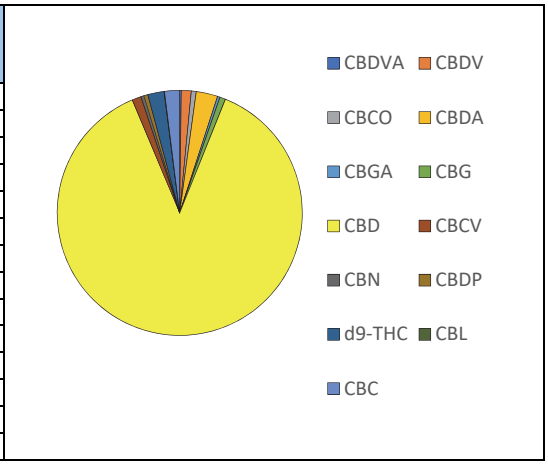


Figure 9: Tincture-Nil profile (A); Tincture 1000x diluted (blue trace) vs. 80 ppm 19-Component Standard (black trace) (B). Results obtained at 5 µL injection volume. Peaks listed in Table 8 in order of retention time.

Table 8: Measured potency for commercial tincture-Nil (container volume: 10mL)

Compound	Measured Conc. (mg/mL)	Amt. (mg) per 10 mL	% of Total
CBDVA	0.15	1.54	0.21
CBDV	0.98	9.82	1.37
CBCO	0.49	4.86	0.68
CBDA	2.15	21.48	3.00
CBGA	0.24	2.42	0.34
CBG	0.58	5.77	0.81
CBD	66.04	660.43	92.20
CBCV	0.91	9.07	1.27
CBN	0.30	3.00	0.42
CBDP	0.40	4.00	0.56
d9-THC	1.61	16.14	2.25
CBL	0.05	0.49	0.07
CBC	1.50	14.98	2.09
Total	71.63	716.30	100.00



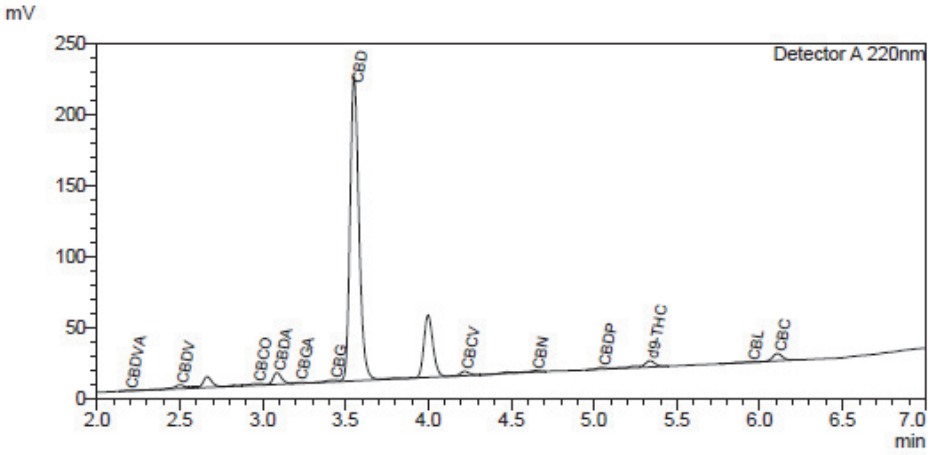
CANNABIS ANALYZER

<Sample Information>

System Administrator

Sample Name	: Tincture Nil_9232020		
Sample ID	: 001		
Method Filename	: HighThroughput.lcm		
Vial #	: 1-18		
Sample Type	: Unknown		
Injection Volume	: 5 uL		
Date Acquired	: 9/24/2020 1:19:15 AM	Acquired by	: System Administrator
Date Processed	: 11/3/2020 3:06:14 PM	Processed by	: System Administrator

<Chromatogram>



<Quantitative Results>

Detector A

ID#	Name	Ret. Time	mg/mL
1	CBDVA	2.193	0.15352
2	CBDV	2.501	0.98152
3	CBCO	2.962	0.48639
4	CBDA	3.087	2.14830
5	CBGA	3.220	0.24162
6	CBG	3.428	0.57680
7	CBD	3.552	66.04347
8	CBCV	4.220	0.90693
9	THCVA	--	0.00000
10	CBN	4.647	0.29982
11	CBDP	5.046	0.39991
12	d9-THC	5.338	1.61380
13	d8-THC	--	0.00000
14	CBL	5.947	0.04857
15	CBC	6.106	1.49842
16	THCA	--	0.00000
17	d8-THCA	--	0.00000
18	CBCA	--	0.00000
19	CBLA	--	0.00000

Total THC	1.61	mg/mL
Total CBD	67.93	mg/mL

Figure 10: Tincture report from Cannabis Analyzer Overlay Software

Table 9 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 679.25 mg CBD (label claimed 500 mg CBD) for a commercially available tincture.

Table 9: Summary of CBD and THC quantitative determination for three samples, using LabSolutions and cannabis analyzer

ID #	Sample Name	Tincture Volume (mL)	Dry Weight (mg)	Extraction Volume (mL)	Dilution	Calculated Dilution Factor	Measured Mean Conc.	
							Total CBD (mg/mL)	Total THC (mg/mL)
1	Flower hemp_074	-	200	20	20	40	2.30	0.10
2	Flower_069	-	102	10	150	150	7.94	0
3	Tincture-Nil	10	-	-	1000	-	67.92	1.61

■ Conclusion

In response to the demand for expanding the number of cannabinoids separation in a high-throughput method in potency testing of hemp, we expanded the list of the cannabinoids to 19 in an 8-minute run (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, and robust potency results for cannabinoid profiles for commercially available dry hemp and tincture oil.

First Edition: November 2020

SHIMADZU Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS
7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800-477-1227/410-381-1227, Fax: 410-381-1222
URL: www.ssi.shimadzu.com