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Application News

No. HPLC-041

High Performance Liquid Chromatography

A Rugged High-Resolution Potency Determination of 18 Cannabinoids in Hemp Plant Material and Finished Tinctures using the Cannabis Analyzer for Potency™

■ Introduction

Non-psychotropic 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.

Research also shows that CBD and other cannabis-based medicine have the potential to be a major tool in the battle against current epidemic abuse and overdose in the United States [1]. Study shows that using medicinal cannabis in those patients with opioid overdose reduced the mortality rate nearly to 25% [2].

We have performed a study in response to the increasing demand for potency testing of many cannabinoids in the run using Shimadzu cannabis analyzer. Prior work in support of the turnkey package, Shimadzu Cannabis Analyzer for Potency™, includes a method for the separation of 11 cannabinoids in a reasonable timeframe of 30 minutes. This study expands the chromatographic target list to 18 cannabinoids in the same amount of run time.

■ 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)	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

Table 2: Initial concentrations for the 18-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
(±)-cannabichromeorcin	CBCO	1,000	Cayman
cannabichromevarin	CBCV	1,000	Cayman
cannabinolic acid	CBNA	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 samples. 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 step 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 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^2) of the linear regression. All calibration curves passed the high-resolution method criteria ($R^2 \geq 0.999$).

Figure 1 shows the calibration curves for the 18 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 show the 18-cannabinoid mixture resolution and repeatability, respectively.

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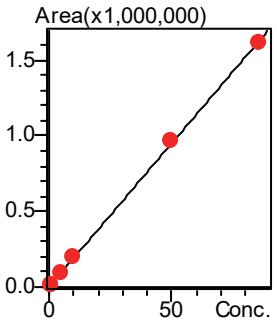
CBDV



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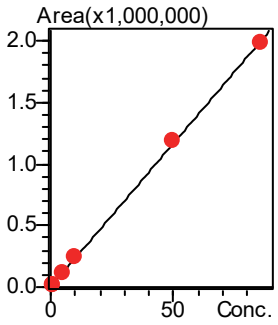
CBDVA



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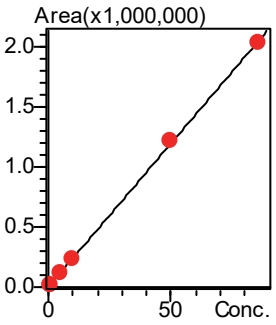
CBCO



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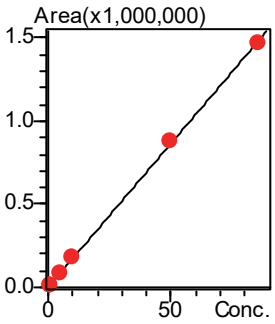
THCV



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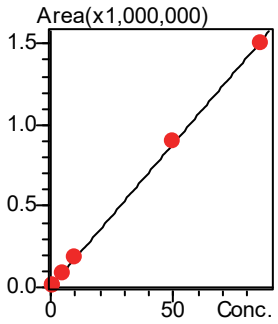
CBD



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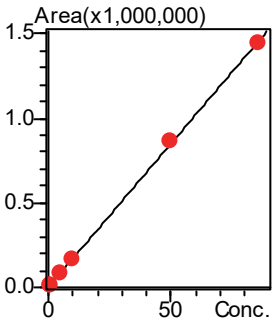
CBG



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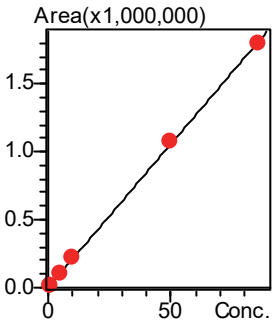

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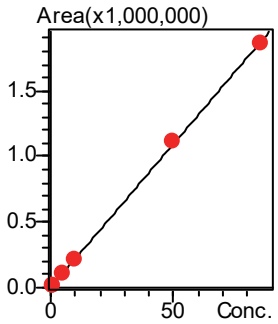
CBCV



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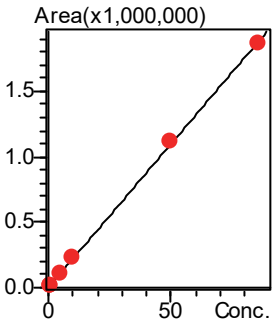
CBGA



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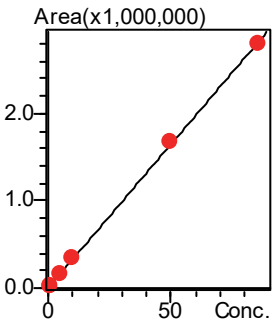
CBN



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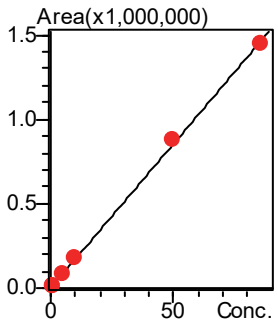
D9-THC



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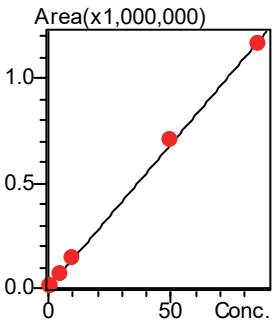
D8-THC



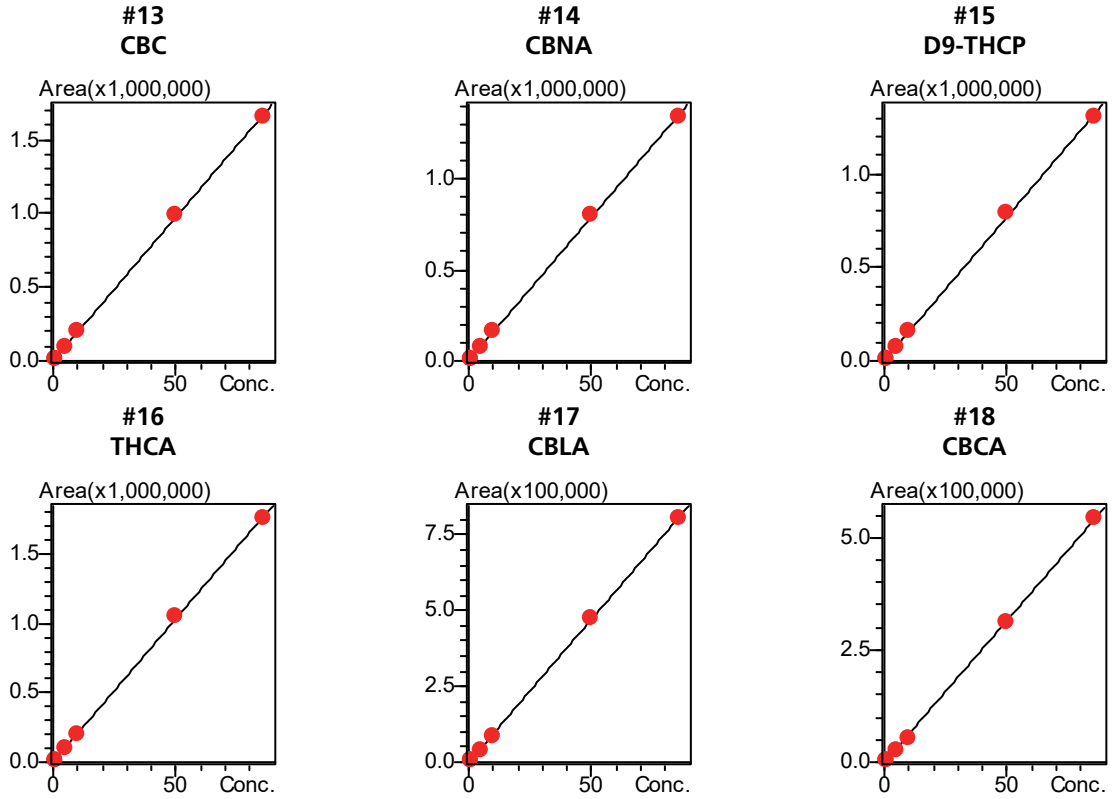


Figure 1: Standard curves for 18 cannabinoids

Table 3: 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)	2.5 ppm (QC Low) (n=7)			25.0 ppm (QC Medium) (n=7)			75.0 ppm (QC High) (n=7)		
		R^2	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

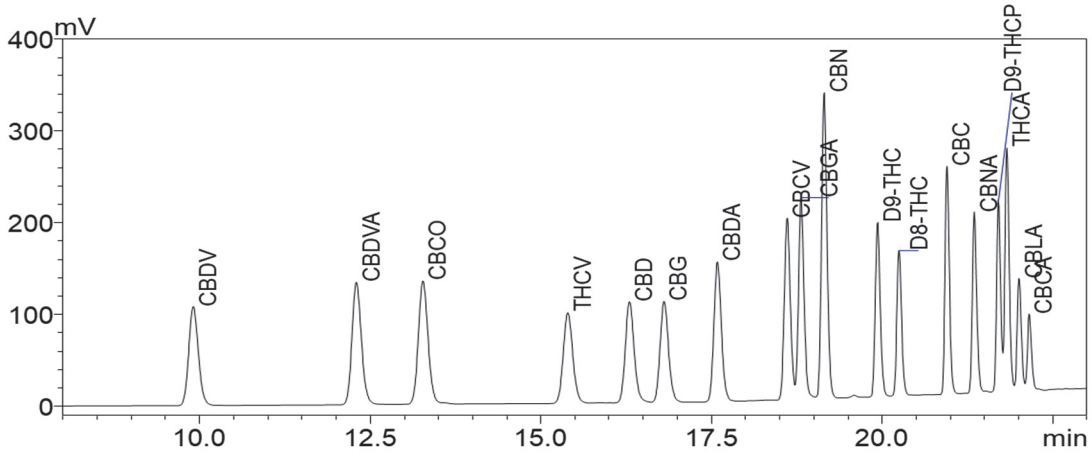


Figure 2: 18-cannabinoid mixture resolution (5 µL injection at 50 ppm)

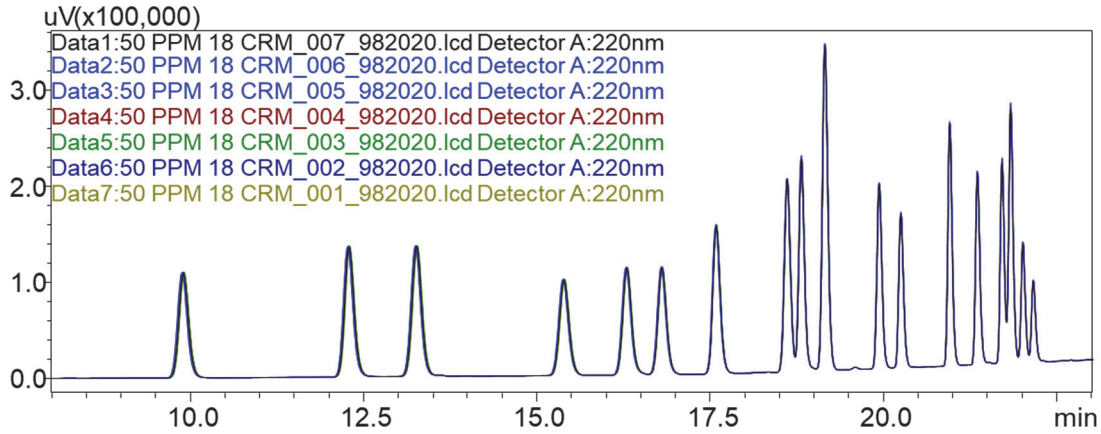


Figure 3: 18-cannabinoid mixture repeatability and overlay of seven-injections (5 µL injection at 50 ppm)

We also established the separation of cannabicitran (CBT) instead of cannabinolic acid (CBNA) under the same conditions. It was observed that CBNA and CBT elude at the same retention time (i.e. 21.3 min) and we selected CBNA between the two for this study. Moreover, cannabidiphorol (CBDP) that can be separated via high sensitivity and a high-throughput method cannot be resolved from delta-9-tetrahydrocannabinol (d9-THC) in this method.

Also, tetrahydrocannabinolic acid (d8-THCA) and cannabichromenic acid (CBCA) elude at the same time (i.e. 22.1 min) and we chose CBCA for this study between the two. Users can choose between CBNA and CBT, and between CBCA and D8-THC based on the cannabinoids present in their mixture. Those results are not presented here.

Figures 4 and 5 show the separation profile for two commercially available hemp dry samples.

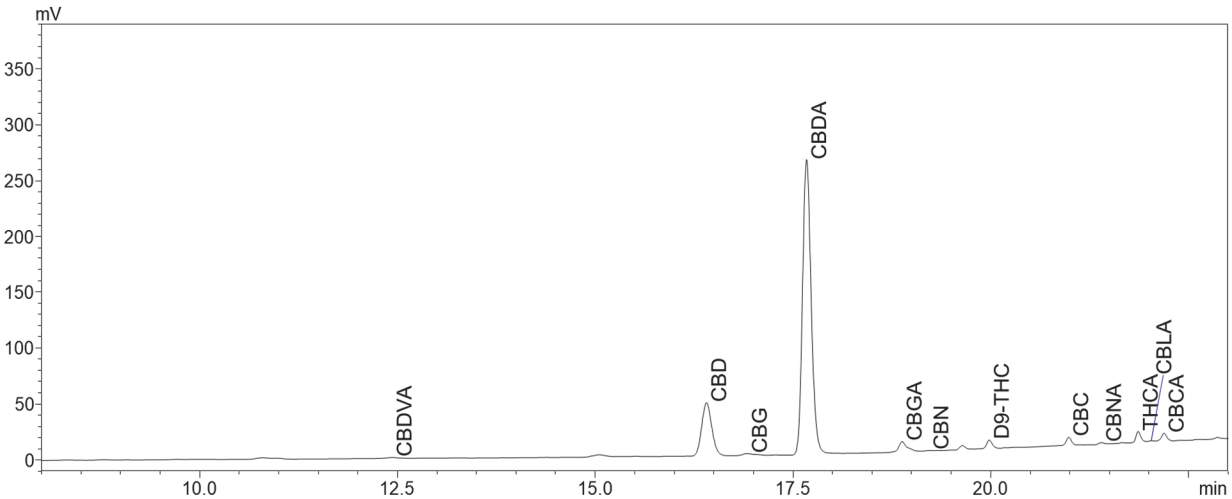


Figure 4: Flower-1 profile. Flower 100x diluted (dry sample). Results obtained at 5 µL injection volume.

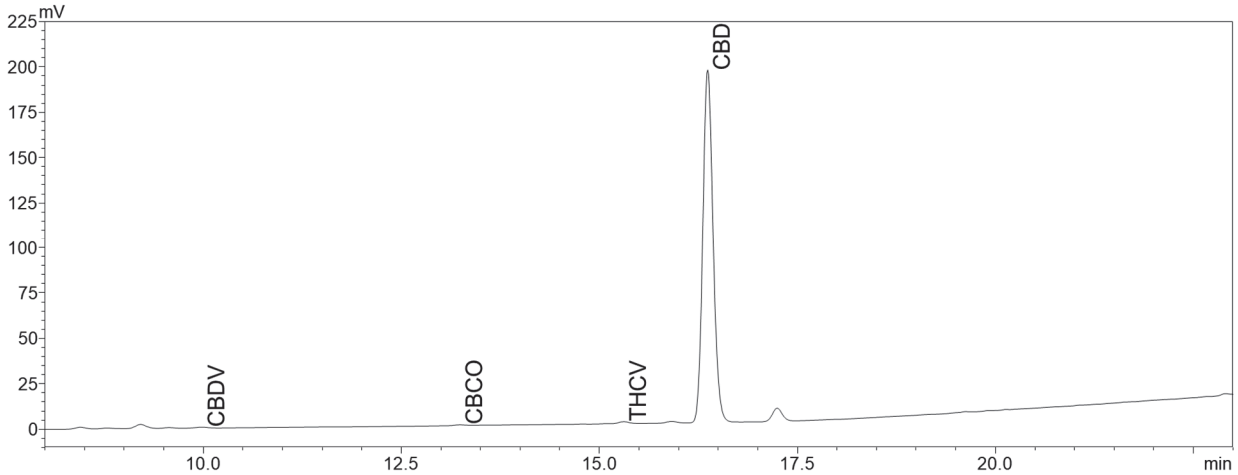


Figure 5: Flower-2 profile (A). Flower 100x diluted (dry sample). Results obtained at 5 µL injection volume.

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[5]+(Conc[7]*0.877))	1	1	1
2	Total THC (%)	(Conc[11]+(Conc[16]*0.877))	1	1	1
3	Total CBD (mg/g)	(Conc[5]+(Conc[7]*0.877))*10	1	1	1
4	Total THC (mg/g)	(Conc[11]+(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]*

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

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

The measured potency for the dry sample (flower-1) is represented in Tables 4 and 5. The results were in consistency with those from the packaging label, as a total-CBD level of more than 90% (wt.%) was expected. This sample does not fall within the definition for "hemp," based on criteria presented in the Farm Bill of 2018, as the total THC exceeds 0.3% (wt. %). On the other hand, flower 2 is considered a hemp sample.

The measured potency for the dry flower-2 is represented in Table 6. A total CBD level of 87.22% (wt. %) or a total 872.22 (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 4: Measured potency for flower-1

Compound	Conc. (wt.%)
CBDVA	0.39
CBD	21.59
CBG	1.03
CBDA	85.19
CBGA	2.66
CBN	0.05
D9-THC	2.11
CBC	1.5
CBNA	0.4
THCA	1.73
CBLA	0.15
CBCA	4.86

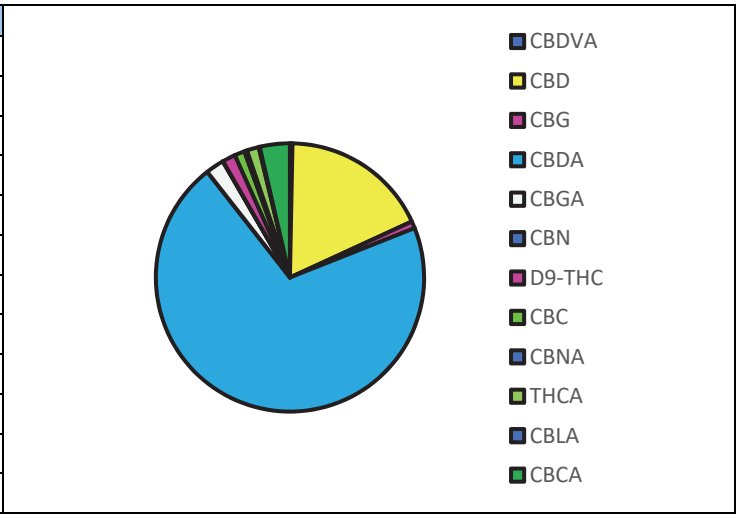


Table 5: Measured CBD:THC potency for flower-1

Total CBD (%)	96.30
Total CBD (mg/g)	963.01
Total THC (%)	3.63
Total THC (mg/g)	36.28

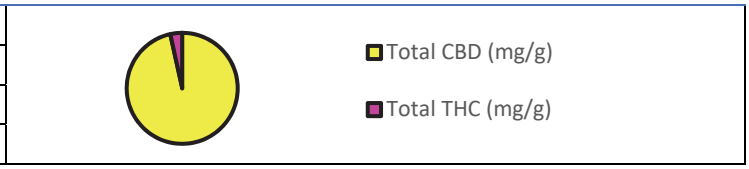
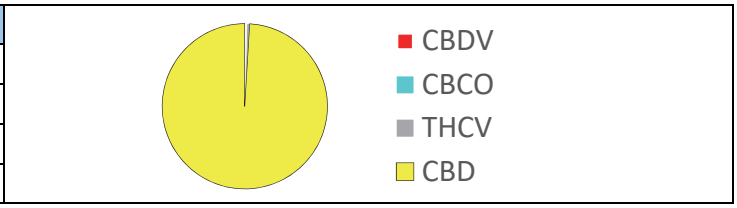


Table 6: Measured potency for flower-2

Compound	Conc. (wt.%)
CBDV	0.26
CBCO	0.10
THCV	0.47
CBD	87.22



<Sample Information>

System Administrator

Sample Name	: Flower_1		
Sample ID	: 001		
Data Filename	: Flower Hemp 1__982020.lcd		
Method Filename	: HighResolution.lcm		
Vial #	: 1-15	Sample Type	: Unknown
Injection Volume	: 5 uL		
Sample Amount	: 100 mg		
Dilution Factor	: 100		
Date Acquired	: 9/10/2020 7:22:30 AM	Acquired by	: System Administrator
Date Processed	: 11/11/2020 2:13:42 PM	Processed by	: System Administrator

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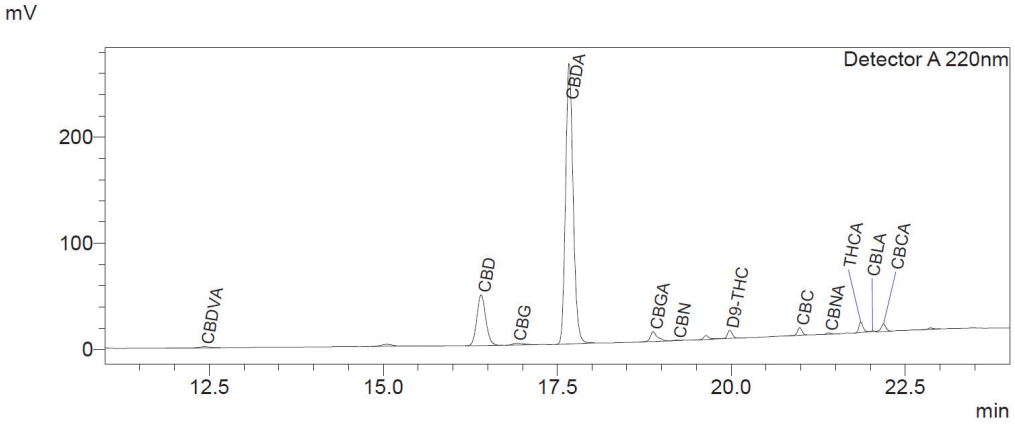

<Quantitative Results>

Detector A

ID#	Name	Ret. Time	Conc.	Unit
1	CBDV	--	--	%
2	CBDVA	12.428	0.395	%
3	CBCO	--	--	%
4	THCV	--	--	%
5	CBD	16.406	21.587	%
6	CBG	16.920	1.033	%
7	CBDA	17.670	85.192	%
8	CBCV	--	--	%
9	CBGA	18.878	2.661	%
10	CBN	19.215	0.047	%
11	D9-THC	19.979	2.109	%
12	D8-THC	--	--	%
13	CBC	20.985	1.500	%
14	CBNA	21.397	0.401	%
15	D9-THCP	--	--	%
16	THCA	21.862	1.732	%
17	CBLA	22.032	0.154	%
18	CBCA	22.189	4.856	%

Total THC	3.63	%
Total THC	36.28	mg/g
Total CBD	96.30	%
Total CBD	963.01	mg/g

Figure 7: Flower-1 dry sample report from Cannabis Analyzer Overlay Software

<Sample Information>

System Administrator
Sample Name : Flower_2
Sample ID : 002
Data Filename : Flower Hemp 2__982020.lcd
Method Filename : HighResolution.lcm
Vial # : 1-13
Sample Type : Unknown
Injection Volume : 5 uL
Sample Amount : 102 mg
Dilution Facor :100
Date Acquired : 9/10/2020 6:21:29 AM
Date Processed : 11/11/2020 2:59:02 PM
Acquired by : System Administrator
Processed by : System Administrator

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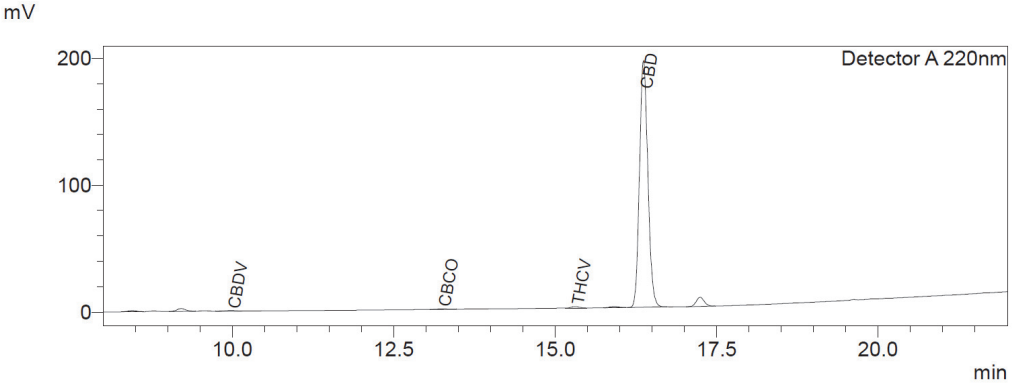


<Quantitative Results>

Detector A

ID#	Name	Ret. Time	Conc.	Unit
1	CBDV	9.982	0.256	%
2	CBDVA	--	--	%
3	CBCO	13.241	0.103	%
4	THCV	15.307	0.476	%
5	CBD	16.366	87.231	%
6	CBG	--	--	%
7	CBDA	--	--	%
8	CBCV	--	--	%
9	CBGA	--	--	%
10	CBN	--	--	%
11	D9-THC	--	--	%
12	D8-THC	--	--	%
13	CBC	--	--	%
14	CBNA	--	--	%
15	D9-THCP	--	--	%
16	THCA	--	--	%
17	CBLA	--	--	%
18	CBCA	--	--	%

Total THC	0.00	%
Total THC	0.00	mg/g
Total CBD	87.23	%
Total CBD	872.31	mg/g

Figure 8: Flower-2 dry sample report from Cannabis Analyzer Overlay Software

Figures 9 and 10 illustrate the chromatograms for two commercially available concentrated CBD tincture hemp. And tables 7 and 8 show the measured potency in (mg/mL) for the quantified cannabinoids in the tinctures.

Figures 11 and 12 illustrate the tincture sample reports.

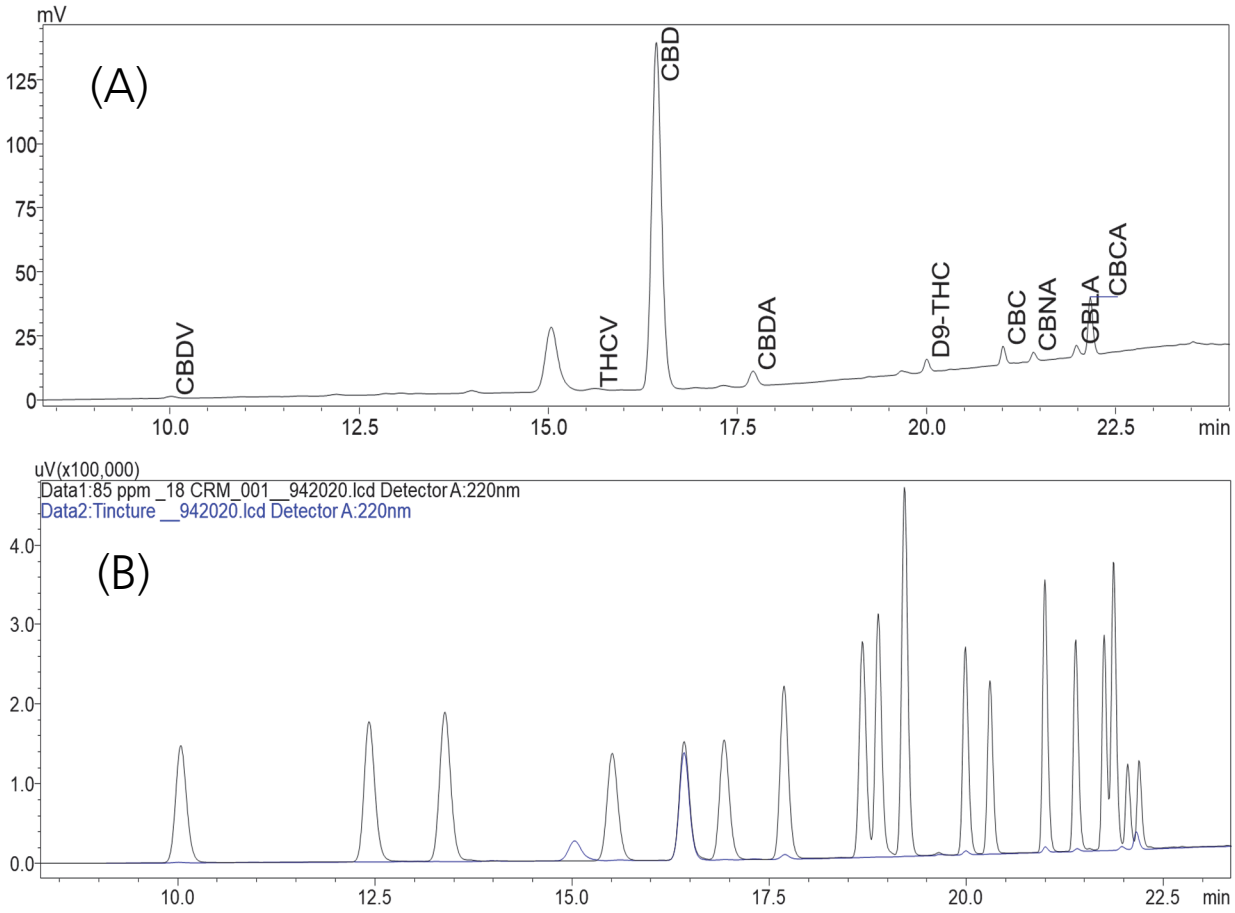
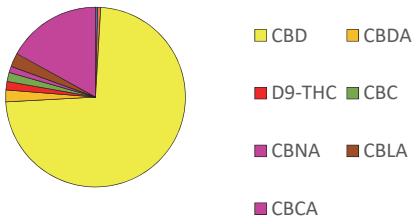


Figure 9: Tincture-1 profile (A); Tincture (blue trace) 1000x diluted vs. 85 ppm 18-Component Standard (black trace) (B). Results obtained at 5 µL injection volume.

Table 7: Measured potency for commercial tincture-1 (container volume: 10 mL or 0.33 oz)

Compound	Measured Conc. (mg/mL)	Amt. (mg) per 10 mL	% of Total
CBDV	0.34	3.40	0.40
THCV	0.45	4.50	0.53
CBD	62.19	621.90	73.21
CBDA	1.81	18.10	2.13
D9-THC	1.3	13.00	1.53
CBC	1.39	13.90	1.64
CBNA	0.96	9.60	1.13
CBLA	2.09	20.90	2.46
CBCA	14.42	144.20	16.97
Total	84.95	849.50	100.00



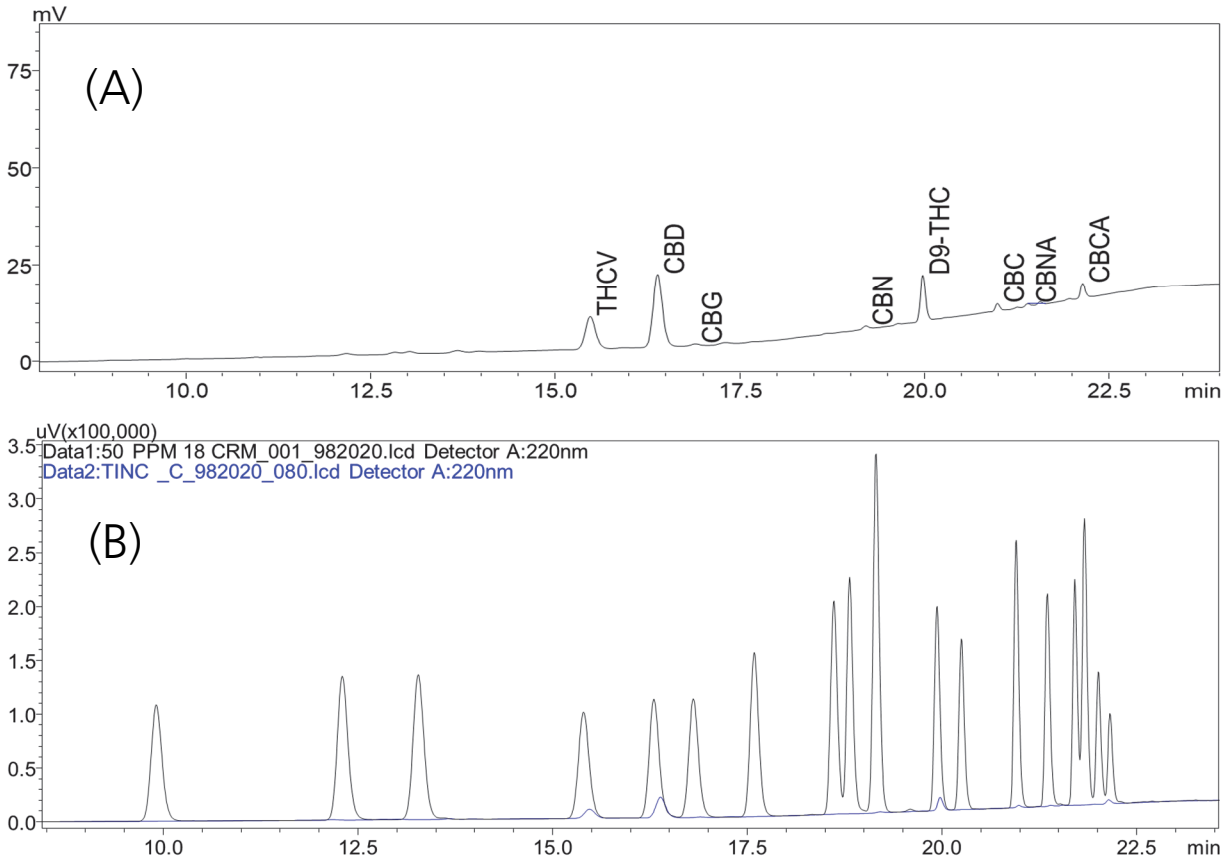
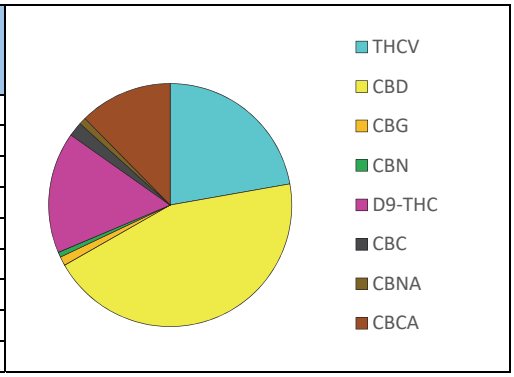


Figure 10: Tincture 2 profile (A); Tincture (blue trace) 1000x diluted vs. 50 ppm 18-Component Standard (black trace) (B). Results obtained at 5 µL injection volume

Table 8: Measured potency for commercial tincture-2 (container volume: 30 mL or 1 oz)

Compound	Measured Conc. (mg/mL)	Amt. (mg) per 30 mL	% of Total
THCV	4.29	128.70	25.34
CBD	8.61	258.30	50.86
CBG	0.23	6.90	1.36
CBN	0.13	3.90	0.77
D9-THC	3.12	93.60	18.43
CBC	0.37	11.10	2.19
CBNA	0.18	5.40	1.06
CBCA	2.39	71.70	12.37
Total	19.32	579.60	100.00



<Sample Information>

System Administrator

Sample Name	: Tincture_942020		
Sample ID	: 001		
Data Filename	: Tincture __942020 - Copy.lcd		
Method Filename	: HighResolution.lcm		
Vial #	: 1-12	Sample Type	: Unknown
Injection Volume	: 5 uL		
Date Acquired	: 9/6/2020 7:08:25 AM	Acquired by	: System Administrator
Date Processed	: 11/11/2020 11:17:26 AM	Processed by	: System Administrator

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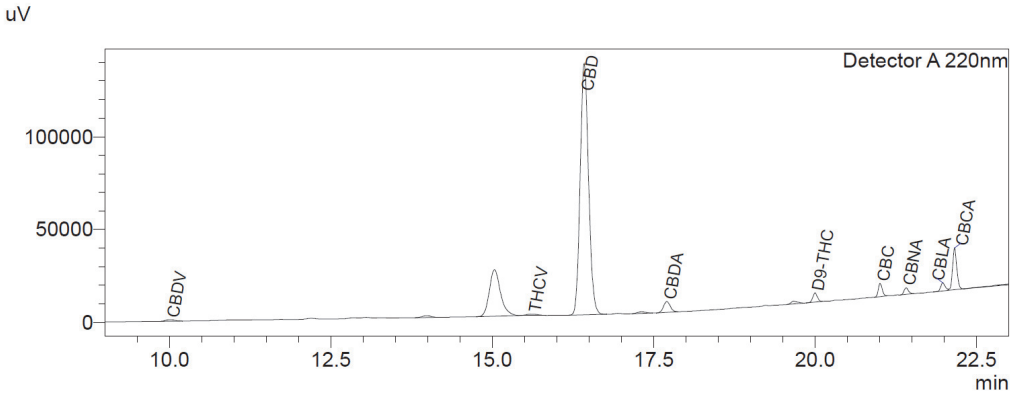


<Quantitative Results>

Detector A

ID#	Name	Ret. Time	mg/mL
1	CBDV	10.011	0.34371
2	CBDVA	--	0.00000
3	CBCO	--	0.00000
4	THCV	15.612	0.45320
5	CBD	16.423	62.19295
6	CBG	--	0.00000
7	CBDA	17.702	1.81195
8	CBCV	--	0.00000
9	CBGA	--	0.00000
10	CBN	--	0.00000
11	D9-THC	19.997	1.29602
12	D8-THC	--	0.00000
13	CBC	21.005	1.38401
14	CBNA	21.408	0.94968
15	D9-THCP	--	0.00000
16	THCA	--	0.00000
17	CBLA	21.976	2.07550
18	CBCA	22.158	14.33589

Total THC	1.30	mg/mL
Total CBD	63.78	mg/mL

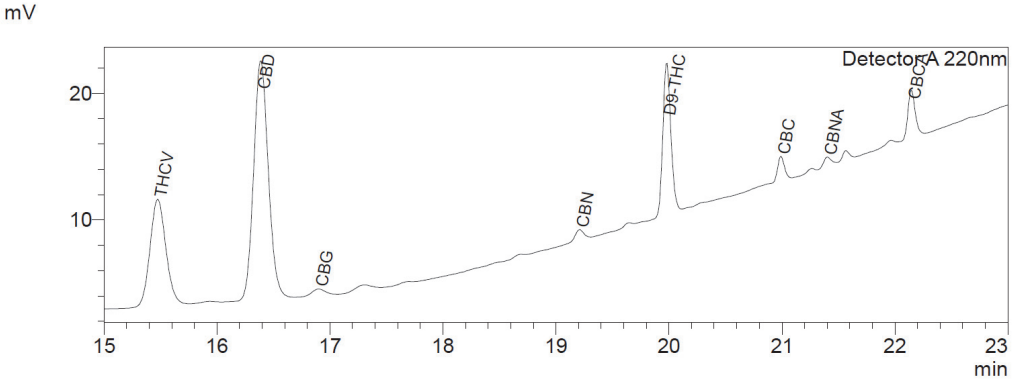
Figure 11: Tincture report for tincture-1 from Cannabis Analyzer Overlay Software

<Sample Information>

System Administrator

Sample Name	: Tincture_C_982020		
Sample ID	: 002		
Data Filename	: Tincture _C_982020_080.lcd		
Method Filename	: HighResolution.lcm		
Vial #	: 1-14	Sample Type	: Unknown
Injection Volume	: 5 uL		
Date Acquired	: 9/10/2020 6:52:00 AM	Acquired by	: System Administrator
Date Processed	: 11/11/2020 9:48:42 AM	Processed by	: System Administrator

<Chromatogram>



<Quantitative Results>

Detector A

ID#	Name	Ret. Time	mg/mL
1	CBDV	--	0.00000
2	CBDVA	--	0.00000
3	CBCO	--	0.00000
4	THCV	15.476	4.29457
5	CBD	16.388	8.61040
6	CBG	16.901	0.22966
7	CBDA	--	0.00000
8	CBCV	--	0.00000
9	CBGA	--	0.00000
10	CBN	19.210	0.12693
11	D9-THC	19.980	3.12098
12	D8-THC	--	0.00000
13	CBC	20.990	0.37219
14	CBNA	21.402	0.17575
15	D9-THCP	--	0.00000
16	THCA	--	0.00000
17	CBLA	--	0.00000
18	CBCA	22.145	2.38510

Total THC	3.12	mg/mL
Total CBD	8.61	mg/mL

Figure 12: Tincture report for tincture-2 from Cannabis Analyzer Overlay Software

Table 9 shows the summary of cannabinoids quantitation. For dry-hemp samples, a 100x dilution was selected so that the response was within the established quantitative dynamic range established for that sample. 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 258.3 mg (label claimed 300 mg CBD), and a total 637.8 mg CBD (label claimed 500 mg CBD), for two commercially available tinctures, respectively.

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	Dilution Factor	Measured Mean Conc.	
							Total CBD (mg/mL)	Total THC (mg/mL)
1	Dry flower sample 1	-	100	10	100	100	9.63	0.17
2	Dry flower sample 2	-	102	10	100	100	8.89	0
3	Tincture 1	10	-	-	1000	-	63.78	1.30
4	Tincture 2	30	-	-	1000	-	8.61	3.12

■ Conclusion

The CBD and other cannabis-based medicine have the potential to be a major tool in the battle against current epidemic abuse and overdose in the United States. In response to the demand for high-resolution chromatography techniques in potency testing of cannabis and hemp, we expanded the list of the cannabinoids to 18 in a 30-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.

■ References

1. Alexandra Sifferlin, "Can Medical Marijuana Help End the Opioid Epidemic?" Time Magazine (July 2016), hhtp://time.com/4419003/can-medical-marijuana-help-end-the-opioid-epidemic/
2. Marcus A. Bachhuber, Brendan Saloner, Chinazo O. Cunningham, and Colleen L. Barry, "Medical Cannabis Laws and Opioid Analgesic Overdose Mortality in the United States, 1999-2010," JAMA Internal Medicine 174, no. 10 (2014): 1668. Doi: 10:1001/jamainternmed.2014.4005.

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