Niloufar Pezeshk - nipezeshk@Shimadzu.com

Differentiating Cannabis and Hemp with the Cannabis Analyzer for Potency™

Niloufar Pezeshk and Craig Young
Shimadzu Scientific Instruments, Inc.

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. Prescriptions of marijuana derived compounds are commonplace in several states with more pharmaceutical treatments to follow.

Hemp-derived CBD products and treatments abound. As of 2020, the only FDA-approved drug for treating epilepsy is Epidiolex, which contains a purified form of CBD. The combination of THC and CBD has shown enhanced immune system in cancer patients, relaxed arteries in patients with heart disease and reduced muscle spasms in patients with Multiple Sclerosis.

The U.S. Hemp Farming Act was signed into law in December, 2018. The law differentiates hemp from cannabis based on its total psychoactive content or Total THC (% THCA + % d9-THC). Hemp is considered < 0.3 wt. %. The purpose for this study is to build a simple and rugged HPLC method for differentiating cannabis and hemp.

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 1 Summary of method and instrument parameters

Item	Description
Standards	Phytocannabinoid Mixture 4 (CRM) solution in ACN, 220-91239-23
HPLC System	Cannabis Analyzer for Potency™, 220-94420-00
Detector	Integrated 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 4 min; 85%-95% B over 0.01 min; 95% B for 0.99 min; 95%-70% B over 0.01 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)	1.6
Oven (°C)	35
Injection Volume (µL)	5

Results and Discussion

A six-point calibration curve ranging from 0.5 to 100 mg/L and two Quality Control (QC) standards, 2.5 mg/L and 25 mg/L, were prepared. Calibration curves and QC standards were evaluated using three 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 4 target cannabinoids. The statistical results were processed via Browser in LabSolutions, version 5.97. Results are shown in Table 2.

Table 2: Statistical analysis of 6-point calibration curve with three replicates for calibration standards and quality control (QC) standards for the 4-cannabinoid mixture

Name	6-point Standard (n=3) ranging from 0.5 to 100 mg/L				2-point Quality Control standards (n=3)							
					QC Low (2.5 mg/L)				QC Medium (25 mg/L)			
	%Dev	Accuracy (%)	RF %RSD	R^2	Mean Conc. (mg/L)	%RSD	%Dev	Accuracy (%)	Mean Conc. (mg/L)	%RSD	%Dev	Accuracy (%)
CBD	3.07	100.0	19.27	0.9999	2.500	0.906	0.64	100.0	25.00	0.33	0.24	100.0
d9-THC	3.30	100.0	11.25	0.9997	2.500	6.342	4.29	100.0	25.00	0.14	0.11	100.0
d8-THC	2.50	100.0	10.45	0.9998	2.500	4.200	3.22	100.0	25.00	0.37	0.27	100.0
THCA	5.46	100.0	10.22	0.9999	2.500	0.525	0.37	100.0	24.00	0.76	0.58	100.0

Figure 1 shows the 4-cannabinoid mixture repeatability. Figure 2 illustrates the chromatograms of two commercially available CBD dry samples and Figure 3 shows an example of a tincture hemp sample chromatogram.

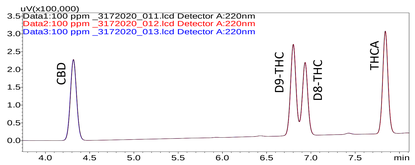

Fig 1. 4-cannabinoid mixture resolution and repeatability

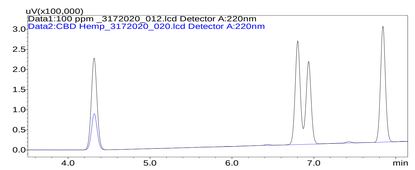

Fig 2. CBD Hemp sample) vs 100 ppm standards

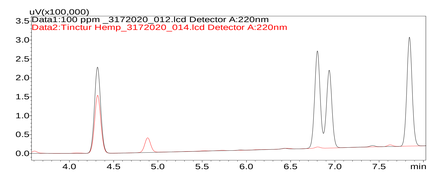

Fig 3. Tincture Hemp vs. 100 ppm Standard.

Table 3 shows the summary of cannabinoids quantitation. Using our method, we obtained an average of 67.47 mg/mL for a commercially available labeled 50 mg/mL of CBD tincture.

Table 3: Summary of CBD and THC quantitative determination for three samples

ID #	Sample Name	Dry Weight (mg)	Extraction Volume (mL)	Dil. Factor	Measured Mean Conc. (mg/mL)		
					CBD	D9-THC	THCA
1	CBD Hemp (200x)	102	12	240	0.04	-	-
2	Flower Hemp (20x)	200	20	40	0.023	0.002	0.002
3	Tincture Hemp (1000x)	-	10	-	67.47	1.50	-

As described earlier, the potency for the tincture/oil is expressed as mg/mL of CBD. The measured potency for the two dry samples is represented in Tables 4 and 5. The results from table 4 was in consistency with those from the manufacturer, as a CBD level of more than 90 (wt.%) was expected. Table 5 suggests that the sample is not defined as a hemp product, i.e. as it contains more than 0.3 (wt. %) total THC.

Table 4. Measured potency for CBD-hemp sample

Name	Average Ret. Time (min)	Average Conc. (wt.%)	Total CBD (wt.%)	Total THC (wt.%)	Total CBD (mg/g)	Total THC (mg/g)
CBD	4.32	92.11	92.11	-	921.09	-

Table 5. Measured potency for flower-hemp sample

Name	Average Ret. Time (min)	Average Conc. (wt.%)	Total CBD (wt.%)	Total THC (wt.%)	Total CBD (mg/g)	Total THC (mg/g)
CBD	4.32	4.52	4.52	0.82	45.29	8.24
d9-THC	6.80	0.42				
THCA	7.84	0.45				

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. The statistical results show retention time and peak area repeatability, quantitative accuracy and sensitivity.