The Potency Determination of 15 Cannabinoids using the Hemp Analyzer

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Introduction

According to a 2020 Grand View Research report[1], the industrial hemp market is expected to exceed 15 billion USD by 2027. This accounts for all segments of the market, including application of the product in pharmaceuticals, medicinal and therapeutic products, skin care products, and fiber/textiles. This growth, and the confidence in hemp products, will require applicable testing to ensure product quality and safety. Chromatography technology will play a large role in this as the technique is used for potency testing in support of manufacturing operations and the associated clinical science. Hemp is available in numerous forms, from dry flower to concentrated oils, and contains over one hundred cannabinoids, making the development of rugged, quantitatively accurate methods a challenge. This study optimizes a quantitative chromatographic determination of 15 cannabinoids using the Shimadzu Hemp Analyzer.

Equipment and Method

For this study a Shimadzu Hemp Analyzer – an integrated HPLC system with built-in UV detector – was used. Table 1 shows the instrument and method parameters summary. To create a 100.0 µg/mL mixture consisting of 15 components mixture. A mixture of 11 cannabinoids (CRM; PN: 220-91239-21) was supplemented with four additional cannabinoid standards (Cerilliant).

Sample A	Sample B
HPLC System	Hemp Analyzer
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 6 min; 85%-95% B over 0.01 min; 95% B for 0.99 min; 95%-70% B over 0.01 min; 70% B for 4.99 min
Column	NexLeaf CBX for Potency, 2.7 um, 4.6 x 150 mm column, 220-91525-70
Guard column	NexLeaf CBXGaurd Column Cartridge, 220-91525-72
Flowrate (mL/min)	1.6
Oven Temperature (°C)	35
Injection Volume (µL)	5

Table 1: Summary of method and instrument parameters

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.

Note: For UHPLC analysis of 16 or more cannabinoids, see Shimadzu Application News No. HPLC-20.

No.	Standard	Compounds	Stock Conc. (mg/L)
1	Shimadzu	CBDV	250
2	Shimadzu	CBDA	250
3	Shimadzu	CBGA	250
4	Shimadzu	CBG	250
5	Shimadzu	CBD	250
6	Shimadzu	THCV	250
7	Shimadzu	CBN	250
8	Shimadzu	d9-THC	250
9	Shimadzu	d8-THC	250
10	Shimadzu	CBC	250
11	Shimadzu	THCA	250
12	Cerilliant	CBDVA	1000
13	Cerilliant	THCVA	1000
14	Cerilliant	CBL	1000
15	Cerilliant	CBCA	1000

Table 2: Initial concentrations for the 15 cannabinoids prior to mixture preparation

Results and Discussion

A six-point calibration curve ranging from 0.5 to 100 µg/mL and three Quality Control (QC) standards, 2.5 µg/mL, 25 µg/mL and 75 µg/mL, 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≥0.999).

Figure 1 shows the calibration curves for the 15 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 Database, version 6.83; results are shown in table 3.

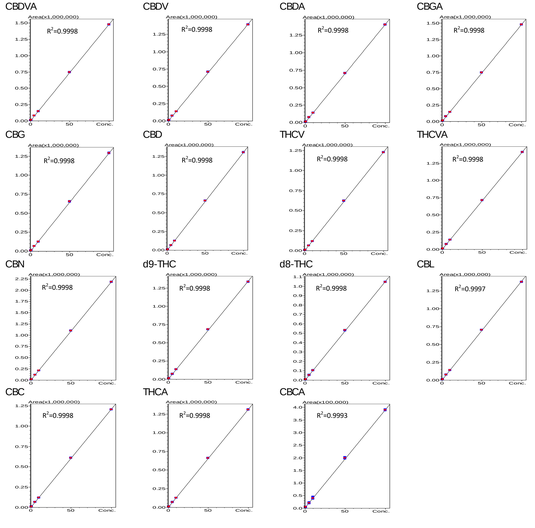

Figure 1: Initial concentrations for the 15 cannabinoids prior to mixture preparation

No.	Compound	Calibration Results		2.5 ppm (QC Low)			25.0 ppm (QC Medium)			75.0 ppm (QC High)		
		RF RSD (%)	R^2	Mean Conc.	RSD (%)	Accuracy (%)	Mean Conc.	RSD (%)	Accuracy (%)	Mean Conc.	RSD (%)	Accuracy (%)
1	CBDVA	3.950	0.9998	2.62	1.265	104.6	25.38	0.206	101.5	77.18	0.665	102.9
2	CBDV	5.002	0.9998	2.63	1.031	105.4	25.74	0.235	102.9	77.83	0.577	103.8
3	CBDA	4.320	0.9998	2.62	1.150	103.6	25.45	0.233	101.8	77.17	0.699	102.9
4	CBGA	4.372	0.9998	2.59	0.904	101.7	25.44	0.137	101.8	76.81	0.668	102.4
5	CBG	6.721	0.9998	2.54	0.708	98.4	25.49	0.201	102.0	78.06	0.616	104.1
6	CBD	4.637	0.9998	2.46	0.689	100.4	25.45	0.250	101.8	78.20	0.742	104.3
7	THCV	4.836	0.9998	2.51	0.766	105.1	25.50	0.198	102.0	77.82	0.512	103.8
8	THCVA	3.557	0.9998	2.63	0.772	105.1	25.50	0.287	102.0	76.38	0.783	101.8
9	CBN	3.587	0.9998	2.63	0.603	98.5	25.41	0.226	101.6	77.83	0.659	103.8
10	d9-THC	9.869	0.9998	2.46	5.300	100.0	25.82	0.383	103.3	77.96	0.531	103.9
11	d8-THC	6.941	0.9998	2.50	5.455	108.3	25.85	0.493	103.4	77.88	0.741	103.8
12	CBL	6.867	0.9997	2.71	2.585	108.3	25.85	0.532	103.4	77.68	0.631	103.6
13	CBC	9.092	0.9998	2.60	2.838	104.0	25.48	0.370	101.9	77.97	0.629	104.0
14	THCA	9.222	0.9998	2.70	3.609	107.9	25.55	0.359	102.2	76.57	0.699	102.1
15	CBCA	38.577	0.9993	2.28	13.645	91.0	25.78	3.193	103.1	75.69	2.017	100.9

Table 3: Statistical analysis of 6-point calibration curve with seven replicates for calibration standards and quality control (QC) standards for the 15-cannabinoid mixture

For the noise/drift calculations as well as detection limit and quantitation limit (Table 4), we selected a specified range from 1.20 min to 2.20 min using the ASTM calculation method. Limits of Detection (LOD) and Quantitation (LOQ) of 3.3 and 10.0 were selected, respectively. LOD and LOQ are terms used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure.

By using the signal-to-noise method, the peak-to-peak noise around the analyte retention time was measured. A signal-to-noise ratio (S/N) of three is generally accepted for estimating LOD and signal-to-noise ratio of ten is used for estimating LOQ. This method is commonly applied to analytical chromatographic methods.[2&3]

ID#	Name	S/N	Detection Limit (LOD)
1	CBDVA	13.68	0.12
2	CBDV	12.60	0.13
3	CBDA	10.95	0.15
4	CBGA	11.09	0.15
5	CBG	9.83	0.18
6	CBD	8.70	0.20
7	THCV	8.22	0.20
8	THCVA	9.85	0.17
9	CBN	16.13	0.10
10	d9-THC	10.58	0.15
11	d8-THC	7.56	0.23
12	CBL	10.23	0.16
13	CBC	9.73	0.18
14	THCA	8.34	0.18
15	CBCA	3.39	0.72

Table 4: Detection limit and quantitative limit for 15 components at 0.5 µg/mL

Figure 2 shows a representative chromatogram for three QC standards. Figure 3 illustrates an overlaid chromatogram of seven injections at 100 ppm for the 15-cannabinoid mixture.

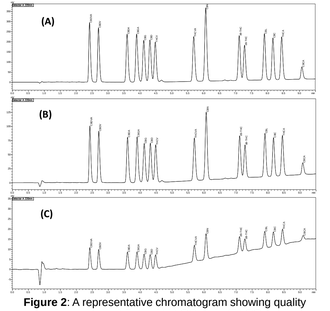

Figure 2: A representative chromatogram showing quality control standards. A 5 µL of (A) QC high, (B) QC medium and (C) QC low

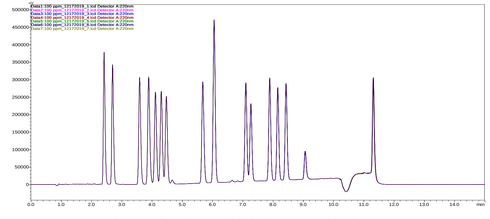

Figure 3: The 15-cannabinoid mixture - Overlay of seven injections (5 µL injection at 100 ppm)

Conclusion

In response to the increasing demand for development of chromatography techniques in potency testing of hemp and hemp, we developed a method that builds on the existing High Sensitivity Method using the Shimadzu Hemp Analyzer, optimized for the quantitative determination of 15 major cannabinoids. The statistical results document rigorous testing for retention time and peak area repeatability, quantitative accuracy and sensitivity.

References

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3. Shrivastava and V.B. Gupta. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Review Article. 2011; 2(1): 21-5.