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Application News

No. HPLC-017

High Performance Liquid Chromatography

Potency Testing in Cannabis Extracts Using a High Throughput Method with the Cannabis Analyzer for Potency

■ Introduction

Cannabis analysis has gained new importance in the USA in light of the legalization of marijuana in several states. Cannabis contains a number of chemical alkaloids known as cannabinoids. Primary cannabinoids of interest to most laboratories are tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). In extracts from the plant, THC and CBD exist as the native acid forms, tetrahydro-cannabinolic acid (THCA) and cannabinolic acid (CBDA). These gradually decarboxylate to THC and CBD through exposure to heat and light.

Cannabis may be analyzed for different purposes, the most common of which is the potency, characterized by the quantitation of THC, CBD and CBN. This application note highlights the use of a high throughput HPLC method to determine the potency of cannabis extracts with the Shimadzu Cannabis Analyzer for Potency. With this method, the most commonly requested cannabinoids may be determined in under 8 minutes.

Cannabis has been legalized in many states in the USA and the District of Columbia for either medical or recreational use or both. Possession is still illegal by Federal statutes. This can influence interstate transportation of cannabis products, but it can also influence laboratory possession of cannabis for testing purposes. Consult state regulatory agencies for proper licensing requirements.

For recreational marijuana, the psychoactive THC is of primary interest. Cannabis grown for recreational use would typically contain high levels of THC and relatively low levels of CBD and CBN. Higher content THC plant material can demand higher prices.

Medical marijuana is often characterized by higher levels of CBD and lower levels of THC. The therapeutic CBD is desirable for medicinal effect but the psychoactive THC may be unnecessary and even undesirable for some patients. Pain mitigation, reduced severity of nausea and seizures are some of the therapeutic benefits reported by medical cannabis patients. This THC/CBD ratio information is of primary importance to the medical personnel prescribing cannabis for medicinal purposes.

■ Calibration of the HPLC system by use of a standard solution

Figure 1 shows the chromatogram of 10.0 mg/L standard mixture. Gradient elution conditions with acid modified water and acetonitrile were employed with a C18 column chemistry to achieve the separation in under 8 minutes.

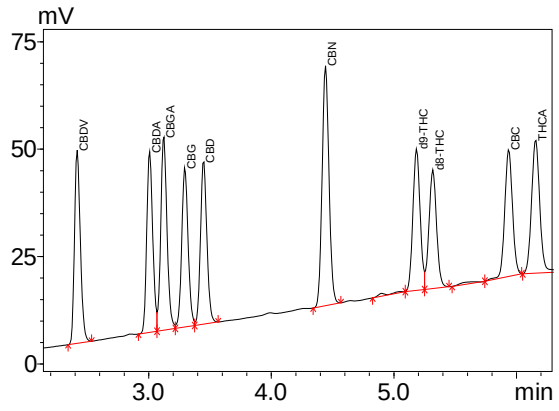

Figure 1: Chromatogram of 10.0 mg/L standard mixture

■ Standard Curves

Standard curves (Fig 2) were prepared for each target analyte with a minimum acceptable correlation coefficient (R^2) of 0.999 over 6 standard levels. A linear dynamic range was established at 0.5 to 100 mg/L (0.05 – 10%) in each analyte except THCA and CBDA. In many cases the abundance of THCA and CBDA in plant material is exceedingly high, therefore the linear dynamic range for those analytes was established from 0.5 to 250 mg/L (0.05 – 25%).

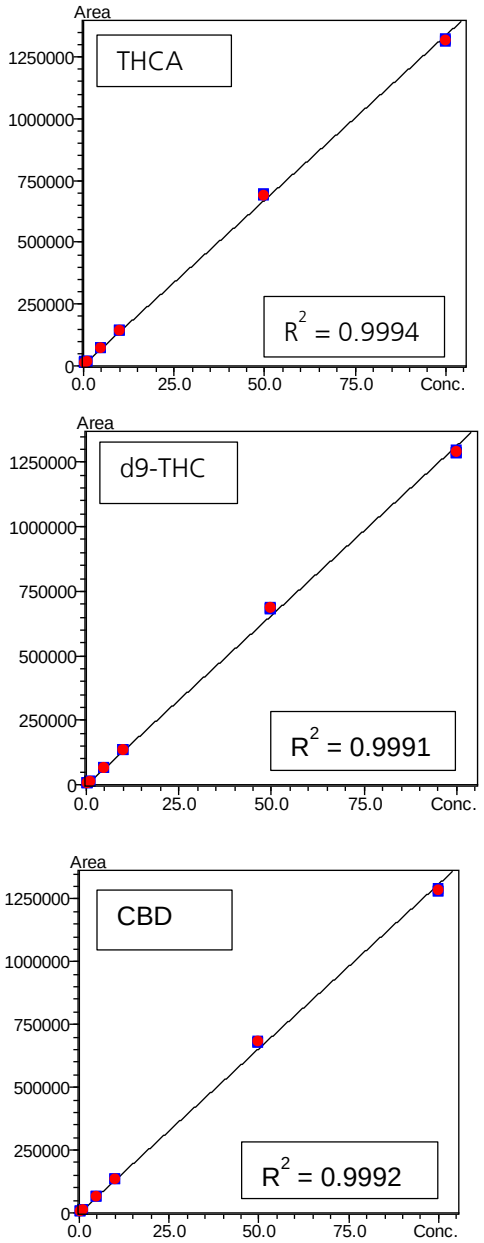

Figure 2: Select standard curves

■ Accuracy% of Standard Curves

Tables 1,2 and 3 show average Accuracy% of low (2.0 mg/L), mid (20 mg/L) and high (70 mg/L) standard mixtures for all analytes comprising the standard curves over 6 runs. The definition of Accuracy% appears below.

- Accuracy% = Cr / Cc x 100
- Cr: Concentration value from the quantitative calculation
- Cc: Standard concentration value of the corresponding level in Compound Table

From the results of Table 1, the quantitation accuracy of all compounds were within ±10 % (THCA was an outlier at ±19%) for low values, and within ±3 % for mid and high quantitation points. Thus, it was confirmed that active ingredients in cannabis flowers could be quantified accurately by using the corresponding calibration curves.

Table 1: Low Standard Accuracy%

2 ppm	Conc. (mg/L)	
#	Name	Avg. Accuracy (%)
1	CBDV	107.25
2	CBDA	105.20
3	CBGA	104.60
4	CBG	107.00
5	CBD	105.80
6	CBN	107.15
7	d9-THC	97.00
8	d8-THC	98.85
9	CBC	105.15
10	THCA	116.15

Table 2: Mid Standard Accuracy%

20 ppm	Conc. (mg/L)	
#	Name	Avg. Accuracy (%)
1	CBDV	105.69
2	CBDA	103.67
3	CBGA	103.43
4	CBG	105.55
5	CBD	105.20
6	CBN	103.83
7	d9-THC	104.40
8	d8-THC	104.71
9	CBC	103.48
10	THCA	104.07

Table 3: High Standard Accuracy%

70 ppm	Conc. (mg/L)	
#	Name	Avg. Accuracy (%)
1	CBDV	103.45
2	CBDA	102.82
3	CBGA	102.20
4	CBG	103.31
5	CBD	103.21
6	CBN	103.28
7	d9-THC	103.19
8	d8-THC	103.78
9	CBC	103.49
10	THCA	102.34

■ Quantitative analysis of cannabinoids in cannabis flowers

Fig. 3 shows the chromatogram of an extract from a THC-rich flower sample.

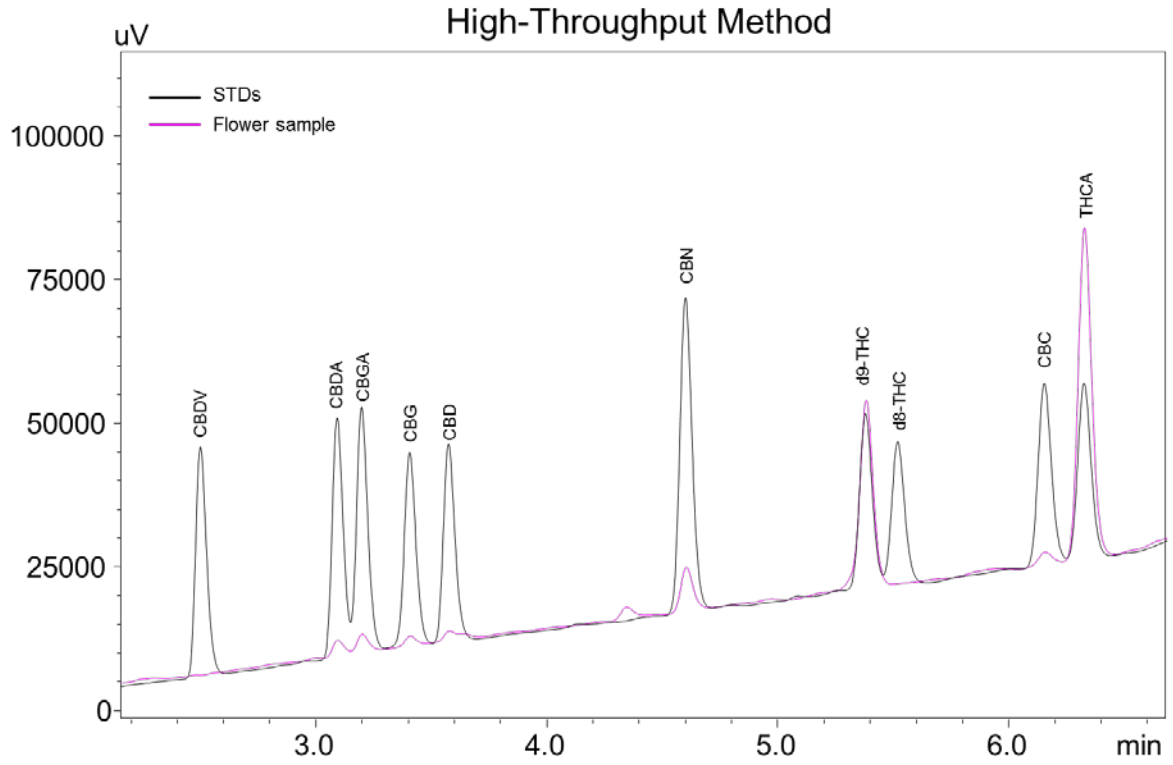

Figure 3: Chromatogram overlay of cannabis flower extract and a 10.0 mg/L standard mixture

■ Potency Calculation

Table 4 shows the quantitative result of each compound as well as a calculation for potency, as defined here.

- %THCA = [THCA] x (DIL) x (VOL/MG) x 100
- Potency: (%THCA x 0.877) + %Δ9-THC

[THCA]: Concentration of THCA, DIL = Dilution Factor, VOL = External Volume
MG = dry sample weight (mg), 0.877 = molecular weight ratio of cannabinoids to cannabinoid acids

#	Compounds	Conc (mg/L)	wt %
1	THCV	--	--
2	CBD	0.852	0.17
3	CBG	0.555	0.11
4	CBDA	0.721	0.14
5	CBGA	0.728	0.14
6	CBN	1.411	0.28
7	D9-THC	11.389	2.28
8	D8-THC	ND	ND
9	CBC	0.602	0.12
10	THCA	19.533	3.90
Potency			5.7%

Table 4: Quantitative Result of Flower Sample

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