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Application News

No. HPLC-015

High Performance Liquid Chromatography

Potency Testing in Cannabis Extracts Using a High Resolution 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 resolution 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 less than 30 minutes with full baseline resolution. Further, the method handles a growing number of cannabinoid targets.

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 complete baseline separation in under 30 minutes.

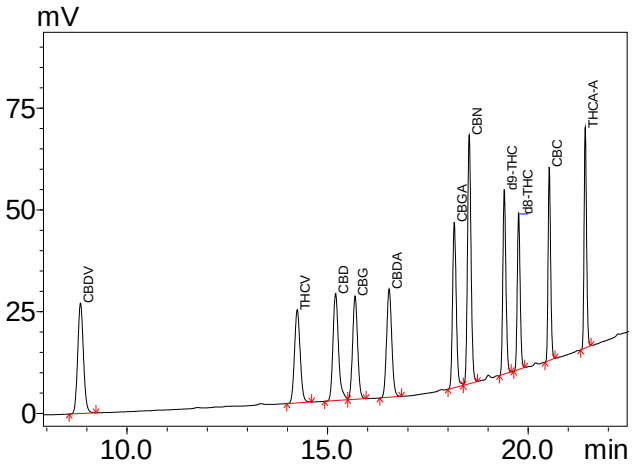

Figure 1: Chromatogram of 10.0 mg/L standard mixture

■ 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%).

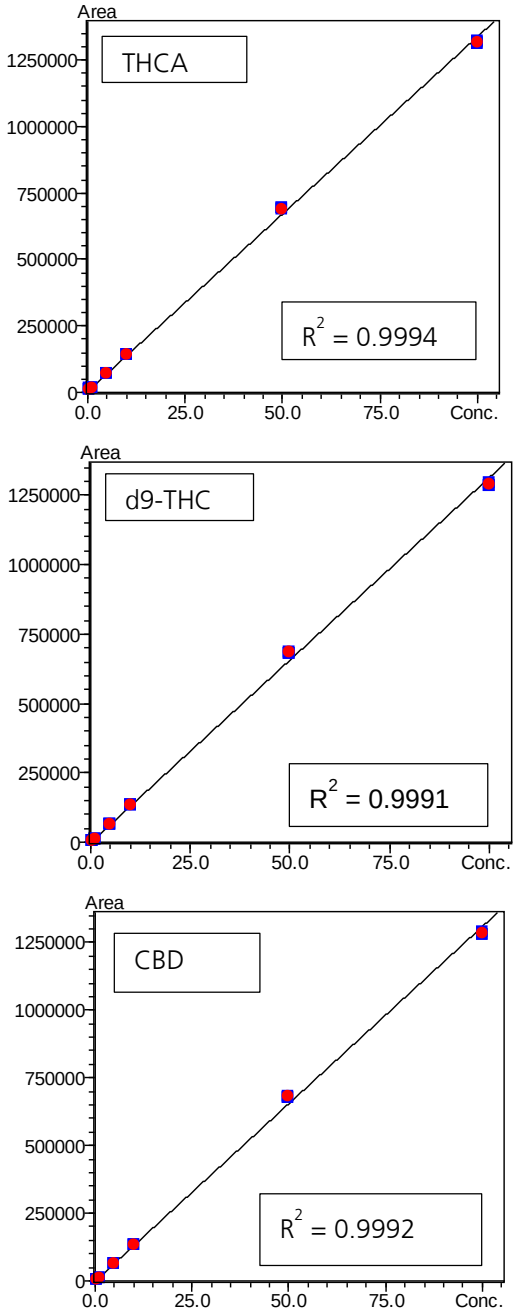

Figure 2: Select standard curves

■ Accuracy% of the Standard Curves

Tables 1 shows average Accuracy% of a low (1.0 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 ±7 % 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%

1 ppm	Conc. (mg/L)	
#	Name	Avg. Accuracy (%)
1	CBDV	--
2	CBDA	102.7
3	CBGA	100.9
4	CBG	98.3
5	CBD	103.0
6	THCV	101.4
7	CBN	103.0
8	d9-THC	102.9
9	d8-THC	103.1
10	CBC	103.5
11	THCA	105.5

■ Quantitative analysis of cannabinoids in cannabis flowers

Fig. 3 shows chromatograms of extracts from a THC-rich and CBD-rich flower samples overlaid with a 10 mg/L standard mixture.

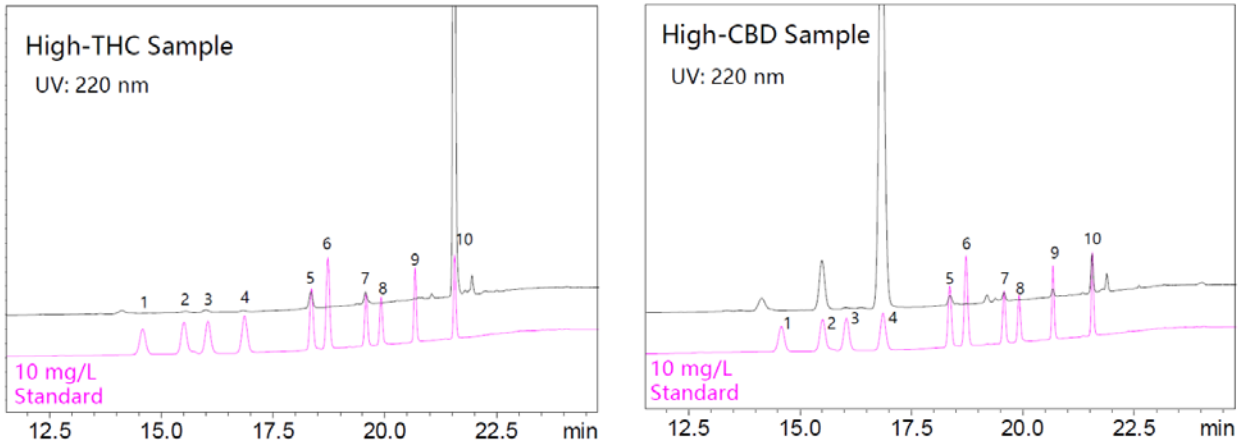

Figure 3: Chromatogram overlays of cannabis flower extract and a 10.0 mg/L standard mixture

■ 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

Table 4: Quantitative Result of THC-rich Flower Sample

#	Compounds	wt %
1	THCV	--
2	CBD	0.05
3	CBG	0.08
4	CBDA	0.03
5	CBGA	0.28
6	CBN	--
7	D9-THC	0.25
8	D8-THC	--
9	CBC	--
10	THCA	13.38
11	CBDV	--
Potency		12%

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