

# Development of an Improved Method for Cannabis Potency Analysis Utilising HPLC/PDA in Preference to UHPLC

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The number of cannabis testing laboratories has expanded, as an increasing number of states have adopted medicinal and/or recreational cannabis use programs. Potency testing is of particular concern when the cannabis is to be used for medical treatment or research. Thus, analytical standards for the primary components of cannabis are of the utmost importance. Historically-used analytical methods have had drawbacks regarding method robustness and long sample turnaround time. This manuscript describes the development of a simple, accurate, and reproducible analytical method for the chromatographic separation and quantitation of seven primary cannabinoids in cannabis extracts by high performance liquid chromatography (HPLC) with photodiode array (PDA) detection, using an SPP (superficially porous particle) column.

## Introduction

In the United States, the federal Controlled Substances Act (CSA) of 1970 makes illegal the use and possession of cannabis for any purpose. Under the CSA, cannabis is classified as a Schedule I substance, meaning it has a high potential for abuse and no accepted medical use, thereby prohibiting even medical use of the drug [1]. At the state level, however, policies regarding the medical and recreational use of cannabis vary greatly, and many state policies conflict with federal law.

In 1996, California became the first state to legalise the medical use of marijuana. Since then many more states have enacted cannabis programs, the most recent being the state of Vermont which legalised adult use of marijuana in January 2018. As of March 2018, 29 states, the District of Columbia, Guam, and Puerto Rico have enacted public medical marijuana and cannabis programs. Another 17 states have approved the medicinal use of products that have high levels of cannabidiol (CBD), a non-psychoactive component of cannabis, as long as the product has low levels of the psychoactive component tetrahydrocannabinol (THC). These 'high

CBD/low THC' programs typically include restrictions on the types of medical conditions for which such products can be used [2]. Between the years 2012 and 2018, nine states and the District of Columbia expanded their programs to include 'retail/adult' recreational use of marijuana [3].

As the legality and use of marijuana has spread across the nation, so has its cultivation and, concurrently, the number of analytical laboratories conducting state-required testing of marijuana crops [4]. Comprehensive cannabis testing covers a range of targets, including THC and CBD, terpenes, microbiological and pesticide contaminants, and heavy metals via absorption from the soil during cultivation. For all of these parameters of concern, reliable and accurate analytical methods are pivotal to ensuring the quality, safety, and potency of cannabis products.

Potency testing is of particular concern when the cannabis is to be used for medical treatment or research. Thus, analytical standards for the primary components of cannabis are of the utmost importance. Naturally occurring cannabinoids, the main biologically active component of the cannabis plant, form a complex group of closely related compounds of

which 113 are known and 70 are well described. The primary focus has been on  $\Delta^9$ -tetrahydrocannabinol (THC), the primary active ingredient in cannabis, due to its psychoactive, pharmacological, and toxicological characteristics, upon which strict legal limits have been enforced. However, analytical labs must also focus on  $\Delta^9$ -tetrahydrocannabinolic acid (THC-A), the naturally occurring precursor to THC that is readily decarboxylated to THC during the drying and/or heating of cannabis.

Many efforts to standardise cannabis testing have used standard solutions of different cannabinoids for method development. Yet, the complex matrix of compounds in cannabis is very different from a pure standard solution and, thus, the development of a reproducible method to be used for whole cannabis testing requires the use of actual cannabis samples [5].

Gas chromatography/mass spectrometry (GC/MS) has historically been used for the separation and quantitation of the compounds of interest in cannabis. However, a limitation of the use of GC for cannabinoid analysis involves distinguishing between THC and THC-A. In order to obtain distinct data for these two compounds, an additional

Table 1. LC Method Parameters.

Column	PerkinElmer Brownlee SPP C18, 2.7 $\mu$ m, 3.0 x 150 mm				
Mobile Phase	Solvent A: 0.1% formic acid in water Solvent B: 0.1% formic acid in acetonitrile Gradient program:				
		Time (min)	Flow Rate (mL/min)	%A	%B
	0 (Equil)	4.5	1.0	30.0	70.0
	1	4.0	1.0	5.0	95.0
	2	2.0	1.0	5.0	95.0
Analysis Time	6.0 min; equilibration time: 4.5 min				
Pressure	4600 psi/317 bar maximum				
Oven Temperature	40°C				
PDA Detection	Wavelength: 228 nm				
Injection Volume	10 $\mu$ L				
Sampling (Data) Rate	10 pts/sec				

process must be completed to derivatise the sample so THC-A is not converted to THC by the heat of injection [6].

This manuscript describes an analytical method for the chromatographic separation and quantitation of seven primary cannabinoids in *C. sativa* extracts by HPLC with PDA detection. This method is simple, accurate, and reproducible, provides improved turnaround time, and precludes some of the challenges posed by GC/MS analysis of cannabis components and costs of UHPLC.

## Experimental

### Hardware/Software

A PerkinElmer® (Shelton, CT, USA) Flexar™ HPLC system was used with a PDA (photodiode array) detector and accompanying CDS system. A PerkinElmer Brownlee™ SPP C18, 2.7  $\mu$ m, 3.0 x 150 mm column was used for all analyses.

### LC Parameters

The LC method parameters are listed in Table 1.

### Solvents, Standards, and Samples

All solvents and diluents used were HPLC grade and filtered via 0.45- $\mu$ m filters. All diluents were 80:20 methanol/water. The 1 mg/mL (in 1 mL of methanol) standards of  $\Delta^9$ -tetrahydrocannabinol (THC),  $\Delta^9$ -tetrahydrocannabinolic acid (THC-A), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabigerol (CBG), cannabinol (CBN), and cannabichromene (CBC) were obtained from Sigma-Aldrich®, Inc. (Allentown, PA, USA) and Restek Corporation (Bellefonte, PA, USA).

A 100- $\mu$ g/mL working standard of the seven standards was prepared by adding 1 mL of each standard to a 10-mL volumetric flask and filling to mark with the 80:20 methanol/water diluent. This working standard also served as the level-6 calibration standard. Calibration standards at 50, 20, 5, 1, and 0.5

$\mu$ g/mL were then prepared via serial dilution of the working standard.

Four 5-mL prepared cannabis extract samples, A through D, were obtained from 3B Analytical® (Portland, OR, USA), a cannabis testing laboratory. The lab prepared the extracts by adding 10 mL of methanol to one gram of dried and ground cannabis flowers. The samples were then vortexed for three minutes followed by filtering of 2 mL of supernatant using a 0.45- $\mu$ m filter. The filtered supernatant was then diluted 3-fold with methanol. This resulted in an overall 30-fold concentration dilution with respect to the initial product. Upon receipt, each sample was further diluted 100-fold with diluent and held under refrigeration.

This considerable dilution was required to stay within the concentration range of the calibrants (0.5-100  $\mu$ g/mL). Cannabinoid standards are commercially (and legally) only obtainable at 1 mg/mL. Therefore, once prepared as part of the calibration mix, the individual analyte concentration at the highest level was 100  $\mu$ g/mL. This level is considerably lower than that expected for some cannabinoids in undiluted cannabis extract, particularly for THC-A; hence, the significant dilution requirement of the samples. All calibrants and samples were subsequently filtered through 0.45- $\mu$ m filters and then injected (4  $\mu$ L).

## Results and Discussion

Figure 1 shows the chromatogram of a standard mixture containing the seven target cannabinoids, all separated in under four minutes. The gradient ramp was due to the relatively low concentrations of the high-end calibrants, again, limited by the obtainable concentrations of the standards. As shown in Figure 2, chromatographic repeatability was demonstrated via ten replicate injections of the 100-ppm standard mixture. The retention time %RSD for all peaks was less than 0.05%. This confirms the reliable performance of this chromatographic

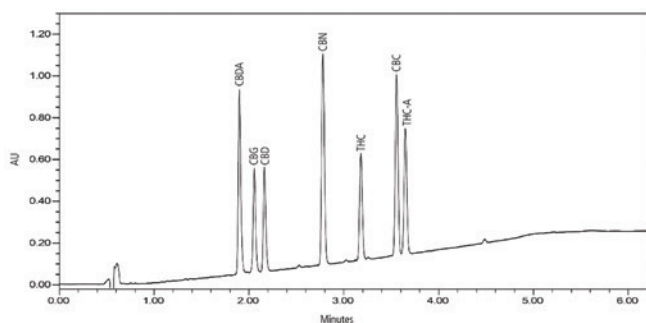


Figure 1. Chromatogram showing separation of the seven target cannabinoids in the 100-ppm standard;  $\lambda$  = 228 nm.

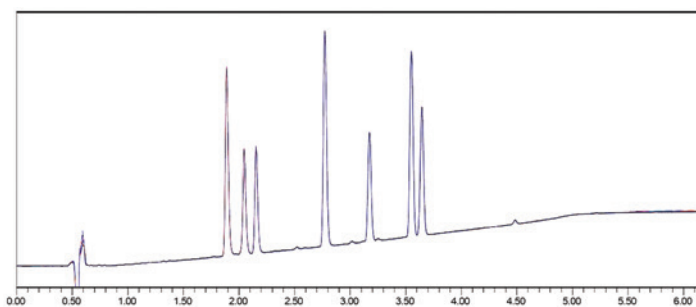


Figure 2. The chromatographic overlay of 10 replicates of the 100-ppm standard, demonstrating the precision of the method.

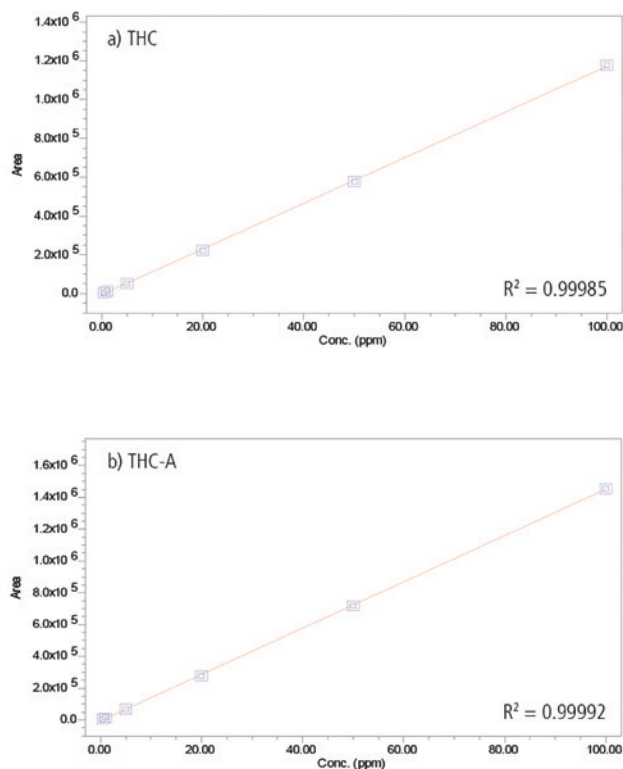


Figure 3. Linearity plots for THC and THC-A, concentrations ranging from 0.5-100  $\mu\text{g/mL}$ .

method, which is essential for ensuring the integrity of the results for medicinal cannabis analysis. In the medicinal cannabis industry, confident product composition is pivotal in helping to ensure the safety of released products.

Representative 6-level linearity plots for THC and THC-A, are shown in Figure 3. The R<sup>2</sup> values for all seven cannabinoids were above 0.999. As listed in Table 2, LOQs (limits of quantitation) were established for each cannabinoid based on calibration standard responses. The LOQs ( $\geq 10$  S/N) were  $\leq 0.10$   $\mu\text{g/mL}$  for all analysed cannabinoids. As cannabinoids are typically tested for high-end potency, these LOQs are well below the current concentrations of interest for the primary cannabinoids being analysed.

Table 2. LOQs for the seven target cannabinoids; PDA at 228 nm.

Cannabinoid	LOQ ( $\mu\text{g/mL}$ )
Cannabigerol (CBG)	0.08
Cannabidiol (CBD)	0.06
Cannabidiolic acid (CBDA)	0.06
Cannabinol (CBN)	0.03
$\Delta^9$ -Tetrahydrocannabinolic (THC)	0.06
Cannabichromene (CBC)	0.04
$\Delta^9$ -Tetrahydrocannabinolic acid (THC-A)	0.09

Figure 4 shows the chromatographic results for each sample. Comparing chromatograms, none of the samples showed any detectable levels of CBN, and only Sample A showed any detectable amount of CBC. Sample A appeared quite different from the others in that it contained a significantly greater proportion of CBDA, and significantly less THC and THC-A. It was also the only sample found to contain quantifiable amounts of CDA and an unknown matrix component, eluting on the backside of CBDA. Otherwise, Samples B, C, and D appeared quite similar to one another.

Table 3 shows the detected concentrations for the seven target cannabinoids in each sample. The detected concentrations were verified by 3B Analytical

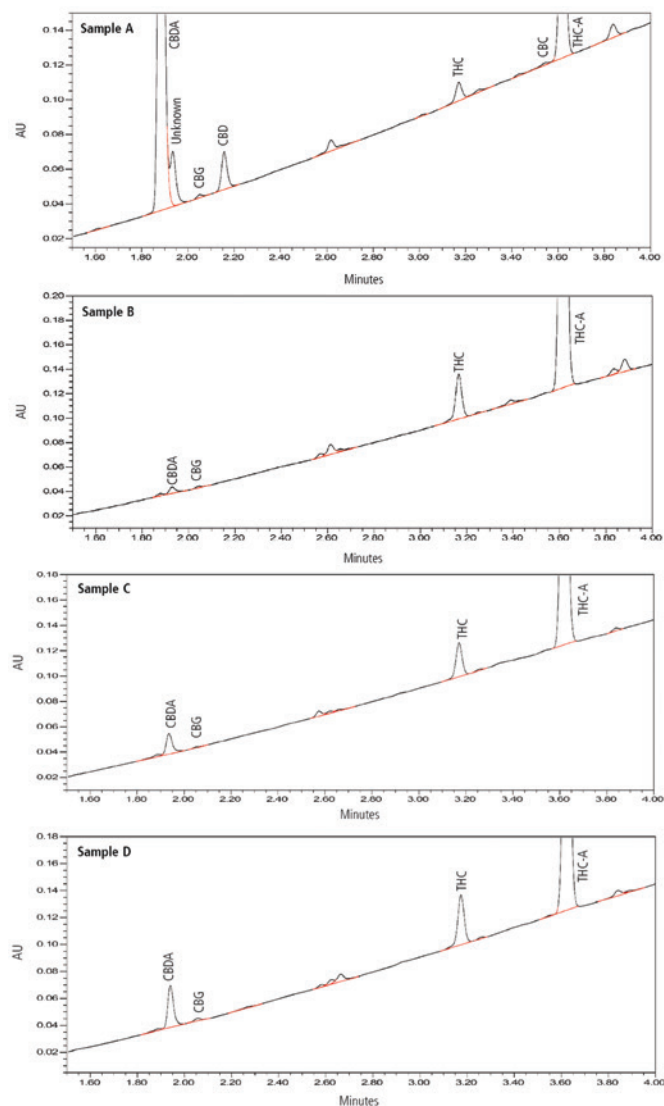


Figure 4. Chromatographic results for Samples A, B, C, and D (expanded views).

Table 3. Cannabinoid concentrations detected in each sample.

Sample	Analyte	Extract Concentration	(Wgt./Vol. %)*	Concentration in Dried Cannabis Sample
A	CBDA	3430	0.343	10.29
	CBG	84	0.008	0.24
	CBD	408	0.041	1.23
	CBN	ND	ND	ND
	THC	221	0.022	0.66
	CBC	62	0.006	0.18
B	THC-A	1500	0.150	4.5
	CBDA	Trace	Trace	Trace
	CBG	77	0.008	0.24
	CBD	Trace	Trace	Trace
	CBN	ND	ND	ND
	THC	650	0.065	1.95
C	CBC	ND	ND	ND
	THC-A	7118	0.712	21.36
	CBDA	Trace	Trace	Trace
	CBG	69	0.007	0.21
	CBD	ND	ND	ND
	CBN	ND	ND	ND
D	THC	473	0.047	1.41
	CBC	ND	ND	ND
	THC-A	4665	0.467	14.01
	CBDA	85	0.009	0.27
	CBG	85	0.009	0.27
	CBD	ND	ND	ND
E	CBN	ND	ND	ND
	THC	640	0.064	1.92
	CBC	Trace	Trace	Trace
	THC-A	4533	0.453	13.59

\*Accounting for 1/100 extract dilution; average of three replicates.

\*\* Accounting for 30-fold dilution during initial sample extraction in lab of origin.

as agreeing with the expected values obtained by an independent GC-based analysis. Sample A exhibited a significantly higher CBDA concentration and, thus, points toward an outlier-type cannabis strain, one that may peak considerable interest for possible medicinal purposes. Sample B showed the highest concentration of THC-A, setting it apart from the other samples and making it a relative front-runner for recreational purposes. Samples C and D were quite similar, both chromatographically and quantitatively, suggesting they were from similar cannabis strains.

## Conclusion

This study demonstrated the effective chromatographic separation and quantitation of seven cannabinoids, including THC and THC-A, in cannabis extracts using HPLC and PDA. The method provided exceptional linearity for each of the seven cannabinoids over the tested concentration range, and the sample results were verified to be consistent with those

obtained by independent GC-analysis.

The separation was accomplished via conventional HPLC, using an SPP column, in less than four minutes, thus reducing turnaround time and avoiding the higher costs associated with UHPLC. PDA quantitation provided excellent sensitivity, precluding the need for quantitation by mass spectrometry.

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