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Application Note

Separation of 16 Cannabinoids in Cannabis Flower and Extracts Using a Reversed Phase Isocratic HPLC Method

Andrew J. Aubin, Catharine Layton, Shawn Helmueller

Waters Corporation

Abstract

This application note presents a simple isocratic separation of 16 cannabinoids that can be used for the analysis of both plant material and concentrates to ensure the quality and safety of cannabis products.

Benefits

The Alliance HPLC System is a highly reliable and robust instrument that can be used for routine analysis of cannabis and cannabis extracts.

Introduction

As the legalization of cannabis for both medicinal and recreational use continues to advance, the need for simple and reliable analytical methods for the analysis of these products is desired by many industry stakeholders (producers, regulators, and consumers). The cannabis plant (*Cannabis sativa*) is a complex natural product that is known to produce at least 100 different cannabinoids.[1] Traditionally, laboratories have

focused their analysis on five primary compounds: delta-9-tetrahydrocannabinol (d9-THC), delta-9-tetrahydrocannabinolic acid (THC-A), cannabidiolic acid (CBD-A), cannabidiol (CBD), and cannabinol (CBN). Recently, more attention has been paid to some of the other minor cannabinoids, particularly in the medicinal market, which requires methods that are capable of higher resolution separations. Many of the minor cannabinoids have shown some medicinal effects so it is important to have the ability to separate and identify them. It is also desirable to have methods that minimize peak co-elutions of minor and major cannabinoids and provide accurate quantitative results. Several industry standardization bodies are currently developing harmonized methods for cannabinoid profiling to minimize the inherent variability in sampling, extraction, and instrumental analysis with an aim to improve potency result accuracy. High Performance Liquid Chromatography (HPLC) is the preferred methodology for potency determination (Total THC, Total CBD, or THC/CBD ratio) as it can identify and measure the structurally similar cannabinoids and their different forms (e.g. free form and corresponding acid forms of THC and CBD) in a single analysis. This application note presents a simple isocratic separation of 16 cannabinoids that can be used for the analysis of both plant material and concentrates to ensure the quality and safety of cannabis products.

Experimental

LC conditions

LC system:	Alliance HPLC
Column:	CORTECS Shield RP18 2.7 µm, 4.6 mm × 150 mm (p/n: 186008685)
Analytical flow rate:	2.0 mL/min
Mobile phase A:	Water with 0.1% TFA
Mobile phase B:	Acetonitrile
Isocratic:	41:59 mobile phase A/mobile phase B
Oven temp.:	35 °C

Detector:	2998 PDA
Detection wavelength:	228 nm at 4.8 nm resolution
Injection volume:	5 µL
Software:	Empower 3 CDS

Standard description

US DEA exempt reference standard solutions were obtained from Cerilliant Corporation, Round Rock, TX. These pre-dissolved solutions have been previously shown to be suitable for the generation of calibration curves[2] when handled in an appropriate manner.[3] Table 1 lists the cannabinoid standards used in this application note.

No.	Cannabinoid	Cannabinoid abbreviation	CAS number
1	Delta-9-tetrahydrocannabinol	d9-THC	1972-08-03
2	Delta-9-tetrahydrocannabinolic acid	THC-A	23978-85-0
3	Cannabidiol	CBD	13956-29-1
4	Cannabidiolic acid	CBD-A	1244-58-2
5	Cannabinol	CBN	521-35-7
6	Delta-8-tetrahydrocannabinol	d8-THC	5957-75-5
7	Cannabigerol	CBG	25654-31-3
8	Cannabigerolic acid	CBG-A	25555-57-1
9	Cannabichromene	CBC	20675-51-8
10	Cannabichromenic acid	CBC-A	185505-15-1
11	Tetrahydrocannabivarin	THCV	31262-37-0
12	Tetrahydrocannabivarinic acid	THCV-A	39986-26-0
13	Cannabidivarin	CBDV	24274-48-4
14	Cannabidivarinic acid	CBDV-A	31932-13-5
15	Cannabicyclol	CBL	21366-63-2
16	Cannabicyclolic acid	CBL-A	40524-99-0

Table 1. Cannabinoids used in the separation.

Standard preparation

Linearity of major cannabinoids d9-THC, THC-A, CBD, CBD-A, and CBN was determined for 10 concentrations between 0.004 mg/mL and 1.000 mg/mL, prepared via serial dilution in methanol using DEA

exempt standards.

Sample preparation

Four representative, pre-prepared samples were obtained from a local cannabis testing laboratory and one from a hemp processing laboratory. Samples were prepared as follows: For flower, a portion of homogenized plant material (Table 2) was added to acetonitrile or ethanol and sonicated for 20 minutes. The subsequent extract was filtered through a 0.22 µm syringe tip filter, diluted with methanol, and placed into a 2 mL sample vial ready for analysis. Concentrates were prepared similarly with isopropanol being used as the extraction solvent.

Sample	Extraction weight (mg)	Extraction volume (mL)	Dilution factor
High CBDA flower	304.6	4 (ACN)	5
High THCA flower	200.1	4 (ACN)	5
CBDA concentrate	17.5	4 (IPA)	5
THCA concentrate	15.9	4 (IPA)	5
Hemp flower	91.0	20 (EtOH)	1

Table 2. Samples used in the study.

Results and Discussion

16 cannabinoids were fully separated in 26 minutes by HPLC using a reversed-phase isocratic method as shown in Figure 1. Resolution of all 16 compounds was calculated 2.5 or greater (Table 3).

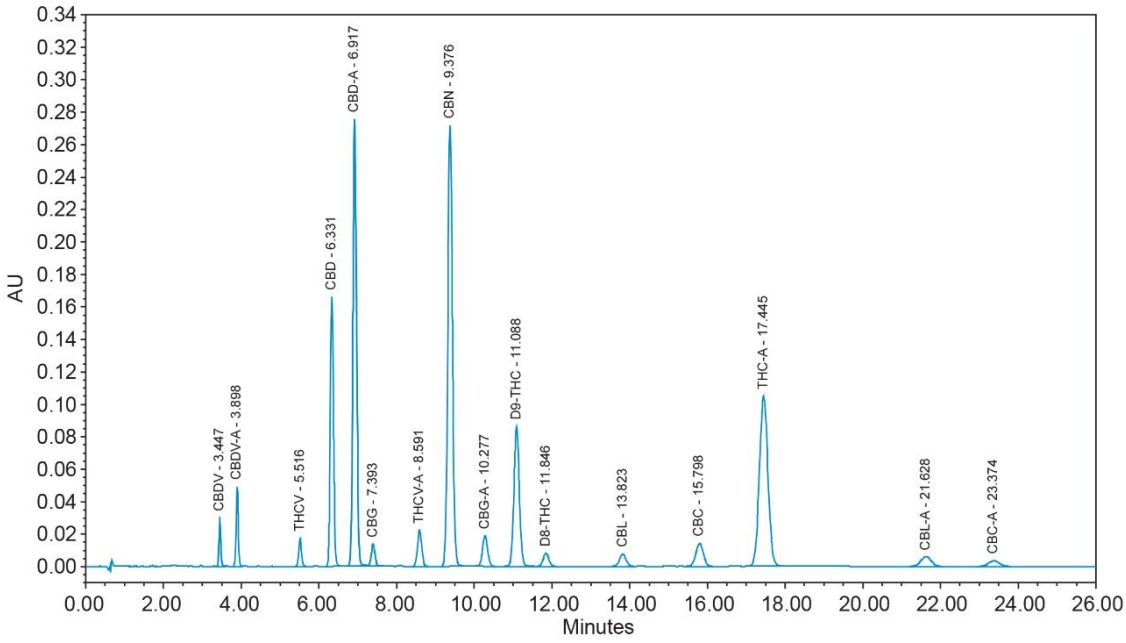

Figure 1. Isocratic separation of 16 cannabinoids.

Peak #	Name	RT (min)	Resolution
1	CBDV	3.44	–
2	CBDV-A	3.90	4.78
3	THCV	5.52	13.97
4	CBD	6.33	5.8
5	CBD-A	6.92	3.66
6	CBG	7.39	2.75
7	THCV-A	8.59	6.35
8	CBN	9.38	3.75
9	CBG-A	10.28	3.88
10	d9-THC	11.09	3.22
11	d8-THC	11.85	2.86
12	CBL	13.82	6.71
13	CBC	15.80	5.80
14	THC-A	17.44	4.28
15	CBL-A	21.63	9.24
16	CBC-A	23.37	3.34

Table 3. Compound resolution and retention times from Figure 1.

Cannabinoid	R^2
CBD	0.9997
d9-THC	0.9998
CBD-A	0.9995
THC-A	0.9993
CBN	0.9995

Table 4. Linear regression of five major cannabinoids (CBD, d9-THC, CBD-A, THC-A, and CBN).

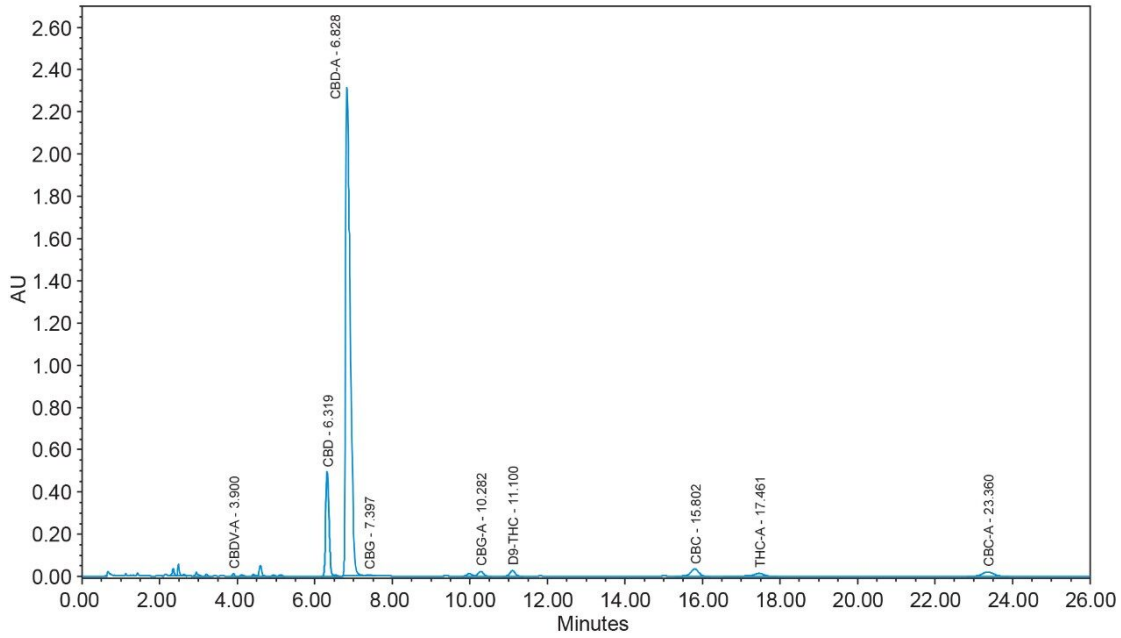

Figure 2. High CBD-A flower sample.

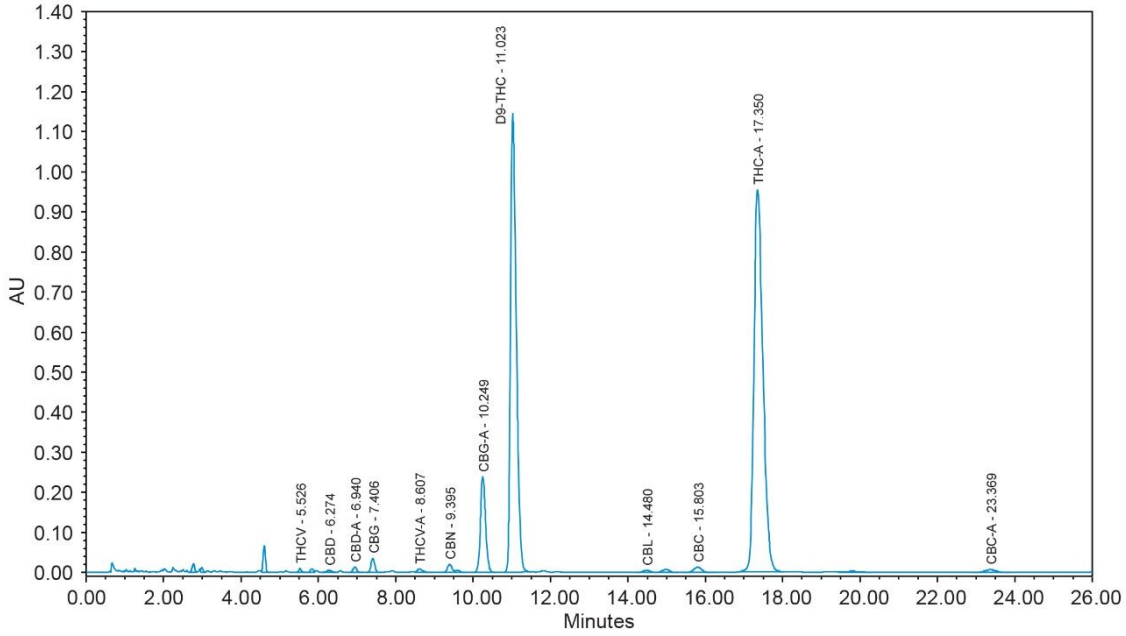

Figure 3. High THC-A flower sample.

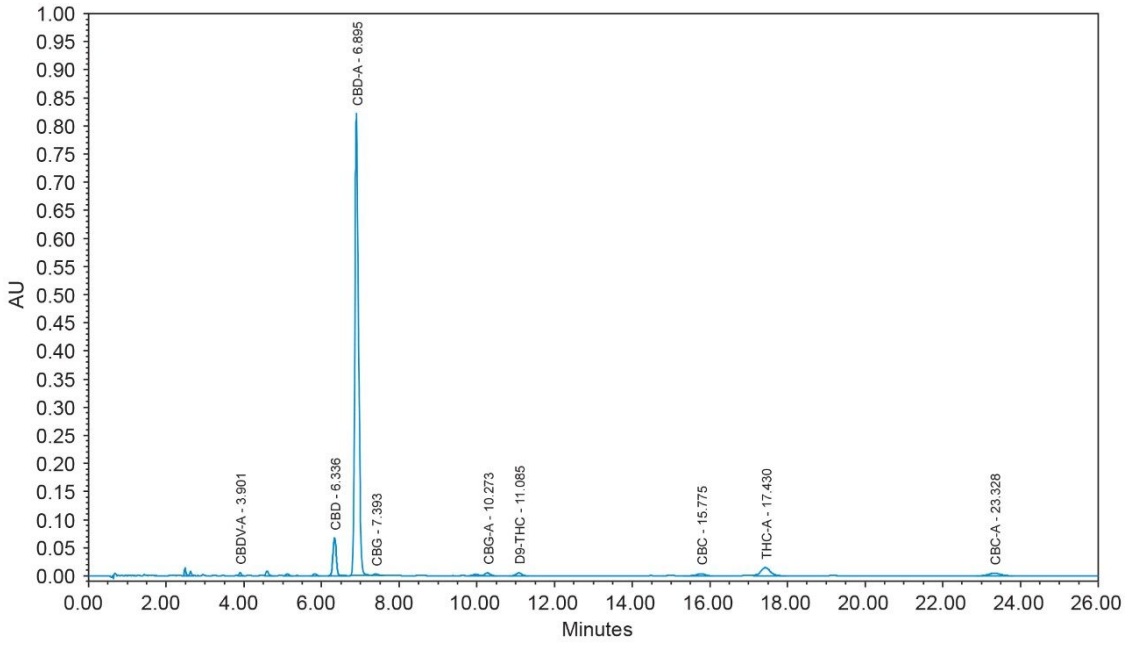

Figure 4. High CBD-A concentrate sample.

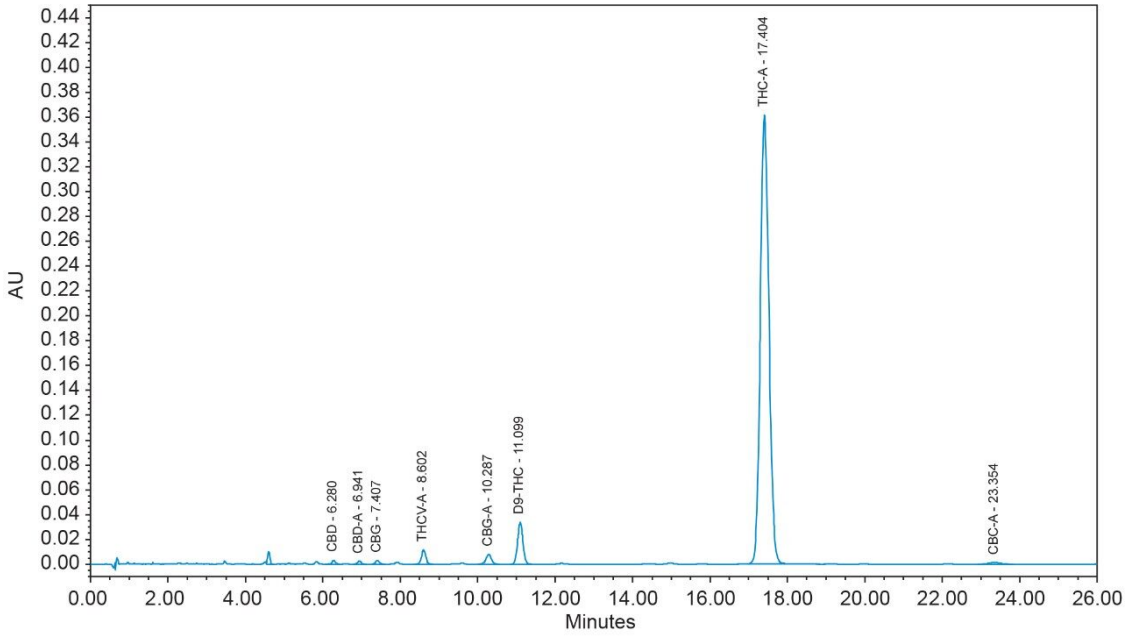

Figure 5. High THC-A concentrate sample.

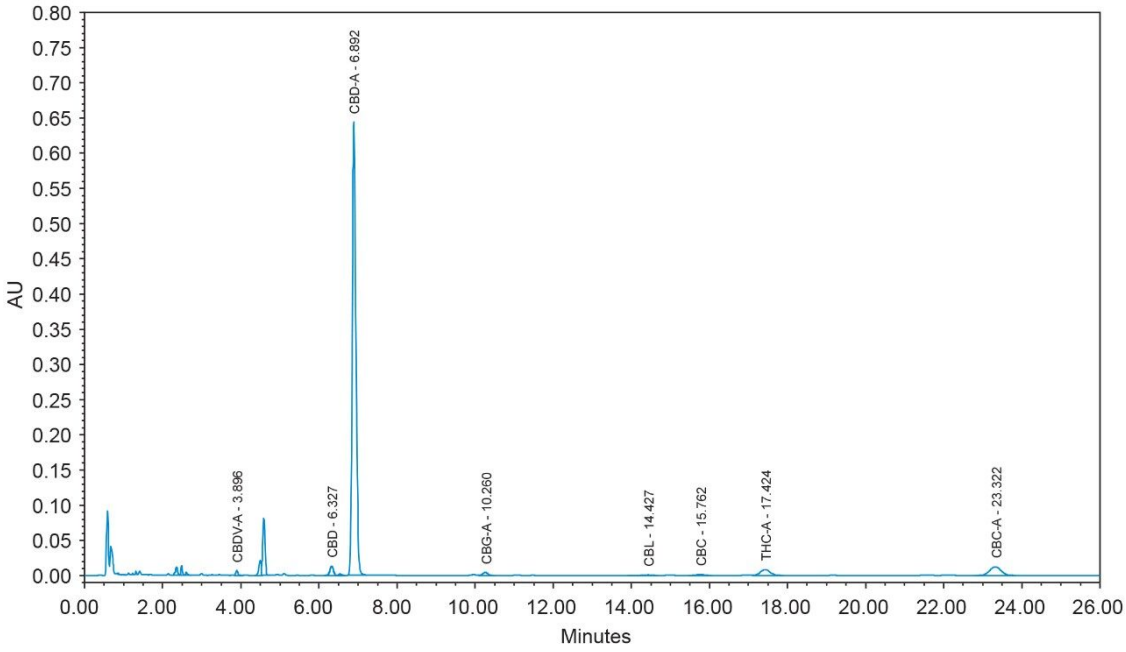

Figure 6. Hemp flower sample.

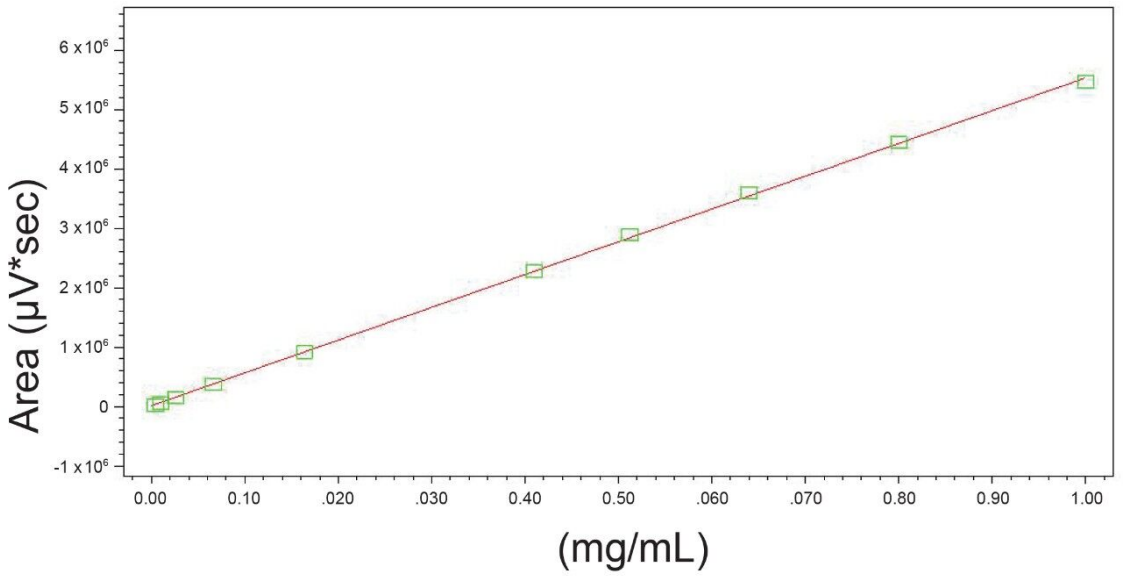

Figure 7. Standard curve for CBD at 10 concentrations between 0.004 mg/mL and 1.000 mg/mL.

Many HPLC methods have been published as the cannabis industry has continued to expand.[4,5,6] These methods are presented using a variety of columns, mobile phase combinations, pH adjustments, and gradient profiles, but more importantly, with only a subset of target cannabinoids. It is possible for any cannabinoid to be present in any cannabis sample. In addition, any cannabis sample can have any cannabinoid as a major or minor constituent; therefore good chromatographic resolution of all cannabinoids of interest is essential. For HPLC methods it is recommended that a resolution of >2 between the peak of interest and the closest potential interfering peak is achieved.[7] To ensure the most accurate and reliable results, every effort should be made to separate as many cannabinoids as possible in a given analysis. Further, methods that are simple, with easy to prepare reagents, reduce operator errors which in turn generates higher quality results. In the method presented here, the mobile phase consists of 0.1% trifluoroacetic acid (TFA) in water and acetonitrile which are mixed using the automated solvent blending capability of the Waters Alliance System.[8] This significantly reduces variability that can happen when manually preparing mixed mobile phases. Detection is accomplished using a Waters 2998 Photodiode Array (PDA) Detector operated in single wavelength mode (228 nm) for simplicity. Even when operated in this mode, full scan PDA data is collected and is available for library matching or peak purity assessment when desired.[9] Separations were achieved using a CORTECS Shield RP18 Column (p/n: 186008685). This solid-core column provides alternative selectivity when compared to typical C_{18} bonded chemistries, especially for phenolic compounds. The instrument was fully controlled and all data were collected and processed using Empower 3 Chromatography Data Software (CDS).

	CBD	CBD-A	CBN	THC	THCA
High CBD-A flower	3.34	11.77	ND	0.35	0.19
High THC-A flower	0.06	0.06	0.10	24.57	15.69
CBD-A concentrate	8.48	65.41	ND	1.19	3.07
THC-A concentrate	0.13	ND	ND	7.58	64.84
Hemp flower	0.28	8.83	ND	ND	0.32

Table 5. Quantitative analysis results of representative samples presented as %dry weight.

ND=Not Detected.

	CBDV	CBDV-A	THCV	CBG	THCV-A	CBG-A	D8-THC	CBL	CBC	CBL-A	CBC-A
High CBD-A flower											
High THC-A flower											
CBD-A concentrate											
THCA concentrate											
Hemp flower											

Table 6. Qualitative analysis results of representative samples. Green shading indicates found, grey shading indicates not found.

Conclusion

- The Alliance HPLC-UV System combined with the CORTECS Shield RP18 Column provided an isocratic separation of 16 cannabinoids in less than 26 minutes.
- All 16 compounds were separated with baseline resolution >2.5.
- Linearity of the five major cannabinoids was shown at 10 concentration levels with R^2 values >0.999.
- This method was demonstrated on five samples representing the most cannabinoid containing sample types. Cannabinoid amounts were generated for those samples.

References

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