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UPLC Separation for the Analysis of Cannabinoid Content in Cannabis Flower and Extracts

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Abstract

This application note presents the transfer of an HPLC separation of 16 cannabinoids to UPLC resulting in a greater than 2-fold increase in productivity while maintaining the linearity, selectivity, and suitability observed in the HPLC separation.

Benefits

The ACQUITY UPLC H-Class System is a highly reliable and robust instrument that works in combination with the CORTECS Column chemistry to provide high throughput analysis of complex cannabinoid mixtures, such as cannabis flower and concentrate.

Introduction

As the legalization of cannabis for both medicinal and recreational use continues to advance, the need for simple, reliable analytical methods for the analysis of these products is desired by many parties (producers, regulators, and consumers).

This application note will present the modification of a simple isocratic HPLC separation of 16 cannabinoids[1] to a separation under UPLC conditions. Conversion of the method to UPLC operation provides a greater than 2 fold increase in testing productivity while maintaining the linearity, selectivity, and suitability observed in the HPLC separation.

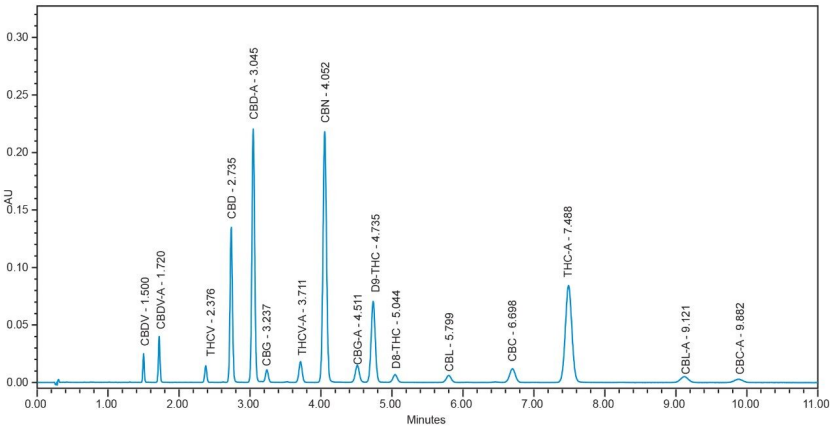

Figure 1. Isocratic separation of 16 cannabinoids (CBD, CBD-A, CBN, (-)Δ^9-THC, THC-A at 0.161 mg/mL) by UPLC. All other cannabinoids at 0.016 mg/mL, 0.7 µL injection, detection at 228 nm.

Experimental

UPLC reversed-phase conditions

LC system:	ACQUITY UPLC H-Class
Analytical column:	CORTECS UPLC Shield RP18, 90 Å, 1.6 µm, 2.1 × 100 mm (p/n: 186008694)
Analytical flow rate:	0.7 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:	ACQUITY UPLC PDA
Detection wavelength:	228 nm at 4.8 nm resolution
Injection volume:	0.7 µL for 1.0 mg/mL reference standard preparations, sample solutions scaled appropriately
Software:	Empower 3 CDS

Solvents, standards, and samples

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 when handled in an appropriate manner.[2,3,4] Table 1 lists the cannabinoids used in this application note.

No.	Cannabinoid	Cannabinoid abbreviation	CAS number
1	Delta-9-tetrahydrocannabinol	(-)Δ9-THC	1972-08-03
2	Delta-9-tetrahydrocannabinolic acid	THC-A	23978-85-0
3	Cannabidiol	CBD	13956-29-1
4	Cannabidiol acid	CBD-A	1244-58-2
5	Cannabinol	CBN	521-35-7
6	Delta-8-tetrahydrocannabinol	(-)Δ8-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.

The HPLC column, flow rate, and injection volume described in the application note, "Separation of 16 Cannabinoids in Cannabis Flower and Extracts Using a Reversed Phase Isocratic HPLC Method"[1] (720006426en) was scaled to UPLC method parameters as described in USP General Chapter <621> titled "System Suitability" for isocratic chromatographic separations.[5] The HPLC method L/d_p ratio (where L is column length and dp is particle diameter) was retained as much as possible, by maintaining the CORTECS Shield RP18, 90 Å particle chemistry and moving from a 2.7 µm 4.6 mm × 150 mm column (p/n: 186008685) to a CORTECS UPLC 1.6 µm particle size with 2.1 mm × 100 mm column (p/n: 186008694) dimensions. The injection volume was scaled proportionally to account for column length and diameter according to the equation for geometric scale-up.[6]

Preparation of standard curves: Linearity of primary cannabinoids (-)Δ^9-THC and CBD were determined for 10 concentrations between 0.004 mg/mL and 1.000 mg/mL, prepared via serial dilution in methanol using the DEA exempt standards as a representative demonstration of method linearity.

Samples: Four representative pre-prepared cannabinoid flower and concentrate samples were obtained from a local testing laboratory in Massachusetts and one sample acquired from a hemp processing laboratory in Vermont. Samples were prepared by the manufacturers as follows: For flower, a portion of homogenized plant material was added to acetonitrile or ethanol and sonicated for 20 minutes. The subsequent extract was filtered through a 0.22 µm syringe tip filter directly into a 2 mL sample vial for analysis. Concentrates were prepared similarly with isopropanol as the extraction solvent.

Results and Discussion

In the method presented, UPLC conditions are utilized to separate 16 cannabinoids in 10.5 minutes using 0.1% trifluoroacetic acid (TFA) in a mixture of water and acetonitrile, under isocratic conditions, combined with a CORTECS UPLC Shield RP18 Column and the ACQUITY UPLC H-Class System (Figure 1). The selectivity observed under HPLC conditions was maintained in the UPLC separation, and the resolution (R_s) of all 16 compounds was >2.0 (Table 3, Figure 2), which meets Rs recommendations for reliable quantitation.[7]

Multi-point calibration curves for two representative cannabinoids (CBD and (-)Δ^9-THC) demonstrated good linearity at R^2 >0.9998. The calibration curve for CBD is presented in Figure 3, as a representative.

Peak #	Name	R. T. (min)	Resolution (R_s)
1	CBDV	1.50	–
2	CDBV-A	1.72	5.3
3	THCV	2.37	13.4
4	CBD	2.73	6.0
5	CBD-A	3.04	4.5
6	CBG	3.23	2.5
7	THCV-A	3.71	5.8
8	CBN	4.05	3.8
9	CBG-A	4.51	4.6
10	(-)Δ^9-THC	4.73	2.1
11	(-)Δ^8-THC	5.04	2.7
12	CBL	5.79	6.2
13	CBC	6.69	6.3
14	THC-A	7.48	4.8
15	CBL-A	9.12	8.6
16	CBC-A	9.88	3.5

Table 2. Retention time and resolution by UPLC.

Parameter	HPLC	UPLC	UPLC savings
Eluent usage	52 mL	7.2 mL	86%
Cycle time	26 min	10.3 min	60%
Sample volume	5 µL	0.7 µL	87%
Sample injections per 24 hrs	55	140	2.5 fold

Table 3. Comparison of the HPLC and UPLC eluent usage, cycle time, sample volume and injections per 24 hrs.

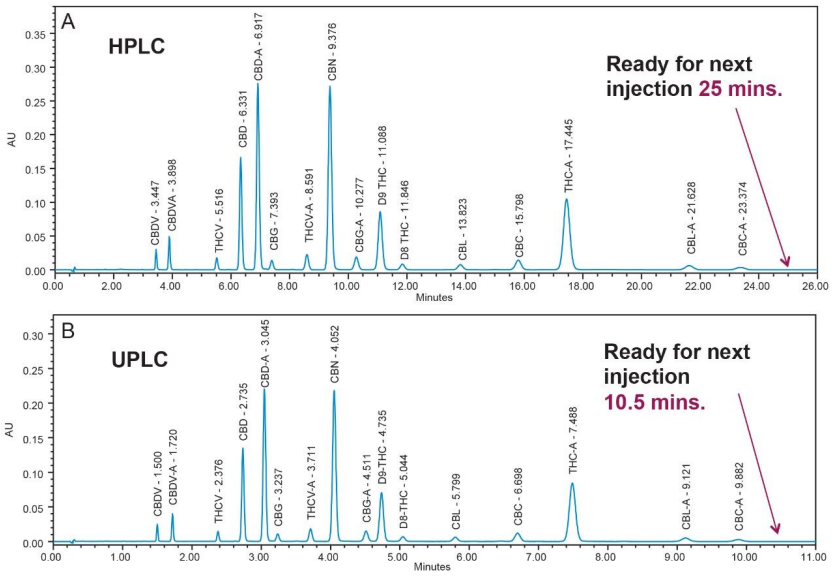

Figure 2. Comparison of selectivity and retention time for the A) HPLC separation scaled to B) UPLC.

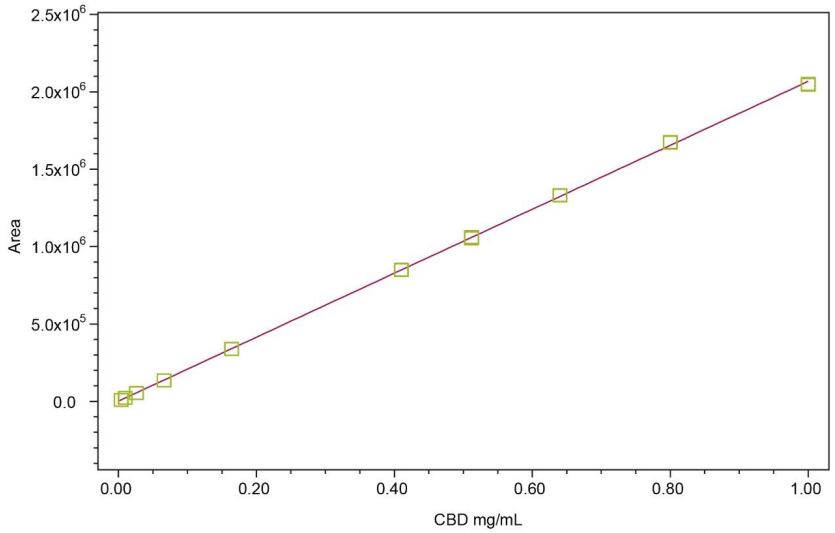

Figure 3. Standard curve for CBD at 10 concentrations between 0.004 mg/mL and 1.000 mg/mL.

Chromatograms for flower and concentrate samples are provided in Figures 4, 5, 6, 7, and 8. The major and minor cannabinoids, as identified by injection of the cannabinoid reference standard solutions, are labeled accordingly.

Cannabinoid separation utilizing UPLC conditions, in combination with the ACQUITY UPLC H-Class System, provide a 2.5 fold increase in the number of samples analyzed per day, plus 85% savings in eluent usage when compared to HPLC (Table 3). Analogous to the HPLC method, the separation employs the CORTECS UPLC Shield RP18 Column under isocratic conditions to further reduce injection cycle time. With the subsequent increase in productivity provided by the conversion to UPLC, the method described can be employed for separation of complex flower and concentrate samples containing major and minor cannabinoids with rapid turn-around time.

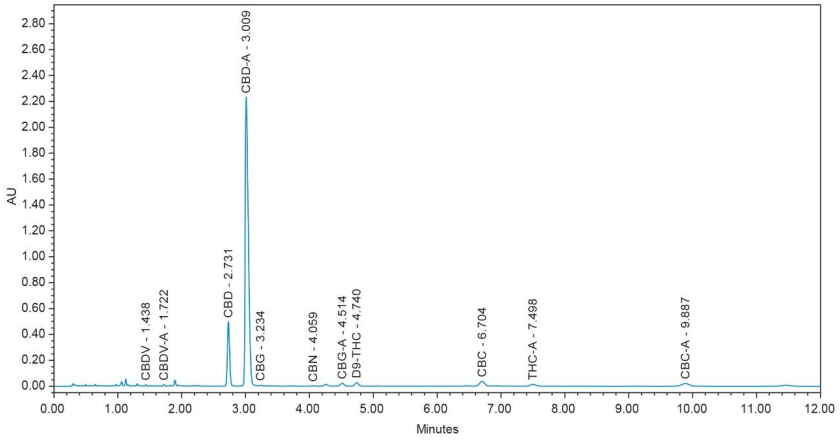

Figure 4. High CBD – A flower sample.

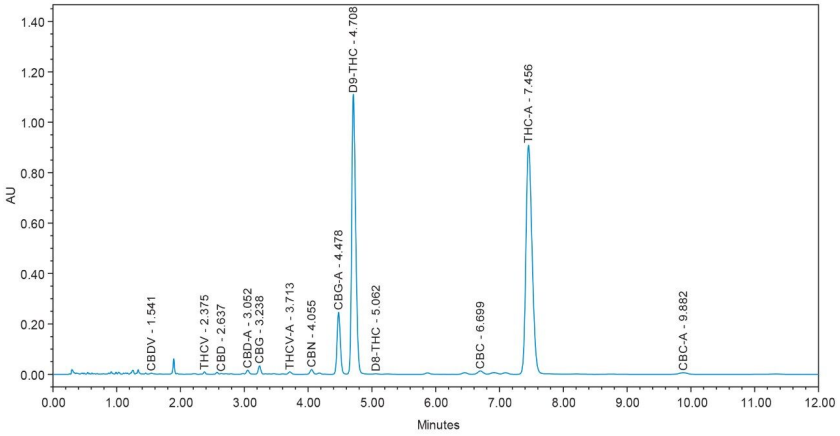

Figure 5. High THC – A flower sample.

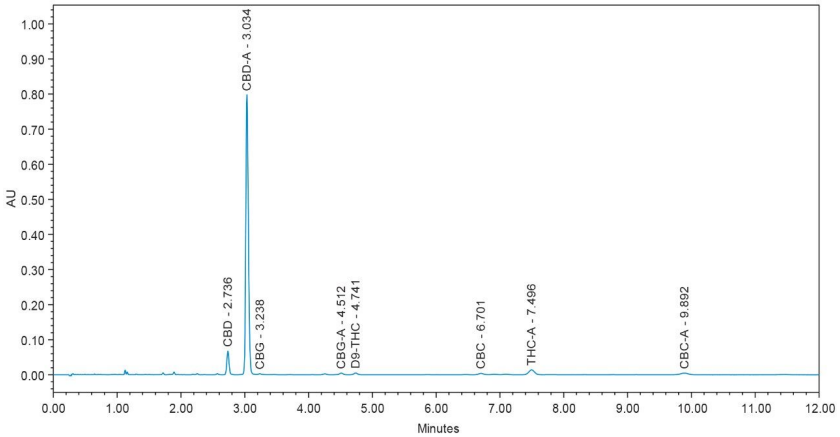

Figure 6. High CBD – A concentrate sample.

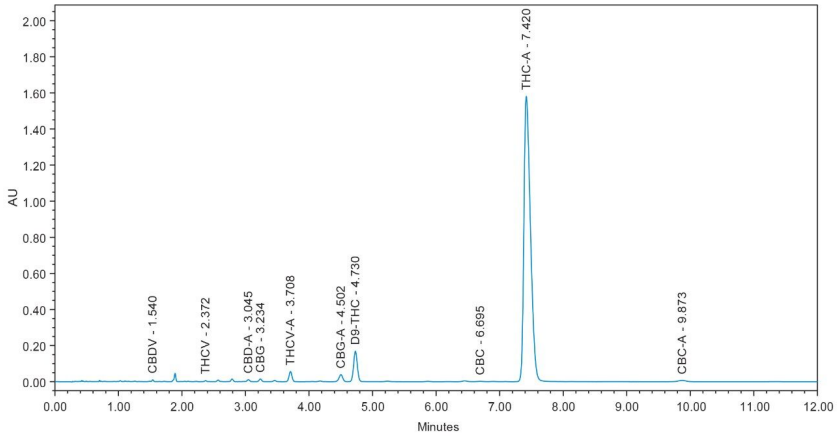

Figure 7. High THC – A concentrate sample.

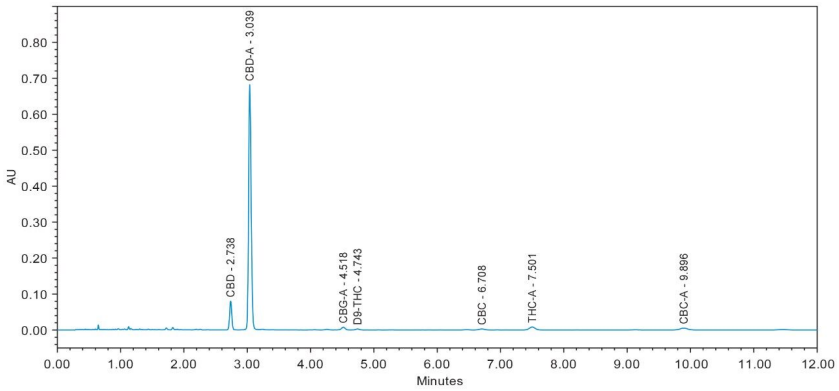

Figure 8. Hemp CBD - A flower sample.

Conclusion

- The ACQUITY UPLC H-Class System combined with the CORTECS UPLC Shield RP18 particle chemistry can be used to provide a UPLC isocratic separation of 16 cannabinoids in a 10.5 minute cycle time.
- A 2.5 fold increase in sample throughput and 86% solvent savings were observed for the UPLC separation compared to HPLC.
- CBD and (-)Δ^9-THC at 10 concentration levels demonstrated linearity with R^2 values $\geqslant$0.999, and the separation of 16 cannabinoids met USP recommended resolution criteria of >2 for accurate quantitation.

- UPLC conditions demonstrated are advantageous for laboratories that require fast turnaround times for the analysis of flower and concentrate samples containing complex cannabinoid mixtures.

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