INCREASING THE THROUGHPUT OF CANNABINOID PROFILING AND POTENCY DETERMINATION USING CHROMATOGRAPHIC GEOMETRIC SCALING PRINCIPLES

Catharine E. Layton and Andrew J. Aubin, Waters Corporation, Milford, MA USA

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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).

The number and diversity of cannabis products moving through the market, has increased demand for the reduction in potency testing result turnaround times. To address the increased demand, testing laboratories have an option to increase productivity by converting HPLC (High-Performance Liquid Chromatography) methods to UPLC (Ultra-Performance Liquid Chromatography) methods.[1]

This poster will present the geometric scaling principles used to transform a simple isocratic HPLC separation of 16 cannabinoids[2] into a UPLC method under US Pharmacopeia (USP) acceptable practices. Conversion to the UPLC method provides a greater than 2 fold increase in testing productivity while maintaining the linearity, selectivity and suitability of the parent HPLC method.

METHOD

UPLC Reversed-Phase Conditions:

Analytical system:	ACQUITY™ UPLC™ H-Class System
Analytical column:	CORTECS™ UPLC Shield RP18 90Å, 1.6µm, 2.1mm x 100mm (part # 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 / B
Oven temperature:	35ºC
ACQUITY UPLC PDA:	Wavelength 228 nm at 4.8 nm res.
Injection volume:	0.7 µL for 1.0 mg/mL reference std preparations, sample solutions scaled appropriately
Software:	Empower 3 Data Software

Solvents, Standards and Samples:

US Drug Enforcement Agency 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.[3,4,5,6,7]

The HPLC column, flow rate and injection volume are described in the application note, "Separation of 16 Cannabinoids in Cannabis Flower and Extracts Using a Reversed Phase Isocratic HPLC Method".[1] For isocratic separations, the particle size and/or the length of the column may be modified for method scaling provided that the ratio of the column length (L) to the particle size (dp) is preserved.[1] The ratio (i.e. linear velocity (L/dp)) was calculated according to Equation 1 for the HPLC method and maintained for the UPLC separation by moving to a 1.6 µm particle size, 2.1mm x 100mm CORTECS Shield RP18 90Å column (Table 1). The HPLC flow rate and injection volume were adjusted accorndingly using the geometric scaling calculations presented in Equation 2 and Equation 3.

Preparation of standard curves: Linearity of major two cannabinoids ($(-)\Delta^9$-THC and CBD were determined for 10 concentrations between 0.004mg/mL and 1.000mg/mL, prepared via serial dilution in methanol using DEA exempt standards.

Equation 1: Linear Velocity

$$Linear\ Velocity = \frac{L}{dp}$$

$$Linear\ Velocity_{HPLC} = \frac{150mm}{2.7\mu m} = 56mm/\mu m$$

Table 1: Comparison of HPLC and UPLC parameters

Mode	CORTECS Shield RP18 Particle Size	Column Length	Linear Velocity
HPLC	2.7 µm	150 mm	56 mm/µm
UPLC	1.6 µm	100 mm	63 mm/µm

Equation 2: Geometric Scaling of Flow Rate

$$Flow_{UPLC} = Flow_{HPLC}\ x\ (\frac{Column\ Diameter_{UPLC}}{Column\ Diameter_{HPLC}})^2$$

$$Flow_{UPLC} = 2.0\ mL/min\ x\ (\frac{2.1\ mm}{4.6\ mm})^2$$

$$Flow_{UPLC} = 0.7\ mL/min$$

Equation 3: Geometric Scaling of Injection Volume

$$Injection\ Volume_{UPLC} = Injection\ Volume_{HPLC}\ x\ (\frac{Column\ Diameter_{UPLC}}{Column\ Diameter_{HPLC}})^2\ x\ (\frac{Column\ Length_{UPLC}}{Column\ Length_{HPLC}})$$

$$Injection\ Volume_{UPLC} = 5\ \mu L\ x\ (\frac{2.1\ mm}{4.6\ mm})^2\ x\ (\frac{100\ mm}{150\ mm})$$

$$Injection\ Volume_{UPLC} = 0.7\ \mu L$$

Samples: Four representative, pre-prepared cannabinoid samples were obtained from a local testing laboratory in Massachusetts and one from a hemp extraction laboratory in Vermont. For flower, a portion of homogenized plant material (Table 2) was added to either acetonitrile or ethanol and sonicated for 20 minutes. The subsequent extract was filtered through a 0.22 micron syringe tip filter directly into a 2 mL sample vial ready for analysis. Concentrates were prepared similarly with isopropanol used 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 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) which meets R_s recommendations for reliable quantitation.[8]

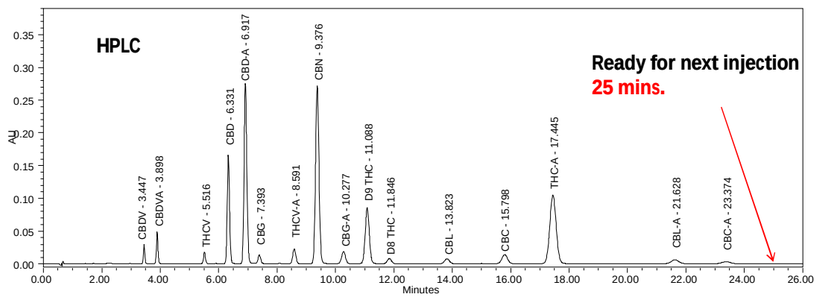

Figure 1: Comparison of the HPLC and UPLC Separation of 16 Cannabinoids

Table 2: UPLC Retention Time and Resolution

Peak #	Name	R.T. (min)	Resolution (Rs)
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	$(-)\Delta^9$-THC	4.73	2.1
11	$(-)\Delta^8$-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

Multi-point calibration curves for two major components (CBD and $(-)\Delta^9$-THC) demonstrated good linearity (R^2 >0.999). Linear regression of the method is demonstrated by the calibration curve for CBD is shown in Figure 2. Overlay chromatograms for flower and concentrate samples are provided in Figures 3 and 4.

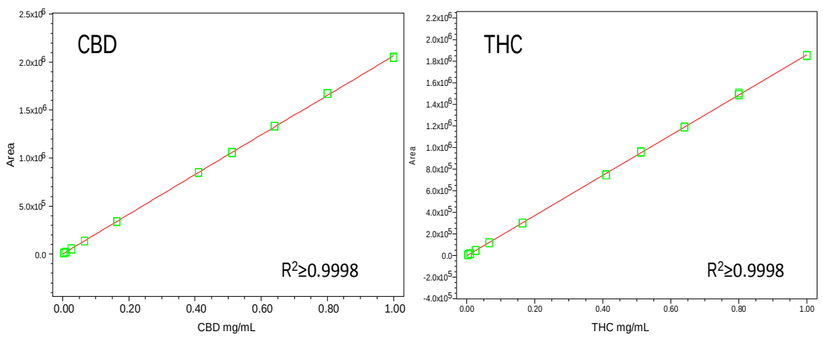

Figure 2: Calibration Curve for CBD and THC for 10 concentrations between 0.004 mg/mL and 1.000mg/mL.

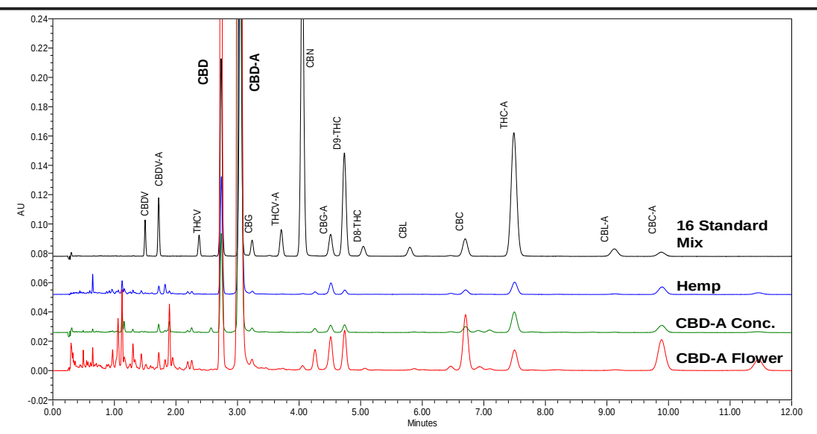

Figure 3: Overlay of the 16 Cannabinoid Mix with High CBD-A Hemp, CBD-A Concentrate and CBD-A flower.

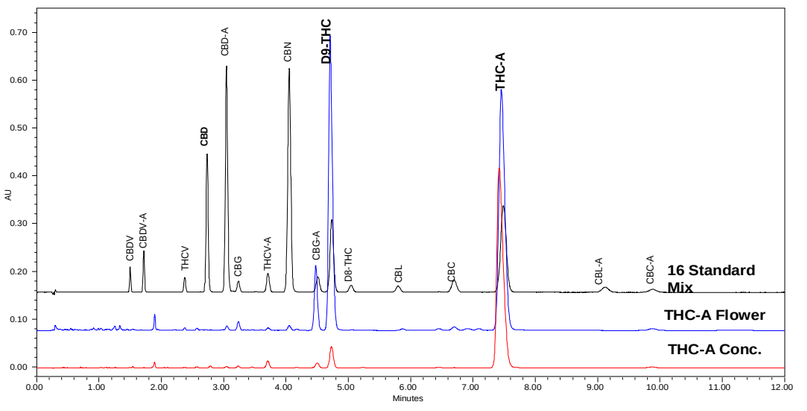

Figure 4: Overlay of the 16 Cannabinoid Mix with High THC Flower and Concentrate

Scaling the cannabinoid separation from HPLC to UPLC conditions, in combination with the ACQUITY H-Class system, provide a 2.5 fold increase in the number of samples analyzed per day, 5 fold decrease in injection volume, plus 85% savings in eluent usage (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 a rapid cycle time.

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

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

CONCLUSIONS

- The ACQUITY H-Class System combined with the CORTECS 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, 5 fold decrease in sample volume, and 86% solvent savings were observed for the UPLC separation compared to HPLC.
- CBD and $(-)\Delta^9$-THC at 10 concentration levels demonstrated linearity with R^2 values ≥0.999, and the separation of 16 cannabinoids met the USP recommended resolution criteria of >2 for accurate quantitation.
- UPLC conditions demonstrated are advantageous for laboratories that require fast turnaround times for analysis of flower and concentrate samples containing complex cannabinoid mixtures.

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