LIQUID CHROMATOGRAPHIC METHOD MODERNIZATION OF AN ISOCRATIC CANNABINOID HIGH PRESSURE LIQUID CHROMATOGRAPHIC SEPARATION

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INTRODUCTION

Cannabis laboratories are commonly pressed to work efficiently while maintaining cost effective analytical techniques. When quantifying cannabinoids in extracts and plant materials, testing most often utilizes HPLC, rather than more expensive, higher pressure UHPLC instrumentation. Modernized HPLCs promote greater flexibility than legacy instrumentation hardware by allowing increased upper backpressure pressure limits. Additionally, this instrumentation can provide improvements in ease-of-use to reduce errors caused by users with less technical training. Instrument modernization results in higher productivity due to faster sample testing turnaround time. This poster will demonstrate the potential increase in testing throughput when using legacy systems versus modernized instrumentation for the HPLC separation of 16 cannabinoids.

METHODS

Instruments:	Alliance™ HPLC System Alliance iS HPLC System
Original Method: 2.7 µm,	CORTECS™ Shield RP18 Column, 4.6 x 150 mm, 2.00 mL/min, 5 µL, UV 220nm
Modernized Method 2.7 µm,	CORTECS Shield RP18 Column, 3.0 x 150 mm, 1.30 mL/min, 2.1 µL, UV 220nm
Mobile Phase A:	0.1% TFA/Water
Mobile Phase B:	Acetonitrile
Flow Conditions:	Isocratic
Software:	Empower™ 3 Chromatography Data System (CDS

Figure 1: The Alliance HPLC System and the Alliance iS HPLC System.

Cannabis and hemp sample solutions comprised of flower and extract were prepared by a cannabis testing laboratory according to the method described in the referenced application note, 720006426EN. A cannabinoid reference standard selectivity mixture was prepared to include the cannabinoids in **Table 1.** Major cannabinoids (CBDA, CBD, THCA, THC, CBN) were prepared at 0.150 mg/mL in methanol, and minor cannabinoids at 0.015 mg/mL by dilution of 1 mg/mL standards with a syringe pipette.

Table 1: Cannabinoid reference standard mixture elution order

Peak #	Cannabinoid Abbreviation	Peak #	Cannabinoid Abbreviation
1	CBDV	9	CBG-A
2	CBDV-A	10	D9-THC
3	THCV	11	D8-THC
4	CBD	12	CBL
5	CBD-A	13	CBC
6	CBG	14	THC-A
7	THCV-A	15	CBL-A
8	CBN	16	CBC-A

RESULTS

The original isocratic separation method operated on the Alliance HPLC System was scaled to the Alliance iS HPLC system using the geometric scaling formulas described in **Figure 2**. Total run time was reduced from 24 minutes to 16 minutes (**Figure 3**), while the flow rate was reduced from 2.00 mL/min to 1.30 mL/min, due to the modernized upper backpressure limit of the Alliance iS HPLC System at 10,000 psi. The integrity of the chromatographic separation was maintained by employing the formulas, as shown by the separation of the cannabinoids in cannabis and hemp, flower and extract sample preparations (**Figure 4, Figure 5**). The reduction in run time and flow rate obtained when using the Alliance iS HPLC System resulted in significant solvent savings (63%) and an increase (45%) in the sample throughput per day.

Original HPLC Method:
4.6 x 150 mm, 2.7 µm
L/d_p = 55,000
Flow rate: 2.000 mL/min

$$F_2 = F_1 x \left[\frac{(d_{c2}^2 \times dp_1)}{(d_{c1}^2 \times dp_2)}\right]$$

Modernized HPLC Method:
3.0 x 150 mm, 2.7 µm
L/d_p = 55,000
Flow rate: 0.860 mL/min

Adjustment range for isocratic methods ± 50% flow rate → 1.30 mL/min ≥ Flow Rate ≥ 0.43 mL/min

F_1 = Monograph flow rate (mL/min)
F_2 = Adjusted flow rate (mL/min)
dc_1 = Internal diameter monograph column (mm)
dc_2 = Internal diameter target column (mm)
dp_1 = Particle size monograph column (µm)
dp_2 = Particle size target column (µm)

Original HPLC Method:
4.6 x 150 mm, 2.7 µm
Inj. Vol.: 5.0 µL

$$V_{inj}2 = V_{inj1} x \left[\frac{(L_2\, dc_2^2)}{(L_1\, dc_1^2)}\right]$$

Modernized HPLC Method:
3.0 x 150 mm, 2.7 µm
Inj. Vol.: 1.4 µL

V_{inj1} = Monograph injection volume (µL)
V_{inj2} = Adjusted injection volume (µL)
L_1 = Length monograph column (mm)
L_2 = Length target column (mm)
dc_1 = Internal diameter monograph column (mm)
dc_2 = Internal diameter target column (mm)

Figure 2: Formulas employed to scale the chromatography from the Alliance HPLC System with the 4.6 x 150 mm column to the Alliance iS HPLC System with a 3.0 x 150 mm column.

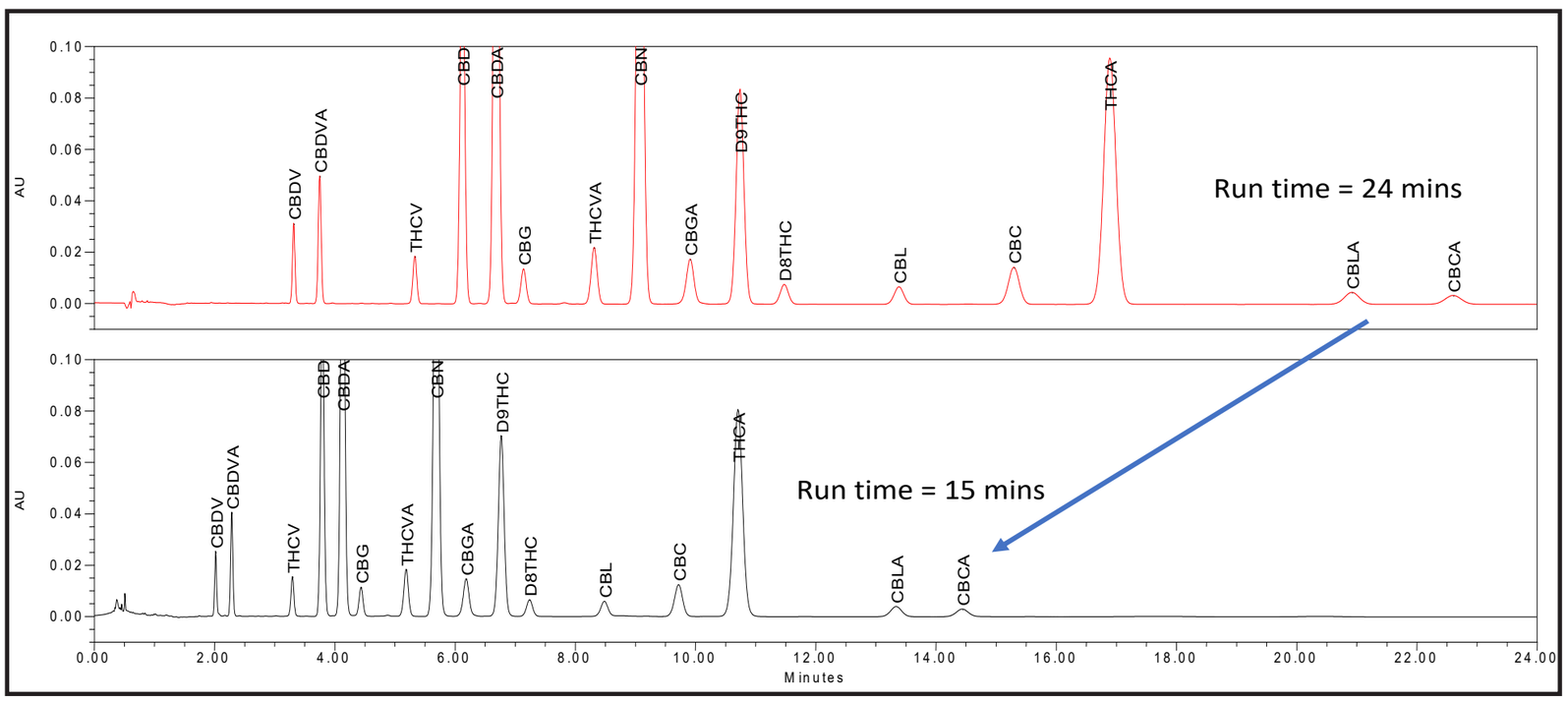

Figure 3: Run time comparison for the Alliance HPLC System method and the modernized HPLC method using the Alliance iS HPLC System.

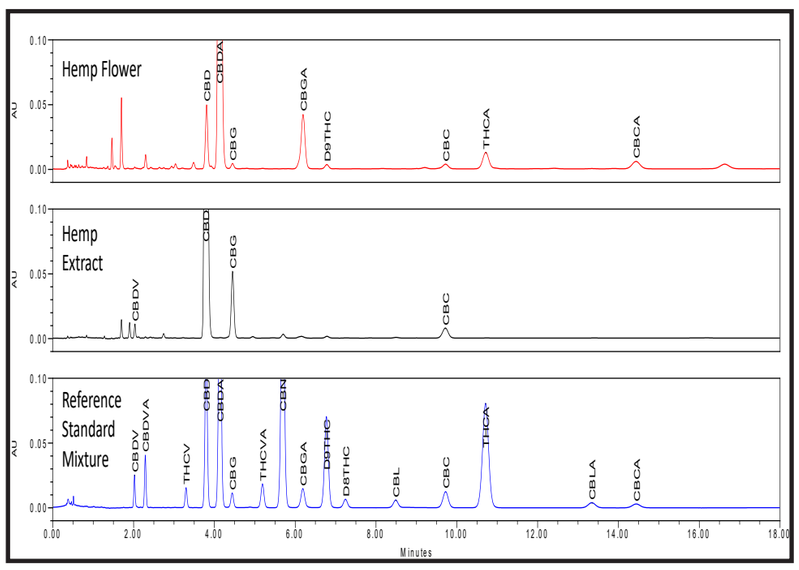

Figure 4: Overlay of hemp flower and hemp extract composed of primarily CBD and CBD-A with the reference standard selectivity mixture.

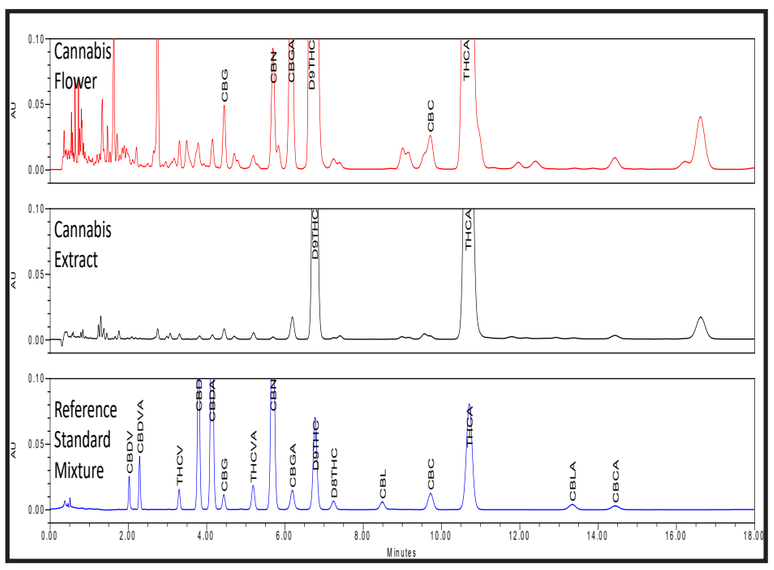

Figure 5: Overlay of cannabis flower and cannabis extract composed of primarily D9-THC and THC-A with the reference standard selectivity mixture.

CONCLUSIONS

Geometric scaling principals can be applied to maintain separation integrity of isocratic cannabinoid HPLC separation methods when scaling to columns of smaller diameter and particle size. Use of columns with smaller diameter as demonstrated and particle size results in less solvent consumption.

These scaling principals can also be utilized to achieve faster run times through utilization of modernized HPLC mode platforms, such as the Alliance iS HPLC System, with a higher upper backpressure limit compared to legacy HPLC platforms. Instrument modernization leads to faster sample testing throughput.

Table 2: Comparison of solvent savings and throughput for the original HPLC method and the modernized Alliance iS HPLC System method.

Parameters	Original HPLC Method	Modernized HPLC Method
HPLC System	Alliance HPLC System	Alliance iS HPLC System
Column Dimensions	4.6 x 150 mm, 2.7 µm	3.0 x 150 mm, 2.7 µm
Sample Run Time per Injection	~26 min	~17 min
Injections per Day	55	85
Solvent Consumption per Injection	2,860 mL	1,806 mL
Savings	-	**63% Reduction in Solvent Consumption** **45% Increase in Testing Throughput per Day**

REFERENCES

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2. **"Separation of 16 Cannabinoids in Cannabis Flower and Extracts Using a Reversed-Phase Isocratic HPLC Method", Andrew J. Aubin, Catharine Layton, and Shawn Helmueller, Application Note, Waters Corporation, 720006426EN, 2018.**
3. **Preparative OBD Column Calculator, www.waters.com, accessed 1/15/2024**
4. **Columns Calculator 2.0, www.waters.com, accessed 1/15/2024**

Special thanks to ProVerde Laboratories, Inc., Milford, MA for cannabis and hemp sample preparations.

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