The Analysis of 21 Cannabinoids by LC-MS

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Abstract & Introduction

The chemical makeup of hemp and cannabis is complex and must be potency tested in order to label products accurately for cannabinoid content. This analysis is typically performed by LC-UV/VIS, as this instrumentation is relatively inexpensive and requires minimal training and maintenance. As cannabis research continues to accelerate, more cannabinoids are likely to be discovered. While LC-UV/VIS is a robust and reliable approach, it has some limitations. LC-UV/VIS is hindered by the need to resolve all analytes and lacks sensitivity when compared to MS. As new cannabinoids are discovered and gain interest in the market, they will likely need to be added into potency testing methods. LC-MS can offer a sensitive and selective analytical solution which enables lower limits of detection and eliminates the need to resolve compounds that are not isobars. In this work, a method was developed for the LC-MS analysis of 21 cannabinoids with a total cycle time of 9 minutes and chromatographic separation of critical pairs.

Newly Discovered Cannabinoids

Two cannabinoids were recently isolated from cannabis and are analogs of THC and CBD.[1,2] These compounds, tetrahydrocannabiphorol (THCP) and cannabidiphorol (CBDP), differ from THC and CBD by the number of carbons on their alkyl side chain. Since THCP has a longer side chain of carbon compounds it has a stronger affinity for CB1 receptor than its THC counterpart, resulting in THCP being 33 times more active than THC.[1]

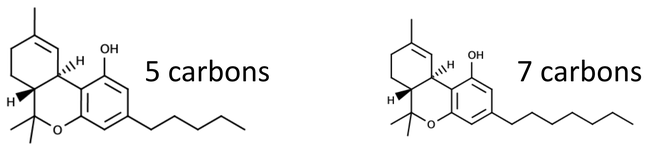

Figure 1: Comparison of the structures of delta-9-tetrahydrocannabinol (THC, left structure) and its analogue tetrahydrocannabiphorol (THCP, right structure) with alkyl chain lengths containing 5 and 7 carbons, respectively.

Isobars

Cannabinoids pose chromatographic challenges, even when an MS detector is utilized. Many isobars are already present within the known, trending cannabinoids and it is likely that these critical pairs will continue to increase as more cannabinoids are discovered and added to routine potency panels. In the 21 cannabinoid panel monitored for these experiments, five groups of isobars exist that all share the same molecular weight and need to be chromatographically separated. Each of the five groups of isobars are outlined below:

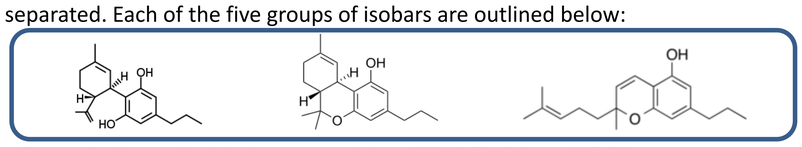

Figure 2: Group 1 of isobars containing CBDV, THCV, and CBCV.

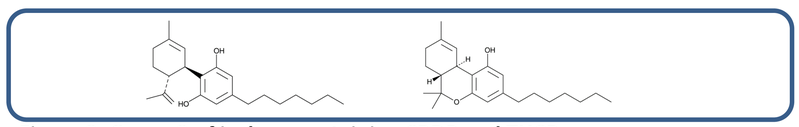

Figure 3: Group 2 of isobars containing CBDP and THCP.

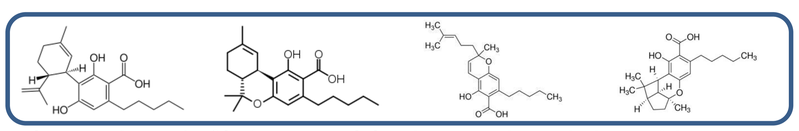

Figure 4: Group 3 of isobars containing CBDA, THCA-A, CBCA, and CBLA.

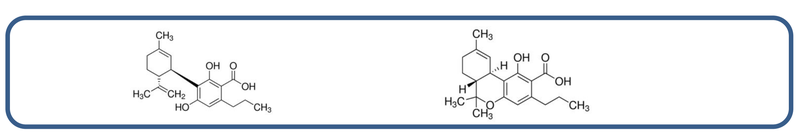

Figure 5: Group 4 of isobars containing CBDVA and THCVA.

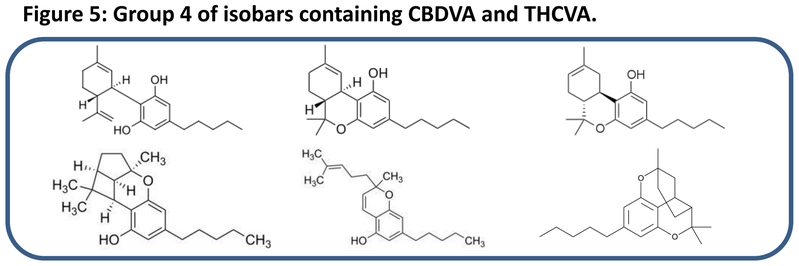

Figure 6: Group 5 of isobars containing CBD, 9-THC, 8-THC (top from left to right) and CBL, CBC, CBT (bottom from left to right).

Method Conditions

	Peaks	Retention Time (min)	Conc. (ng/mL)	SIM	ESI
1.	Cannabidivarin (CBDV)	1.44	500	287.0	+
2.	Cannabidivarinic acid (CBDVA)	1.56	500	331.0	+
3.	Cannabidiol (CBD)	2.14	500	315.0	+
4.	Cannabigerol (CBG)	2.15	500	317.0	+
5.	Cannabidiolic acid (CBDA)	2.26	500	357.0	-
6.	Tetrahydrocannabivarin (THCV)	2.44	500	287.0	+
7.	Cannabigerolic acid (CBGA)	2.70	500	359.0	-
8.	Cannabichromevarin (CBCV)	3.15	500	287.0	+
9.	Cannabinol (CBN)	3.43	500	311.0	+
10.	Cannabidiphorol (CBDP)	3.62	500	343.5	+
11.	Tetrahydrocannabivarinic acid (THCVA)	3.97	500	331.0	+
12.	Δ9-Tetrahydrocannabinol (Δ9-THC)	4.25	500	315.0	+
13.	Δ8-Tetrahydrocannabinol (Δ8-THC)	4.55	500	315.0	+
14.	Cannabinolic acid (CBNA)	4.91	500	353.0	-
15.	Cannabicyclol (CBL)	4.94	500	315.0	+
16.	Cannabichromene (CBC)	5.73	500	315.0	+
17.	Tetrahydrocannabinolic acid A (THCA-A)	7.52	500	357.0	-
18.	Cannabicitran (CBT)	7.55	500	315.0	+
19.	Tetrahydrocannabiphorol (THCP)	8.28	500	343.5	+
20.	Cannabichromenic acid (CBCA)	8.31	500	357.0	-
21.	Cannabicyclolic acid (CBLA)	8.79	500	357.0	-

Table 1. Analyte list with their observed retention time, concentration, SIM, and ESI polarity.

Column:	Raptor ARC-18			
Dimension:	150 mm x 2.1 mm			
Particle Size:	2.7 µm			
Pore Size:	90 A			
Guard Column:	Raptor ARC-18 EXP 5 mm x 2.1 mm ID, 2.7 µm			
Temperature:	30 °C			
Dilutent:	Water:Acetonitrile 20:80			
Concentration:	500 ng/mL			
Injection Volume:	2 uL			
Mobile Phase:				
A:	Water, 0.1% formic acid, 12 mM ammonium formate			
B:	Acetonitrile:methanol (50:50), 0.1% formic acid			
	Time (min)	**Flow (mL/min)**	**%A**	**%B**
	0	0.5	20	80
	9	0.5	20	80
Detector:	MS			
Acquisition Type:	SIM			
Interface:	ESI +/-			
Instrument:	UHPLC			

Table 2. Method conditions for the analysis of 21 cannabinoids by LC-MS.

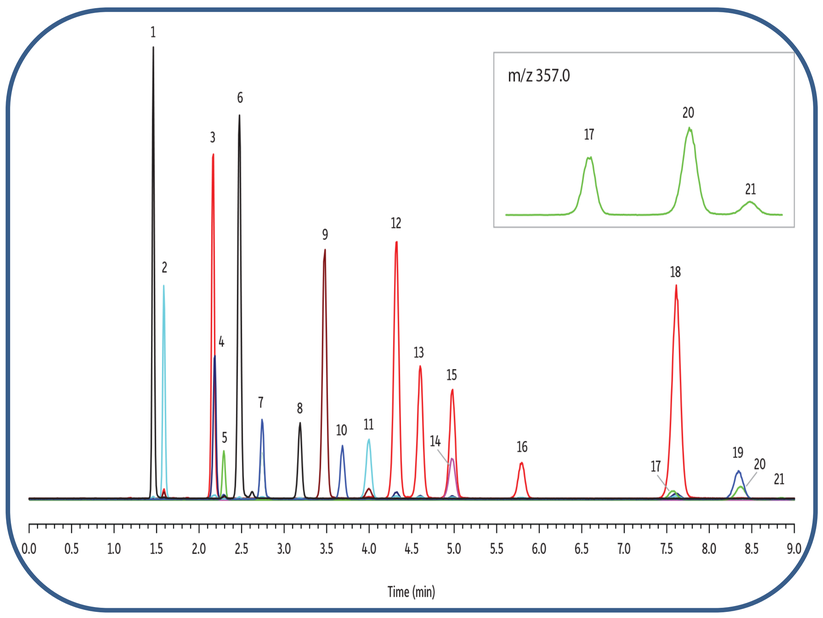

Figure 7. Chromatogram for the detection of 21 cannabinoids in solvent by LC-MS.

Isobar Separation

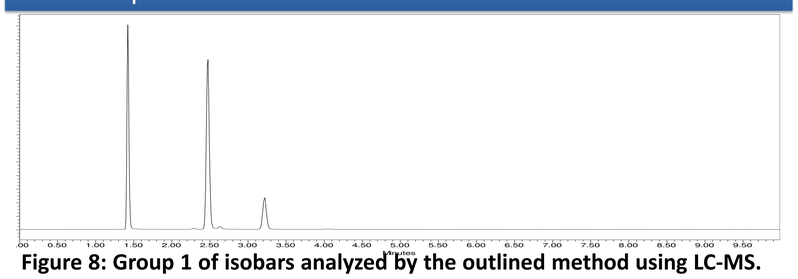

Figure 8: Group 1 of isobars analyzed by the outlined method using LC-MS. Analyte order: CBDV, THCV, and CBCV detected using *m/z* 287.0.

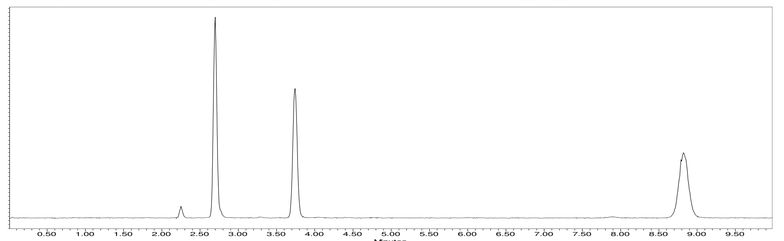

Figure 9: Group 2 of isobars analyzed by the outlined method using LC-MS. Analyte order: CBDP (second peak) and THCP (third peak) detected using *m/z* of 343.5.

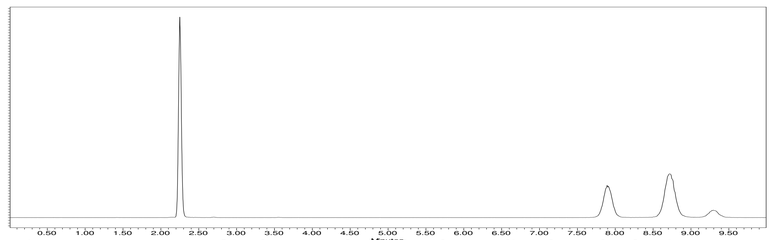

Figure 10: Group 3 of isobars analyzed by the outlined method using LC-MS. Analyte order: CBDA, THCA-A, CBCA, and CBLA detected using *m/z* of 357.0.

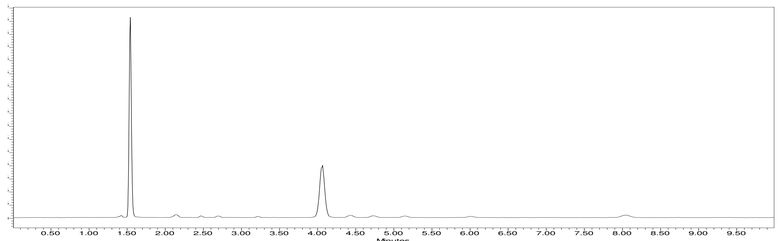

Figure 11: Group 4 of isobars analyzed by the outlined method using LC-MS. Analyte order: CBDVA and THCVA detected using *m/z* of 331.0.

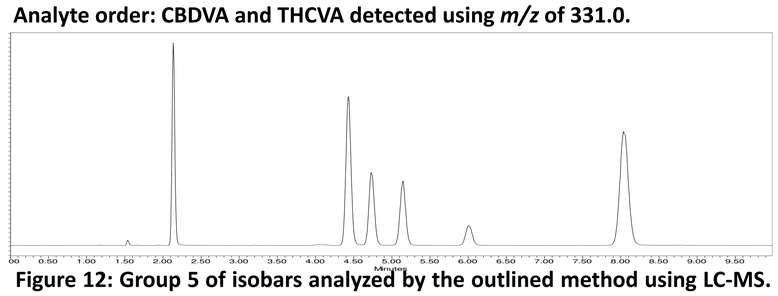

Figure 12: Group 5 of isobars analyzed by the outlined method using LC-MS. Analyte order: CBD, 9-THC, 8-THC, CBL, CBC, and CBT detected using *m/z* of 315.0.

Conclusions

- Potency testing cannabis and hemp products is becoming more challenging by LC-UV detection as the analyte list of cannabinoids continues to grow.
- LC-MS is able to overcome these challenges by eliminating the need to resolve all compounds, as only isobar separation is required.
- Herein, an LC-MS method was developed using a Raptor ARC-18 150 x 2.1 mm, 2.7 µm analytical column that achieves baseline separation for all isobars.
- 21 cannabinoids were able to be detected using isocratic mobile phase conditions with a 9 minute cycle time, allowing for high throughput of samples.
- Additional cannabinoids can be added to the analyte list as more are discovered with optimization only required for compounds that are isobars.

References

1. Linciano, P., et al. Journal of Natural Products 2020 83 (1), 88-98.
2. Citti, C., et al. Data in Brief 2019 26, 1-16.