Creating Custom *Cannabis* CRM Mixtures: Forty-seven Phytocannabinoids, One HPLC Method

Roxanne Franckowski, Marc Gregerson, PhD, Kathleen Calati, and Kirk W. Hering, PhD

Cayman Chemical Company, 1180 E. Ellsworth Road, Ann Arbor, MI 48108 USA

KEY FINDING Custom phytocannabinoid CRM mixtures require a robust method, consideration of coeluting components, and stability considerations.

INTRODUCTION

Production of certified reference materials (CRMs) is prescribed by the conformity assessment standard ISO 17034:2016. During production planning, the reference material producer shall address the "validation of measurement procedures" and assessment of stability, among other criteria.[1,2] Creating multicomponent mixtures can present arduous challenges to ensure these criteria are met. To validate a method for the characterization of the multicomponent CRM, the analytes of interest must be resolved. The final CRM solutions must have stability which is fit for the intended purpose of the end user.

To increase efficiency and reduce the necessity of developing individual methods for independent mixtures, a general method has been developed to separate several phytocannabinoid compounds. This poster will address the challenges of separating 47 (12 acid and 35 neutral) phytocannabinoid compounds for method validation and the considerations for the stability of the final CRM solutions.

METHOD

Final Method Conditions			
Instrument	Shimadzu Nexera LC-40 with photodiode array detector		
Column	Millipore Ascentis® Express C18, 150 x 3 mm, 2.7 µm		
Mobile Phase A	70:30 ACN: H_2O with 0.1% HCO_2H and 5 mM NH_4HCO_2	Column Temp	25°C
Mobile Phase B	CH_3OH with 0.1% HCO_2H	Sample Temp	4°C
Flow Rate	0.5 ml/min	Injection Volume	4 µl
Gradient	Time (min)	%A	%B
	0	100	0
	5.5	100	0
	19.5	15	85
	21	15	85
	21.1	100	0
Run Time	24 min	Linear Range	0.05 - 1.2 mg/ml
Wavelength (nm)	Acids 305	Neutrals	275

Table 1 – Final Method Conditions.

Abbrev.	Name
CBCO	(±)-Cannabichromeorcin
CBCV	(±)-Cannabichromevarin
CBCVA	(±)-Cannabichromevarinic Acid
CBC	(±)-Cannabichromene
CBCA	(±)-Cannabichromenic Acid
CBT	Cannabicitran
CBDO	Cannabidiorcin
CBDE	Cannabidiethanol
CBDV	Cannabidivarin
CBDVA	Cannabidivarinic Acid
CBDB	Cannabidibutol
CBD	Cannabidiol
CBDA	Cannabidiolic Acid
CBDH	Cannabidihexol
CBDP	Cannabidiphorol
CBDPA	Cannabidiphorolic Acid
CBD-C8	Cannabidiol-C8
CBE	Cannabielsoin
CBGV	Cannabigerovarin
CBGVA	Cannabigerovarinic Acid
CBG	Cannabigerol
CBGA	Cannabigerolic Acid
CBL	(±)-Cannabicyclol
CBLA	(±)-Cannabicyclolic Acid
CBN	Cannabinol
CBNA	Cannabinolic Acid
9(R)-HHC	9(R)-Hexahydrocannabinol
9(S)-HHC	9(S)-Hexahydrocannabinol
9(R)-$\Delta^{6a,10a}$-THC	9(R)-$\Delta^{6a,10a}$-Tetrahydrocannabinol
9(S)-$\Delta^{6a,10a}$-THC	9(S)-$\Delta^{6a,10a}$-Tetrahydrocannabinol
9(R)-Δ^{7}-THC	9(R)-Δ^{7}-Tetrahydrocannabinol
9(S)-Δ^{7}-THC	9(S)-Δ^{7}-Tetrahydrocannabinol
Δ^{8}-THC	Δ^{8}-Tetrahydrocannabinol
Δ^{8}-THC-C8	Δ^{8}-Tetrahydrocannabinol-C8
Δ^{8}-THC Acetate	Δ^{8}-Tetrahydrocannabinol Acetate
THCV	Δ^{9}-Tetrahydrocannabivarin
THCVA	Δ^{9}-Tetrahydrocannabivarinic Acid
Δ^{9}-THCB	Δ^{9}-Tetrahydrocannabibutol
Δ^{9}-THC	Δ^{9}-Tetrahydrocannabinol
THCA-A	Δ^{9}-Tetrahydrocannabinolic Acid A
THCH	Δ^{9}-Tetrahydrocannabihexol
THCP	Δ^{9}-Tetrahydrocannabiphorol
Δ^{9}-THCPA	Δ^{9}-Tetrahydrocannabiphorolic Acid
Δ^{9}-THC-C8	Δ^{9}-Tetrahydrocannabinol-C8
(6aR,9R)-Δ^{10}-THC	(6aR,9R)-Δ^{10}-Tetrahydrocannabinol
(6aR,9S)-Δ^{10}-THC	(6aR,9S)-Δ^{10}-Tetrahydrocannabinol
exo-THC	exo-Tetrahydrocannabinol

Table 2 – Abbreviations.

CHROMATOGRAPHY

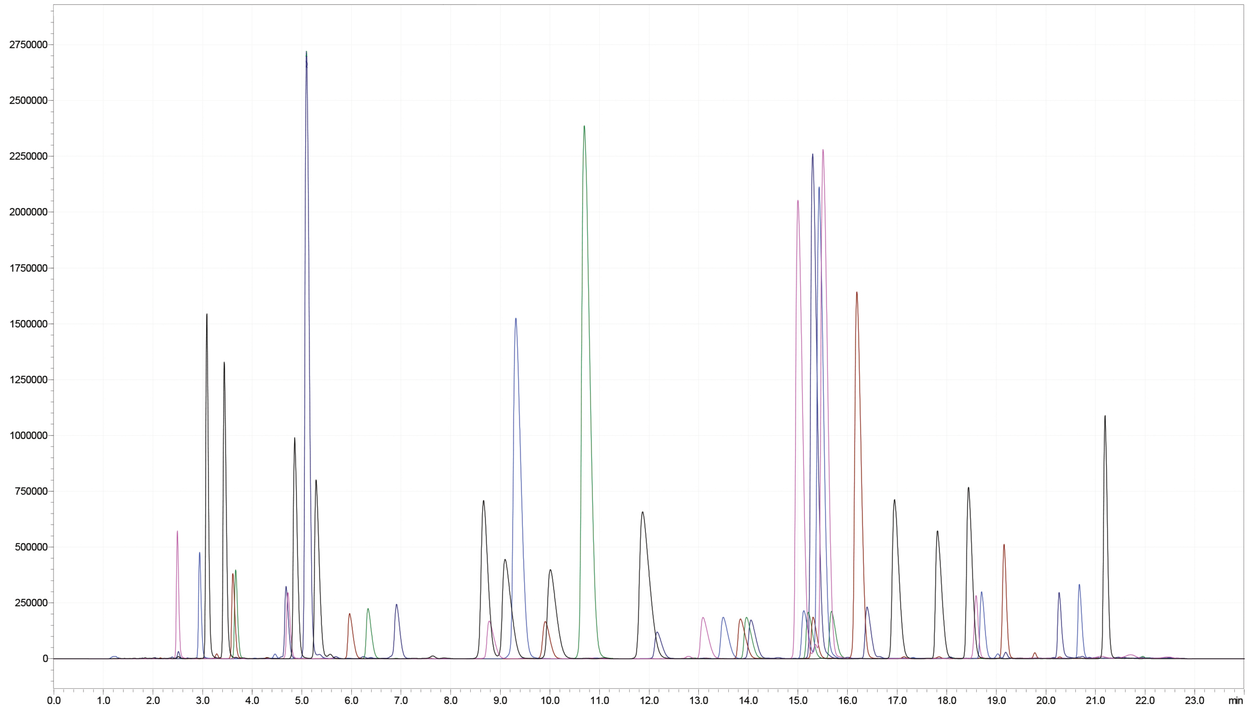

Figure 1 – Overlay chromatogram. This chromatogram displays resolution of all 47 analytes. There were six groups of co-eluting analytes identified.

Analyte Retention Time											
Analyte	RT (min)	Analyte	RT (min)	Analyte	RT (min)	Analyte	RT (min)	Analyte	RT (min)	Analyte	RT (min)
CBDO	2.49	CBDA	4.92	CBDPA	9.29	Δ^{9}-THC	13.50	CBD-C8	15.31	CBLA	18.53
CBDE	2.94	CBCO	5.10	CBCV	9.31	9(R)-Δ^{7}-THC	13.84	9(R)-$\Delta^{6a,10a}$-THC	15.43	CBT	18.59
CBDVA	3.11	CBGA	5.37	Δ^{9}-THCB	9.91	Δ^{8}-THC	13.97	(6aR,9R)-Δ^{10}-THC	15.51	THCP	18.70
CBGVA	3.48	CBG	5.97	CBCVA	10.18	9(S)-Δ^{7}-THC	14.06	9(R)-HHC	15.68	Δ^{8}-THC Acetate	19.16
CBGV	3.61	CBD	6.34	CBN	10.70	(6aR,9S)-Δ^{10}-THC	15.00	CBC	16.19	Δ^{8}-THC-C8	20.27
CBDV	3.67	THCV	6.91	CBNA	12.06	CBL	15.12	THCH	16.40	Δ^{8}-THC-C8	20.67
CBDB	4.68	CBDH	8.78	CBDP	12.16	9(S)-HHC	15.21	THCA-A	17.05	Δ^{9}-THCPA	21.24
CBE	4.72	THCVA	8.80	exo-THC	13.09	9(S)-$\Delta^{6a,10a}$-THC	15.30	CBCA	17.94		

Table 3 – Analyte Retention Time. The six groups of co-eluting analytes are grouped by color in the table. Method validation for the neutral phytocannabinoid compounds was accomplished by separating co-eluting analytes into five different solutions.

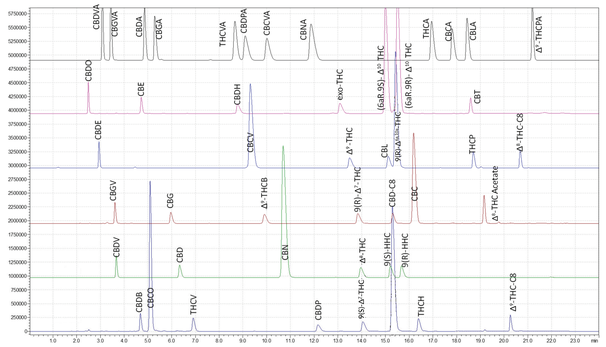

Figure 2 – Stacked Chromatogram. This figure represents the composition of the independent phytocannabinoid acid and neutral solutions used to validate the HPLC method. Co-eluting compounds were separated among the mixtures.

DISCUSSION

The goal was to create a quick turn method which included several dozen phytocannabinoid compounds. The resulting method allows for timely production of customized multicomponent phytocannabinoid CRMs.

Upon development, notable co-elutions were observed early in the run between CBGV and CBDV, CBDB and CBE, and later between the HHC and Δ^{10}-THC isomers. The method was validated by placing the neutral phytocannabinoids in five (5) groups to separate co-eluting compounds.

Based on experience of producing multicomponent phytocannabinoid mixtures, the phytocannabinoid acids were separated from the neutral compounds to promote enhanced stability of the final multicomponent CRM. The diluent for the multicomponent phytocannabinoid acid solutions is stabilized with DIPEA and ascorbic acid.

CONCLUSIONS

- Cayman has validated a method which allows for quick turn production of custom phytocannabinoid CRMs.
- The co-eluting neutral compounds have been identified which will aid in timely production planning of multicomponent CRM solutions.
- This method meets the ISO 17034:2016 requirements of the use of a validated method for characterization and considerations of stability of the final CRM solutions.

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References

1. International Organization for Standardization. (2016) *General requirements for the competence of reference material producers* (ISO Standard No. 17034:2016). https://www.iso.org/standard/29357.html
2. International Organization for Standardization. (2017) *General requirements for the competence of testing and calibration laboratories* (ISO Standard No. 17025:2017). https://www.iso.org/standard/66912.html