Separation of 11-Hydroxy-THC Metabolites and Quantitation of 18 Total Cannabinoids in Whole Blood by UHPLC-MS/MS

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INTRODUCTION

Despite changes in legal status of medical and recreational Δ9-Tetrahydrocannabinol (Δ9-THC), employer restrictions and regional accessibility continue to reinforce the popularity of Δ8-Tetrahydrocannabinol (Δ8-THC) among cannabis users. While toxicology testing for Δ9- and Δ8-Carboxy-THC-metabolites has become more routine, in many cases the determination of the psychoactive metabolites, 11-Hydroxy-Δ9-THC and 11-Hydroxy-Δ8-THC, is of higher importance. The method developed by our laboratory provides a detailed analysis of whole blood specimens, separating Δ9- and Δ8-11-Hydroxy-THC-metabolites, and quantitatively evaluating 18 cannabinoids at concentrations from 0.500-200 ng/mL.

OBJECTIVE

Develop an analytical method for the extraction, detection, and quantitation of (-)-Δ9-THC, (-)-Δ8-THC, 11-Hydroxy-Δ9-THC (11-OH-Δ9-THC), 11-Hydroxy-Δ8-THC (11-OH-Δ8-THC), Δ9-Carboxy-THC (Δ9-COOH-THC), Δ8-Carboxy-THC (Δ8-COOH-THC), Δ9-Tetrahydrocannabivarin (Δ9-THCV), Δ8-Tetrahydrocannabivarin (Δ8-THCV), Δ9-Carboxy-Tetrahydrocannabivarin (Δ9-COOH-THCV), Δ8-Carboxy-Tetrahydrocannabivarin (Δ8-COOH-THCV), Cannabidiol (CBD), 7-Carboxy-Cannabidiol (7-COOH-CBD), 7-Hydroxy-Cannabidiol (7-OH-CBD), Cannabidiolic Acid (CBDA), Cannabinol (CBN), Cannabigerol (CBG), Cannabicyclol (CBL), and Cannabichromene (CBC) in whole blood by LC-MS/MS for a controlled-dosing research study.

EXTRACTION METHOD

Specimens were prepared by mixing a 0.200 mL aliquot of whole blood sample with 20 µL of internal standard solution and 0.1M Sodium Phosphate in appropriately labeled silanized glass culture tubes. While vortexing, 500 µL of ice-cold Acetonitrile was added to each tube. Samples were then centrifuged and the supernatant was decanted from the blood protein pellet. A liquid-liquid extraction was performed using this supernatant and 9:1 Hexanes: Ethyl Acetate, and the organic components were subsequently dried and reconstituted with 0.1% Formic Acid in 50:50 DI H2O: Methanol.

INSTRUMENT PARAMETERS

Table 1: UHPLC-MS/MS Parameters

UHPLC System	Shimadzu Nexera	LC-40D X3 Pumps
		SIL-40C X3 Auto Sampler
		SCL-40 System Controller
		CTO-40C Column Oven
		DGU-405 Degassing Unit
Injection Volume	25 µL	
Analytical Column	(2) Waters CORTECS C18+, 90Å, 2.7 µm, 2.1 mm x 150 mm (Waters Part No. 186007398)	
Guard Column	Waters CORTECS C18+ VanGuard, 90Å, 2.7 µm, 2.1 mm x 5 mm (Waters Part No. 186007685)	
Column Temp.	40°C	
Mobile Phase	Aqueous: 0.1% Acetic Acid in DI H2O	
	Organic: 0.1% Acetic Acid in Acetonitrile	
Flow Rate	0.700 mL/min	
Run Time	27.00 minutes	
Mass Spectrometer	Sciex API7500 Triple Quad	
Ionization	ESI	Positive and Negative
Source Temp.	550°C	
Scheduled MRM	120-240-sec detection window	
	Target cycle time	1000 milliseconds

Positive Ionization

Analyte	Internal Standard	Precursor Ion	Product Ion Quantifier	Product Ion Qualifier	Retention Time (± 0.8 mins)
Δ9-THCV	THCV-D5	287.2	165.1	123.0	19.90
THCV-D5		292.2	170.1	123.1	19.84
Δ8-THCV	THCV-D5	287.2	165.1	123.0	20.16
CBD	CBD-D3	315.2	193.1	135.0	20.31
CBD-D3		318.2	196.1	135.0	20.28
CBN	CBN-D3	311.2	208.0	223.0	22.24
CBN-D3		314.2	208.0	195.0	22.19
(-)-Δ9-THC	(-)-Δ9-THC-D3	315.2	193.1	123.0	23.66
(-)-Δ9-THC-D3		318.2	196.0	123.0	23.60
(-)-Δ8-THC	(-)-Δ8-THC-D9	315.2	193.1	123.0	24.08
(-)-Δ8-THC-D9		324.2	202.1	123.0	23.91

Negative Ionization

Analyte	Internal Standard	Precursor Ion	Product Ion Quantifier	Product Ion Qualifier	Retention Time (± 0.8 minutes)
7-COOH-CBD	7-COOH-CBD-D3	343.1	297.1	231.1	5.17
7-COOH-CBD-D3		346.2	300.2	234.2	5.13
Δ8-COOH-THCV	7-COOH-CBD-D3	315.2	271.1	163.0	5.51
7-OH-CBD	7-OH-CBD-D3	329.2	299.2	268.1	5.66
7-OH-CBD-D3		332.2	302.2	271.1	5.61
Δ9-COOH-THCV	7-COOH-CBD-D3	315.2	271.1	163.0	5.92
11-OH-Δ9-THC	11-OH-Δ9-THC-D3	329.2	173.0	268.1	13.48
11-OH-Δ9-THC-D3		332.2	173.0	271.1	13.34
11-OH-Δ8-THC	11-OH-Δ8-THC-D3	329.2	173.0	268.1	13.91
11-OH-Δ8-THC-D3		332.2	173.0	271.1	13.76
Δ8-COOH-THC	Δ8-COOH-THC-D6	343.1	245.1	191.1	14.50
Δ8-COOH-THC-D6		349.2	251.1	191.1	14.14
Δ9-COOH-THC	Δ9-COOH-THC-D9	343.1	299.1	245.1	15.54
Δ9-COOH-THC-D9		352.2	308.1	254.2	14.98
CBG	CBG-D3	315.0	191.1	136.0	20.17
CBG-D3		318.0	194.0	136.0	20.14
CBDA	CBDA-D3	357.2	245.1	179.0	20.74
CBDA-D3		360.2	248.1	182.0	20.71
CBL	CBC-D9	313.2	191.0	203.0	24.66
CBC	CBC-D9	313.2	191.0	203.0	25.17
CBC-D9		322.3	200	212	25.06

UHPLC Mobile Phase Gradient

Time	Aqueous (%)	Organic (%)
0.00	52.0	48.0
0.50	52.0	48.0
16.75	52.0	48.0
17.00	30.0	70.0
19.50	28.0	72.0
23.00	28.0	72.0
23.25	20.0	80.0
25.00	20.0	80.0
25.01	52.0	48.0
27.00	STOP	

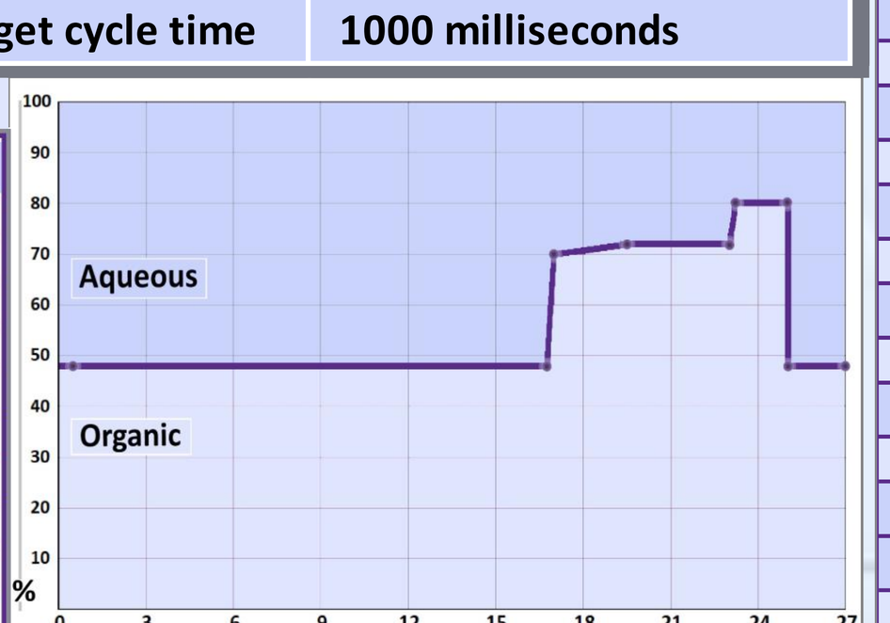
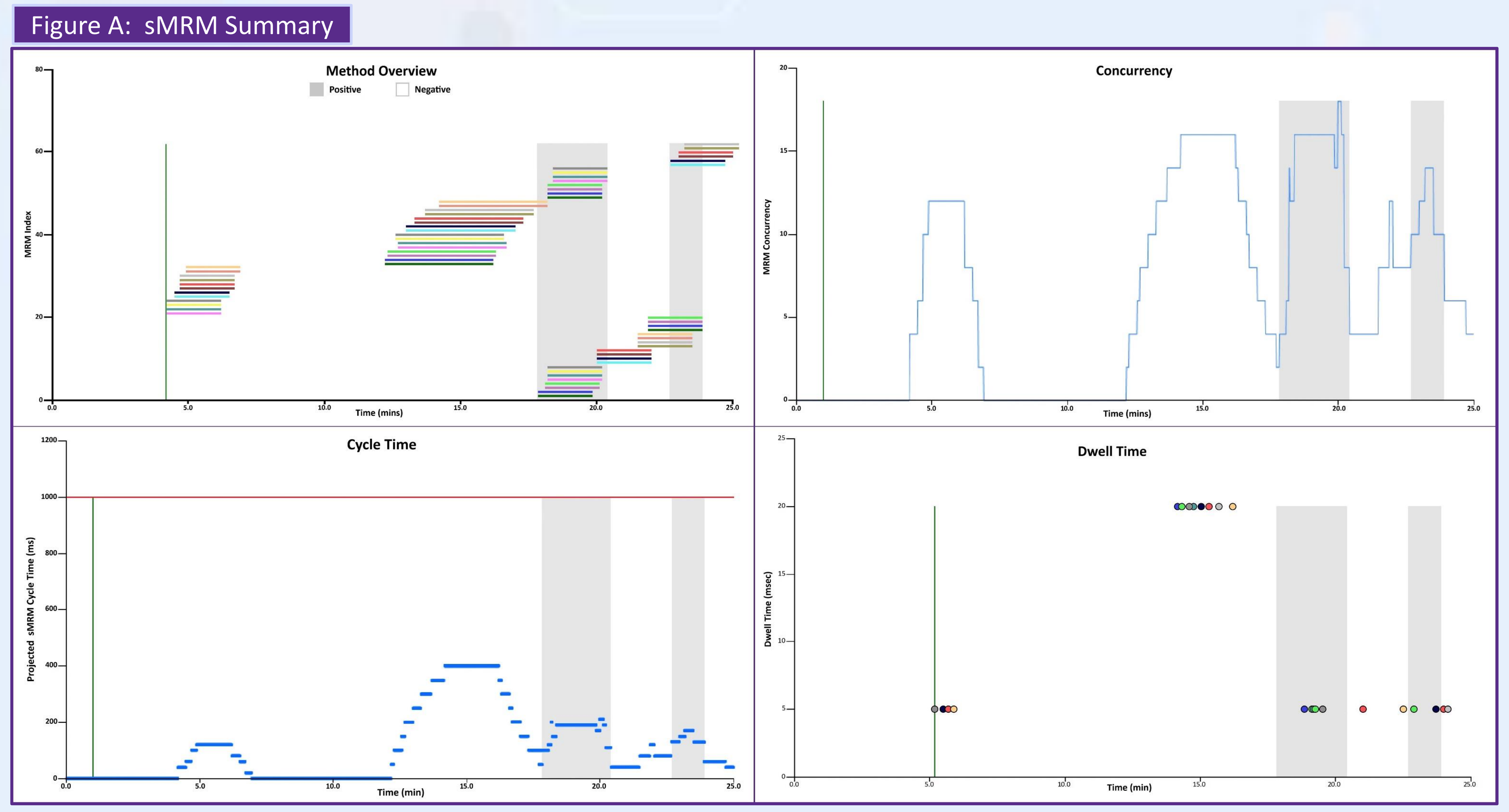


Figure A: sMRM Summary

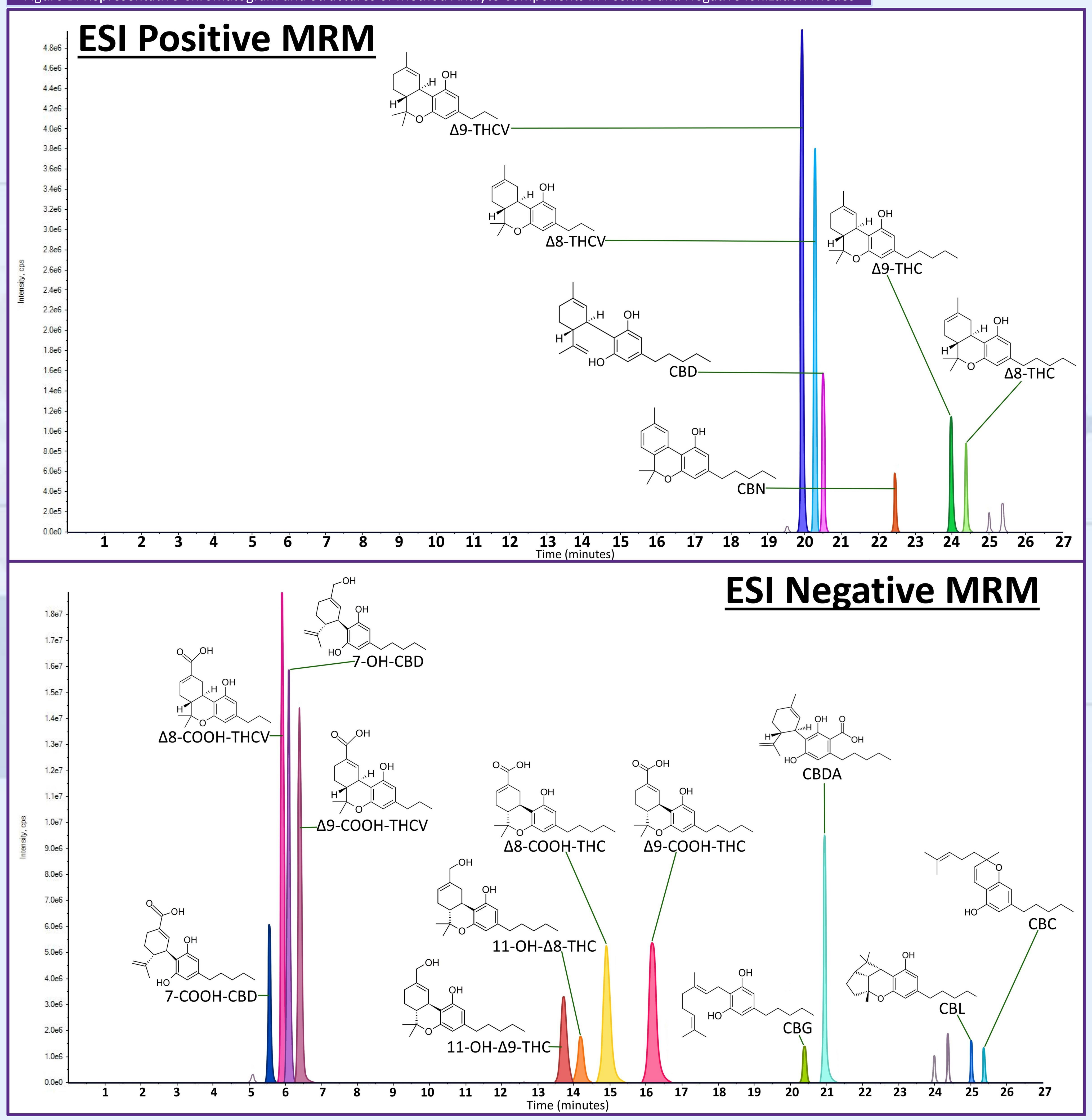


RESULTS / DISCUSSION

A single-point calibrator at 5.0 ng/mL was used for quantitation. A low control at 2.0 ng/mL (40% of the calibrator), two positive controls at 6.25 ng/mL (125% of the calibrator), and two negative controls were run with each analytical batch, with one of the negative controls and one of the positive controls injected at the end of the batch to bracket donor samples. In addition to the low and positive controls, a conversion control was included in each batch to monitor the potential conversion of CBD and its metabolites to Δ9-THC and Δ8-THC and corresponding metabolites; the conversion control contained CBD, 7-OH-CBD, 7-COOH-CBD, and CBDA at 5.0 ng/mL.

Linearity was determined and assay limits of detection and quantitation (LOD/LOQ) and upper limit of linearity (ULOL) were established through the analysis of cannabinoid-analyte spiked UTAK negative whole blood samples at concentrations ranging from 0.250 ng/mL to 200 ng/mL. Accuracy and precision were calculated for 3 replicates of each of 14 concentration levels, including 40%, 50%, 100%, 125%, 150%, and 200% of the calibrator concentration. Qualitative acceptance was based on mean analyte concentrations within ±20% of target values with a coefficient of variation (CV) of <10%; due to the absence of a labeled internal standard for CBL, Δ9-COOH-THCV, and Δ8-COOH-THCV, accuracy within ±25% and CV <20% were accepted. For LOD/LOQ assessment, at the 0.250 ng/mL level, no analytes had reproducible quantitative results within ±20% of target with all replicates also passing qualitative acceptance criteria (see Table 2). Replicates for all analytes met both quantitative and qualitative acceptance criteria at the 0.5 ng/mL level. At the upper limit of linearity, replicates for 7-OH-CBD, Δ9-COOH-THCV, and Δ8-COOH-THCV met acceptance criteria at 100 ng/mL, and replicates for all other analytes met full acceptance criteria at 200 ng/mL. No carryover was observed at the highest concentrations.

Figure B: Representative Chromatogram and Structures of Method Analyte Components in Positive and Negative Ionization Modes



Interference was investigated with 54 compounds, including over-the-counter, illicit, and commonly prescribed drugs. Throughout the study, no interference was detected with chromatography or internal standard recovery, and no erroneous peaks were observed that were greater than assay LOQ, which could create possible quantitation or identification issues. The potential of sample matrix components to interfere with the analytical method was evaluated by testing ten random negative whole blood patient samples, and continually monitored through the analysis of hundreds of study patient samples. Results showed no indication of methodic ion suppression or enhancement, and analyte and internal standard recovery was consistent. All samples passed with acceptable chromatography as no qualitative issues were observed, and no interfering peaks were present in the negative samples that could be problematic in quantitation or identification.

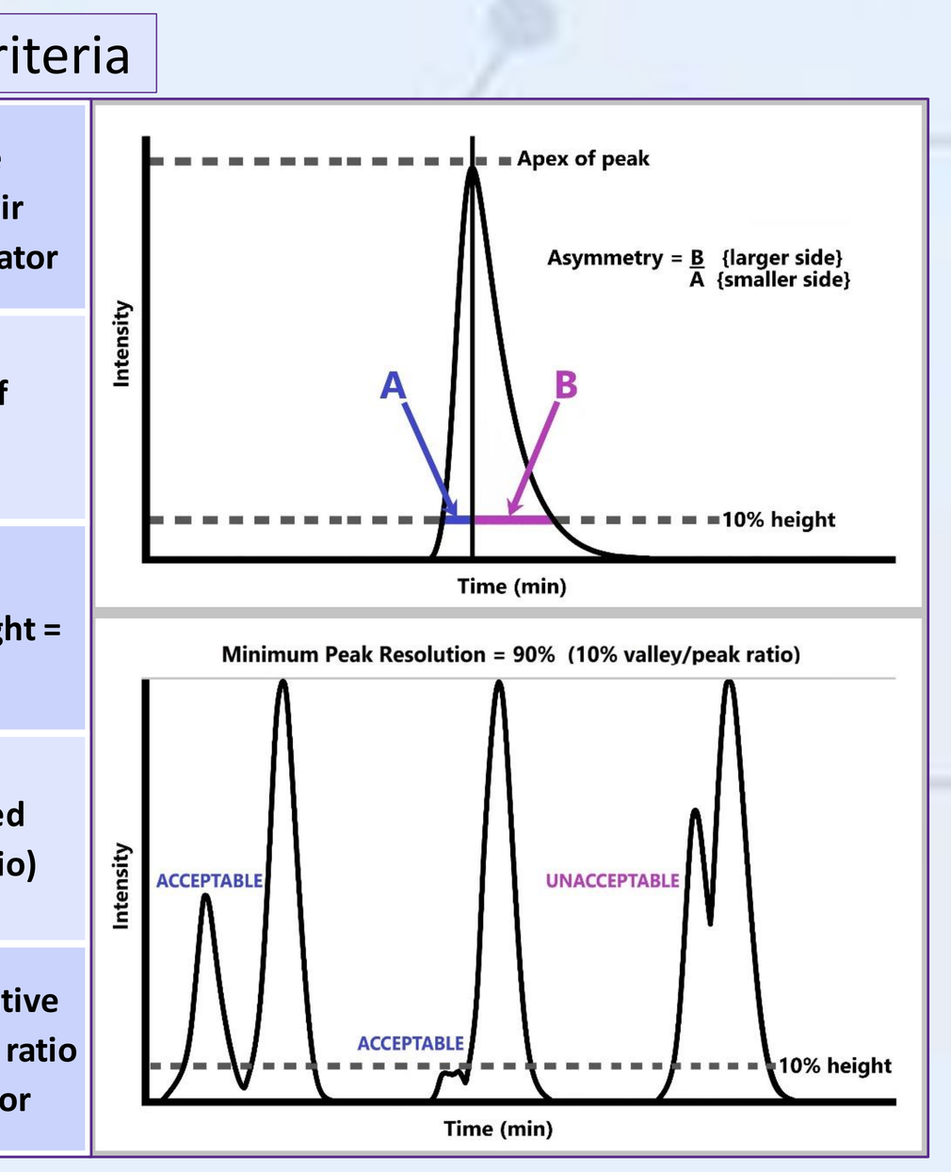
Figure C: Analyte Linearities

Table 2: Analyte LOD/LOQ and ULOL

Analyte	LOD/LOQ (ng/mL)	ULOL (ng/mL)
Δ9-THCV	0.500	200
Δ8-THCV	0.500	200
CBD	0.500	200
CBN	0.500	200
(-)-Δ9-THC	0.500	200
(-)-Δ8-THC	0.500	200
7-COOH-CBD	0.500	200
Δ8-COOH-THCV	0.500	100
7-OH-CBD	0.500	100
Δ9-COOH-THCV	0.500	100
11-OH-Δ9-THC	0.500	200
11-OH-Δ8-THC	0.500	200
Δ8-COOH-THC	0.500	200
Δ9-COOH-THC	0.500	200
CBG	0.500	200
CBDA	0.500	200
CBL	0.500	200
CBC	0.500	200

Table 3: Quantitative Acceptance Criteria

Relative Retention Time (RRT)	±2% of expected RRT of the analyte/internal standard pair established by the batch calibrator
Internal Standard (IS) Response	Total IS peak area = ≥10% of calibrator IS peak area
Symmetry / Peak Shape	Gaussian peaks; asymmetry at 10% of peak height = <3.0 for IS and quant peaks
Resolution	Adjacent peaks ≥90% resolved (≤ 10% valley/peak height ratio)
Ion Ratios (Qualifiers)	Ratio of abundance of quantitative to qualifier ion = ±20% of target ratio established by batch calibrator



CONCLUSION

This analytical method effectively separated 11-OH-Δ9-THC and 11-OH-Δ8-THC and demonstrated selectivity, accuracy, and reproducibility for the analysis of hundreds of samples in federally-sponsored controlled dosing research studies. Its application reliably identified and quantitated 18 cannabinoids at pg/mL levels, contributing to the scientific knowledge of cannabinoid metabolism and distribution in whole blood.

REFERENCES

Adams R., Cain C.K., McPhee W.D., Wearn R.B. (1941). Structure of cannabidiol. XII. Isomerization to tetrahydrocannabinols. *Journal of the American Chemical Society*, *63*(8), 2209–2213. https://doi: 10.1021/ja01853a052.

Applied Biosystems API 7500 LC-MS/MS Hardware Manual

Cayman Chemical Reference Materials

Cerilliant Analytical Reference Materials

Coulter, C., & Wagner, J.R. (2021). Cannabinoids in Oral Fluid: Limiting Potential Sources of Cannabidiol Conversion to Δ9- and Δ8-Tetrahydrocannabinol. *Journal of analytical toxicology*, *45*(8), 807–812. https://doi.org/10.1093/jat/bkab074

Day, D., Kuntz, D., Feldman, M. (2011). *Oral Detection Test for Cannabinoid Use* (U.S. Patent No. 7,977,107; Pat. No. 7,816,143 (2008); Pat. No. 7,465,586 (2004). https://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=7977107.PN.&OS=PN/7977107&RS=PN/7977107

Merrick, J., Lane, B., Sebree, T., Yaksh, T., O'Neill, C., & Banks, S.L. (2016). Identification of Psychoactive Degradants of Cannabidiol in Simulated Gastric and Physiological Fluid. *Cannabis and cannabinoid research*, *1*(1), 102–112. https://doi.org/10.1089/can.2015.000

Pourseyed Lazarjani, M., Torres, S., Hooker, T., Fowlie, C., Young, O., & Seyfoddin, A. (2020). Methods for quantification of cannabinoids: a narrative review. *Journal of cannabis research*, *2*(1), 35. https://doi.org/10.1186/s42238-020-00040-2

Sholler, D.J., Spindle, T.R., Cone, E.J., Goffi, E., Kuntz, D., Mitchell, J.M., Winecker, R.E., Bigelow, G.E., Flegel, R.R., & Vandrey, R. (2022). Urinary Pharmacokinetic Profile of Cannabidiol (CBD), Δ9-Tetrahydrocannabinol (THC) and Their Metabolites following Oral and Vaporized CBD and Vaporized CBD-Dominant Cannabis Administration. *Journal of analytical toxicology, 46*(5), 494-503. https://doi.org/10.1093/jat/bkab059

Substance Abuse and Mental Health Services Administration (SAMHSA), Department of Health and Human Services. (2019). Mandatory Guidelines for Federal Workplace Drug Testing Programs—Oral/Fluid, 42 CFR Chapter I. *Federal Register, 84*(207), 57554-57600. https://www.govinfo.gov/content/pkg/FR-2019-10-25/pdf/2019-22684.pdf

Swortwood, M.J., Newmeyer, M.N., Andersson, M., Abulseoud, O.A., Scheidweiler, K.B., & Huestis, M.A. (2017). Cannabinoid disposition in oral fluid after controlled smoked, vaporized, and oral cannabis administration. *Drug testing and analysis*, *9*(6), 905–915. https://doi.org/10.1002/dta.2092

Watanabe K., Itokawa Y., Yamaori S., Funahashi T., Kimura T., Kaji T., Usami, N., Yamamoto, I. (2007) Conversion of cannabidiol to delta-9-tetrahydrocannabinol and related cannabinoids in artificial gastric juice, and their pharmacological effects in mice. *Forensic Toxicology, 25*(1), 16–21. https://doi: 10.1007/s11419-007-0021-y

DISCLOSURE

No relevant financial or nonfinancial relationships to disclose.

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