Development of an LC-MS/MS Method for the Analysis of Δ8-THC, Δ9-THC, and Their Metabolites in Whole Blood

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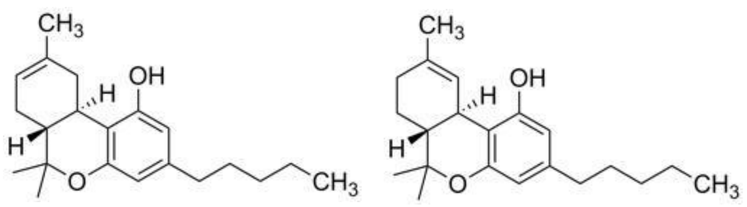
Introduction

The testing of whole blood samples for tetrahydrocannabinol (Δ9-THC) consumption is routine and has been around for many decades. Δ-9-THC is metabolized into 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-Δ9-THC) and further into 11-nor-9-carboxy-Δ9-THC (Δ9-THC-COOH). It is important to test for the parent and both metabolites to properly monitor for THC usage.[1] As more isomers of Δ9-THC become available on the market, testing becomes more complicated and novel methods are needed to achieve isomeric resolution. One such isomer, Δ8-THC, has gained popularity as a recreational product in recent years. This compound forms its own hydroxylated and carboxylated metabolites, (11-OH-Δ8-THC and Δ8-THC-COOH), that must be resolved from their isomeric metabolites. The resolution of these metabolites is key in reporting accurate results. Poor resolution, especially when one isomer is in much greater abundance than the other, can result in invalid data. In this study, three different column chemistries were investigated to see if separation of the three isomer pairs was feasible. An LC-MS/MS method was then developed to adequately resolve the parent, hydroxy, and carboxy isomer compounds in whole blood.

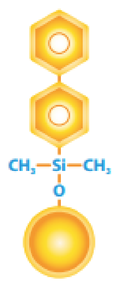


Δ8-THC

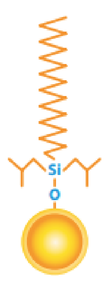
Δ9-THC

Column Chemistries

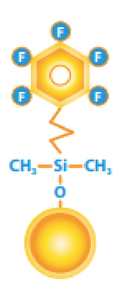
Three stationary phases were scouted: **Biphenyl**, **ARC-18**, and **FluoroPhenyl**. The ligand for each stationary phase is shown below.



Biphenyl



ARC-18



FluoroPhenyl

Method Scouting

A sample containing all 6 compounds was analyzed using the following run conditions on a column from each of the three stationary phases.

Method Scouting Conditions			
Stationary Phases	Raptor **Biphenyl** Raptor **ARC-18** Raptor **FluoroPhenyl**		
Column Dimension	100 x 2.1 mm, 2.7 µm		
Mobile Phase A	0.1% formic acid in H_2O		
Mobile Phase B	0.1% formic acid in MeOH		
Column Temperature	40°C		
Diluent	50:50 MPA:MPB (v/v)		
Injection Volume	5 µL		
Flow Rate	0.5 mL/min		
Gradient	Time (min)	%A	%B
	0.00	40	60
	6.00	20	80
	9.00	20	80
	9.01	40	60
	10.00	40	60

Results

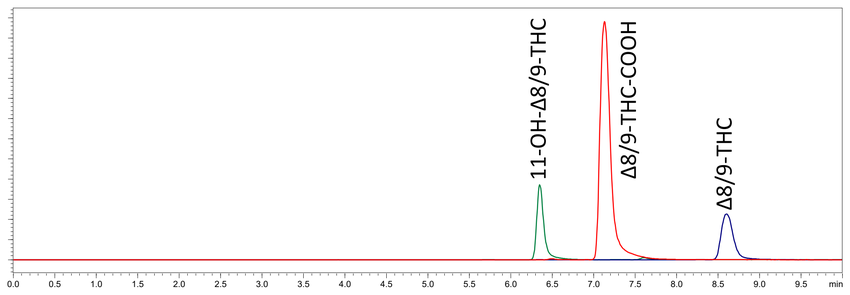


Figure 1. Isomers analyzed on a **Biphenyl** column. No selectivity is shown for the isomers, with all 3 pairs co-eluting.

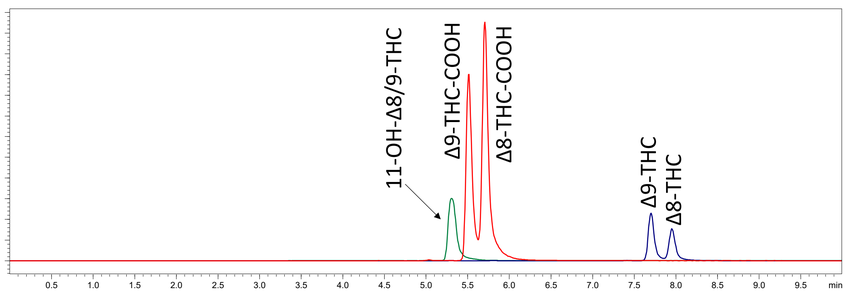


Figure 2. Isomers analyzed on an **ARC-18** column. Δ8/9-THC are well resolved on this phase. The carboxy analytes also show separation, but the hydroxy isomers are still coeluting.

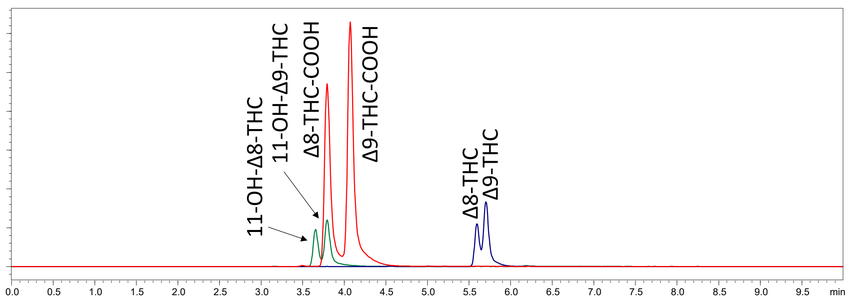


Figure 3. Isomers analyzed on a **FluoroPhenyl** column. All isomers showed separation on this phase. Further method development must be performed to fully separate the compounds.

Optimized Method Conditions			
Stationary Phase	Raptor **FluoroPhenyl**		
Column Dimension	100 x 3 mm, 2.7 µm		
Guard Column	Raptor EXP guard cartridge, 5 x 3 mm, 2.7 µm		
Mobile Phase A	0.1% formic acid in H_2O		
Mobile Phase B	0.1% formic acid in MeOH		
Column Temperature	40°C		
Diluent	40:60 MPA:MPB (v/v)		
Injection Volume	5 µL		
Flow Rate	0.8 mL/min		
Gradient	Time (min)	%A	%B
	0.00	36	64
	6.50	36	64
	6.60	32	68
	13.00	32	68
	13.10	0	100
	14.00	0	100
	14.10	36	64
	16.00	36	64

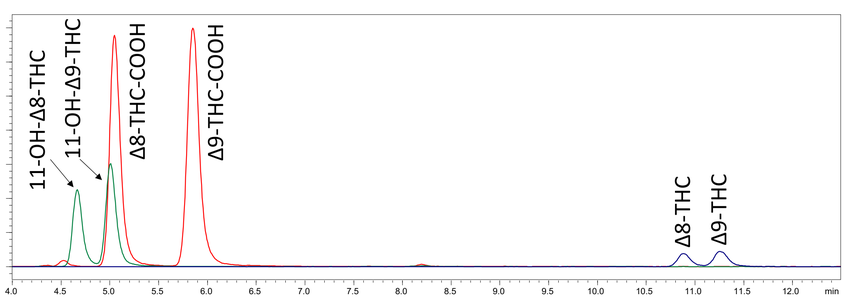
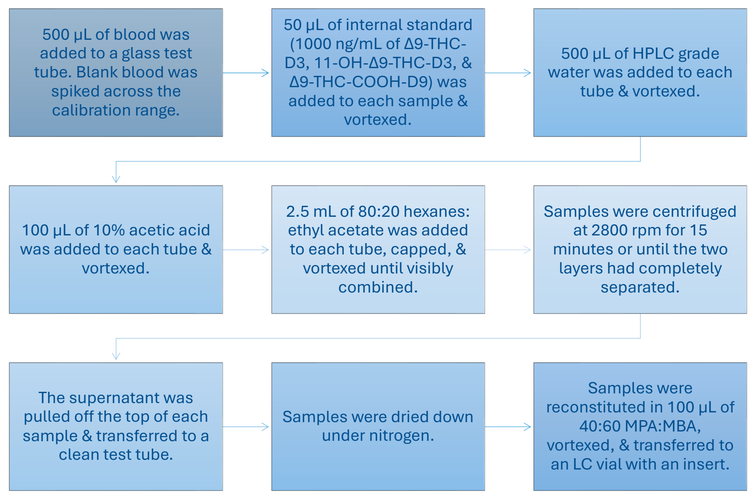


Figure 4. Optimized method conditions on a **FluoroPhenyl** column. All isomers are baseline resolved. A larger column ID was selected to increase resolution capabilities. The flow rate was adjusted for the larger column ID. A guard cartridge was added to protect the analytical column. The gradient was altered to optimize separation of the isomers, and the sample diluent was changed to reflect starting conditions.

Sample Preparation

The developed method was applied to whole blood. Samples were prepared using the liquid-liquid extraction procedure shown below. The extraction procedure was adapted from Tiscione, Nicholas B et al. (2016).[2]



Method Performance

Linearity, Precision, Accuracy

Linearity was demonstrated using a 1/x weighted linear regression. All analytes showed acceptable r^2 values (≥0.99). The calibration range was made up of six calibrators (range shown below). The calibration ranges encompass typical concentration levels for these analytes in whole blood specimens. Precision and accuracy were assessed at four different concentrations (LLOQ, Low QC, Medium QC, High QC) and evaluated within a day and as an average of three days (n=9). The results demonstrated that the method was accurate and precise.

Analyte	Calibration Range (ng/mL)	r^2
11-OH-Δ8-THC	0.5 – 100	0.9932
11-OH-Δ9-THC	0.5 – 100	0.9984
Δ8-THC-COOH	2.5 – 500	0.9956
Δ9-THC-COOH	2.5 – 500	0.9964
Δ8-THC	0.5 – 100	0.9976
Δ9-THC	0.5 – 100	0.9950

Interferences

Potential interferences from 12 other commonly encountered/structurally similar cannabinoids was also investigated. A sample containing CBDV, CBD, CBG, THCV, exo-THC, 9(S)-HHC, CBL, 9(R)-HHC, CBN, 9(S)-Δ6a,10a-THC, CBC, and THCA-A was analyzed using the developed method. All compounds were resolved from the analytes of interest. CBL elutes in the same MRM window as Δ9-THC but has a notable difference in retention time so no misidentification should occur.

Conclusion

In this work, a method was developed for Δ8-THC, Δ9-THC, and their hydroxy and carboxy metabolites. Three column chemistries were scouted to determine which phase showed the most selectivity for the isomers. Between Biphenyl, ARC-18, and FluoroPhenyl, the FluoroPhenyl phase showed the best selectivity for the isomers. A method was optimized on this column chemistry and applied to whole blood. The developed method was shown to be robust and reproducible across the calibration range. This work demonstrates the importance of evaluating alternate column chemistries for difficult separations.

References

1. Karschner, E. L.; Swortwood-Gates, M. J.; Huestis, M. A. Identifying and Quantifying Cannabinoids in Biological Matrices in the Medical and Legal Cannabis Era. Clinical Chemistry 66:7 (2020) 888-914.
2. Tiscione NB, Miller R, Shan X, Sprague J, Yeatman DT. An Efficient, Robust Method for the Determination of Cannabinoids in Whole Blood by LC-MS-MS. J Anal Toxicol. 2016;40(8):639-648. doi:10.1093/jat/bkw063