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Quantitative Separation of THC Isomers and Metabolites from Whole Blood

Using Agilent Captiva Enhanced Matrix Removal (EMR) cartridges and LC/MS/MS

Authors

Peter J. W. Stone,
Natalie N. Rasmussen,
Timothy Bolduc, and
Tina Chambers
Agilent Technologies, Inc.

Abstract

The ability to distinguish psychoactive tetrahydrocannabinol (THC) isomers (Δ8-, Δ9-, and Δ10-THC) and their metabolites from nonpsychoactive compounds such as cannabidiol (CBD) and exo-THC is increasingly important in forensic toxicology. This study presents a 15-minute Agilent 6475 LC/TQ method for the separation and quantification of these analytes from whole blood extracts. The workflow uses Agilent Captiva EMR–Lipid cartridges for sample preparation and an Agilent InfinityLab Poroshell 120 PFP column for chromatographic separation. The workflow achieves low ng/mL sensitivity, excellent linearity, and high analyte recovery, making it a suitable basis for possible driving under the influence of drugs (DUID) investigations.

Introduction

THC isomers (Δ8- and Δ9-THC, in particular) are psychoactive compounds of increasing interest in forensic toxicology, where accurate identification and quantification of these individual isomers and their respective metabolites are essential for legal and clinical assessments. However, an analytical challenge derives from their structural or empirical similarity and the presence of potential isobaric interferences.

Isobaric compounds are molecules that are empirically identical, share the same nominal mass, but differ in structure. In the context of THC analysis, CBD and exo-THC are notable isobaric interferences. These compounds can coelute or produce overlapping mass spectral signals, complicating the interpretation of results. CBD, a nonpsychoactive cannabinoid, shares an identical molecular weight and similar fragmentation pattern with THC isomers, making it difficult to distinguish using conventional mass spectrometry alone. Exo-THC, another structural analog, further adds to the complexity for the same reasons.

Without adequate chromatographic separation, these isobaric interferences can lead to false positives or inaccurate quantitation. This is particularly problematic in forensic settings, where precise identification of psychoactive substances is critical. Therefore, a robust analytical method that can resolve these compounds and potential interferents is desirable.

This study introduces a workflow that combines enhanced matrix removal (EMR) with LC/MS/MS to achieve baseline separation of THC isomers and their metabolites from the isobaric interferences that were examined within the scope of this application note. The method enables confident separation, identification, and quantification in a single 15-minute analysis.

Experimental

Sample preparation

The sample preparation protocol was designed to efficiently extract Δ8-, Δ9-, and Δ10-THC isomers and their respective hydroxy and carboxy metabolites from whole blood while minimizing matrix effects. A volume of 500 µL of room-temperature sheep whole blood was directly applied to a 3 mL Captiva EMR–Lipid cartridge (part number 5190-1003), which had been preloaded into a positive pressure extraction manifold. The cartridge material is engineered to remove phospholipids and other matrix components that can interfere with downstream LC/MS/MS analysis.

To initiate protein precipitation and analyte extraction, 1.2 mL of cold acetonitrile/methanol (85:15, v:v) was added to the blood in the cartridge. The mixture was allowed to stand for at least five minutes to ensure thorough interaction between the solvent and the blood matrix. Thereafter, positive pressure was applied to the manifold at a controlled rate of approximately 4 to 6 drops per second. This step facilitated the elution of the analytes while retaining unwanted matrix components within the cartridge.

The eluent was collected directly into 2 mL conical autosampler vials positioned beneath the cartridges. These vials were then subjected to evaporation under a gentle stream of nitrogen to remove organic solvents. The dried residue was reconstituted in 100 µL of 50:50 v:v methanol/water to ensure optimal solubility and compatibility with LC/MS/MS analysis. The vials were capped and briefly vortexed to ensure complete dissolution of the analytes before being placed in the autosampler for injection.

This streamlined sample preparation workflow (summarized in Figure 1) offers high analyte recovery, minimal matrix interference, and compatibility with high-throughput toxicology workflows.

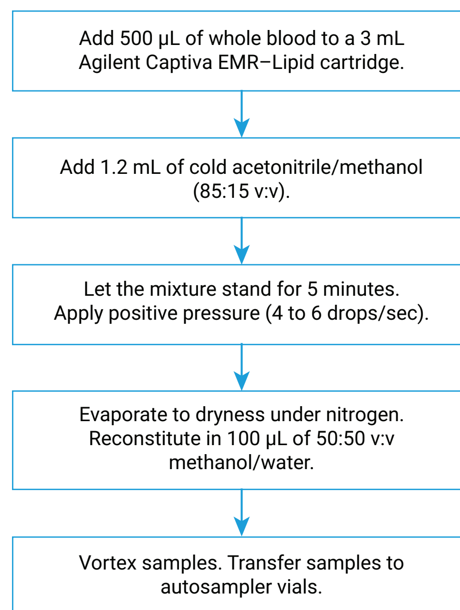

Figure 1. Sample preparation workflow.

Chromatographic separation and LC/MS/MS detection

This procedure can be accomplished using different instrument configurations. Chromatographic separation was verified using both an Agilent 1260 Infinity II LC and an Agilent 1290 Infinity II LC. Analyte detection and quantitation were performed via both an Agilent 6475 triple quadrupole LC/MS and an Agilent Ultivo triple quadrupole LC/MS. Method parameters are detailed in Tables 1 and 2. Detailed MRM transition settings are outlined in Appendix A.

Table 1. Method parameters for LC separation.

Parameter	Value
Analytical and Guard Columns	Agilent InfinityLab Poroshell 120 PFP, 2.1 × 100 mm, 2.7 µm (p/n 695775-408) Agilent InfinityLab Poroshell 120 PFP, 2.1 mm, 2.7 µm, UHPLC guard (p/n 821725-915)
Injection Volume	5 µL
Column Temperature	55 °C
Needle Wash	Methanol (100%)
Mobile Phase	A) LC/MS-grade water with 0.1% formic acid B) LC/MS-grade methanol (100%)
Flow Rate	0.5 mL/min
Run Time	13.5 min
Post Time	Agilent 1290 Infinity II LC: 1.0 min Agilent 1260 Infinity II LC: 2.5 min
Gradient	Time (min) %B 0.00 55 5.85 59 6.50 59 6.60 64 10.50 64 11.50 100 13.50 100

Table 2. Method parameters for MS detection.

Parameter	Value
Ion Mode	Agilent Jet Stream Technology ion source (AJS), ESI+
Drying Gas Temperature	325 °C
Drying Gas Flow	11 L/min
Nebulizer Pressure	35 psi
Capillary	4,500 V
Sheath Gas Temperature	350 °C
Sheath Gas Flow	11 L/min
Nozzle Voltage	500 V
Dwell Time	Variable (dMRM mode) 12 ms (MRM mode)

Results and discussion

The LC/MS/MS method outlined herein successfully achieved baseline chromatographic separation of Δ8-, Δ9-, and Δ10-THC isomers and their respective hydroxy and carboxy metabolites in whole blood. Using an InfinityLab Poroshell 120 PFP column, the method provided clear resolution of these structurally similar analytes within a single 15-minute run. Figure 2 illustrates the separation profiles for all target compounds, including panels A through D, which show the separation of all analytes (A), isobaric THC isomers and interferents (B), carboxy metabolites (C), and hydroxy metabolites (D). These chromatograms demonstrate the ability of the method to distinguish psychoactive THC isomers from nonpsychoactive interferences such as CBD and exo-THC, which are known to complicate forensic analyses due to their similar mass and retention behavior.

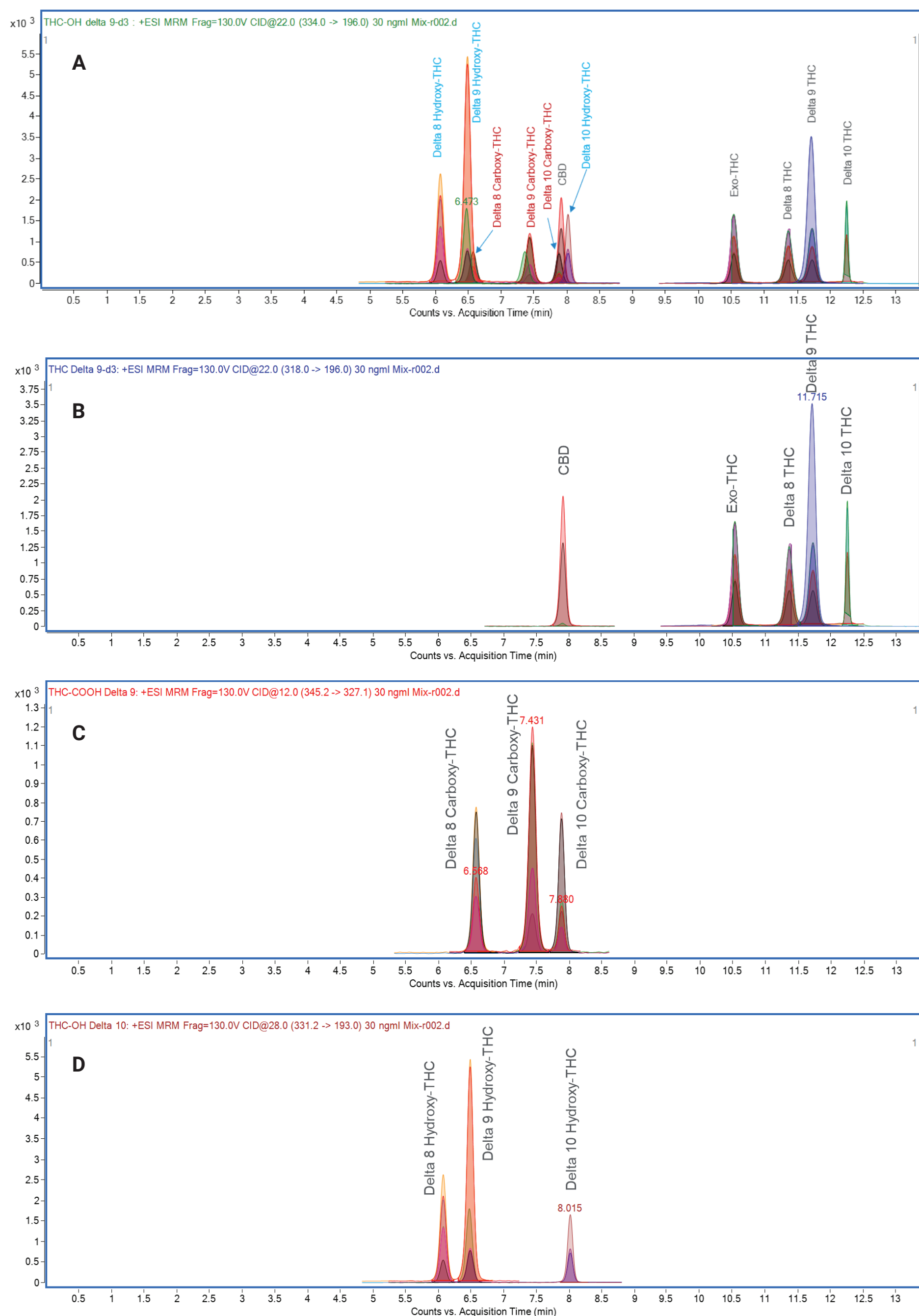

Figure 2. Chromatographic separation of (A) all analytes, (B) isobaric delta-THCs and interferents only, (C) isobaric delta-carboxy-THCs, and (D) isobaric delta-hydroxy-THCs.

Sensitivity was a key performance metric in this study. The method achieved low nanogram-per-milliliter (ng/mL) detection limits for all THC isomers and their metabolites. Specifically, the working range for Δ8-, Δ9-, and Δ10-THC and their hydroxy metabolites was 1 to 80 ng/mL in-vial concentration (or 5 to 400 ng/mL original-blood concentration), while carboxy metabolites were quantified across a broader range of 5 to 400 ng/mL in-vial concentration (or 25 to 2,000 ng/mL original-blood concentration). Linearity was excellent across all analytes, with correlation coefficients (R^2) exceeding 0.998, as shown in Figure 3, which presents a typical calibration curve for Δ9-THC. This high degree of linearity supports the reliability of the method for quantitative analysis in forensic toxicology.

Figure 4 illustrates the chromatographic separation and response under these method conditions for matrix calibrator 1, which was an in-vial concentration of 1 ng/mL (5 ng/mL blood equivalence).

Recovery studies further validated the robustness of the sample preparation workflow.[1] Using Captiva EMR–Lipid cartridges, analyte recoveries ranged between 80 to 120%, indicating efficient extraction and minimal loss during processing. These results were consistent across multiple replicates and were confirmed using whole sheep blood as a surrogate matrix to verify separation and quantitation performance. The use of EMR technology effectively removed many lipid and protein interferences, contributing to cleaner extracts and improved signal clarity.

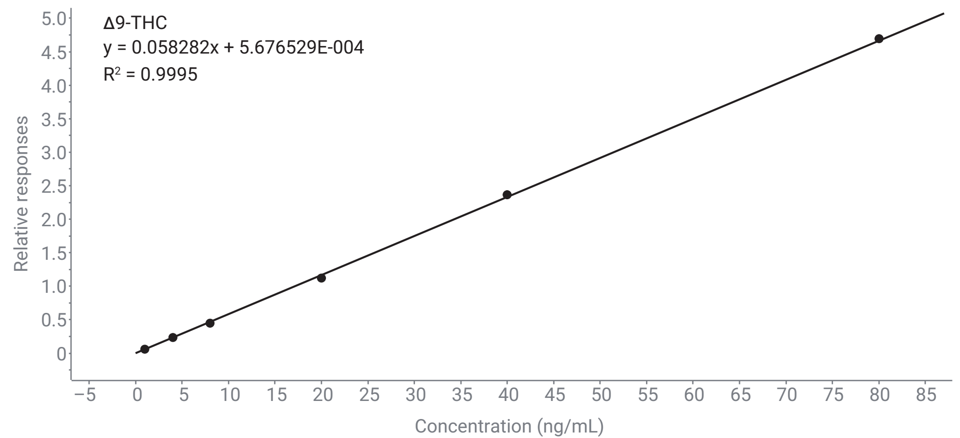

Figure 3. Typical Δ9-THC quantitative linearity.

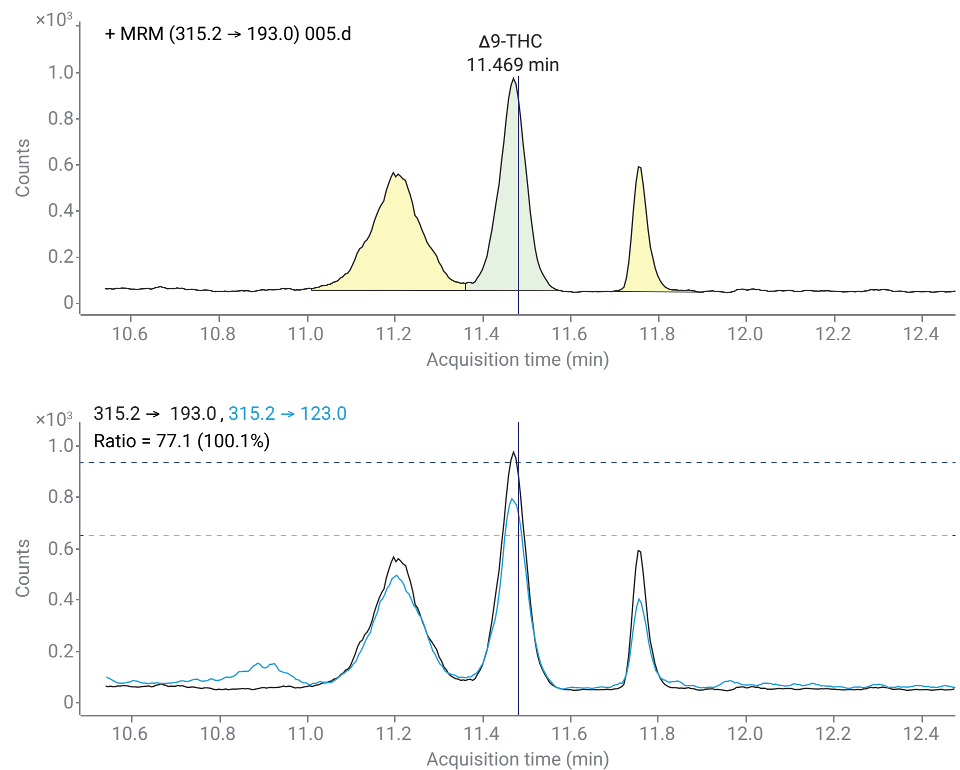

Figure 4. Typical chromatography and Δ9-THC response of matrix calibrator 1 (1 ng/mL in-vial concentration).

Mass spectrometry conditions were optimized to ensure high sensitivity and selectivity. Operating in positive ion mode with the Jet Stream Technology ion source, the system maintained stable ionization and efficient analyte transmission. Dynamic MRM mode allowed dwell times to adjust automatically, improving detection across a complex panel of THC isomers and metabolites.

Overall, the method demonstrated strong performance across all critical analytical metrics—separation, sensitivity, linearity, and recovery. Its ability to resolve the isobaric interferences CBD and exo-THC and quantify multiple THC isomers and metabolites in a single run makes it a valuable basis for toxicology laboratories. The streamlined workflow and relatively short analysis time support high-throughput applications, particularly in scenarios where rapid and accurate results are essential.

Conclusion

This study presents an effective, integrated workflow for the separation and quantification of THC isomers and metabolites in whole blood. Agilent Captiva EMR–Lipid cartridges removed sufficient matrix interferences to enable clean extraction and high analyte recovery. Chromatographic separation using an Agilent InfinityLab Poroshell 120 PFP column provided reliable resolution of isobaric compounds, including CBD and exo-THC. Coupled with Agilent LC/MS/MS systems featuring Agilent Jet Stream ionization technology, the method delivered consistent sensitivity and selectivity across a complex panel of analytes. Together, this methodology offers the basis of a streamlined workflow for the separation of Δ8-, Δ9-, and Δ10-THC isomers and their respective hydroxy and carboxy metabolites and potential interferents from whole blood.

Acknowledgements

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Reference

1. Stevens, J.; Zhao, L. Efficient Quantitative Analysis of THC and its Metabolites in Whole Blood Using Agilent Captiva EMR–Lipid and LC-MS/MS. *Agilent Technologies application note*, publication number 5991-8635EN, **2020**.

Appendix A

Dynamic MRM transitions and retention times shown for the 1260 configuration

Compound Name	Compound Formula	Ion Species	ISTD?	Precursor (*m/z*)	MS1 Res	Product (*m/z*)	MS2 Res	RT (min)	RT Window (min)	Fragmentor (V)	CAV (V)	CE (V)	Polarity	Cycle Time (ms)
CBD	$C_{21}H_{30}O_2$	$(M+H)^+$	No	315.2	Unit	193	Unit	7.7	2	130	5	22	+	330
CBD	$C_{21}H_{30}O_2$	$(M+H)^+$	No	315.2	Unit	123	Unit	7.7	2	130	5	38	+	330
CBD-d_3	$C_{21}H_{27}D_3O_2$	$(M+H)^+$	Yes	318.1	Unit	145	Unit	7.7	2	130	5	22	+	330
EXO-THC	$C_{21}H_{30}O_2$	$(M+H)^+$	No	315.2	Unit	259.1	Unit	10.4	2	140	5	17	+	330
EXO-THC	$C_{21}H_{30}O_2$	$(M+H)^+$	No	315.2	Unit	193.1	Unit	10.4	2	140	5	22	+	330
EXO-THC	$C_{21}H_{30}O_2$	$(M+H)^+$	No	315.2	Unit	123	Unit	10.4	2	140	5	38	+	330
THC Delta 10	$C_{21}H_{30}O_2$	$(M+H)^+$	No	315.2	Unit	193	Unit	12.35	2	100	5	22	+	330
THC Delta 10	$C_{21}H_{30}O_2$	$(M+H)^+$	No	315.2	Unit	123	Unit	12.35	2	100	5	38	+	330
THC Delta 8	$C_{21}H_{30}O_2$	$(M+H)^+$	No	315.2	Unit	259	Unit	11.17	2	140	5	17	+	330
THC Delta 8	$C_{21}H_{30}O_2$	$(M+H)^+$	No	315.2	Unit	193	Unit	11.17	2	140	5	22	+	330
THC Delta 8	$C_{21}H_{30}O_2$	$(M+H)^+$	No	315.2	Unit	123	Unit	11.17	2	140	5	38	+	330
THC Delta 9	$C_{21}H_{30}O_2$	$(M+H)^+$	No	315.2	Unit	193	Unit	11.5	2	130	5	22	+	330
THC Delta 9	$C_{21}H_{30}O_2$	$(M+H)^+$	No	315.2	Unit	123	Unit	11.5	2	130	5	38	+	330
THC Delta 9-d_3	$C_{21}H_{27}D_3O_2$	$(M+H)^+$	Yes	318	Unit	196	Unit	11.5	2	130	5	22	+	330
THC-COOH Delta 10	$C_{21}H_{28}O_4$	$(M+H)^+$	No	345.2	Unit	327.1	Unit	7.61	2	130	5	17	+	330
THC-COOH Delta 10	$C_{21}H_{28}O_4$	$(M+H)^+$	No	345.2	Unit	299.1	Unit	7.61	2	130	5	22	+	330
THC-COOH Delta 10	$C_{21}H_{28}O_4$	$(M+H)^+$	No	345.2	Unit	257.1	Unit	7.61	2	130	5	22	+	330
THC-COOH Delta 8	$C_{21}H_{28}O_4$	$(M+H)^+$	No	345.2	Unit	299.1	Unit	6.32	2	130	5	17	+	330
THC-COOH Delta 8	$C_{21}H_{28}O_4$	$(M+H)^+$	No	345.2	Unit	193.1	Unit	6.32	2	130	5	28	+	330
THC-COOH Delta 9	$C_{21}H_{28}O_4$	$(M+H)^+$	No	345.2	Unit	327.1	Unit	7.16	2	130	5	12	+	330
THC-COOH Delta 9	$C_{21}H_{28}O_4$	$(M+H)^+$	No	345.2	Unit	299.1	Unit	7.16	2	130	5	22	+	330
THC-COOH Delta 9	$C_{21}H_{28}O_4$	$(M+H)^+$	No	345.2	Unit	41	Unit	7.16	2	130	5	55	+	330
THC-COOH delta 9-d_9	$C_{21}H_{19}D_9O_4$	$(M+H)^+$	Yes	354	Unit	307	Unit	7.16	2	130	5	22	+	330
THC-OH Delta 10	$C_{21}H_{30}O_3$	$(M+H)^+$	No	331.2	Unit	193	Unit	7.8	2	130	5	28	+	330
THC-OH Delta 10	$C_{21}H_{30}O_3$	$(M+H)^+$	No	331.2	Unit	43	Unit	7.8	2	130	5	42	+	330
THC-OH Delta 10	$C_{21}H_{30}O_3$	$(M+H)^+$	No	331.2	Unit	41	Unit	7.8	2	130	5	60	+	330
THC-OH Delta 8	$C_{21}H_{30}O_3$	$(M+H)^+$	No	331.2	Unit	313.1	Unit	5.83	2	130	5	12	+	330
THC-OH Delta 8	$C_{21}H_{30}O_3$	$(M+H)^+$	No	331.2	Unit	201	Unit	5.83	2	130	5	27	+	330
THC-OH Delta 8	$C_{21}H_{30}O_3$	$(M+H)^+$	No	331.2	Unit	43	Unit	5.83	2	130	5	35	+	330
THC-OH Delta 9	$C_{21}H_{30}O_3$	$(M+H)^+$	No	331.2	Unit	313.1	Unit	6.23	2	120	5	10	+	330
THC-OH Delta 9	$C_{21}H_{30}O_3$	$(M+H)^+$	No	331.2	Unit	193	Unit	6.23	2	120	5	27	+	330
THC-OH Delta 9	$C_{21}H_{30}O_3$	$(M+H)^+$	No	331.2	Unit	43	Unit	6.23	2	120	5	38	+	330
THC-OH delta 9-d_3	$C_{21}H_{27}D_3O_3$	$(M+H)^+$	Yes	334	Unit	196	Unit	6.23	2	130	5	22	+	330

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