Semi-Synthetic Cannabinoids: The Newest Challenge in Analyzing THC Isomers

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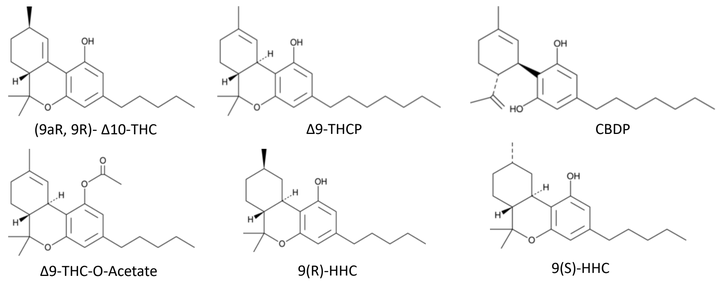
Introduction

Over the past several years, Δ8-THC has made headlines as a popular "legal" alternative to Δ9-THC. The rise in popularity of Δ8-THC led to a new analytical challenge for toxicology laboratories—differentiating between the Δ8 and Δ9 isomer forms. For labs performing this testing by LC-MS/MS, complete separation of the isomers and their metabolites is necessary for accurate quantitation. While HPLC stationary phases like Biphenyl and C18 have traditionally been used for toxicological analysis of cannabinoids, they lack the selectivity needed to adequately separate these isomers. FluoroPhenyl phase columns have emerged as a superior choice for analyzing THC isomers in biological matrices due to their unique selectivity capabilities.

Figure 1. Structures of emerging semi-synthetic cannabinoids.



Recently, several new cannabinoids have emerged on the market. These compounds are often referred to as semi-synthetic cannabinoids. The term comes from the fact that many of these compounds are found in low abundance in cannabis or hemp; as a result, they are often synthetically made and marketed as naturally occurring, or their chemical structures are slightly modified to produce a new substance entirely. In response to these new compounds, The Center for Forensic Science Research & Education (CFSRE) added a semi-synthetic cannabinoids category to their quarterly scope recommendations, making labs aware of these new cannabinoids and enables them to add theses to their testing scope. Integrating new compounds into an existing method is an efficient way to expand testing scopes without increasing the number of panels that must be run. This work explores adding 6 semi-synthetic cannabinoids to a pre-existing method that has been verified for the LC-MS/MS analysis of THC isomers in whole blood.

Materials and Methods

The original method was developed for the quantitative analysis of Δ8-THC, Δ9-THC and their isomeric metabolites in whole blood by LC-MS/MS. The analytes included in the original method are listed in Table 1 below. In Q3 of 2025, the CFSRE identified the six semi-synthetic cannabinoids shown below in Table 2 as scope recommendations based on current detection trends. A sample of all 12 compounds was prepared at a concentration of 100 ng/mL. The diluent was 50:50 water:methanol (v/v), both containing 0.1% formic acid.

Table 1. Analytes included in the original method.

11-OH-Δ8-THC	11-OH-Δ9-THC
Δ8-THC-COOH	Δ9-THC-COOH
Δ8-THC	Δ9-THC

Table 2. Semi-synthetic cannabinoids to be integrated into the existing method.

CBDP	Δ9-THCP
9(S)-HHC	9(R)-HHC
(9aR, 9R)-Δ10-THC	Δ9-THC-O-Acetate

The conditions utilized in the original method are shown in Table 3. The prepared sample was analyzed using these method conditions. Based on the results, the method conditions were altered to improve the chromatographic separation of the analytes. Full resolution of all isomers was required. Compounds which are isomers have been highlighted in the retention time results tables.

Results

Table 3. Original method conditions for the analysis of six THC isomers in whole blood.

Analytical Column	Raptor FluoroPhenyl, 100 x 3 mm, 2.7 µm		
Mobile Phase A	Water, 0.1% formic acid		
Mobile Phase B	Methanol, 0.1% formic acid		
Column Temperature	40 °C		
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 2. Separation of six THC isomers using original method conditions. All three pairs of isomers are fully resolved for accurate quantitation.

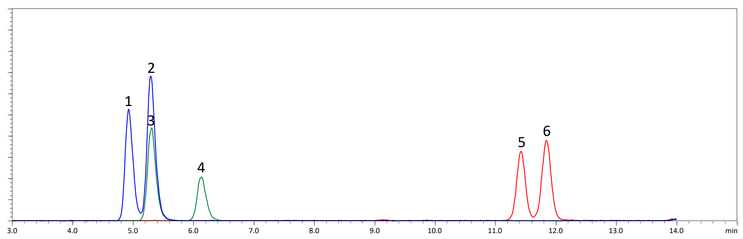


Table 4. Peak ID and retention times of six THC isomers using original method conditions.

Peak #	Analyte	RT (min)	Peak #	Analyte	RT (min)
1	11-OH-Δ8-THC	4.94	**4**	Δ9-THC-COOH	6.15
2	11-OH-Δ9-THC	5.31	**5**	Δ8-THC	11.45
3	Δ8-THC-COOH	5.32	**6**	Δ9-THC	11.88

Figure 3. Separation of six THC isomers + six semi-synthetic cannabinoids using original method conditions. While all isomers are resolved, three of the semi-synthetic cannabinoids are highly retained on the column and do not elute until ~14 minutes, after the gradient has ramped up to 100% organic. This causes poor peak shape and potential interference with hydrophobic/lipophilic matrix components.

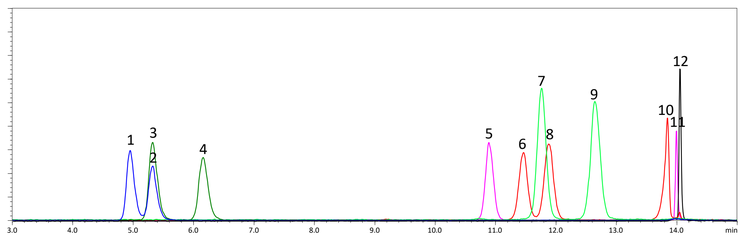


Table 5. Peak ID and retention times of six THC isomers and six semi-synthetic cannabinoids using original method conditions.

Peak #	Analyte	RT (min)	Peak #	Analyte	RT (min)
1	11-OH-Δ8-THC	4.94	**7**	9(S)-HHC	11.76
2	11-OH-Δ9-THC	5.31	**8**	Δ9-THC	11.88
3	Δ8-THC-COOH	5.32	**9**	9(R)-HHC	12.64
4	Δ9-THC-COOH	6.15	**10**	(6aR, 9R)-Δ10-THC	13.83
5	CBDP	10.88	**11**	Δ9-THCP	13.99
6	Δ8-THC	11.45	**12**	Δ9-THC-O-Acetate	14.05

Results

Table 6. Updated method conditions for the analysis of six THC isomers and six semi-synthetic cannabinoids. The gradient has been modified and the column temperature decreased from 40 °C to 30 °C.

Analytical Column	Raptor FluoroPhenyl, 100 x 3 mm, 2.7 µm		
Mobile Phase A	Water, 0.1% formic acid		
Mobile Phase B	Methanol, 0.1% formic acid		
Column Temperature	30°C		
Flow Rate	0.8 mL/min		
Gradient	Time (min)	%A	%B
	0.00	34	66
	5.50	34	66
	5.60	29	71
	10.50	29	71
	11.00	15	85
	13.00	0	100
	14.00	0	100
	14.10	34	66
	16.00	34	66

Figure 4. Separation of six THC isomers and six semi-synthetic cannabinoids using updated method conditions. All isomers are fully resolved, and the three highly retained semi-synthetic cannabinoids are now eluting prior to the gradient ramping up to 100% organic, improving resolution and peak shape of these compounds.

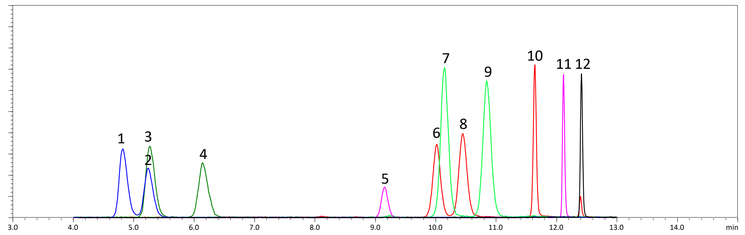


Table 7. Peak ID and retention times of six THC isomers and six semi-synthetic cannabinoids using updated method conditions.

Peak #	Analyte	RT (min)	Peak #	Analyte	RT (min)
1	11-OH-Δ8-THC	4.81	**7**	9(S)-HHC	10.14
2	11-OH-Δ9-THC	5.24	**8**	Δ9-THC	10.45
3	Δ8-THC-COOH	5.27	**9**	9(R)-HHC	10.84
4	Δ9-THC-COOH	6.15	**10**	(6aR, 9R)-Δ10-THC	11.63
5	CBDP	9.15	**11**	Δ9-THCP	12.11
6	Δ8-THC	10.02	**12**	Δ9-THC-O-Acetate	12.40

Discussion

Results show, six emerging semi-synthetic cannabinoids were successfully incorporated into an existing method for the analysis of THC isomers. This work highlights the importance of choosing a stationary phase which shows optimal selectivity for the analyte class being analyzed, allowing new analytes to easily be integrated into the method as they emerge.

While the original method could resolve all critical isomer pairs, however, three of the semi-synthetic cannabinoids were strongly retained on the stationary phase and eluted after the gradient had ramped up to 100% organic. A hold of 100% organic is often employed in HPLC methods to elute or "wash" everything off the column, such as hydrophobic/highly lipophilic matrix components. This step helps to reduce carryover in the next sample. It is not ideal to have analytes eluting in this area, as hydrophobic matrix components can co-elute and cause interference, as well as compromise peak shape. By adjusting the original gradient and lowering the column temperature, separation of all critical pairs was achieved, and the late eluting compounds were moved out of this "wash" region without needing to extend the method run time.