Analysis of Δ⁸ and Δ⁹-THC Metabolites and Other Cannabinoids in Urine

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INTRODUCTION

In recent years, there has been a notable increase in the use and commercialization of Δ⁸-THC. This increase stems from the passing of the Farm Bill of 2018 which legalized hemp. The main cannabinoid of hemp is CBD which can be converted into Δ⁸-THC. Labs worked to be able to separate parent THC isomers Δ⁸ and Δ⁹-THC. However, tetrahydrocannabinol (THC) is extensively metabolized by the body into 11-nor-9-carboxy-THC (COOH-THC) and 11-hydroxy-THC (OH-THC). Therefore, quantitation and identification of THC's metabolites is critical for proper identification and interpretation.The newest challenge many laboratories face is the separation of the isomeric metabolites Δ⁸-COOH-THC, Δ⁹-COOH-THC and Δ⁸-OH-THC and Δ⁹-OH-THC.

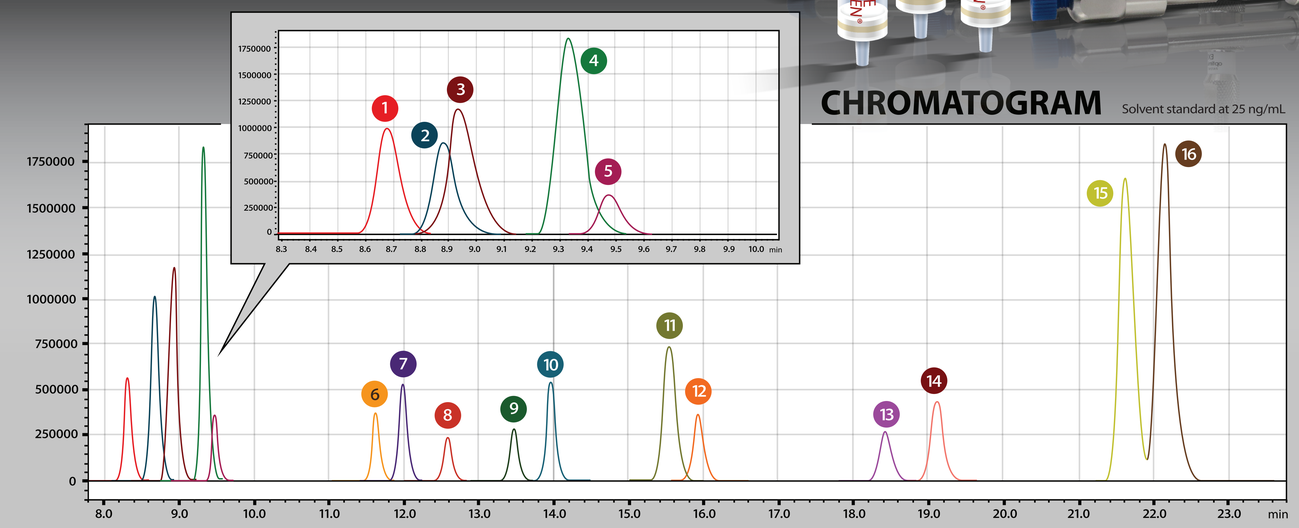
Additionally with the expanding cannabis market, new cannabinoids continue to emerge. One example includes, Δ^{10} and $\Delta^{6a,10a}$-THC which occur naturally in marijuana at low levels, but can be artificially synthesized. THC-O-acetate (THC-O) is a new semi-synthetic cannabinoid that is synthesized by adding acetic anhydride to THC. These three examples are included in the panel with other emerging cannabinoids.

INSTRUMENT PARAMETERS

LC-MS/MS System	Shimadzu Nexera LC-30AD with MS-8050
HPLC Column	SelectraCore® PFPP Column 100 x 2.1 mm, 2.7 µm (PN: SCS27-PFP1021)
Guard Column	SelectraCore® PFPP Guard Column 5 x 2.1 mm, 2.7 µm (PN: SCS27-PFPGDC21)
Column Temperature	35°C
Flow Rate	0.3 mL/min
Injection Volume	10 µL
Mobile Phase A	5 mM Ammonium Formate + 0.1% Formic Acid in DI H_2O
Mobile Phase B	Methanol
Gradient	Conc. B 45% (0 min) - 70% (6 min) - 75% (19-24.5 min) - 45% (24.6-27.6 min)

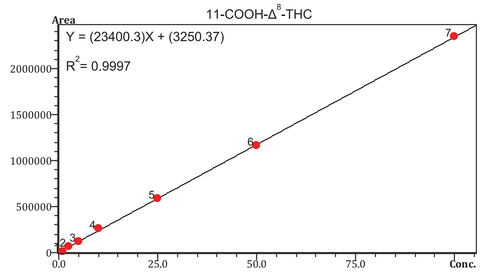
CHROMATOGRAM

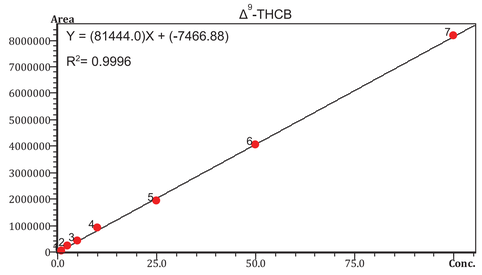
Solvent standard at 25 ng/mL

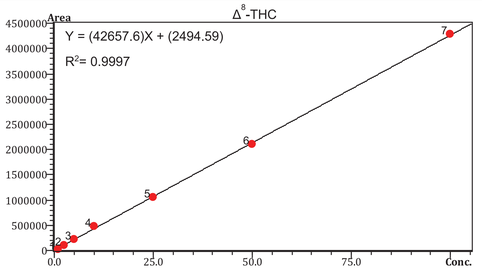


1 11-OH-Δ⁸-THC	5 CBD	9 Δ⁸-THC	13 Δ⁸-THCP
2 11-COOH-Δ⁸-THC	6 Δ⁸-THCB	10 Δ⁹-THC	14 Δ⁹-THCP
3 11-OH-Δ⁹-THC	7 Δ⁹-THCB	11 Δ¹⁰-THC / Δ⁶ᵃ,¹⁰ᵃ-THC	15 Δ⁸-THC-O-Acetate
4 11-COOH-Δ⁹-THC	8 exo-THC	12 CBN	16 Δ⁹-THC-O-Acetate

CALIBRATION CURVES







*Example 7 point solvent calibration curves with linear equation and r² value [1, 2.5, 5, 10, 25, 50, 100 ng/mL]

SPE PROCEDURE

Styre Screen® HLB 60 mg, 6 mL (PN: SSHLB066)

SAMPLE PREP
1 mL urine sample + ISTD + 1 mL ACN + 1 mL of 100 mM phosphate buffer pH 7.0, vortex

CONDITION
3 mL MeOH
3 mL 100 mM phosphate buffer pH 7.0

LOAD
Load sample and aspirate 1-2 mL/min

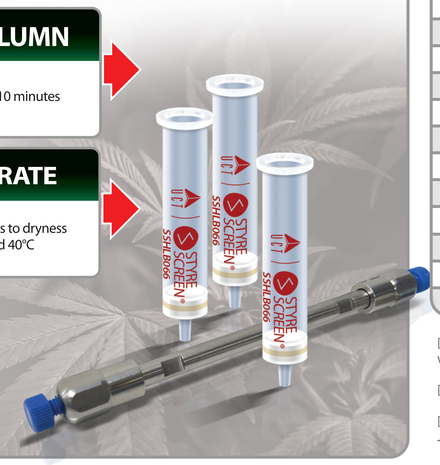
WASH
3 mL DI H_2O
3 mL 40:60 MeOH:DI H_2O

DRY COLUMN
Dry for at least 10 minutes

ELUTE
3 mL of 60:40 MeOH:Hexane
*make elution solvent daily and shake/vortex well before use

EVAPORATE
Evaporate samples to dryness at 10 psi and 40°C

RECONSTITUTE
1 mL of 45:55 MeOH:DI H_2O or other appropriate solvent and volume



RESULTS

URINE n=3	5 ng/mL			25 ng/mL			50 ng/mL		
	Recovery	Matrix Effect	RSD	Recovery	Matrix Effect	RSD	Recovery	Matrix Effect	RSD
11-OH-Δ⁸-THC	104%	-20%	3%	83%	-6%	11%	88%	-3%	10%
11-COOH-Δ⁸-THC	99%	-16%	4%	83%	-4%	8%	84%	-1%	6%
11-OH-Δ⁹-THC	99%	-11%	2%	83%	0%	14%	86%	6%	8%
11-COOH-Δ⁹-THC	105%	-21%	13%	82%	-9%	10%	86%	-8%	8%
CBD	118%	-24%	6%	85%	-12%	8%	85%	-7%	8%
Δ⁸-THCB	106%	-14%	4%	85%	-8%	11%	83%	4%	8%
Δ⁹-THCB	106%	-18%	4%	84%	-9%	13%	84%	7%	9%
exo-THC	104%	-8%	7%	86%	-1%	15%	84%	4%	9%
Δ⁸-THC	108%	-13%	5%	87%	2%	13%	86%	6%	7%
Δ⁹-THC	106%	-20%	3%	86%	3%	10%	84%	7%	9%
Δ¹⁰-THC/Δ⁶ᵃ,¹⁰ᵃ-THC	110%	14%	2%	83%	25%	9%	90%	11%	6%
CBN	97%	-2%	3%	80%	5%	13%	78%	3%	7%
Δ⁸-THCP	111%	-15%	4%	85%	-4%	11%	83%	-2%	9%
Δ⁹-THCP	109%	-16%	2%	88%	-7%	10%	85%	-6%	9%
Δ⁸-THC-O-Acetate	117%	-13%	1%	95%	-8%	3%	87%	-11%	3%
Δ⁹-THC-O-Acetate	117%	-19%	1%	96%	-16%	4%	90%	-3%	4%

[1] "5 Things to Know about Delta-8 Tetrahydrocannabinol – Delta-8 THC." U.S. Food and Drug Administration, 4 May 2022, www.fda.gov/consumers/consumer-updates/5-things-know-about-delta-8-tetrahydrocannabinol-delta-8-thc.

[2] Mallen, Briana. "Is Delta 10 Natural or Synthetic?" Secret Nature, 1 Sept. 2021, secretnaturecbd.com/blogs/cbd/is-delta-10-natural-or-synthetic.

[3] Alaina K Holt and others, Δ⁸-THC, THC-O Acetates and CBD-di-O Acetate: Emerging Synthetic Cannabinoids Found in Commercially Sold Plant Material and Gummy Edibles, Journal of Analytical Toxicology, Volume 46, Issue 8, October 2022,Pages 940–948, https://doi.org/10.1093/jat/bkac036

CONCLUSION

Questions / Comments: **methods@unitedchem.com**

UCT's SelectraCore® PFPP column and methanol as mobile phase B proved to be the best combination to achieve separation of the four isomeric metabolites. This method separated a total of 16 cannabinoids. Unfortunately, the PFPP column was not able to separate Δ^{10}-THC and $\Delta^{6a,10a}$-THC. Additionally, baseline separation of the THC-O-Acetate isomers were also not achieved. Analytes were extracted from urine utilizing UCT's Styre Screen® HLB solid phase extraction column. After optimization, the extraction's recovery, matrix effect, and relative standard deviation were evaluated at three concentrations (5, 25, 50 ng/mL). Recoveries for all analytes were acceptable, above 75% (range 78-118%). Matrix effects and relative standard deviations were within ANSI/ASB Standard 063 guidelines. Matrix effects were within ± 25% and the RSDs were less than 20% (range 1-15%).