Enhanced LC-MS/MS Separation of Δ[8] and Δ[9] -THC Metabolites and Other Cannabinoids Extracted from Urine Samples using a Polymeric SPE Column

UCT Part Numbers

SSHLB066
Styre Screen® HLB
60 mg, 6 mL

SCS27-PFPGDC21
SelectraCore® PFPP Guard Column 5 x 2.1 mm, 2.7 µm

SPHPHO7001-10
Select pH buffer pouch
100 mM Phosphate Buffer pH 7.0

SCS27-PFP1021
SelectraCore® PFPP Column
100 x 2.1 mm, 2.7 µm

SLGRDHLDR-HPOPT
Selectra® Direct Connect Guard Holder

Introduction:

In recent years, there has been a notable increase in the use and commercialization of Δ[8] -THC products. The rise in numbers is attributed to the legalization of hemp through the Farm Bill of 2018. The main cannabinoid of hemp is CBD which can be converted into Δ[8] -THC.[1] This resulted in drug testing laboratories, needing to be able to separate parent THC isomers Δ[8] and Δ[9] -THC. However, tetrahydrocannabinol (THC) is extensively metabolized by the body into 11-nor-9-carboxyTHC (COOH-THC) and 11-hydroxy-THC (OH-THC). Therefore, quantitation and identification of THC's metabolites are critical for proper identification and interpretation. The newest challenge many laboratories face is the separation of the isomeric metabolites. Δ[8] -COOH-THC and Δ[9] -COOH-THC can be separated on a core-shell C18 column; however, Δ[8] -OH-THC and Δ[9] -OH-THC continue to be a challenge.

Additionally, with the expanding cannabis market, new cannabinoids continue to emerge. One example includes Δ[10] and Δ[6a,10a] -THC which occur naturally in marijuana at low levels but can be artificially synthesized to obtain higher concentrations.[2] THC-O-Acetate (THC-O) is a new semi-synthetic cannabinoid that is synthesized by adding acetic anhydride to THC.[3] These three examples are included in the panel with other emerging cannabinoids. This application note introduces an LC-MS/MS method that separates 16 cannabinoids using UCT's SelectraCore® PFPP column. This includes the separation of the isomeric metabolites of Δ[8] and Δ[9] -THC. A solid phase extraction from urine is also introduced using UCT's Styre Screen® HLB column.

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Sample Pretreatment:

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

Note: Include a hydrolysis procedure to recover conjugated analytes.

SPE Procedure:

1. Condition Column:
- a) 1 x 3 mL MeOH
- b) 1 x 3 mL 100 mM phosphate buffer pH 7.0

2. Load Sample:
- a) Load sample and aspirate 1-2 mL/min

3. Wash Column:
- a) 1 x 3 mL DI H_2O
- b) 1 x 3 mL 40:60 MeOH: DI H_2O

4. Dry Column:
- a) Dry for at least 10 minutes at full vacuum or pressure

5. Elute Analytes:
- a) 1 x 3 mL of 60:40 MeOH:Hexane

Note: Make elution solvent daily and shake/vortex well before use

6. Evaporate:
- a) Evaporate samples to dryness at 10 psi and 40°C

7. Reconstitute:
- a) Reconstitute samples in 1 mL of 45:55 MeOH:DI H_2O or other appropriate solvent and volume

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Instrument Parameters	
LC-MS/MS System	Shimadzu Nexera LC-30AD with MS-8050
UHPLC 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

Gradient Program		
Time (min)	**Mobile Phase A (%)** 5 mM Ammonium Formate + 0.1% Formic Acid in DI H_2O	**Mobile Phase B (%)** Methanol
0	55	45
6	30	70
19-24.5	36	74
24.6-27.6	55	45

MRM Table						
Analyte	**R.T. (min)**	**Parent Ion (m/z)**	**Product Ion 1 (m/z)**	**CE (V)**	**Product Ion 2 (m/z)**	**CE (V)**
11-OH-Δ⁸ -THC	8.67	330.7	313.1	-16	201.0	-26
11-COOH-Δ⁸ -THC	8.87	344.7	299.2	-21	327.2	-18
11-OH-Δ⁹ -THC	8.93	300.9	313.2	-15	267.2	-20
11-COOH-Δ⁹ -THC	9.33	344.9	327.2	-17	299.2	-19
CBD	9.47	314.9	193.2	-23	123.1	-35
Δ⁸ -THCB	11.61	301.1	179.3	-22	123.0	-35
Δ⁹ -THCB	11.98	301.1	179.1	-24	23.0	-32
exo-THC	12.58	314.7	193.1	-24	123.0	-33
Δ⁸ -THC	13.47	314.9	193.1	-23	123.1	-35
Δ⁹ -THC	13.95	314.9	193.1	-24	123.1	-35
Δ^{10} /$\Delta^{6a,10a}$-THC	15.54	314.9	193.25	-24	123.1	-37
CBN	15.90	311.2	223.2	-21	293.2	-16
Δ⁸ -THCP	18.41	342.7	221.1	-25	122.9	-35
Δ⁹ -THCP	19.10	342.7	221.1	-24	123.0	-35
Δ⁸ -THC-O-Acetate	21.62	356.7	315.2	-18	193.1	-30
Δ⁹ -THC-O-Acetate	22.15	356.7	315.2	-17	193.1	-29

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Chromatogram:

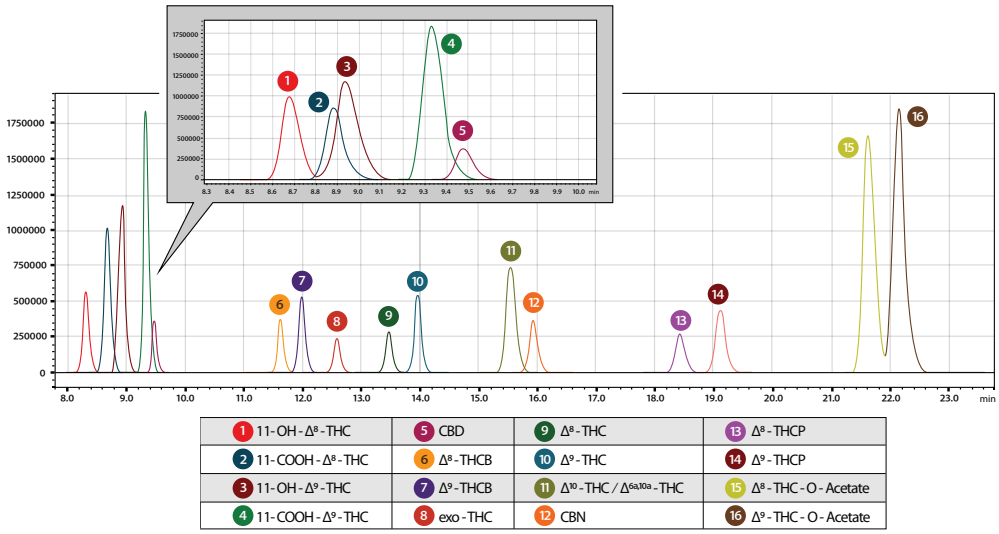
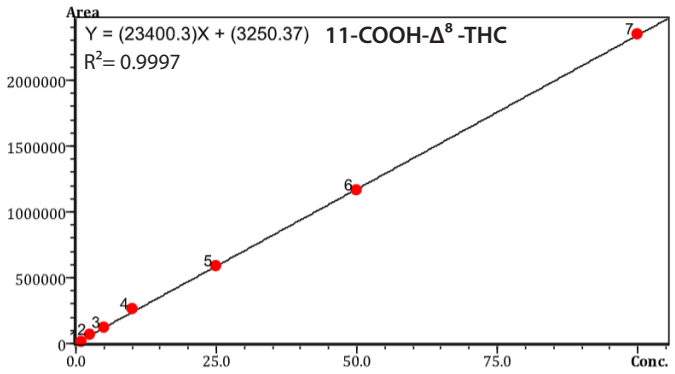
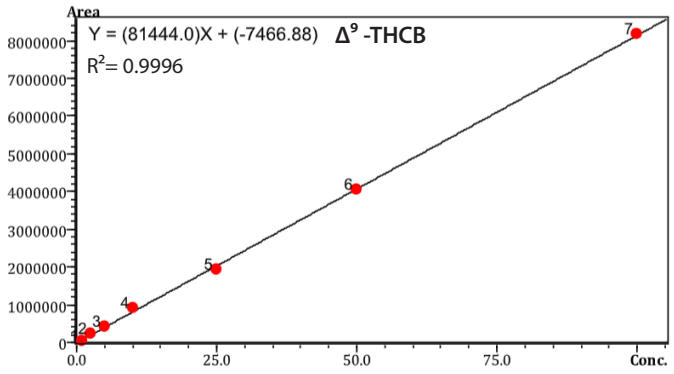


Figure 1: Chromatogram of Solvent Standard Mix Prepared at 25 ng/mL

Example Solvent Calibration Curves:





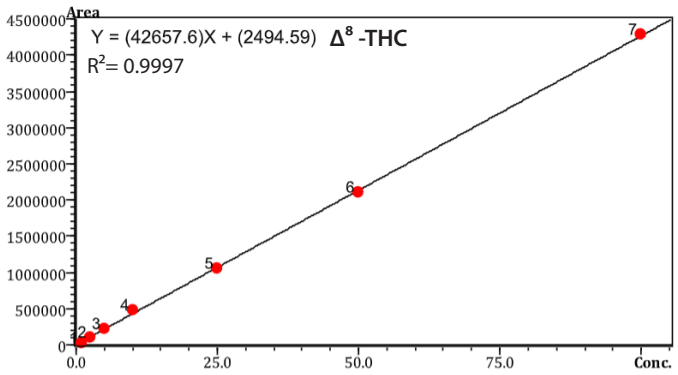


Figure 2: A 7-point solvent calibration curve for 11-COOH-Δ⁸ -THC, Δ⁹ -THCB, and Δ⁸ -THC with linear equation and R^2 Value (1, 2.5, 5, 10, 25, 50, and 100 ng/mL)

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Results									
n=3	5 ng/mL			25 ng/mL			50 ng/mL		
Analyte	**Recovery**	**Matrix Effects**	**RSD**	**Recovery**	**Matrix Effects**	**RSD**	**Recovery**	**Matrix Effects**	**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%

**Recoveries were calculated using a pre and post-spiked sample technique. Matrix effects were calculated by comparing post-spiked and solvent standards.*

Conclusion:

UCT's SelectraCore® PFPP column with 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. The PFPP column was not able to separate Δ¹⁰-THC and Δ⁶ᵃ,¹⁰ᵃ -THC. Additionally, baseline separation of the THC-O-Acetate isomers was 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, and 50 ng/mL). Recoveries for all analytes were 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%).

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References:

[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

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