# Separation of $\Delta^8$ and $\Delta^9$ THC Metabolites and Other Cannabinoids in Urine

## UCT Part Numbers

**SSHLB066**
Styre Screen®
HLB 60 mg, 6 mL

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

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

**SLGRDHLDR-HPOPT**
Selectra® Direct Connect
Guard Holder

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

## Introduction:

In recent years, there has been a notable increase in the use and commercialization of $\Delta^8$ -THC products. 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 $\Delta^8$ -THC [1]. This resulted in drug testing laboratories, needing to be able to separate parent THC isomers $\Delta^8$ and $\Delta^9$ -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 are critical for proper identification and interpretation. The newest challenge many laboratories face is the separation of the isomeric metabolites. $\Delta^8$ -COOH-THC and $\Delta^9$ -COOH-THC can be separated on a core-shell C18 column; however, $\Delta^8$ -OH-THC and $\Delta^9$ -OH-THC continue to be a challenge.

Additionally, with the expanding cannabis market, new cannabinoids continue to emerge. One example includes $\Delta^{10}$ and $\Delta^{6a,10a}$ -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 $\Delta^8$ and $\Delta^9$ THC. A solid phase extraction from urine is also introduced using UCT's Styre Screen® HLB column.

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# SPE Extraction:

**Sample Preparation:** 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.

**Condition:**
1 x 3 mL MeOH
1 x 3 mL 100 mM phosphate buffer pH 7.0

**Load:**
Load sample and aspirate 1-2 mL/min

**Wash:**
1 x 3 mL DI $H_2O$
1 x 3 mL 40:60 MeOH: DI $H_2O$

**Dry Column:**
Dry for at least 10 minutes

**Elute:**
1 x 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:**
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 |
| **Mobile Phase A** | 5 mM Ammonium Formate + 0.1% Formic Acid in DI $H_2O$ |
| **Mobile Phase B** | Methanol |

| Gradient | | |
|---|---|---|
| **Time (min)** | **Mobile Phase A (%)** | **Mobile Phase B (%)** |
| 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-$\Delta^8$-THC | 8.67 | 330.7 | 313.1 | -16 | 201.0 | -26 |
| 11-COOH-$\Delta^8$-THC | 8.87 | 344.7 | 299.2 | -21 | 327.2 | -18 |
| 11-OH-$\Delta^9$-THC | 8.93 | 300.9 | 313.2 | -15 | 267.2 | -20 |
| 11-COOH-$\Delta^9$-THC | 9.33 | 344.9 | 327.2 | -17 | 299.2 | -19 |
| CBD | 9.47 | 314.9 | 193.2 | -23 | 123.1 | -35 |
| $\Delta^8$-THCB | 11.61 | 301.1 | 179.3 | -22 | 123.0 | -35 |
| $\Delta^9$-THCB | 11.98 | 301.1 | 179.1 | -24 | 23.0 | -32 |
| exo-THC | 12.58 | 314.7 | 193.1 | -24 | 123.0 | -33 |
| $\Delta^8$-THC | 13.47 | 314.9 | 193.1 | -23 | 123.1 | -35 |
| $\Delta^9$-THC | 13.95 | 314.9 | 193.1 | -24 | 123.1 | -35 |
| $\Delta^{10}$ /$\Delta^{6a,10a}$-THC | 15.54 | 314.9 | 193.25 | -24 | 123.1 | -37 |
| CBN | 15.90 | 311.2 | 223.2 | -21 | 293.2 | -16 |
| $\Delta^8$-THCP | 18.41 | 342.7 | 221.1 | -25 | 122.9 | -35 |
| $\Delta^9$-THCP | 19.10 | 342.7 | 221.1 | -24 | 123.0 | -35 |
| $\Delta^8$-THC-O-Acetate | 21.62 | 356.7 | 315.2 | -18 | 193.1 | -30 |
| $\Delta^9$-THC-O-Acetate | 22.15 | 356.7 | 315.2 | -17 | 193.1 | -29 |

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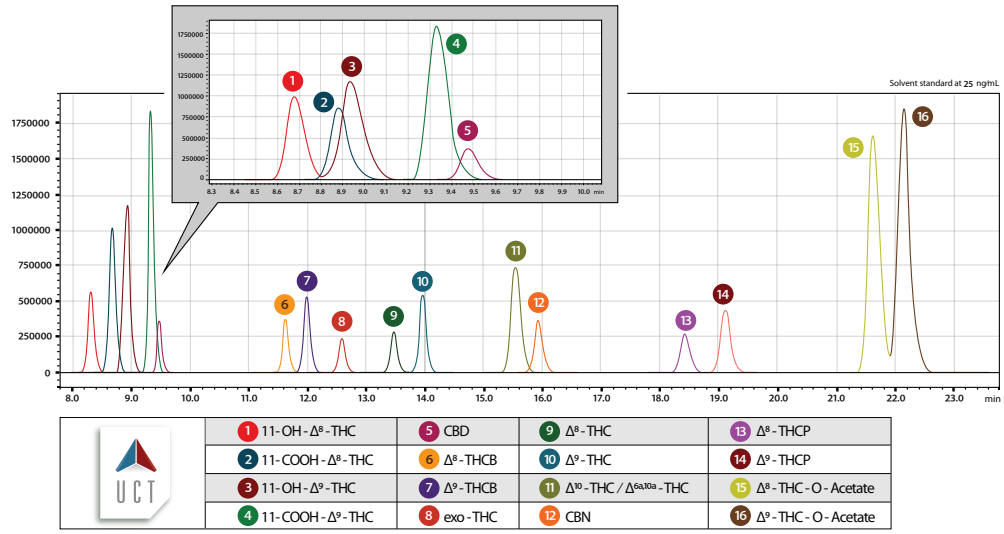
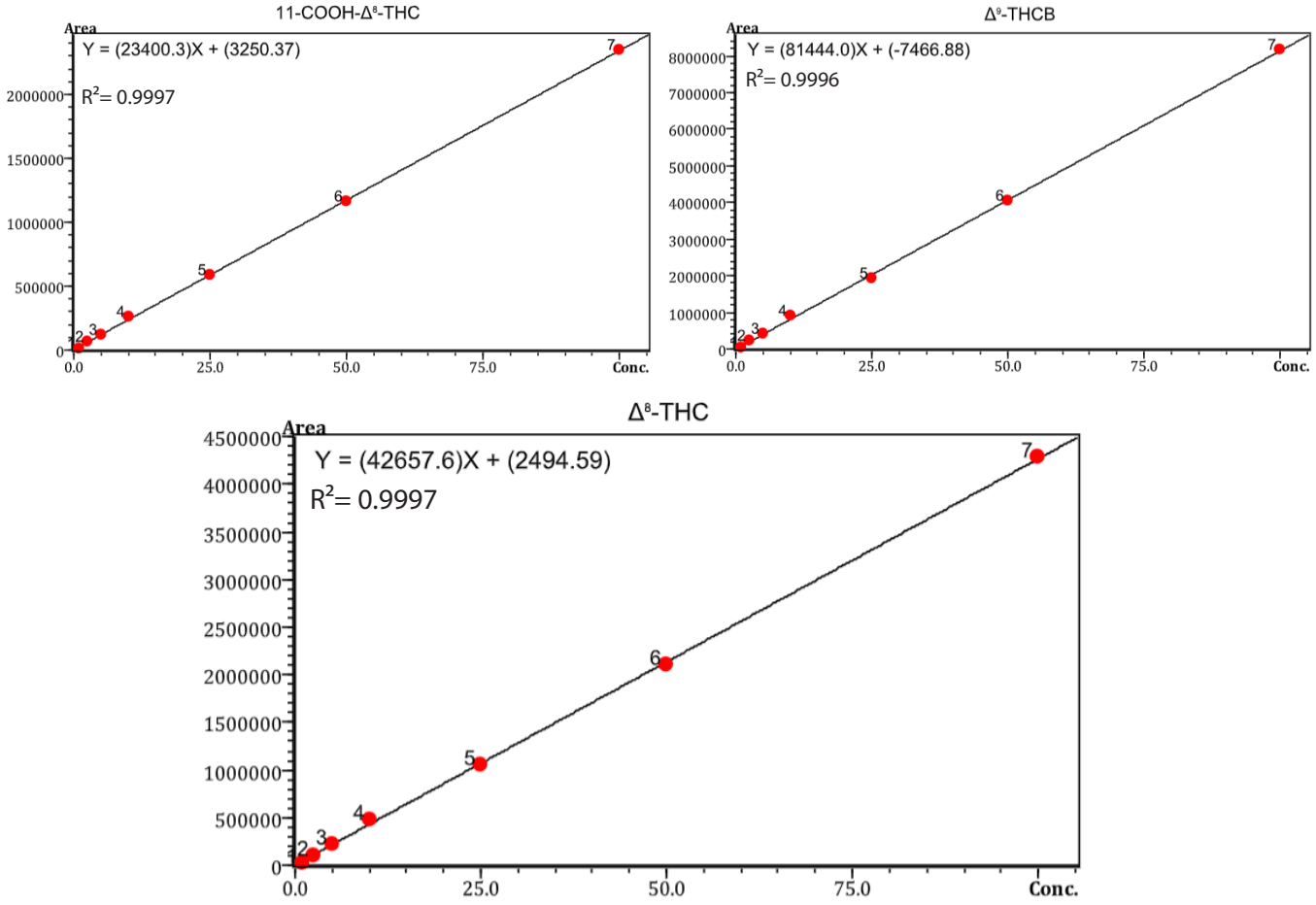
# Chromatogram:




| | | | |
|---|---|---|---|
| 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 r 2 value [1, 2.5, 5, 10, 25, 50, 100 ng/mL]*

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| Results | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Urine n=3** | **5 ng/mL** | | | **25 ng/mL** | | | **50 ng/mL** | | |
| | **Recovery** | **Matrix Effects** | **RSD** | **Recovery** | **Matrix Effects** | **RSD** | **Recovery** | **Matrix Effects** | **RSD** |
| 11-OH-$\Delta^8$-THC | 104% | -20% | 3% | 83% | -6% | 11% | 88% | -3% | 10% |
| 11-COOH-$\Delta^8$-THC | 99% | -16% | 4% | 83% | -4% | 8% | 84% | -1% | 6% |
| 11-OH-$\Delta^9$-THC | 99% | -11% | 2% | 83% | 0% | 14% | 86% | 6% | 8% |
| 11-COOH-$\Delta^9$-THC | 105% | -21% | 13% | 82% | -9% | 10% | 86% | -8% | 8% |
| CBD | 118% | -24% | 6% | 85% | -12% | 8% | 85% | -7% | 8% |
| $\Delta^8$ -THCB | 106% | -14% | 4% | 85% | -8% | 11% | 83% | 4% | 8% |
| $\Delta^9$ -THCB | 106% | -18% | 4% | 84% | -9% | 13% | 84% | 7% | 9% |
| exo-THC | 104% | -8% | 7% | 86% | -1% | 15% | 84% | 4% | 9% |
| $\Delta^8$ -THC | 108% | -13% | 5% | 87% | 2% | 13% | 86% | 6% | 7% |
| $\Delta^9$ -THC | 106% | -20% | 3% | 86% | 3% | 10% | 84% | 7% | 9% |
| $\Delta^{10}$-THC/$\Delta^{6a,10a}$-THC | 110% | 14% | 2% | 83% | 25% | 9% | 90% | 11% | 6% |
| CBN | 97% | -2% | 3% | 80% | 5% | 13% | 78% | 3% | 7% |
| $\Delta^8$ -THCP | 111% | -15% | 4% | 85% | -4% | 11% | 83% | -2% | 9% |
| $\Delta^9$ -THCP | 109% | -16% | 2% | 88% | -7% | 10% | 85% | -6% | 9% |
| $\Delta^8$ -THC-O-Acetate | 117% | -13% | 1% | 95% | -8% | 3% | 87% | -11% | 3% |
| $\Delta^9$ -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 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. The PFPP column was not able to separate $\Delta^{10}$-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%).

## 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, $\Delta^8$ -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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