

# Analysis of Natural Cannabinoids and Metabolites from Urine Using Styre Screen® HLB SPE Column and SelectraCore® C18 on LC-MS/MS



## UCT Part Numbers

**SSHLB063**Styre Screen® HLB  
3 mL, 60 mg sorbent**SCS27-C181021**SelectraCore® C18 Column  
100 X 2.1 mm, 2.7 µm**SPPHO7001-10**Select pH buffer pouch  
100 mM phosphate pH 7.0**SLGRDHLDR-HPOPT**UHPLC Direct Connect  
Guard Holder**SCS27-C18GDC21**SelectraCore® C18 Guard Column  
5 X 2.1 mm, 2.7 µm

## Introduction:

Natural cannabinoids are chemical compounds that can be found in the *Cannabis* plant. Over a hundred different cannabinoids have been identified so far. The legal definition of marijuana encompasses all parts of the *Cannabis* plant, whether they are growing or not, and contain more than 0.3% dry weight of  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC).<sup>1</sup>  $\Delta^9$ -THC is known for its psychoactive and euphoric effects and is abundant in the *Cannabis Sativa* subspecies.<sup>2</sup> Marijuana is widely used as a recreational drug in the United States and across the globe. Although marijuana is federally illegal in the United States, Colorado became the first state to legalize it for recreational use in 2012, and many other states have followed suit by legalizing it for both recreational and medical purposes. As new state laws emerge, it is crucial to have accurate and precise methods for quantifying cannabinoids from biological samples.

This application note presents a solid phase extraction (SPE) procedure for extracting cannabinoids from urine samples, along with a 12-minute liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for analyzing four natural cannabinoids and the metabolites of  $\Delta^9$ -THC. The panel includes the isomers  $\Delta^9$ -THC and  $\Delta^8$ -THC, which were successfully separated using the innovative SelectraCore® C18 core-shell column.



## Sample Pretreatment:

- To 1 mL of urine add internal standard, 1 mL of acetonitrile, and 1 mL of pH 7 phosphate buffer
  - Vortex and centrifuge samples for 10 minutes at 3000 rpm
- Note:** Include a hydrolysis procedure if glucuronide compounds are to be recovered

## SPE Procedure:

### 1. Condition Column

- a) 1 x 2 mL of MeOH
- b) 1 x 2 mL of pH 7 phosphate buffer

### 2. Load Sample

- a) Load at 1 to 2 mL/minute

### 3. Wash Column

- a) 1 x 3 mL deionized water
- b) 1 x 3 mL 50% MeOH in deionized water

### 4. Dry Column

- a) Dry column for at least 10 minutes under full pressure or vacuum

### 5. Elute Analytes

- a) 1 x 3 mL of 60:40 MeOH: Hexane
- Note:** shake or vortex elution solvent well before use

### 6. Dry Eluate

- a) Evaporate eluate under a constant gentle stream of nitrogen  $\leq 40^{\circ}\text{C}$

### 7. Reconstitute

- a) Reconstitute in 1 mL of MeOH
- Note:** Alternative compatible solvents and volumes can be used



LC-MS/MS Parameters		
LC-MS/MS System	Shimadzu Nexera LC-30AD with MS-8050	
UHPLC Column	SelectraCore® C18 Column 100 x 2.1 mm, 2.7 µm (UCT P/N: <b>SCS27-C181021</b> )	
Guard Column	SelectraCore® C18 5 x 2.1 mm, 2.7 µm (UCT P/N: <b>SCS27-C18GDC21</b> )	
Column Temperature	40°C	
Flow Rate	0.4 mL/min	
Injection volume	10 µL	
Gradient Program		
Time (min)	% Mobile Phase A: 0.1% formic acid in DI H <sub>2</sub> O	% Mobile Phase B: 0.1% formic acid in MeOH
0	50	50
3	20	80
7.5	10	90
8	0	100
9	0	100
9.1	50	50
12	50	50

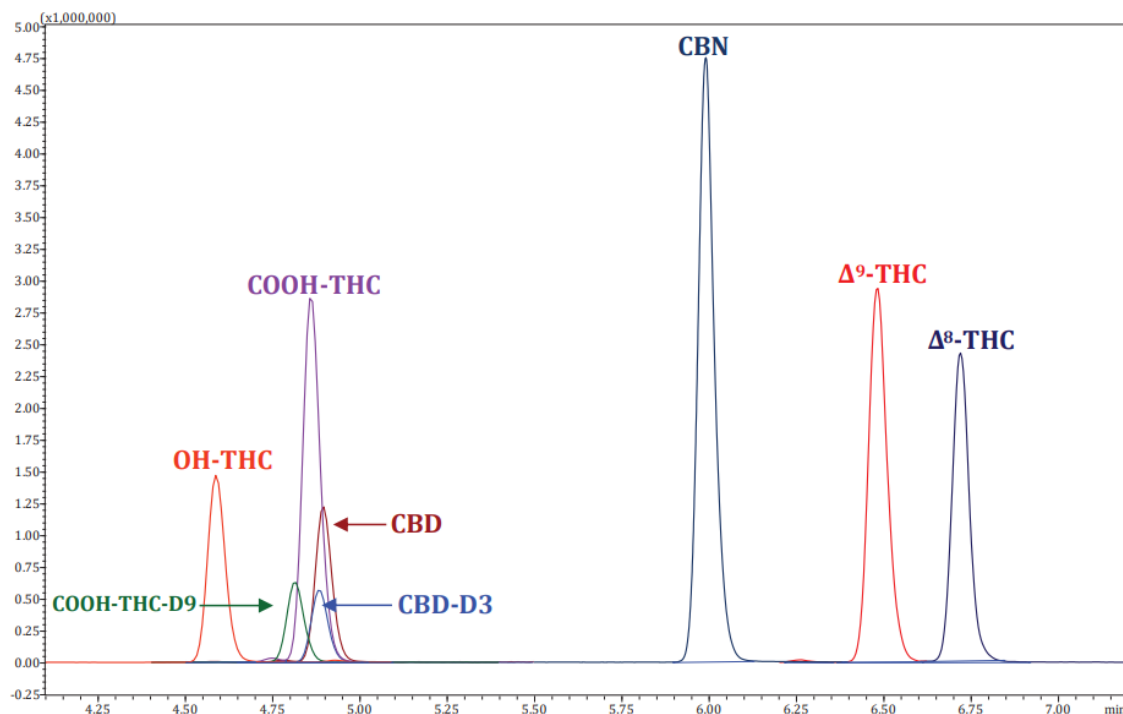
**MRM Table:**

Analyte	Parent ion (m/z)	Product ion 1 (m/z)	CE (v)	Product ion 2 (m/z)	CE (v)	RT (mins)
$\Delta^9$ -THC	314.9	193.1	24	283.1	11	6.42
$\Delta^8$ -THC	314.9	193.1	23	123.1	35	6.72
COOH-THC	344.9	327.2	17	299.2	19	4.86
OH-THC	330.9	201.2	23	193.0	26	4.58
Cannabidiol (CBD)	314.9	193.2	23	282.9	14	4.89
Cannabinol (CBN)	311.2	223.2	21	241.1	18	5.99
COOH-THC D9	354.2	336.0	16	308.2	21	4.81
CBD-D3	318.2	196.1	23	122.9	30	4.88

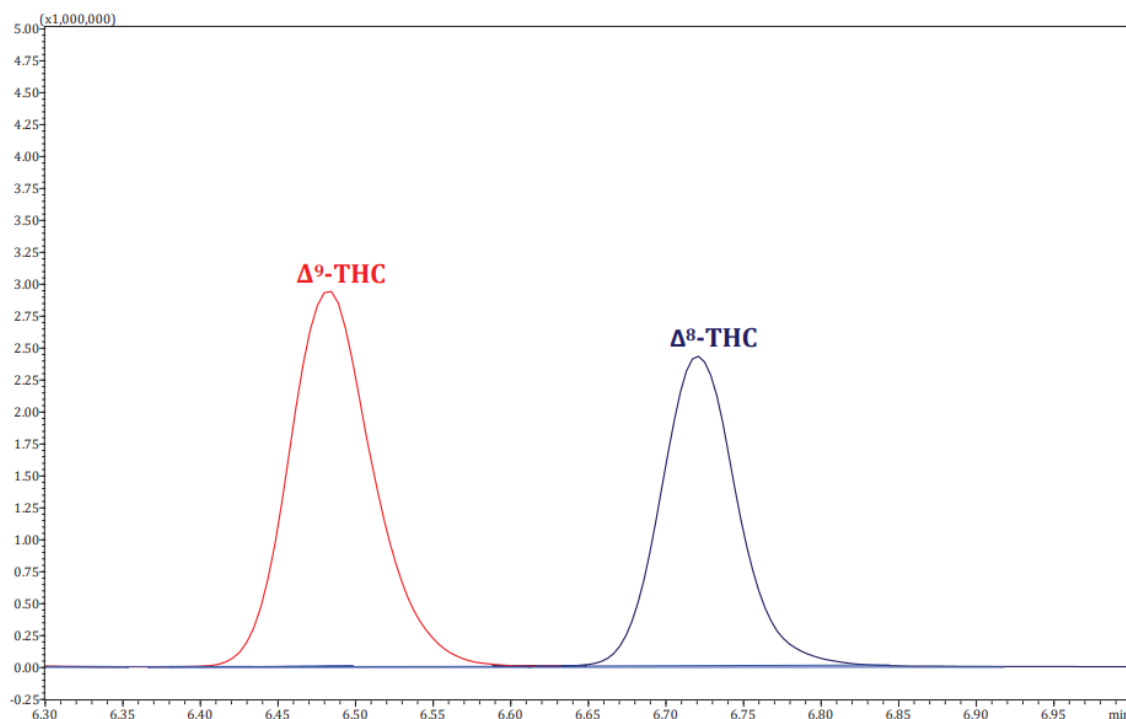
\*CE=collision energy, RT=retention time



## Chromatogram:



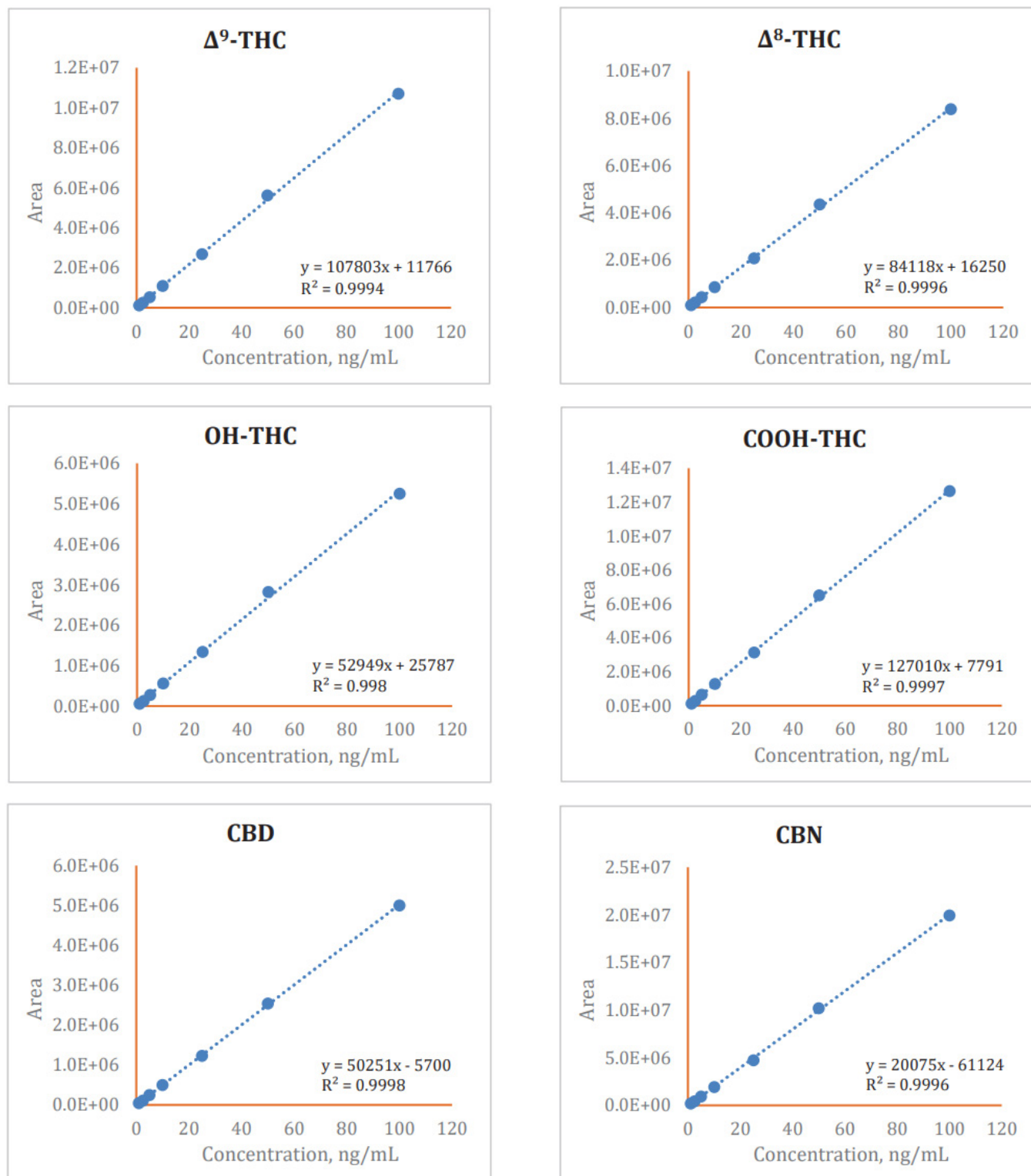
**Figure 1.** Chromatogram of extracted 50 ng/mL urine sample, minutes 4.10 – 7.20



**Figure 2.** Zoomed in Chromatogram of extracted 50 ng/mL urine sample showing the complete separation of the THC isomers, minutes 6.30 - 7.00



## Calibration Curves:



**Figure 3.** 7-point neat calibration curve for all analytes with linear equation and R<sup>2</sup> value. [1, 2.5, 5, 10, 25, 50, and 100 ng/mL]



## Results:

Absolute Recovery (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	50 ng/mL	RSD
$\Delta^9$ -THC	97%	5%	99%	2%	98%	2%
$\Delta^8$ -THC	95%	4%	96%	3%	90%	3%
OH-THC	80%	4%	84%	5%	86%	6%
COOH-THC	95%	5%	97%	4%	94%	3%
CBD	103%	6%	99%	1%	97%	2%
CBN	97%	4%	99%	1%	95%	1%

**Table 1:** Extracted samples were compared to a solvent calibration curve

Extraction Efficiency (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	50 ng/mL	RSD
$\Delta^9$ -THC	98%	4%	97%	2%	99%	2%
$\Delta^8$ -THC	93%	4%	94%	2%	95%	1%
OH-THC	103%	3%	99%	1%	105%	3%
COOH-THC	94%	7%	95%	3%	99.7%	2%
CBD	96%	4%	98%	1%	99.8%	1%
CBN	99%	4%	93%	2%	96%	1%
CBD-D3	98%	3%	96%	2%	91%	0%
COOH-THC-D9	93%	8%	94%	4%	92%	2%

**Table 2:** The Peak area of pre-spiked samples was compared to post-spiked samples

Matrix Effects (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	50 ng/mL	RSD
$\Delta^9$ -THC	-1.2%	5%	-0.4%	2%	-5.1%	2%
$\Delta^8$ -THC	-4.1%	4%	-2.1%	3%	-8.1%	3%
OH-THC	-19.4%	6%	-15.3%	5%	-22.0%	3%
COOH-THC	-12.9%	3%	-1.8%	4%	-7.6%	5%
CBD	-3.9%	6%	0.8%	1%	-4.4%	2%
CBN	-0.2%	4%	4.1%	1%	-3.2%	1%
CBD-D3	1.3%	2%	3.9%	2%	6.8%	4%
COOH-THC-D9	1.2%	3%	3.2%	2%	7.7%	3%

**Table 3:** The peak area of post-spiked samples compared to respective solvent standard in curve



## Conclusions:

A LC-MS/MS and SPE extraction method was developed for the analysis of four natural cannabinoids and the two major  $\Delta^9$ -THC metabolites in urine (OH-THC and COOH-THC). The sticky nature of these compounds can make them difficult to work with and result in low recoveries. The addition of 1 mL of acetonitrile in the sample preparation helps prevent analytes from sticking to the test tube when transferring the sample to the SPE cartridge. The LC-MS/MS method was able to successfully analyze samples in 12 minutes. Additionally, UCT's new SelectraCore® C18 core-shell column was able to separate THC isomers,  $\Delta^9$ -THC and  $\Delta^8$ -THC.

All analytes were extracted from urine using Styre Screen® HLB, a water wettable polymeric sorbent. For all the analytes in the panel, the absolute recovery from urine was equal to or greater than 80% with a relative standard deviation of less than 6%. The extraction efficiency of all analytes at low, medium & high concentrations was greater than 90% with a relative standard deviation of less than 8%. Matrix effects were minimized by washing the sorbent with deionized water and 50% methanol before eluting the compounds. Apart from COOH-THC and OH-THC, all other analytes had matrix effects between +10% and -10%. Although the matrix effects for COOH-THC and OH-THC were significant, the absolute recoveries were found to be equal to or greater than 80%. The simple and robust extraction method described in this application note can be readily implemented in high throughput forensic and clinical laboratories.

## References:

[1] 21 U.S.C. § 802 (16) (2022)

[2] Pellati, Federica et al. "Cannabis sativa L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer." BioMed research international vol. 2018 1691428. 4 Dec. 2018, doi:10.1155/2018/1691428

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