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

UCT Part Numbers

SSHLB063
Styre Screen® HLB
3mL, 60mg sorbent

SPHPHO7001-10
Select pH buffer pouch
100mM phosphate pH 7.0

SCS27-C181021
SelectraCore® C18 Column
100 mm x 2.1 mm, 2.7 µm

SCS27-C18GDC21
SelectraCore® C18 Guard Column
5 mm X 2.1 mm, 2.7 µm

SLGRDHLDR-HPOPT
UHPLC Direct Connect
Guard Holder

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Introduction:

Natural cannabinoids are compounds found in the Cannabis plant. More than a hundred cannabinoids have already been identified. The legal definition of marijuana is all parts of the Cannabis plant whether growing or not, have Δ9-tetrahydrocannabinol (Δ⁹-THC) more than 0.3% dry weight. [1] Known for its psychoactive and euphoric effects, Δ⁹-THC has the highest levels in the subspecies Cannabis Sativa. [2] Marijuana is one of the most popular drugs in the United States and around the world. In the United States, marijuana is a federally illegal drug. However, in 2012 Colorado became the first state to legalize marijuana for recreational use. Many other states have also legalized it for recreational and medical use. With new state laws, it is important to be able to accurately and precisely quantitate cannabinoids from biological matrices.

This application note outlines a solid phase extraction (SPE) procedure from urine and a 12-minute liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for four natural cannabinoids and the metabolites of Δ⁹-THC. Included in the panel are isomers Δ⁹-THC and Δ⁸-THC, which were successfully separated using the new SelectraCore® C18 core-shell column.

Sample Pretreatment:

To 1mL of urine add internal standard, 1mL of acetonitrile, and 1mL 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

Extraction Procedure:

1. **Condition Column**
   1 x 2mL of MeOH
   1 x 2mL of pH 7 phosphate buffer

2. **Load Sample**
   Load at 1 to 2 mL/minute

3. **Wash Column**
   1 x 3mL deionized water
   1 x 3mL 50% MeOH in deionized water

4. **Dry Column**
   Dry column for at least 10 minutes under full pressure or vacuum

5. **Elution**
   1 x 3mL of 60:40 MeOH: Hexane
   Note: shake or vortex elution solvent well before use

6. **Dry Eluate**
   Evaporate eluate under a constant gentle stream of nitrogen ≤ 40°C

7. **Reconstitute**
   Reconstitute in 1mL of MeOH
   Alternative compatible solvents or volumes can be used

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LC-MS/MS Parameters:

LC-MS/MS System: Shimadzu Nexara LC-30AD w/MS-8050
UHPLC Column: SelectraCore® C18 Column 100 x 2.1 mm, 2.7 µm
Guard Column: SelectraCore® C18 5 x 2.1 mm, 2.7 µm
Column Temperature: 40°C
Flow Rate: 0.4 mL/min
Injection Volume: 10 uL

Gradient Program:

Time (min.)	% Mobile Phase A: 0.1% formic acid in DI H_2O	% 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	Product ion 1	CE	Product ion 2	CE	RT (min.)
Δ^9-THC	314.9	193.1	24	283.1	11	6.42
Δ^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

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Chromatogram:

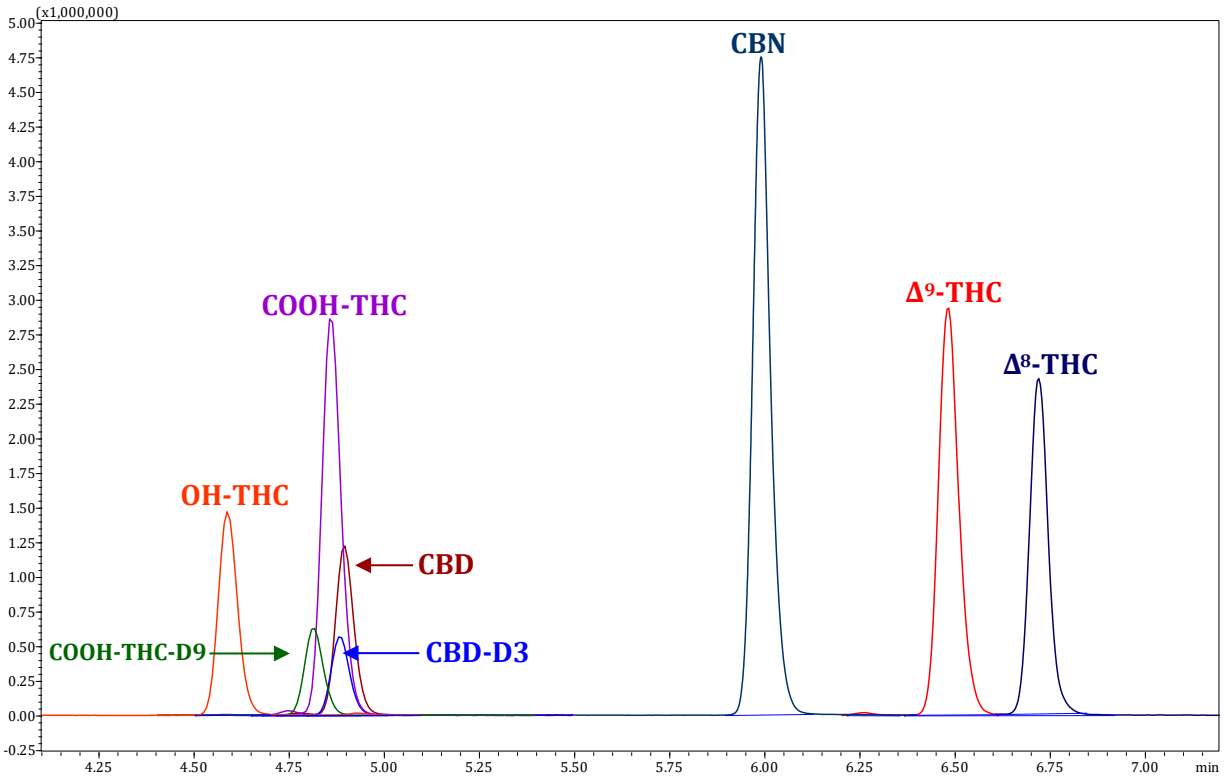

Figure 1. Chromatogram of extracted 50 ng/mL urine sample, minutes 4.10 – 7.20

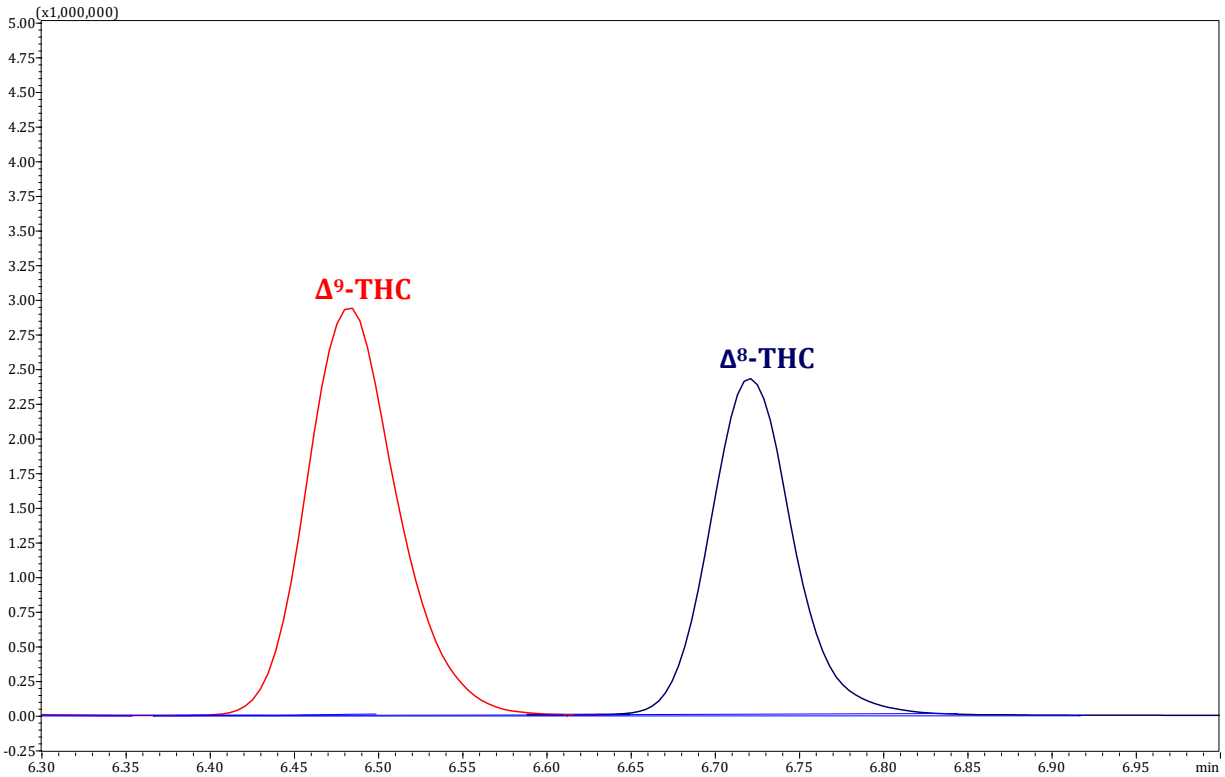

Figure 2. Zoomed in chromatogram of extracted 50 ng/mL urine sample showing the complete separation of the THC isomers, minutes 6.30 – 7.00

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Calibration Curves:

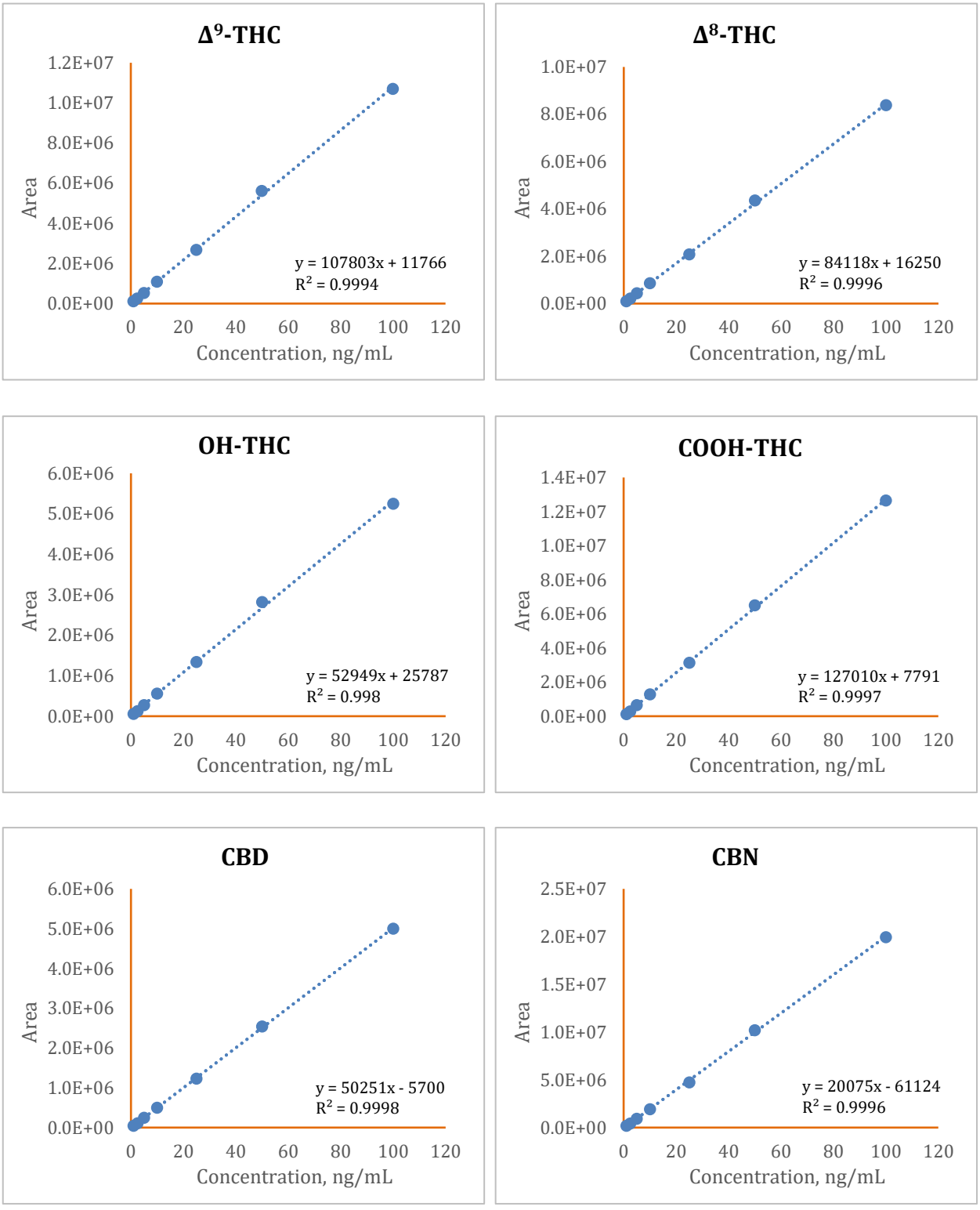

Figure 3. 7-point neat calibration curve for all analytes with linear equation and R^2 value. [1, 2.5, 5, 10, 25, 50, 100 ng/mL]

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Results:

Absolute Recovery (n=5)

Analyte	5 ng/mL	RSD	25 ng/mL	RSD	50 ng/mL	RSD
Δ^9-THC	97%	5%	99%	2%	98%	2%
Δ^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
Δ^9-THC	98%	4%	97%	2%	99%	2%
Δ^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
Δ^9-THC	-1.2%	5%	-0.4%	2%	-5.1%	2%
Δ^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 Δ⁹-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, Δ⁹-THC and Δ⁸-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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