Analysis of Natural Cannabinoids and Metabolites from Blood Using Clean Screen® THC SPE Column and SelectraCore® C18 on LC-MS/MS

UCT Part Numbers

CSTHC206
Clean Screen® THC
6 mL, 200 mg sorbent

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

SPHPHO7001-10
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:

Marijuana refers to parts or products derived from the *cannabis* plant that contains a concentration of Δ^9-THC greater than 0.3%. The *cannabis* plant contains various cannabinoids, with Δ^9-Tetrahydrocannabinol (Δ^9-THC) being the most desired compound due to the resulting effects that include altered perception of time and space, euphoria, and increased appetite.[1] With the growing number of states legalizing marijuana for recreational and medical purposes, each state has established its own laws regarding drug-impaired driving while under the influence of marijuana. Consequently, it is crucial for forensic laboratories to develop accurate and precise testing protocols.

Protein precipitation is a widely utilized technique to eliminate interferences from blood samples. It involves precipitating the proteins in the blood by modifying the pH or the hydrophobicity of the aqueous environment. Common reagents used for protein precipitation include acids, organic solvents, salts, and metals.

This application note provides a detailed procedure for protein precipitation sample preparation followed by a solid phase extraction (SPE) method to extract four natural cannabinoids and the two major metabolites of Δ^9-THC from blood samples. The analytes were extracted using the Clean Screen® THC SPE column. The LC-MS/MS parameters are also outlined which were optimized for the separation of isomers Δ^8-THC and Δ^9-THC using a SelectraCore® C18 core-shell column.

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Sample Pretreatment:

- To 0.5 mL of blood add internal standard(s) and 2 mL of ACN:Acetone (75:25)
- Vortex well and centrifuge for 10 minutes at 3000 rpm
- Decant sample into 3 mL of pH 7 phosphate buffer leaving behind blood pellet
- Vortex sample

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) 2 x 3 mL deionized water
b) 2 x 3 mL 40% 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 ACN:MeOH:Acetic Acid (89:9:2)

6. Dry Eluate

a) Evaporate eluate under a constant gentle stream of nitrogen ≤ 40°C

7. Reconstitute

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

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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: **SCS27-C181021**)
Guard Column	SelectraCore® C18 5 x 2.1 mm, 2.7 µm (UCT P/N: **SCS27-C18GDC21**)
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_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 (m/z)	Product ion 1 (m/z)	CE (v)	Product ion 2 (m/z)	CE (v)	RT (mins)
Δ^9-THC	314.9	193.1	24	283.1	11	6.57
Δ^8-THC	314.9	193.1	23	123.1	35	6.81
COOH-THC	344.9	327.2	17	299.2	19	4.94
OH-THC	330.9	201.2	23	193.0	26	4.65
Cannabidiol (CBD)	314.9	193.2	23	282.9	14	4.98
Cannabinol (CBN)	311.2	223.2	21	241.1	18	6.08
COOH-THC D9	354.2	336.0	16	308.2	21	4.88
CBD-D3	318.2	196.1	23	122.9	30	4.97

*CE=collision energy, RT=retention time

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Chromatogram:

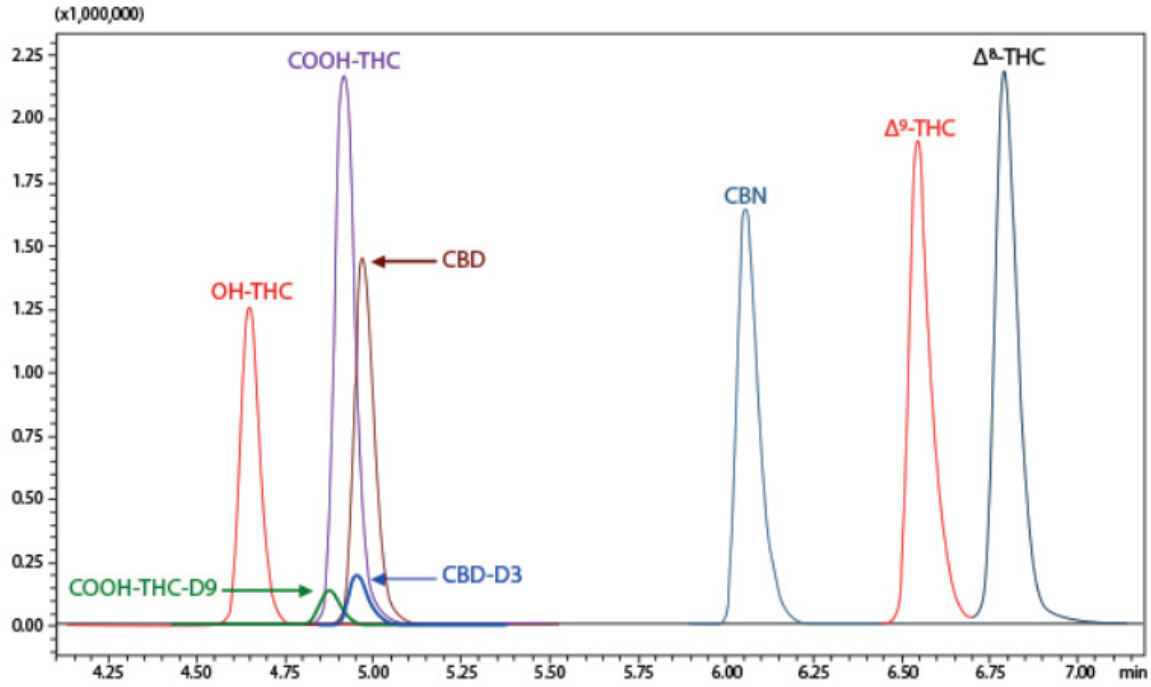

Figure 1. Chromatogram of extracted 50 ng/mL blood sample, minutes 4.10-7.20

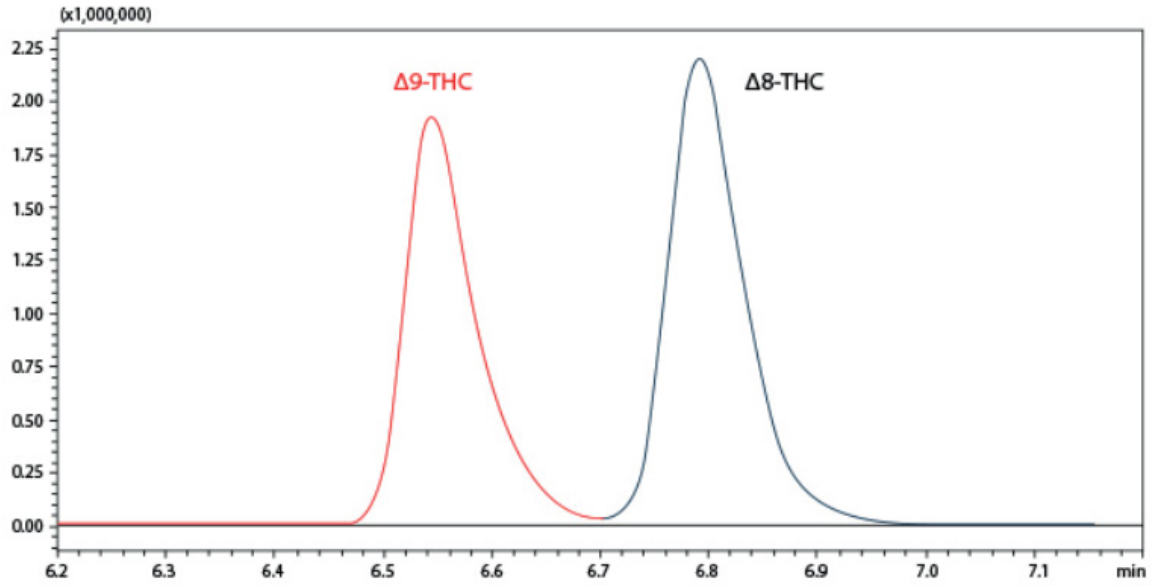

Figure 2. Zoomed in chromatogram of 50 ng/mL extracted blood sample showing separation of THC isomers, minutes 6.20-7.20

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Calibration Curves:

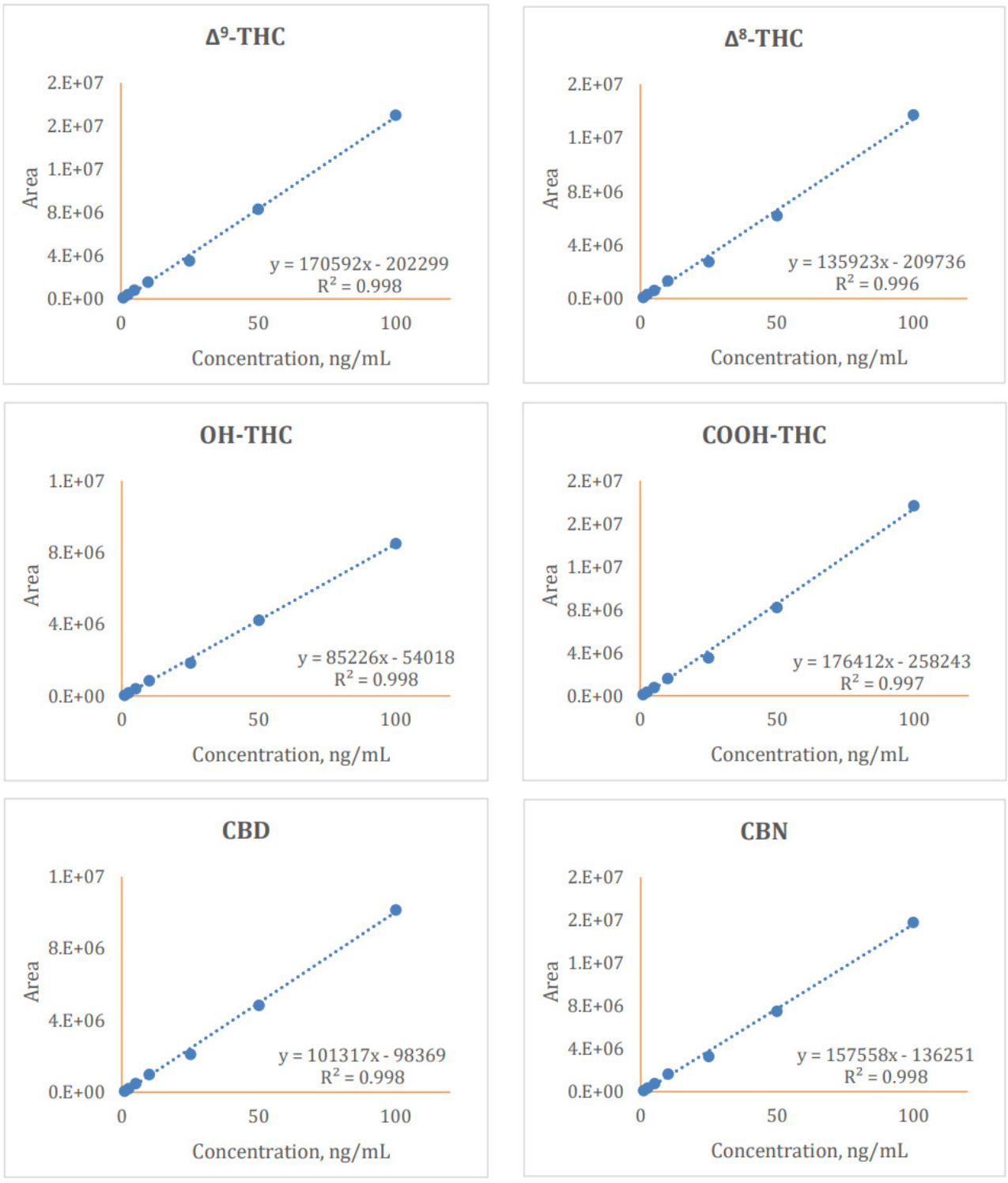

Figure 3. 7-point solvent calibration curve for all analytes with linear equation and R^2 value. [1, 2.5, 5, 10, 25, 50 and 100 ng/mL]

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Results:

Recovery (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	50 ng/mL	RSD
Δ^9-THC	85%	2%	74%	3%	76%	2%
Δ^8-THC	85%	1%	74%	3%	75%	1%
OH-THC	89%	3%	84%	2%	87%	3%
COOH-THC	85%	2%	80%	1%	80%	2%
CBD	84%	2%	80%	3%	81%	2%
CBN	83%	3%	75%	2%	76%	2%

Table 1: The peak areas of pre-spiked samples were compared to the peak area of post-spiked samples

Matrix Effects (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	50 ng/mL	RSD
Δ^9-THC	-26%	4%	-8%	3%	-3%	5%
Δ^8-THC	-26%	3%	-7%	3%	0%	5%
OH-THC	-16%	1%	-6%	2%	0%	3%
COOH-THC	-10%	1%	6%	3%	11%	3%
CBD	-23%	3%	-5%	2%	-2%	6%
CBN	-25%	4%	-4%	2%	2%	3%

Table 2: The peak areas of post-spiked samples were compared to the respective solvent standard in the calibration curve

Conclusions:

An extraction method was developed for the detection of four cannabinoids and the two major Δ^9-THC metabolites in blood (OH-THC and COOH-THC). The sticky nature of these compounds can make them difficult to work with and result in low recoveries. The acetonitrile: acetone (75:25) protein precipitation in the sample preparation has two purposes: First, as a solvent to precipitate and remove potential matrix interferences from blood. Second, to prevent the cannabinoids from sticking to the test tube when transferring the sample to the SPE column. An LC-MS/MS method was optimized that allowed successful analysis of samples in 12 minutes. Additionally, UCT's new SelectraCore® C18 core-shell column was able to separate isomers, Δ^9-THC, and Δ^8-THC eliminating the need for a chiral column. Washes were optimized to produce the highest recoveries with the lowest matrix effects. Recovery for analytes at low, medium, and high concentrations range from 74-89% with low relative standard deviation < 6%. Matrix effects for blood samples were within ± 26%.

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References:

[1] Carlini E. A. (2004). The good and the bad effects of (-) trans-delta-9-tetrahydrocannabinol (Delta 9-THC) on humans. *Toxicon : official journal of the International Society on Toxinology,* 44(4), 461–467. https://doi.org/10.1016/j.toxicon.2004.05.009

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