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

UCT Part Numbers

CSTHC206
Clean Screen® THC
6mL, 200mg 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:

Marijuana is parts or products derived from the *Cannabis* plant with Δ^9-THC content greater than 0.3%. The *Cannabis* plant contains several compounds called cannabinoids, but the most desired is Δ^9-Tetrahydrocannabinol (Δ^9-THC). The resulting effects of this drug includes alteration of perception of time and space, euphoria, and increased appetite.[1] As more states legalize marijuana for recreational and medical use, each has written their own marijuana drug-impaired driving laws. For this reason, it is important for forensic laboratories to have accurate and precise testing protocols.

Protein precipitation is a commonly used method to help remove matrix interferences from blood. The proteins in blood can be precipitated by either changing the pH or the hydrophobicity of the aqueous environment. Common reagents for protein precipitation include acids, organic solvents, salts, and metals.

This application note outlines a protein precipitation sample preparation followed by a solid phase extraction (SPE) method for four natural cannabinoids and the two major metabolites of Δ^9-THC from blood. Analytes were extracted from blood using the Clean Screen® THC SPE column. 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.

Sample Pretreatment:

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

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**
    - 2 x 3mL deionized water
    - 2 x 3mL 40% 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 ACN:MeOH:Acetic Acid (89:9:2)
6. **Dry Eluate**
    - Evaporate eluate under a constant gentle stream of nitrogen ≤ 40°C
7. **Reconstitute**
    - Reconstitute in 1mL of MeOH
    - Alternative compatible solvents and 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 HPLC 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 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	Product ion 1	CE	Product ion 2	CE	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:

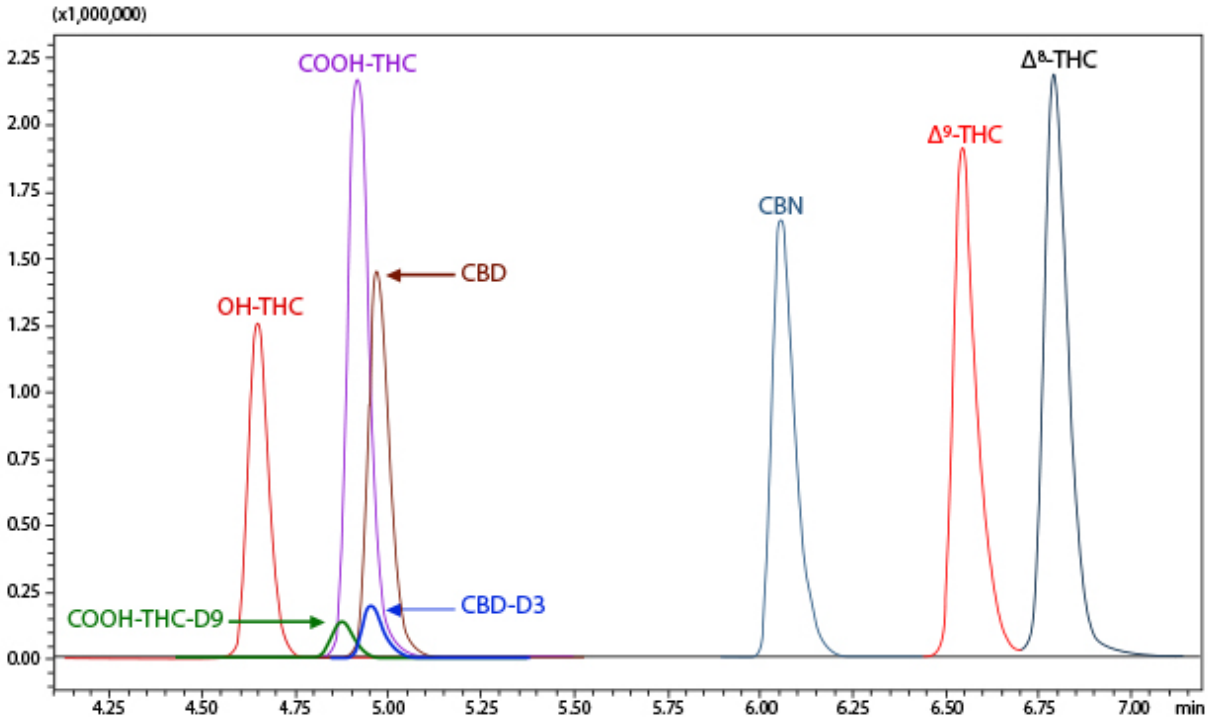

Figure 1. Chromatogram of extracted 50 ng/mL blood sample

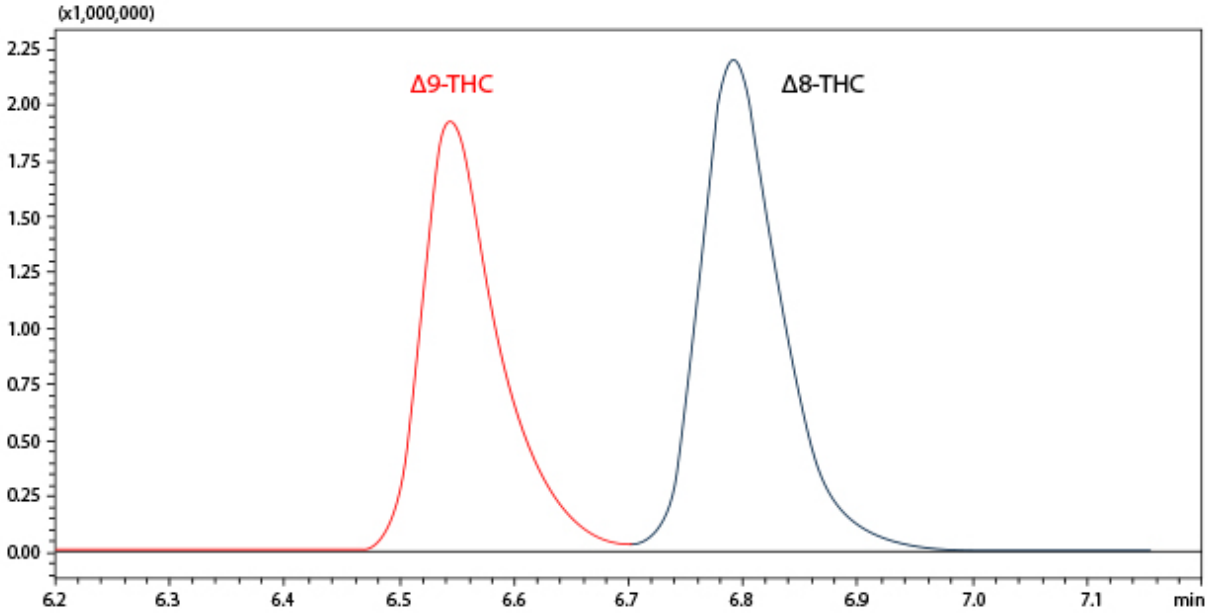

Figure 2. Zoomed in chromatogram of 50 ng/mL extracted blood sample showing separation of THC isomers

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Calibration Curves:

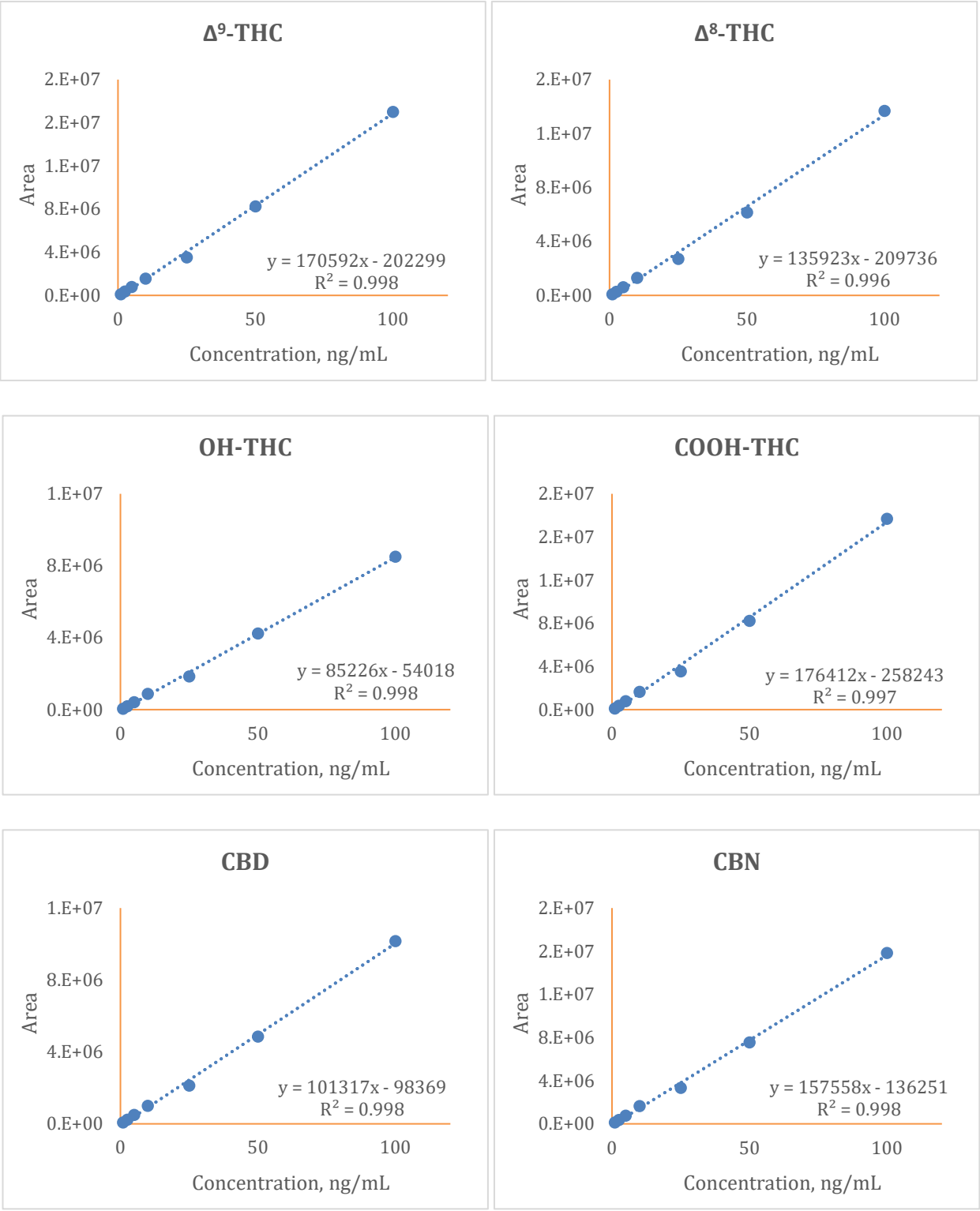

Figure 3. 7-point solvent calibration curve for all analytes with linear equation and R^2 value. [1, 2.5, 5, 10, 25, 50, 100 ng/mL]

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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 Effect (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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