Solid Phase Extraction of Natural Cannabinoids and Metabolites from Blood and Urine

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INTRODUCTION

The *Cannabis* plant contains over a hundred identified natural cannabinoids with Δ⁹ - tetrahydrocannabinol (Δ⁹ -THC), Δ⁸ -tetrahydrocannabinol (Δ⁸ -THC), and Cannabidiol (CBD) being some of the most well-known. Both marijuana and hemp are forms of *Cannabis*, only differentiated by their Δ⁹ -THC content. *Cannabis* is considered to be marijuana when all parts of the plant, whether growing or not, have a Δ⁹ -THC content above 0.3% of its dry weight [1]. It's becoming increasingly more important to both accurately identify and quantitate cannabinoids in biological matrices due to the changing legal status of marijuana across the U.S. and the rise in popularity of cannabinoids for medical and recreational use [2].

While cannabinoids and their analysis are not novel, there is much room for improvement as they continue to persist as a problem for toxicology laboratories due to their "sticky" nature. SPE procedures generally yield low recoveries and liquid-liquid extractions can be tedious for analysts to perform and generally require large amounts of solvent. This poster outlines two separate extraction methods for four natural cannabinoids and Δ⁹ -THC metabolites from blood and urine using UCT's Clean Screen® THC and Styre Screen® HLB respectively. Using a Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer, all six analytes were separated in a short convenient 12-minute method. Equipped with UCT's SelectraCore® C18 core-shell column, this LC method successfully separates isomers Δ⁸ -THC and Δ⁹ -THC. Both solid-phase extraction procedures can be easily implemented with good recoveries and low matrix effects into high throughput laboratories.

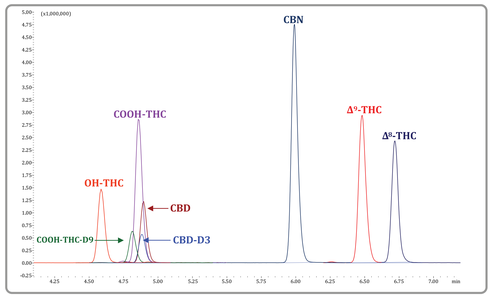
[1] 21 U.S.C. § 802 (16) (2022)

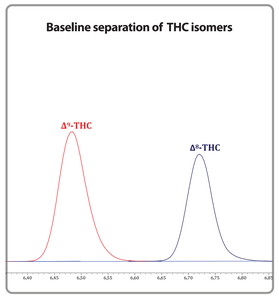
[2] Pellati, Federica et al. "*Cannabis sativa* L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer." *BioMed research international* vol.2018 1691428. 4 Dec. 2018, doi:10.1155/2018/1691428

INSTRUMENT 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 µL
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in methanol
Gradient	Conc. B 50% (0 min) – 80% (3 min) – 90% (7.5 min) – 100% (8 to 9 min) – 50% (9.1 to 12 min)

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





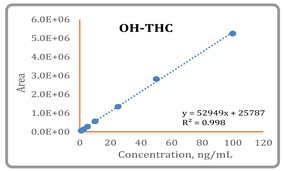
SPE PROCEDURE

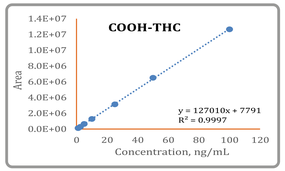
Urine Extraction — Styre Screen HLB 3mL, 60mg (PN: SSHLB063)

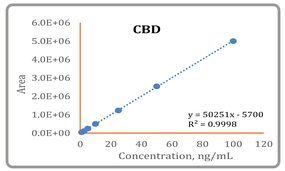
Blood Extraction — Clean Screen THC 6mL, 200mg (PN: CSTHC206)

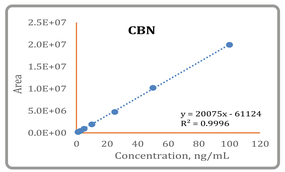
	Urine Extraction	Blood Extraction
1 Sample Prep	• 1mL sample + ISTD + 1mL of ACN + 1ml phosphate buffer • Vortex and centrifuge	• 0.5mL sample + ISTD + 2mL cold ACN: Acetone (75:25) • Vortex well and centrifuge • Decant into 3mL pH 7 phosphate buffer
2 Condition	• 1 x 2mL of MeOH • 1 x 2mL of pH 7 phosphate buffer	• 1 x 2mL of MeOH • 1 x 2mL of pH 7 phosphate buffer
3 Load	• Load at 1 to 2 mL/minute	• Load at 1 to 2 mL/minute
4 Wash	• 1 x 3mL deionized water • 1 x 3mL 50% MeOH in deionized water	• 2 x 3mL deionized water • 2 x 3mL 40% MeOH in deionized water
5 Dry	• Dry column for at least 10 minutes under full pressure or vacuum	• Dry column for at least 10 minutes under full pressure or vacuum
6 Elution	• 1 x 3mL of 60:40 MeOH: Hexane Note: shake or vortex elution solvent well before use	• 1 x 3mL of 89:9:2 ACN:MeOH:Acetic Acid
7 Dry Elute	• Evaporate eluate under a constant gentle stream of nitrogen ≤ 40°C	• Evaporate eluate under a constant gentle stream of nitrogen ≤ 40°C
8 Reconstitute	• Reconstitute in 1mL of MeOH Alternative compatible solvents or volumes can be used	• Reconstitute in 1mL of MeOH Alternative compatible solvents or volumes can be used

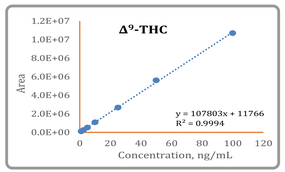
CALIBRATION CURVES

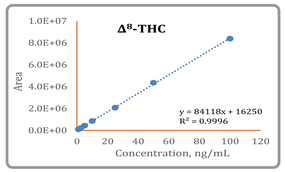








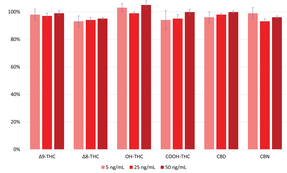


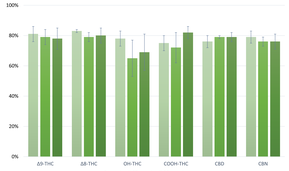


RESULTS

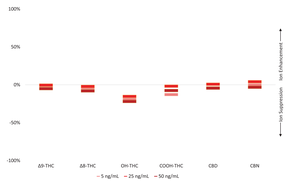
Urine | **Blood**

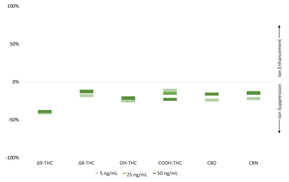
Recoveries





Matrix Effects





Styre Screen® HLB and Clean Screen® THC were utilized to extract natural cannabinoids from urine and blood respectively. Urine extractions resulted in excellent recoveries and low matrix effects. All analytes at three different concentrations had recoveries > 90% and matrix effects within ±25%. Blood extractions also resulted in good recoveries with low matrix effects for most analytes. All analytes at three different concentrations had recoveries of about 70% and matrix effects less than -25% except for Δ⁹ -THC.

CONCLUSION

Questions / Comments: **methods@unitedchem.com**

Separate solid-phase extraction methods have been developed for the analysis of natural cannabinoids from blood and urine. The short 12-minute LC-MS/MS method using a SelectraCore® C18 core-shell column can separate isomers, Δ⁸ -THC and Δ⁹-THC . Cannabinoids were extracted from urine using Styre Screen® HLB with high recoveries and low matrix effects. After a protein precipitation, Clean Screen® THC was used to extract analytes from blood. The extraction had good recoveries and low matrix effects except for Δ⁹ -THC. Future work will include looking into the LC-MS/MS method with the hope to separate the analyte from its interference.

Disclosure: The speaker, author, moderator, planning member and/or presenter/s do have financial relationships with UCT, Inc. , as defined in the AACC policy on potential bias or conflict of interest. The specific product/s : Styre Screen® HLB, Clean Screen® THC and the SelectraCore® C18 column will be mentioned and/or discussed.

FORENSICS

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