

# 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  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC),  $\Delta^8$ -tetrahydrocannabinol ( $\Delta^8$ -THC), and Cannabidiol (CBD) being some of the most well-known. Both marijuana and hemp are forms of *Cannabis*, only differentiated by their  $\Delta^9$ -THC content. *Cannabis* is considered to be marijuana when all parts of the plant, whether growing or not, have a  $\Delta^9$ -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  $\Delta^9$ -THC metabolites from blood and urine using UCT's Clean Screen<sup>®</sup> THC and Styre Screen<sup>®</sup> 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<sup>®</sup> C18 core-shell column, this LC method successfully separates isomers  $\Delta^8$ -THC and  $\Delta^9$ -THC. Both solid-phase extraction procedures can be easily implemented with good recoveries and low matrix effects into high throughput laboratories.

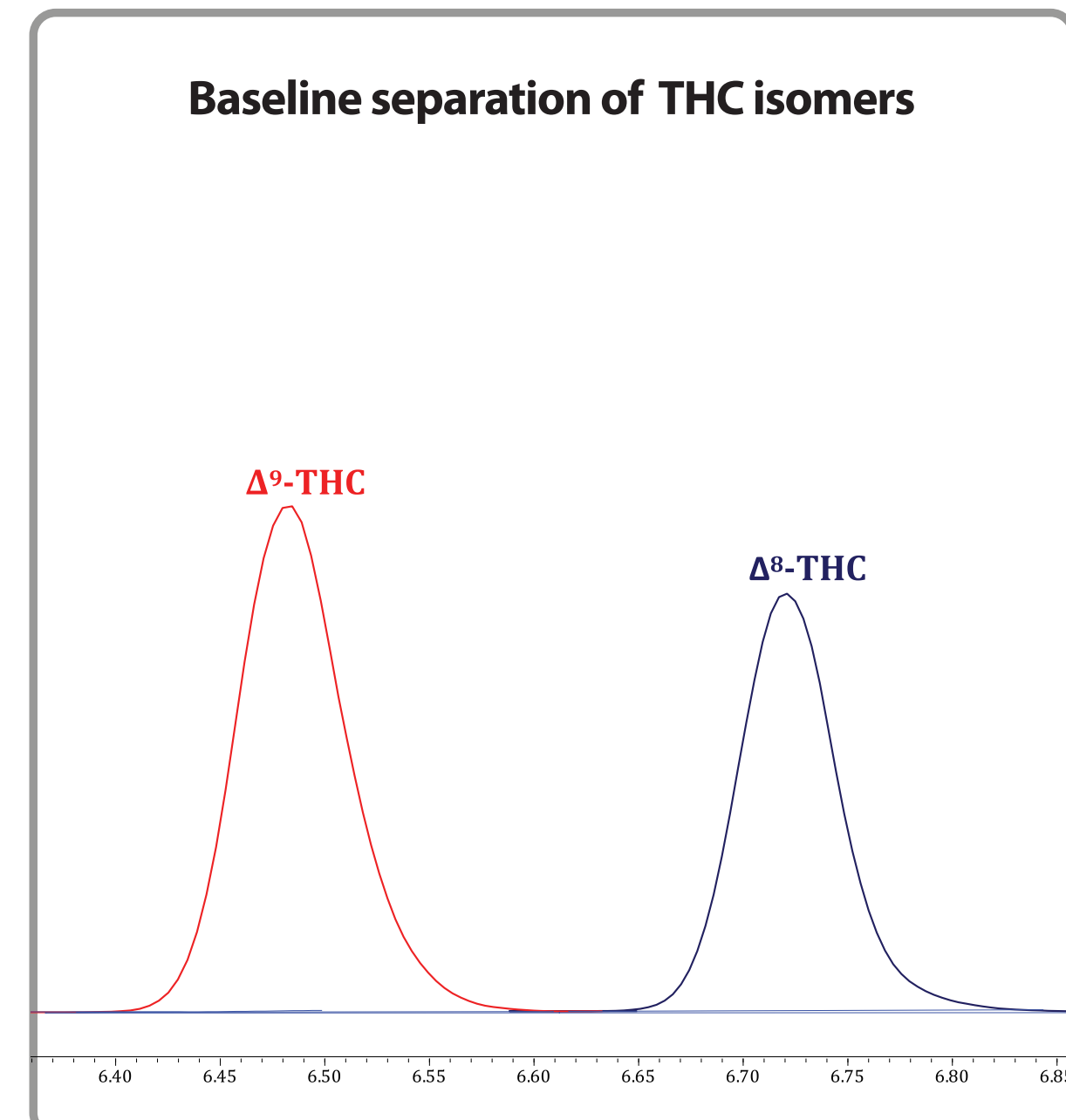
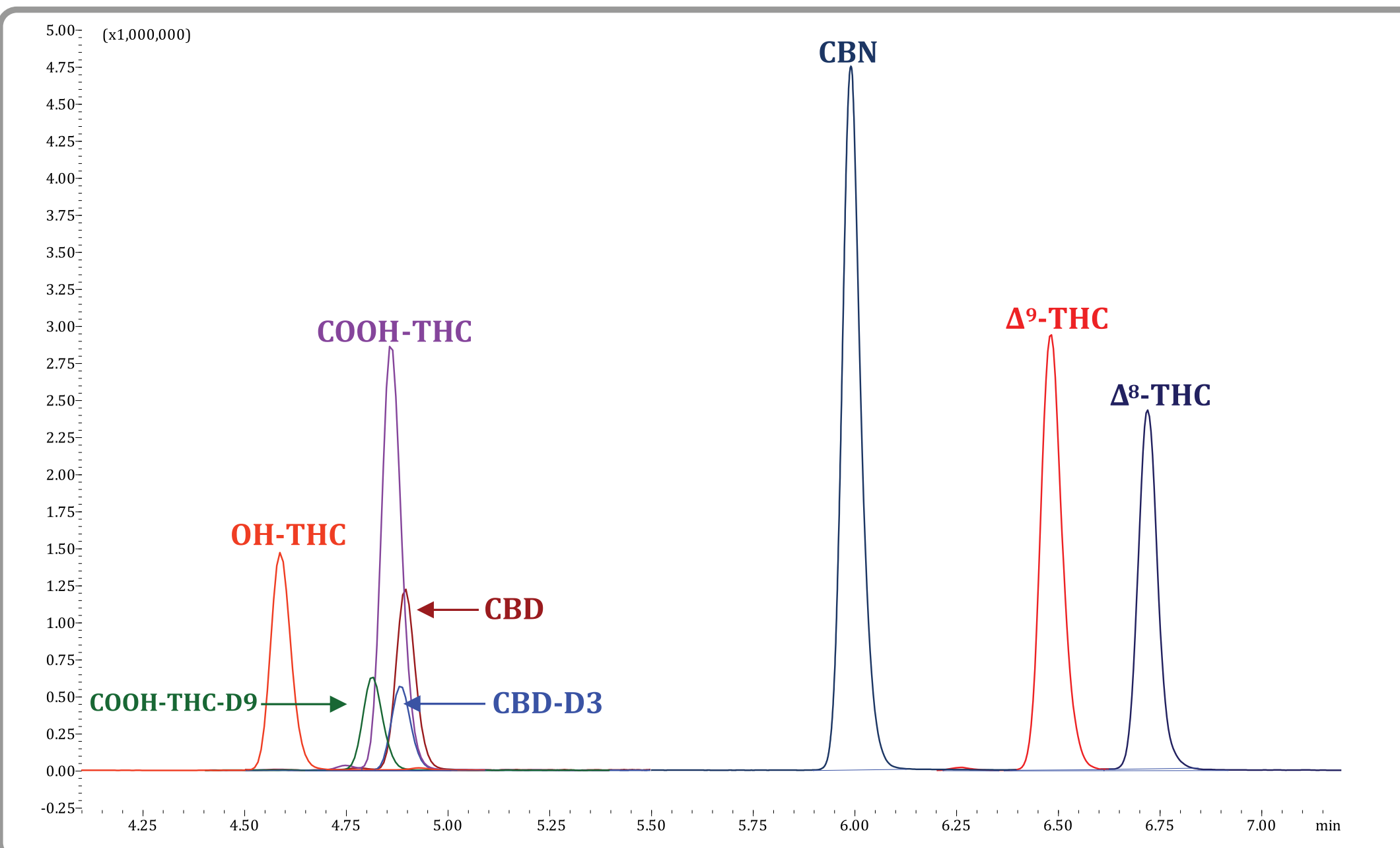
[1] 21 U.S.C. § 802 (16) (2022)

[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 Nexera LC-30AD w/ MS-8050
UHPLC Column	SelectraCore <sup>®</sup> C18 Column 100 x 2.1 mm, 2.7 $\mu$ m
Guard Column	SelectraCore <sup>®</sup> C18 5 x 2.1 mm, 2.7 $\mu$ m
Column Temperature	40°C
Flow Rate	0.4 mL/min
Injection Volume	10 $\mu$ L
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



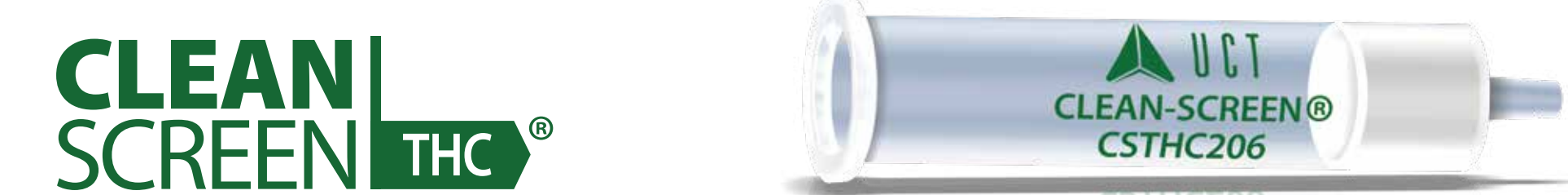
## SPE PROCEDURE

### Urine Extraction Styre Screen HLB 3mL, 60mg (PN: S5HLB063)



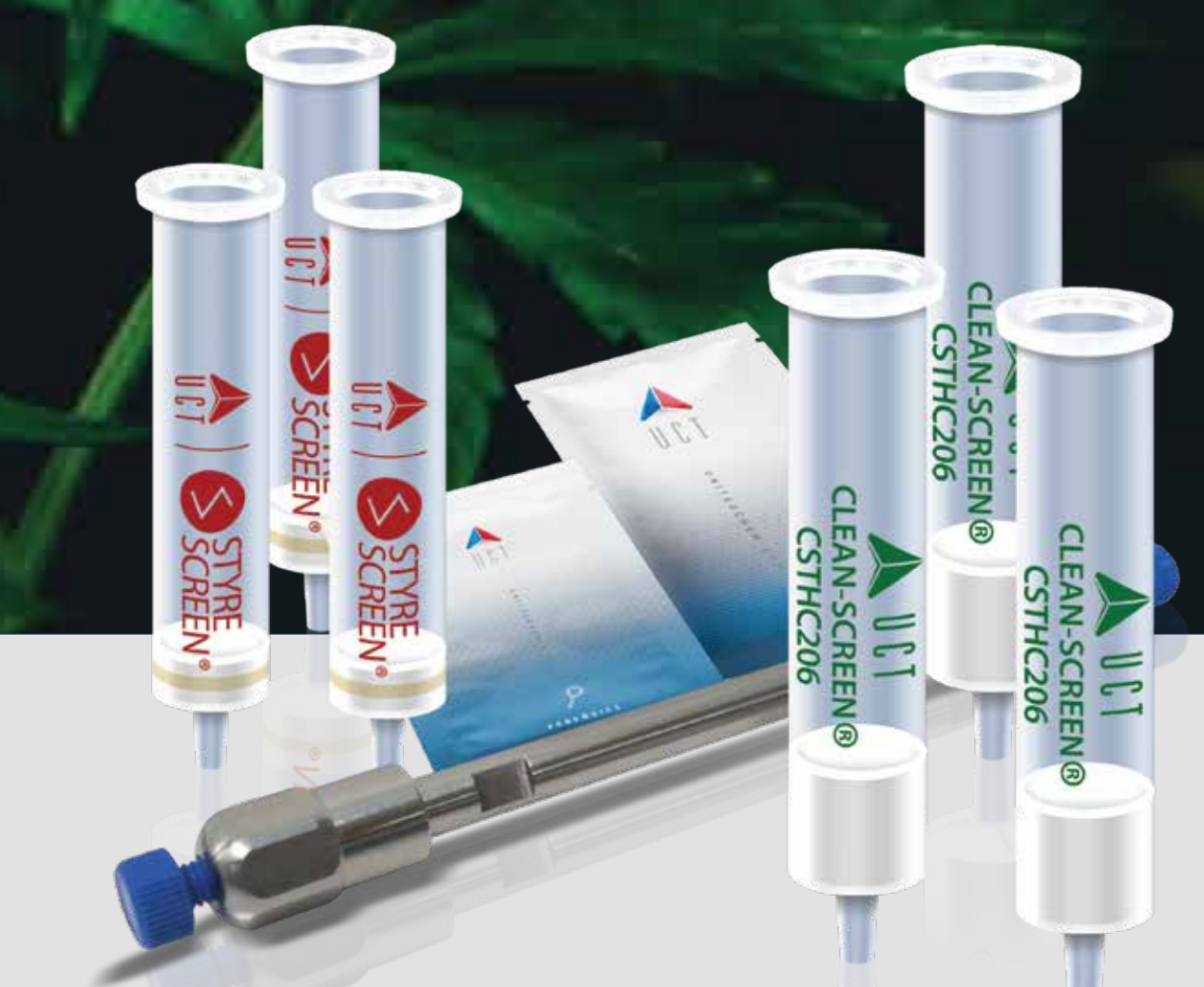
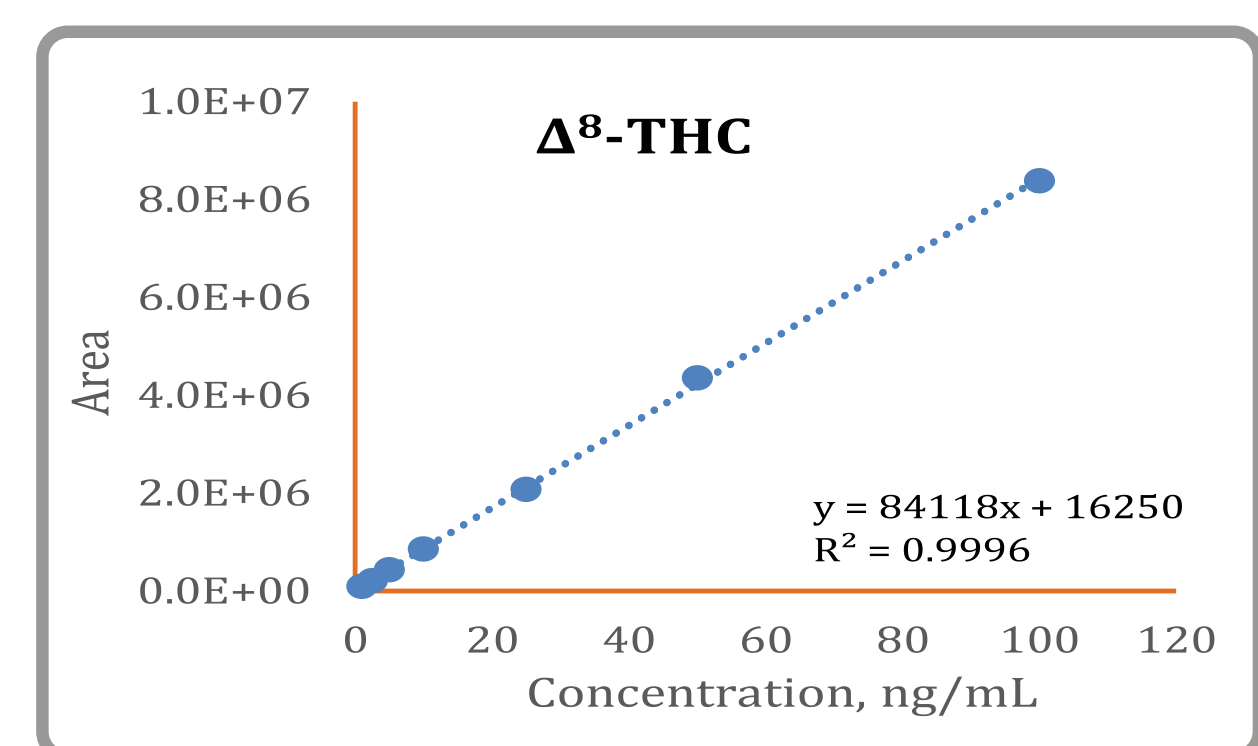
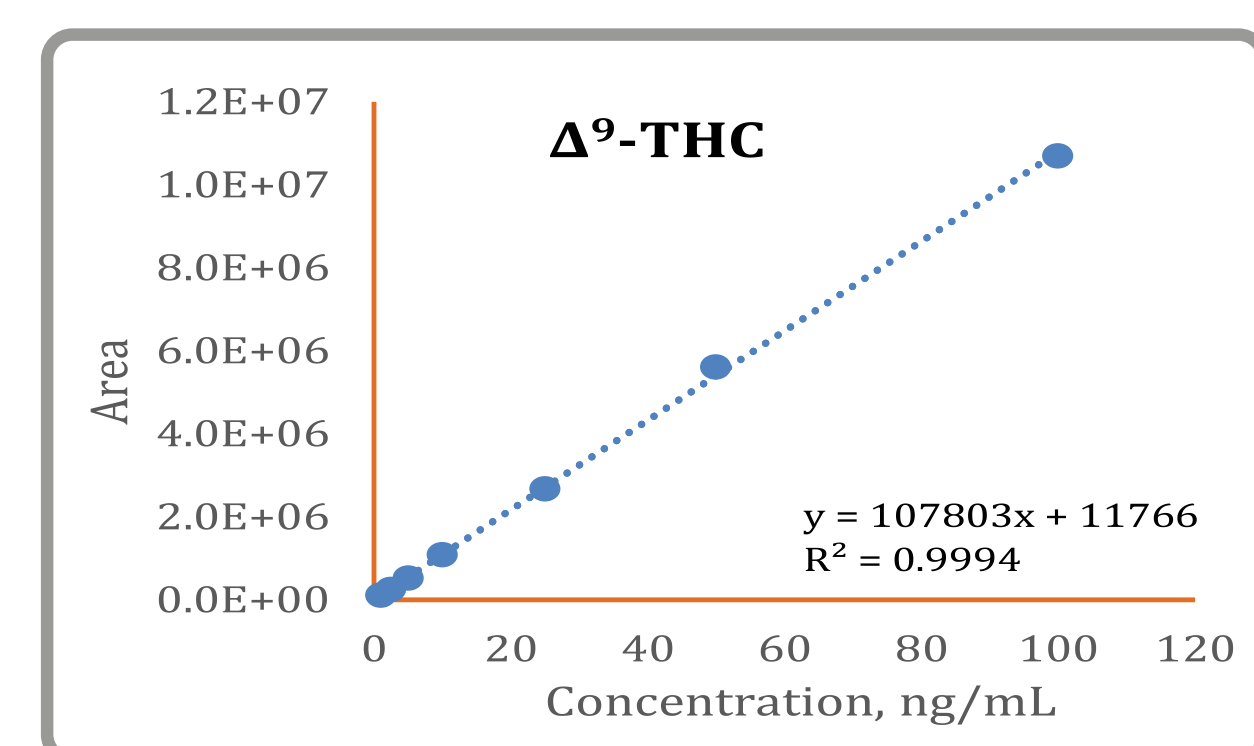
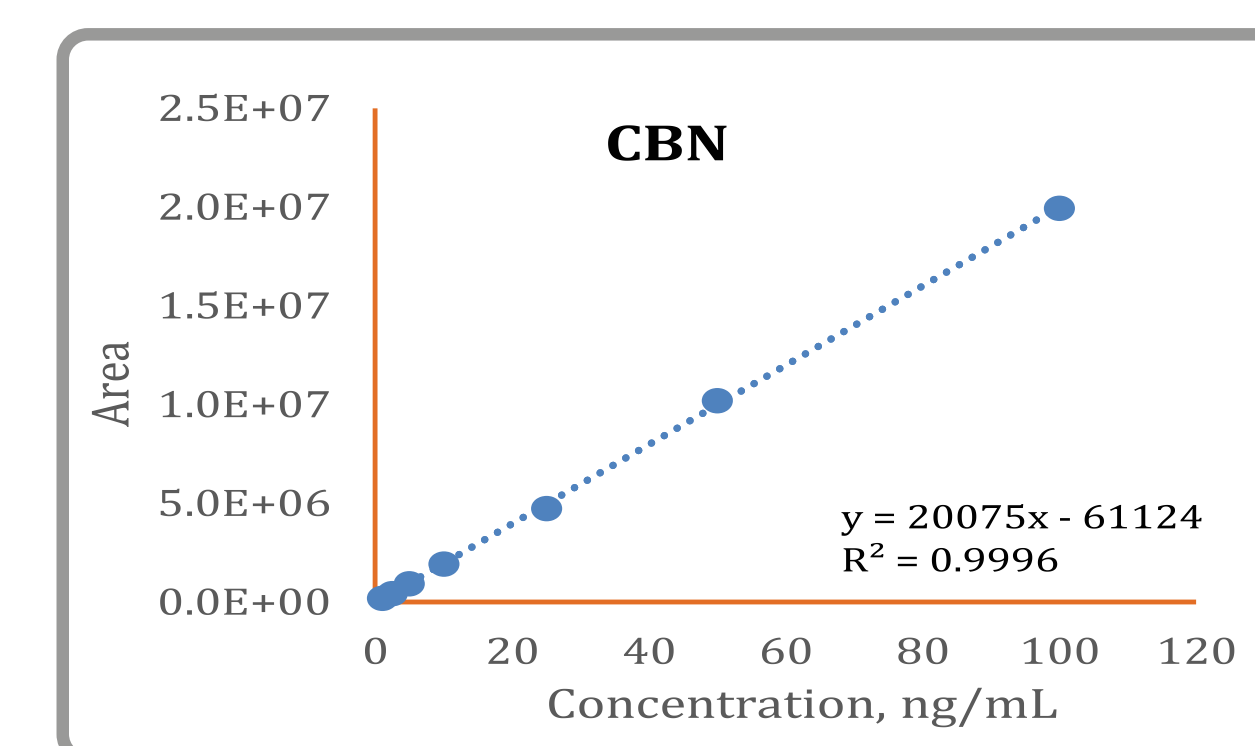
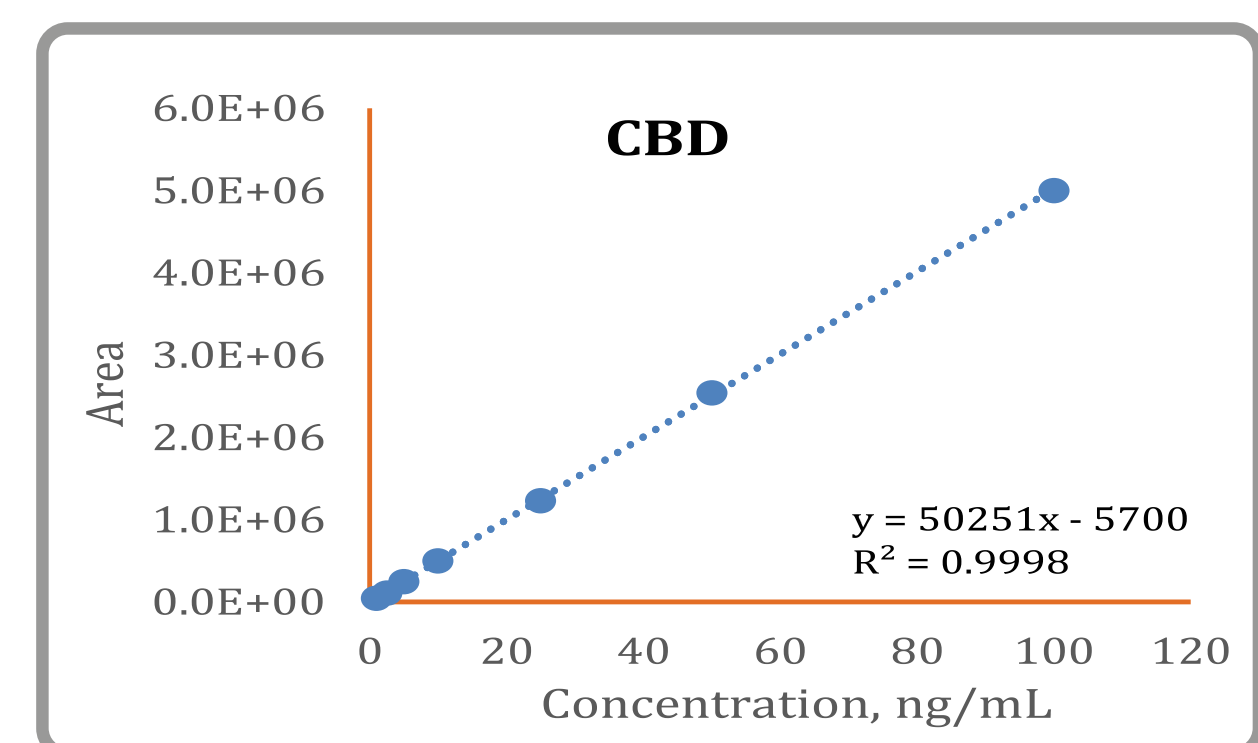
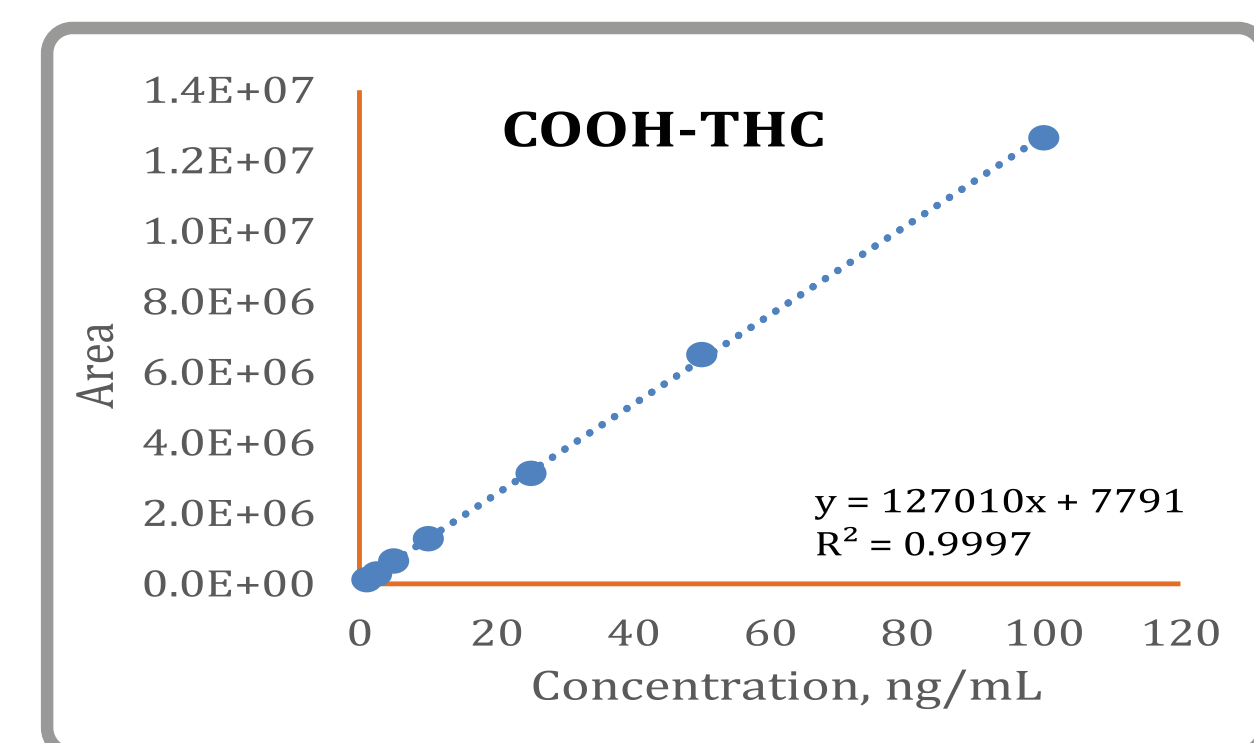
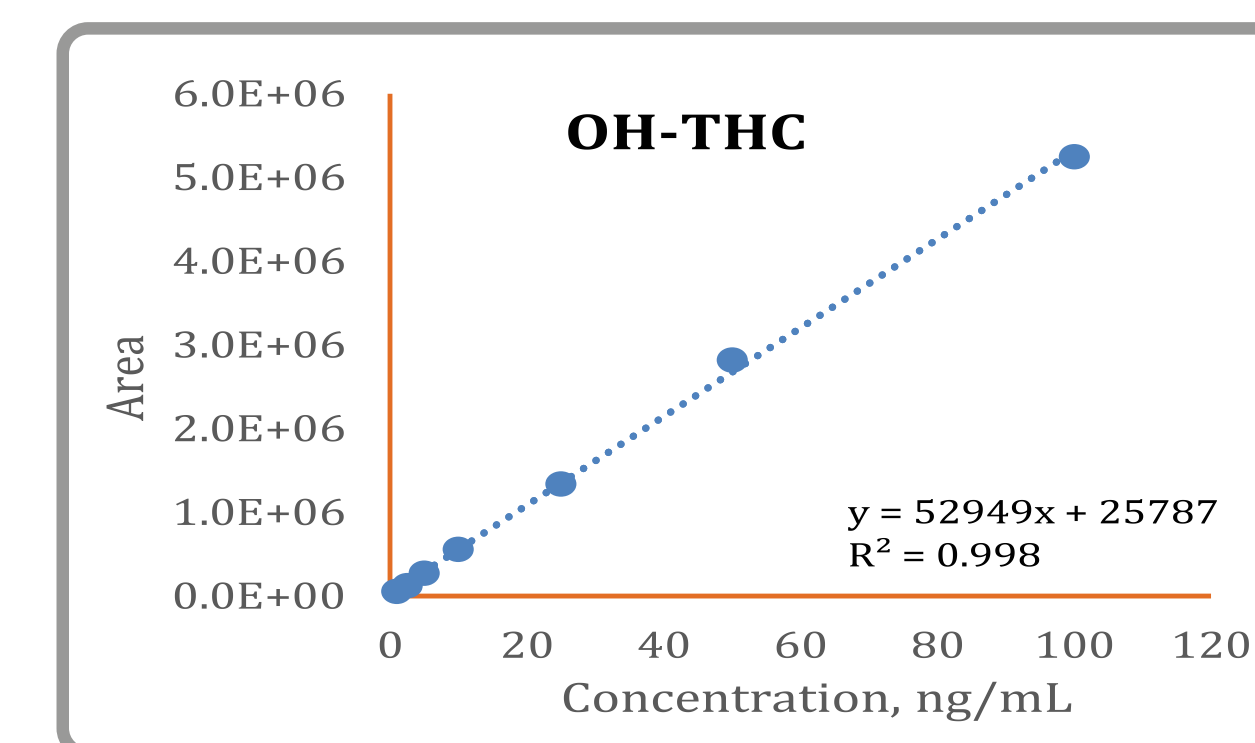
- Sample Prep**
  - 1 mL sample + ISTD + 1 mL of ACN + 1 mL phosphate buffer
  - Vortex and centrifuge
- Condition**
  - 1 x 2 mL of MeOH
  - 1 x 2 mL of pH 7 phosphate buffer
- Load**
  - Load at 1 to 2 mL/minute
- Wash**
  - 1 x 3 mL deionized water
  - 1 x 3 mL 50% MeOH in deionized water
- Dry**
  - Dry column for at least 10 minutes under full pressure or vacuum
- Elution**
  - 1 x 3 mL of 60:40 MeOH: Hexane
  - Note: shake or vortex elution solvent well before use
- Dry Elute**
  - Evaporate eluate under a constant gentle stream of nitrogen  $\leq 40^\circ\text{C}$
- Reconstitute**
  - Reconstitute in 1 mL of MeOH
  - Alternative compatible solvents or volumes can be used

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



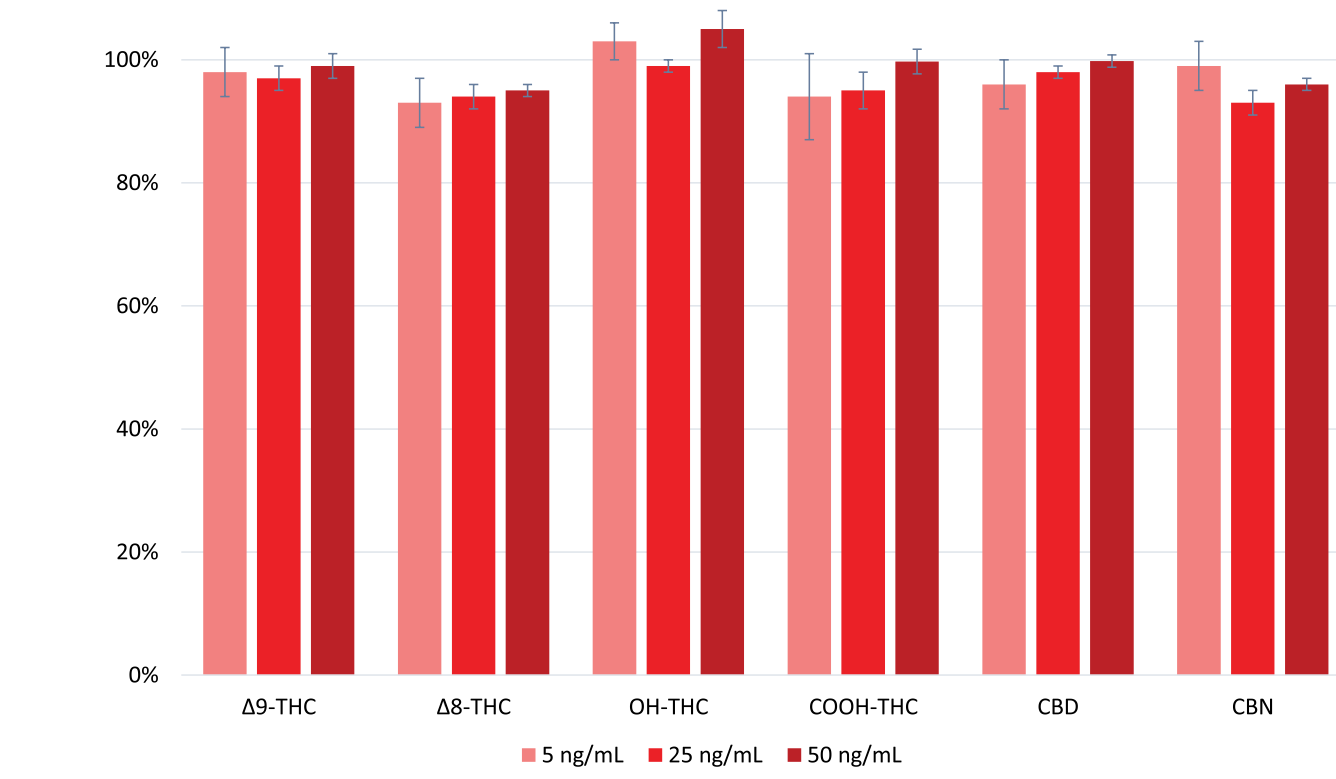
- Sample Prep**
  - 0.5 mL sample + ISTD + 2 mL cold ACN: Acetone (75:25)
  - Vortex well and centrifuge
  - Decant into 3 mL pH 7 phosphate buffer
- Condition**
  - 1 x 2 mL of MeOH
  - 1 x 2 mL of pH 7 phosphate buffer
- Load**
  - Load at 1 to 2 mL/minute
- Wash**
  - 2 x 3 mL deionized water
  - 2 x 3 mL 40% MeOH in deionized water
- Dry**
  - Dry column for at least 10 minutes under full pressure or vacuum
- Elution**
  - 1 x 3 mL of 89:9:2 ACN:MeOH:Acetic Acid
- Dry Elute**
  - Evaporate eluate under a constant gentle stream of nitrogen  $\leq 40^\circ\text{C}$
- Reconstitute**
  - Reconstitute in 1 mL of MeOH
  - Alternative compatible solvents or volumes can be used

## CALIBRATION CURVES

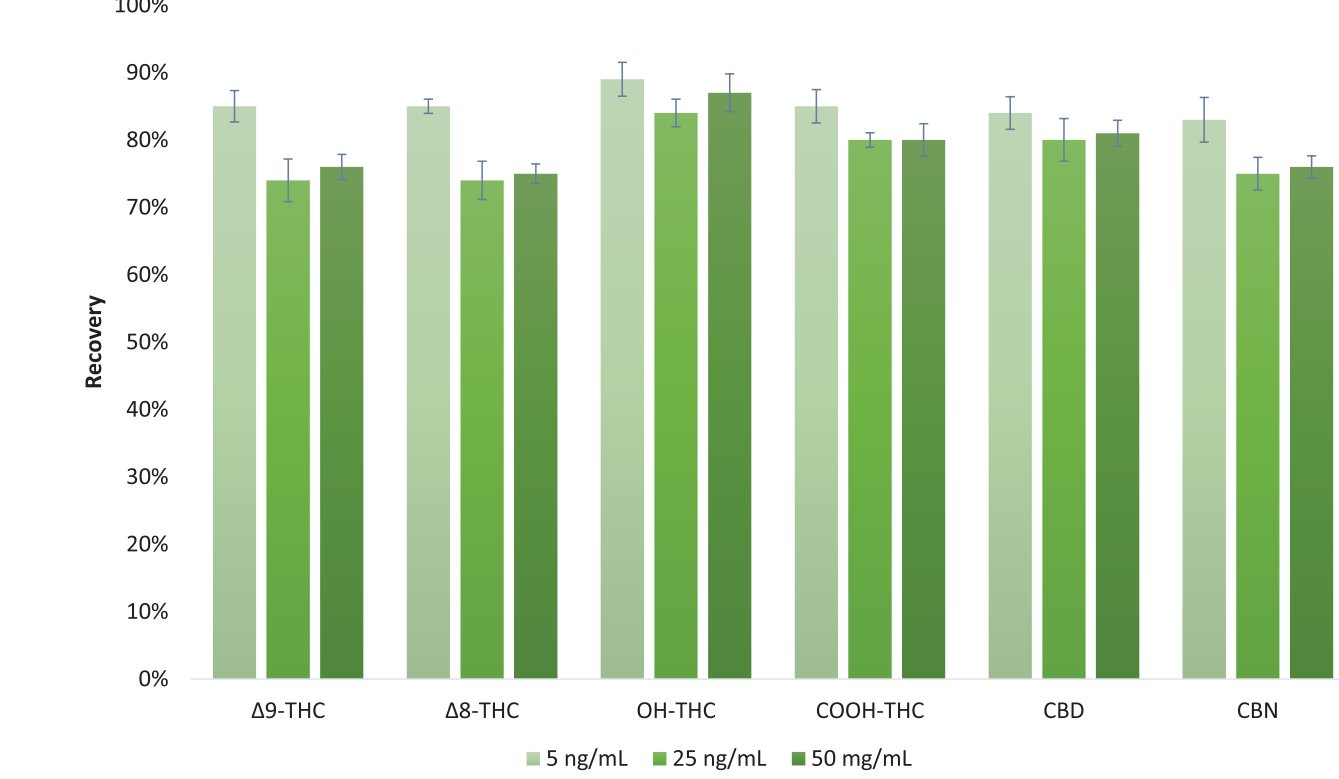


## RESULTS

### Urine

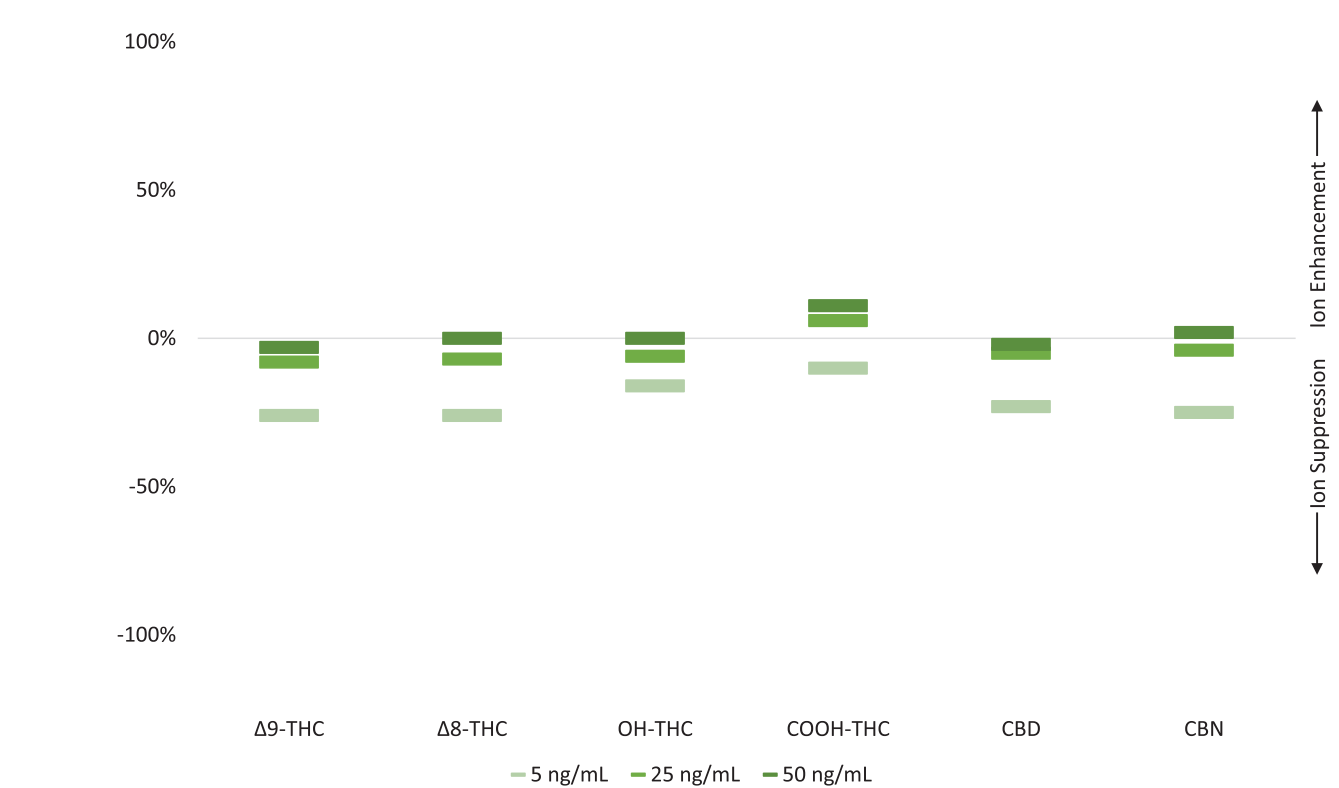
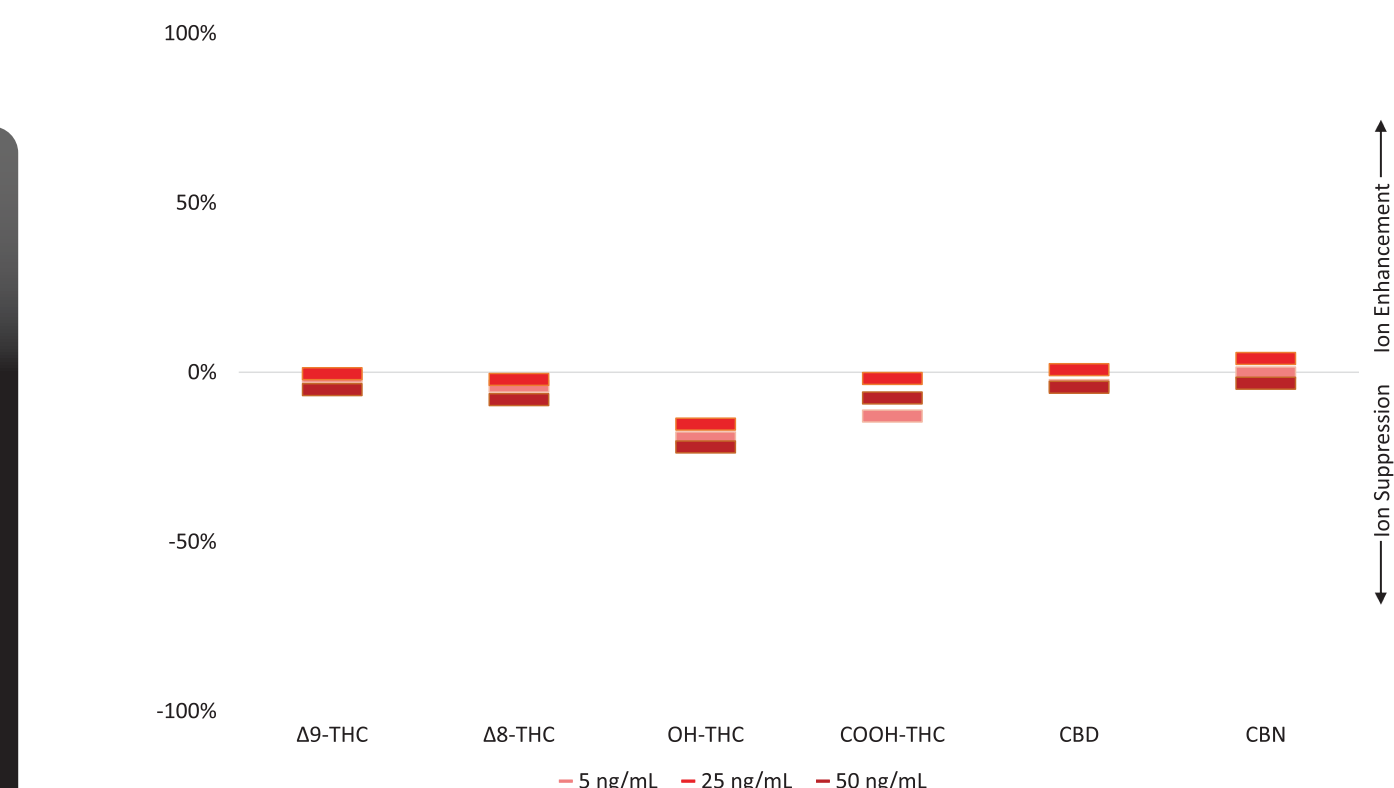


### Blood



Recoveries

Matrix Effects



Styre Screen<sup>®</sup> HLB and Clean Screen<sup>®</sup> 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  $\pm 25\%$ . Blood extractions also resulted in good recoveries with low matrix effects for most analytes. All analytes at three different concentrations had recoveries > 74% and matrix effects within  $\pm 26\%$ .

## CONCLUSION

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Two different solid-phase extraction methods have been developed for the analysis of natural cannabinoids from blood and urine. A short 12-minute LC-MS/MS method using a SelectraCore<sup>®</sup> C18 core-shell column can separate isomers,  $\Delta^8$ -THC and  $\Delta^9$ -THC. Cannabinoids were extracted from urine using Styre Screen<sup>®</sup> HLB with high recoveries and low matrix effects. The addition of 1 mL of acetonitrile (ACN) during sample preparation of the urine samples was optimized to prevent analytes from sticking to the sample test tube and loads more analyte onto the SPE cartridge. This results in high recoveries. After a protein precipitation, Clean Screen<sup>®</sup> THC was used to extract analytes from blood. The extracts had good recoveries and low matrix effects for all analytes. Using ACN:acetone (75:25) as the protein precipitation solvent resulted in the best compromise of high recoveries and lower matrix effects compared to other solvents such as ACN and ACN with 0.1% formic acid.

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



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