



THC, THC-OH, AND CARBOXY-THC IN EQUINE URINE FOR GC/MS CONFIRMATIONS

Part Numbers:

XRDAH206 - 200 mg XtrackT[®] DAU Extraction Column in 6 mL cartridge

SPHACE5010-10 - Select pH Buffer Pouches 1M Acetate pH 5.0

SBSTFA-1-1 – SELECTRA-SIL[®] BSTFA w/1% TMCS

BETA-GLUC-10 - SELECTRAZYME[®] β – Glucuronidase

1. PREPARE SAMPLE - ENZYMATIC AND BASE HYDENZYMATIC AND BASE HYDROLYSIS OF GLUCURONIDES:

To 1 mL of urine add internal standard (s) and 50 µL of Beta Glucuronidase solution (*Haliotis rufescens*), add 2 mL of 1 M Acetate buffer pH= 5.

Mix and incubate at 65 °C for 3 hours.

Cool to room temperature

Add 100 of 10 M NaOH. Mix/vortex

Hydrolyze for 20 minutes at 60°C. Cool before proceeding

Adjust sample pH to 3.0 with approx. 1.0 mL of glacial acetic acid. Check pH to insure that the pH value is ~ 3.0

Centrifuge as appropriate

2. CONDITION XTRACTT[®] DAU EXTRACTION COLUMN:

1 x 3 mL CH₃OH

1 x 3 mL D.I. H₂O

1 x 1 mL Acetate buffer (pH= 3.0)

NOTE: Aspirate at full vacuum or pressure

3. APPLY SAMPLE:

Load at 1 to 2 mL/ minute

4. WASH COLUMN:

1 x 2 mL D.I. H₂O

1 x 2 mL 100 mM HCl: Acetonitrile (95:5)

Dry column (5-10 minutes at full vacuum or pressure)

1 x 200 1 mL Hexane; Aspirate. (Additional step to remove any residual moisture)

5. ELUTE CANNABINOIDS:

1 x 3 mL Hexane/ Ethyl Acetate/ Glacial Acetic Acid (49:49:2)

Collect eluate at 1 to 2 mL/minute

NOTE: Before proceeding, ensure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing efficiency

6. DRY ELUATE:

Evaporate to dryness at < 40°C

7. DERIVATIZE:

Add 50 µL Ethyl Acetate and 50 µL BSTFA w/1% TMCS

Mix/vortex

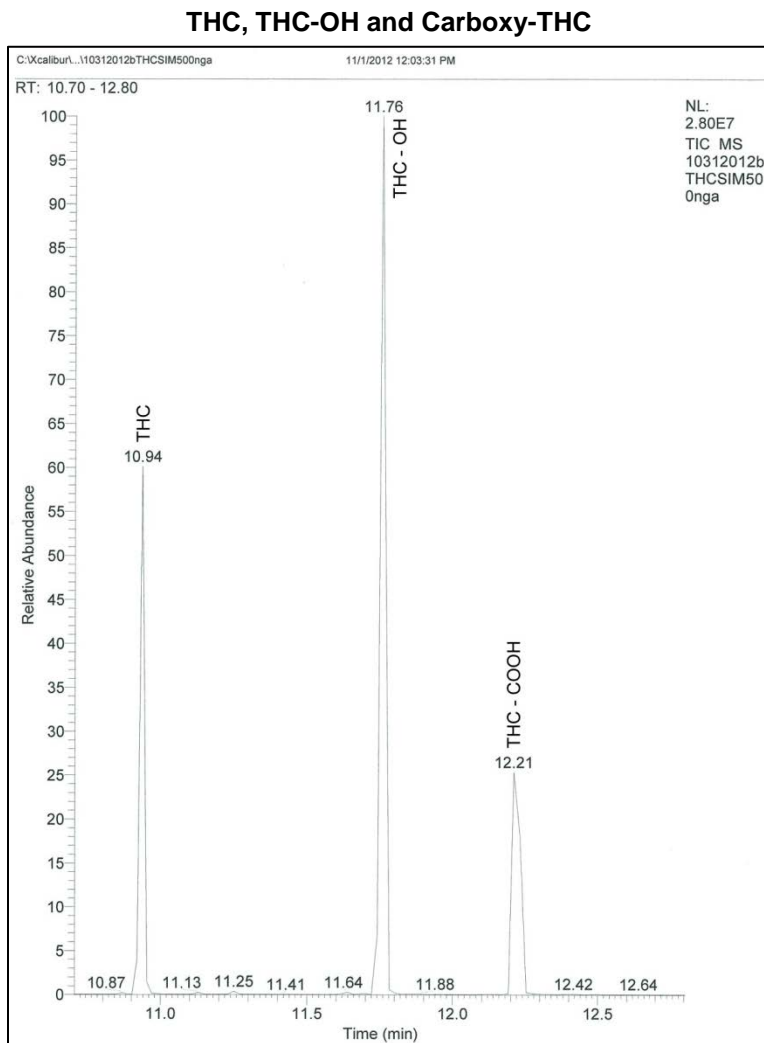
React 20 minutes at 70°C

Remove from heat source to cool

NOTE: Do not evaporate BSTFA

Inject 1 to 2 µL onto gas chromatograph

CHROMATOGRAM



Mass Spec Table

Compound	Primary Ion*	Secondary Ion	Tertiary Ion
THC-TMS	371	343	366
THC-D ₃ -TMS [†]	374	346	889
THC-OH-TMS	371	459	474
THC-OH-D ₃ -TMS [†]	374	462	471
THC-COOH-TMS	371	473	488
THC-COOH-D ₃ -TMS [†]	374	476	491

*Quantitation Ion

[†]Suggested internal standard for GC/M