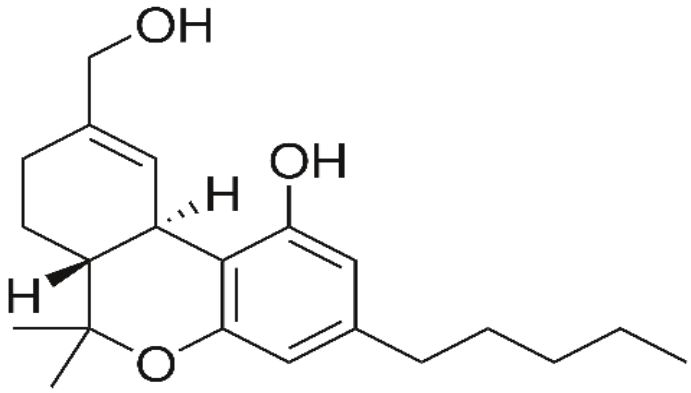
THC, THC-OH, and Carboxy-THC In Equine Urine for GC/MS Confirmations



UCT Part Numbers

XRDAH206
XtrackT DAU
200 mg, 6 mL

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

Procedure:

1. Prepare Sample

a) 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.
b) Mix and incubate at 65 °C for 3 hours.
c) Cool to room temperature.
d) Add 100 of 10 M NaOH. Mix/vortex.
e) Hydrolyze for 20 minutes at 60°C. Cool before proceeding.
f) 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 XTRACKT® DAU Extraction Column

a) 1 x 3 mL CH_3OH.
b) 1 x 3 mL D.I. H_2O.
c) 1 x 1 mL Acetate buffer (pH= 3.0).

Note: Aspirate at full vacuum or pressure.

3. APPLY SAMPLE

a) Load at 1 to 2 mL/ minute.

4. Wash Column

a) 1 x 2 mL D.I. H_2O.
b) 1 x 2 mL 100 mM HCl/acetonitrile (95:5).
c) Dry column (5-10 minutes at full vacuum or pressure).
d) 1 x 200 1 mL hexane; Aspirate. (Additional step to remove any residual moisture)

5. Elute Cannabinoids

a) 1 x 3 mL hexane/ethyl acetate/ glacial acetic acid (49:49:2).
b) Collect eluate at 1 to 2 mL/minute.

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

6. Dry Eluate

a) Evaporate to dryness at < 40 °C.

7. Derivatize

a) Add 50 µL ethyl acetate and 50 µL BSTFA w/1% TMCS.
b) Mix/vortex.
c) React 20 minutes at 70°C.
d) Remove from heat source to cool.

Note: Do not evaporate BSTFA

e) Inject 1 to 2 µL onto gas chromatograph.

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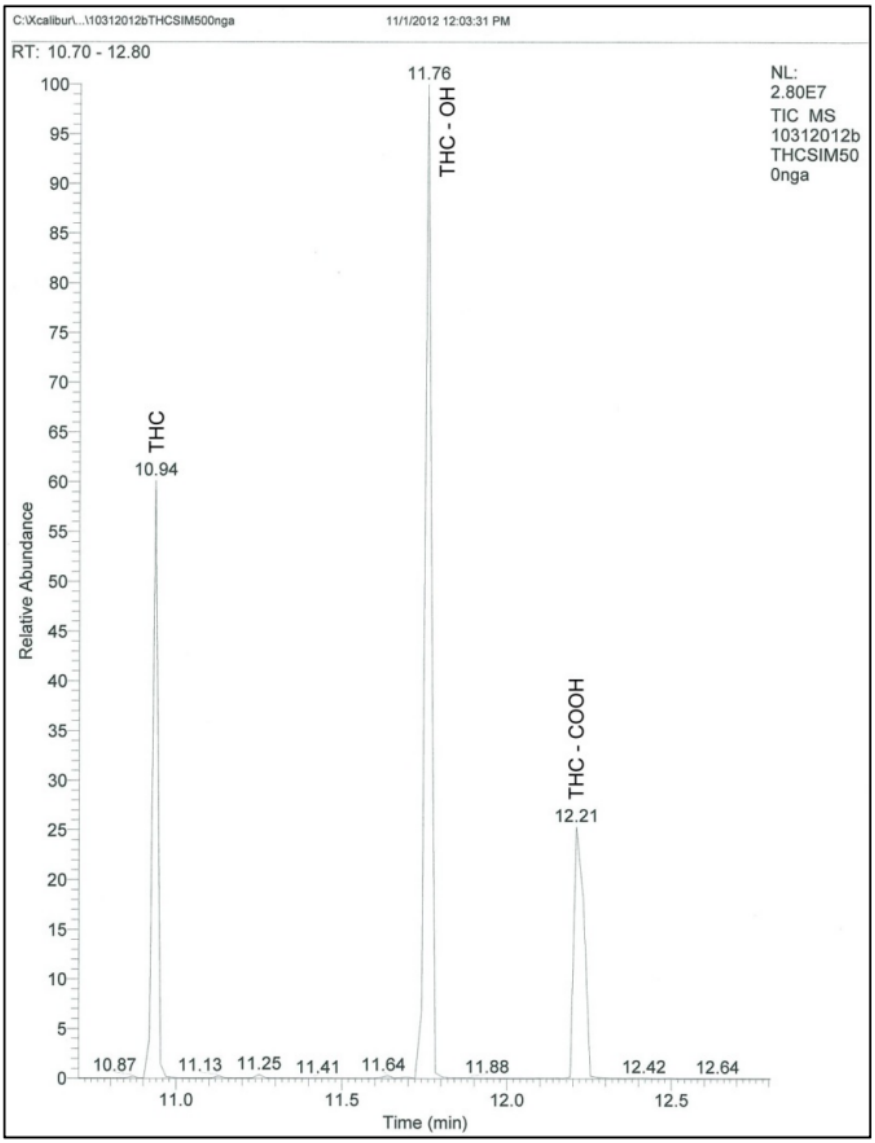
Chromatogram:

THC, THC-OH, and THC-COOH



Mass Spec Table

Compound	Primary Ion*	Secondary Ion	Tertiary Ion
THC-TMS	371	343	366
THC-D3-TMS†	374	346	889
THC-OH-TMS	371	459	474
THC-OH-D3-TMS†	374	462	471
THC-COOH-TMS	371	473	488
THC-COOH-D3-TMS†	374	476	491

*Quantitation Ion
†Suggested internal standard for GC/MS

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4107-03-01

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