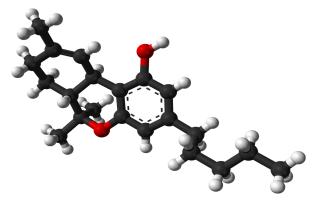
delta 9-THC (parent), delta 9-Hydroxy THC, Carboxy- delta 9-THC In Whole Blood For GC/MS Confirmations Using: 200 mg Styre Screen® SSTHC Extraction Column



UCT Part Numbers

ZSTHC020

Clean Screen® THC Extraction Column 200 mg 10 mL

CSDAU206

Clean Screen® DAU Extraction Column 200 mg 6 mL

Procedure:

1. Prepare Sample

- a) To 1-2 mL of whole blood add internal standards*.
- b) Mix/vortex.
- Add dopwise whilst vortexing, 1 mL of Ice Cold acetonitrile.
- d) Centrifuge and transfer acetonitrile to a clean tube.
- e) Adjust sample pH to 3.0 ± 0.5 with approx. 2.0 mL of 100 mM Sodium Acetate buffer.

Note: Check pH of buffer to insure that the pH value is ~ 3.0 .

2. Condition Clean Screen® Extraction Column

- a) 1 x 3 mL CH₃OH.
- b) 1 x 3 mL D.I. H₂O.
- c) 1 x 1 mL Acetate buffer (pH=3.0).

Note: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. Apply Sample

a) Load at 1 to 2 mL/minute.

4. Wash Column

- a) $1 \times 2 \text{ mL D.I. } H_2O$.
- b) 1 x 2 mL 100 mM HCI/acetonitrile (95:5).
- c) Dry column (5-10 minutes at greater than 10 inches Hg/ Full Flow for Positive Pressure manifold). 1 x 200 μL hexane; Aspirate. (Additional step to remove any residual moisture. Could substitute 200 μL MeOH for hexane.)

Optional: Dry column (5 minutes at greater than 10 inches Hg/ Full Flow for Positive Pressure manifold). Note: The delta-9-THC (parent) will elute in hexane so special attention must be paid to not use more than 200 μL hexane in the wash/ dry step. The 200 μL hexane wash step can be eliminated if the column is allowed to dry longer under vacuum or by positive pressure gas flow.

5. Elute THC (metabolites)

- a) 1 x 2 ml hexane (optional, contains delta-9-THC).
- b) 1 x 3 mL hexane/ethyl acetate (50:50).
- c) Collect eluate at 1 to 2 mL/minute.

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

6. Dry Eluate

a) Evaporate to dryness at < 40 °C.

7. Derivatize

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

Note: Do not evaporate BSTFA.

8. Quantitate

- a) Inject 2 μL onto gas chromatograph.
- b) For MSD monitor the following ions:







ANALYTE (TMS) Primary Ion / Secondary / Tertiary				
Compound	Primary ion	Secondary	Tertiary	Cerilliant #
Carboxy- delta 9-THC-D3 TMS*	374	476	491	T-008
Carboxy- delta 9-THC-D9-TMS*	380	479	497	T-007
Carboxy-delta 9-THC	371	473	488	T-018
delta 9-THC-D3-TMS*	374	389		T-003
delta 9-THC-TMS	371	386		T-005
Hydroxy- delta 9-THC-D3-TMS*	374	462	477	H-041
Hydroxy- delta 9-THC-TMS	371	459	474	H-027
(303, 315, 330, 343)**				

^{*} Suggested internal standard for GC/MS: D₀-Carboxy-delta 9-THC, D₃-Hyroxy- delta 9-THC, D₃-delta 9-THC

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^{**} lons common to deuterated delta-9 THC and non-deuterated compounds.