



**CARBOXY- delta 9-THC (pKa = 4.5) IN URINE FOR
GC/MS CONFIRMATIONS USING:
200 mg CLEAN SCREEN® EXTRACTION COLUMN**
Part #: ZSTHC020 or CSDAU206

1. PREPARE SAMPLE - BASE HYDROLYSIS OF GLUCURONIDES

To 2 mL of urine add internal standard* and 100 µL 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.

2. CONDITION CLEAN SCREEN® 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 < 3 inches Hg to prevent sorbent drying.

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 greater than 10 inches Hg/ Full Flow for Positive Pressure manifold).

1 x 200 µL hexane; Aspirate. (Additional step to remove any residual moisture.)

5. ELUTE CARBOXY THC

1 x 3 mL hexane/ethyl acetate (50:50).

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 efficiency.

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

Add 50 µL ethyl acetate and 50 µL BSTFA (with 1% TMCS).

Mix/vortex.

React 20 minutes at 70°C.

Remove from heat source to cool.

NOTE: Do not evaporate BSTFA.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.

For MSD monitor the following ions:

<u>ANALYTE (TMS)</u>	<u>Primary Ion**</u>	<u>Secondary</u>	<u>Tertiary</u>	<u>Cerilliant #</u>
Carboxy-delta 9-THC-D3*	374	476	491	T-008
Carboxy-delta 9-THC-D9*	380	479	497	T-007
Carboxy-delta 9-THC	371	473	488	T-019

* Suggested internal standard for GC/MS: -Carboxy-delta 9-THC-D9