

**CARBOXY- delta 9-THC (pKa =4.5) IN URINE
FOR GC/MS CONFIRMATIONS using
THCA Extraction Columns.**

**1. PREPARE SAMPLE -BASE HYDROLYSIS OF
GLUCURONIDES**

To 2 mL of urine add internal standard* and 100 µL of 10 N NaOH.
Mix/vortex.
Hydrolyze for 20 minutes at 60°C. Cool before proceeding.
Adjust sample pH to 6.5 with approx. 0.5 mL of
glacial acetic acid. Check pH to insure that the pH value is ~ 6.5± 0.5.
(Note: After pH adjusted to ~ 6.5, add 500 uL of Methanol to sample.)

2. APPLY SAMPLE

Load at 1 to 2 ml/minute.

3. WASH COLUMN

1 x 2 mL DI Water/MeOH/NH₄OH (90:5:5)
Dry column (5-10 minutes at greater than 10 inches Hg (vacuum)/ Full Flow for Positive Pressure manifold).

4. ELUTE CARBOXY THC

1 x 3 mL Methanol/ NH₄OH (98:2).
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 derivitizing efficiency.

5. DRY ELUATE

Evaporate to dryness at < 40°C.

6. 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.

7. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

ANALYTE (TMS) Primary Ion Secondary Tertiary**

D₃-Carboxy- delta 9-THC* 374 476 491

D₉-Carboxy- delta 9-THC* 380 479 497

Carboxy-delta 9-THC 371 473 488

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