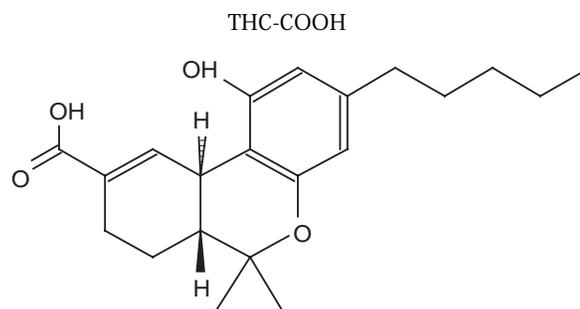


Extraction of THC-COOH from Urine using Strata™ Screen-C

This method is optimized for the extraction and clean-up of 11-Nor-delta-9-Tetrahydrocannabinol-9-Carboxylic Acid (THC-COOH), the metabolite of cannabis/marijuana used for SAMSHA drug confirmation, from urine. Strata Screen-C, a mixed-mode sorbent (C8 + SCX), successfully retains THC-COOH and any of the parent THC compound using hydrophobic interactions with the C8 portion of the sorbent. Urinary salts and very nonpolar contaminants are effectively removed by a series of aqueous and organic washes. The cationic amine interferences are irreversibly retained via ionic interactions with the SCX portion of the sorbent and never eluted. A strong organic elution solvent disrupts the analyte-sorbent hydrophobic interaction, resulting in a very clean, concentrated THC-COOH (+ parent THC) extract.



Specimen preparation:

Base Hydrolysis: To 2-4mL of urine add internal standard(s) + 300µL 10M potassium hydroxide. Mix/Vortex. Heat to 60°C for 20 min. Allow solution to cool. Add glacial acetic acid to adjust pH to 4-5.

Suggested internal standard for GC/MS:

d₃-Carboxy-Δ⁹-THC; d₃-THC.

SPE method:

Condition
1) 2mL methanol
2) 2mL 50mM phosphoric acid
Load
1) Apply the sample at a rate ≤2mL/min.
Wash/Dry
1) 3mL DI water
2) 3mL 50mM phosphoric acid/methanol (70:30)
3) Dry column 30-60 sec at full vacuum (>10" Hg).
4) 200µL hexane. Allow solvent to soak into sorbent for 15-30 sec, then aspirate.
Elute THC-COOH
1) With the vacuum turned off, apply 2mL hexane/ethyl acetate solution (50:50). Allow solvent to slowly soak into sorbent for 15-30 sec before applying vacuum. Optimal flow rate of elution solvent is ≤2mL/min.

(Important: The volumes shown are for 150mg sorbent mass. The method can be optimized for smaller or larger bed masses, by adjusting the solvent volumes.)



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Extraction of THC-COOH from Urine using Strata™ Screen-C

Derivatize:

Evaporate to dryness at $\leq 40^{\circ}\text{C}$. Add 50 μL ethyl acetate + 50 μL BSTFA (with 1% TMCS). Cap, mix/vortex and heat for 20 min at 70°C . Allow the solution to cool. Important: Do not evaporate the BSTFA solution.

BSTFA = *N,O*-bis(trimethylsilyl)trifluoroacetamide
TMCS = trimethylchlorosilane

Analysis:

Inject 1-2mL derivatized sample onto GC column. (recommended: Zebron ZB-5, 15m x 0.25mm x 0.25 μm).

Monitor the following ions (MSD):

Carboxy- Δ^9 -THC	d_3 -Carboxy- Δ^9 -THC	THC	d_3 -THC
371	374	303	306 ◀ quantification ions
473	476	315	318
488	491	386	389

Ordering Information:

Order No.	Description	Unit
8B-S016-EAK	Screen-C Tubes (100mg/1mL)	100/Box
8B-S016-EBJ	Screen-C Tubes (100mg/3mL)	50/Box
8B-S016-SBJ	Screen-C Tubes (150mg/3mL)	50/Box
8B-S016-RBJ	Screen-C Tubes (300mg/3mL)	50/Box
8B-S016-SCH	Screen-C Tubes (150mg/6mL)	30/Box
8B-S016-HCH	Screen-C Tubes (500mg/6mL)	30/Box
8E-S016-CGB	Screen-C 96-Well Plate (25mg/well)	2/Box
8E-S016-DGB	Screen-C 96-Well Plate (50mg/well)	2/Box
7EG-G002-11	Zebron ZB-5 (15m x 0.25m x 0.25 μm)	1/Box

Extraction Tips!

- Preparing solutions
 - 50mM phosphoric acid
Add 3.4mL of phosphoric acid to 950mL DI water in 1L volumetric flask. Mix and the bring volume up to the mark with DI water.
 - 10M potassium hydroxide
Add 140g of potassium hydroxide to an empty 250mL volumetric flask. Add 150mL DI water to dissolve the solid. Bring the volume up to the mark with DI water.
- Do not allow the sorbent to dry between the conditioning steps or prior to loading the sample. Excessive drying of the sorbent causes "deconditioning" which may lead to significantly lower and erratic recoveries. To ensure a properly solvated sorbent, apply each solvent immediately after the previous solvent.
- Always condition the sorbent with the strongest solvent used in the method to ensure the cleanest extraction of target analyte(s). In this method, 50mM phosphoric acid is used after methanol.
- During the wash step, drying the sorbent removes any residual water and will ensure optimal analyte recovery. Too strong of organic wash solvent will remove THC-COOH (+ parent THC compound), resulting in low recoveries.

Questions? Please contact your Phenomenex Technical Representative.

This method is designed as a convenient starting point for further investigation. Phenomenex makes no guarantee regarding the accuracy or completeness of the method.