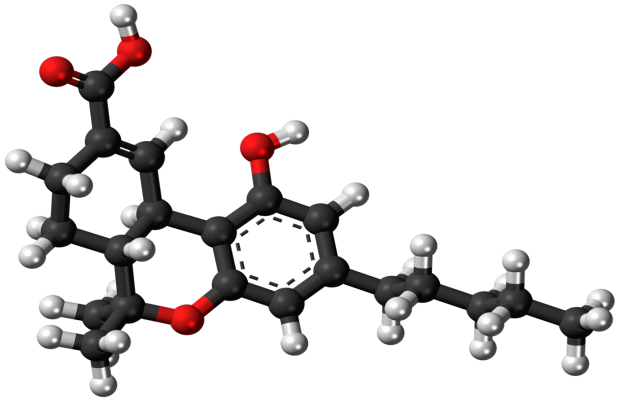
Determination of 11-nor-9-Carboxy-THC in Human Urine by QuEChERS and LC-MS/MS



UCT Part Numbers

ECQUUS1115CT
15 mL centrifuge tube containing 800 mg $MgSO_4$ and 200 mg NaCl

CUMC182CT
2 mL dSPE tube containing 150 mg $MgSO_4$ and 50 mg C18

SLDA100ID21-3UM
Selectra® DA LC column 100 x 2.1 mm, 3 µm

SLDAGDC21-3UM
Selectra® DA Guard Column 10 x 2.1 mm, 3 µm

SLGRDHLDR
Guard Column Holder

Summary:

11-nor-9-carboxy-THC also known as carboxy-THC or COOH-THC, is the main secondary metabolite of THC (the active component of marijuana) formed in the human body [1]. COOH-THC is excreted in urine in the form of glucuronide conjugates. COOH-THC is not psychoactive but has a long half-life of up to several days or even weeks in very heavy users, thus determination of COOH-THC in urine plays an important role in confirmation of marijuana consumption. The Substance Abuse and Mental Health Services Administration (SAMHSA) has set the COOH-THC cutoff concentration of confirmatory testing at 15 ng/mL. Typical sample preparation methods for COOH-THC in urine include liquid-liquid extraction (LLE) and solid phase extraction (SPE). This application utilizes a novel sample preparation technique, QuEChERS to effectively quantitate COOH-THC levels in human urine.

2 mL of a urine sample is hydrolyzed by sodium hydroxide (NaOH) to release COOH-THC from glucuronide conjugates. The sample is adjusted to pH 6-7 and the released COOH-THC is extracted with 2 mL of acetonitrile (MeCN). 800 mg magnesium sulfate (MgSO4) and 200 mg sodium chloride (NaCl) are used to enhance the phase separation and the partition of COOH-THC into the MeCN layer. After shaking and centrifuging, 1 mL of the supernatant is purified using a 2-mL dispersive SPE tube containing 150 mg $MgSO_4$ and 50 mg C18. $MgSO_4$ absorbs residual water in the extract, while C18 removes the non-polar matrix co-extractives, resulting in a clean extract for LC-MS/MS analysis.

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Sample Pretreatment:

1. Add 2 mL human urine to a clean test tube; add appropriate amount of COOH-THC spiking solution for calibration standards and spiked samples.
2. Add 100 µL of 10 M NaOH to each sample, and hydrolyze the urine samples at 65 °C for 20 min.
3. Allow the samples cool, then adjust sample pH to 6-7 using 6 M HCl.

QuEChERS Extraction:

1. Add 2 mL MeCN containing 200 ng/mL COOH-THC D3 (IS) to the 15 mL centrifuge tube containing pre-packed 800 mg $MgSO_4$ and 200 mg NaCl.
2. Transfer the pretreated urine sample to the 15 mL centrifuge tube.
3. Cap and shake for 1 min manually or use a Spex 2010 Geno-Grinder at 1000 strokes/min.
4. Centrifuge at 3000 g for 5 min.

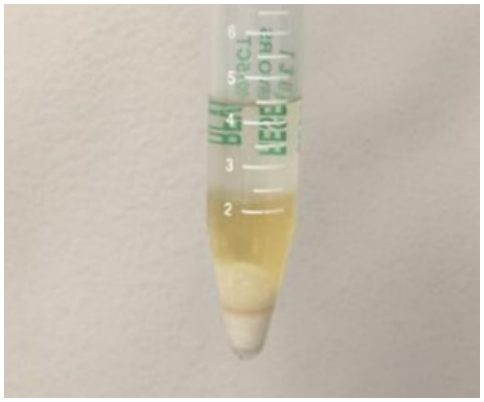

Figure 1. Sample after centrifugation
COOH-THC extracted into the upper clear layer

dSPE Cleanup:

1. Transfer 1 mL of the supernatant to a 2-mL centrifuge tube containing 150 mg $MgSO_4$ and 50 mg C18.
2. Shake 1 min manually or use the Spex 2010 Geno-Grinder at 1000 strokes/min.
3. Centrifuge at 3000 g for 5 min.
4. Transfer 0.4 mL of the cleaned extract into a 2-mL auto-sampler vial, add 0.4 mL of reagent water, and vortex for 30 sec.
5. The samples are ready for LC-MS/MS analysis.

LC-MS/MS Parameters:

System	AB Sciex API 4000 QTrap MS/MS with Agilent 1200 Binary Pump SL
Column	UCT Selectra® DA, 100 x 2.1 mm, 3 µm (**PN: SLDA100ID21-3UM**)
Guard Column	UCT Selectra® DA, 10 x 2.1 mm, 3 µm (**PN: SLDAGDC21-3UM**)
Column Temperature	40 °C
Column Flow Rate	0.3 mL/min
Injection Volume	15 µL

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Gradient Program		
Time (min)	A% (0.1% Formic Acid in H_2O)	B% (0.1% Formic Acid in MeOH)
0	90	10
2	0	100
10.0	0	100
10.2	90	10
15.0	90	10

MRM transitions (ESI -, dwell time: 100 ms)					
Compound	Rt (min)	Q1 ion	Q3 ion 1	Q3 ion 2	Linearity (R^2)
COOH-THC D3	7.27	346.1	302.1	247.9	NA
COOH-THC	7.28	343.1	299.0	245.0	0.9990

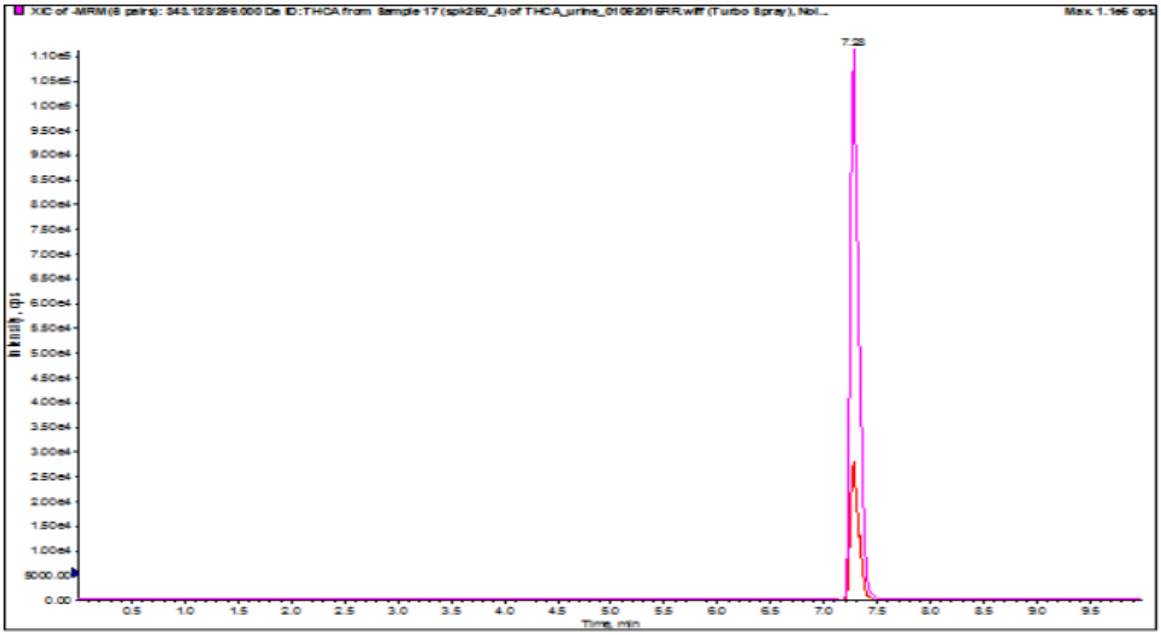

Figure 2. Chromatogram of a human urine sample spiked with 250 ng/mL COOH-THC

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Results:

Recovery and RSD% from Spiked Urine Samples				
	10 ng/mL		250 ng/mL	
Analyte	Recovery %	RSD % (n=6)	Recovery %	RSD % (n=6)
COOH-THC	115.3	2.0	101.1	3.4

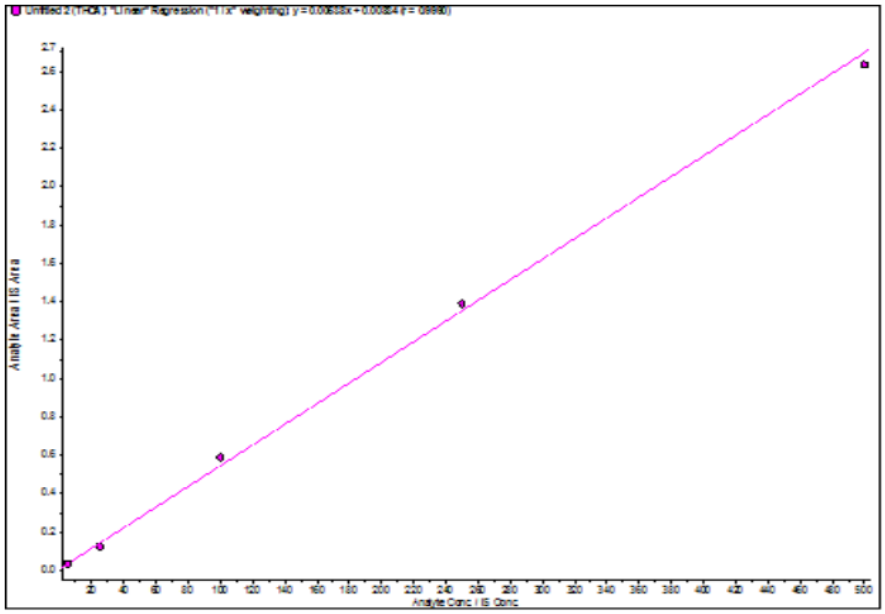

Figure 3. Example of a five-point matrix-matched calibration curve (R^2=0.9990).

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