



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

UCT Part Numbers

ECQUUS1115CT

Enviro-Clean® 15 mL CT tube
800 mg MgSO₄ and 200 mg NaCl

—

CUMC182CT

Enviro-Clean® 2 mL dSPE tube
150 mg MgSO₄ 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 2.0 mm, 3 µm

—

SLGRDHLDR

Guard Column Holder



Summary:

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

2 mL of a urine sample is hydrolyzed by sodium hydroxide (NaOH) to release THCA from its glucuronide conjugates. The sample is adjusted to pH 6-7 and the released THCA 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 THCA into the MeCN layer. After shaking and centrifugation, 1 mL of the supernatant is purified using a 2-mL dispersive SPE tube containing 150 mg MgSO₄ and 50 mg C18. MgSO₄ 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.



Sample Pretreatment:

1. Add 2 mL human urine to a clean test tube; add appropriate amount of THCA spiking solution for calibration standards and spiked samples.
2. Add 100 μ L of 10 M NaOH to each sample, and hydrolyze the urine samples at 65 $^{\circ}$ 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 THCA 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 THCA 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 (p/n: SLDA100ID21-3UM)		
Guard Column: UCT Selectra® DA, 10 x 2.1 mm, 3 µm (p/n: SLDAGDC21-3UM)		
Column Temperature: 40 °C		
Column Flow Rate: 0.3 mL/min		
Injection Volume: 15 µL		
Gradient Program:		
Time (min)	% Mobile Phase A (0.1% Formic Acid in Water)	% Mobile Phase 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)
THCA D3	7.27	346.1	302.1	247.9	NA
THCA	7.28	343.1	299.0	245.0	0.9990

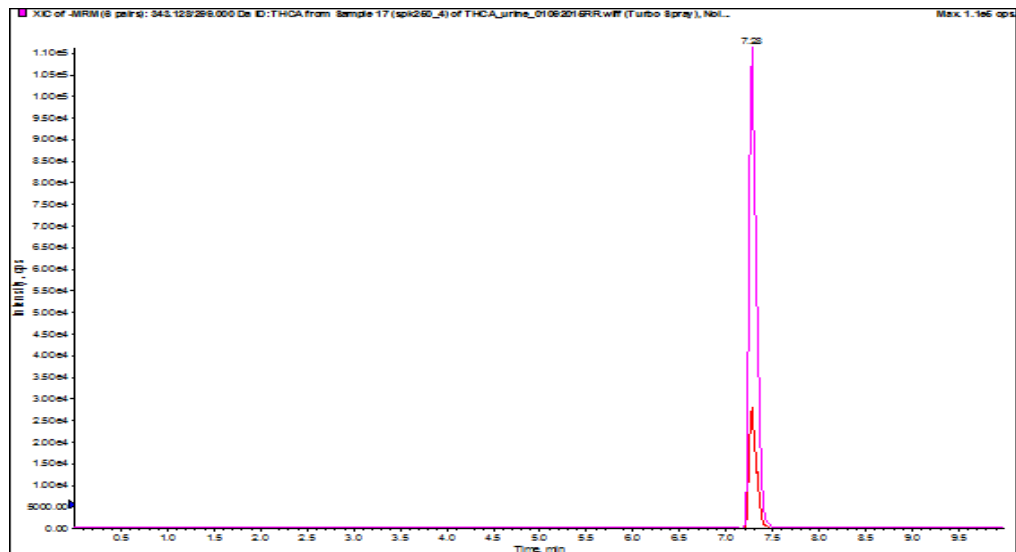


Figure 2. Chromatogram of a Human Urine Sample Spiked with 250 ng/mL THCA

Results:

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

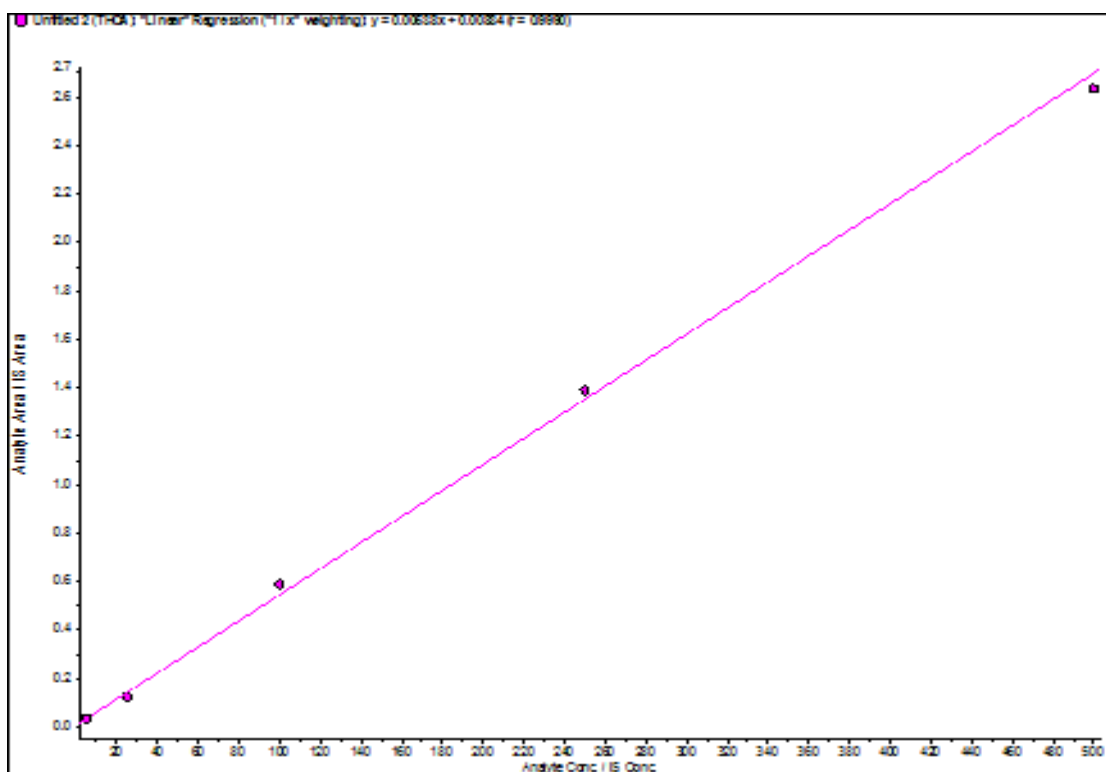


Figure 3. Example of a five-point matrix-matched calibration curve ($R^2=0.9990$).



5102-01-02

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 •
215.781.9255 www.unitedchem.com Email: methods@unitedchem.com
©UCT, LLC 2016 • All rights reserved

