



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

UCT Part Numbers:

**ECQUUS1115CT** - Enviro-Clean<sup>®</sup> 15 mL centrifuge tube with 800 mg MgSO<sub>4</sub> and 200 mg NaCl

**CUMC182CT** - Enviro-Clean<sup>®</sup> 2 mL dSPE tube with 150 mg MgSO<sub>4</sub> and 50 mg C18

**SLDA100ID21-3UM** - Selectra<sup>®</sup> DA LC column, 100 x 2.1 mm, 3 μm

**SLDAGDC21-3UM** - Selectra<sup>®</sup> DA guard column, 10 x 2.0 mm, 3 μm

**SLDGRDHLDR** - Guard cartridge holder

January 2015

### 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 (MgSO<sub>4</sub>) 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 MgSO<sub>4</sub> and 50 mg C18. MgSO<sub>4</sub> 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.

## Procedure:

### 1. Sample pretreatment

- a) Add 2 mL human urine to a clean test tube; add appropriate amount of THCA spiking solution for calibration standards and spiked samples.
- b) Add 100  $\mu\text{L}$  of 10 N NaOH to each sample, and hydrolyze the urine samples at 65  $^{\circ}\text{C}$  for 20 min.
- c) Allow the samples cool, then adjust sample pH to 6-7 using 6 N HCl.

### 2. QuEChERS extraction

- a) Add 2 mL MeCN containing 200 ng/mL THCA D3 (IS) to the 15-mL centrifuge tube containing pre-packed 800 mg  $\text{MgSO}_4$  and 200 mg NaCl (UCT p/n **ECQUUS1115CT**).
- b) Transfer the pretreated urine sample to the 15-mL centrifuge tube.
- c) Cap and shake for 1 min manually or use a Spex 2010 Geno-Grinder at 1000 strokes/min.
- d) Centrifuge at 3000 g for 5 min.

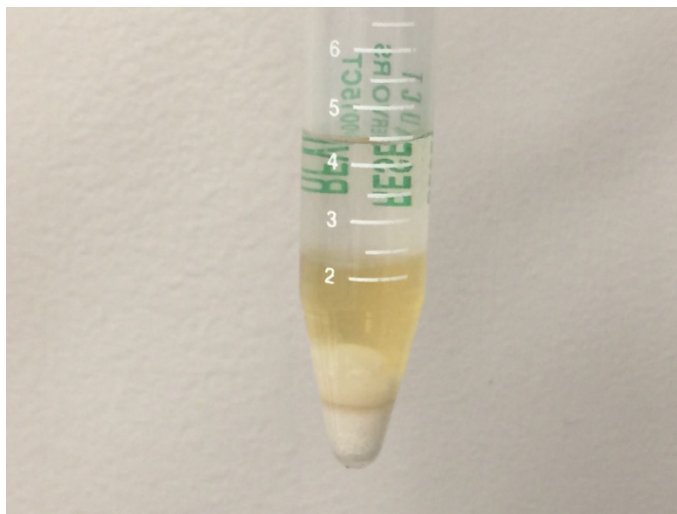


Figure 1. Sample after centrifugation  
THCA extracted into the upper clear layer

### 3. dSPE cleanup

- a) Transfer 1 mL of the supernatant to a 2-mL centrifuge tube containing 150 mg MgSO<sub>4</sub> and 50 mg C18 (UCT p/n **CUMC182CT**).
- b) Shake 1 min manually or use the Spex 2010 Geno-Grinder at 1000 strokes/min.
- c) Centrifuge at 3000 g for 5 min.
- d) 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.
- e) The samples are ready for LC-MS/MS analysis.

### LC-MS/MS method:

<b>HPLC:</b>	Agilent 1200 Binary Pump SL	
<b>Detector:</b>	AB Sciex API 4000 QTrap MS/MS	
<b>Column:</b>	UCT Selectra DA LC Column, 100 x 2.1 mm, 3 µm	
<b>Guard Column:</b>	UCT Selectra <sup>®</sup> DA guard column, 10 x 2.0 mm, 3 µm	
<b>Column Temperature:</b>	40 °C	
<b>Column Flow Rate:</b>	0.300 mL/min	
<b>Injection Volume:</b>	15 µL	
<b>Gradient Program:</b>		
<b>Time (min)</b>	<b>% Mobile Phase A (0.1% FA in water)</b>	<b>% Mobile Phase B (0.1% FA in methanol)</b>
0	90	10
2	0	100
10	0	100
10.2	90	10
15	90	10

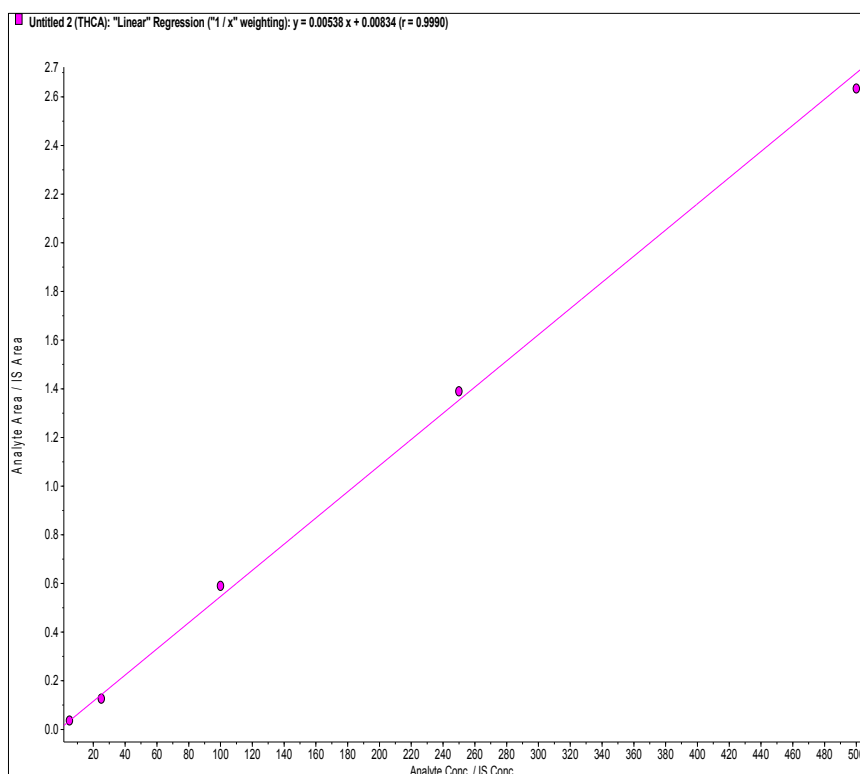
MRM transitions (ESI -, dwell time: 100 ms)					
Compound	Rt (min)	Q1 ion	Q3 ion 1	Q3 ion 2	Linearity (R <sup>2</sup> )
THCA D3	7.27	346.1	302.1	247.9	NA
THCA	7.28	343.1	299.0	245.0	0.9990

## Results:

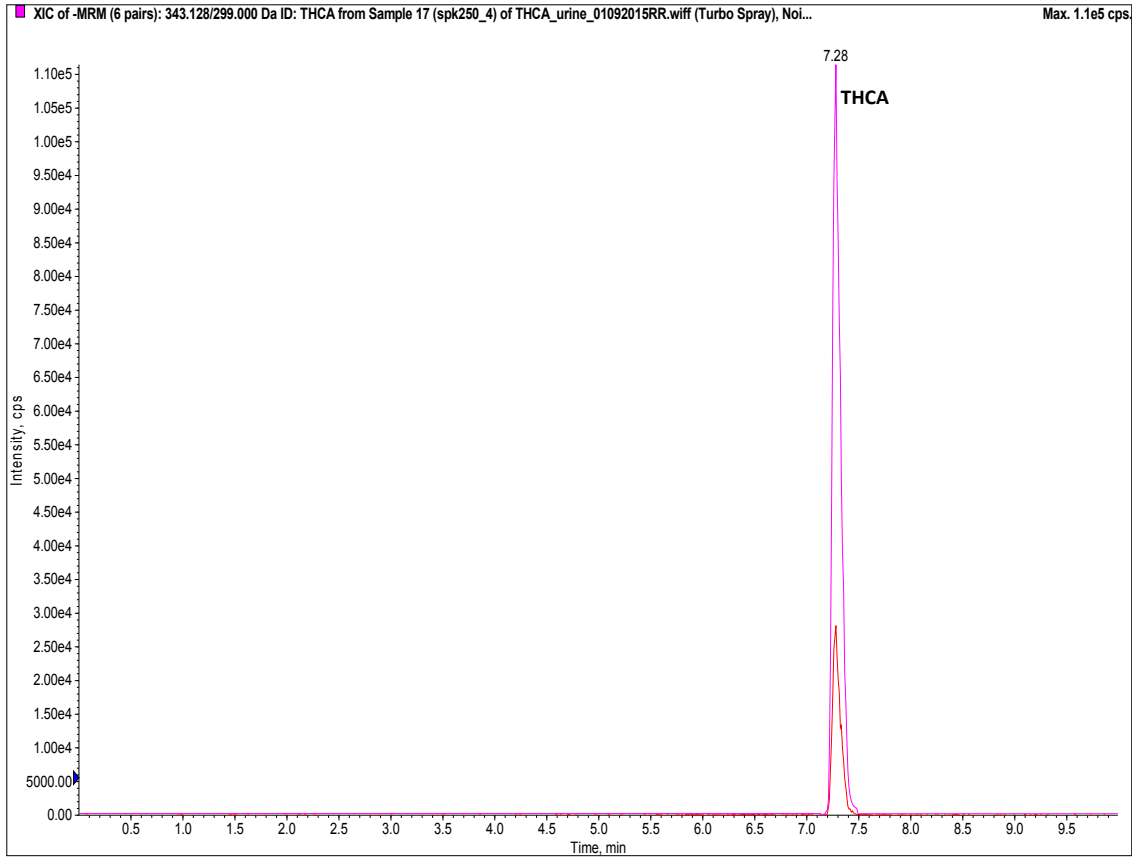
### Recovery and RSD% from Spiked Urine Samples

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

### Calibration Curve of THCA (R<sup>2</sup>=0.9990)



# Chromatogram of a Human Urine Sample Spiked with 250 ng/mL THCA



## Reference:

[1] <http://en.wikipedia.org/wiki/11-nor-9-Carboxy-THC>

5102-01-01

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 • 800.385.3153 • 215.781.9255 • [www.unitedchem.com](http://www.unitedchem.com)  
Email: [methods@unitedchem.com](mailto:methods@unitedchem.com) • ©UCT, LLC 2015 • All rights reserved