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 $MgSO_4$ and 200 mg NaCl

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CUMC182CT
Enviro-Clean® 2 mL dSPE tube
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 2.0 mm, 3 µm

SLGRDHLDR
Guard Column Holder

CLINICAL FORENSICS

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 ($MgSO_4$) 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 $MgSO_4$ 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.

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 µL of 10 M NaOH to each sample, and hydrolyze the urine samples at 65 ºC 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.

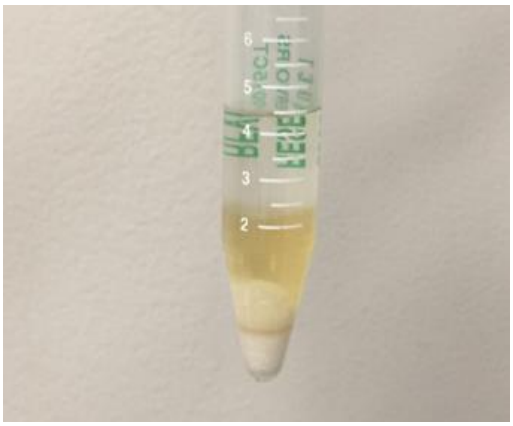

Figure 1. Sample after centrifugation
THCA extracted into the upper clear layer

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.

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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

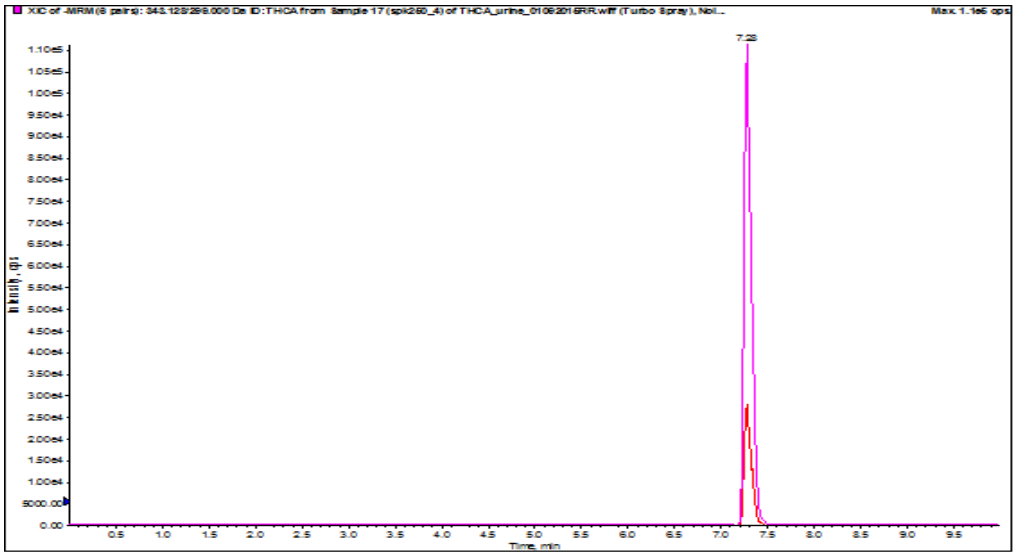

Figure 2. Chromatogram of a Human Urine Sample Spiked with 250 ng/mL THCA

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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

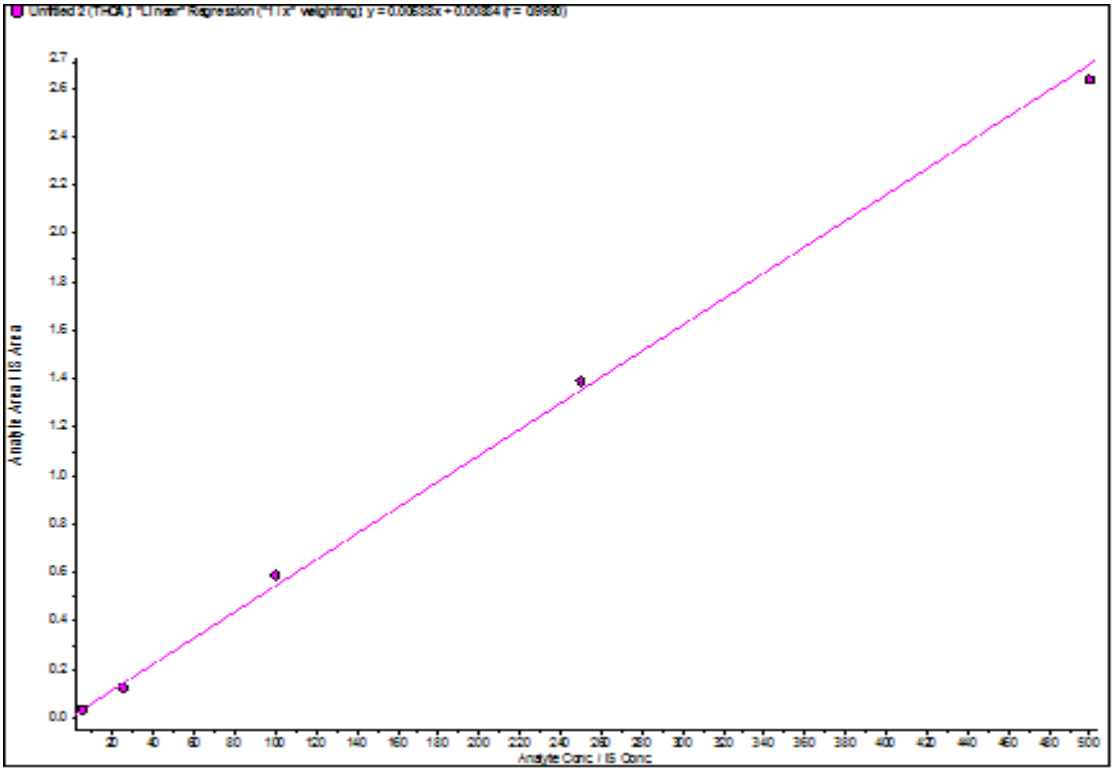

Figure 3. Example of a five-point matrix-matched calibration curve (R2=0.9990).

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MZ-Analysentechnik GmbH, Barcelona-Allee 17• D-55129 Mainz
Tel +49 6131 880 96-0, Fax +49 6131 880 96-20
e-mail: info@mz-at.de, www.mz-at.de

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