

CARBOXY-THC IN URINE BY LC-MS/MS OR GC-MS USING CLEAN SCREEN XCEL® II EXTRACTION COLUMN



UCT Featured Products

CSXCE2106
CLEAN SCREEN XCEL® II
130 mg 6 mL Tube

SMSTFA-1-1
SELECTRA-SIL® MSTFA
w/ 1% TMCS

SBSTFA-1-1
SELECTRA-SIL® BSTFA
w/ 1% TMCS

SLDA50ID21-5UM
Selectra® DA HPLC Column,
50 x 2.1 mm, 5 µm

Procedure:

1) Prepare Sample-Base Hydrolysis of Glucuronides

To 2 mL of urine add internal standard and 50 µL of 10 M NaOH.
Mix/vortex.
Hydrolyze for 15 minutes at 60-70 °C.
Cool before proceeding.
Adjust sample pH to 7.0 with 50 µL of 1:1 H₂O: Glacial Acetic Acid.
Add 200 µL pH 7.0 100mM Phosphate Buffer (pH should be ~7.0)

2) Apply Sample

Load at 1 to 2 mL/minute
Dry column (2 minutes at full vacuum or pressure)

3) Wash Column

1 x 2 mL Hexane
Dry Column at full vacuum or pressure for 10 minutes

4) Elute Analyte

1 x 3 mL Hexane/ Ethyl Acetate/ Glacial Acetic Acid (49: 49:2). Collect at 1-2 mL/ minute.

5) Dry Eluate

Evaporate to dryness at < 40 °C.

6) Reconstitute / Derivatize

LC-MS/MS: Reconstitute sample in 100 µL of mobile phase. Inject 20 µL.

GC-MS: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL MSTFA (with 1%TMCS). Overlay with N₂ and cap. Mix/ vortex. React 30 minutes at 70 °C; Cool and inject 1 µL.

Alternate Derivatization

Form TMS Derivatives by adding 50 µL BSTFA w/ 1% TMCS and 50 µL of Ethyl Acetate; React 45 minutes at 70 °C



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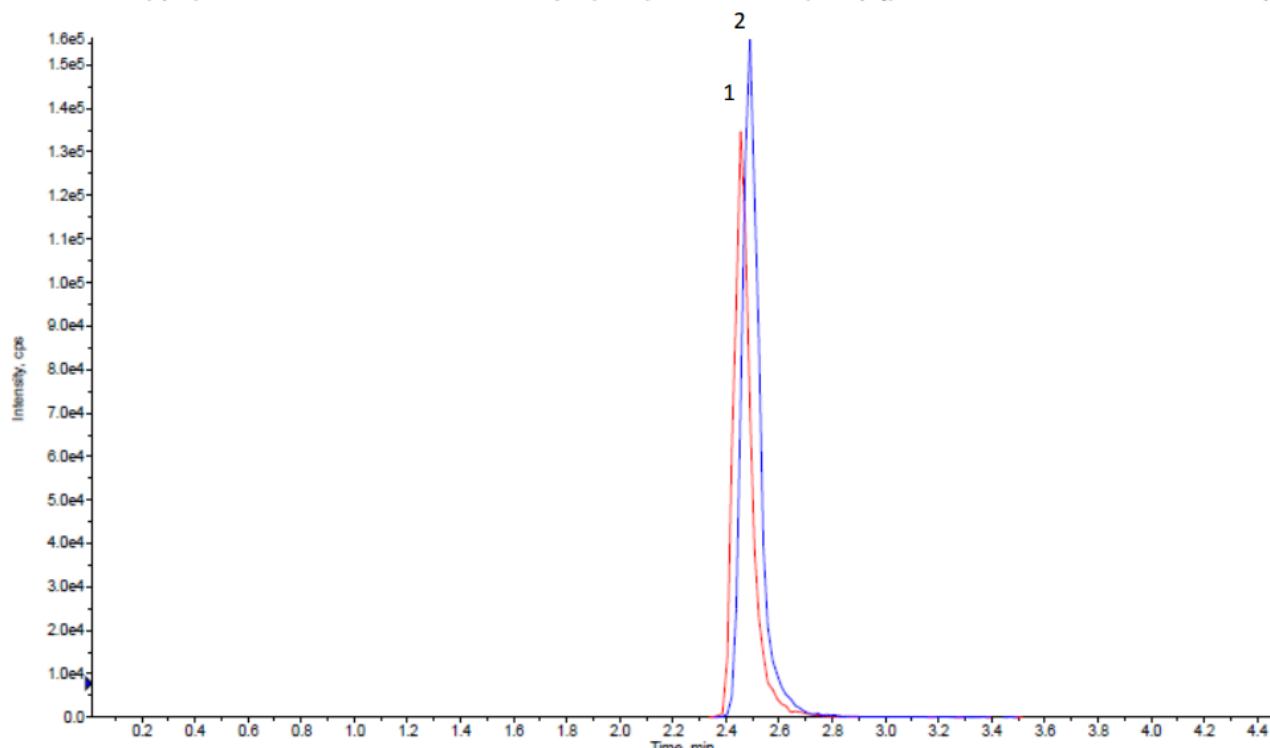
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INSTRUMENT CONDITIONS (LC-MS/MS):

■ XIC of -MRM (5 pairs): 343.0/299.3 Da ID: THC-DELTA 9-COOH 1 from Sample 2 (THC 60) of 010314-THCUR.wiff (Turbo Spray)

Max. 1.6e5 cps



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
THC-DELTA ₉ -COOH D ₉	352	308	2.44
THC-DELTA 9-COOH	343	299	2.49

LC-MS/MS Parameters	
Mobile Phase A	0.1% Formic Acid in D.I. H ₂ O
Mobile Phase B	0.1% Formic Acid in Methanol
Flow Rate	0.5 mL/minute
Reconstitute	100 µL
Instrument	API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC
LC Column	Selectra® DA HPLC Column 50 x 2.1 mm 5 µm
Polarity	Negative
Injection Volume	20 µL

Gradient		
Time	%A	%B
0.00	60	40
2.00	30	70
2.50	10	90
2.51	60	40
4.00	STOP	STOP



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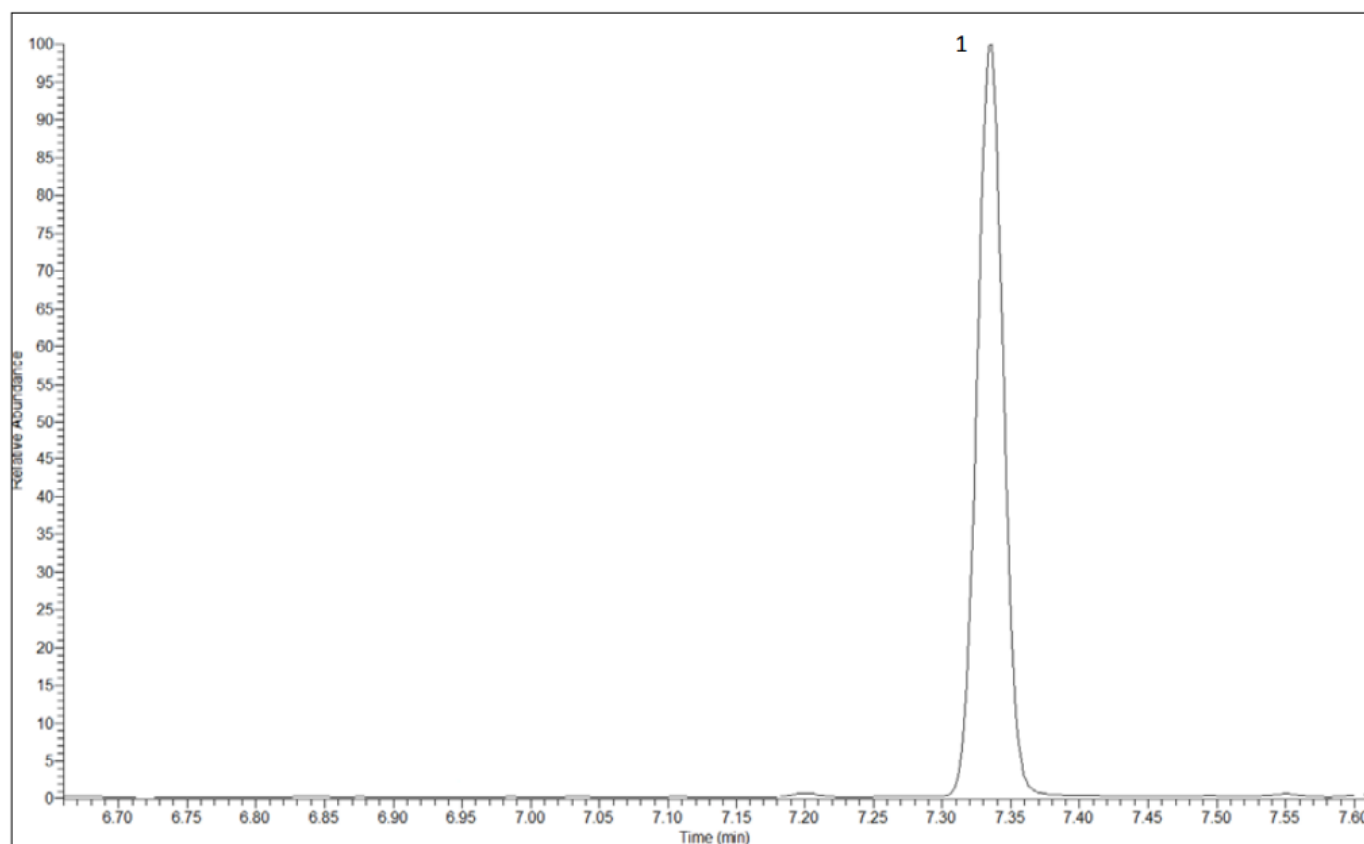


INSTRUMENT CONDITIONS (GC/MS):

GC/MS Parameters	
GC/MS	Thermo ISQ Trace 1300
GC capillary column	30 m x 0.25 mm (0.25 μ m) TG-1MS
Injector	1 μ L Splitless, 250 °C
Oven temperature program	170 °C (1) to 310 °C (30 °C/ minute): hold (5 minutes)
Carrier gas	Helium (1.2 mL/ minute)
MSD condition	Aux temperature: 280 °C, MS Source: 350 °C, MS Quad: 150 °C

MSTFA/BSTFA TMS IONS:

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
THC-COOH	371	473	488	7.34
THC-COOH D ₃	374	476	491	7.31



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