



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

Part #

ZSTHC020 – CLEAN SCREEN® THC 200 mg 10 mL Tube

or

CSDAU206 – CLEAN SCREEN® DAU 200 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

1. PREPARE SAMPLE-BASE HYDROLYSIS OF GLUCURONIDES:

To 2 mL of urine add internal standard and 100 µL of 10 M NaOH.

Mix/vortex.

Hydrolyze for 20 minutes at 60 °C. Cool before proceeding.

Adjust sample pH to 3.0 with approx. 1.0 mL Glacial Acetic Acid. (pH should be ~3.0)

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN:

1 x 3 mL CH₃OH.

1 x 3 mL D.I. H₂O.

1 x 1 mL Acetate buffer (pH 3.0)

NOTE: Aspirate at full vacuum or pressure

3. APPLY SAMPLE:

Load at 1 to 2 mL/minute.

4. WASH COLUMN:

1 x 2 mL D.I. H₂O

1 x 2 mL 100 mM HCl/Acetonitrile (95:5)

Dry column (10 minutes at full vacuum or pressure)

1 x 200 µL Hexane

Aspirate at full vacuum or pressure (Additional step to remove any residual moisture)

5. ELUTE ANALYTE:

1 x 3 mL Hexane/ Ethyl Acetate (50:50)

Collect eluate at 1 to 2 mL/minute

NOTE: Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing efficiency

6. DRY ELUATE:

Evaporate to dryness at < 40 °C.

7. RECONSTITUTE / DERIVATIZE:

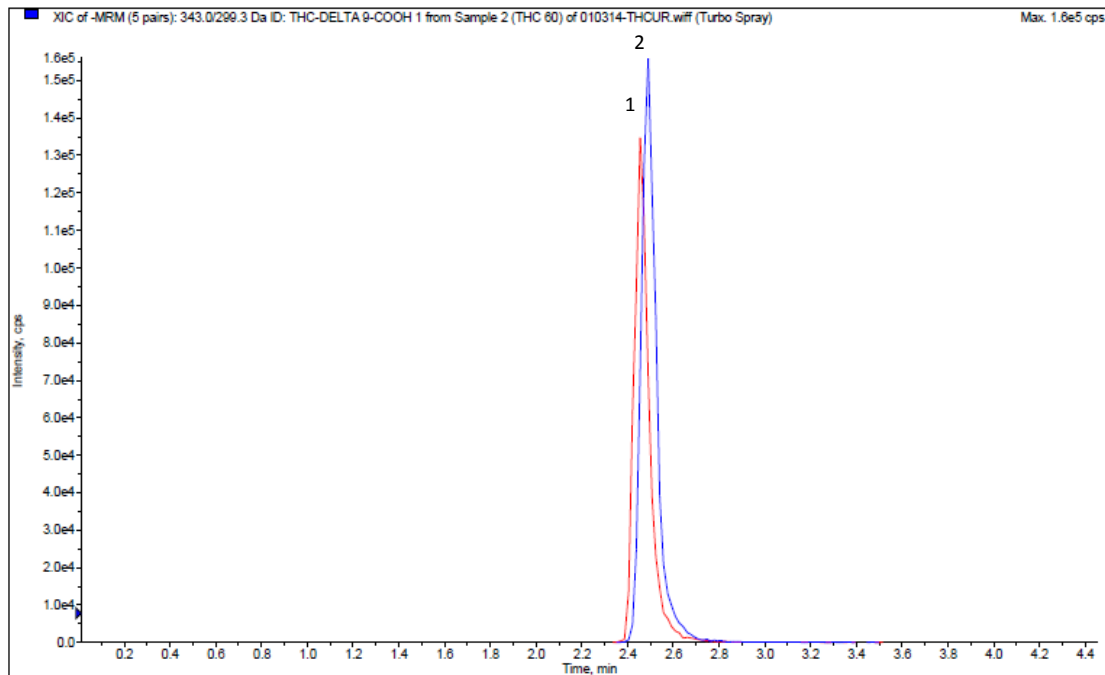
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 20 µL.
- **GC-MS:** Dissolve residue in 50 µL of Ethyl Acetate and
50 µL MSTFA w/1%TMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 µL

Alternate Derivatization

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

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



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

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H₂O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Negative

Reconstitute: 100 µL

Injection Volume: 20 µL

LC Column: Selectra[®] DA HPLC Column 50 x 2.1 mm 5 µm

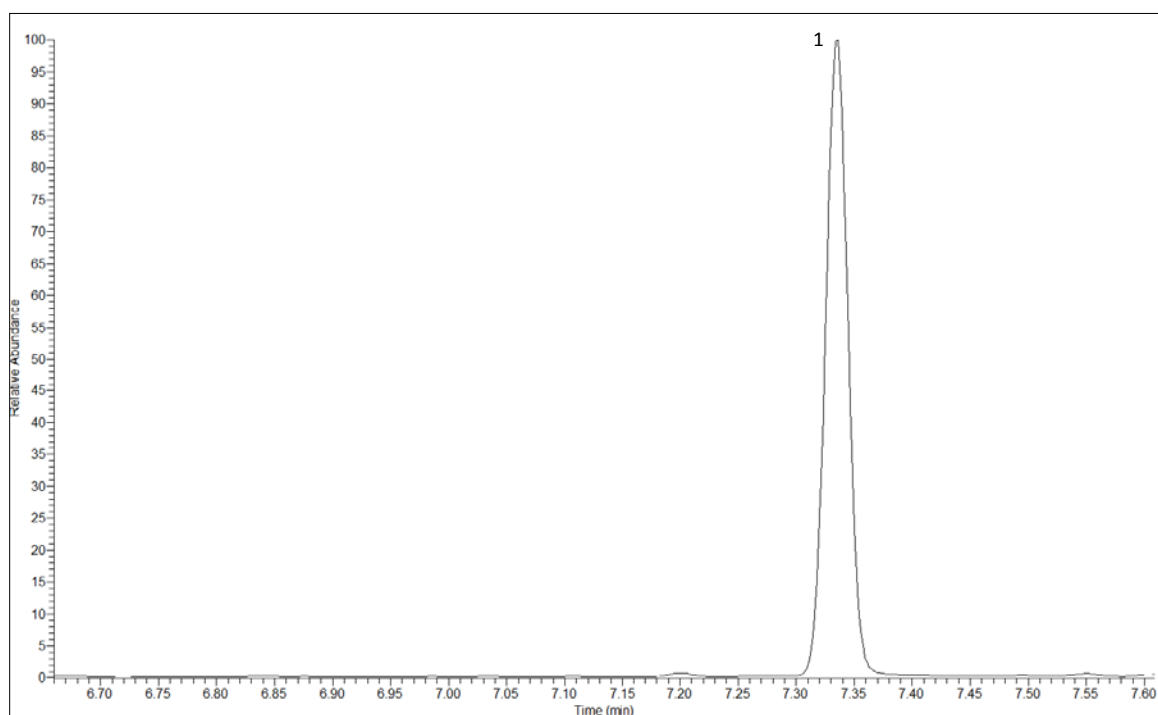
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

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

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



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

PARAMETERS

GC/MS: Thermo ISQ Trace 1300

GC capillary column: 30 m x 0.25 mm (0.25 µm) TG-1MS

Injector: 1 µL 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