THC-COOH FROM ORAL FLUIDS BY LC-MS/MS OR GC-MS
CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #
ZSTHC020 - CLEAN SCREEN® THC 200 mg, 10 mL Tube
or
CSDAU206 - CLEAN SCREEN® THC 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:**
   To 1 mL of oral fluid specimen 50ng/mL internal standard (THCA-D9) and let sit for ten minutes at room temperature
   Mix/vortex for 10 seconds
   Add 0.5 mL of Glacial Acetic Acid and vortex for 10 seconds

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 0.1N HCl.
   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. H_2O
   1 x 2 mL 0.1 M HCI: Acetonitrile (70:30)
   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 2 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 MSTFA 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 N_2 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

Contributed by:
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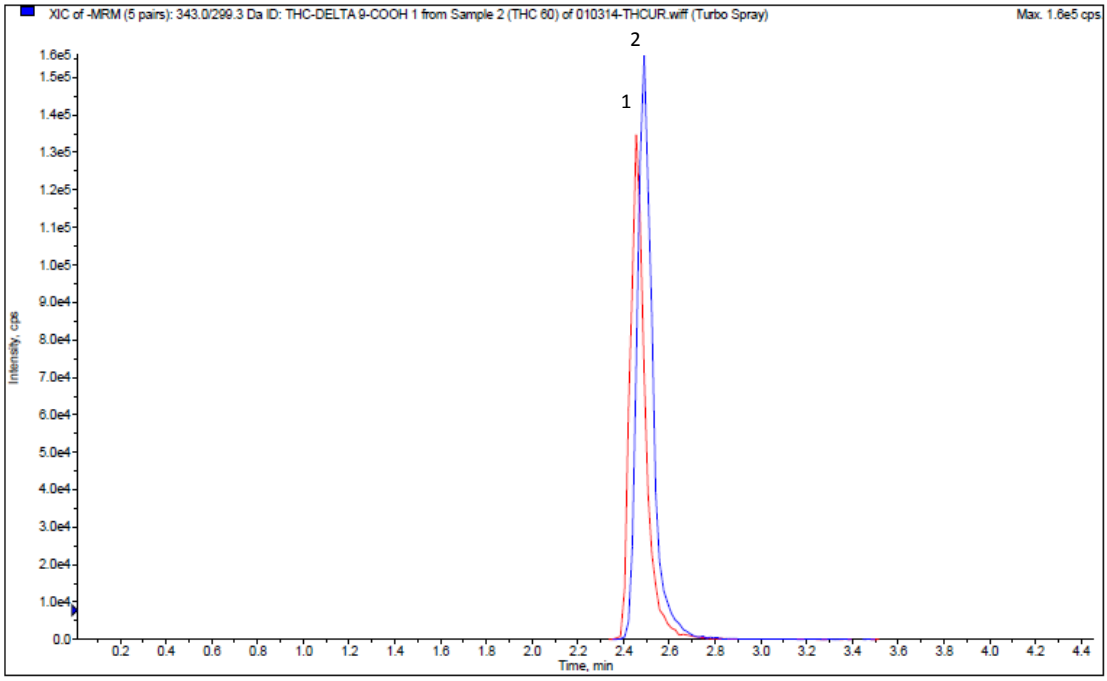
INSTRUMENT CONDITIONS (LC-MS/MS):



Analyte	MRM Transitions		Relative Retention Time (minutes)
	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. H_2O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Negative

Reconstitute: 100 µL

Injection Volume: 20 µL

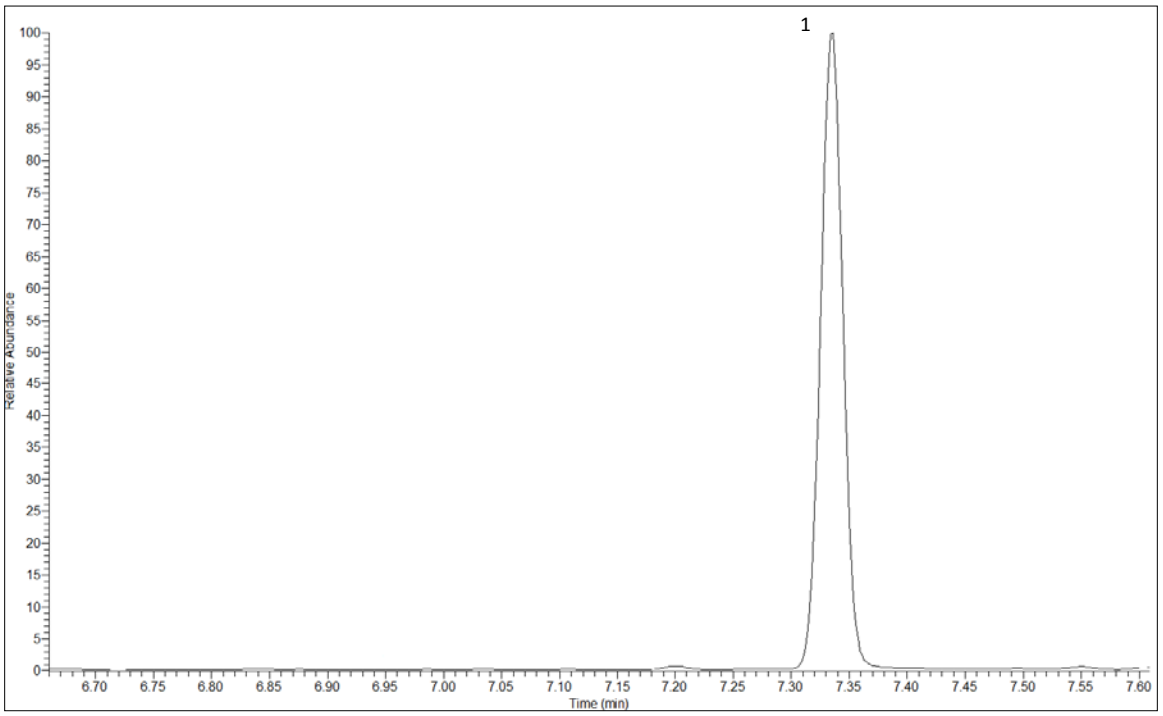
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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):



MSTFA/BSTFA TMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. 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 ºC, MS Source: 350 ºC, MS Quad: 150 ºC