



CARBOXY-THC IN URINE BY LC-MS/MS OR GC-MS USING 30mg STYRE SCREEN[®] DBX EXTRACTION COLUMN

Part #:

SSDBX033 without Tips - Styre Screen[®] DBX 30 mg 3 mL Tube

or

SCDBX033 with CLEAN-THRU[®] Tips – Styre Screen[®] DBX w/ CLEAN THRU[®]
Tips 30 mg 3 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 μ m

1. PREPARE SAMPLE-BASE HYDROLYSIS OF GLUCURONIDES:

To 2 mL of urine add internal standard and 100 μ L 10M NaOH.
Mix/vortex. Hydrolyze for 20 mins at 60 °C. Cool before proceeding.
Adjust sample pH to 3.5 \pm 0.5 with 1.0 mL glacial acetic acid.

2. APPLY SAMPLE TO DBX COLUMN:

Load at a rate of 1 to 2 mL/min.

3. WASH COLUMN:

1 x 1 mL D.I. H₂O.
1 x 1 mL 0.1M HCl/Acetonitrile (70/30).
Dry column (3 mins at > 10 inches Hg).
1 x 200 μ L Hexane.

4. ELUTE CARBOXY-THC:

2 x 0.5 mL Hexane/ Ethyl Acetate (50:50); Collect eluate at 1 to 2 mL/min.
Evaporate eluate to dryness at < 40 °C.

5. DRY ELUATE:

Evaporate to dryness at < 40 °C.

6. RECONSTITUTE / DERIVATIZE:

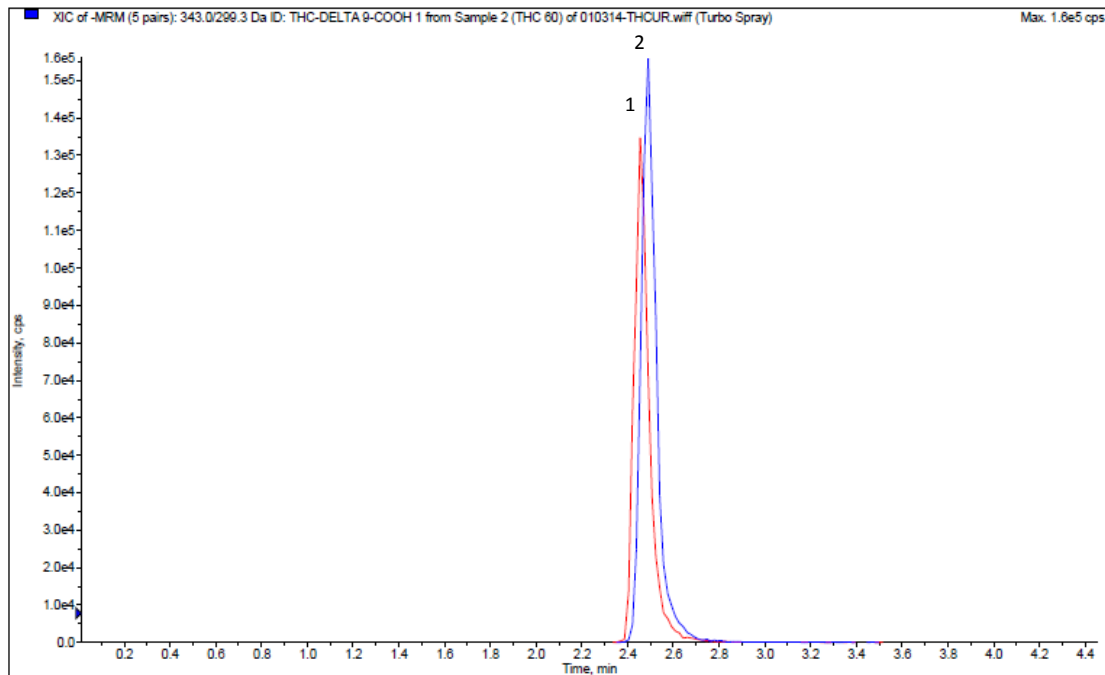
- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 20 μ L.
- **GC-MS:** Dissolve residue in 50 μ L of Ethyl Acetate and 50 μ L MSTFA w/
1%TMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 μ L

Alternate Derivatization

1. Form TMS Derivatives by adding 50 μ L BSTFA w/ 1% TMCS and 50 μ L 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

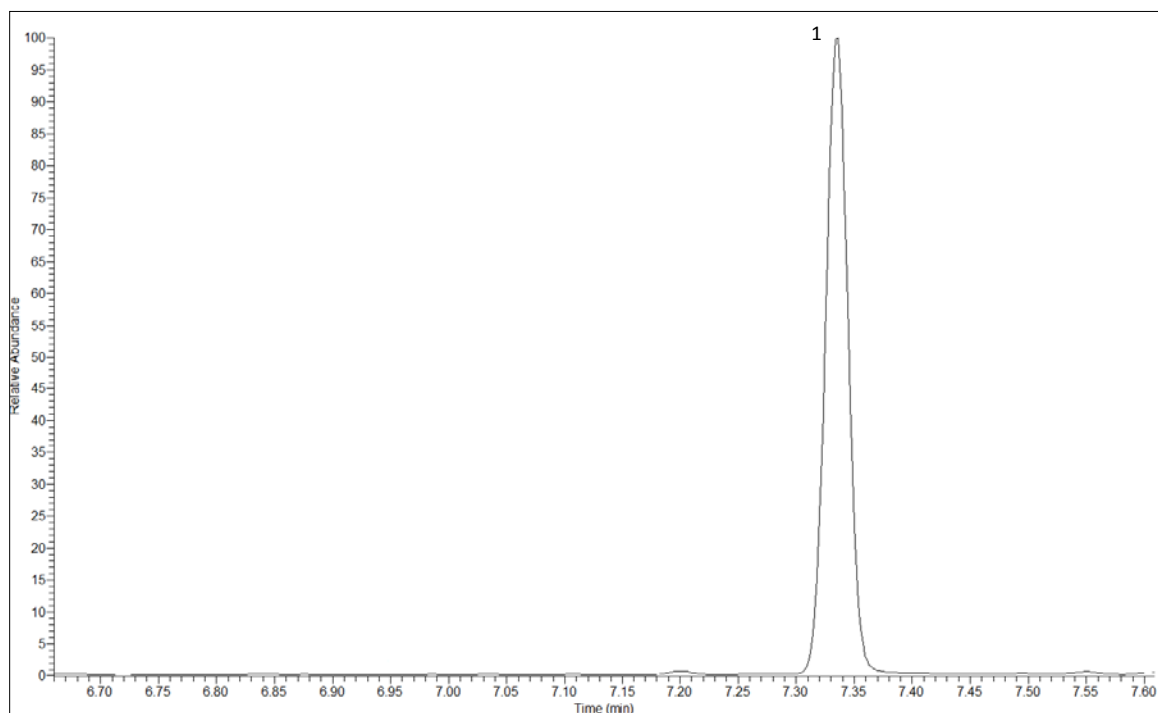
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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)
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 °C, MS Source: 350 °C, MS Quad: 150 °C