Δ^8-THC by GC-MS: Ion Ratio Failure Investigation

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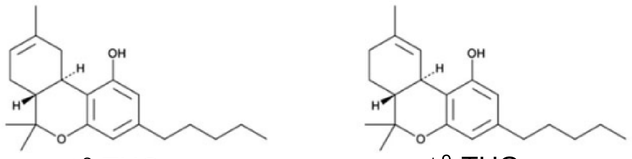
OBJECTIVE

- Add Δ^8-tetrahydocannabinol (THC) metabolite 11-nor-9-carboxy-Δ^8-THC (Δ^8-THCCOOH) to our existing Δ^9-THC urine confirmation GC-MS assay



Δ^8-THC Δ^9-THC

Figure 1: Δ^8-THC differs from its isomer Δ^9-THC in the location of a single double bond.

PROBLEM

- Ion ratio failures of Δ^8-THCCOOH due to multiple possible factors

METHOD

- Base hydrolysis:
  - 1.0 mL patient urine 50 µL internal standard undergo alkaline hydrolysis with 200 µL 10 M KOH
  - Vortex; incubate at 60°C for 20 minutes; cool 5-10 minutes at ambient temperature
  - Add 1.5 mL glacial acetic acid and vortex
- Extraction:
  - Condition 10 mL UCT THC Clean Screen cartridges on positive pressure manifold
  - Pour into column reservoir, slowly increase flow (1-2 mL/min)
  - Add 3 mL DI H2O; 2 mL THC Wash Solution (100 mM HCl with 5% acetonitrile); air dry 5 min; 200 uL hexane
  - Elute with 2 mL 1:1 hexane and ethyl acetate
  - Evaporate under nitrogen gas (LABCONCO RapidVap Vertex Evaporator) at 12 psi and dry bath at 60°C for approx. 30 min
- Derivatization:
  - Add 100 µL BTSFA with 1% TMCS to the dry residue; cap
  - Heat at 70 °C for 30 minutes; cool 5-10 min; transfer to vials with micro inserts, crimp seal
- GC-MS analysis:
  - Confirmation with Agilent MSD system/THC_ACQ.m program. Selected ion monitoring (SIM) mode analyzes ions in Table 1
  - 1 uL aliquots of derivatized samples are injected onto the column by the autosampler
  - Agilent Technologies 15m HP-5MS, 0.25mm i.d., 0.25 µm film thickness fused silica capillary column
  - GC oven temp. program: 175°C isothermal for 1 minute, then increase at 25°C /minute to 280°C, held at 280° for 2.5 minutes on the MSD 5975 and 175°C increasing at 25°C/min to 280°C, held at 280°C for 2.5 minutes on the MSD 5977
  - Helium is used as carrier gas
  - Mass Spec Conditions: Positive Ion Electron Impact (EI).
  - Transfer line temp. = 280°C on MSD 5975, 310°C on MSD 5977
  - MS Source temp. = 230°C for MSD 5975, 300°C for MSD 5977

Analyte	Product Ion (m/z)	Role
Δ^8-THCCOOH	303	Quantifier
Δ^8-THCCOOH	488	Qualifier
Δ^8-THCCOOH	432	Qualifier
Δ^9-THCCOOH	371	Quantifier
Δ^9-THCCOOH	473	Qualifier
Δ^9-THCCOOH	488	Qualifier
Δ^9-THCCOOH-d3	374	Internal Standard
Δ^9-THCCOOH-d3	476	Internal Standard
Δ^9-THCCOOH-d3	491	Internal Standard

Table 1: Ion m/z values

Δ^8-THCCOOH: WHAT IS EXPECTED

- Qualifier ions 488 and 432 are used with quantifier ion 303 to form ion ratios used to determine acceptability (Figure 2)
- These ratios must be within +/-20% of the corresponding ion ratios of the 100 ng/mL standard for Δ^8-THCCOOH

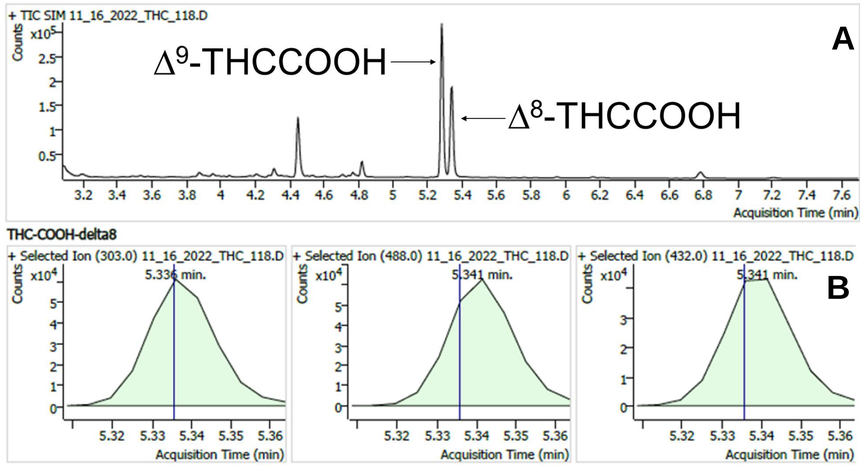


Figure 2: A, Δ^8-THCCOOH has a slightly longer retention time than Δ^9-THCCOOH. **B**, SIM at Δ^8-THCCOOH product ions

Δ^9-THC: GETTING TOO HIGH IS A PROBLEM

- In specimens with Δ^9-THCCOOH quantities higher than the analytical measurement range (>1000ng/mL), 488 and 432 ion ratios have a higher rate of failure (Figures 3 and 4)

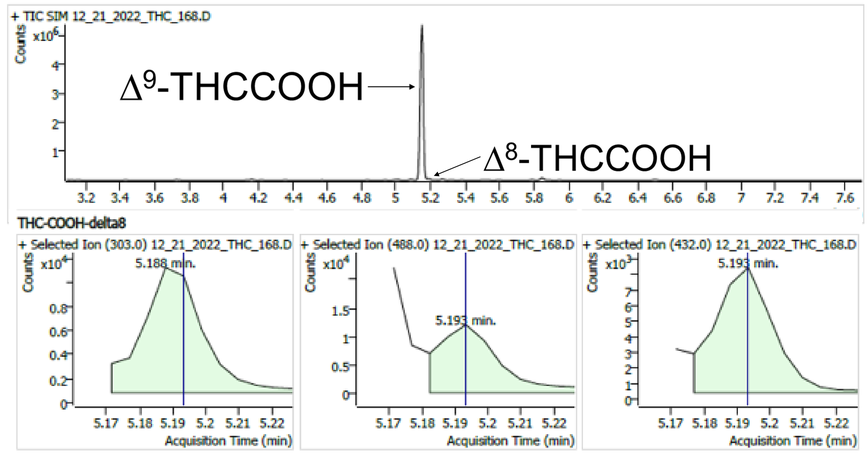


Figure 3: Δ^8-THCCOOH peaks merging with adjacent peaks, causing 488 ratio failure in context of >1000 ng/mL Δ^9-THCCOOH

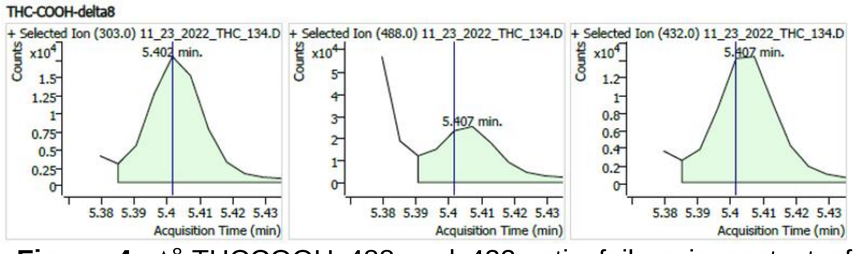


Figure 4: Δ^8-THCCOOH 488 and 432 ratio failure in context of >1000 ng/mL Δ^9-THCCOOH

432 ION AND MYSTERY PEAKS, ETC.

- Peaks of unknown identity with retention time (RT) between 4.4-4.6 minutes are seen with 432 ion ratio failures (n=4, Figure 5)
- Ion ratio failures seen with delayed RT peak (not shown)
- Δ^8-THCCOOH >1000 ng/mL is associated with 432 ion ratio failures (n=5, Figure 6)

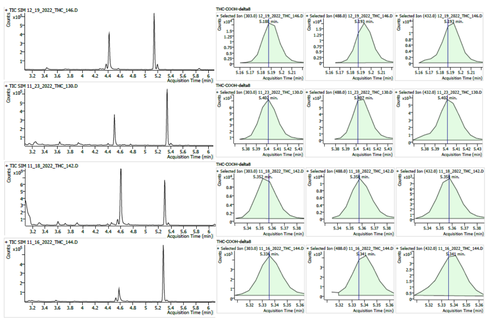


Figure 5: Peaks at ~4.5 min and Δ^8-THCCOOH ion peaks

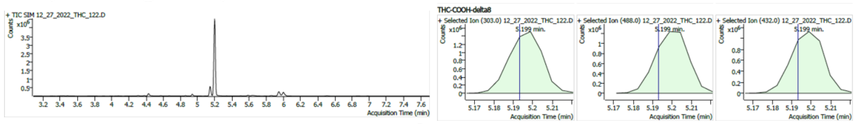


Figure 6: Representative high Δ^8-THCCOOH example

HIGH Δ^9-THC AND PEAK PARAMETERS

- Of 304 samples run from Nov 2022 to Dec 2023, 112 quantitated positive for Δ^8-THCCOOH, with 72 (64.2%) meeting criteria including appropriate RT and ion ratios

	Δ^9-THCCOOH Quantity (AMR 10-1000 ng/mL)					
	Negative (n=20)	10-99 ng/mL (n=33)	100-549 ng/mL (n=17)	550-1000 ng/mL (n=12)	>1000 ng/mL (n=30)	Δ^9:Δ^8 quantity ratio
Δ^8-THCCOOH: ratio acceptable (n=70)	15	26	13	9	7	9.6
Δ^8-THCCOOH: ratio unacceptable (n=42)	5	7	4	3	23	52.2
432 failure only (n=22)	5	4	4	2	7	19.4
488 failure only (n=11)	0	0	0	0	11	119.6
432 & 488 failure (n=9)	0	3	0	1	5	50.1

Table 2: Ion ratio acceptability by Δ^9-THCCOOH quantity in Δ^8-THCCOOH positive cases. Column on right shows the ratio between Δ^9-THCCOOH and Δ^8-THCCOOH quantities.

- Greater peak width and increased signal to noise ratio (S/N) are seen in cases with ion ratio failures (Table 3)

	Sample Count	Δ8-THCCOOH Quantity (ng/mL)	Δ9-THCCOOH Quantity (ng/mL)	Height	Width	FWHM	Noise	S/N
Δ8-THCCOOH: ratio acceptable	72 (64.2%)	409	379	460300	0.077	0.018	95	5650
Δ8-THCCOOH ratio unacceptable:	42 (37.5)	471	1544	1543029	0.096	0.022	165	15048
432 ratio failure	22 (19.6%)	814	546	801309	0.081	0.020	132	8060
488 ratio failure	11 (8.8%)	47	3829	2952021	0.118	0.029	188	28381
432 & 488 ratio failures	9 (8.0%)	69	1237	1263878	0.110	0.022	278	10088

Table 3: Ion ratio failure categories by mean analyte quantity and peak characteristics

FUTURE STUDIES

- Consider creative options to enhance chromatographic separation of Δ^8-THCCOOH and Δ^9-THCCOOH
- Investigate cases with peaks of unknown identity with untargeted LC-MS/MS and library matching
- Consider increasing ion ratio acceptability criteria to +/-30% of Δ^8-THCCOOH 100ng/mL standard ion ratio
- Perform side-by-side runs on second instrument (MSD 5977)
- Consider ion ratio acceptability by height

Access this poster and references:

UVA Health

Disclosures: The authors have no relevant disclosures.