Investigation of the composition of a Δ^8-THC-acetate containing sample Amota Processing THCO-D8 Acetate 8.02.21 (SA-08042021-3132)

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Introduction

A sample was submitted for analysis by Amota Processing (THCO-D8 Acetate 8.02.21 – SA-08042021-3132) that was purported to contain Δ^8-THC-acetate. The sample was analyzed to verify this claim. Although a reference standard for Δ^9-THC-acetate is commercially available, a reference standard for Δ^8-THC-acetate is not available. Therefore, we could not verify the identity of the submitted material as Δ^8-THC-acetate by comparison to a certified reference standard. However, the sample was analyzed by GC-MS and HPLC-PDA and the resulting data were interpreted and compared to published data for Δ^8-THC-acetate and laboratory data for Δ^9-THC-acetate.

Note that Δ^9-THC acetate and Δ^9-THC-O-Acetate are used interchangeably to refer to the same substance.

Experimental

The following samples were prepared for HPLC-PDA and GC-MS analysis:

Δ^9-THC-acetate solution:

- In a vial, 10 µL of Δ^9-THC-O-Acetate solution (Cerilliant T151-1ML, lot FE03192002, 1 mg/ml) was pipetted.
- The solution was diluted with 990 µL of acetonitrile.
- The final concentration of Δ^9-THC-acetate was 10 µg/mL.

Sample solutions:

- In a 50-mL centrifuge tube, 25±0.1 mg of sample was weighed out.
- The sample was dissolved in 25 mL of acetonitrile.
- The sample concentration of the intermediate solution was 1 mg/mL.
- Into a vial, 10 µL of intermediate solution was pipetted.
- To the vial, 990 µL of acetonitrile was added.
- The sample concentration of the final solution was 10 µg/mL.

Mixed solution:

- Into a vial, 100 µL of Δ^9-THC-acetate solution was pipetted.
- To the vial, 100 µL of sample solution was pipetted.
- The final concentrations were 5 µg/mL of Δ^9-THC-acetate and 5 µg/mL of sample or Δ^8-THC-acetate (assuming the sample is pure Δ^8-THC-acetate).

All samples were analyzed by HPLC-PDA to determine the overall cannabinoid content and by GC-MS to collect mass spectral information for further identification of any acetylated Δ^8-THC.

Results and Discussion

HPLC-PDA Results

During analysis of sample SA-08042021-3132 by HPLC-PDA for cannabinoids, minor cannabinoids eluting in the RT range and with similar absorbance to Δ9-THC and Δ8-THC were observed. A major constituent eluted at 11.98 min (Fig. 1) but its retention time and DAD spectrum (Fig. 2) did not match those of any current cannabinoid reference standard evaluated by KCA Labs. Without a reference standard for

comparison, the identity of this unknown substance could not be ascertained by HPLC. The compound in question had a relative peak area contribution of 64.56 % compared to the total areas of all peaks in the chromatogram (minus the peak represented by the internal standard).

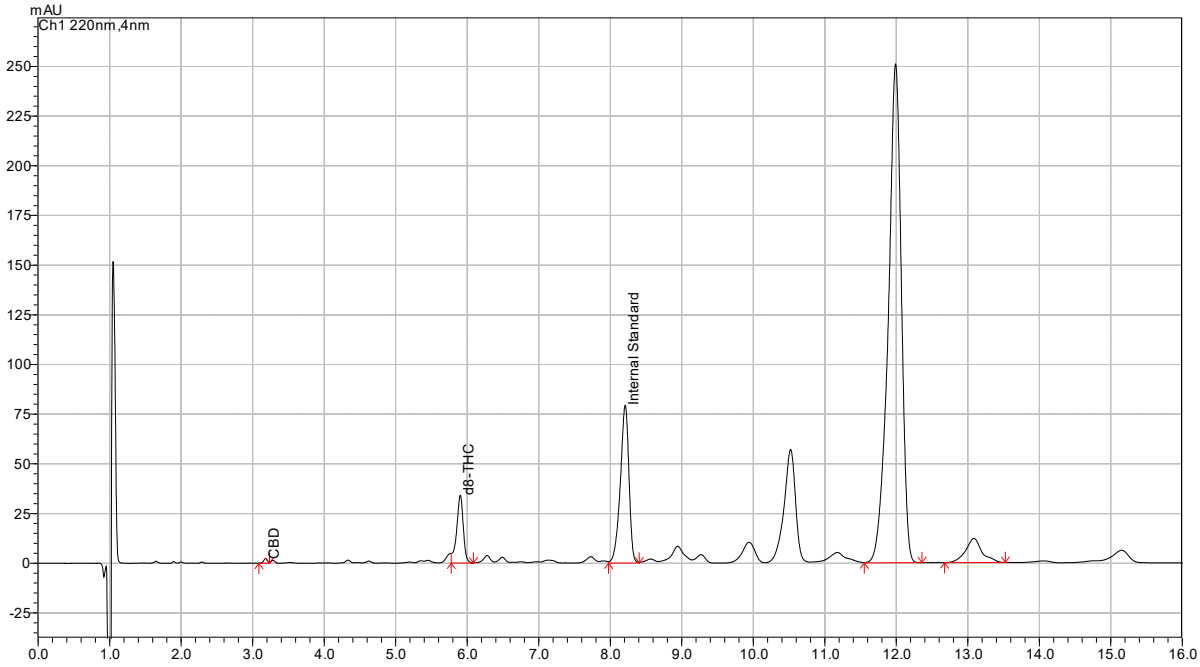

Figure 1: HPLC-PDA Chromatography of Sample SA-08042021-3132

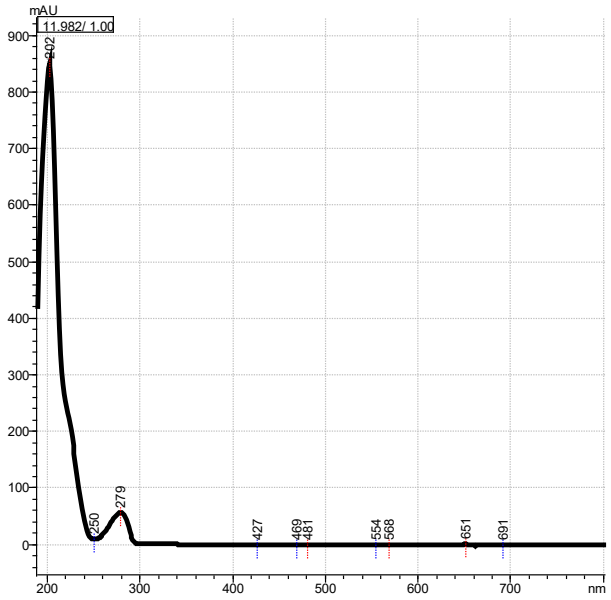

Figure 2: PDA Spectrum of unknown peak eluting at 11.98 min

GC-MS Results

The unidentified peak in the HPLC analysis of the sample was believed to be Δ^8-THC-acetate based on client communication. To assist in determination, the available Δ^9-THC-acetate reference standard was analyzed and evaluated for suitability for identification. In the full-scan TIC (total ion chromatogram) of the Δ^9-THC-acetate solution (Fig. 3), a signal was observed at 6.07 min. The mass spectrum at this retention time (Fig. 4) was consistent with that of Δ^9-THC-acetate through a library match (Fig. 5).

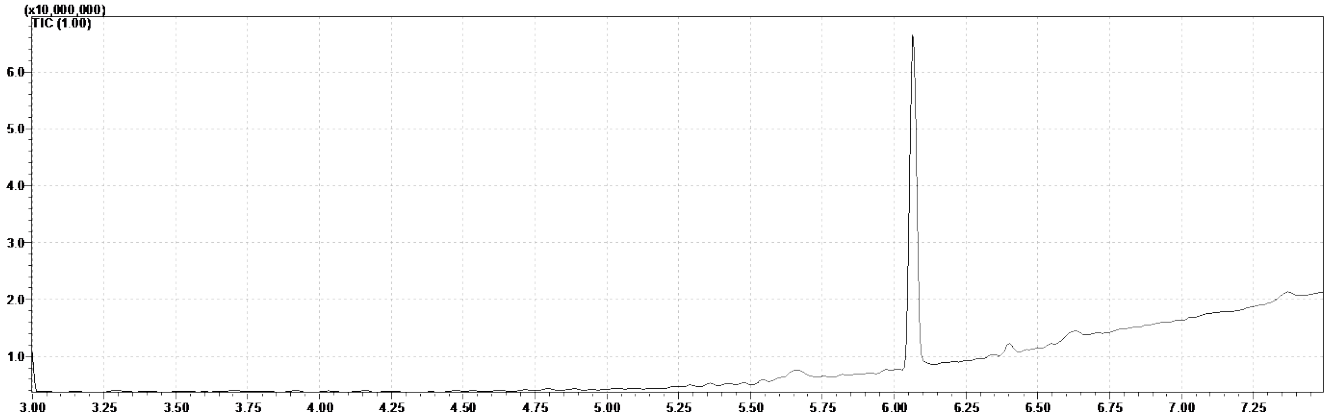

Figure 3: TIC of the Q3-scan of the Δ^9-THC-acetate standard

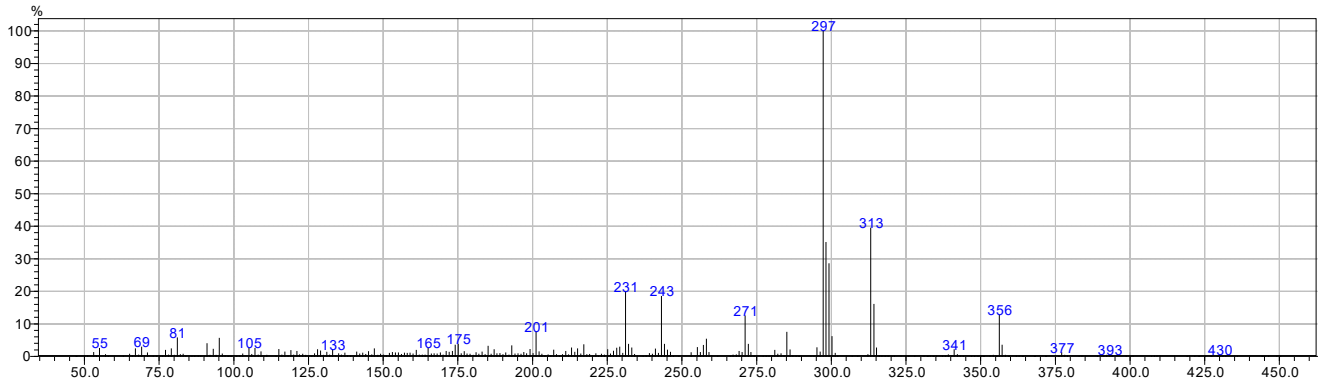

Figure 4: Mass spectrum of the signal at retention time 6.07 min of the Δ^9-THC-acetate standard

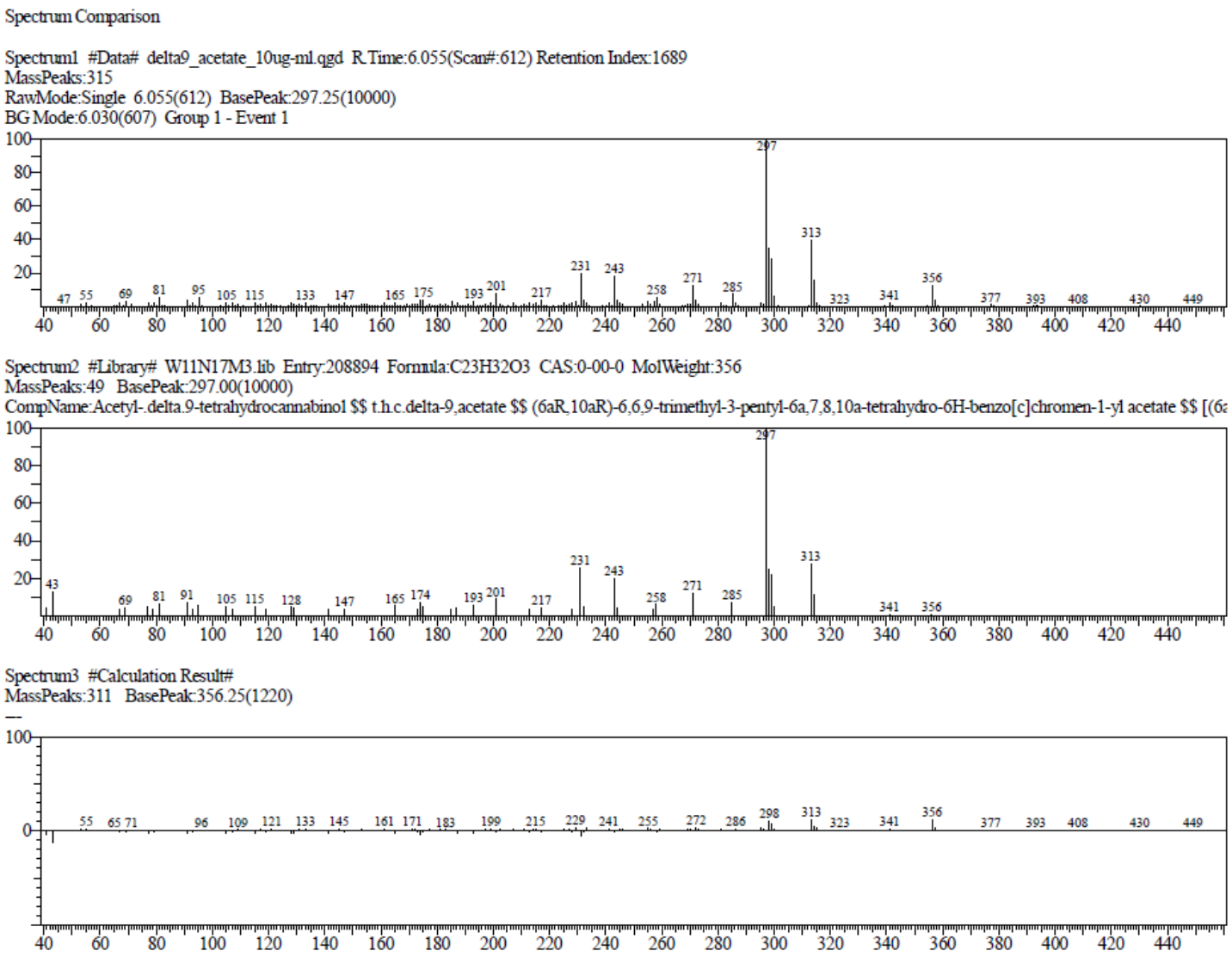

Figure 5: Library match of the mass spectrum at retention time 6.07 min of the Δ^9-THC-acetate standard

The sample was extracted and analyzed without the addition of Δ^9-THC-acetate standard. TIC chromatography indicated the presence of the major component consistent with that observed by HPLC-PDA (Fig. 6). The mass spectrum (Fig. 7) was consistent with the published reference spectrum of Δ^8-THC-Acetate (Fig. 8). No Δ^9-THC-acetate was observed in the sample (a small peak eluted at 6.04 minutes, but this was determined to be system noise when compared to a blank). Fortification of the sample extract with Δ^9-THC-acetate produced chromatographic separation consistent with what we observed for Δ^8/ Δ^9-THC (Fig. 9).

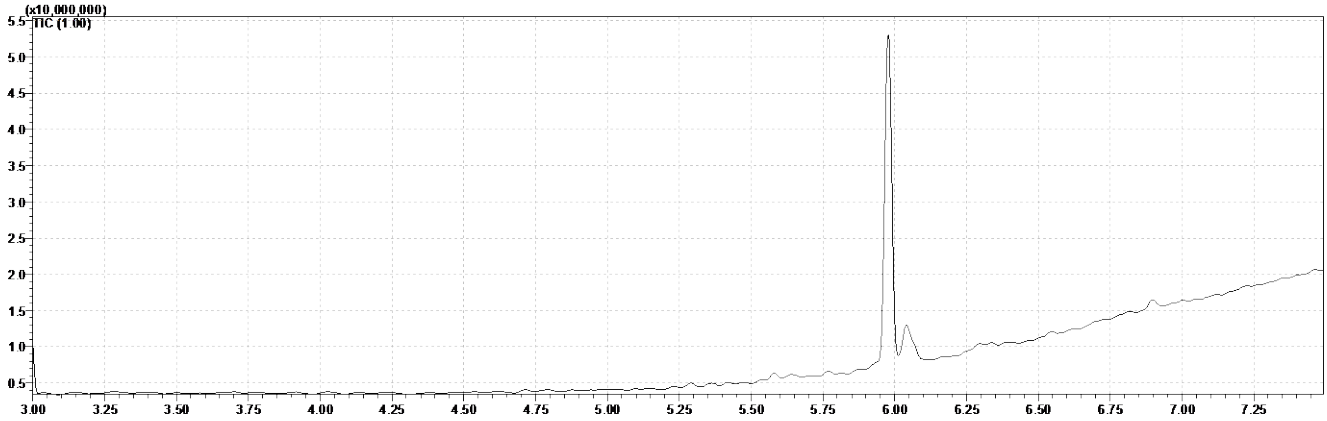

Figure 6: TIC of the Q3-scan of sample SA-08042021-3132 extract

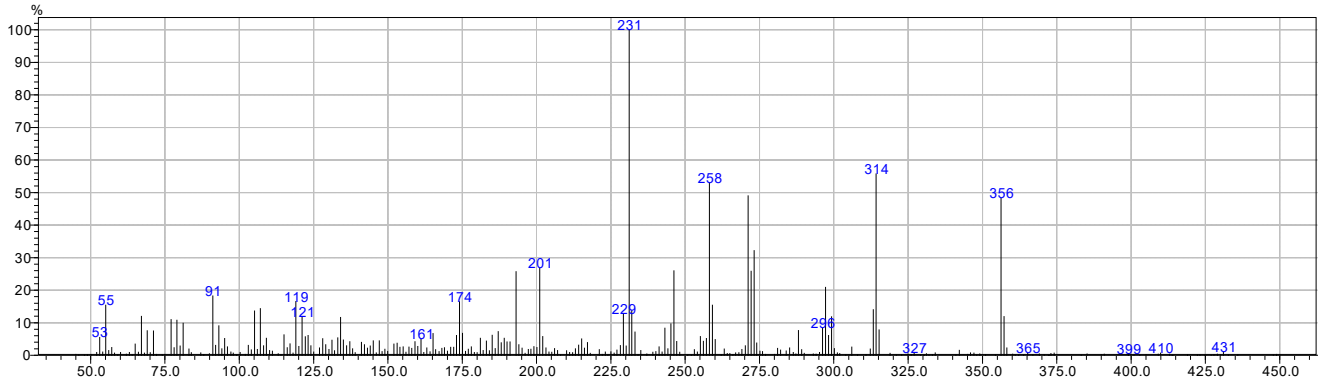

Figure 7: Mass spectrum of the signal at retention time 5.98 min of sample SA-08042021-3132 extract

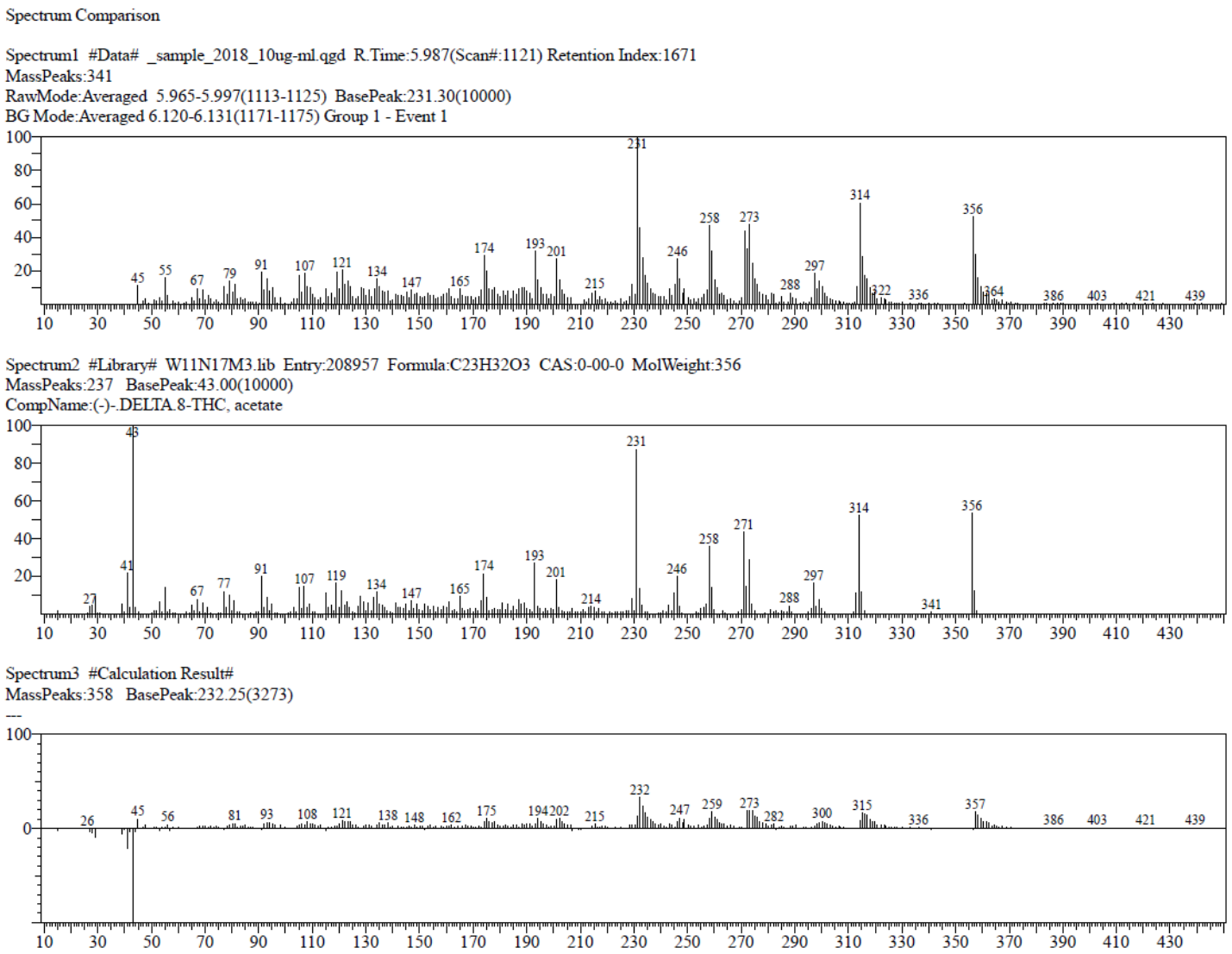

Figure 8: Library match of the mass spectrum at retention time 5.98 min of sample SA-08042021-3132 extract

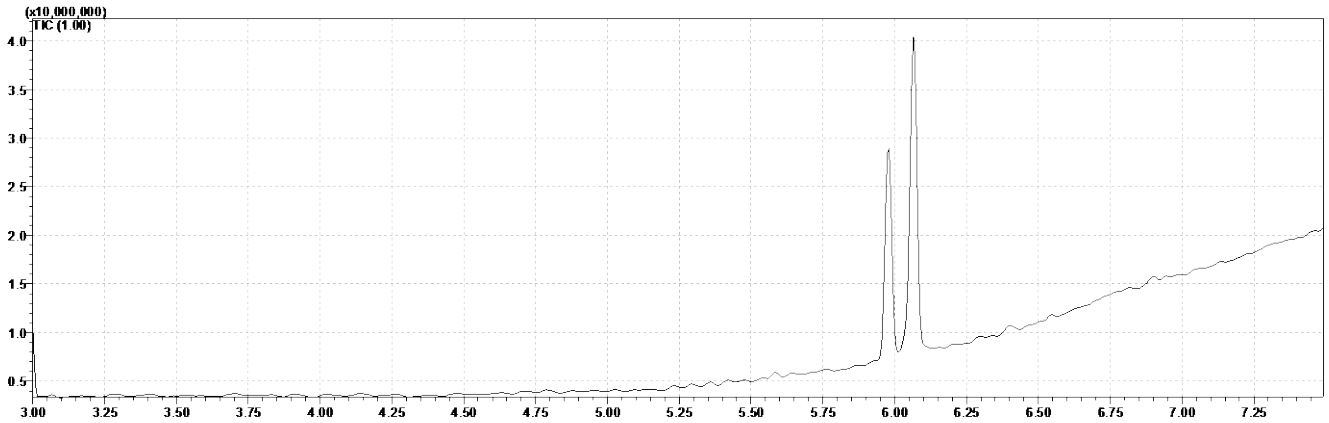

Figure 9: Sample SA-08042021-3132 extract that was fortified with Δ^9-THC-acetate as a retention marker

The sample was assayed for via GC-MS analysis using external calibrators and an internal standard. A calibration curve of seven points ranging from 2 µg/mL to 10 µg/mL was constructed and used for quantification of Δ9-THC and Δ8-THC. The intermediate sample solution (1 mg/mL) was aliquoted and analyzed for minor constituents by known MRM mass transitions. Identities were of minor constituents were assessed by both relative retention to the internal standard and relative ion ratios compared to those of reference standards.

Conclusions

GC-MS analysis and spectral library matching indicate that Amota Processing THCO-D8 Acetate 8.02.21 (SA-08042021-3132) contained Δ^8-THC-acetate and Δ^9-THC and Δ^8-THC as minor constituents (< 1 % total). Due to the unavailability of a certified reference standard for Δ^8-THC-acetate, it was not possible to quantify the amount of Δ^8-THC-acetate in the sample. However, the area of the peak in HPLC-PDA analysis contributed 64.56 % of all subtances detected. It should be noted that this number is an estimate only and can vary based on the PDA signal of the target compound although molar absorptivities of cannabinoids at the wavelength used for quantification are similar. Identification of Δ8-THC acetate as the major component in the sample was inferred from comparison of its relative retention to that of a certified reference standard of Δ9-THC acetate and comparison of the electron impact ionization spectrum of that of the major component to that of Δ8-THC acetate in published paper (Inayama et al., 1976).

References

1. SEIICHI INAYAMA, AIKO SAWA, EIKICHI HOSOYA, Mass Spectrometry of Oxidation Products of Δ1- and Δ6-Tetrahydrocannabinols, Chemical and Pharmaceutical Bulletin, 1976, Volume 24, Issue 9, 2209-2218, Released March 31, 2008, Online ISSN 1347-5223, Print ISSN 0009-2363, https://doi.org/10.1248/cpb.24.220