CHARACTERIZATION OF DELTA-8 THC DISTILLATES USING HIGH RESOLUTION MASS SPECTROMETRY (HRMS)

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INTRODUCTION

The use of Δ^8-THC (delta-8 tetrahydrocannabinol) in consumer products has caused safety concerns in the US.[1,2] Though Δ^8-THC is the main pharmacologically active component in the cannabis plant, its psychoactive isomer, Δ^8-THC, naturally occurs in the cannabis plant at low levels. Bulk Δ^8-THC is typically produced from hemp derived CBD (<0.3% Δ^9-THC US/Canada) which many producers consider legal under the 2018 US Farm Bill. The regulations governing the use of synthetic components derived from hemp are not clearly addressed which has created a growing market for Δ^8-THC production and use. The conversion of CBD to Δ^8-THC requires harsh conditions leading to multiple reaction byproducts which need to be characterized to enhance the chemical understanding of the components produced.[3-7] There are several known double bond isomers of Δ^8-THC, compounded with the different stereoisomers that can be present, could create a considerable separation challenge as well as potential legal problems if the analytical identification of the regulated Δ^9-THC is ambiguous.[8]

In this study, Δ^8-THC distillates were analysed by UHPLC and both PDA and quadrupole time-of-flight (QToF) mass spectrometry using data independent analysis. In the HRMS analysis, the software highlighted a predicted m/z 315.23186 as the base peak for several unknowns with proposed elemental compositions of $C_{21}H_{30}O_2$. The fragmentation data suggests the components share structural characteristics with the C_{21} neutral cannabinoids including Δ^9-THC. A chlorinated component with a proposed elemental composition corresponding to $C_{21}H_{31}ClO_2$ and common fragments with Δ^9-THC and its isomers, was also observed in a purified distillate sample.[8]

METHODS

Sample Preparation. Distillate samples were dissolved and diluted with acetonitrile.

Instrumentation and Software

ACQUITY UPLC I-Class Plus System and Xevo G3 QToF with the UNIFI Scientific Information Software

MS Conditions

Ionization mode: ESI+; Capillary Voltage: 1.0 kV; Cone Voltage: 15 V
Source/Desolvation Temp.: 100 °C/450 °C;
Cone/ Desolvation Gas Flow-rate: 100/1000 L/hr
Mass range: 50 to 1200 Da; Low CE: 4 eV; High CE: 15-45 eV ramp
MS Experiment: Simultaneous collection of high and low collision energy (CE) spectra (MS^E mode).

UPLC Conditions

Column: CORTECS C_{18}, 2.1 x 100 mm, 1.6 µm Column
Solvent A: 0.1% formic acid in water. Solvent B: 0.1% formic acid in acetonitrile. Flow rate: 0.56 mL/min: Column temp.: 25°C
Injection volume: 0.5-1 µL; PDA detection: 210 to 400 nm

Xevo G3 QToF

Time (min)	Flow Rate (mL/min)	%A	%B
0.0	0.560	29.0	71.0
6.0	0.560	29.0	71.0
7.0	0.560	1.00	99.0
8.0	0.560	1.00	99.0
8.1	0.560	29.0	71.0

RESULTS

Analysis of Δ^8-THC Distillate Sample A

The presence of Δ^8-THC , exo-THC and Δ^8-THC were detected in the PDA data using retention time (t_R) and spectral matching. Several unknown components were detected in the UV with Area% values exceeded 0.1% (Figure 1A and 1B). Investigation of the unknown components detected in the PDA using the HRMS data showed multiple components with a base peak of m/z 315.23 eluting in the region preceding the main Δ^8-THC peak at 3.28 min (Figure 1B). In-house cannabinoid reference libraries were used to assign putative identities to the compounds detected in the distillate samples. The libraries consisted of accurate masses of precursor, fragment ions, isotope patterns and, in cases where reference standards are available, additional chromatographic properties such as t_R, which were used to confidently assign putative identifications to the components (Figure 2). Unidentified major components visible in both the PDA and MS data were then evaluated using the structural elucidation tools.
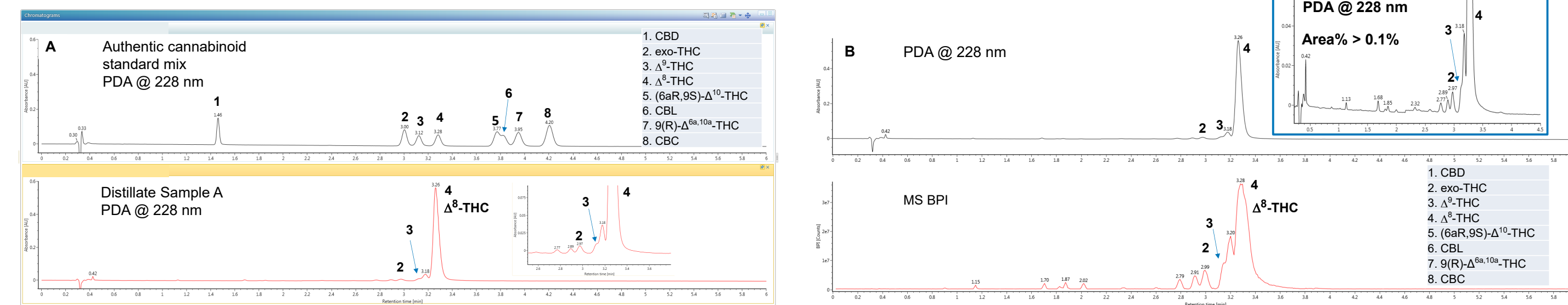

Figure 1A. PDA chromatogram of an authentic standard mix of 8 isomeric cannabinoids at 228 nm (top) (10 µg/mL, 0.5 µL), and (beneath) Δ^8-THC distillate sample A (1 mg/mL, 0.5 µL). Three components can be identified in the sample based on t_R; Figure 1B. PDA chromatogram at 228 nm (top) showing expanded view of UV baseline and the MS BPI (beneath).
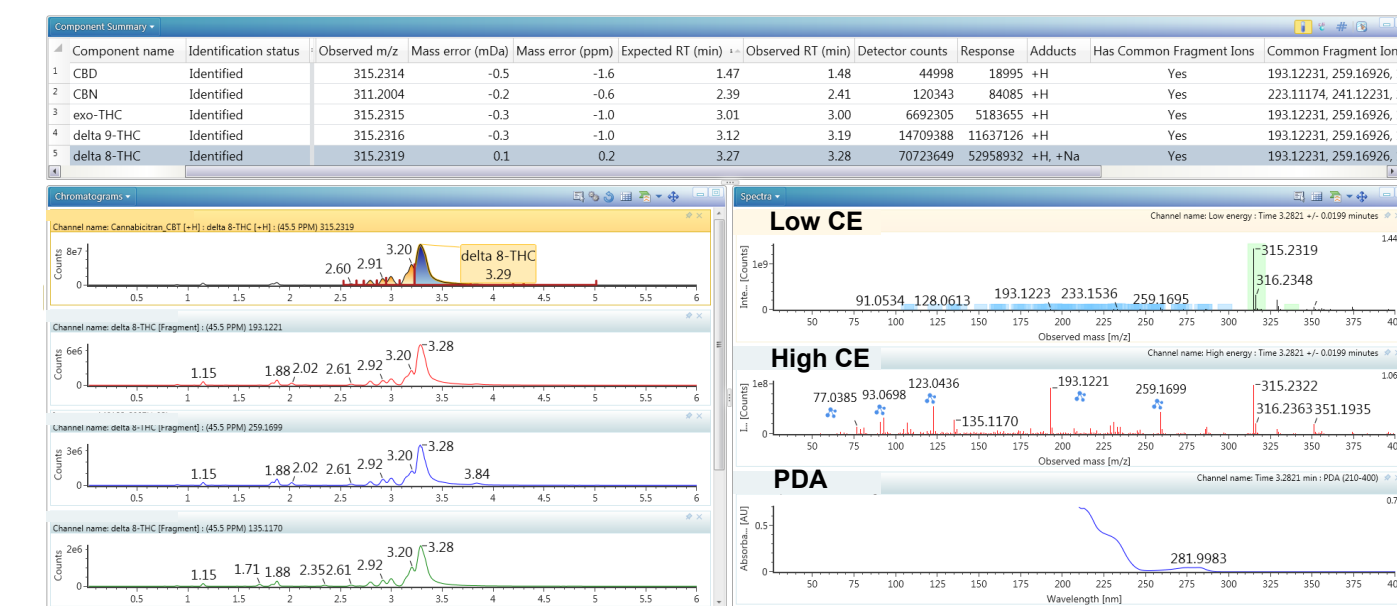

Figure 2. Summary of components identified by the library (top table). The extracted ion chromatograms (XIC) are visible for the selected precursor (Δ^8-THC) and fragments identified from the library (left). The low and high CE fragmentation spectra, and UV spectrum for Δ^8-THC are shown in the spectrum window (right).

Figure 3. Selected high CE spectra from unknown components identified as having a base peak of m/z 315.23. Fragments observed could be matched with those observed in authentic standards of Δ^8-THC, Δ^9-THC and other isomers.

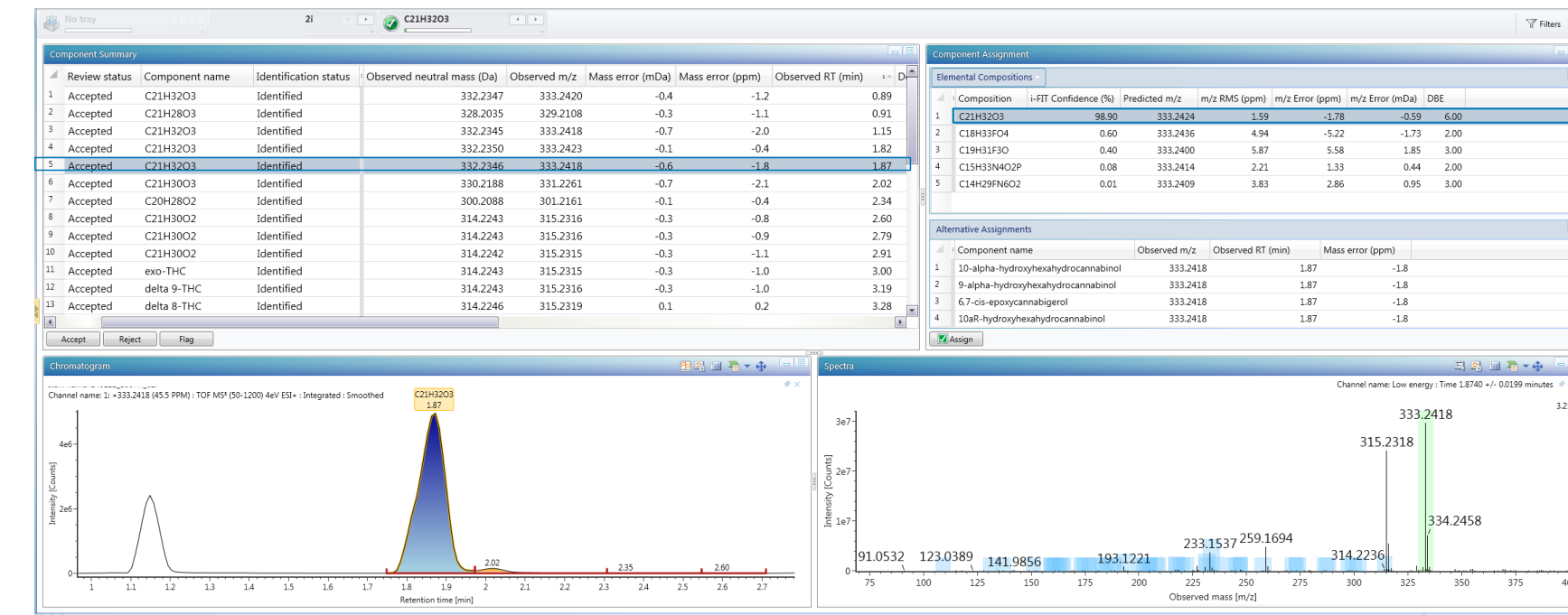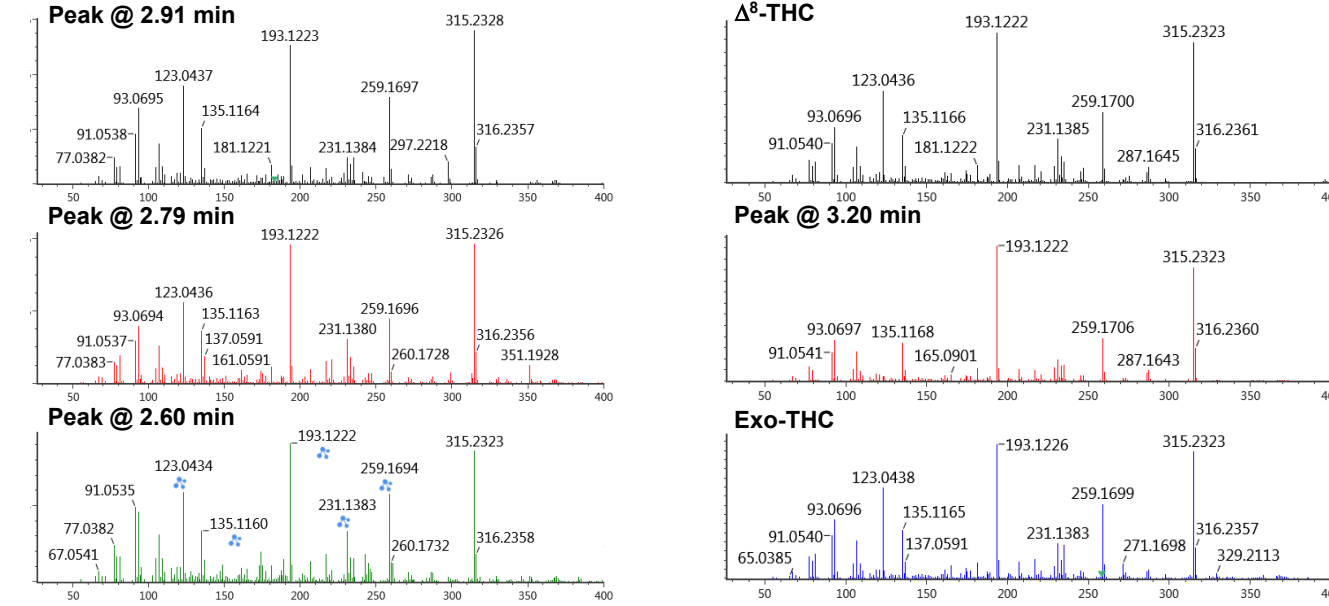Figure 4. Summary of high intensity components identified by the library (top table) or with tentative elemental compositions assigned.

In addition to Δ^9-THC , exo-THC, and Δ^8-THC, CBN and CBD were identified in the distillate sample and could be verified using the t_R, precursor and fragment ion information from the MS library (Figure 2, table). Selected high CE spectra for unknown components with a base peak of m/z 315.23 are shown in Figure 3. The proposed elemental composition for each of these unknown component precursors was $C_{21}H_{30}O_2$ (mass error -0.3 mDa) (Figure 4 table). In addition, many common fragments were observed when the spectra were compared to that of an authentic standard of Δ^8-THC, indicating the likelihood that the unknown components are structurally related and potential isomers. The detection of isomers presents an analytical challenge which requires chromatographic resolution. Structural .mol files based on components reported in recent publications on CBD conversion products were included in the internal library to aid in the tentative assignment of unknown components detected in the distillate samples.[3-6] The proposed elemental compositions for several of the other high intensity unknown peaks eluting in the region between 0.8 and 3.0 min could be matched with the elemental compositions and fragments of components reported in the recent journal articles. Alternative assignments for each of the components are proposed by the library based on the analytical data, however, authentic standards were not available for confirmation (Figure 4).

Analysis of Δ^8-THC Distillate Sample B

In distillate sample B, two major components were identified in the PDA at 228 nm. Δ^8-THC was identified as the primary component at a t_R of 3.27 min with a purity of 79%. An additional component was observed in the PDA data at a t_R of 3.10 min, and an Area% of 19.4% (Figure 5). The UV spectrum for the unknown component matched that of Δ^8-THC, and eluted in close proximity to the t_R of an authentic standard of Δ^9-THC.
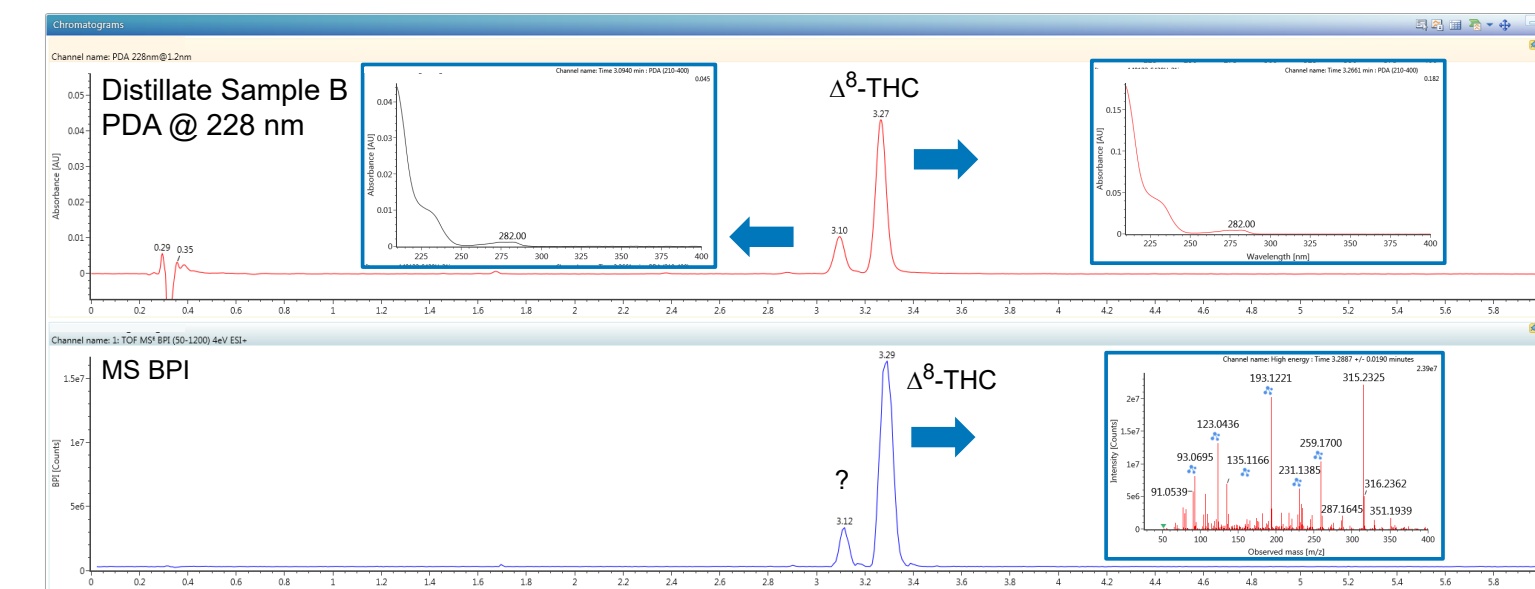

Figure 5. PDA chromatogram of Δ^8-THC distillate sample B at 228 nm (top). PDA spectra for detected peaks are shown (inset). MS BPI chromatogram (beneath) with high CE spectrum for component identified as Δ^8-THC showing most abundant fragments.
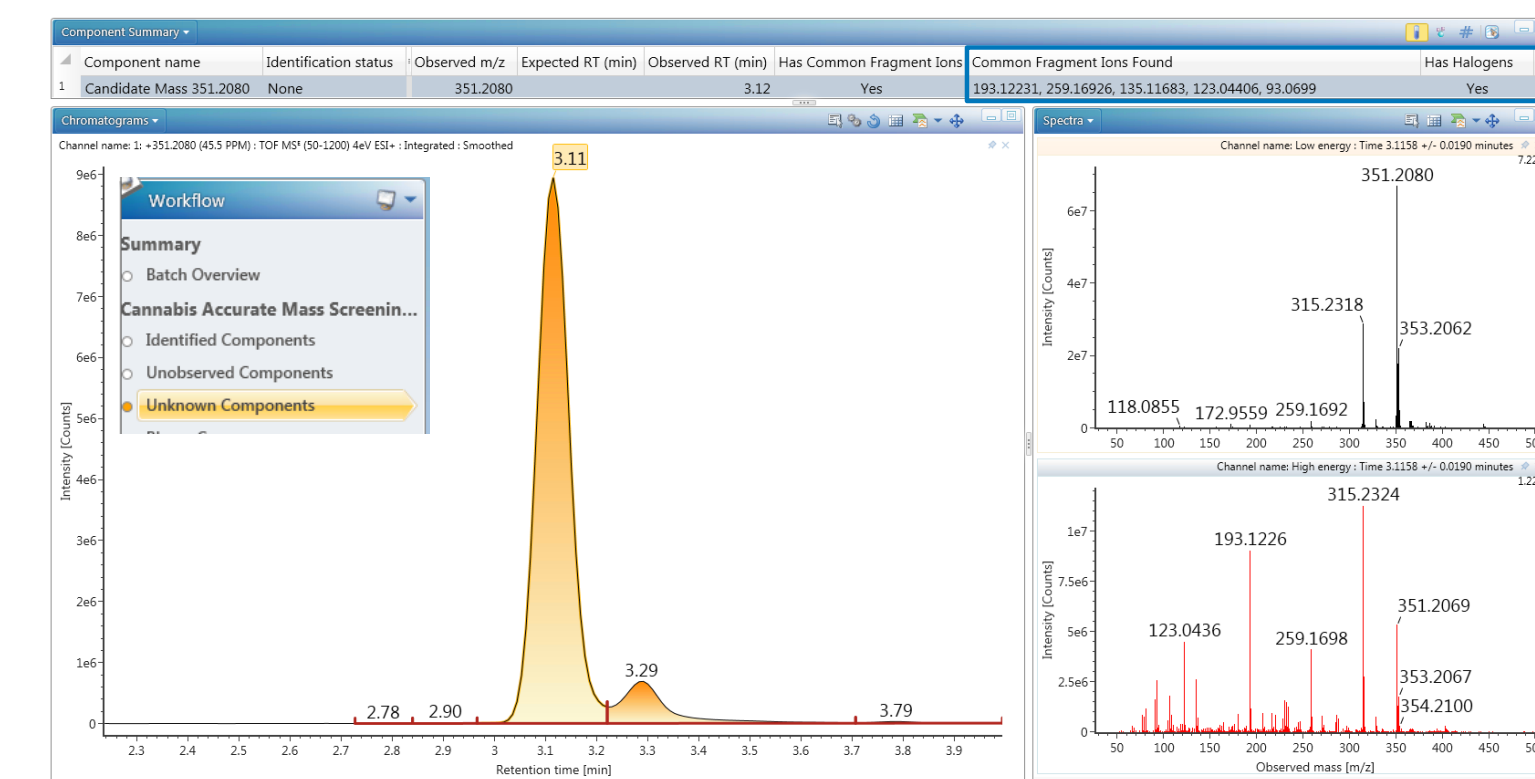

Figure 6. Unknown component at t_R 3.11 min with m/z 351.2080 (top table), common fragments and halogens noted by the software. XIC for m/z 351.2080 (left). High and low CE spectra (right).
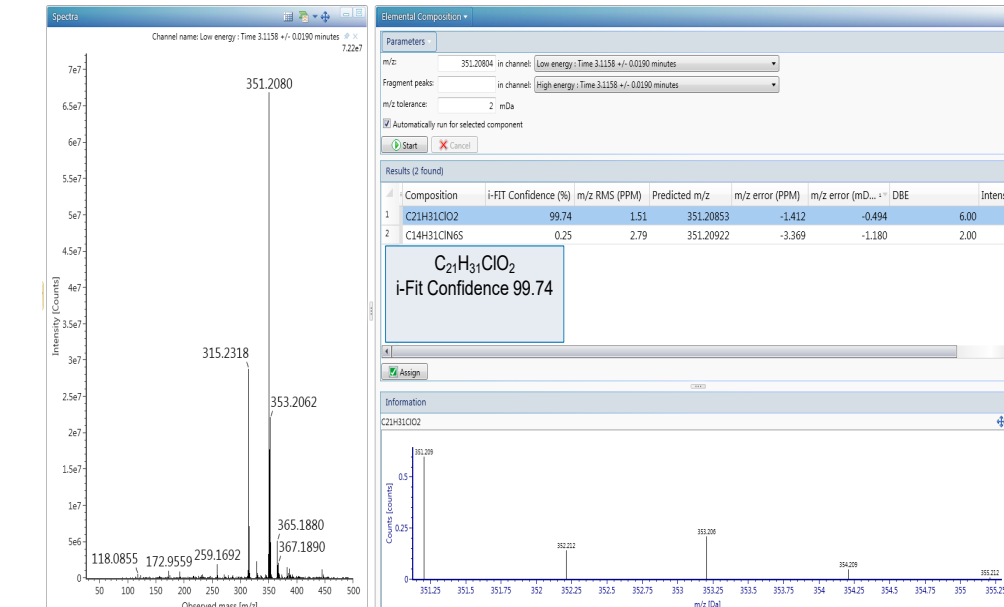

Figure 7. Proposed elemental composition of unknown component with m/z 351.2080, $C_{21}H_{31}ClO_2$ (mass error −0.49 mDa).

In the MS data, the base peak of the unknown component had a m/z 351.2080 (Figure 6). Notably the observed isotopic pattern indicated the presence of a chlorine in the chemical structure. As well as halogens, the software flagged the presence of fragments common to Δ^9-THC and its isomers. The proposed elemental composition of the unknown was $C_{21}H_{31}ClO_2$ (mass error −0.49 mDa) with an iFit confidence of 99.7% indicating the agreement between the measured and the theoretical isotopic patterns. (Figure 7).

Confirmatory MS/MS experiments using a 1 Da window were performed to ensure that the fragments were not isotopes, and originated from m/z 351 (Figure 8A). In addition, a second quad resolution set to pass Cl-37 was used (Figure 8B). Both MS/MS experiments confirmed that the fragments observed in the initial high CE fragmentation analysis were the same (Figure 8).
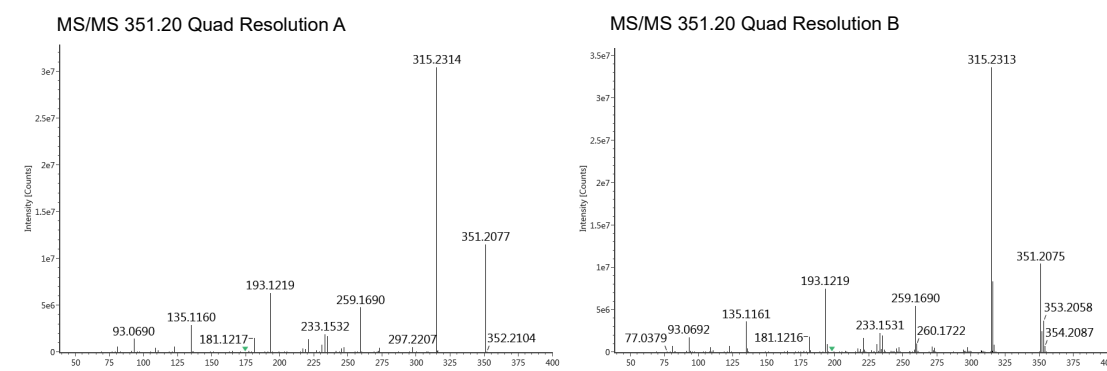

Figure 8. Targeted MS/MS of m/z 351.2080 with two quad resolution settings.

The analytical data suggests the unknown to be mono chlorinated with fragments common to Δ^8-THC isomers. Mono and dihalogenated derivatives of CBD and Δ^8-THC have been reported previously[9-11] though the observed m/z and proposed elemental composition does not support the detection of monochlorinated CBD or Δ^8-THC, which would have an elemental composition of $C_{21}H_{29}ClO_2$.

CONCLUSION

- Several known cannabinoids were identified using a compound library with compound assignment based on multifactor identification, including t_R, precursor, fragment ions, and isotopic patterns.
- Several unknown components with a base peak of m/z 315.23, and proposed elemental composition of $C_{21}H_{30}O_2$ were detected in the distillate samples. The analytical results including common fragment ions suggest that they are potential structural isomers of Δ^8-THC. The Area% for these components measured in the UV at 228 nm ranged from 0.13-4.9%.
- An unknown component detected in the PDA with an Area% of 19.4%, and a proposed elemental composition of $C_{21}H_{31}ClO_2$ was detected in distillate sample B, the fragmentation data suggests a structural relationship with Δ^8-THC and its isomers. The purity of distillate B was less than 80%.
- Multiple unknown components were detected in the distillates. Characterization of components with unknown activity or toxicology data is important to enhance understanding of the complex chemistry and ensure consumer safety.
- Analysis of distillate samples using QToF MS provides insights into the elemental composition and other structural information that can aid in improving understanding of the structural relationships between the unidentified components detected.

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