Nota de aplicación

Characterization of Δ^8-THC Distillates using Non-Targeted Screening with High Resolution Mass Spectrometry

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Abstract

The use of delta-8 tetrahydrocannabinol (Δ^8-THC) has caused consumer safety concerns in the US. Delta-9 tetrahydrocannabinol (Δ^9-THC) is the main intoxicating component in the cannabis plant. Its isomer, Δ^8-THC, is also intoxicating and naturally occurs in the cannabis plant at trace levels. Products that contain Δ^8-THC are formulated using Δ^8-THC made from the chemical conversion of hemp-derived cannabidiol (CBD), which was defined and legalized under the 2018 US Farm Bill. Regulations governing the use of synthetic components derived from hemp are not clearly addressed which has created a growing market for Δ^8-THC. Conversion of CBD to Δ^8-THC often requires harsh conditions leading to reaction byproducts. This study demonstrates a workflow to characterize Δ^8-THC distillates using non targeted screening and high-resolution mass spectrometry (HRMS). Cannabinoid libraries were generated with available authentic standards, which included accurate mass fragments and additional properties such as retention times and were used to identify target components. The purity of the distillate samples measured by UV ranged from 79.0–93.6%. Several unidentified peaks were detected in the UV data. The UV spectra indicated that there may be structural similarities between the unknown components and the primary compound in the distillates, Δ^8-THC. During HRMS analysis, the software highlighted *m/z* 315.2318 as a base peak for several unknowns with proposed elemental compositions of $C_{21}H_{30}O_2$. Fragmentation data suggest that the components share structural characteristics with the C_{21} neutral cannabinoids including Δ^9-THC. A chlorinated component with a proposed elemental composition of $C_{21}H_{31}ClO_2$ and common fragments with many isomers of Δ^9-THC was also observed in a purified distillate.

Benefits

- Enhanced confidence in compound identifications using an analytical solution that simultaneously collects accurate mass precursor and product ion data combined with compound libraries increasing confidence in the results for known and emerging components
- Structural elucidation tools such as common fragment searching can be useful in identifying structurally related components

Introduction

The rapidly increasing use of Δ^8-THC in consumer products has caused safety concerns in the US.[1–4] Δ^9-THC is the main compound responsible for inducing an intoxicating effect in the cannabis plant and is regulated at levels exceeding 0.3% which is the legal definition of marijuana under the federal Controlled Substances Act. Delta-8 tetrahydrocannabinol is a double bond isomer of Δ^9-THC, which also elicits an intoxicating effect, and naturally occurs in the cannabis plant at trace levels.[5–7] However recently, bulk Δ^8-THC is being manufactured from hemp derived CBD (<0.3% Δ^9-THC US/Canada) which many producers consider legal under the Agriculture Improvement Act of 2018 (commonly referred to as the 2018 Farm Bill).[8] 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 increased use of Δ^8-THC has led both the CDA and the FDA to issue warnings due to reports of multiple adverse reactions directly linked to Δ^8-THC.[3,4] The conversion of CBD to Δ^8-THC requires conditions that can lead to multiple reaction byproducts which need to be characterized to enhance the chemical understanding of the components produced.[9–13]

In this study, a workflow for the determination of components in Δ^8-THC distillates will be demonstrated using data from non-targeted analysis by ultra-performance liquid chromatography (UPLC) and high-resolution time-of-flight mass spectrometry (ToF-MS) in addition to PDA detection. In-house cannabinoid reference libraries were used to assign identities to the compounds detected. These libraries were generated in-silico and from experimental data. Mass spectral information is predicted from chemical structures of known components supported by experimental data generated through the analysis of available authentic reference standards. The libraries consisted of accurate masses of molecular ions ($[M+H]^+$), fragment ions, isotope patterns, and in cases where reference standards are available, additional chromatographic properties such as retention times, were

used to identify components. Then, data from remaining components was evaluated using additional software tools including common fragment searching.

Experimental

Sample Preparation

A mixture of authentic cannabinoid standards was sequentially diluted in acetonitrile to a concentration of 100 µg/mL. Distillate samples were weighed out in glass scintillation vials, dissolved, and diluted with acetonitrile to a concentration of 1 mg/mL in preparation for injection.

Method Conditions

LC system:	ACQUITY UPLC I-Class PLUS
Detection:	Xevo G3 QTof Mass Spectrometer
Vials:	Glass autosampler vial (p/n: 186002802) and sealed with a polyethylene cap (p/n: 186005826)
Column(s):	CORTECS $C_{18,}$ 2.1 x 100 mm, 1.6 µm (p/n: 186007095) Column
Column temp.:	25 °C
Sample temp.:	8 °C
Injection volume:	0.5 µL
Flow rate:	0.560 mL/min

Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
0.0	0.56	29	71	–
6.0	0.56	29	71	6
7.0	0.56	1.0	99	6
8.0	0.56	1.0	99	6
8.1	0.56	29	71	1

MS Conditions

MS system:	Xevo G3 QTof Mass Spectrometer
Ionization mode:	ESI+
Acquisition range:	50–1200 Da, 0.1 sec
Capillary voltage:	1.0 kV
Cone voltage:	15 V
Collision energy (CE):	Low CE: 4 eV; High CE: 15–45 eV ramp
Desolvation temp:	450 °C
Source temp.:	100 °C

Desolvation gas flow:	1000 (L/Hr)
Cone gas:	100 (L/Hr)
Lock mass reference:	Leucine Enkephalin, 200 ng/mL

Data Management

Informatics:	UNIFI™ App

A data independent acquisition mode, known as MS^E, was used to collect accurate mass measurements from precursor and product ions in a single injection.[14] The incidence of false positives is significantly reduced when using multiple attributes (***i.e.***, retention time, accurate mass, expected fragments) to search entries in a compound library, greatly increasing confidence in the identifications. The processed data files from the analysis of available authentic standards in combination with the relevant structural .mol files, were used to create a custom library of compounds of interest. Data import is available which will then be converted to the library within the software. Additional structures of compounds found in the literature, for which no reference standards were available, were also added to the library.[9–12] The library entry for Δ^8-THC is shown in Figure 1.[15]

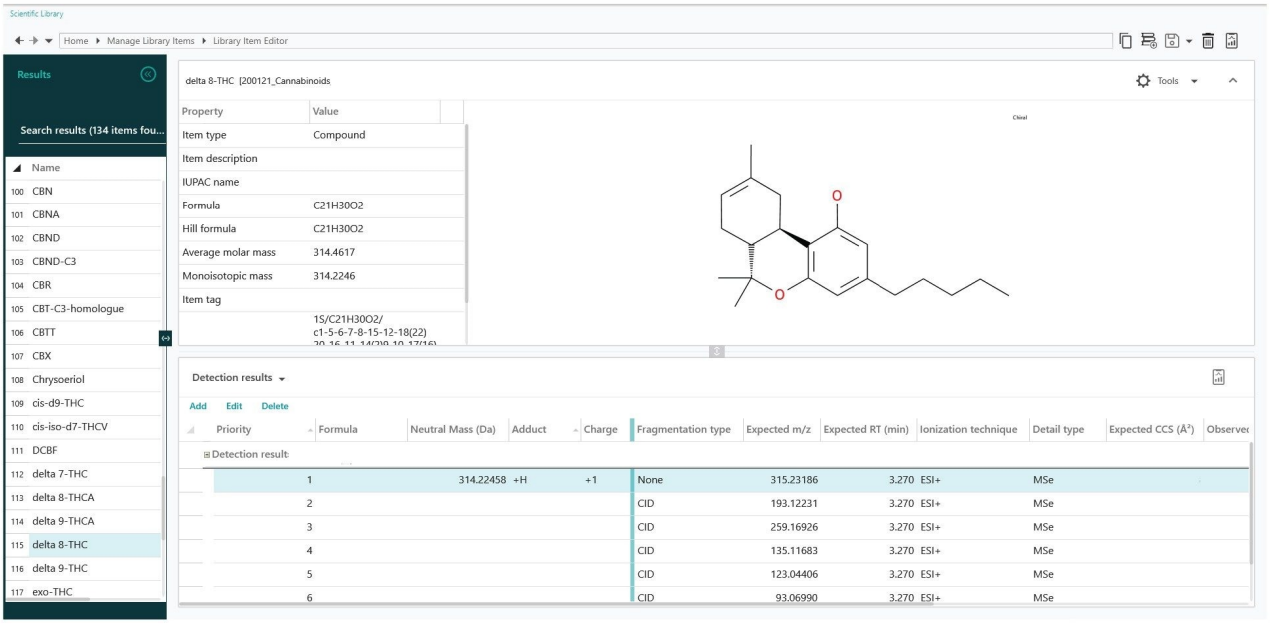

Figure 1. An example of the library entry for Δ^8-THC showing the expected m/z of precursors, fragments, adducts and retention time.

Results and Discussion

Analysis of Delta-8-THC Distillate Sample A

The chromatographic separation of an authentic standard mixture of eight isomeric cannabinoids (Figure 2) using PDA detection at 228 nm is shown in Figure 3A.

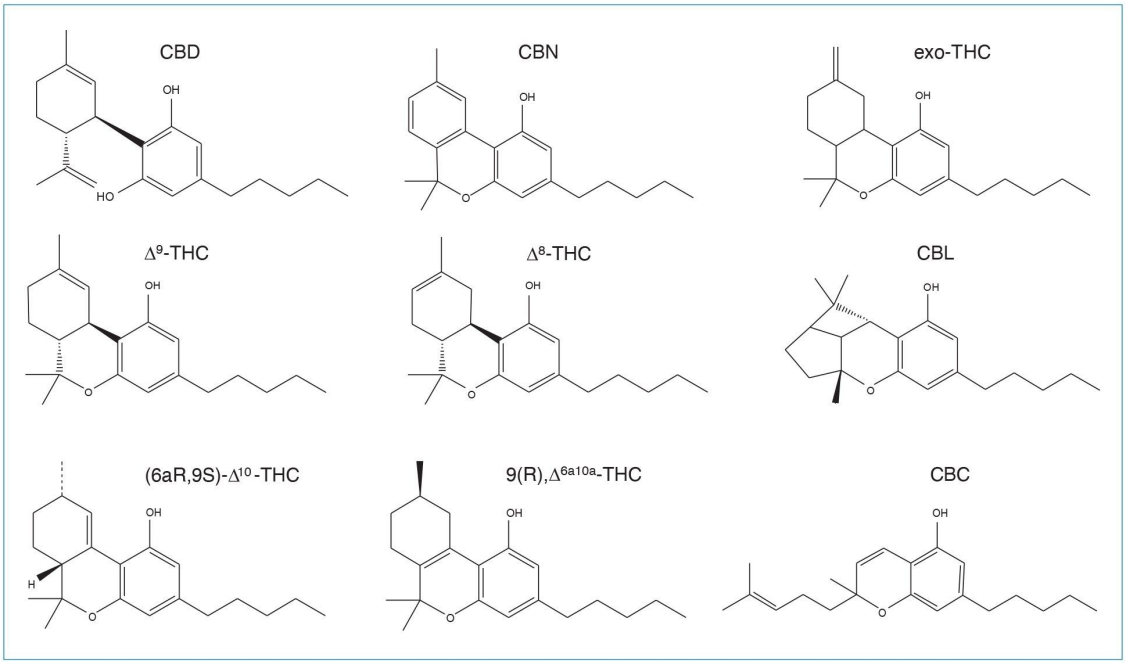

Figure 2. Structures of cannabinoids used or identified during the study.

In the PDA data, the presence of Δ^9-THC, exo-THC, and Δ^8-THC were detected in a Δ^8-THC distillate sample using retention time (t_R) and spectral matching. Several unknown components were detected in the UV with Area% values significantly exceeding 0.1% (Figure 3A and 3B). Investigation of the unknown components detected in the PDA using the HRMS data showed multiple components with a base peak of *m/z* of 315.2318 eluting in the region preceding the main Δ^8-THC peak at 3.28 minutes (Figure 3B).

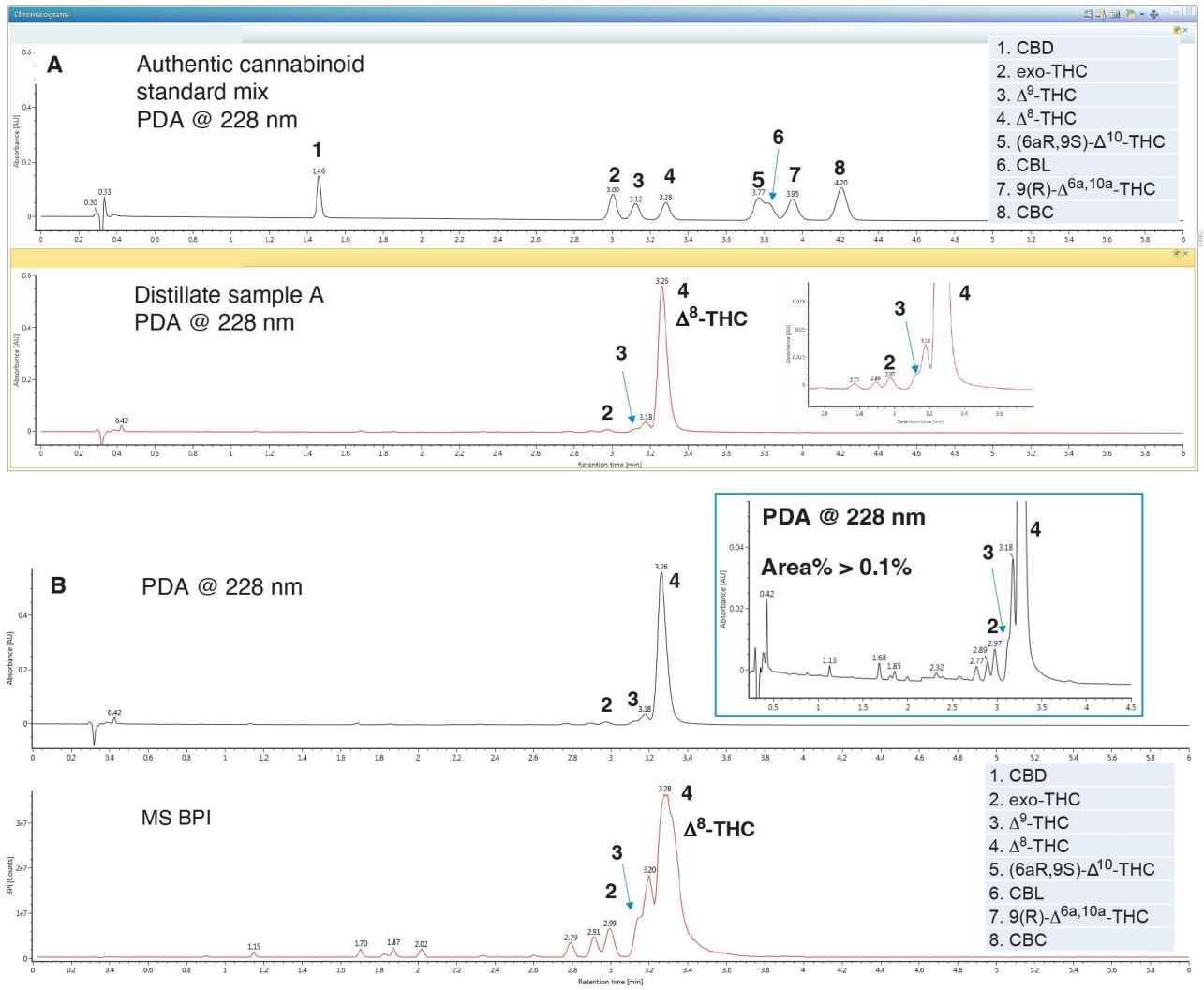

Figure 3A. PDA chromatogram of an authentic standard mix of 8 isomeric cannabinoids at 228 nm (top) (100 mg/mL, 0.5 mL), and (beneath) Δ^8-THC distillate sample A (1 mg/mL, 0.5 mL). Three components can be identified in the sample based on t_R;

Figure 3B. PDA chromatogram at 228 nm (top) showing expanded view of UV baseline and the MS BPI (beneath).

In-house cannabinoid reference libraries were used to assign putative identities to the compounds detected in the distillate samples. Unidentified major components visible in both PDA and mass spectrometry (MS) data were evaluated using the structural elucidation tools.

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 4, table). A customizable workflow is shown on the left side of the figure. The workflow items can be arranged in a sequence to aid with data review. The Identified Components step is selected, automatically displaying all library components that have been identified by the software in the component summary table. The component summary table shows information pertaining to the identified analyte including, *m/z,* mass error, retention time and detected adduct. The entry for Δ^8-THC is highlighted, displaying the precursor and fragment extracted ion chromatograms (XIC), associated mass and UV spectral data and structural assignments.

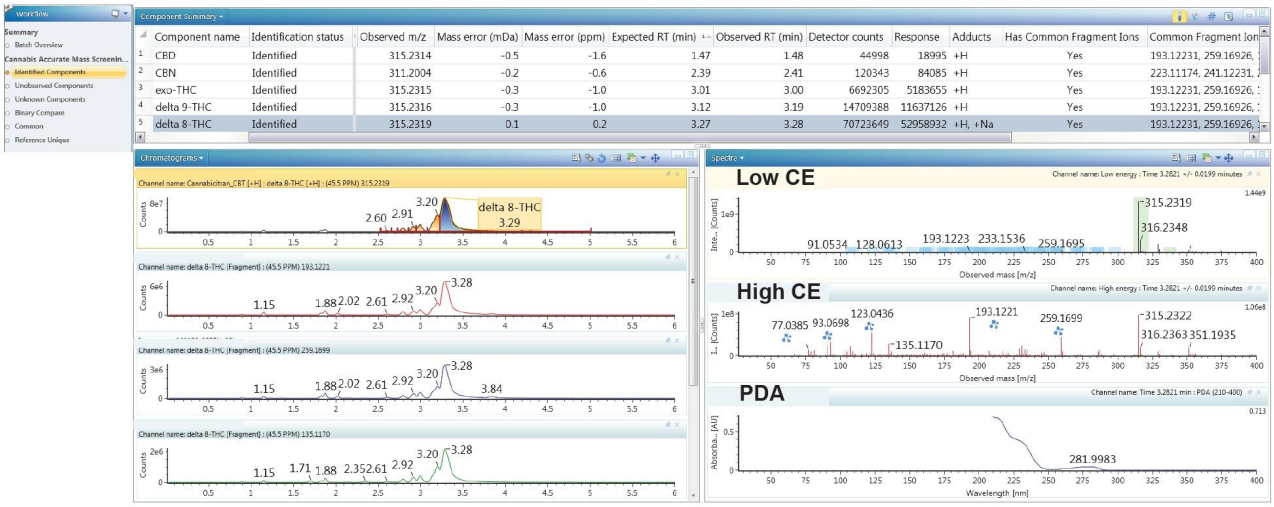

Figure 4. Summary of components identified by the library (top table). The extracted ion chromatograms (XIC) are visible for the selected precursor (Δ^8-THC) and fragments identified from the library (left). The low and high CE fragmentation spectra, and UV spectrum for Δ^8-THC are shown in the spectrum window (right).

UNIFI Software has a suite of tools that can aid in identifying and elucidating components present in samples, that are absent from in-house libraries. The tools include common fragments, neutral loss, and mass defect searching. Detected unknown components that share common structural features can be flagged automatically in the component summary table. In the case of common fragment searching, known fragments can be entered in the search criteria of the processing method for automated searching of these shared structural features. In Figure 5 several components have been flagged as having common fragments (highlighted in blue) with Δ^9-THC and its isomers.

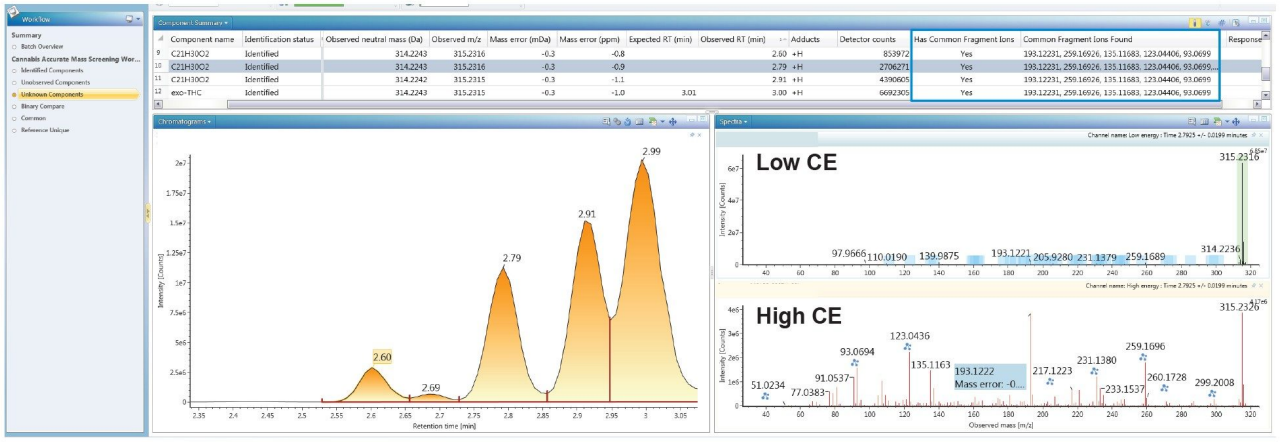

Figure 5. Unknown components flagged by the software as having common fragments. The low and high CE fragmentation spectra, for the selected unknown at 2.79 minutes are shown in the spectrum window (right)

Selected high CE spectra for unknown components with an exact *m/z* 315.2318 at high intensity are shown in Figure 6. The proposed elemental composition for each of these unknown component precursors was $C_{21}H_{30}O_2$ (mass error -0.3 mDa). 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 these unknown components are structurally related and possibly isomers. The detection of isomers presents an analytical challenge which requires chromatographic resolution.

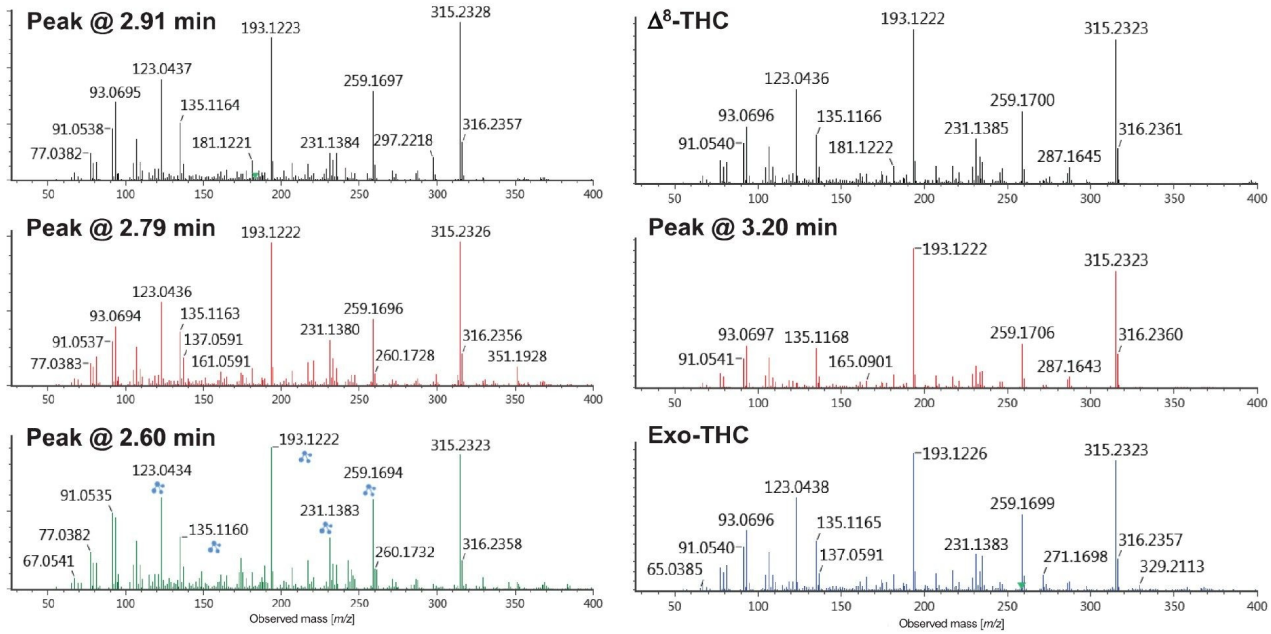

Figure 6. Selected high CE spectra from high intensity unknown components with an exact m/z 315.2318. Fragments observed could be matched with those observed in authentic standards of Δ^8-THC, Δ^9-THC, and other isomers.

Structural .mol files based on components reported in recent publications on CBD conversion products were included in the library to aid in tentative assignment of unknown components detected in the distillate samples.[9–12] The proposed elemental compositions for several of the other high intensity unknown peaks eluting in the region between 0.8 and 3.0 minutes 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 7).

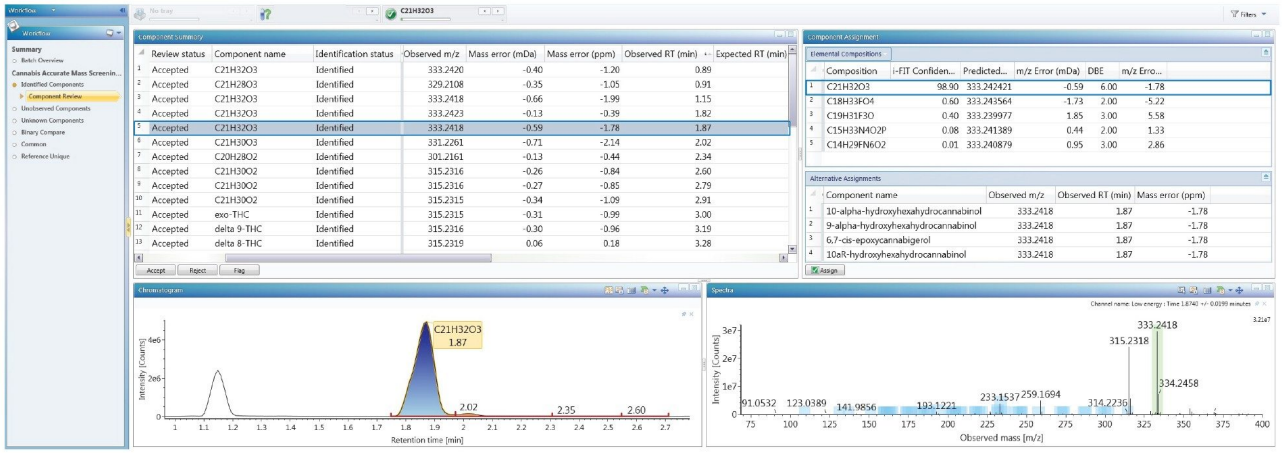

Figure 7. Summary of high intensity components identified by the library (top table) or with tentative elemental compositions assigned.

Analysis of Delta-8-THC Distillate Sample B

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

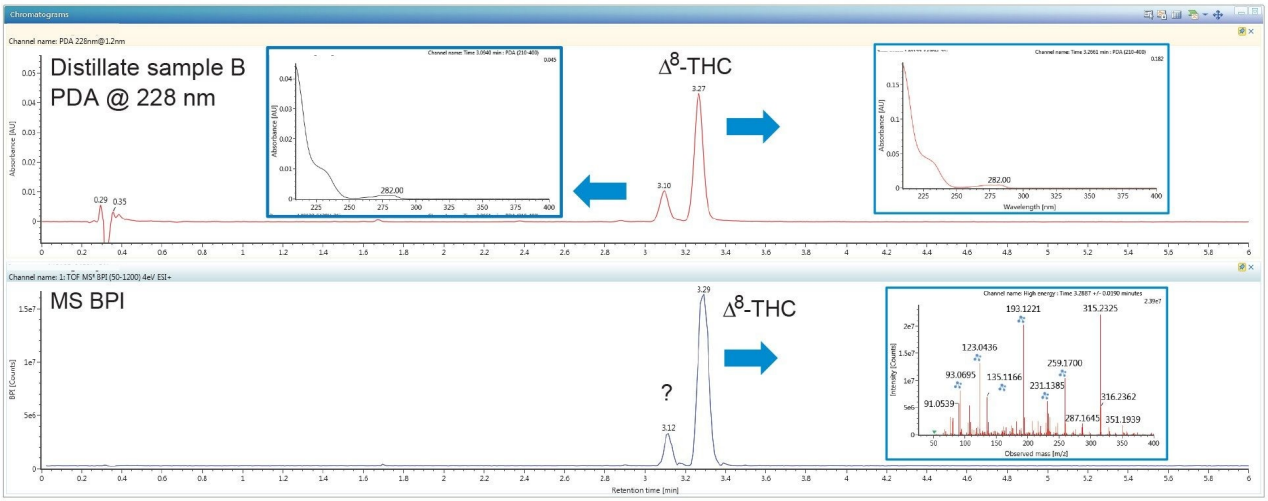

Figure 8. PDA chromatogram of Δ^8-THC distillate sample B at 228 nm (top). PDA spectra for detected peaks are shown (inset). MS BPI chromatogram (beneath) with high CE spectrum for component identified as Δ^8-THC showing most abundant fragments.

In the MS data, the base peak of the unknown component had a *m/z* 351.2080 (Figure 9). Notably, the observed isotopic pattern indicated the presence of chlorine in the chemical structure. The software also 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 10).

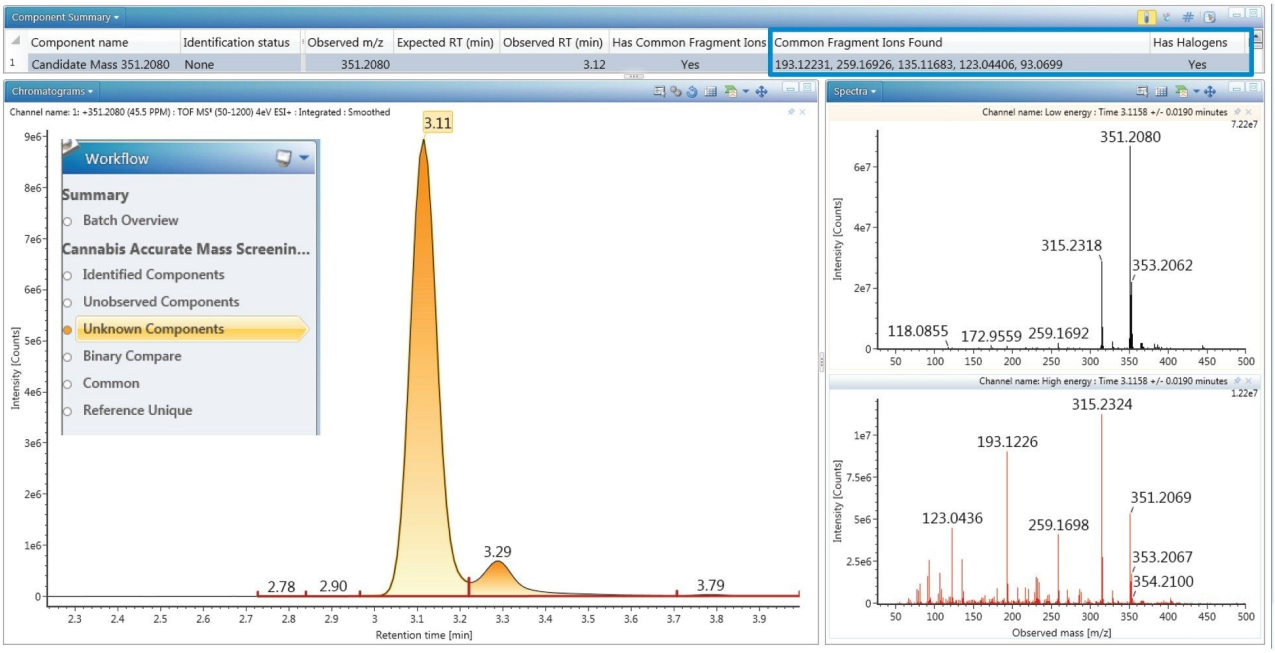

Figure 9. Unknown component at t_R 3.11 minutes with m/z 351.2080 (top table), common fragments and halogens noted by the software. XIC for m/z 351.2080 (left). High and low CE spectra (right).

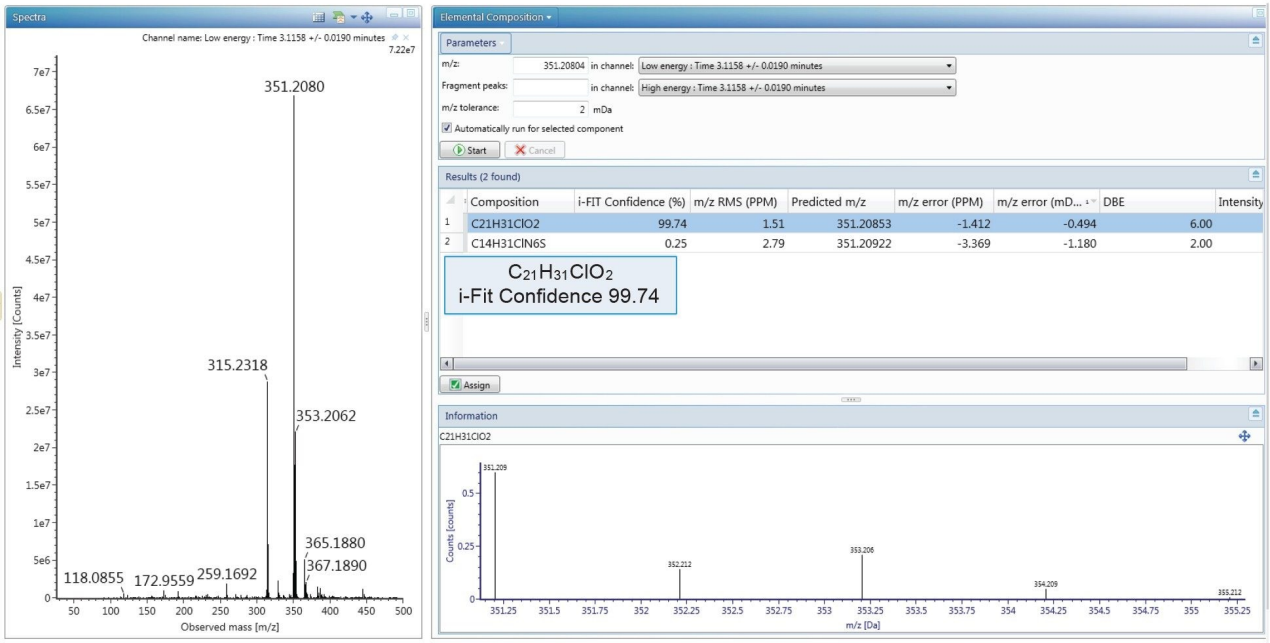

Figure 10. Proposed elemental composition of unknown component with m/z 351.2080, $C_{21}H_{31}ClO_2$ (mass error –0.49 mDa).

Confirmatory targeted tandem mass spectrometry (MS/MS) experiments using a 1 Da quadrupole window were performed to ensure that the fragments were not isotopes and originated from *m/z* 351 (Figure 11A). In addition, a second quadrupole resolution set to pass Cl-37 was used (Figure 11B). Both MS/MS experiments confirmed that the fragments observed in the initial high CE fragmentation analysis were the same (Figure 9).

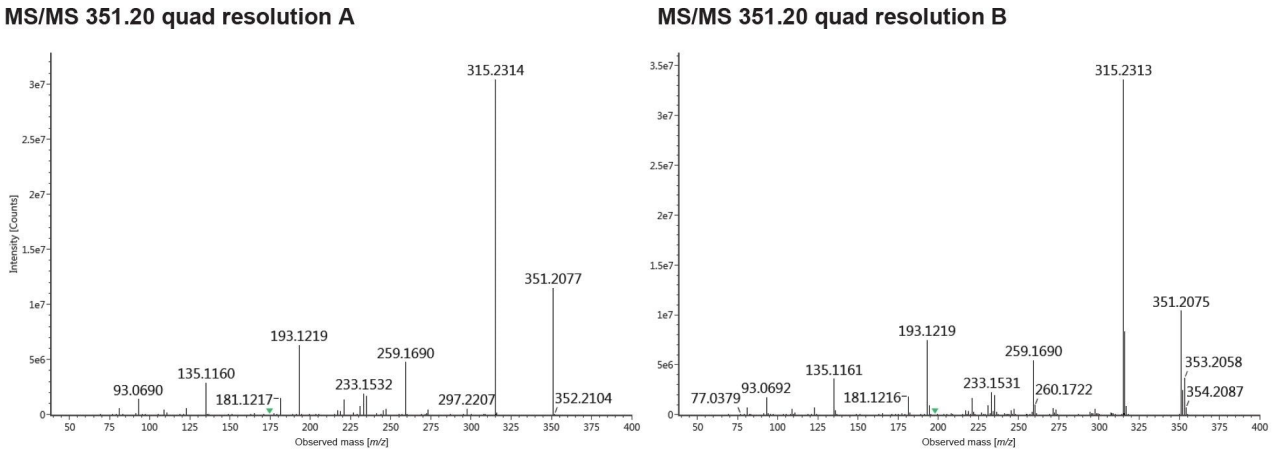

Figure 11. Targeted MS/MS of m/z 351.2080 with two quadrupole resolution settings.

These data suggest that the unknown is monochlorinated with structural similarity to Δ^9-THC isomers. Mono- and dihalogenated derivatives of CBD and Δ^9-THC have been reported previously, though the observed *m/z* and proposed elemental composition does not support the detection of monochlorinated CBD or Δ^9-THC, which would have an elemental composition of $C_{21}H_{29}ClO_2$.[16–18]

Conclusion

Several known cannabinoids were identified using a compound library which greatly aided with compound assignment as it was based on multiple characteristics including t_R, precursor mass, fragment ions, and isotopic patterns.

Several unknown components with a significant *m/z* 315.2318 and proposed elemental composition of $C_{21}H_{30}O_2$ were detected in the distillate samples using a non-targeted screening approach. Analytical results including common fragment ions suggest that they are potential structural isomers of Δ^9-THC.

The Area% ranged from 0.13–4.9% and were derived from UV signals at 228 nm. UNIFI aids in the identification of unknown components that are not present in a library through the structural elucidation toolkit, which includes common fragments, neutral loss, and mass defect searching. These search features allow for easy

filtering of components that exhibit structural similarity.

An unknown component discovered 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, and the fragmentation data suggests structural similarity with Δ^9-THC and its isomers. The purity of distillate B was less than 80%.

Multiple unknown components were detected in the distillates using a non-targeted screening approach. The discovery and characterization of unknown components is important to enable enhanced understanding of the complex chemistry and to ensure consumer product safety.

Analysis of distillate samples using UPLC-QTof-MS provides insights into elemental composition and other structural information that can aid in improving understanding of the structural relationships between unidentified components and knowns.

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