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Chromatography, Detection, and Software Tools to Aid in the Exploration of Delta-8 THC Distillates

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

The expanding use of delta-8 tetrahydrocannabinol (Δ^8-THC) which is manufactured from hemp derived CBD is causing safety concerns in the United States (US). The conversion of CBD to Δ^8-THC can lead to multiple reaction byproducts which need to be characterized to enhance understanding of the chemical components produced and their potential risks to consumer safety.

The following work describes the analysis of Δ^8-THC distillates using High-Performance Liquid Chromatography (HPLC) with photodiode array (PDA), single quadrupole mass spectrometry (MS) detection and tools within Empower™ Chromatography Data Software that aid in sample exploration.

Several known cannabinoids were identified in the distillate sample analyzed based on retention time and UV spectra, including Δ^8-THC and Δ^9-THC. However, several unidentified peaks were also 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. In the MS analysis, the software highlighted *m/z* 315 as the base

peak for several unknowns. Cannabinoid spectral libraries were generated using authentic standards. The spectra and retention times, were used to aid in confirming the identity of the target components and highlighting potential structurally related unknowns by spectral matching.

Benefits

- Enhanced confidence in the detection of cannabinoids using both PDA and MS detection
- PDA spectral libraries increase confidence in compound assignments and enable possible isomer identification
- Peak Purity can be used to aid in the identification of co-eluting components
- Additional structural information using in-source fragmentation of precursor to product ions

Introduction

Expanding cannabis legalization has driven innovation in mechanisms of consumption for cannabis and its derived products. The importance of correctly identifying cannabinoids is of increasing significance as the safety of various routes of ingestion are unknown. 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. The Δ^8-THC found in consumer products is produced from hemp-derived cannabidiol (CBD), which is a non-intoxicating cannabinoid. Interpretation of the Unites States Agriculture Improvement Act (2018 US Farm Bill) has led to varying discourse about Δ^8-THC legality; many feel that Δ^8-THC is legal, leading to a steady increase in US sales. 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. Synthetic conversion of CBD to Δ^8-THC leads to multiple reaction byproducts which need to be characterized to enhance both the chemical understanding of the resulting components and their safety profiles.[1–7]

Recently, there have been reports of product labeling inaccuracies associated with Δ^8-THC consumer products such as incorrect labels and undeclared reaction byproducts, including isomers.[4–6] There are several known double bond isomers of Δ^9-THC with differing stereochemistry that can be present, which could create a considerable separation challenge. This could also cause legal issues if the analytical identification of the regulated Δ^9-THC is ambiguous.[8]

The following work describes the analysis of Δ^8-THC distillates using HPLC with PDA and single quadrupole MS detection and tools within Empower Chromatography Data Software that aid in sample exploration.

Results and Discussion

The chromatographic separation of an authentic standard mixture of ten neutral cannabinoids (Figure 1) using PDA detection at 228 nm is shown in Figure 2. The mixture contains several isomers of Δ^9-THC including CBD, exo-THC, Δ^8-THC, CBL, and CBC.
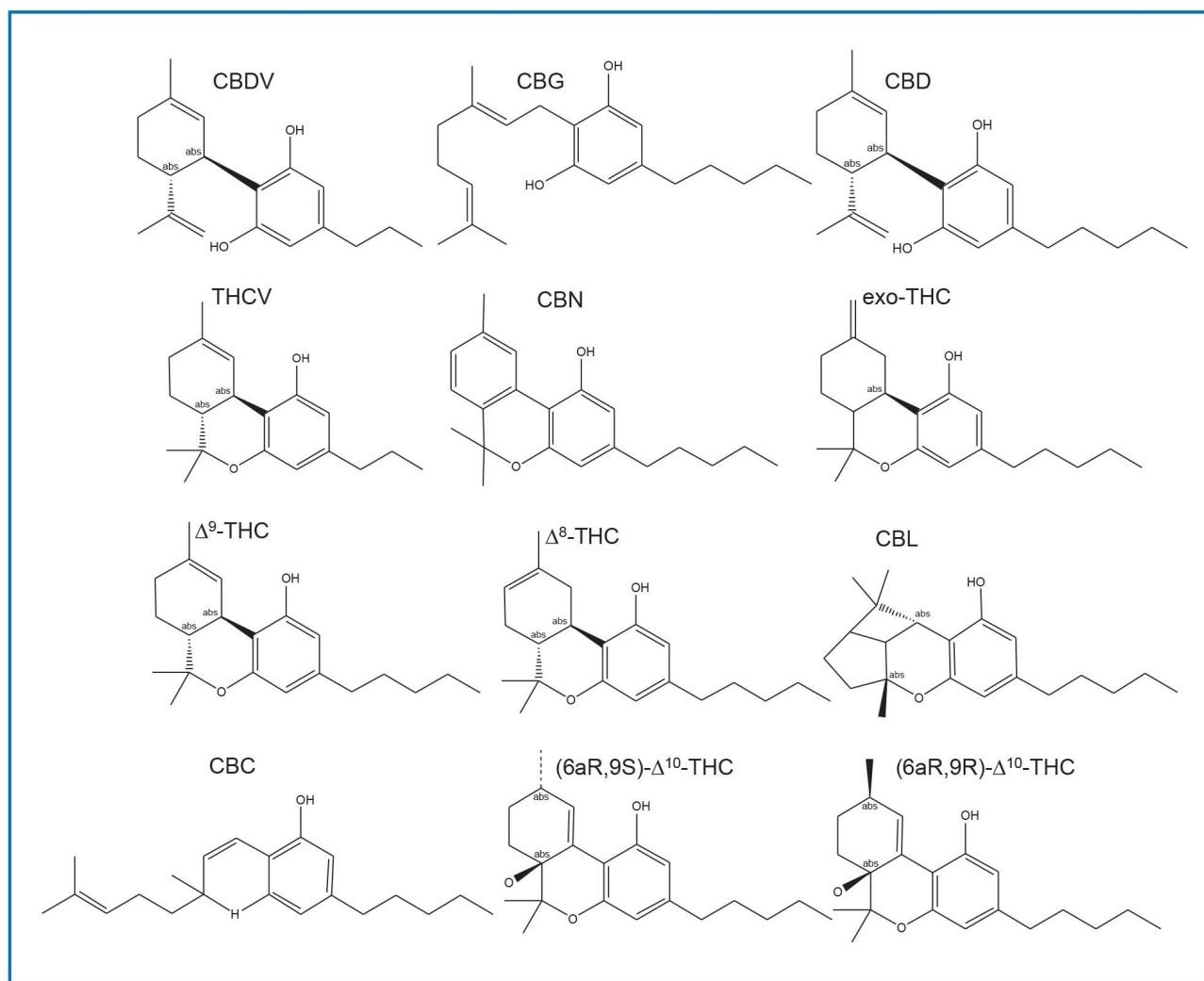
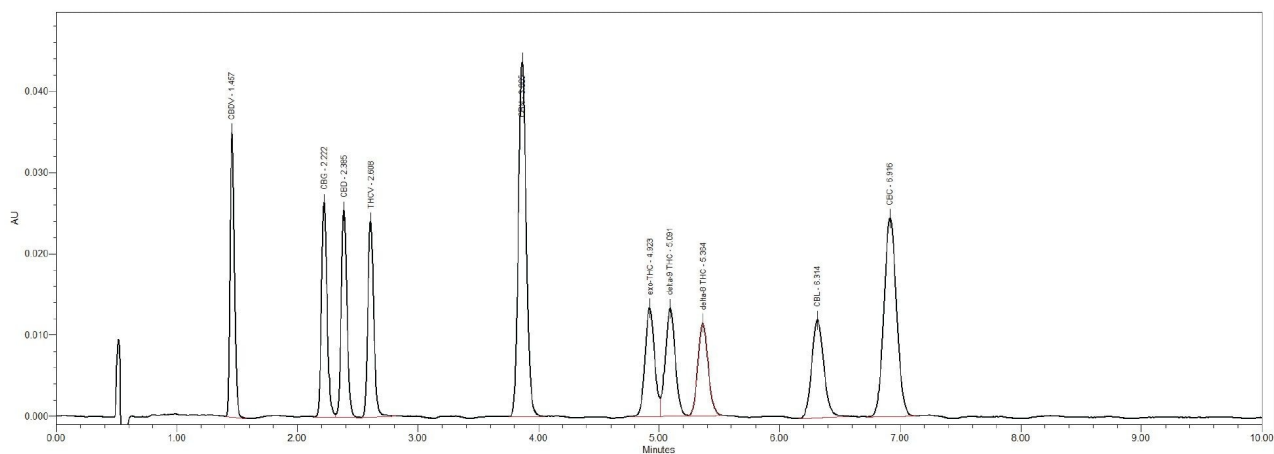*Figure 1. Structures of cannabinoids used in the study.*

Figure 2. UV chromatogram at 228 nm resulting from the separation of an authentic standard mixture of neutral cannabinoids, 12.5 µg/mL, 5 µL.

Following the analysis of a cannabis distillate sample, several compounds were identified based on retention times (t_R) recorded in the Empower processing method: CBD, CBN, exo-THC, Δ^9-THC, and Δ^8-THC. Two components eluting after the main Δ^8-THC peak with Area% values of 2% and 12%, respectively, were integrated but not identified by the processing method.

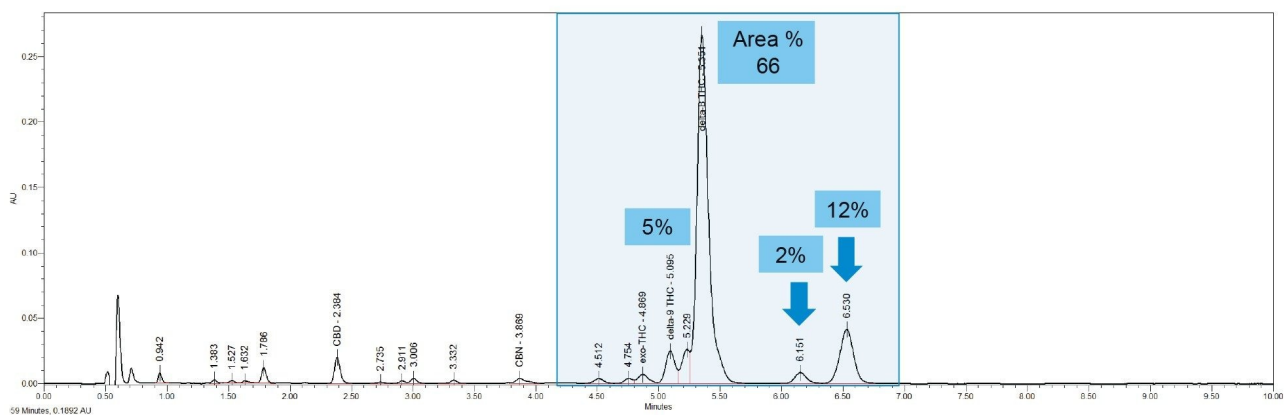The main component, identified as Δ^8-THC (at t_R 5.351 minutes), was calculated to have an Area% of 66% (Figure 3).

Figure 3. UV chromatogram at 228 nm resulting from the analysis of a distillate sample, 1 µg/mL, 5 µL.

In the region of the chromatogram between 4.4 to 5.3 minutes (the peaks preceding the Δ^8-THC peak at t_R 5.351 minutes), the UV and MS spectral data for the unknown component peaks showed close similarities to those of the identified components. The protonated ion [M+H]$^+$ *m/z* 315 was observed for each of the identified components: exo-THC, Δ^9-THC, and Δ^8-THC. The base peak for each of the unknown component peaks at t_R 4.512 min, 4.754 minutes and 5.229 minutes was also *m/z* 315 Figure 4. These data suggest that the components may be structurally related and possible isomers.

The UV spectra for the two components eluting after the main Δ^8-THC peak at t_R 6.151 min and 6.530 minutes differed significantly from the six components described in Figure 4, despite also having a base peak of *m/z* 315 (Figure 5).
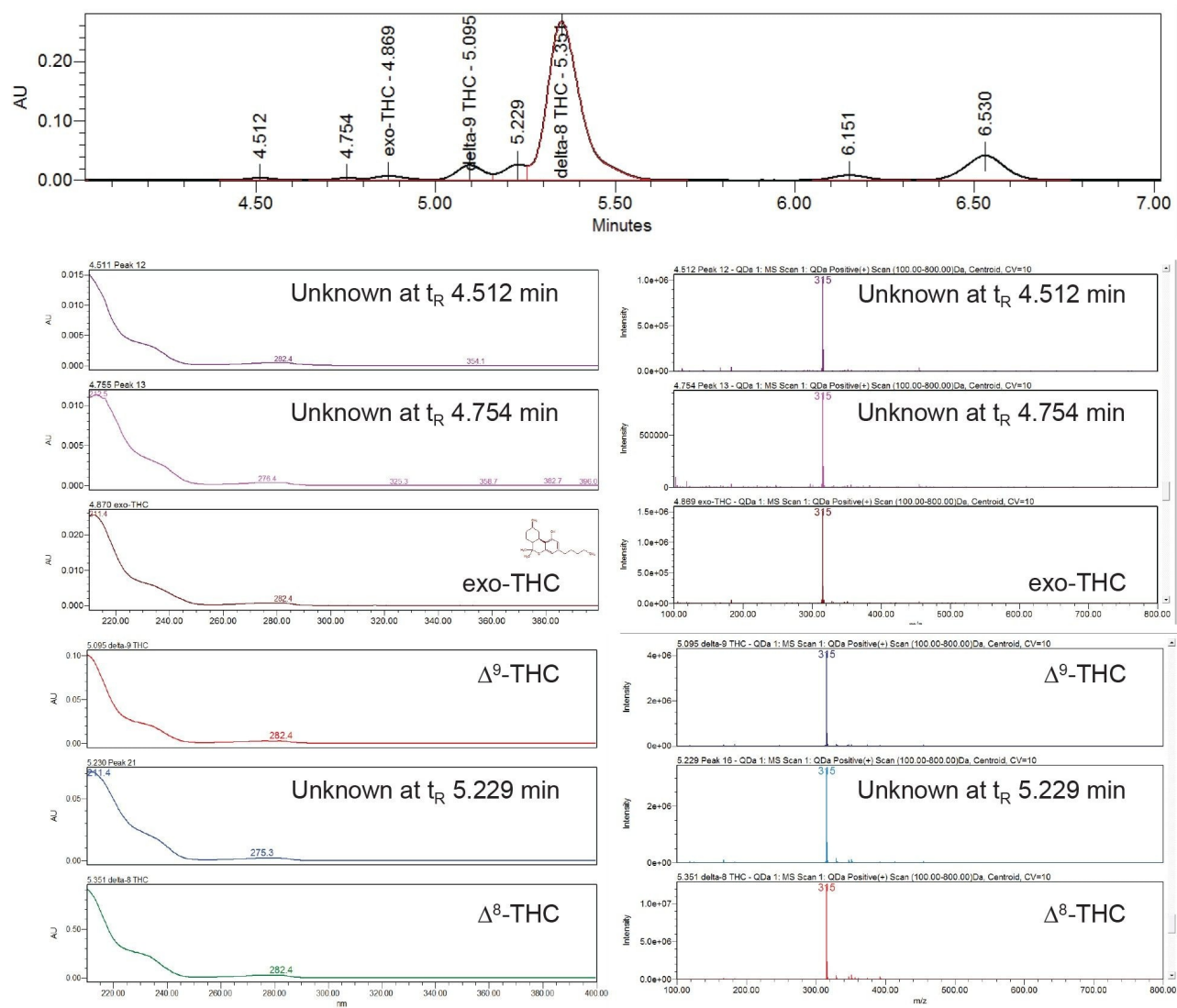
Figure 4. Comparison of the PDA and mass spectra for identified and unidentified components detected in the Δ^8-THC distillate sample and eluting in the defined chromatographic region. The same UV spectra (left) and mass spectra (right) were observed.
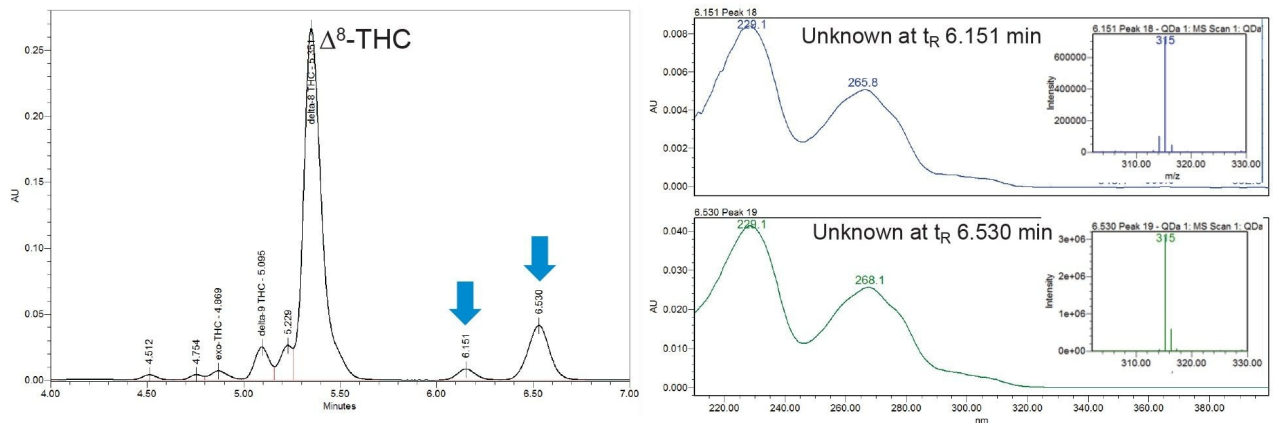
Figure 5. Comparison of the UV spectra (right) and mass spectra (right inset) for the unknown components at 6.151 min and 6.530 min.

The UV spectra for all integrated peaks can be compared in a single view using the Empower Spectrum Index plot (Figure 6). The clear spectral differences between the last two eluting components and the previous six can be observed in Figure 6.
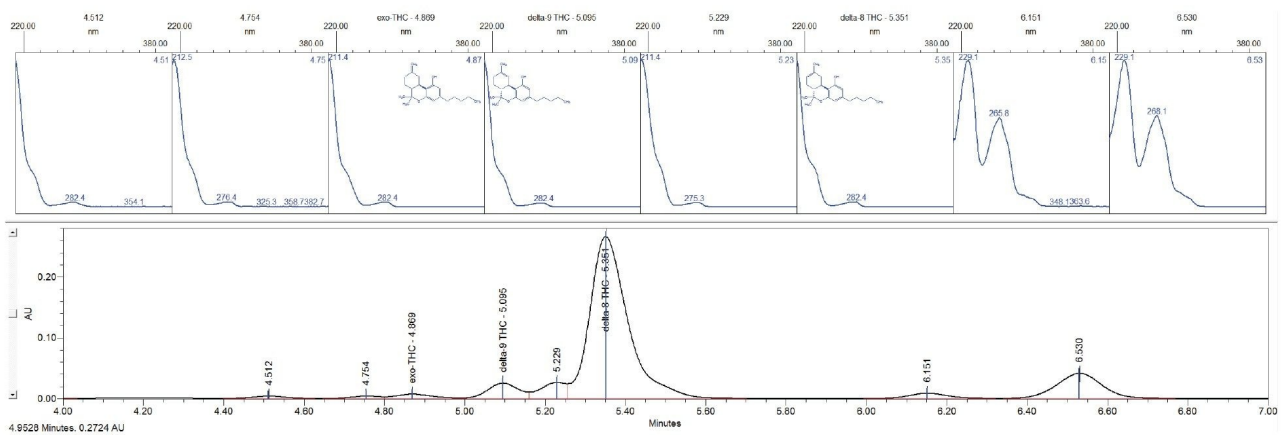

Figure 6. PDA Spectrum index plot comparison for identified and unidentified components eluting between 4.0 and 7.0 min.

Empower library searching can be used to match unknown spectra from peaks with PDA or mass spectral data

stored in a library. The library spectra are first collected using authentic standards and saved. A cannabinoid PDA spectral library was compiled using available authentic standards for both acidic and neutral components. PDA spectra and retention times are recorded in the library. Libraries can be searched manually or automatically as part of the processing method.

When the cannabinoid library was searched with a retention time window of 5% set in the processing method, one library match was proposed for each of the unknown components at t_R 6.151 minutes and 6.530 minutes.

The library match results indicate the best possible match for the unknown component at 6.151 minutes to be (6aR,9S)-Δ^{10}-THC (highlighted in Figure 7). Using the spectral contrast algorithm in Empower, the Match 1 Angle was observed to be less than the Match 1 Threshold.[9–13] The smaller the match angle, the better the match. In the Results window, the PDA Triple Plot, displays the spectrum from the unknown component along with the best possible spectral match, and the difference between them (lower spectrum, Figure 7). To aid visual inspection of the spectra, they can be superimposed to highlight any spectral differences. Figure 8 shows the superimposed spectral for the unknown component at 6.151 minutes and the library spectrum, indicating an observed close match.

The library search for the unknown at 6.530 minutes indicated the closest match to be (6aR,9R)-Δ^{10}-THC. However, the Match 1 Angle was greater that the Match 1 Threshold, in this case. In addition, there was a purity flag which required further investigation.
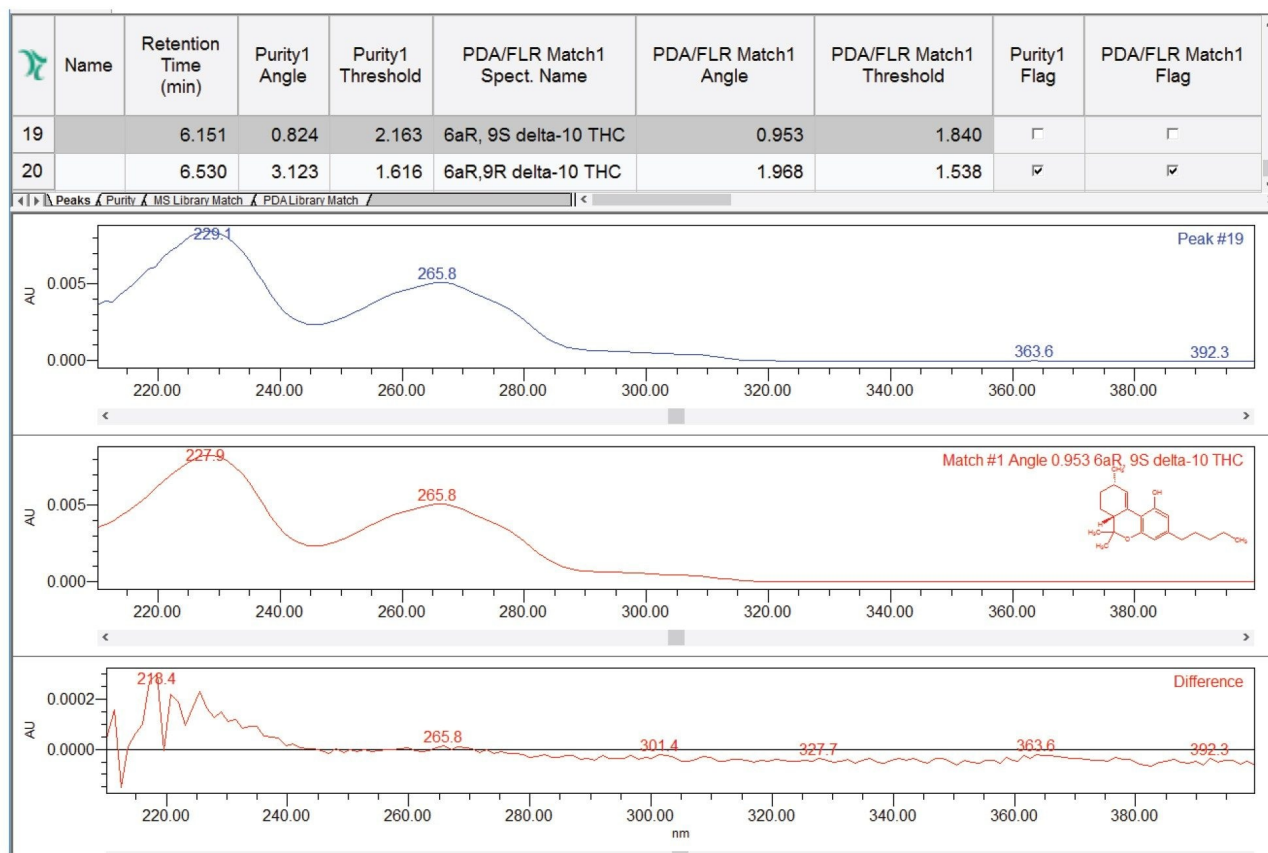
	Name	Retention Time (min)	Purity1 Angle	Purity1 Threshold	PDA/FLR Match1 Spect. Name	PDA/FLR Match1 Angle	PDA/FLR Match1 Threshold	Purity1 Flag	PDA/FLR Match1 Flag
19		6.151	0.824	2.163	6aR, 9S delta-10 THC	0.953	1.840	☐	☐
20		6.530	3.123	1.616	6aR,9R delta-10 THC	1.968	1.538	☑	☑

◄ ► � Peaks ⅄ Purity ⅄ MS Library Match ⅄ PDA Library Match ⅄

Figure 7. PDA Triple Plot displaying the library matches for the unknowns at t_R 6.151 minutes, and 6.530 minutes. A comparison of the spectrum for the selected unknown, the library proposal, and the difference spectrum is also shown.
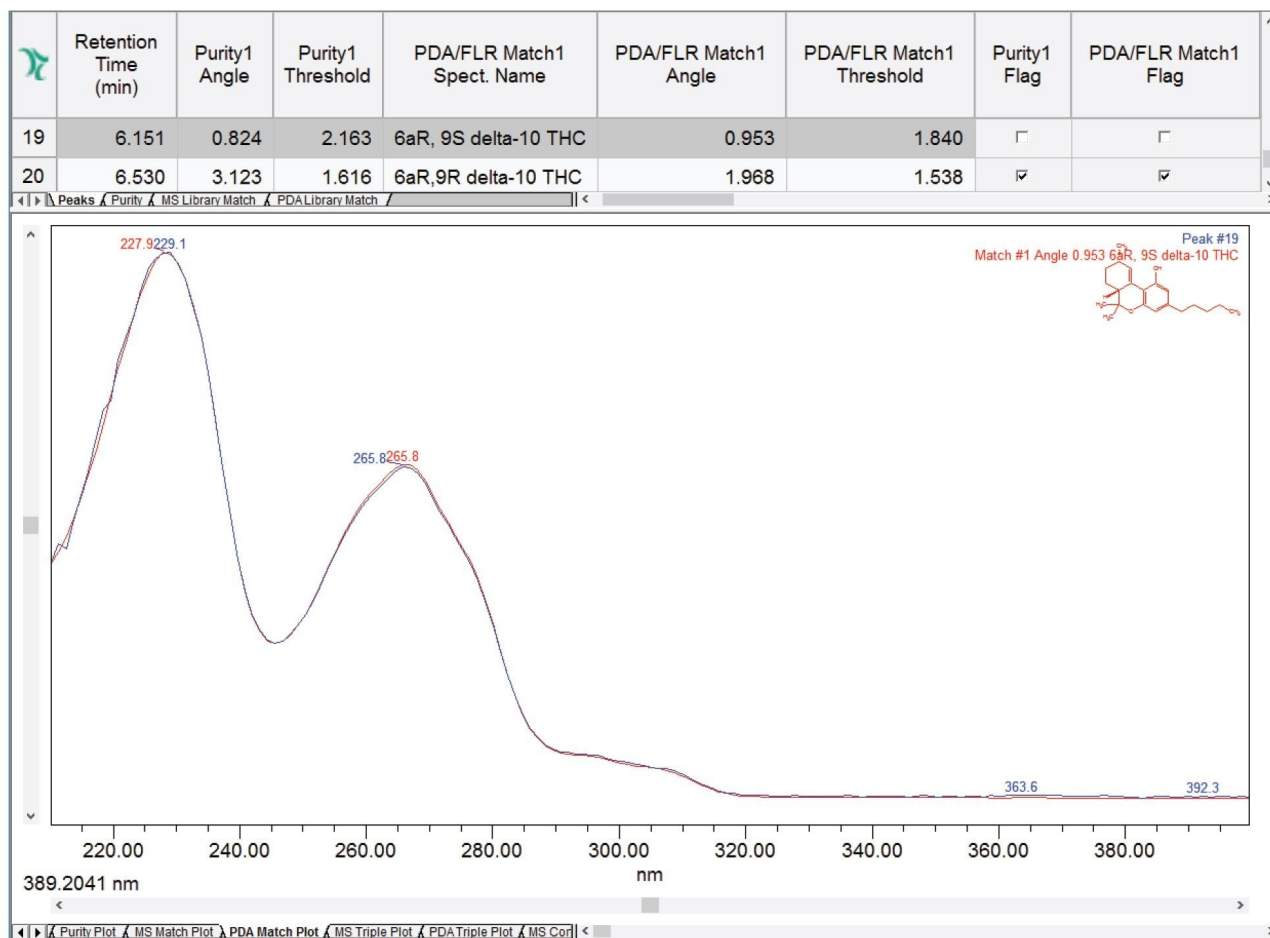
	Retention Time (min)	Purity1 Angle	Purity1 Threshold	PDA/FLR Match1 Spect. Name	PDA/FLR Match1 Angle	PDA/FLR Match1 Threshold	Purity1 Flag	PDA/FLR Match1 Flag
19	6.151	0.824	2.163	6aR, 9S delta-10 THC	0.953	1.840	☐	☐
20	6.530	3.123	1.616	6aR,9R delta-10 THC	1.968	1.538	☑	☑

◄│►│\ Peaks ⟨ Purity ⟨ MS Library Match ⟨ PDA Library Match /   ‖ ‹

Figure 8. PDA Match Plot displaying the superimposed spectra for the unknown at tR 6.151 minutes and the closest library match.

Peak Purity

The Purity Plot can be used for evaluating co-eluting components.[14–15] Each spectrum across the integrated peak is evaluated. In the Purity Plot (Figure 9), the Purity 1 Angle is greater than the Purity 1 Threshold indicating the presence of an impurity. The vertical line annotated with the letter M indicates the point in the peak with the maximum value for the ratio of purity angle/purity threshold. In this case, the leading edge of the peak.

Comparison of the t_R and UV spectra between authentic standards of (6aR,9S)-Δ^{10}-THC and (6aR,9R)-Δ^{10}-THC and the unknown components in the distillate sample showed close similarities providing further confidence in
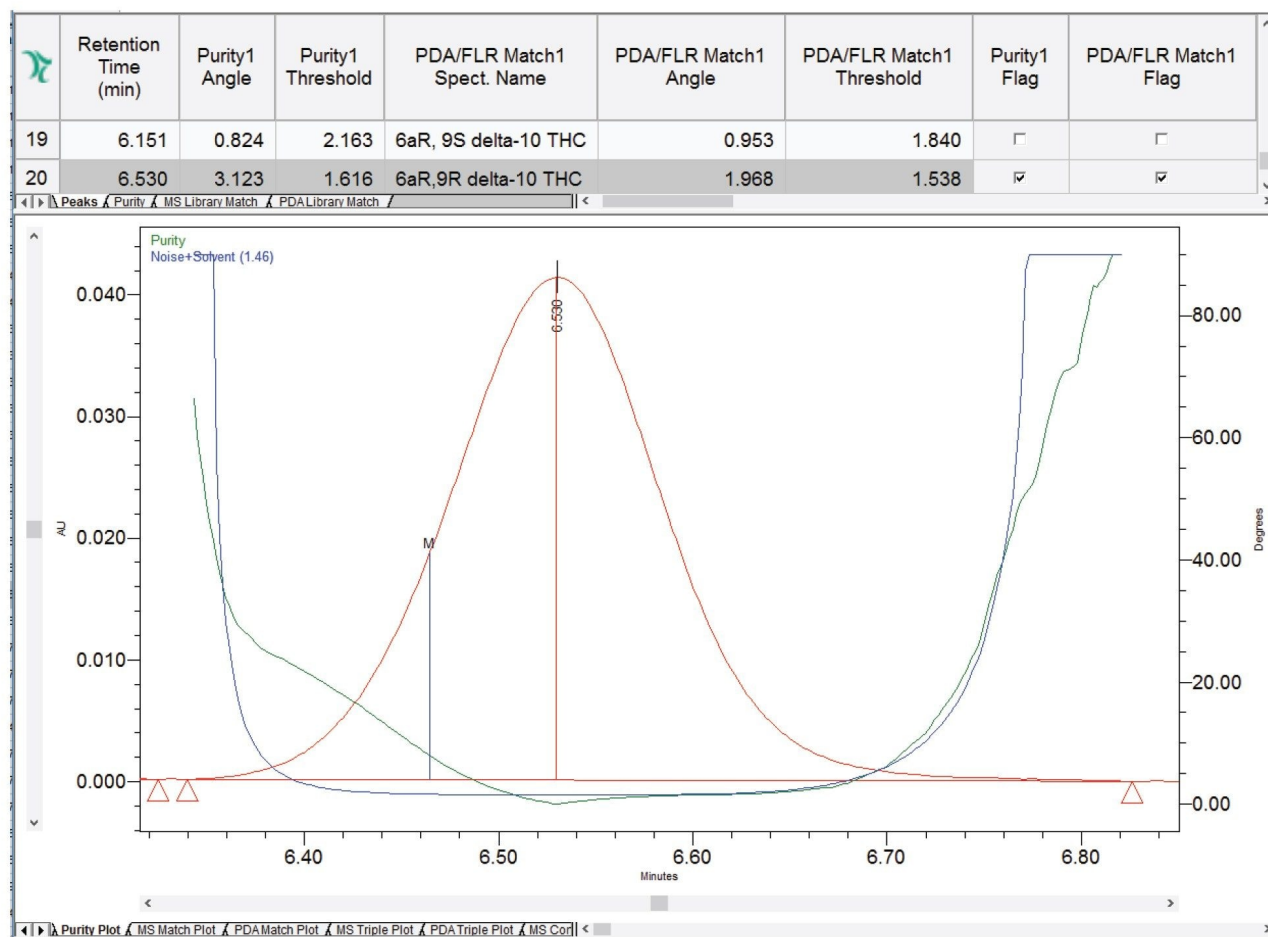
the identifications.

	Retention Time (min)	Purity1 Angle	Purity1 Threshold	PDA/FLR Match1 Spect. Name	PDA/FLR Match1 Angle	PDA/FLR Match1 Threshold	Purity1 Flag	PDA/FLR Match1 Flag
19	6.151	0.824	2.163	6aR, 9S delta-10 THC	0.953	1.840	☐	☐
20	6.530	3.123	1.616	6aR,9R delta-10 THC	1.968	1.538	☑	☑

◄│►│▐ Peaks 〈 Purity 〈 MS Library Match 〈 PDA Library Match ╱ ║ <

Figure 9. Purity Plot for the unknowns at t$_R$ 6.530 minutes.

In-source Fragmentation

MS data adds confidence to component identification. Precursor and product ion data are frequently used together to further increase confidence in the identification of unknowns. In-source fragmentation experiments with a single quadrupole mass spectrometer can provide additional structural information. However, unlike tandem mass spectrometry, this mode of fragmentation is non-specific and can include contributions from multiple components in the event of compound co-elution.

The method can be configured to simultaneously acquire data using two MS scan functions at two different cone

voltages: a low value (15 V) and a high value (45 V). The high cone voltage function induces fragmentation due to ion acceleration and collision with gaseous species including the desolvation gas and solvent vapor.

During the analysis of the distillate sample using the low cone voltage experiment, the precursor ion $[M+H]^+$ *m/z* 315 for the peak identified as (6aR,9R)-Δ^{10}-THC was observed. During the high cone voltage experiment, several product ions with *m/z* typical of Δ^9-THC and its isomers were observed including *m/z* 259, 193, 135, and 123. There was good agreement between the fragmentation patterns observed for the high cone voltage experiment when an authentic standard of (6aR,9R)-Δ^{10}-THC was compared with the peak in the sample at t_R at 6.530 minutes (Figure 10).
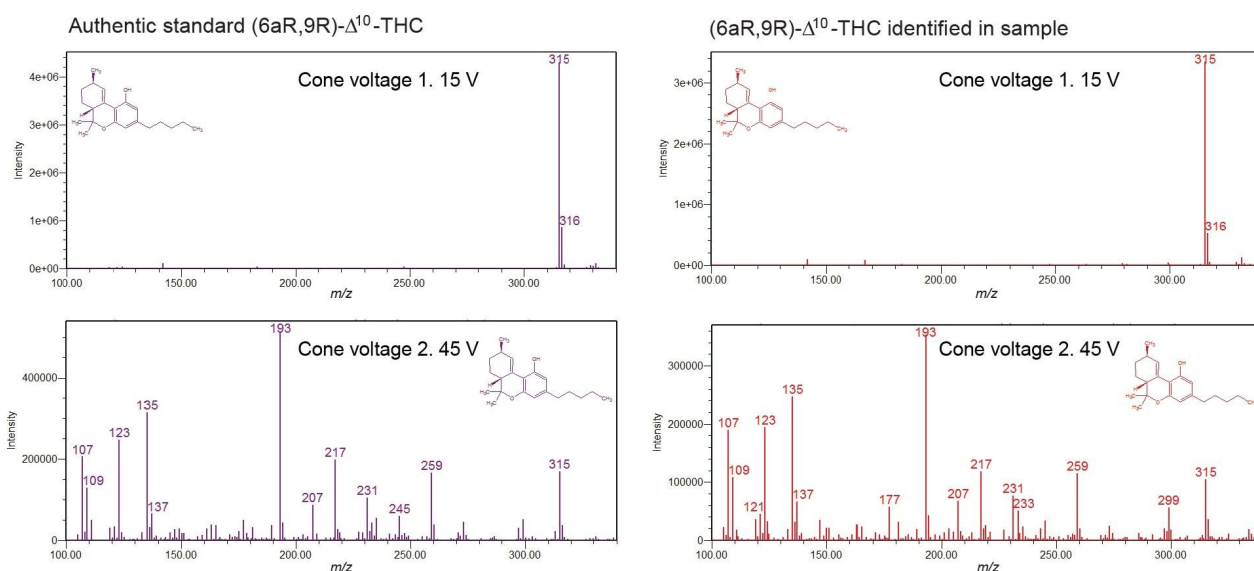

Figure 10. Comparison of low and high cone voltage spectra for an authentic standard of (6aR,9R)-Δ^{10}-THC (left) and the peak at the same t_R in the distillate sample (right). The same product ions were observed.

Conclusion

· Several unidentified components with a base peak of *m/z* 315 were observed eluting in the region between 4.0 minutes and 7.0 minutes in the distillate sample. HPLC-PDA-MS data suggest that they are possible

structural isomers of Δ^9-THC.

- Unknown components were tentatively identified using a cannabinoid PDA spectral library with compound assignment based on PDA spectral matching.

- The Purity Plot can be used for evaluating peak purity, indicating the presence of co-eluting components.

- Authentic standards of (6aR,9S)-Δ^{10}-THC and (6aR,9R)-Δ^{10}-THC were used to confirm the identifications based on t_R, precursor, and product ions.

- Multiple unknown components were detected in the distillate samples. Characterization of components with unknown biological activity or toxicological data is important to enhance understanding and ensure consumer safety.

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