CHARACTERISATION OF Δ^8-THC DISTILLATES USING HPLC WITH PDA AND MASS SPECTROMETRY DETECTION

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INTRODUCTION

The use of delta-8 tetrahydrocannabinol (Δ^8-THC) has caused consumer safety concerns in the US.[1-2] 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 typically formulated using Δ^8-THC made from the chemical conversion of hemp-derived cannabidiol (CBD), which was defined and legalised 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.[3-7] 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 Empower software tools that aid in sample exploration.

METHODS

Sample Preparation.

Distillate samples and standards were dissolved and diluted with acetonitrile.

Instrumentation and Software

Arc™ HPLC System

2998 Photodiode Array (PDA) Detector

ACQUITY QDa™ mass detector

Empower™ Chromatography Data Software

MS Conditions

Ionization mode: ESI+

Mass range: 100 to 800 Da

Low Cone voltage: 15 V; High Cone voltage: 45 V

PDA detection: 210 to 400 nm; Single wavelength @ 228 nm

HPLC Conditions

Column: CORTECS™ C_{18}, 4.6 x 100 mm, 2.7 µm Column

Solvent A: 0.1% formic acid in water. Solvent B: 0.1% formic acid in acetonitrile; Flow rate: 1.592 mL/min: Column temp.: 25°C

Starting composition 71%B until 10.13 min. At 11.81 ramp to 99% B. Hold until 13.5 min. Return to initial conditions.

Injection volume: 5 µL.

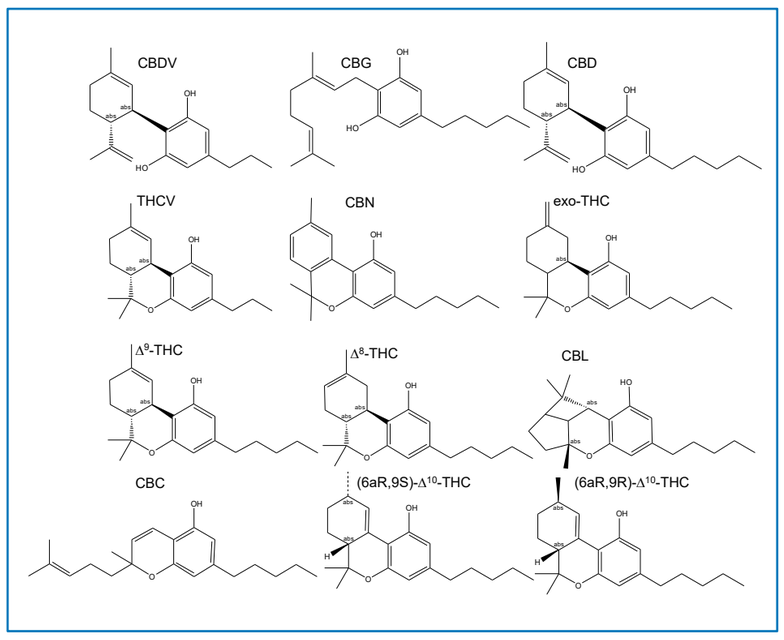


Figure 1. *Structures of cannabinoids used in the study.*

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.

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.

RESULTS AND DISCUSSION

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

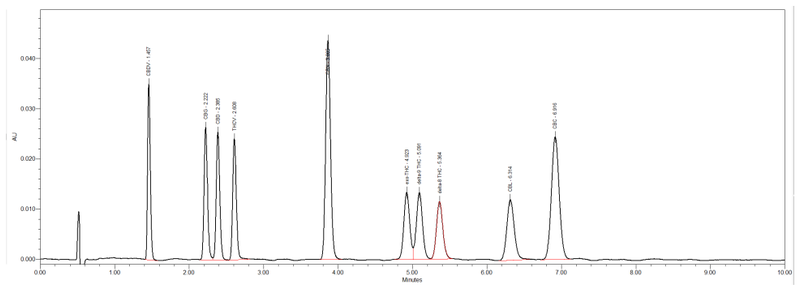


Figure 2. *UV chromatogram at 228 nm resulting from the separation of an authentic standard mixture of neutral cannabinoids, 12.5 µg/mL, 5 µL.*

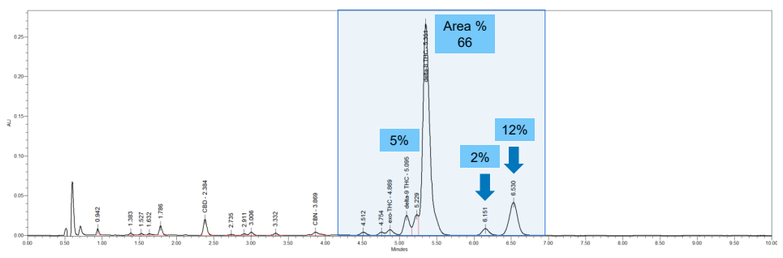


Figure 3. *UV chromatogram at 228 nm resulting from the analysis of a distillate sample, 1 mg/mL, 5 µL*

In the region of the chromatogram between 4.4 to 5.3 min (the peaks preceding the Δ^8-THC peak at t_R 5.351 min), 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 min and 5.229 min 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 min differed significantly from the 6 components described in Figure 4, despite also having a base peak of *m/z* 315 (Figure 5).

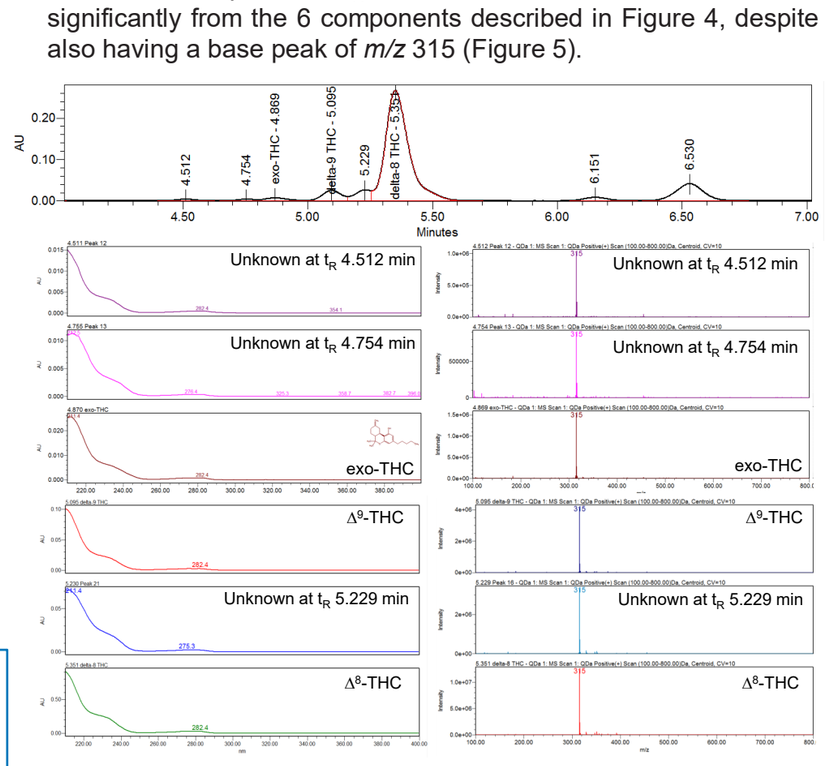


Figure 4. *Comparison of the PDA and mass spectra for identified and unidentified components detected in the defined chromatographic region. The same UV spectra (left) and mass spectra (right) were observed.*

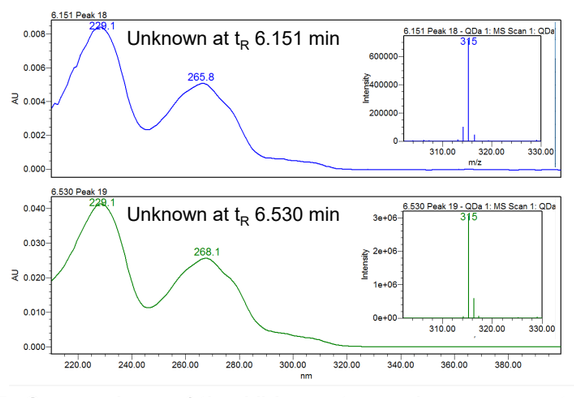


Figure 5. *Comparison of the UV spectra and mass spectra for the unknown components at 6.151 min and 6.530 min.*

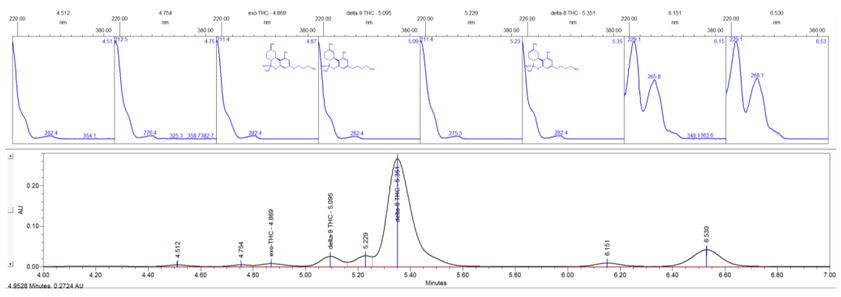
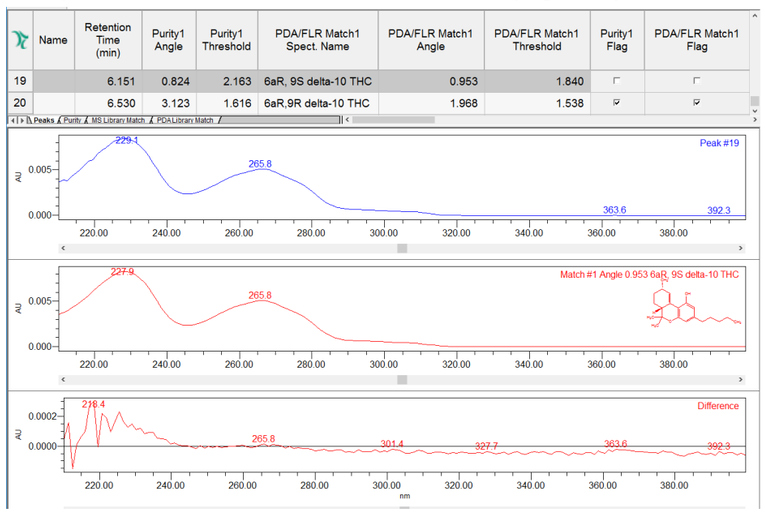


Figure 6. *PDA Spectrum Index Plot comparison for identified and unidentified components between 4.0 and 7.0 min.*

The UV spectra for all integrated peaks can be compared in a single view using the Spectrum Index Plot (Figure 6). The spectral differences between the first six components and the last two eluting in the chromatographic region specified, can clearly be observed.

Library Searching

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. 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 min and 6.530 min (Figure 7).



	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	☑	☑

Figure 7. *PDA Triple Plot displaying the library matches for the unknowns at t_R 6.151 min, and 6.530 min. A comparison of the spectrum for the selected unknown, the library proposal, and the difference spectrum is also shown.*

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.

When the distillate sample was analysed using a low cone voltage experiment (15V), the precursor ion [M+H]+ *m/z* 315 was observed for the component identified as (6aR,9R)-Δ^{10}-THC in the distillate sample. During the high cone voltage experiment (45V), several product ions with *m/z* typical of Δ^9-THC 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 min (Figure 8).

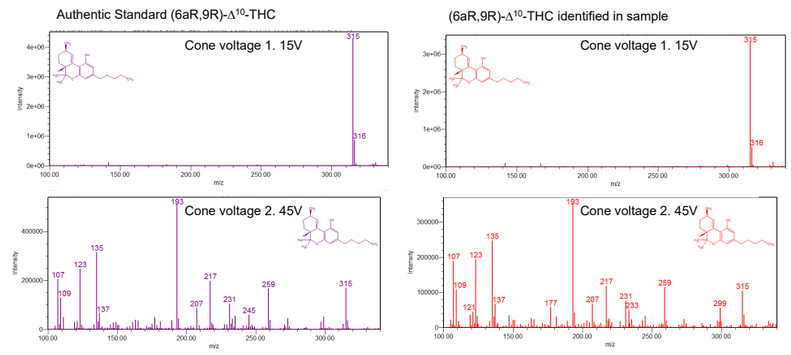


Figure 8. *Comparison of low and high cone voltage spectra for an authentic standard of (6aR,9R)-Δ^{10}-THC (left) and the peak at the same t_R in the distillate sample (right).*

CONCLUSION

- **Several unidentified components with a base peak of *m/z* 315 were observed eluting in the region between 4.0 min and 7.0 min in the cannabis 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.**
- **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. Characterisation of components with unknown biological activity or toxicological data is important to enhance understanding and ensure consumer safety.**

References

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