CHARACTERISATION OF DELTA-8 THC DISTILLATES USING HIGH RESOLUTION MASS SPECTROMETRY (HRMS) AND CYCLIC ION MOBILITY SPECTROMETRY COUPLED WITH 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] Bulk Δ^8-THC is typically produced from hemp derived CBD. 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 characterised to enhance the chemical understanding of the components produced.[3-7]

In the Quadrupole time-of-flight (QTOF) analysis, the software highlighted a predicted *m/z* 315.23186 as the base peak for several unknowns with proposed elemental compositions of $C_{21}H_{30}O_2$, and common fragments with Δ^9-THC. Ion mobility spectrometry (IMS), which separates species on the basis of size, shape, and charge suggests that these species are additional isomeric forms of the C_{21} neutral cannabinoids. The presence of isomers can present separations challenges. The addition of ion mobility enhances system peak capacity and improves the resolution of isomeric and isobaric components. Resolution was achieved using a multi-pass travelling-wave cyclic IM (cIM) device where increased mobility resolution is achieved with successive number of passes (Figure 1).[8]

METHODS

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

Ionization mode: ESI+

UPLC and QToF Instrumentation and Software

ACQUITY™ UPLC™ I-Class Plus and Xevo™ G3 QToF with UNIFI™Software

UPLC and IMS Instrumentation and Software

ACQUITY UPLC I-Class Plus and Select Series™ Cyclic™ IMS. Data acquired in MassLynx™ and Processed in UNIFI

QToF MS Experiment: Simultaneous collection of high and low collision energy (CE) spectra (MS^E mode).

Cyclic IMS Experiment: Ion mobility separation was achieved using single ($HDMS^E$) and multi-pass (targeting MS/MS *m/z* 315.2) experiments

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

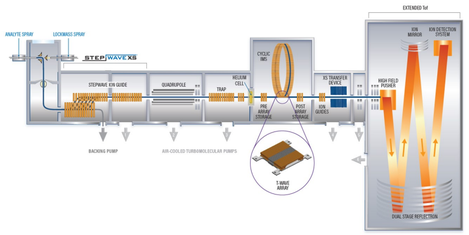

Figure 1. Schematic for Waters SELECT SERIES cIMS system. Ions can be maintained in the cyclic mobility separator for multiple passes and higher resolution. IMS^n experiments are also possible.

QTOF RESULTS

In distillate sample A, the presence of Δ^8-THC , exo-THC and Δ^9-THC were detected in the PDA data using retention time (t_R) and spectral matching (data not shown). Several unknown components were detected in the UV with Area% values ranging from below 0.1% to 4.9%. Investigation of the unknown components detected in the PDA using the HRMS data showed multiple components with a base peak of *m/z* of 315.23 eluting in the region preceding the main Δ^8-THC peak at 3.28 min (Figure 2). In-house cannabinoid reference libraries were used to assign putative identities to the compounds detected in the distillate samples based on multifactor identification. Compounds were verified by the library using accurate masses of precursor, fragment ions, isotope patterns and t_R. Unidentified major components visible in both the PDA and MS data were then evaluated using the structural elucidation tools (Figure 4).

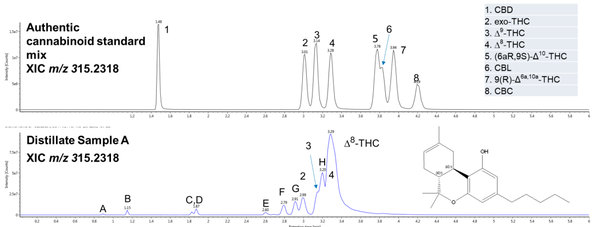

Figure 2. Extracted Ion Chromatogram (XIC) 315.2318 of an authentic standard mix of 8 isomeric cannabinoids (top) (10 µg/mL, 0.5 µL) and (beneath) Δ^8-THC distillate sample A (1 mg/mL, 0.5 µL).

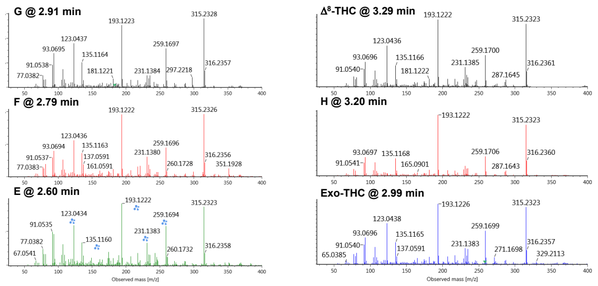

Figure 3. Selected high CE spectra from unknown components identified as having a base peak of m/z 315.23. Fragments observed could be matched with those observed in authentic standards of Δ^8-THC, Δ^9-THC, and other isomers.

The fragmentation spectra for unknown components with a base peak of *m/z* 315.23 eluting in the region before the main Δ^8-THC peak at 3.29 min are shown in Figure 3. The proposed elemental composition for each of the unknown component precursors was $C_{21}H_{30}O_2$ (mass error -0.3 mDa). 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 in the absence of orthogonal techniques. The resolution of isomers can be possible using ion mobility spectrometry which separates species on the basis of size, shape and charge (Figures 5-8).

In distillate sample B, an unknown component with *m/z* 351.2080, and an observed isotopic pattern indicating the presence of a chlorine in the chemical structure was detected. The software flagged the presence of fragments common to Δ^9-THC and its isomers indicating a likely structural relationship. The Area% for the component was 19.4%. The purity of the distillate was <80%. The proposed elemental composition of the unknown was $C_{21}H_{31}ClO_2$ (mass error –0.49 mDa) (Figure 4). Targeted MS/MS of *m/z* 351.20 confirmed the fragmentation data.

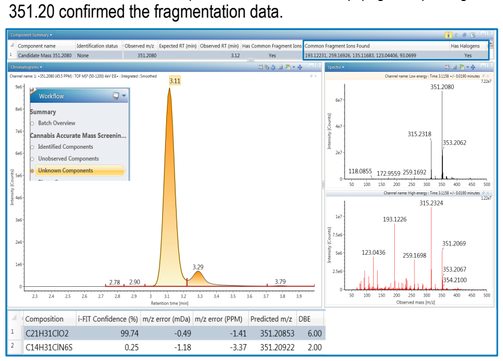

Figure 4. Unknown component at t_R 3.11 min with m/z 351.2080 (top table), common fragments and halogens noted by the software. XIC for m/z 351.2080 (left). High and low CE spectra (right). Proposed elemental composition $C_{21}H_{31}ClO_2$ (mass error –0.49 mDa).

CYCLIC IMS RESULTS

In the chromatographic separation of 8 isomers of Δ^9-THC using the specified conditions, CBL is not resolved from (6aR,9S)-Δ^{10}-THC (Figure 5 inset). In a single pass experiment, the two isomers are also unresolved as shown in the drift time (Y axis) Vs. t_R (X-axis) plot (Figure 5). However, with multi-pass MS/MS experiments targeting *m/z* 315.2 using the travelling-wave cIM device, where increased mobility resolution is achieved with increasing number of passes, and ion residence time, the isomers can be completely resolved (Figure 6). The data in Figure 6 demonstrates the ability to resolve isomers in the mobility dimension despite the chromatographic co-elution. Time aligned product ion spectra from mobility separated precursor ions is also possible since collision induced dissociation (CID) occurs post IM separation in the transfer region (Figures 1 and 6). Clear differences in the isomer MS/MS spectra were observed.

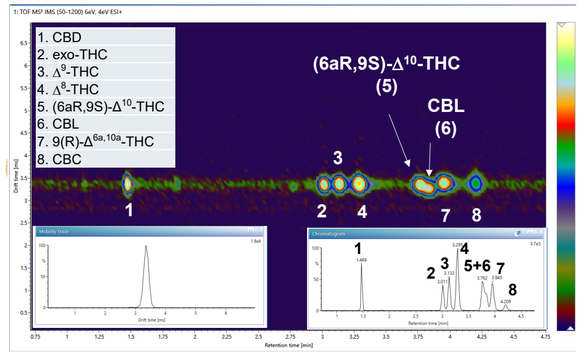

Figure 5. Drift time Vs. t_R plot for the analysis of an authentic standard mix of 8 isomers of Δ^9-THC using a single pass experiment. CBL and (6aR,9S)-Δ^{10}-THC are not resolved in the chromatographic (t_R axis, X) or mobility dimensions (drift time axis, Y).

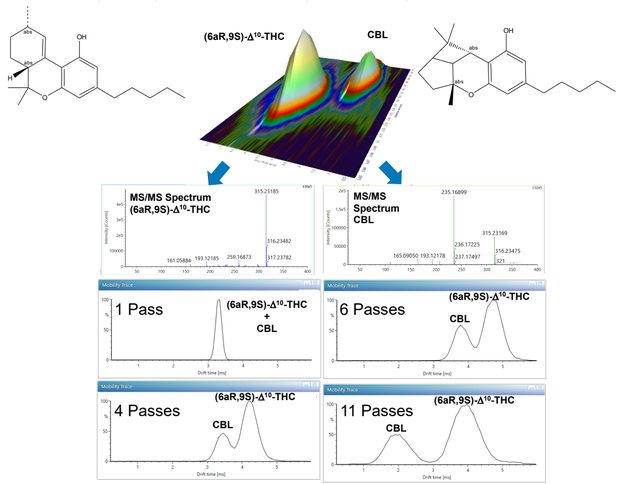

Figure 6. Cyclic Ion Mobility separation for single (1 Pass) and multi-pass experiments, showing the increased IM resolution of CBL and (6aR,9S)-Δ^{10}-THC. Mobility separated MS/MS spectra for each isomer separated using 11 passes also shown (top).

In the QToF analysis of distillate sample A, several unknown components, and potential isomers eluting in the region preceding the main Δ^8-THC peak at 3.28 min were observed (Figures 2 and 3). The analysis of sample A using a single pass experiment revealed five peaks in the t_R range of 2.4– 3.05 min, one of which was identified as exo –THC (Peak 5, Figure 7), however, using a 6 pass MS/MS experiment targeting *m/z* 315.2, revealed two additional components previously undifferentiated by chromatography but resolved in the mobility dimension due to the increased mobility resolution that is possible (Figure 7).

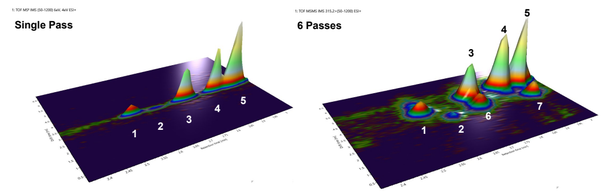

Figure 7. Cyclic Ion Mobility single and 6 pass experiments. Two additional components previously undifferentiated (6 and 7) were observed in the 6 pass experiment.

The two groups of component peaks (3, 6, and 5, 7) which are not separated chromatographically or in the single pass mobility experiments, resolve using a 6 pass experiment. The results shown in Figure 8 also demonstrate the ability of the cIM to provide product ion spectra from mobility separated precursor ions as CID occurs after the IM separation in the transfer region which aids in structural elucidation. Differences in MS/MS spectra were observed for peaks 5 and 7, including varying fragmentation ratios which can indicate structure differences.

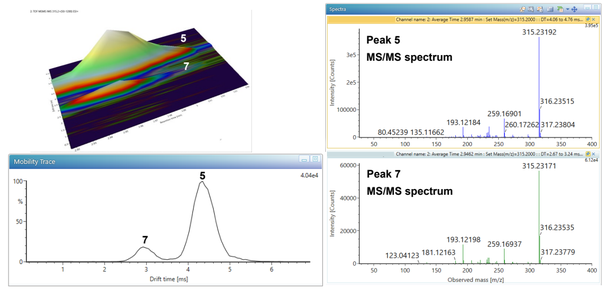

Figure 8. Cyclic ion mobility separation of peaks 5 and 7, and MS/MS spectra.

CONCLUSION

- **Analysis of the Δ^8-THC distillates using QToF MS revealed several components that appear to be structurally related and potential isomers.**
- **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 suggesting a structural relationship, was observed in a purified distillate sample.**
- **Further work using cIM showed evidence of co-eluting isomeric components illustrating the complexity of the distillate samples.**
- **Isomers of Δ^9-THC, CBL and (6aR,9S)-Δ^{10}-THC resolved after 6 passes.**
- **Product ion spectra from mobility separated precursor ions is possible as CID occurs post IM separation.**
- **Combining GC or LC chromatography and MS with IMS technologies, provides a more thorough characterisation of very complex samples.**

References

1. Erickson BE. (2021). "Delta-8-THC craze concerns chemists." C&EnNews. 99(31).(https://cen.acs.org/biological-chemistry/natural-products/Delta-8-THC-craze-concerns/99/i31
2. Hudalla. C. (2021). "We believe in unicorns (and Delta-8)". The Cannabis Scientist. https://thecannabisscientist.com/testing-processing/we-believe-in-unicorns-and-delta-8
3. Golombek, P., et al. (2020). "Conversion of Cannabidiol (CBD) into Psychotropic Cannabinoids Including Tetrahydrocannabinol (THC): A Controversy in the Scientific Literature." Toxics 8(2).
4. Kiselak, T. D., et al. (2020). "Synthetic route sourcing of illicit at home cannabidiol (CBD) isomerization to psychoactive cannabinoids using ion mobility-coupled-LC-MS/MS." Forensic Sci Int **308**: 110173.
5. Watanabe, K., et al. (2007). "Conversion of cannabidiol to Δ9-tetrahydrocannabinol and related cannabinoids in artificial gastric juice, and their pharmacological effects in mice." Forensic Toxicology **25**(1): 16-21.
6. Meehan-Atrash, J. and I. Rahman (2022). "Novel Δ8-Tetrahydrocannabinol Vaporizers Contain Unlabeled Adulterants, Unintended Byproducts of Chemical Synthesis, and Heavy Metals." Chemical Research in Toxicology **35**(1): 73-76.
7. Webster GRB, Sarna, LP, Mechoulam. US Patent Application Publication. Pub. Np.: https://patentimages.storage.googleapis.com/ed/9f/1b/58e43cc4792306/US20040143126A1.pdf
8. Giles, K., et al. (2019). "A Cyclic Ion Mobility-Mass Spectrometry System." Analytical Chemistry **91**(13): 8564-8573.

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