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Note d'application

Forced Degradation of Cannabidiol

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Abstract

This Application note demonstrates the Use of Orthogonal Methods to Monitor the Major Degradation Products of Cannabidiol (CBD).

Through a preliminary forced degradation study of crystalline CBD, we demonstrate how UPLC and UPC^2 can be applied collectively to monitor solution stability. The ACQUITY UPLC H-Class instrument uses conventional reversed-phase chromatography, while the ACQUITY UPC^2 system provides a stereo-chemical separation of important structural isomers via convergence chromatography. When these techniques are coupled with photo diode array (PDA) and mass spectrometry (MS), the chemical and structural identity of important CBD degradation products is determined.

Benefits

- The ACQUITY UPLC H-Class and the ACQUITY UPC^2 Systems are highly reliable instruments that can be used to orthogonally monitor the stability of CBD by reversed-phase liquid chromatography (RPLC) and UltraPerformance Convergence Chromatography, respectively.
- The ACQUITY UPLC H-Class System separates closely eluting Δ^9-THC and Δ^8-THC by reversed-phased chromatography, while the ACQUITY UPC^2 System further separates THC compounds based upon their stereochemistry.
- The ACQUITY UPLC PDA Detector and ACQUITY QDa Detectors are useful tools that provide spectral and mass information for the identification of unknown compounds and degradation products.

- The Waters Fraction Manager – Analytical (WFMA) can be used to collect degradation product peaks separated at the UPLC scale for additional analysis.

Introduction

The debate surrounding the use of cannabinoids for medicinal purposes has been in the news for several years. Although there are at least 85 active substances identified in cannabis, many people associate the bio-botanical with the psychoactive compound tetrahydrocannabinol (THC). Recent attention has shifted to the non-psychoactive compound cannabidiol (CBD), as evidence of the medical benefits continue to grow.[1]

Some CBD preparations are marketed as dietary supplements and claim efficacy against a range of medical conditions. For manufacturers of these preparations, it is important to monitor product stability. A change in this parameter risks consumer health and safety when toxic or unexpected degradation products form over time resulting in the delivery of a different CBD dose than expected.[2]

Extracts prepared from cannabis products can pose a significant challenge to chromatographers due to the vast number of naturally occurring cannabinoid structural isomers.[3] When analyzing complex mixtures, it is beneficial to employ multiple strategies and utilize various analytical tools to provide a more comprehensive understanding of the components within the mixture. For example, when applying different modes of separation, one may gain additional knowledge of both the compounds present and their structural composition.

Through a preliminary forced degradation study of crystalline CBD, we demonstrate how UPLC and UPC^2 can be applied collectively to monitor solution stability. The ACQUITY UPLC H-Class instrument uses conventional reversed-phase chromatography, while the ACQUITY UPC^2 system provides a stereo-chemical separation of important structural isomers via convergence chromatography. When these techniques are coupled with Photo diode array (PDA) and mass spectrometry (MS), the chemical and structural identity of important CBD degradation products is determined.

Experimental

UPLC Reversed-Phase Method

Analytical column:	ACQUITY UPLC CSH C_{18}, 130Å, 1.7 µm, 2.1 x 150 mm
Analytical flow rate:	0.60 mL/min
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Gradient:	Starting conditions at 73% mobile phase B with a 1 minute hold time, linear increase to 90% mobile phase B over 6 minutes
Oven temp.:	30 °C
PDA detector:	Wavelength 225 nm at 4.8 nm resolution, 3D data scan range Wavelength 225 nm at 4.8 nm resolution, scan range 200–400 nm
MS detector:	ACQUITY QDa ionization mode ESI+ mass range 100–600 Da, cone voltage 15 V, capillary temperature 500 °C, capillary voltage 0.80 kV
Injection volume:	2 µL
Software:	Empower3 Chromatography Data Software

UPC2 Method (Figure 7, Figure 8, and Figure 9)

Analytical column:	Trefoil Cel1, 2.5 µm, 3.0 x 150 mm

Analytical flow rate:	2.0 mL/min
Mobile phase A:	CO_2
Mobile phase B:	200 proof ethanol
Gradient:	Starting conditions at 3% mobile phase B, linear increase to 13% mobile phase B over 9 minutes
ABPR:	1800 psi
Oven temp.:	50 °C
PDA detector:	Wavelength 225 nm at 4.8 nm resolution, Compensated reference 310–410 nm, 3D data scan range 200–400 nm
MS detector:	ACQUITY QDa ionization mode ESI+ mass range 100–600 Da, cone voltage 10 V, capillary temperature 550 °C, capillary voltage 1.5 kV
Make up solvent:	8:2 methanol/water with 0.1% formic acid at 0.75 mL/min
Injection volume:	2 µL
Software:	Empower3 Chromatography Data Software

Solvents, Standards and Samples

Approximately 165 mg of crystalline CBD were dissolved in exactly 10 mL of 190 proof ethanol. Three aliquots were prepared to contain 0.1 M acid, 0.1 M base, and 3.0% oxidizing agent. One aliquot was set aside to serve as the control. The samples were stored in glass vials and incubated at 60 °C for 24 hours.

All solvents and diluents were HPLC-MS grade and filtered via 0.45-µm filters.

Reference standard solutions of cannabidiol (CBD) (p/n C6395), cannabinol (CBN) (p/n C6888), cannabichromene (CBC) (p/n C143), cannabigerol (CBG) (p/n C141), (±)Δ^9-THC (p/n T-047), (-)Δ^9-THC (p/n T-032) and (-)Δ^8-THC (p/n T-032) were obtained from Sigma-Aldrich Inc. Allentown, PA.

Results and Discussion

Cannabinoid reference standards were fully separated within 5.5 minutes by reversed-phase UPLC, as shown in the Figure 1 normalized overlay. As described, crystalline CBD was degraded under acidic, basic, and oxidative conditions. The reversed-phase analysis is shown in Figure 2. Degradation products that eluted at the same retention time as the reference standards were investigated for this study.

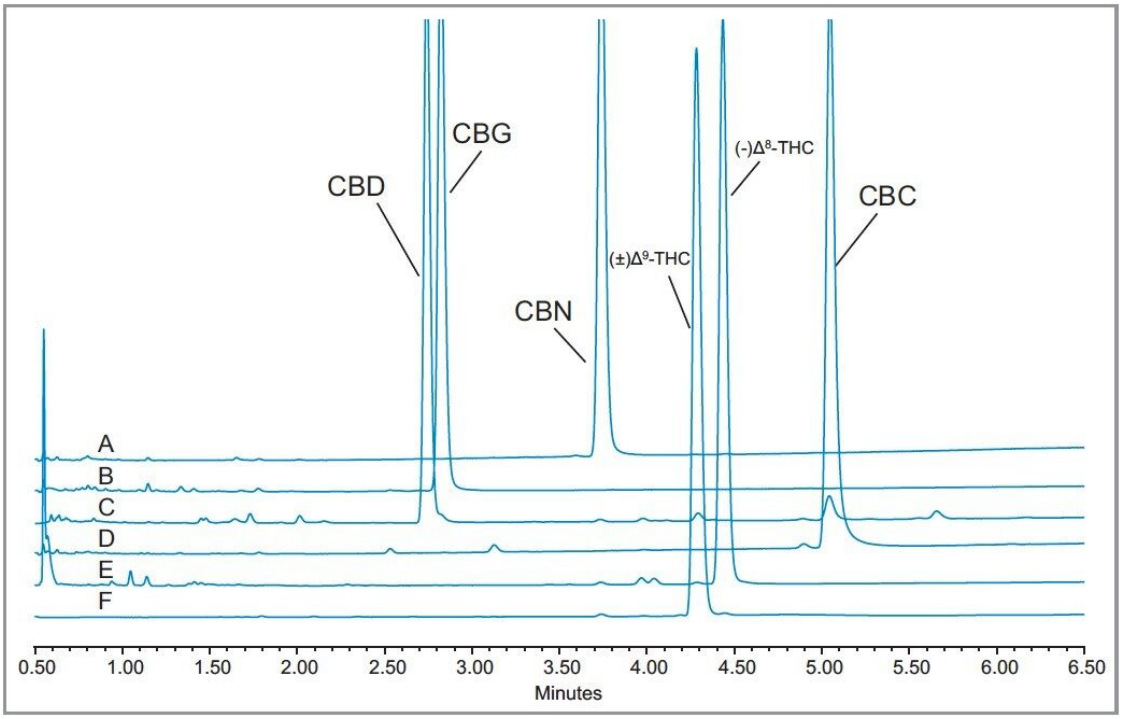

Figure 1. Normalized overlay of cannabinoid reference standards; A) CBN, B) CBG, C) CBD, D) CBC, E) (-)Δ^8-THC, and F) (±)Δ^9-THC.

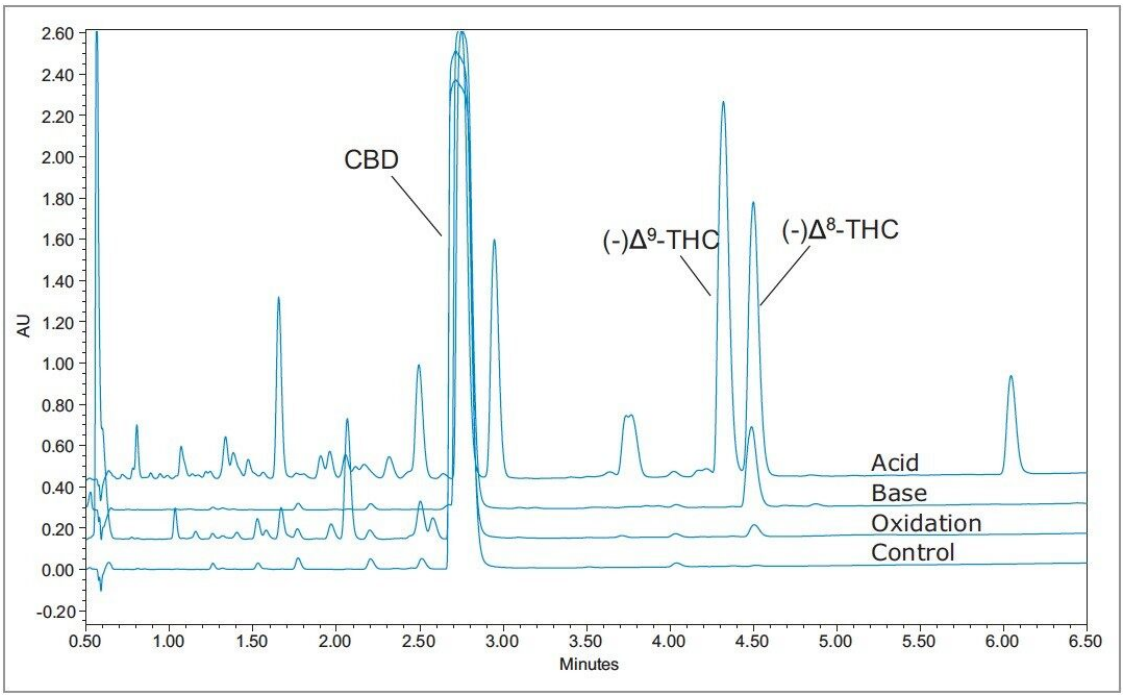

Figure 2. Overlay of the control, oxidation, base, and acid stress results for CBD by reversed-phase.

In comparison to the control, oxidative conditions (3.0% hydrogen peroxide) generated degradation products that eluted t 0.5 and 2.1 minutes. These degradation products did not correlate to the retention time of the reference standards and were not pursued at this time as peaks of interest.

Under basic (0.1 M NaOH) stressed conditions, a degradation product eluted at 4.5 minutes which corresponded to the retention time of (-)Δ^8-THC. The degradation product had a *m/z* of 329 Da and a lambda max of 269.1 nm (Figure 3) which did not match that of (-)Δ^8-THC (*m/z* 315 Da). As a result, based upon the MS data and UV data, it was determined to be an unknown, rather than (-)Δ^8-THC.

Significant degradation products were observed after exposure to acidic (0.1 M HCl) conditions. Degradation products that eluted at 2.9, 4.3, and 4.5 minutes corresponded to reference standard retention times for CBG, (-)Δ^9-THC and (-)Δ^8-THC, respectively. UV and MS data was generated using the PDA and QDa detectors to gain further information regarding peak identity.

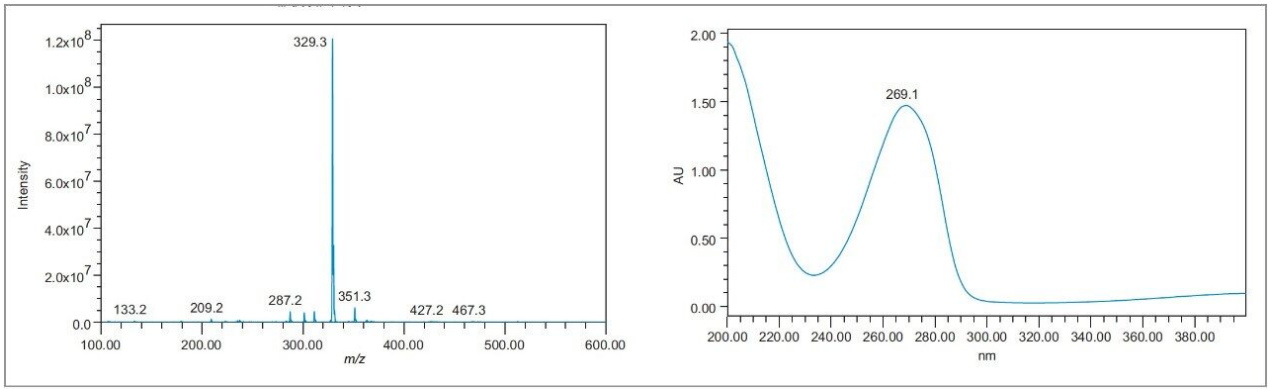

Figure 3. QDa-MS and UV-PDA data for the base degradation peak eluting at 4.5 minutes in the acid degradation chromatogram, Figure 2.

The acid degradation product eluting at approximately 2.9 minutes, was identified as an unknown with a *m/z* of 333 Da, rather than CBG (*m/z* 317 Da) which shares the same retention time. The acid degradation products at 4.3 and 4.5 minutes (Figure 4) had the same mass at 315 Da and comparable UV-PDA spectra with lambda max at 222.6 nm and 218.9 nm, respectively. Based upon the retention time, mass, and UV spectra; the degradation products at 4.3 and 4.5 minutes were tentatively identified as (-)Δ^9-THC and (-)Δ^8-THC (Figure 5).

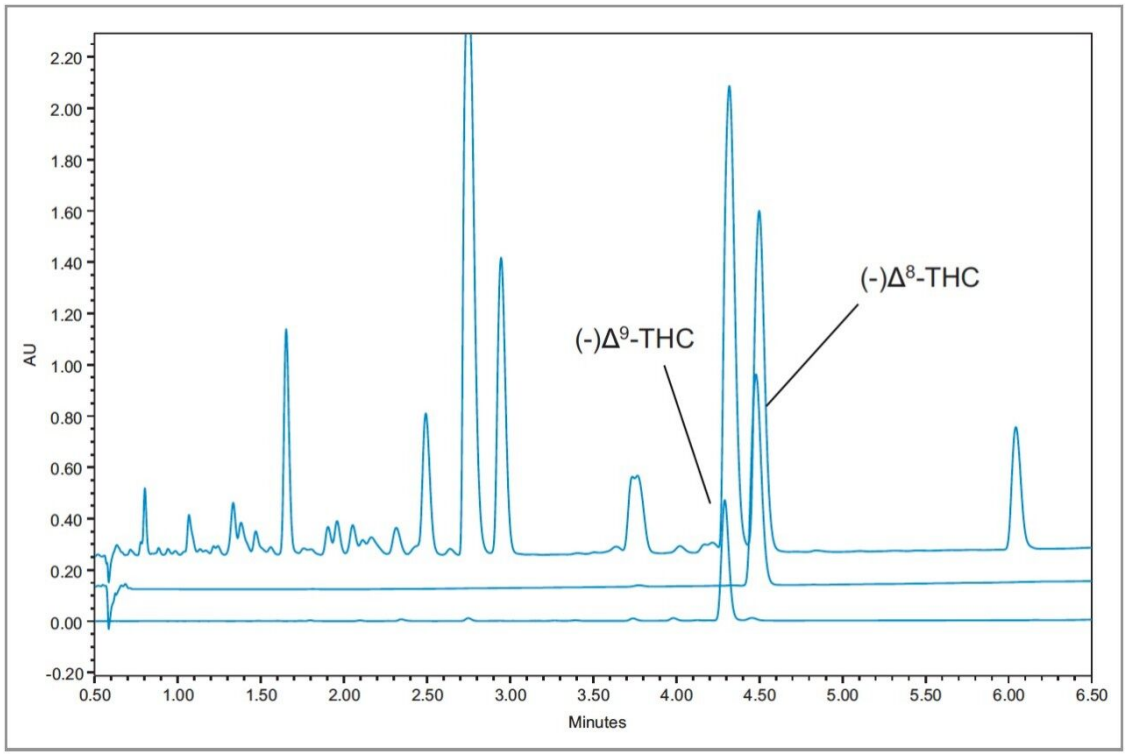

Figure 4. Overlay of the 0.1 M HCl stressed results with (-)Δ^9-THC and (-)Δ^8-THC reference standards by reversed-phase.

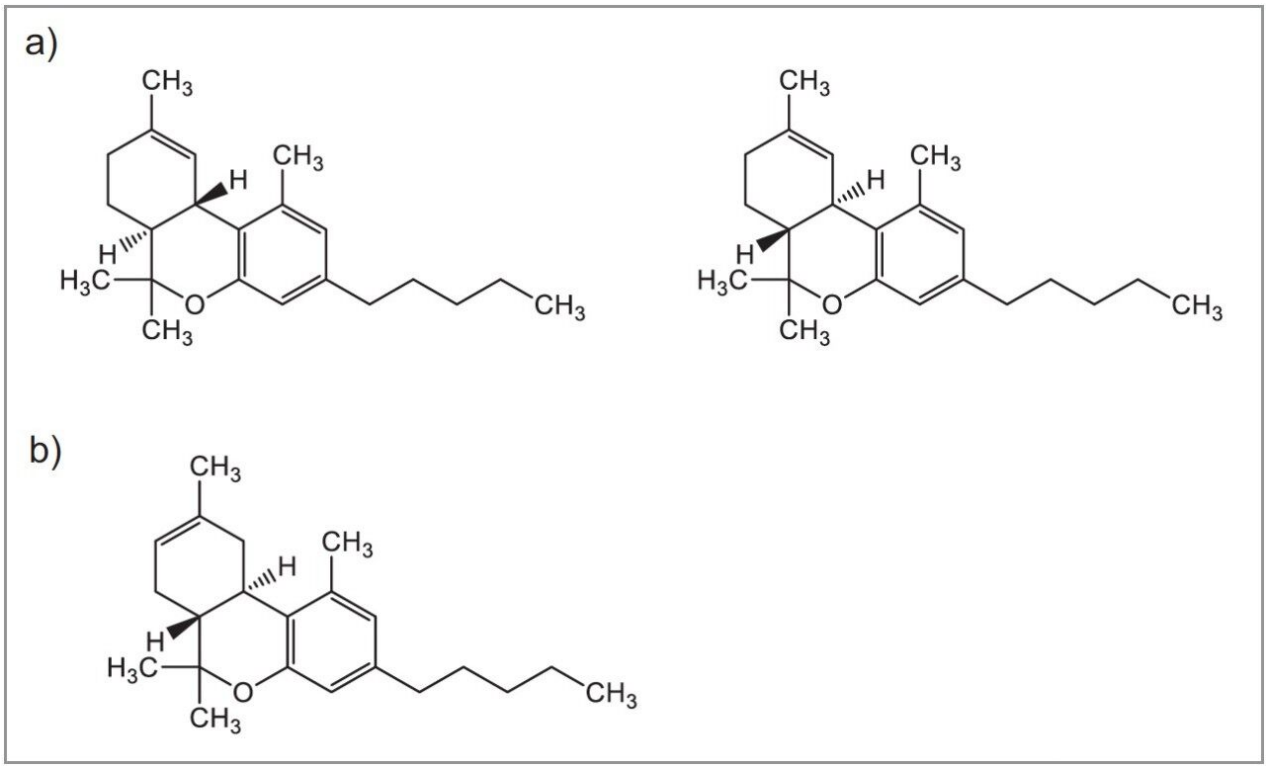

Figure 5. Structures of a) (+)Δ^9-THC and (-)Δ^9-THC, respectively, and b) (-)Δ^8-THC standards.

Information regarding the stereo-chemical purity of the degradation products identified as (-)Δ^9-THC and (-) Δ^8 THC was not determined by reversed-phase given that the technique does not typically distinguish between stereo-isomeric forms. The two degradation products were collected by the Waters Fraction Manager – Analytical (Figure 6) in separate aliquots. The samples were then re-injected onto the ACQUITY UPC^2 System for determination of the stereo-chemical composition by convergence chromatography.

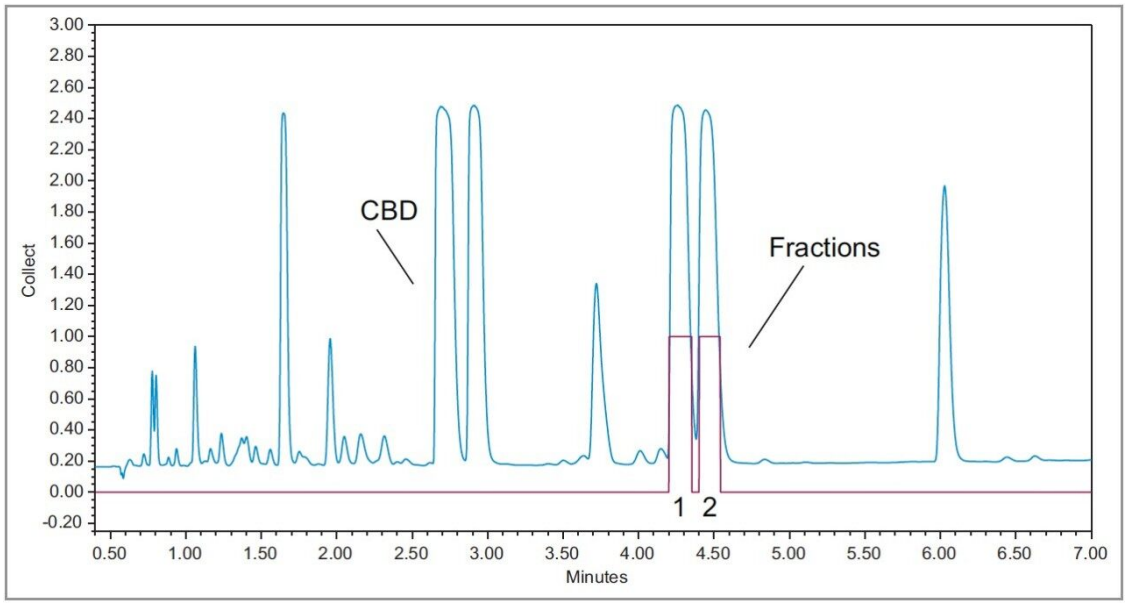

Figure 6. Reversed-phase fractions collected by WFM-A. Fraction 1 was collected at approximately 4.3 minutes, and fraction 2 at 4.5 minutes.

Figure 7 shows UPC^2 or UltraPerformance Convergence Chromatography of cannabinoid standards ( )Δ^8-THC, CBN, (±)Δ^9-THC and CBD. Of highest significance, the main naturally occurring, psychoactive ( )Δ^9-THC isomer, is fully separated from the less common isomer, (+) Δ^9-THC.[4] As a result, this method could potentially be used as a tool to gather information regarding Δ^9-THC potency since (-)Δ^9-THC is documented to be 6–100 times more potent than (+)Δ^9-THC.[5] Figure 7 shows UPC^2 or UltraPerformance Convergence Chromatography of cannabinoid standards ( )Δ^8-THC, CBN, (±)Δ^9-THC and CBD. Of highest significance, the main naturally occurring, psychoactive ( )Δ^9-THC isomer, is fully separated from the less common isomer, (+) Δ^9-THC.[4] As a result, this method could potentially be used as a tool to gather information regarding Δ^9-THC potency since (-)Δ^9-THC is documented to be 6–100 times more potent than (+)Δ^9-THC.[5]

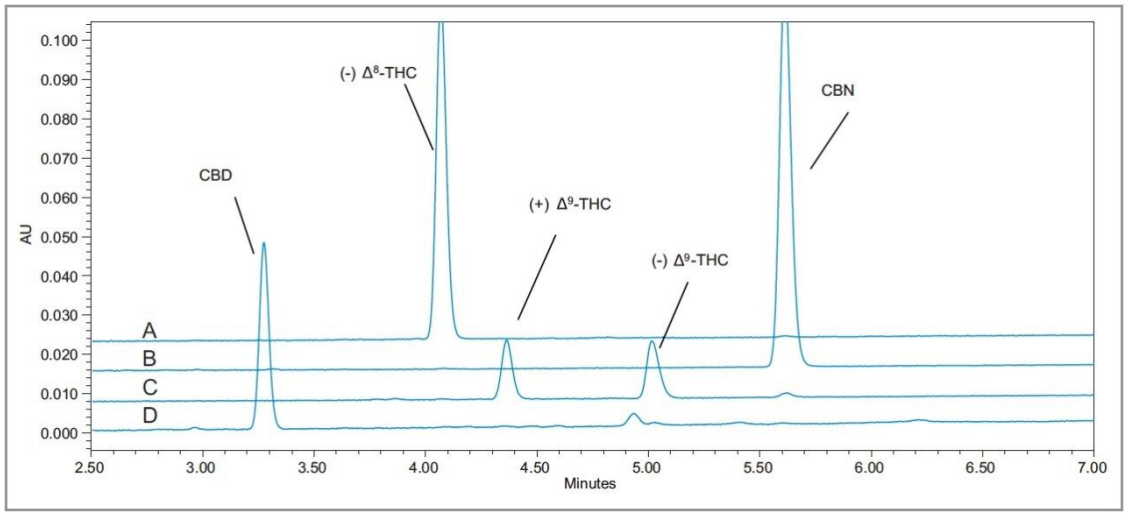

Figure 7. Overlay of A) (-)Δ^8-THC, B) CBN, C) (±)Δ^9-THC, and D) CBD standards by convergence chromatography.

In Figure 8, the UPC^2 chromatogram shows that the acid degradation peak collected as Fraction 1 matches the retention time of the (-) Δ^9-THC isomeric reference standard, rather than the retention time of the less potent form, (+)Δ^9-THC. Fraction 2 was confirmed to be (-)Δ^8-THC by comparison with the isomeric reference standard, shown in Figure 9. The UV spectra and *m/z* of both fractions were similar to the respective reference standards.

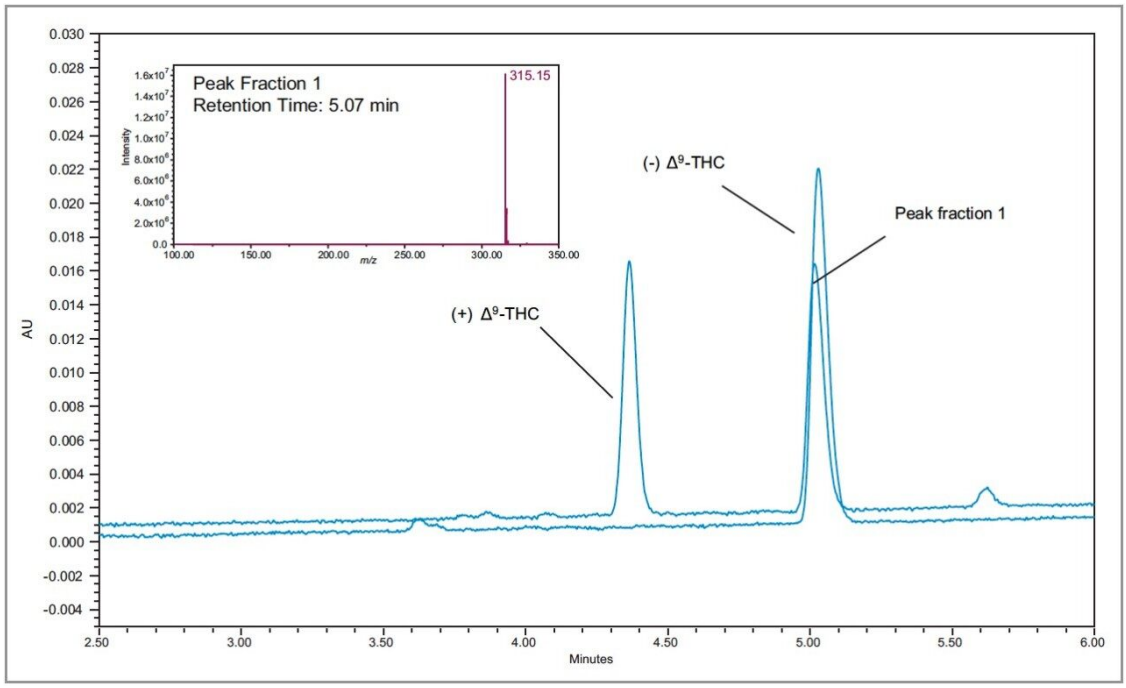

Figure 8. Overlay of the acid degradation peak fraction 1 (m/z 315.15) compared to the (±) Δ^9-THC standard by convergence chromatography.

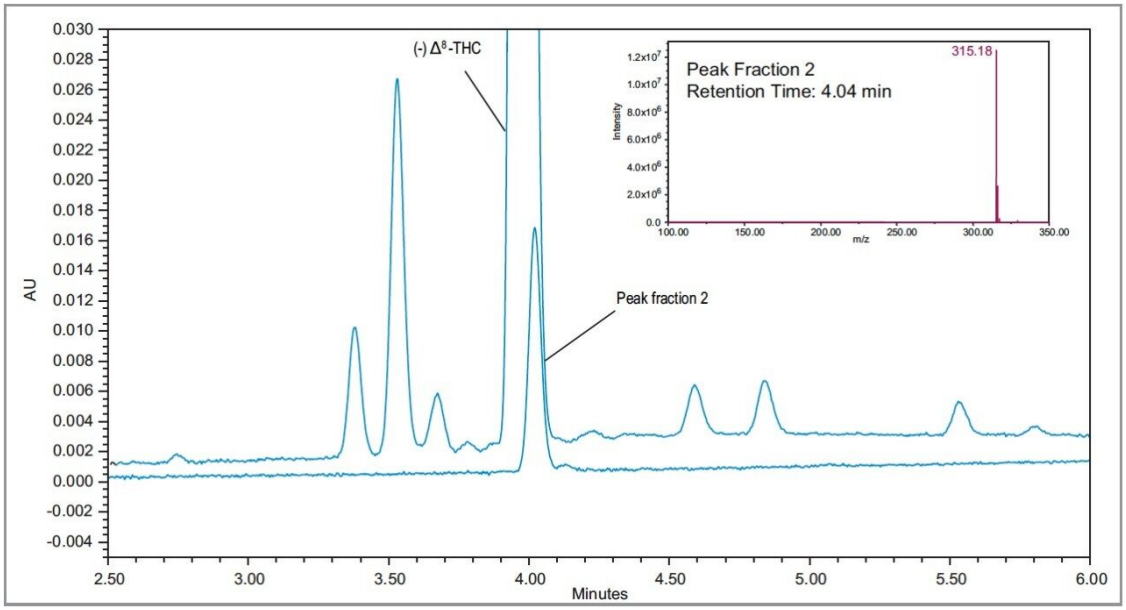

Figure 9. Overlay of peak fraction 2 (m/z 315.18) compared to the (-)Δ^8-THC standard by convergence chromatography.

The separation demonstrated that convergence chromatography can be used as a tool, combined with UV and MS, to confirm the identity of degradation products predicted by reversedphase. Most importantly, it can be utilized to verify stereo-chemical composition of (-)Δ^9-THC and (-)Δ^8-THC by comparison with isomeric reference standards (pending patent application).

The data provided by the degradation of crystalline CBD demonstrates two separation tools that can be used orthogonally for the chemical and structural verification of important cannabinoid compounds. Identity can be determined by reversed-phase coupled with PDA and QDa, and the stereo-chemical composition can be confirmed by convergence chromatography. The data also reveals the utility of MS and UV detectors as important tools to prevent the misidentification of degradation products that have the same retention time as reference standards.

As more pharmacological data becomes available and regulatory requirements are established, chemical and structural characterization of the active ingredients within CBD containing products will become essential for the documentation of product purity and stability.

Conclusion

- The ACQUITY UPLC H-Class System and the ACQUITY UPC^2 System are highly reliable instruments that can be used in tandem to orthogonally separate naturally occurring cannabinoids, including cannabidiol (CBD), (±})Δ^9-THC, and (-)Δ^8-THC and their stereoisomers by reversed-phase and convergence chromatography, respectively.
- The Waters Fraction Manager – Analytical (WFM-A) can be easily used at a UPLC scale to isolate and collect degradation product peaks for further analysis.
- The UV-PDA and QDa-MS detectors are useful tools that can be coupled with reversed-phase and convergence chromatography to confirm the identity of degradation products.
- When analyzing CBD and THC formulations, (-)Δ^9-THC and (-)Δ^8-THC elute at close retention times by reversed-phase chromatography, therefore special diligence should be implemented to ensure adequate separation of these compounds to avoid misidentification.

References

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