THE SEPARATION OF Δ⁸-THC, Δ⁹-THC, AND THEIR STEREOISOMERS BY UPC² USING TREFOIL CHIRAL COLUMNS

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INTRODUCTION

Cannabinoids, such as tetrahydrocannabinol (THC), have gained considerable attention over the past decade for use in treating various conditions. Depending on how it is derived and handled, THC exists in many isomeric forms.[1] Four (trans) isomers are predominant including (+)-Δ⁸-THC, (-)-Δ⁸-THC, (+)-Δ⁹-THC, and (-)-Δ⁹-THC (structures in Figure 1). The major naturally-occurring isomer is (-)-Δ⁹-THC; considered the most therapeutically active cannabinoid present in cannabis.[1] Under acidic conditions, cannabidiol (CBD) can convert to Δ⁹-THC and other THC isomers.[2,3]

The FDA requires that stereoisomeric composition be quantified for active chiral pharmaceutical compounds. Consumable products also need to be monitored for mixtures of positional and stereoisomers that can form resulting in changes in potency, pharmacological activity, or toxicity.[3] Chiral analysis of THC is also applicable in forensic drug profiling.[4]

To that end, the separation of Δ⁸-THC, Δ⁹-THC and their stereoisomers, was investigated using Waters ACQUITY UPC² technology and Trefoil chiral columns. After optimization, calibration and repeatability was performed for each isomer. The method was then used to determine the THC composition of a commercially available CBD product, before and after undergoing acidic conversion to THC.

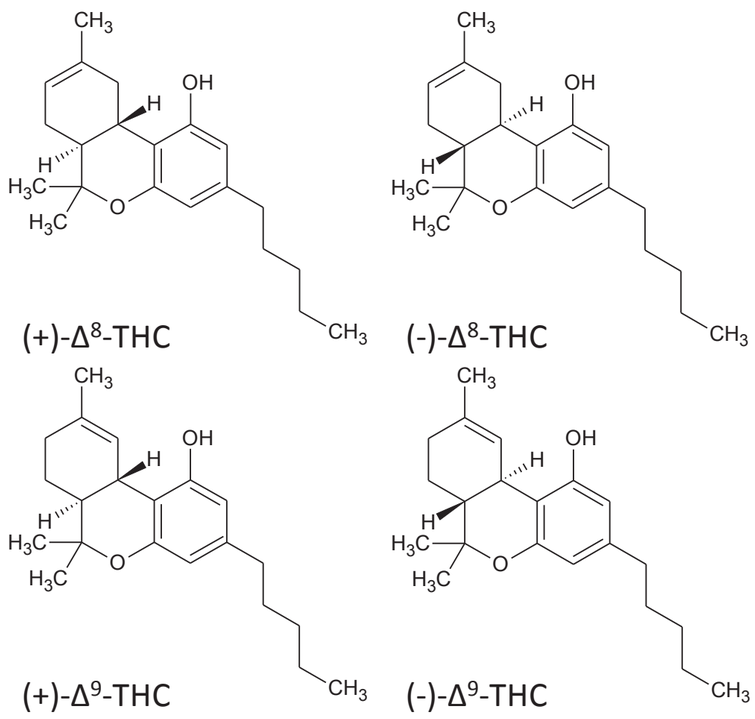

Figure 1: *Positional and stereo isomeric structures of THC*

METHODS

Standards

(Obtained from Cerilliant, exempt standards)

- (±)Δ⁸-THC at 0.1 mg/ml in heptanes
- (±)Δ⁹-THC at 0.1 mg/ml in heptanes
- (-)Δ⁸-THC at 1 mg/ml in methanol
- (-)Δ⁹-THC at 1 mg/ml in methanol

A 50:50 mix of the (±)Δ⁸-THC and (±)Δ⁹-THC standards was used for method development.

The (-)Δ⁸-THC and (-)Δ⁹-THC standards were diluted 1:10 in 200 proof ethanol and were used to determine peak order.

Serial dilutions of the (±)Δ⁸-THC and (±)Δ⁹-THC standards in 200 proof ethanol were used for calibration.

CBD Acid Conversion

A commercial CBD oil extract was used as an example product. Three aliquots of the CBD oil were treated using the following conditions:

- Sample 1: 15.65 mg of CBD Oil in 3mL 200-proof ethanol, heated overnight at 55°C
- Sample 2: 15.26 mg of CBD Oil in 3mL 0.1M HCl in 200-proof ethanol, heated overnight at 55°C
- Sample 3: 15.40 mg of CBD Oil in 3mL 200-proof ethanol, room temperature

All three samples were filtered and diluted 1:10 in ethanol before injection on the UPC²

UPC² screening conditions

System: ACQUITY UPC² system with an ACQUITY PDA detector
Columns: (3 mm X 150 mm, 2.5 µm)
ACQUITY UPC² Trefoil AMY1 Column (AMY1)
ACQUITY UPC² Trefoil CEL1 Column (CEL1)
ACQUITY UPC² Trefoil CEL2 Column (CEL2)
Mobile Phase A: Carbon Dioxide
Mobile Phase B: 200-proof ethanol
Gradient: 2 to 20% B over 5 minutes
Column temperature: 50°C
Injection volume: 1 µL
Flow rate: 2 mL/min
ABPR: 2000 psi
PDA absorbance: 228nm
Compensation reference: 500-600nm
Optimized conditions: noted on figures

RESULTS & DISCUSSION

Initially, the three Trefoil columns were screened, which are amylose (AMY1) and cellulose (CEL1 & CEL2) based chiral stationary phases that have a wide range of applicability. The results of the screen (Figure 2) showed that all three columns provided good separation of the THC isomers. Different elution order and selectivity were observed, which can be beneficial when separating these compounds from matrix interferences. Even though all three separations could be optimized, the separation obtained on the AMY1 column was selected due to higher retention and resolution than on the two CEL columns.

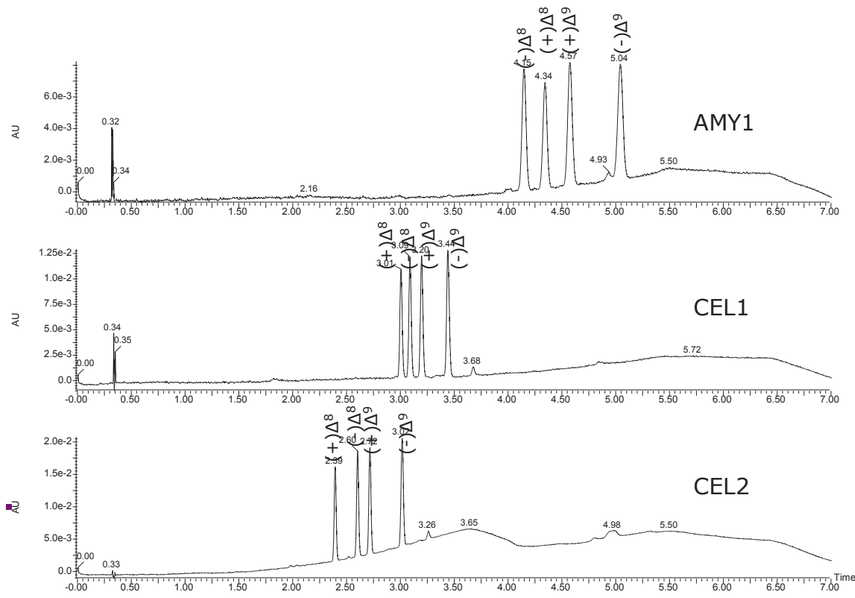

Figure 2*:Screening of the four THC isomers on the three Trefoil chiral columns using a 2-20% gradient over 5 minutes*

In order to optimize the separation, the co-solvent percentage at elution was determined from the screening separation. With a gradient delay of 0.34 min, gradient slope of 3.6%/min, and 2% starting percentage, the co-solvent percentage at elution of the first peak at 4.15 minutes was calculated using the following equation:

%Co-solvent at Elution =
(retention time – gradient delay) x gradient slope + starting %
(4.15 – 0.34 min) x 3.6%/min + 2%
15.7%

Figure 3 shows separation of the THC isomers at 15% and 10% ethanol conditions. At 15%, the separation of the positional and stereo isomers of THC is achievable in less than 3 minutes. Comparatively, the liquid chromatography separation of these isomers is 23 minutes and does not achieve baseline resolution.

In this case, the separation would be used to analyze THC content in a sample containing cannabidiol (CBD). As a result, the isocratic methods were investigated to separate the THC isomers from CBD. The 10% method provided acceptable resolution and was therefore used for calibration and repeatability. Figure 4 shows calibration curves for the four THC isomers on the AMY1 column using the 10% isocratic method.

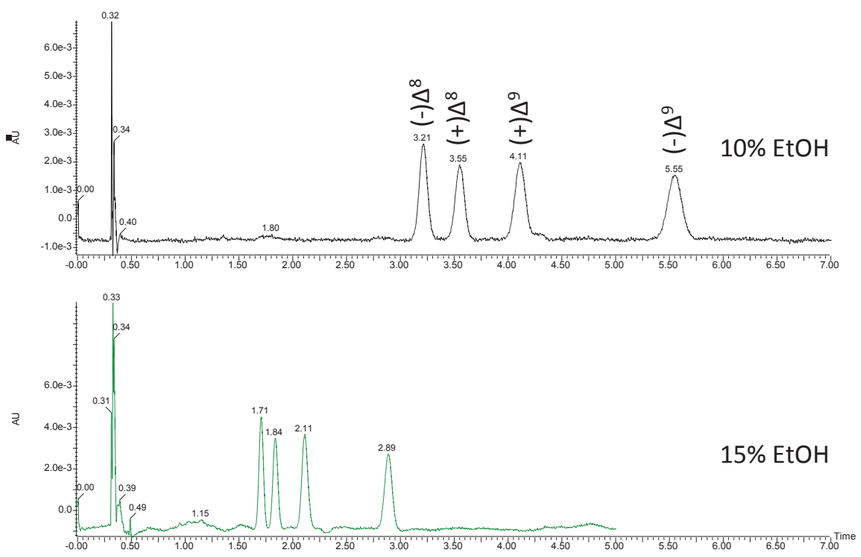

Figure 3*: Isocratic separations of the four THC isomers on the AMY1 column at 10% co-solvent (top) and 15% co-solvent (bottom) conditions*

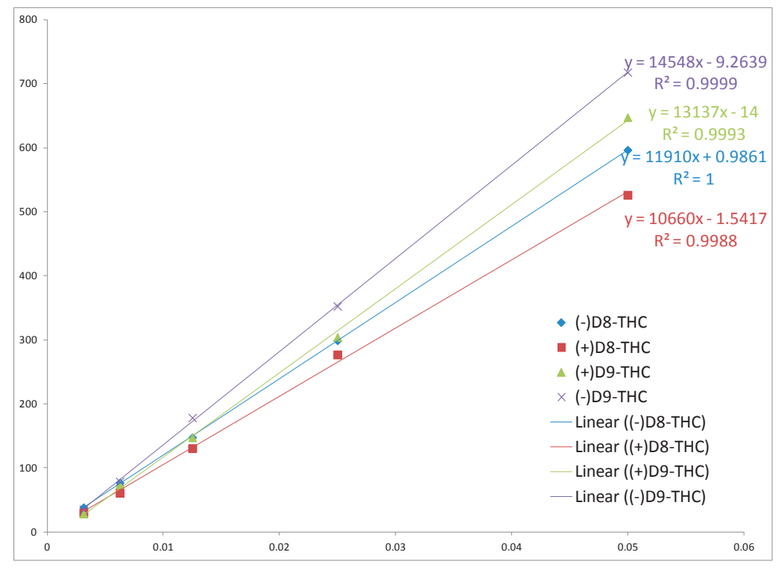

Figure 4*: Calibration curves for the four THC isomers on the AMY1 column at 10% isocratic conditions. The calibration curve concentrations were 0.003125, 0.00625, 0.0125, 0.025 and 0.05 mg/mL, and the injection volume was 1 µL*

Repeatability was determined using the 0.025 mg/mL sample. The calibration curves were linear (R^2 > 0.998), and the peak areas showed good reproducibility, with area count RSD values less than 2% (n=7).

THC content is of particular interest for purposes of product quality control. Under acidic conditions, CBD can convert to Δ⁹-THC and other THC isomers.[3] Once the method was calibrated, and shown to be reproducible, the separation was applied to the analysis of the three CBD oil extract samples (Figure 5).

Results of the analysis showed a very small amount of THC in the initial product and no change in the heated product. Under acidic conditions, a significant amount of (-)Δ⁹-THC was detected, calculated to be 1.16mg or approximately 7.6% of the initial 15.26 mg sample, along with a very small detectable amount of (-)Δ⁸-THC.

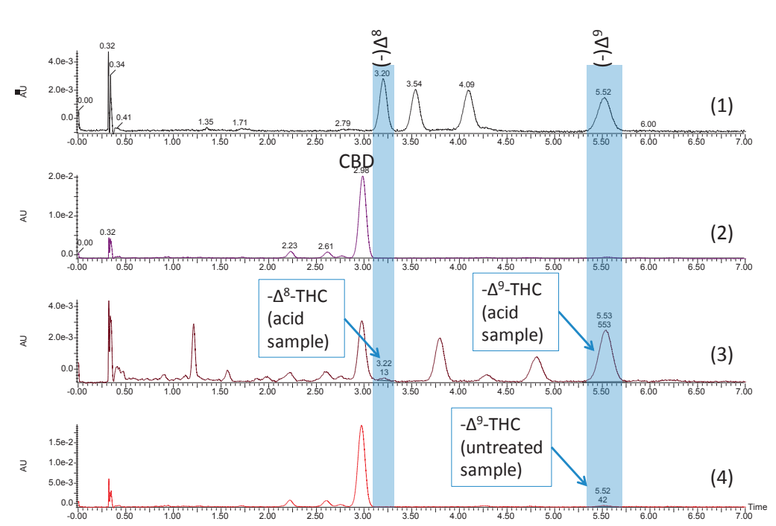

Figure 5*: UPC² chromatograms showing (1) separation of the THC isomer standards, (2) analysis of CBD oil Sample 1 (heat only), (3) analysis of CBD oil Sample 2 (heat + acid), and (4) analysis of CBD oil Sample 3 (control). THC isomers are as indicated. The separations were achieved at 2 mL/min and 10% ethanol on the AMY1 column using 1µL injections.*

CONCLUSION

- **The separation of (±)Δ⁸-THC and (±)Δ⁹-THC and their stereoisomers was accomplished in less than 3 minutes on the Waters ACQUITY UPC² system, using the Trefoil AMY1 column at 15% ethanol mobile phase conditions.**
- **The THC isomers were well separated on all three Waters Trefoil chiral stationary phases. The columns also exhibited different selectivity, which is beneficial when resolving these THC isomers from matrix interferences.**
- **The ACQUITY UPC² separation of the four THC isomers showed excellent repeatability and the calibration curves showed good linearity. As a result, the methodology can be used for quantitative analysis of THC in cannabis products.**

References

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4) L. Ambach, F. Penitschka, A. Broillet, S. Konig, W. Weinmann, W. Bernhard, Simultaneous quantification of delta-9-THC, THC-acid A, CBN and CBD in seized drugs using HPLC-DAD, *Forensic Science International*, 243 (2014) 107-111

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