

Simple Method Development for the Separation of Chiral Synthetic Cannabinoids using Ultra High Performance Supercritical Fluid Chromatography

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Ultra high performance supercritical fluid chromatography (UHPSFC) technology presents an alternative and orthogonal solution to GC and HPLC for the separation of synthetic cannabinoids. In particular, it offers excellent selectivity for structural analogs and stereoisomers. Here, a simple method development strategy is demonstrated for the chiral analysis of selected synthetic cannabinoids including: HU-210, HU-211, (\pm)-CP 47,497, (\pm)-epi CP 47,497, (\pm)-CP 55,940 and (\pm)-5-epi CP 55,940. Fast stereoisomeric separations, including related enantiomers and diastereomers, are achieved on the Waters ACQUITY UPC² System using the Waters Trefoil chiral columns. The efficiency of UHPSFC is demonstrated for rapid enantiomeric separation of chiral synthetic cannabinoids.

Synthetic cannabinoids (so named because they are structurally related to naturally occurring compounds found in the marijuana plant) refer to a large and growing number of man-made compounds that are often sprayed on dried, shredded plant material so they can be smoked. Mixtures of synthetic cannabinoids are sold under various brand names such as 'Spice' and 'Smoke' and are becoming more widely used as illicit, abused drugs [1]. Many of these analogues are being produced in order to circumvent laws banning their use as recreational drugs [2]. However, some synthetic cannabinoids have been used for medicinal purposes including rimonabant, nabilone, and dronabinol [3]. In addition, efforts have been ongoing to find selective analogues of tetrahydrocannabinol (THC) that separate beneficial effects, such as pain treatment, from psychotropic effects [4]. Since pharmacological studies involving structure–activity relationships, receptor binding, and detailed mechanisms of activity for these drugs is required, it is necessary to fully characterise these molecules.

The series of synthetic cannabinoids possess a wide structural variability and potent cannabimimetic pharmacological activity, and bind to the same cannabinoid receptors as THC. The modifications made to these compounds result in structural analogues, structural homologues, positional isomers and stereoisomers [1,4]. Just as with other active pharmaceutical ingredients, stereochemistry of synthetic cannabinoids affects pharmacological activity. For example, HU-210 is a synthetic cannabinoid

that has neuroprotective effects and psychoactivity 100 to 800x more potent than THC, while HU-211 (the enantiomer of HU-210) also has slightly different neuroprotective effects without the psychotropic effects.

Due to the growing popularity and hazardous potential of uncharacterised synthetic cannabinoids, the ingredient analysis of these products is required in forensic toxicology, regulatory environments, and pharmaceutical development [1]. Specifically, chiral separation of the cannabinoid stereoisomers is important for pharmaceutical development, whether for identification and characterization of impurities, or for purification purposes. Chiral analysis is also important for screening in forensic laboratories, where achiral analysis

alone will not result in comprehensive qualitative analysis. A variety of analytical techniques have been used for the comprehensive screening of drugs of abuse, including immunoassay, GC-MS and LC-MS. The immunoassay solution suffers from low selectivity, while GC is limited to volatile compounds and requires derivatisation [1]. LC-MS offers high sensitivity and specificity for cannabinoids, however, another complementary methodology is needed to obtain high-precision, qualitative analysis of these products. As laboratories struggle to keep pace with the proliferation of these newly emerging drugs, simple method development strategies that can be quickly applied to obtain the necessary qualitative information are urgently needed [2].

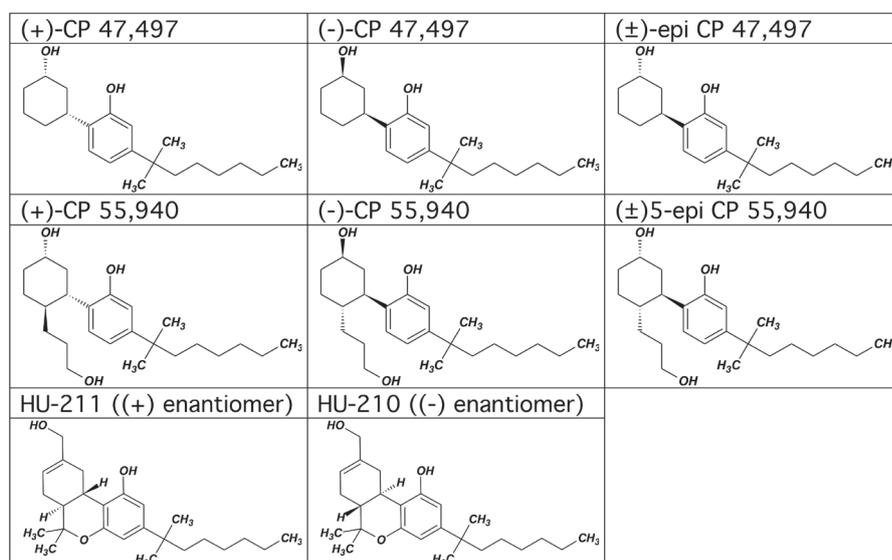


Figure 1: Stereochemical structures of selected synthetic cannabinoids

Supercritical fluid chromatography (SFC), is the underlying principle behind UHPSFC technology, and presents an alternative and orthogonal solution for the separation of synthetic cannabinoids. UHPSFC takes advantage of smaller particle columns and allows for very efficient and rapid separations, up to four times faster than UHPLC. This is attributable to a mobile phase that is more diffusive and has lower viscosity, favouring the ability to run at higher linear mobile phase velocities than with UHPLC. In particular, since SFC can be operated under normal phase conditions, it generally offers greater selectivity for structural analogues and stereoisomers [2]. Here, simple method development will be demonstrated for the chiral analysis of selected synthetic cannabinoids, including separating HU-210 from its enantiomer HU-211, and the stereoisomers of cis and trans (epi) CP 47,497, and CP 55,940. Structures for these compounds are shown in Figure 1, including stereochemical configurations.

Experimental

Federally exempt synthetic cannabinoid standards were obtained from Cayman Chemical (Ann Arbor, MI) including: HU-210, HU-211, (±)-CP 47,497, (±)-epi CP 47,497, (±)-CP 55,940 and (±)-5-epi CP 55,940. The standards were received in solution at 5 mg in 500 µL methanol and were subsequently diluted to 1 mg/mL with ethanol. (-)-CP 47,497 and (-)-CP 55,940 standards were used to determine order of elution for those separations. 200 proof HPLC grade ethanol (Sigma-Aldrich) was used as the co-solvent and all injections were 1 µL.

All separations were performed on a Waters ACQUITY UPC² System equipped with a PDA (UV) detector and a QDa (MS) detector. Achiral analysis was performed using a 3x150 mm ACQUITY UPC² Torus 1-AA column with 1.7 µm particles. This column was chosen based on a previously described achiral column screening of synthetic cannabinoids [2]. ACQUITY UPC² Trefoil chiral columns, including the amylose (AMY1) and cellulose (CEL1 & CEL2) based chiral stationary phases (Waters Corporation) were used for chiral screening and method development. All three Trefoil columns were 3x150 mm and were packed with 2.5 µm particles.

For screening, a generic gradient was used from 2% to 20% ethanol over 5 minutes, followed by a 1 minute hold at 20%, before returning to initial 2% conditions. The total flow was 2 mL/min, the back pressure was 2000 psi, and the column temperature was

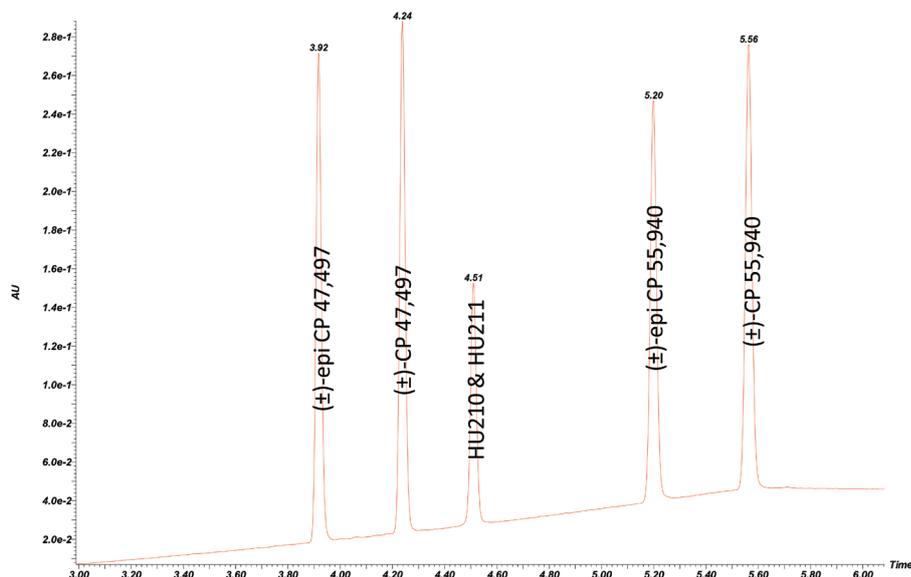


Figure 2: Achiral separation of synthetic cannabinoids on the ACQUITY UPC² Torus 1-AA column. Conditions as follows: 1.5 mL/min total flow, 2-20% ethanol gradient over 5 minutes, temperature at 40°C, and pressure at 2000 psi

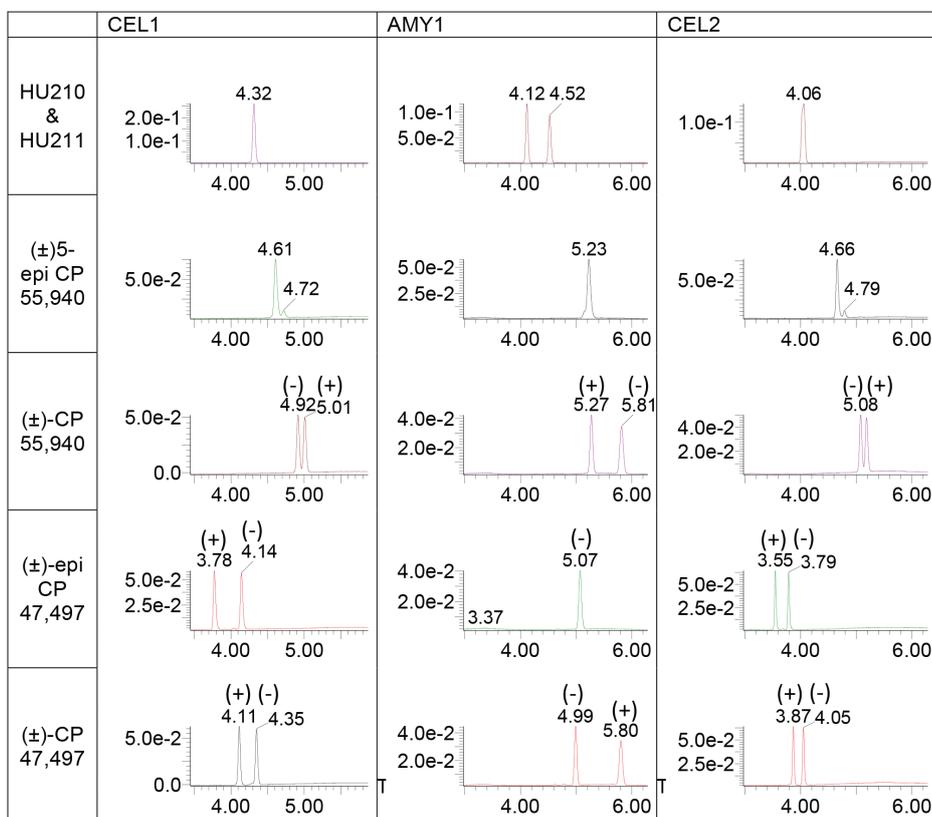


Figure 3: Chromatograms of the five chiral synthetic cannabinoids screened on the three Trefoil chiral columns. Conditions as follows: 2 mL/min total flow, 2-20% ethanol gradient over 5 minutes, temperature at 40°C, and pressure at 2000 psi

40°C. The PDA data was obtained using an absorbance compensated channel at 228 nm with a compensation reference from 500 to 600 nm. The same conditions were used for the achiral analysis, with the exception of the flow rate, which was 1.5 mL/min. After a review of initial column screening, optimised methods were developed. Those conditions are described in the respective figures.

Results and Discussion

Achiral separation of the five synthetic cannabinoids is displayed in Figure 2. The peaks are well separated under generic gradient conditions (2-20% ethanol) on the ACQUITY UPC² Torus 1-AA column, including the separation of two sets of diastereomers; cis and trans (epi) CP 47,497, and cis and trans (5-epi) CP 55,940.

This demonstrates the chromatographic efficiency of SFC using small particle size columns for separating closely related structural isomers, even while using achiral stationary phases under simple generic method conditions. However, each of the peaks in this chromatogram actually represents two different pharmaceutically active enantiomers. As such, the achiral separation alone is not adequate for comprehensive qualitative analysis of these synthetic cannabinoids.

In normal phase chromatography, and SFC in particular, the first step in method development involves column screening. In this case, the columns used in the screening were amylose (AMY1) and cellulose (CEL1 & CEL2) based chiral stationary phases, which are commonly used because of their wide range of applicability. In particular, the three Trefoil columns provide orthogonal selectivity, which is optimal for successful chiral method development. Much of the work involving cannabis quality control and post-extraction processing uses ethanol as the preferred solvent in order to avoid toxicity from solvent contamination in any final product or consumable. Ethanol has also been shown to be an effective co-solvent for the separation of natural cannabinoids (pending patent application). Consequently, ethanol was chosen as the sample diluent and co-solvent for method development. Methanol and isopropanol are also appropriate co-solvents for the analytical application; however for simplicity, ethanol was used throughout the study. As is typical in column screening, a generic

gradient from 2% to 20% ethanol was utilised. Figure 3 shows chromatograms of the column screen for the five synthetic cannabinoids. Many of the chiral separation screening results showed excellent peak shape and resolution.

A few observations that were made during the screen warrant some discussion. For the cyclohexylphenol (CP) compounds, specifically (\pm)-CP 47,497, (\pm)-epi CP 47,497, and (\pm)-CP 55,940, orthogonality was observed between the cellulose (CEL1 and CEL2) and amylose (AMY1) based stationary phases, where the elution order was reversed, but the enantiomers were still very well resolved. This has advantages for analysis of these compounds, especially in cases where there are matrix interferences. On the AMY1 column, the (\pm)-epi CP 47,497 cannabinoid enantiomers appear to be co-eluting. However, the peak at 5.07 is, in fact, only one of the enantiomers, the second peak failed to elute under the conditions of the gradient. Further investigation confirmed that the second peak eluted quite a bit later than the first, even under high co-solvent percentages. Another unexpected observation was that the (\pm)-epi CP 55,940 sample appears to have a significant enantiomeric excess. Based on the UV and MS spectra, the small peak eluting at 4.72 on the CEL1 (4.79 on CEL2) appears to be the enantiomer of the larger peak in that particular sample.

Even though the generic screening conditions resulted in acceptable chromatography for many of these

compounds, in a fast paced analytical environment, it is always beneficial to decrease run times and simplify methodology. Also, since (\pm)-CP 47,497 and (\pm)-epi CP 47,497, and (\pm)-CP 55,940 and (\pm)-epi CP 55,940 are actually diastereomer pairs, it would be more advantageous if all four stereoisomers (enantiomers and diastereomers) could be separated in a single run. To that end, the separations were optimised for speed and resolution of the four stereoisomers. Optimisation of the mobile phase conditions usually involves either focusing the gradient or running isocratically. Gradients usually result in better peak shape, however focusing gradients in SFC is more complicated than in HPLC because the effect of increasing co-solvent percentage (or %B in HPLC) on retention times is not linear, and the resulting chromatography is harder to predict. Isocratic methods are ideal because they are easy to develop based on the screening results and no equilibration is required between runs, improving productivity. Using retention times and gradient slope, and compensating for system and column volume delay, the co-solvent percentages at elution were determined for each compound. In SFC, usually the best starting point for optimisation is 5% below the calculated percentage.

Using the HU-210 and HU-211 separation on the AMY1 column (Figure 4A) as an example, with a gradient delay of 0.46 min, gradient slope of 3.6%/min, and 2% starting percentage, the co-solvent percentage at elution of the first peak at 4.12 minutes was calculated using the following equation:

$$\begin{aligned} \% \text{Co-solvent at Elution} = & \\ (\text{retention time} - \text{gradient delay}) & \\ \times \text{gradient slope} + \text{starting \%} & \end{aligned}$$

$$\begin{aligned} \% \text{Co-solvent at Elution} = & \\ (4.12 \text{ min} - 0.46 \text{ min}) \times 3.6\%/\text{min} + 2\% & \end{aligned}$$

$$\% \text{Co-solvent at Elution} = 15\%$$

Therefore, after subtracting the 5%, 10% isocratic co-solvent conditions were used as a starting point for optimisation (Figure 4B). The resulting chromatography showed good separation; but by increasing the co-solvent fraction of the mobile phase back to 15%, effective separation was achieved in approximately 2 minutes (Figure 4C).

This same method optimisation strategy was used to develop fast methods to separate all four stereoisomers of the two cyclohexylphenol (CP) synthetic cannabinoids. For both sets of stereoisomers, the retention time of

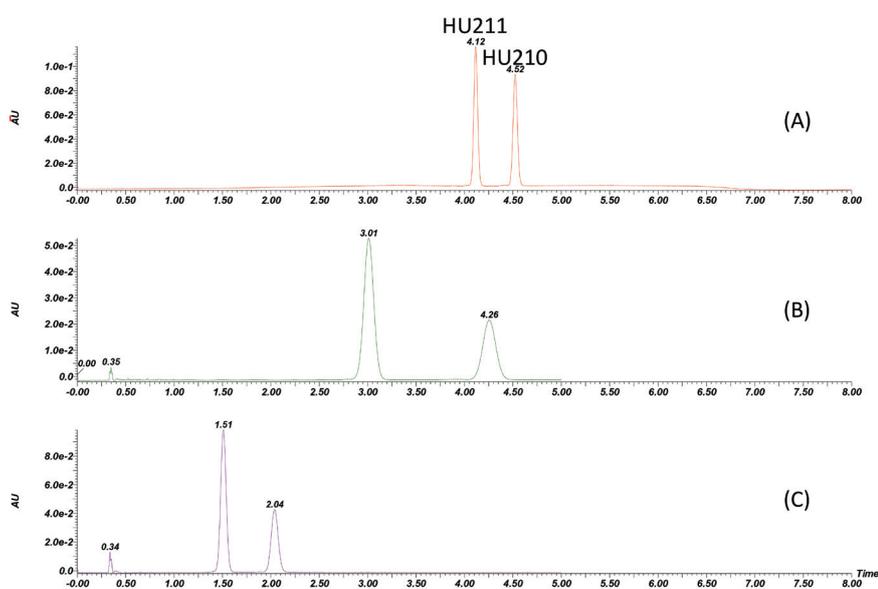


Figure 4: Chromatograms showing optimization of the HU-210 and HU-211 separation on the Trefoil AMY1 column. Conditions are as follows: (A) 2%-20% ethanol gradient over 5 minutes (B) 10% ethanol isocratic method and (C) 15% ethanol isocratic method.

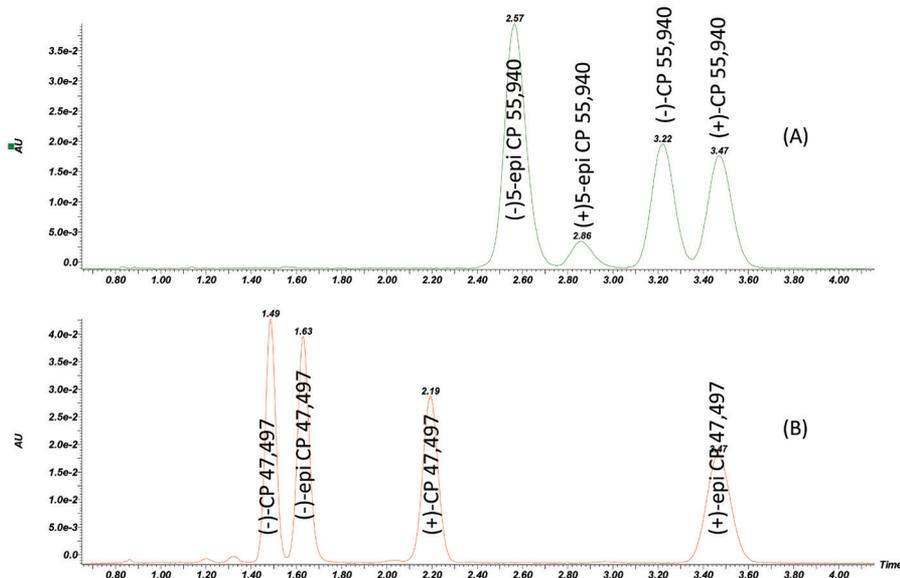


Figure 5: Separations of the stereoisomers of (A) (±)-CP 55,940 and (±)5-epi CP 55,940 on the Trefoil CEL1 column at 13% ethanol isocratic conditions, and (B) (±)-CP 47,497 and (±)-epi CP 47,497 on the Trefoil AMY1 column at 20% ethanol isocratic conditions.

the latest eluting peak in the screen was used to calculate the isocratic method conditions. The CEL1 column was selected for the separation of (±)-CP 55,940 and (±)5-epi CP 55,940 because it showed the best resolution of the (±)5-epi CP 55,940 enantiomers. Based on the retention time of the (+)-CP 55,940 enantiomer at 5.01 min, the co-solvent percentage at elution was 18%, after subtracting 5%, the optimised separation was achieved at 13% isocratic conditions. The AMY1 column was used to separate the (±)-CP 47,497 and (±)-epi CP 47,497 stereoisomers because it showed the most resolution for both sets of enantiomers. As stated earlier, the (+)-epi CP 47,497 didn't elute during the screen, but it was well separated from the (-)-epi CP 47,497. Because the last two peaks eluted during or well after the 20% hold, 20% isocratic conditions were used for the optimised method. The optimised chromatography can be seen in Figure 5, where all four stereoisomers (enantiomers and diastereomers) of the two cyclohexylphenol (CP) compounds were

separated in approximately 3.5 minutes using Trefoil stationary phases at the calculated isocratic mobile phase conditions.

Conclusion

Synthetic cannabinoids possess a wide range of structural variability, which include structural analogues, structural homologues, positional isomers and stereoisomers [1,4]. Just as with other active pharmaceutical ingredients, the stereochemistry of synthetic cannabinoids affects pharmacological activity. As a result, comprehensive ingredient analysis of these products, including their stereoisomers, is required in regulated environments, forensic laboratories and for pharmaceutical development [1]. In pharmaceutical development, chiral separation is important for the identification and characterisation of impurities, or for purification purposes. Chiral analysis is also important for screening in forensic laboratories, where achiral analysis alone will not result in comprehensive qualitative and quantitative analysis.

UHPSFC technology presents an alternative and orthogonal solution for the separation of synthetic cannabinoids. In particular, when paired with Trefoil column chemistries, UHPSFC offers excellent selectivity for structural analogues and stereoisomers [2]. Here, simple method development was demonstrated for the chiral analysis of selected synthetic cannabinoids including: HU-210, HU-211, (±)-CP 47,497, (±)-epi CP 47,497, (±)-CP 55,940 and (±)5-epi CP 55,940. Fast stereoisomeric separations, for both enantiomers and diastereomers, were achieved using the Waters ACQUITY UPC² System with Waters Trefoil chiral columns. Isocratic conditions derived from screening gradients effortlessly resolved the enantiomers of several chiral synthetic cannabinoids and increased analysis speed and efficiency.

References

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