A Validated Chiral HPLC Method for Resolution of $\Delta^8$ and $\Delta^9$-tetrahydrocannabinol Enantiomers

Introduction

Background
- $\Delta^8$THC, unlike $\Delta^9$THC, is an API known as Dronabinol
- (+)-THC enantiomer has no or minimal effects
- $\Delta^8$THC may be synthesized from $\Delta^9$THC
- FDA guidance, stereoselective comparison must be quantified for chiral API materials used in pharmacological, toxicological, and clinical studies

Literature
- Actra analysis of $\Delta^8$THC, $\Delta^9$THC is well-documented
- USP Monograph for Dronabinol is also available
- Some chiral systems have been illustrated for analysis of these related cannabinoids, but none were validated.

Challenges
- Validated chiral method needed with the ability to resolve 4 isomers
- New material is difficult to handle
  - Glassy solid at room temperature
  - ($\Delta^9$THC) is light and sensitive
- High purity $\Delta^8$THC and $\Delta^9$THC reference material not commercially available
  - Needed for method development, validation, and ongoing use as system suitability standards
- ($\Delta^9$THC), synthesis was chiral and therefore a new synthetic route had to be created for racemic material
- Synthesized, purified, and certified at Cerilliant

No method demonstrates simultaneous separation of all four $\Delta^8$THC & $\Delta^9$THC enantiomers

Analytical Method

- Normal Phase Chiral LC
  - Optimized RPLC: (+)-$\Delta^8$THC, (+)-$\Delta^9$THC, (-)-$\Delta^8$THC, (-)-$\Delta^9$THC
  - Used to determine % enantiomeric excess

- Conditions
  - Chromatographic column: 4.6 x 250 mm, 5 μm
  - Mobile phase: 11:0:95, acetonitrile:methanol:hexane
  - 0.7 ml/min, 40°C, 228 nm, 5 μl injection

Baseline separation of all four $\Delta^8$THC & $\Delta^9$THC enantiomers within 25 minutes

System Suitability

Ensures that sensitivity, resolution, and reproducibility of the chromatographic system are adequate for the analysis to be performed as intended.

USP calculations for Peak Resolution and Tailing were used to determine System Suitability.

Robustness

Measure of the method’s capacity to remain unaffected by small but deliberate variations in parameters.
- Provides an indication of reliability during normal usage
- Performance reference injections at unmacho conditions with each analysis

Accuracy

The accuracy of an analytical method is the closeness of the results obtained by the method to the true value or an accepted reference value.
- The intended use of this method is to determine $\Delta^9$THC by comparing relative peak areas of the (+) and (-) enantiomers within a sample.

Sample Preparation
- Samples prepared in triplicate for each study
- (+) enantiomers @ 100%, 120% (Nominal = 25 μg/ml) clinical samples
- (-) enantiomers @ 80%, 100%, 120% (Nominal = 20 μg/ml) clinical samples
- Racemic material used to evaluate the accuracy of (+) enantiomers

Method's ability to produce results that are directly proportional to the concentration of the analyte in the sample within a given range.
- (+) enantiomers:
  - 200 μg/ml for (+) enantiomers
  - 25 μg/ml for (-) enantiomers
- (-) enantiomers:
  - 80% to 120% for (+) enantiomers
  - 100% to 105% for (-) enantiomers

Summary of Data for $\Delta^8$THC Linearity, LOD and LOQ

LOD/LOQ

Lowest concentration of an enantiomer that can be detected or quantitated reliably.
- Based on $S/N$ for peak height
  - Limit of Detection = 3:1 $S/N$
  - Limit of Quantitation = 10:1 $S/N$
- LODs verified - Samples prepared in triplicate

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Method’s ability to produce results that are directly proportional to the concentration of the analyte in the sample within a given range.
- Method's accuracy across 5 levels:
  - 80% to 120% for (+) enantiomers
  - 100% to 105% for (-) enantiomers

Summary of Data for $\Delta^8$THC Linearity, LOD and LOQ

Linearity and Range

Method's ability to produce results that are directly proportional to the concentration of the analyte in the sample within a given range.
- Method's accuracy across 5 levels:
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  - 100% to 105% for (-) enantiomers

Summary of Data for $\Delta^8$THC Linearity, LOD and LOQ

References

CONCLUSIONS

- The chiral method developed demonstrates simultaneous separation of all four $\Delta^8$THC & $\Delta^9$THC enantiomers.
- Method was successfully validated and is robust to a wide concentration range from 2 to 250 μg/ml.
- Method is suitable for use in determining $\Delta^9$THC of Dronabinol, USP.

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