A Validated Chiral HPLC Method for Resolution of Δ⁸ and Δ⁹-tetrahydrocannabinol Enantiomers

**Introduction**

- **Background:**
  - Enantiomers are a pair of substances that are mirror images of each other and cannot be superimposed.
  - Chiral substances are found in nature and can exhibit different biological effects.
- **USP Monograph:**
  - Monograph for Dronabinol is also available.
- **Challenges:**
  - Validated chiral methods are needed with the ability to resolve four isomers.
  - Pure material is difficult to handle.
  - Δ⁸-THC is less potent and sensitive.
  - High purity Δ⁹-THC and Δ⁹-THC reference material is not commercially available.
  - Needed for method development, validation, and ongoing system suitability standards.
  - Δ⁸-THC synthesis was carried and therefore a new synthetic route had to be created for racemic material.
  - Synthesized, purified, and certified at Certilliant.

No method demonstrates simultaneous separation of all four Δ⁸-THC & Δ⁹-THC enantiomers.

**Analytical Method**

- **Optical Phase Chiral LC:**
  - Separates best at Δ⁸-THC, Δ⁹-THC, Δ⁹-THC, Δ⁹-THC
  - Used to determine % enantiomeric excess
- **Conditions:**
  - Capillary ADH column, 4.6 x 250 mm, Syp.
  - 131-195% isopropyl methyl ether (IPME). 0.7 ml/min, 40°C, 228 nm, 5 µl injection

Baseline separation of all four Δ⁸-THC & Δ⁹-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.

**Verified System Suitability Criteria**

<table>
<thead>
<tr>
<th>Acceptance Criteria</th>
<th>Results measured</th>
<th>Acceptance Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak resolution</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**Robustness**

Measure of the method’s capacity to remain unaffected by small but deliberate variations in parameters.
- Provides indication of reliability during normal usage.
- Performed interference injections at unintended conditions with each analyte.

**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 performance:**
  - 200 ng/µL for Δ⁸-THC enantiomers
  - 25 ng/µL for Δ⁹-THC enantiomers
  - 10% to 20% for Δ⁹-THC enantiomers.

**Summary of Data for Δ⁸-THC Linearity, IOD and LOQ**

- **LOQ:**
  - Lowest concentration of Δ⁸-THC 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
- **IOD & LOQ verification:**
  - Samples prepared in triplicate.

**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 %ee by comparing relative peak areas of the (+) and (-) enantiomers within a sample.
- **Sample preparation:**
  - Samples were prepared in triplicate for each study.
  - (+) enantiomers @ LOQ, 100%, 120% (Nominal = 25 µg/mL)
  - (-) enantiomers @ 80%, 100%, 120% (Nominal = 200 µg/mL)

**Precision**

Expresses the agreement between a series of measurements obtained from multiple analyses of the same homogenous sample under the prescribed conditions.
- **Bias:**
  - Intermediate precision, i.e., ruggedness.
  - Samples: (+) Δ⁸-THC and (-) Δ⁹-THC.

**Repeatability**

Precision under the same operating conditions over a short period of time.
- **High method precision for a chiral analysis with RSD’s < 2.0% for response factors and < 0.50% for %ee determination.**

**CONCLUSIONS**

- The chiral method developed demonstrates simultaneous separation of all four Δ⁸-THC & Δ⁹-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 %ee of Dronabinol, USP.