A Validated Chiral HPLC Method for Resolution of Δ^8 and Δ^9-tetrahydrocannabinol Enantiomers

Introduction

Analytical Method

Baseline separation of all four Δ^8- & Δ^9-THC enantiomers within 25 minutes

System Suitability

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

Verification of System Suitability Criteria

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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 Tee by comparing relative peak areas of the (+) and (-) enantiomers within a sample.

Samples Preparation

- Samples were prepared in triplicate for each study
- (+) enantiomer @ IOQ, 100%, 120% (Nominal = 25 µg/mL)
- (-) enantiomer @ 80%, 100%, 120% (Nominal = 20 µg/mL)
- Racemic material used to evaluate the accuracy of (+) enantiomers.

Racemic Resolution Standard

Linear Range

Method’s ability to produce results that are directly proportional to the concentration of the analyte in the sample within a given range:

- **Methodology**
  - 25 µg/mL for (-) enantiomers
  - 100% to 200% for (+) enantiomers

Summary of Data for Δ^8-THC Linearity, LOD and LOQ

Summary of Data for Δ^9-THC Linearity, LOD and LOQ

Robustness

Measures the method’s capacity to remain unaffected by small but deliberate variations in parameters.

- Provides an indication of reliability under normal usage.
- Performed reference injections at unmapped conditions with each analyte.

Calibration parameters:
- column temperature (42±2°C)
- flow rate (0.7±0.1 mL/min)
- injection volume (5±2 µL)

Change in Resolution of Enantiomers

Change in RRT of Enantiomers

- No measurable effect on RRT (0±0.0)

Method robust to slight variations in column temperature, mobile phase flow rate, and injection volume

Conclusions

- The chiral method developed demonstrates simultaneous separation of all four Δ^8- & Δ^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 Tee of Dronabinol, USP.

References