

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

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Introduction

Background

- (-)- Δ^9 -THC is an API known as Dronabinol
- (+)- Δ^9 -THC enantiomer has little or no clinical effect^{1,2}
- (-)- Δ^9 -THC may be synthesized from (+)- Δ^8 -THC³
- FDA guidance: stereoisomeric composition must be quantitated for chiral API materials used in pharmacological, toxicological, and clinical studies⁴

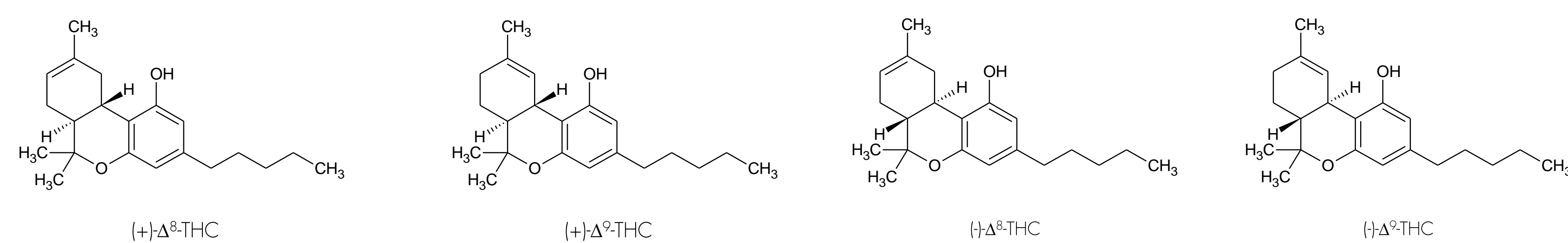
Literature

- Chiral analysis of Δ^8 -THC & Δ^9 -THC is well-documented^{5,6}
- USP Monograph for Dronabinol is also achiral
- Some chiral systems have been illustrated for analysis of these and related cannabinoids, but none were validated⁷⁻¹⁰

Challenges

- Validated chiral method is needed with the ability to resolve four isomers
- Neat material is difficult to handle
 - Glassy solid at room temperature
 - (-)- Δ^9 -THC is light and air sensitive
- High purity racemic Δ^9 -THC and Δ^8 -THC reference material not commercially available
 - Needed for method development, validation and ongoing use as system suitability standards
 - (-)- Δ^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 Δ^9 -THC & Δ^8 -THC enantiomers

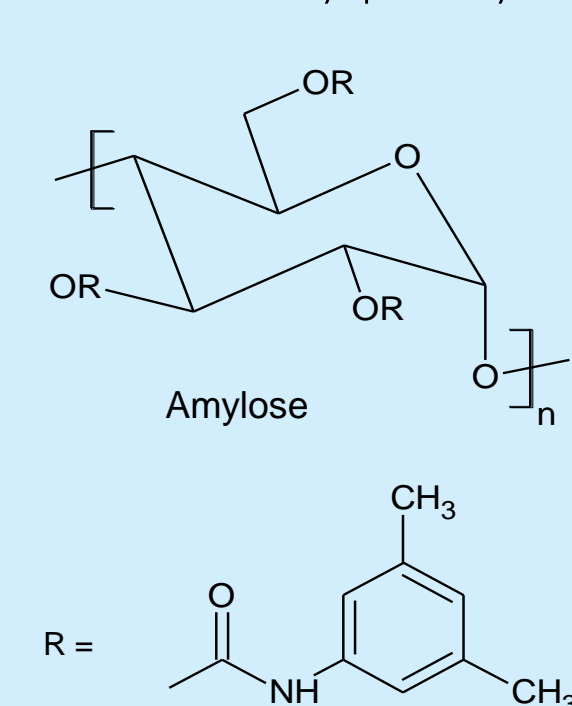


Analytical Method

- Normal Phase Chiral LC
 - Order of elution: (impurities first) (+)- Δ^8 -THC, (+)- Δ^9 -THC, (-)- Δ^8 -THC, (-)- Δ^9 -THC
 - Used to determine % enantiomeric excess
- Conditions
 - Chiralpak ADH column, 4.6 x 250 mm, 5 μ
 - (3:1:9:95:0) isopropanol:methanol:n-heptane
 - 0.7 ml/min, 40°C, 228 nm, 5 μ l injection

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

Chiralpak ADH
(amylose tris 3,5-dimethylphenyl carbamate)



Enantiomeric Excess

- Absolute difference between the mole fractions of each enantiomer.
- Expressed as % enantiomeric excess (%ee)
- Methodology
 - Quantitate each enantiomer individually
 - Calculate %ee of (-)- Δ^9 -THC or (+)- Δ^8 -THC
 - Equation:

$$\% \text{ Enantiomeric excess} = \left(\frac{\% \text{ Area}_{(-)\text{-}\Delta^9\text{-THC}} - \% \text{ Area}_{(+)\text{-}\Delta^8\text{-THC}}}{\% \text{ Area}_{(-)\text{-}\Delta^9\text{-THC}} + \% \text{ Area}_{(+)\text{-}\Delta^8\text{-THC}} \right) \times 100$$

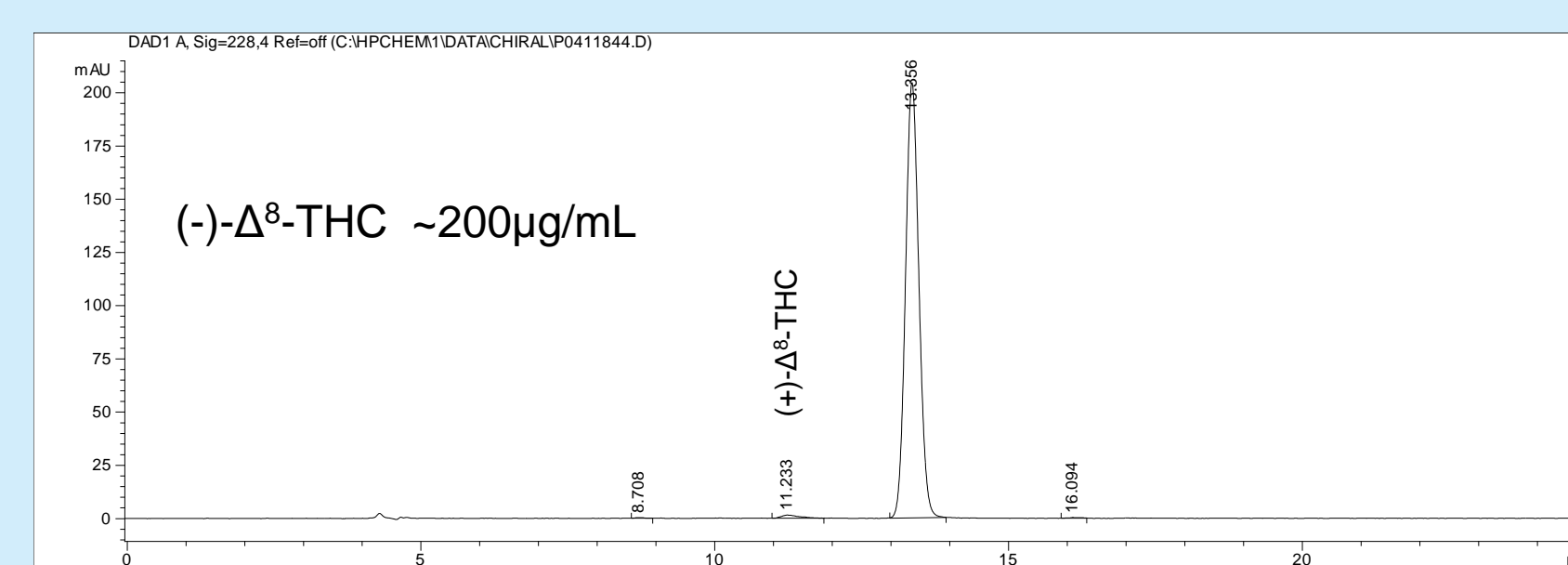
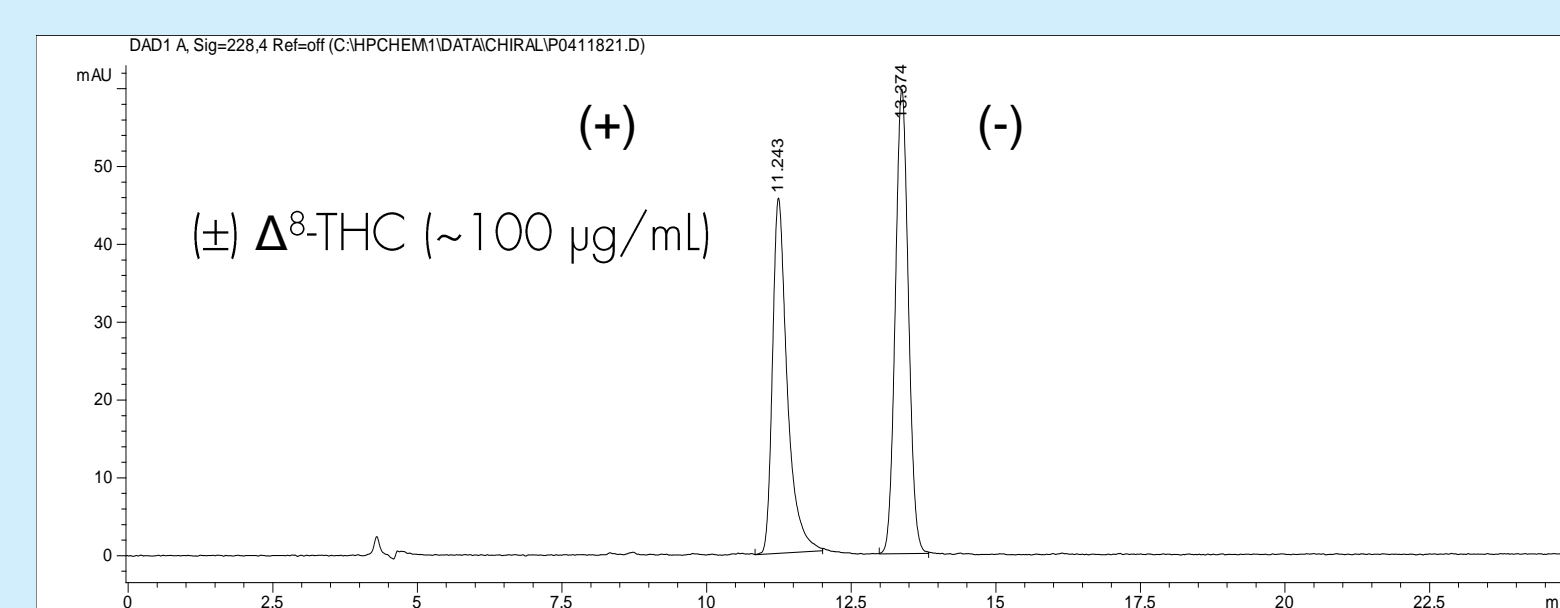
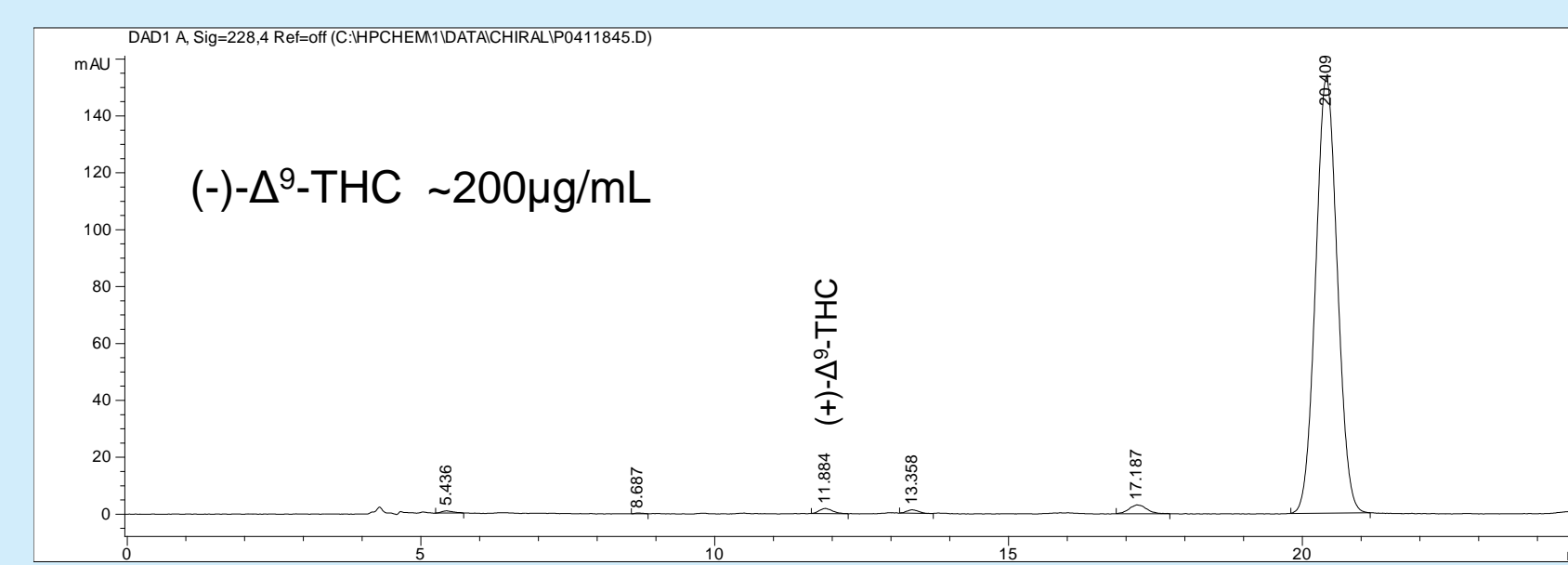
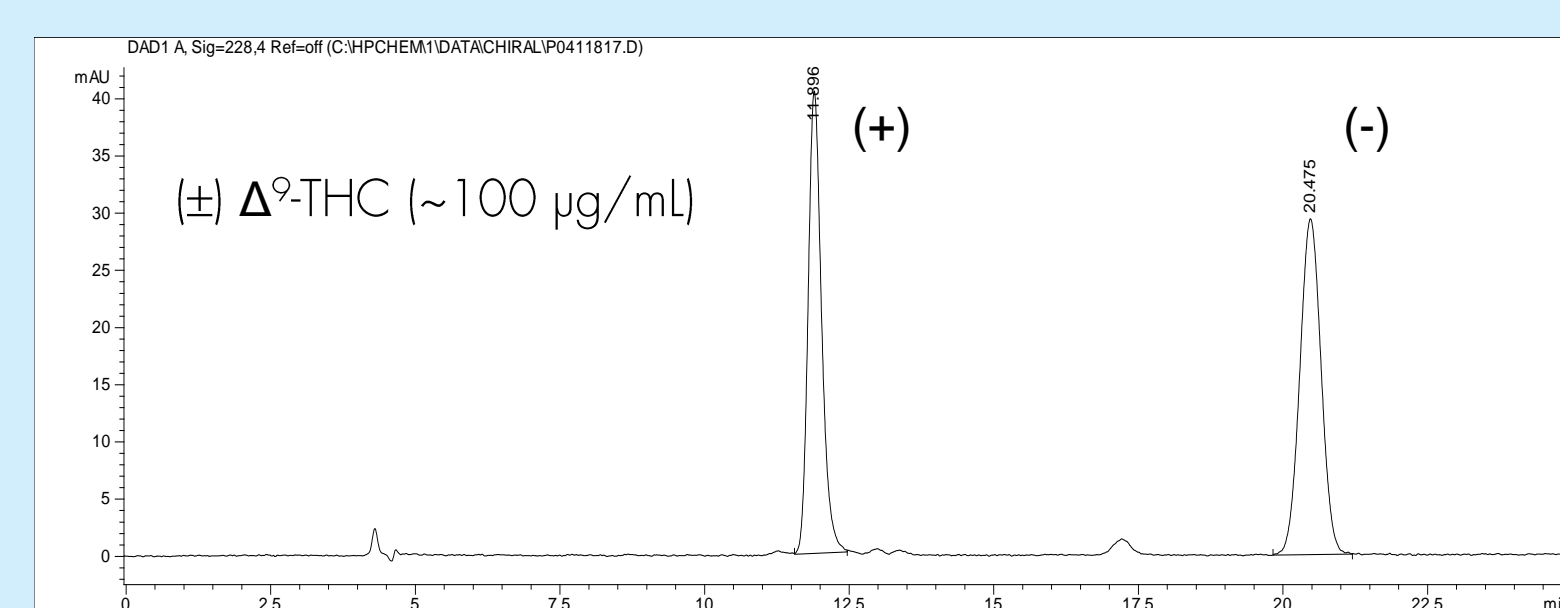
System Suitability

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

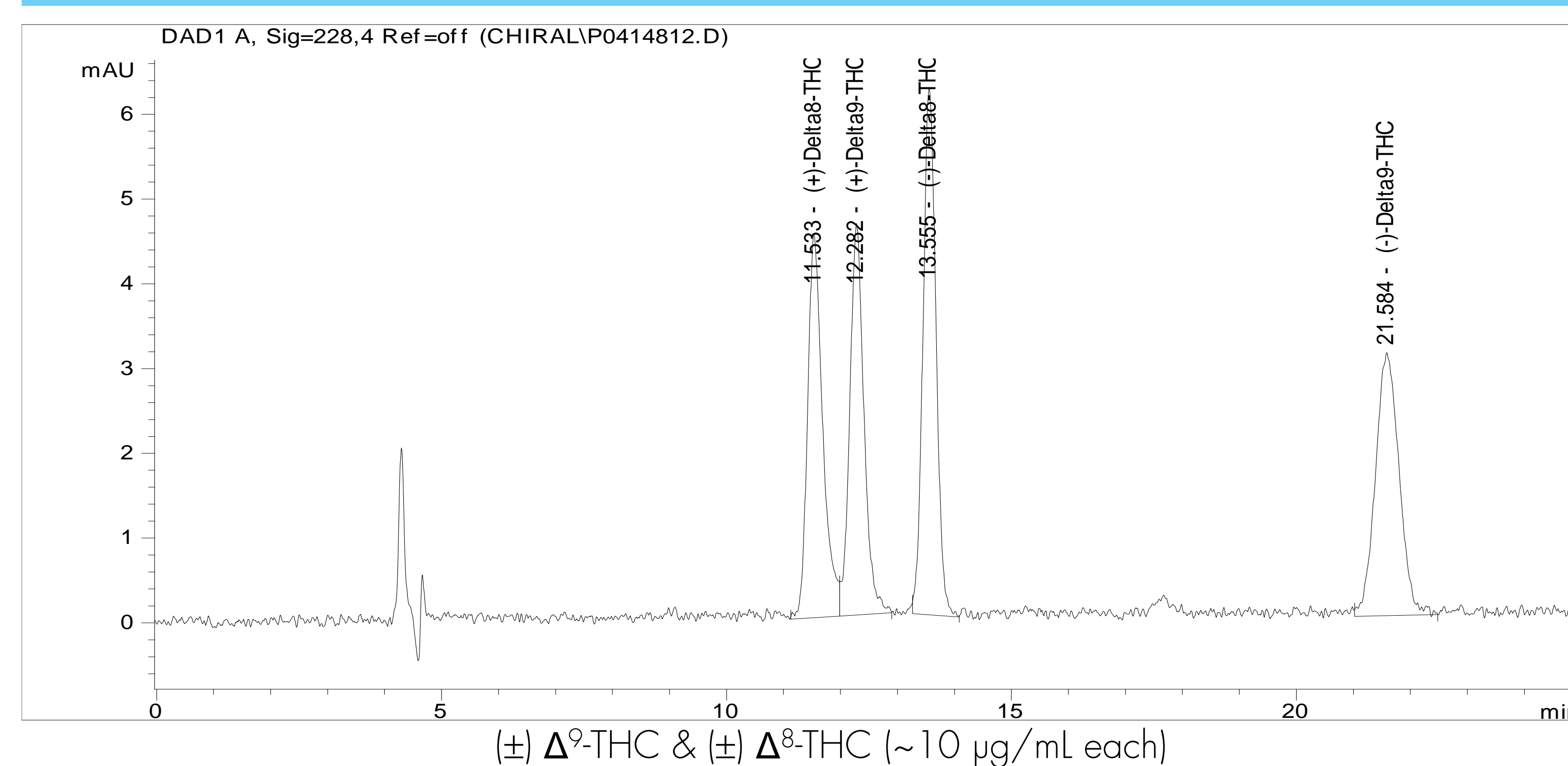
| | Acceptance Criteria | | Results (average) |
|------------|--|-------------|-------------------|
| | Resolution | Results | (average) |
| Resolution | (+)- Δ^9 -THC, (-)- Δ^8 -THC | ≥ 12.0 | 14.4 |
| | (+)- Δ^8 -THC, (-)- Δ^9 -THC | ≥ 3.0 | 5.1 |
| | (+)- Δ^8 -THC, (+)- Δ^9 -THC | ≥ 1.2 | 1.2 |
| | (+)- Δ^9 -THC, (-)- Δ^8 -THC | ≥ 1.8 | 4.0 |
| Tailing | (+)- Δ^9 -THC | ≤ 2.0 | 1.3 |
| | (+)- Δ^8 -THC | ≤ 2.0 | 1.6 |



References

- Edey, H.; Grunfeld, Y.; Ben-Zvi, Z.; Mechoulam, R. *Ann. N.Y. Acad. Sci.* **1971**, *191*, 40.
- Pertwee, R. G.; *Br. J. Pharmacol.* **2006**, *147*, S163-S171.
- Mechoulam, R.; Braun, P.; Gaoni, Y. *J. Am. Chem. Soc.* **1972**, *94*, 6159-6165.
- FDA, *Development of New Stereoisomeric Drugs*, last updated 07-06-05, <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm122883.htm>, retrieved 07-19-09.
- Wojtasik, E.; Anyszewska, M.; Arent, I. *J. Liq. Chrom. & Rel. Technol.* **2002**, *25*, 949-959.
- Hazekamp, A.; Peltenburg, A.; Verpoorte, R. *J. Liq. Chrom. & Rel. Technol.* **2005**, *28*, 2361-2382.
- Okamoto, Y.; Kawashima, M.; Hatada, K. *J. Am. Chem. Soc.* **1984**, *106*, 5357-5359.
- Levin, S.; Abu-Lafi, S.; Zahalka, J.; Mechoulam, R. *J. Chromatogr. A* **1993**, *654*, 53-64.
- Abu-Lafi, S.; Sterin, M.; Levin, S. *J. Chromatogr. A* **1994**, *679*, 47-58.
- Levin, S.; Sterin, M.; Abu-Lafi, S. *Chirality* **1995**, *7*, 140-146.

Racemic Resolution Standard



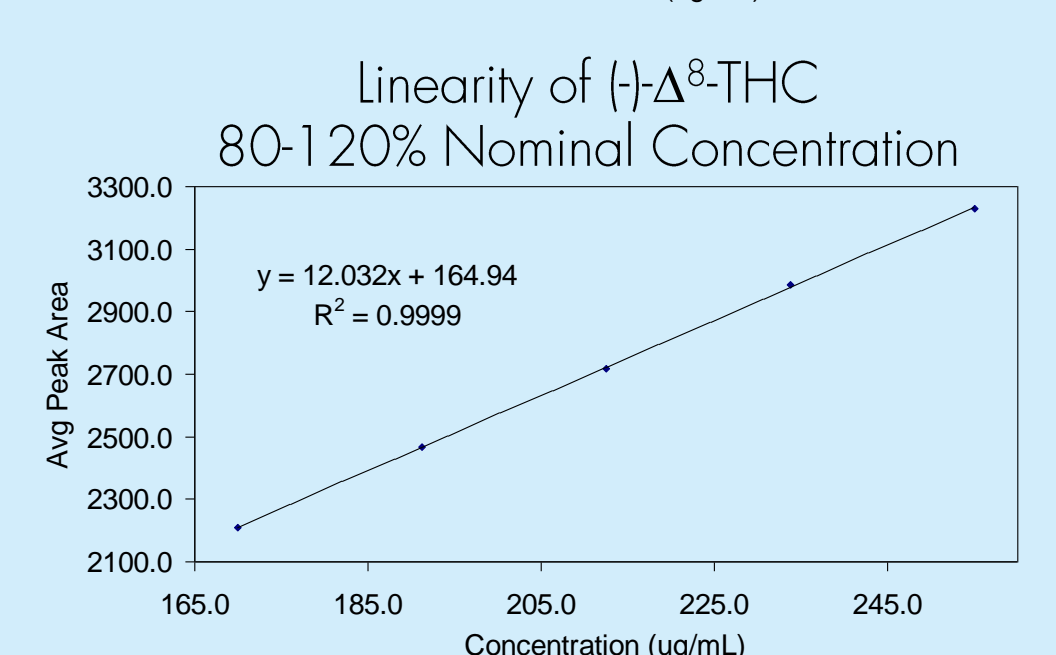
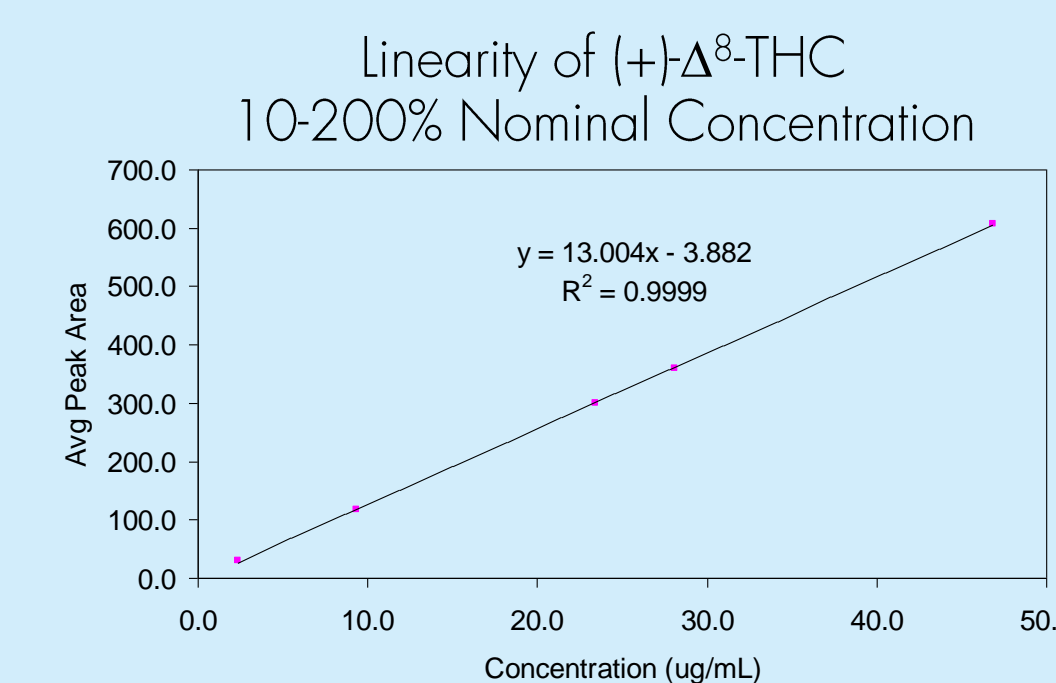
Linearity and Range

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

- Nominal concentrations
 - 200 μ g/mL for (-) enantiomers
 - 25 μ g/mL for (+) enantiomers
- Linearity demonstrated across 5 levels
 - 80% to 120% for (-) enantiomers
 - 10% to 200% for (+) enantiomers

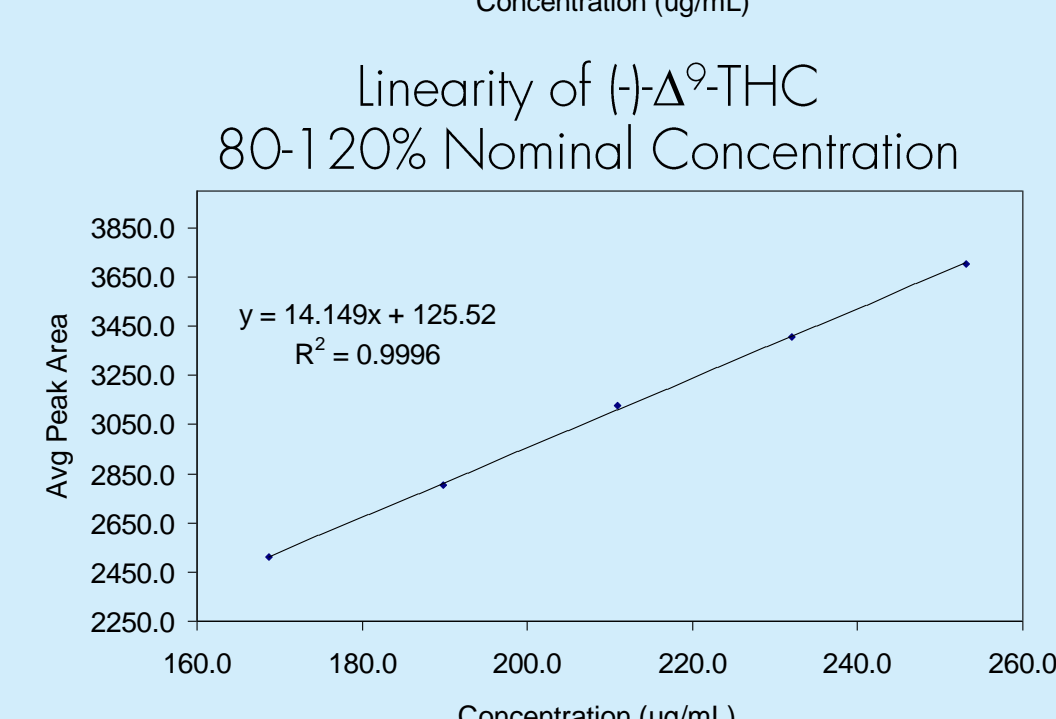
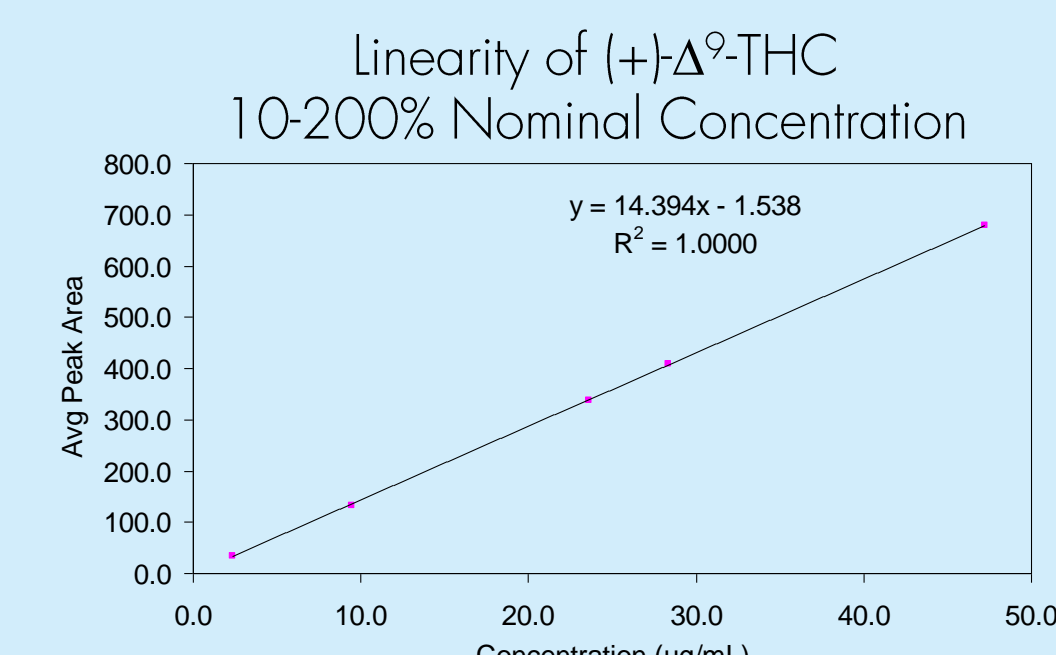
Summary of Data for Δ^8 -THC Linearity, LOD and LOQ

| (+)- Δ^8 -THC | | | | | (-)- Δ^8 -THC | | | | |
|----------------------|---------------------|----------------|------|-----------------|----------------------|---------------------|----------------|------|-----------------|
| % Nominal | Conc. (μ g/mL) | Avg. Peak Area | %RSD | Response Factor | % Nominal | Conc. (μ g/mL) | Avg. Peak Area | %RSD | Response Factor |
| 2% | 0.47 | 5.383 | 2.5% | 11.5 | 0.25% | 0.53 | 6.624 | 3.6% | 12.5 |
| 10% | 2.3 | 28.910 | 1.0% | 12.3 | 1.25% | 2.7 | 34.278 | 0.8% | 12.9 |
| 40% | 9.4 | 117.243 | 1.2% | 12.5 | 5% | 10.6 | 137.312 | 1.0% | 12.9 |
| 100% | 23.4 | 299.484 | 0.9% | 12.8 | 13% | 26.6 | 344.422 | 0.7% | 13.0 |
| 120% | 28.1 | 359.787 | 0.9% | 12.8 | 15% | 31.9 | 411.040 | 0.4% | 12.9 |
| 200% | 46.9 | 607.749 | 0.3% | 13.0 | 25% | 53.1 | 684.592 | 0.3% | 12.9 |
| 500% | 117.2 | 1539.293 | 0.4% | 13.1 | 63% | 132.8 | 1727.076 | 0.5% | 13.0 |
| 640% | 150.0 | 1971.394 | 0.9% | 13.1 | 80% | 170.0 | 2209.418 | 1.0% | 13.0 |
| 720% | 168.8 | 2200.336 | 0.8% | 13.0 | 90% | 191.2 | 2466.224 | 0.9% | 12.9 |
| 800% | 187.5 | 2427.350 | 1.9% | 12.9 | 100% | 212.5 | 2718.952 | 1.9% | 12.8 |
| 880% | 206.3 | 2664.305 | 1.0% | 12.9 | 110% | 233.7 | 2984.539 | 1.0% | 12.8 |
| 960% | 225.0 | 2882.662 | 1.6% | 12.8 | 120% | 255.0 | 3228.562 | 1.6% | 12.7 |



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

| (+)- Δ^9 -THC | | | | | (-)- Δ^9 -THC | | | | |
|----------------------|---------------------|----------------|------|-----------------|----------------------|---------------------|----------------|-------|-----------------|
| % Nominal | Conc. (μ g/mL) | Avg. Peak Area | %RSD | Response Factor | % Nominal | Conc. (μ g/mL) | Avg. Peak Area | %RSD | Response Factor |
| 2% | 0.47 | 8.208 | 3.7% | 17.4 | 0.25% | 0.53 | 12.351 | 15.1% | 23.4 |
| 10% | 2.4 | 32.993 | 5.6% | 14.0 | 1.25% | 2.7 | 46.305 | 6.5% | 17.6 |
| 40% | 9.5 | 132.957 | 4.0% | 14.1 | 5% | 10.6 | 157.696 | 5.8% | 15.0 |
| 100% | 23.6 | 338.185 | 3.2% | 14.3 | 13% | 26.4 | 382.873 | 3.4% | 14.5 |
| 120% | 28.4 | 408.995 | 4.5% | 14.4 | 15% | 31.6 | 472.356 | 3.7% | 14.9 |
| 200% | 47.3 | 677.769 | 3.0% | 14.3 | 25% | 52.7 | 763.755 | 3.1% | 14.5 |
| 300% | 118.2 | 1746.506 | 2.2% | 14.8 | 63% | 131.9 | 1987.912 | 2.2% | 15.1 |
| 640% | 151.2 | 2229.964 | 1.2% | 14.7 | 80% | 168.8 | 2510.832 | 1.3% | 14.9 |
| 720% | 170.1 | 2474.275 | 0.9% | 14.5 | 90% | 189.9 | 2804.956 | 0.8% | 14.8 |
| 800% | 189.0 | 2770.245 | 0.9% | 14.7 | 100% | 211.0 | 3126.682 | 0.8% | 14.8 |
| 880% | 207.9 | 3028.419 | 1.5% | 14.6 | 110% | 232.1 | 3407.203 | 1.4% | 14.7 |
| 960% | 226.8 | 3289.817 | 0.9% | 14.5 | 120% | 253.2 | 3702.129 | 0.9% | 14.6 |



Method is linear from 2.5 to 50 μ g/mL for (+) enantiomers and from 170 to 250 μ g/mL for (-) enantiomers

LOD/LOQ

Lowest concentration of (+) 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
- LOQ's verified - Samples prepared in triplicate

| Analyte | Dilution Level (mg/mL) | Linear Equation | R ² | LOD (mg/mL) | LOQ (mg/mL) | LOQ Verification (n=3) | | |
|----------------------|--------------------------|------------------------|----------------|-------------|-------------|------------------------|-----------|------|
| | | | | | | mg/mL | Peak Area | %RSD |
| (+)- Δ^8 -THC | 2 to 200% [0.47 - 46.88] | $y = 13.004x - 3.882$ | 0.9996 | 0.54 | 0.93 | 0.94 | 11.486 | 3.1 |
| (+)- Δ^9 -THC | 2 to 200% [0.47 - 47.26] | $y = 14.394x - 1.5377$ | 0.9999 | 0.23 | 0.60 | 0.53 | 6.6351 | 3.2 |

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.
- Performed reference injections at unmodified conditions with each analysis.
- Modifications
 - column temperature (40 \pm 2 C)
 - flow rate (0.7 \pm 0.1 mL/min)
 - injection volume (5 \pm 2 μ l)

Change in Resolution of Enantiomers

| Modification | (+)- Δ^8 -THC, (+)- Δ^9 -THC | (+)- Δ^9 -THC, (-)- Δ^8 -THC | (-)- Δ^8 -THC, (-)- Δ^9 -THC |
|------------------------------|--|--|--|
| Column @ 38°C | -0.03 | -0.14 | -0.13 |
| Column @ 42°C | 0.02 | 0.13 | 0.10 |
| Flow Rate @ 0.6 mL/min | 0.03 | 0.17 | 0.01 |
| Flow Rate @ 0.8 mL/min* | -0.05 | -0.15 | -0.55 |
| Injection Volume @ 3 μ l | 0.00 | 0.01 | 0.00 |
| Injection Volume @ 7 μ l | -0.01 | 0.00 | -0.17 |

Change in RRT of Enantiomers

- No measured effect on RRT (Δ <0.00)

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

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 μ g/mL)
 - (-) enantiomers @ 80%, 100%, 120% (Nominal = 200 μ g/mL)
- Racemic material used to evaluate the accuracy of (+) enantiomers

| (-) Enantiomer Accuracy | | (+) Enantiomer Accuracy | |
|--|---|---|---|
| Sample | Reference | Sample | Reference |
| Neat material prepared at 120% and diluted to 100% and 80% nominal concentration | Racemic stock standard diluted to nominal concentration of (-) enantiomer | Racemic standard spiked into (-) enantiomer accuracy samples to achieve LOQ, 100%, and 120% nominal concentration | Racemic standard diluted to nominal concentration of (+) enantiomer |

Recovery of (-)- Δ^8 -THC (n=3)

| Level | Theoretical % (based on wt.) | % Recovery | Absolute Difference |
|-------|------------------------------|------------|---------------------|
| 80% | 80.9% | 79.6% | 1% |
| 100% | 101.0% | 98.0% | 3% |
| 120% | 121.4% | 119.1% | 2% |

Summary of %ee and RF for (-)- Δ^8 -THC (n=3)

| Level | % Enantiomeric Excess | | Response Factor | |
|---------|-----------------------|-------|-----------------|------|
| | Average | %RSD | Average | %RSD |
| 80% | 98.946% | 0.08% | 12.98 | 0.1% |
| 100% | 98.944% | 0.07% | 12.80 | 1.5% |
| 120% | 98.848% | 0.06% | 12.95 | 0.1% |
| Overall | 98.913% | 0.08% | 12.91 | 1.0% |

Recovery of (+)- Δ^8 -THC (n=3)

| Level | Theoretical % (based on wt.) | % Recovery | Absolute Difference |
|-------|------------------------------|------------|---------------------|
| LOQ | 7.6% | 6.1% | 2% |
| 100% | 95.7% | 95.1% | 1% |
| 120% | 114.8% | 113.0% | 2% |

Summary of %ee and RF for (+)- Δ^8 -THC (n=3)

| Level | Peak Area | | Observed % ee | | Theoretical % ee | Difference in % ee |
|-------|-----------|------|---------------|-------|------------------|--------------------|
| | Average | %RSD | Average | %RSD | | |
| LOQ | 20.801 | 3.3% | 98.994% | 0.05% | 98.081% | -0.3% |
| 100% | 324.225 | 0.3% | 67.528% | 0.17% | 68.089% | -0.6% |
| 120% | 385.240 | 1.0% | 63.060% | 0.43% | 63.215% | -0.2% |

Recovery of (-)- Δ^9 -THC (n=3)

| Level | Calculated % (based on wt.) | % Recovery | Absolute Difference |
|-------|-----------------------------|------------|---------------------|
| 80% | 67.9% | 66.0% | 2% |
| 100% | 84.8% | 82.0% | 3% |
| 120% | 101.9% | 99.9% | 2% |

Summary of %ee and RF for (-)- Δ^9 -THC (n=3)

| Level | % Enantiomeric Excess | | Response Factor | |
|---------|-----------------------|-------|-----------------|------|
| | Average | %RSD | Average | %RSD |
| 80% | 98.730% | 0.01% | 13.82 | 0.5% |
| 100% | 98.755% | 0.03% | 13.75 | 0.6% |
| 120% | 98.763% | 0.01% | 13.95 | 0.5% |
| Overall | 98.749% | 0.02% | 13.84 | 0.8% |

Recovery of (+)- Δ^9 -THC (n=3)

| Level | Calculated % (based on wt.) | % Recovery | Absolute Difference |
|-------|-----------------------------|------------|---------------------|
| LOQ | 5.8% | 5.8% | 0% |
| 100% | 96.5% | 97.0% | |