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

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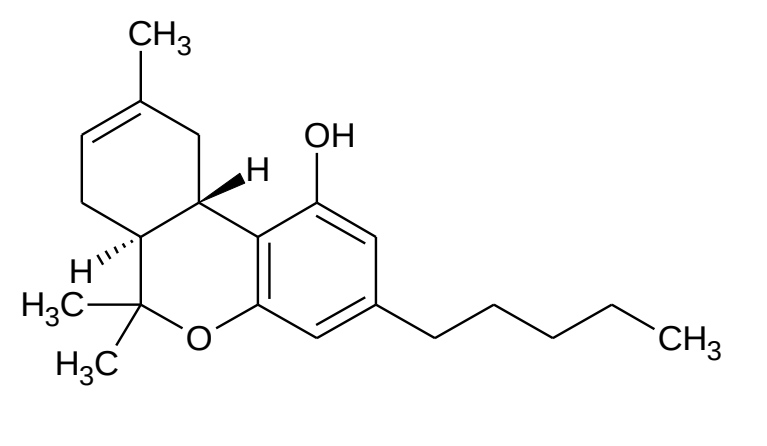
Introduction

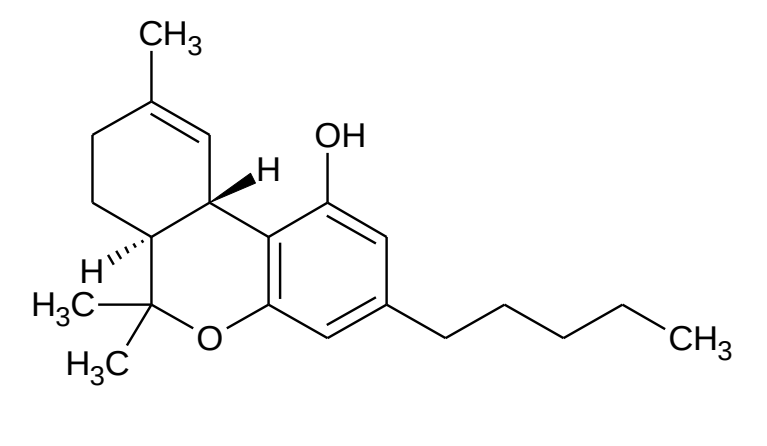
Background
- (-)-Δ^9-THC is an API known as Dronabinol
- (+)-Δ^9-THC enantiomer has little or no clinical effect[1,2]
- (-)-Δ^9-THC may be synthesized from (-)-Δ^8-THC[3]
- FDA guidance: stereoisomeric composition must be quantitated for chiral API materials used in pharmacological, toxicological, and clinical studies[4]

Literature
- Achiral 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 validated[7-10]

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


(+)-Δ^8-THC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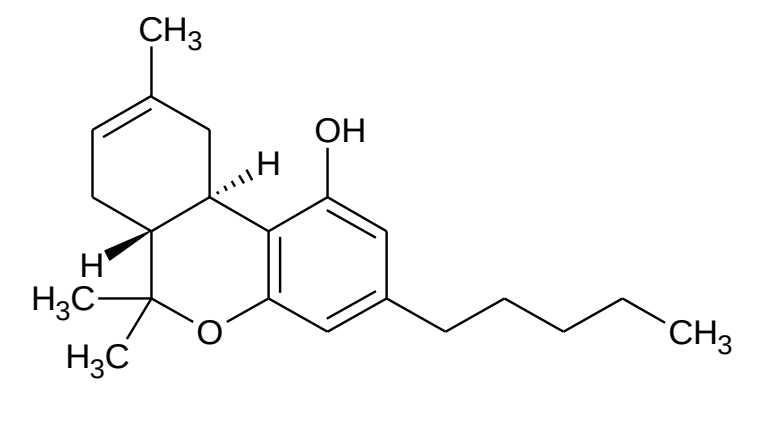
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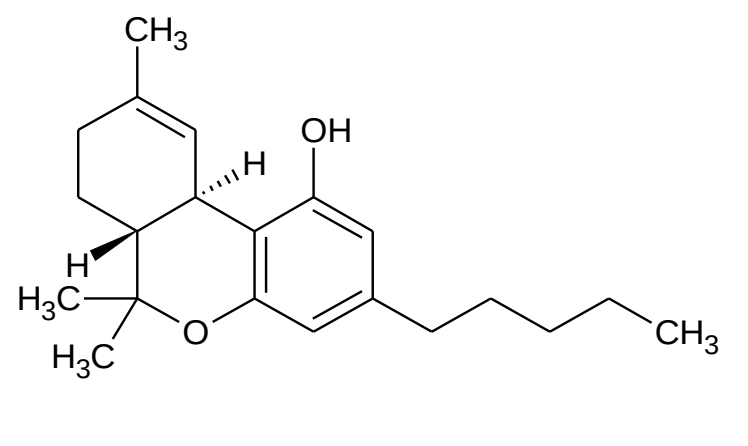
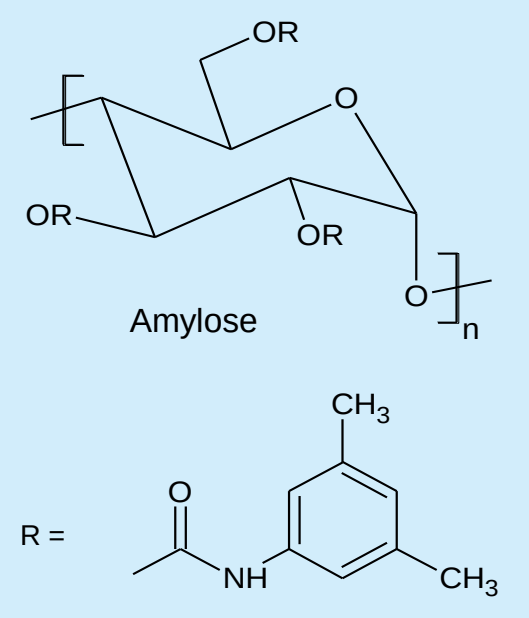

(-)-Δ^9-THC

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 AD-H column, 4.6 x 250 mm, 5µ
  - (31:9:950) isopropanol:methanol:n-heptane
  - 0.7 ml/min, 40°C, 228 nm, 5 µl injection

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

Chiralpak AD-H
(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:

$$\%\,Enantiomeric\ excess = \left(\frac{\%Area_{(-)\text{-}\Delta^x\text{-THC}} - \%Area_{(+)\text{-}\Delta^x\text{-THC}}}{\%Area_{(-)\text{-}\Delta^x\text{-THC}} + \%Area_{(+)\text{-}\Delta^x\text{-THC}}}\right) \times 100$$

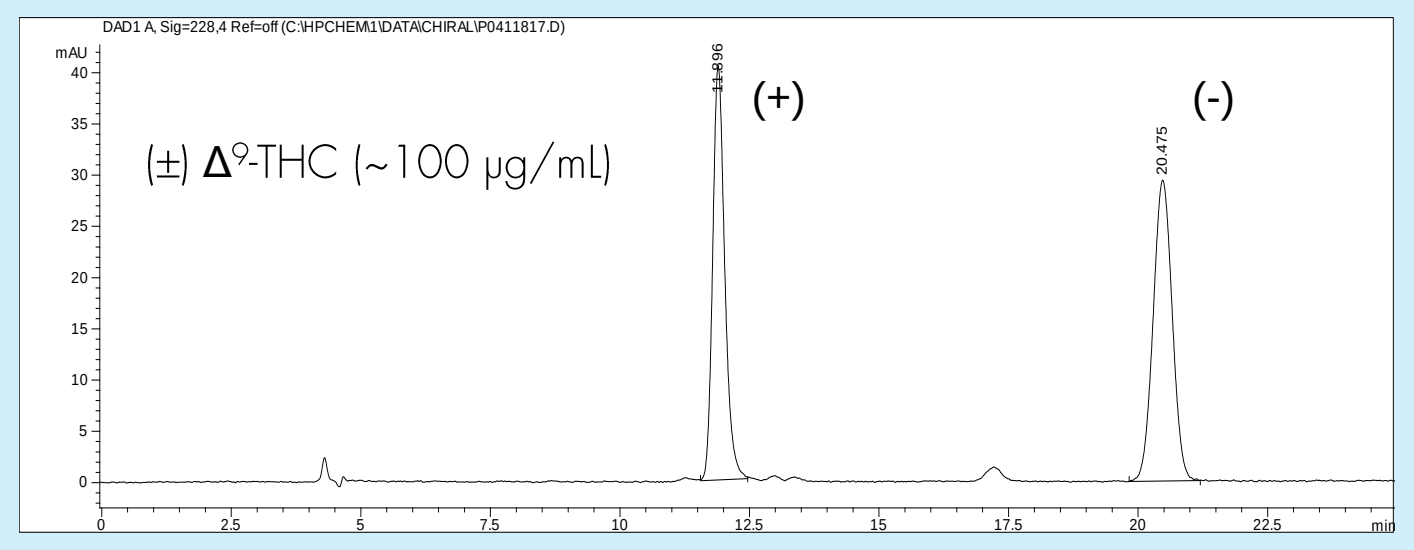
System Suitability

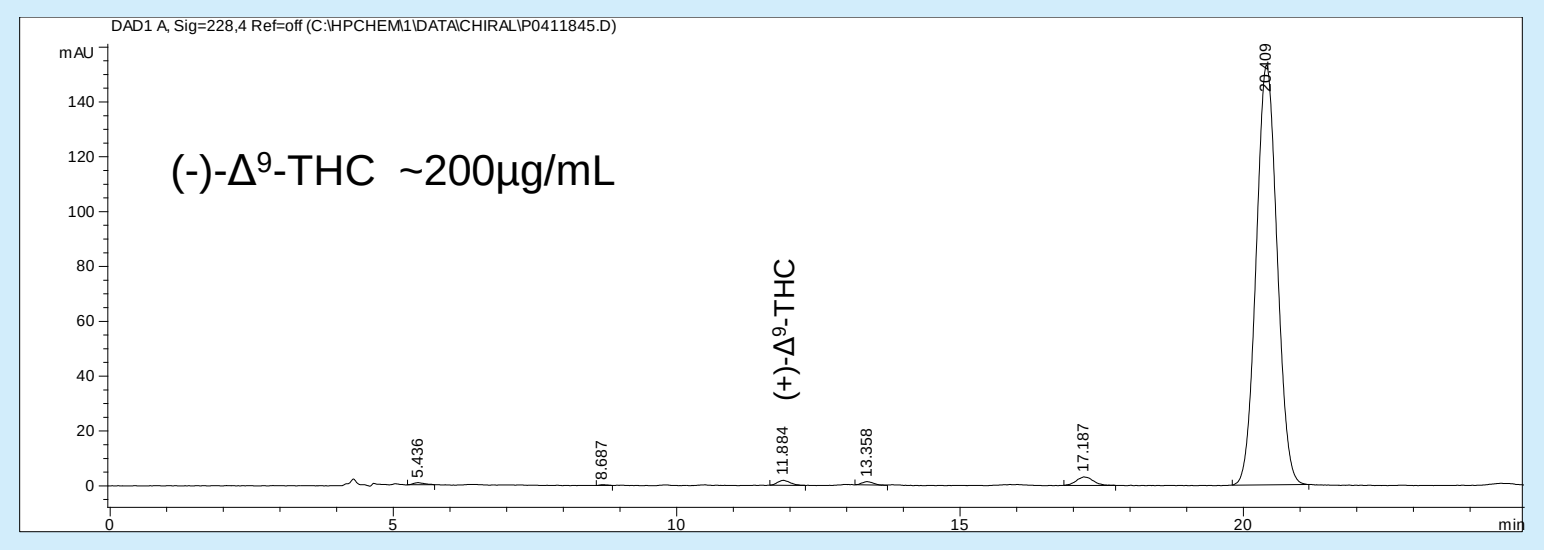
Ensures that sensitivity, resolution, and reproducibility of the chromatographic system are adequate for the analysis to be performed as intended.

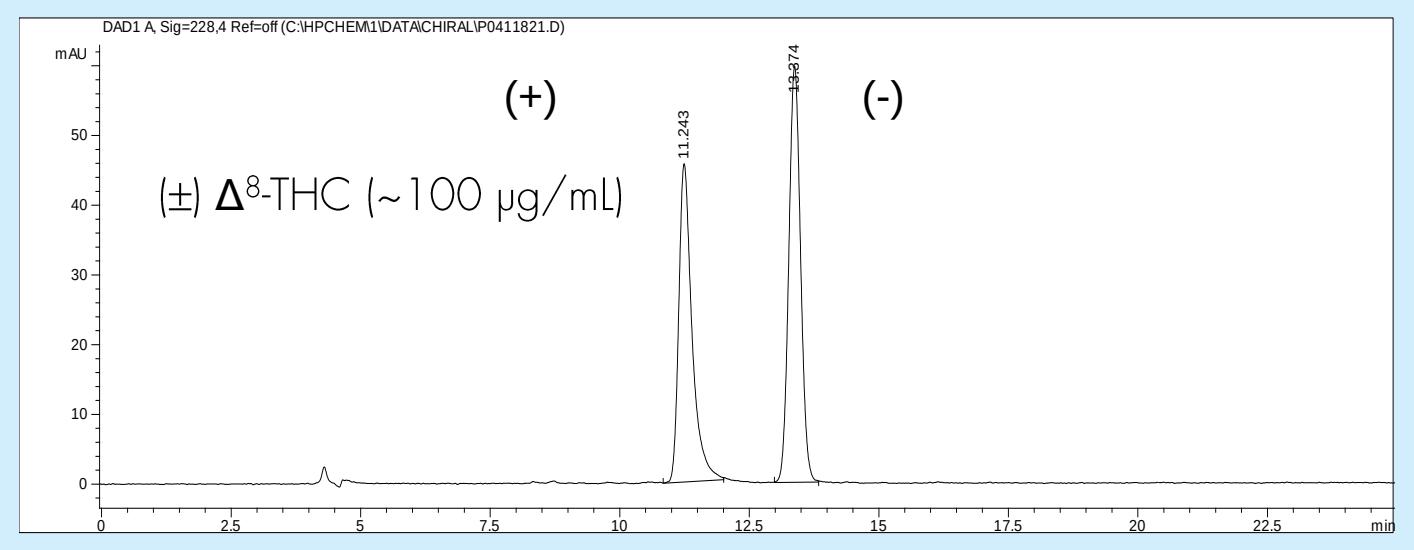
USP calculations for Peak Resolution and Tailing were used to determine System Suitability.

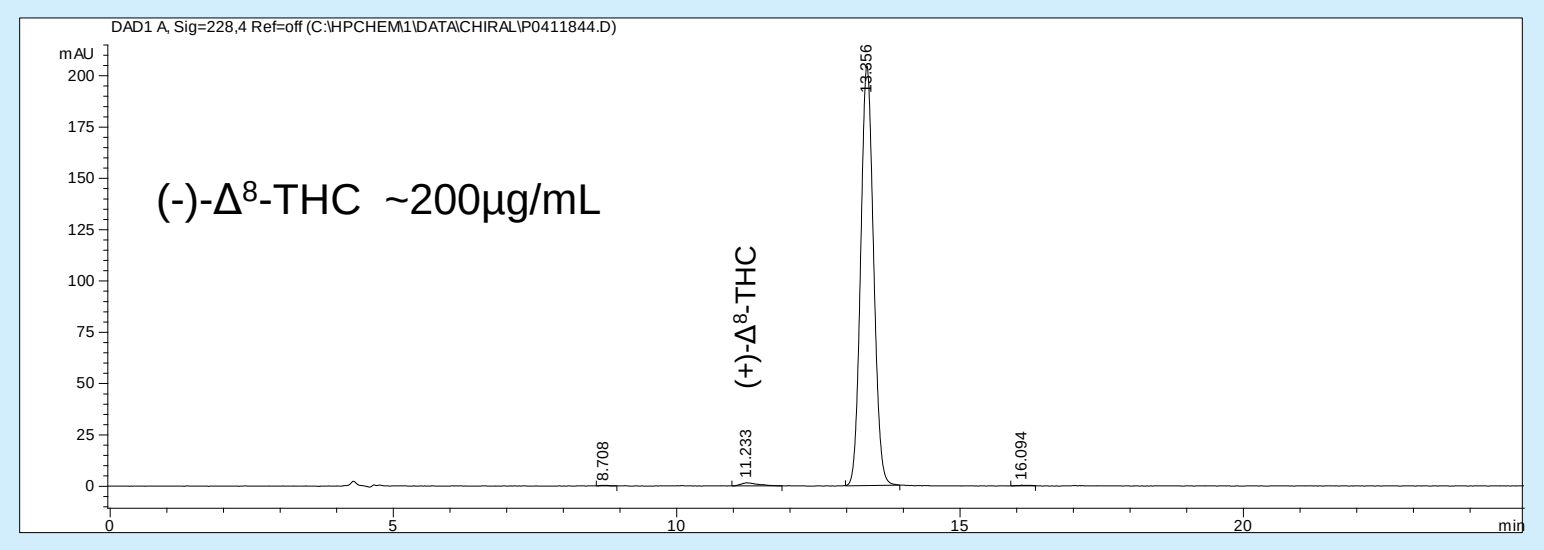
Verified System Suitability Criteria

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

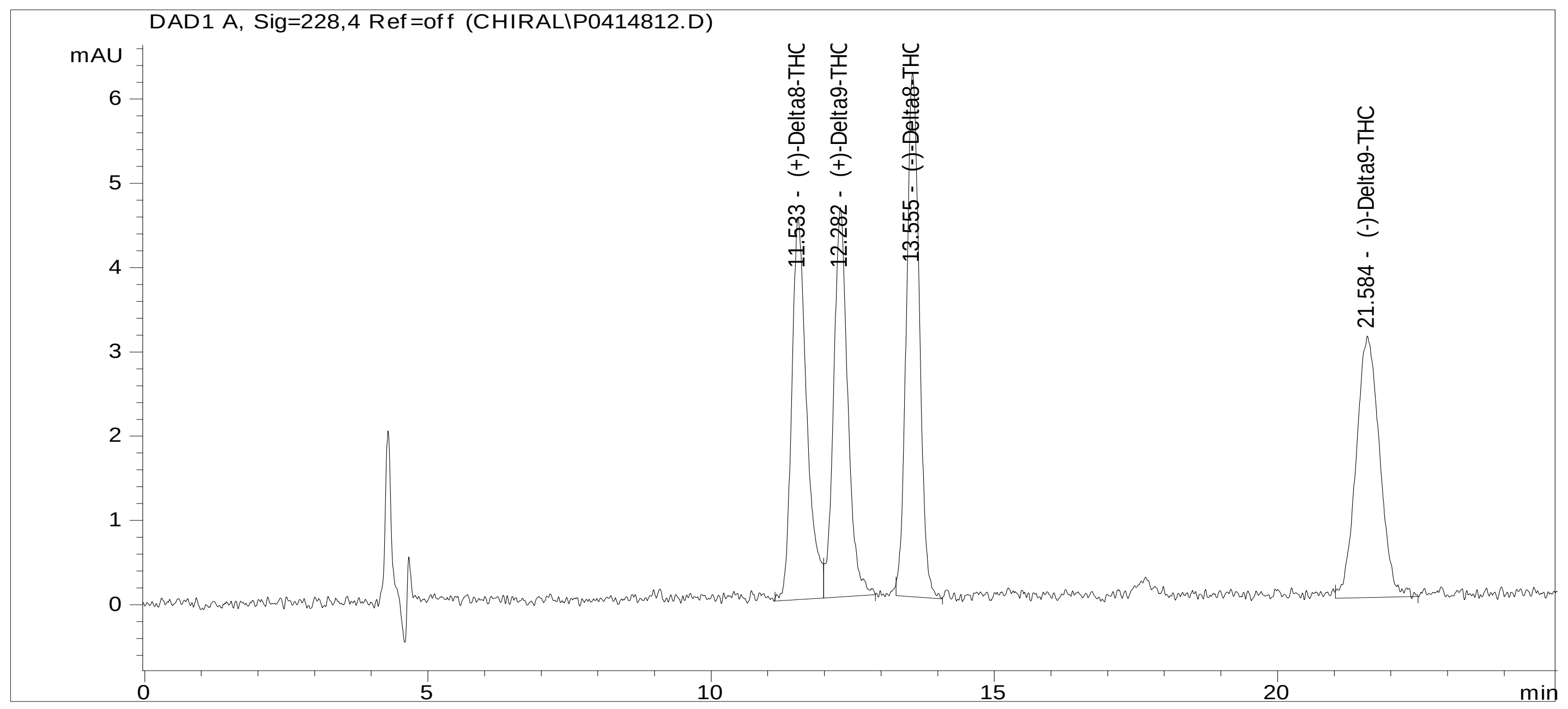








Racemic Resolution Standard



(±) Δ^9-THC & (±) Δ^8-THC (~10 µg/mL each)

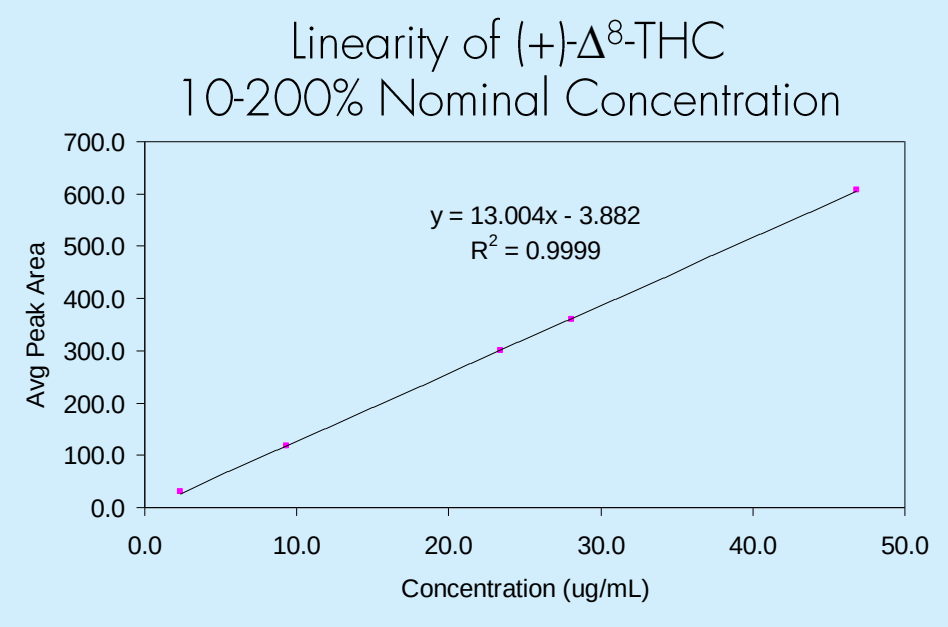
Linearity and Range

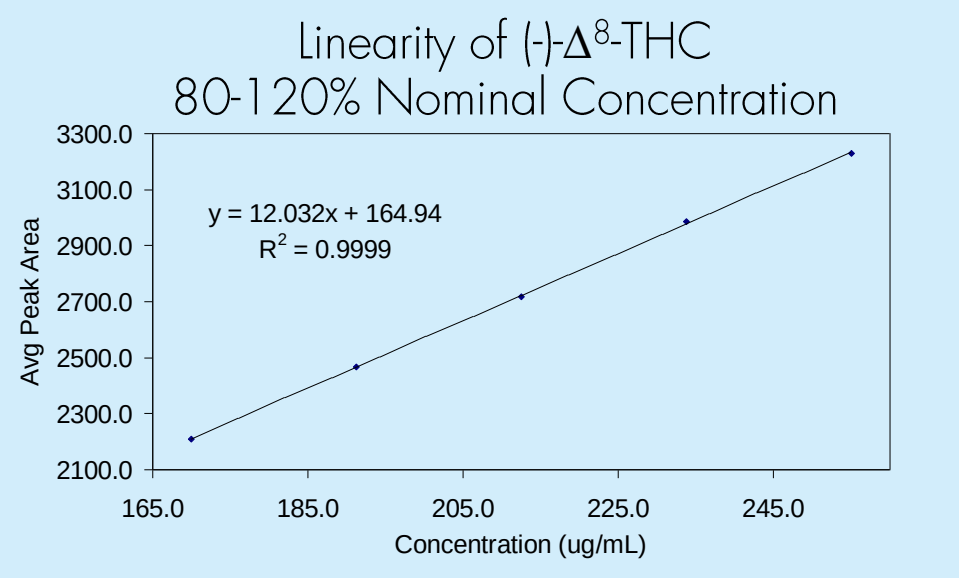
Method's ability to produce results that are directly proportional to the concentration of the analyte in the sample within a given range.

- Nominal concentrations
  - 200 µg/mL for (-) enantiomers
  - 25 µg/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

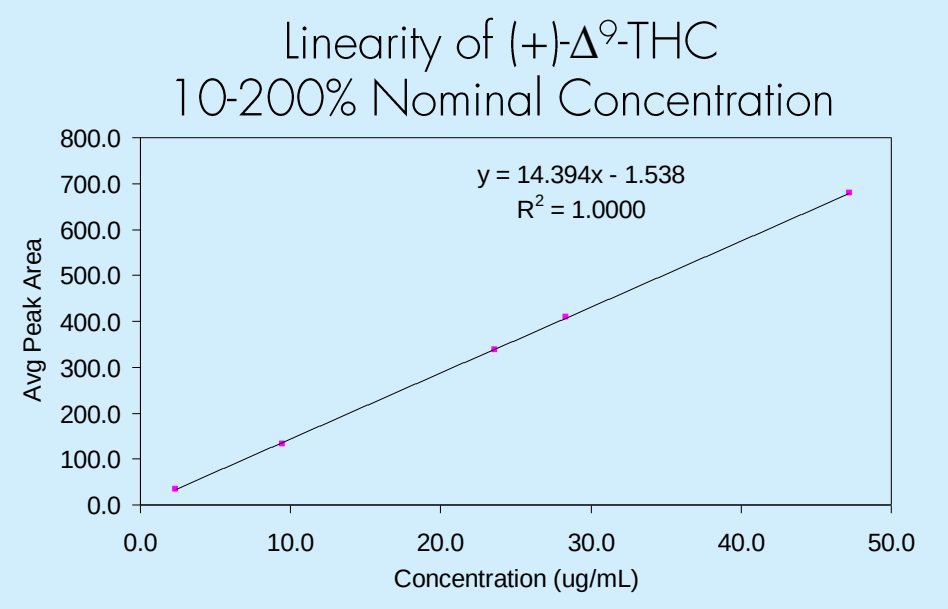
(+)-Δ^8-THC					(-)-Δ^8-THC				
% Nominal	Conc. (µg/mL)	Avg. Peak Area	%RSD	Response Factor	% Nominal	Conc. (µg/mL)	Avg. Peak Area	% RSD	Response Factor
2%	0.47	5.383	2.5%	11.5	0.25%	0.53	6.624	3.6%	12.5
10%	2.3	28.910	1.0%	12.3	1.25%	2.7	34.278	0.8%	12.9
40%	9.4	117.243	1.2%	12.5	5%	10.6	137.312	1.0%	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

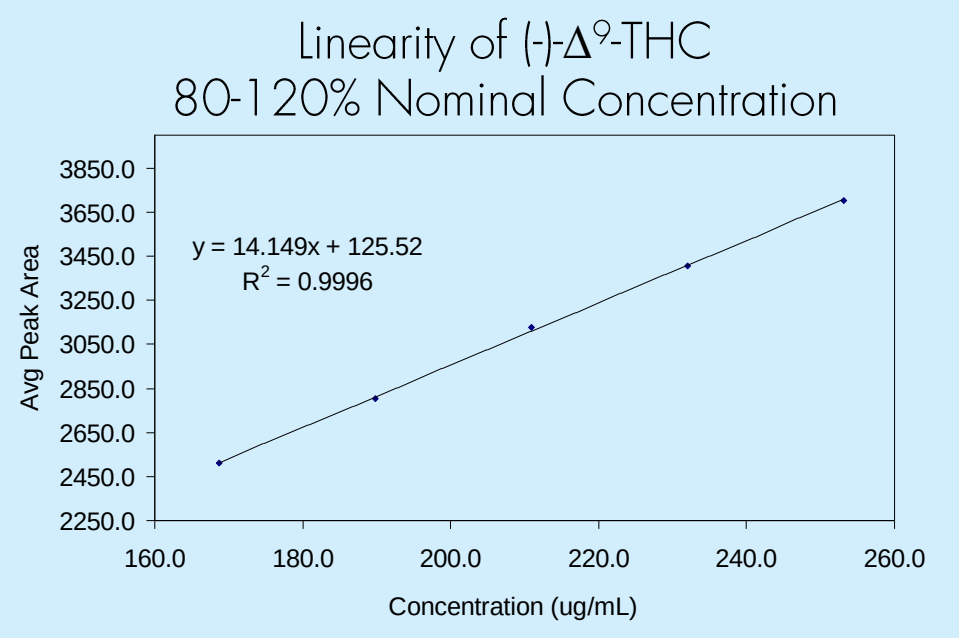




Summary of Data for Δ^9-THC Linearity, LOD and LOQ

(+)-Δ^9-THC					(-)-Δ^9-THC				
% Nominal	Conc. (µg/mL)	Avg. Peak Area	%RSD	Response Factor	% Nominal	Conc. (µg/mL)	Avg. Peak Area	% RSD	Response Factor
2%	0.47	8.208	37%	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
500%	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 µg/mL for (+) enantiomers and from 170 to 250 µg/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 ± 2 C)
  - –flow rate (0.7 ± 0.1 mL/min)
  - –injection volume (5 ± 2µL)

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 µL	0.00	0.01	0.00
Injection Volume @ 7 µL	-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 µg/mL)
  - (-) enantiomers @ 80%, 100%, 120% (Nominal = 200 µg/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%	98.394%	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%	1%
120%	115.3%	114.2%	1%

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

Level	Peak Area		Observed % Enantiomeric Excess		Theoretical % Enantiomeric Excess	Absolute Difference
	Average	%RSD	Average	%RSD		
LOQ	21.981	3.8%	98.037%	0.08%	98.187%	-0.1%
100%	366.527	1.7%	61.720%	0.50%	62.790%	-1.1%
120%	431.496	0.8%	56.478%	0.49%	57.771%	-1.3%

Precision

Expresses the agreement between a series of measurements obtained from multiple analyses of the same homogeneous sample under the prescribed conditions.
- Two Levels of Precision Evaluated
  - Repeatability
  - Intermediate precision, i.e.- Ruggedness
- Samples
  - (-)-Δ^8-THC and (-)-Δ^9-THC
  - Triplicate independent sample preparations

Repeatability

Precision under the same operating conditions over a short period of time.

Analyte	% Enantiomeric Excess		Response Factor	
	Average	%RSD	Average	%RSD
(-)-Δ^8-THC	99.24%	0.3%	12.81	0.7%
(-)-Δ^9-THC	98.65%	0.2%	12.37	1.6%

Intermediate Precision

Precision using different analysts, instruments, column and same lots of materials.

Analyte	% Enantiomeric Excess		Response Factor	
	Average	%RSD	Average	%RSD
(-)-Δ^8-THC	99.55%	0.02%	13.11	1.2%
(-)-Δ^9-THC	98.63%	0.03%	12.80	1.0%

High method precision for a chiral analysis with RSD's < 2.0% for response factors and < 0.50% for %ee determination.

Absolute difference in %ee < 0.50% for both enantiomers despite different analysts, columns, and instruments.

CONCLUSIONS

- The chiral method developed demonstrates simultaneous separation of all four Δ^9-THC & Δ^8-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.

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