

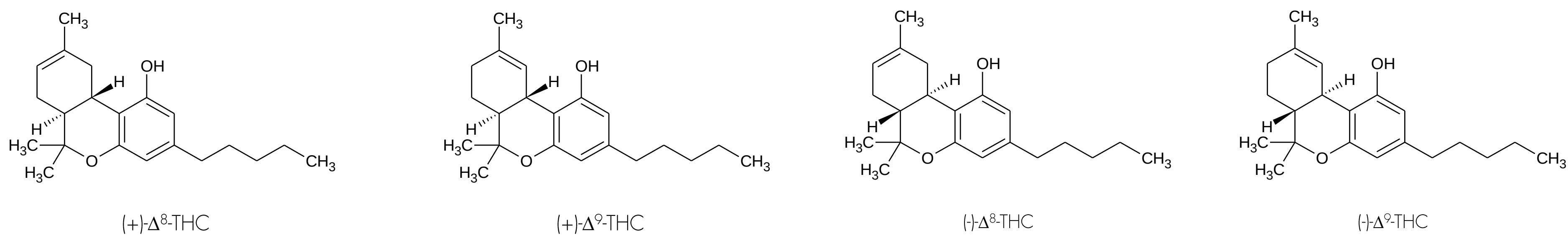
A Validated Chiral HPLC Method for Resolution of Δ⁸ and Δ⁹-tetrahydrocannabinol Enantiomers

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Introduction

- Background**
- (+)-Δ⁹-THC is an API known as Dronabinol
 - (+)-Δ⁹-THC enantiomer has little or no clinical effect^{1,2}
 - (+)-Δ⁹-THC may be synthesized from (±)-Δ⁸-THC³
 - FDA guidance: stereoisomeric composition must be quantitated for chiral API materials used in pharmacological, toxicological, and clinical studies⁴
- Literature**
- Achiral analysis of Δ⁸-THC & Δ⁹-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
 - (±)-Δ⁹-THC is light and air sensitive
 - High purity racemic Δ⁹-THC and Δ⁸-THC reference material not commercially available
 - Needed for method development, validation and ongoing use as system suitability standards
 - (±)-Δ⁹-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 Δ⁹-THC & Δ⁸-THC enantiomers

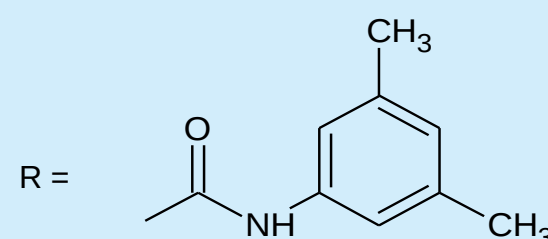
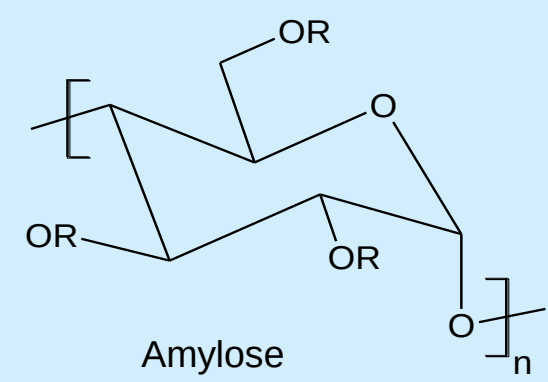


Analytical Method

- Normal Phase Chiral LC
 - Order of elution: (impurities first) (+)-Δ⁸-THC, (+)-Δ⁹-THC, (-)-Δ⁸-THC, (-)-Δ⁹-THC
 - Used to determine % enantiomeric excess
- Conditions
 - Chiralpak ADH 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 Δ⁹-THC & Δ⁸-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 (-)-Δ⁹-THC or (+)-Δ⁸-THC
 - Equation:

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

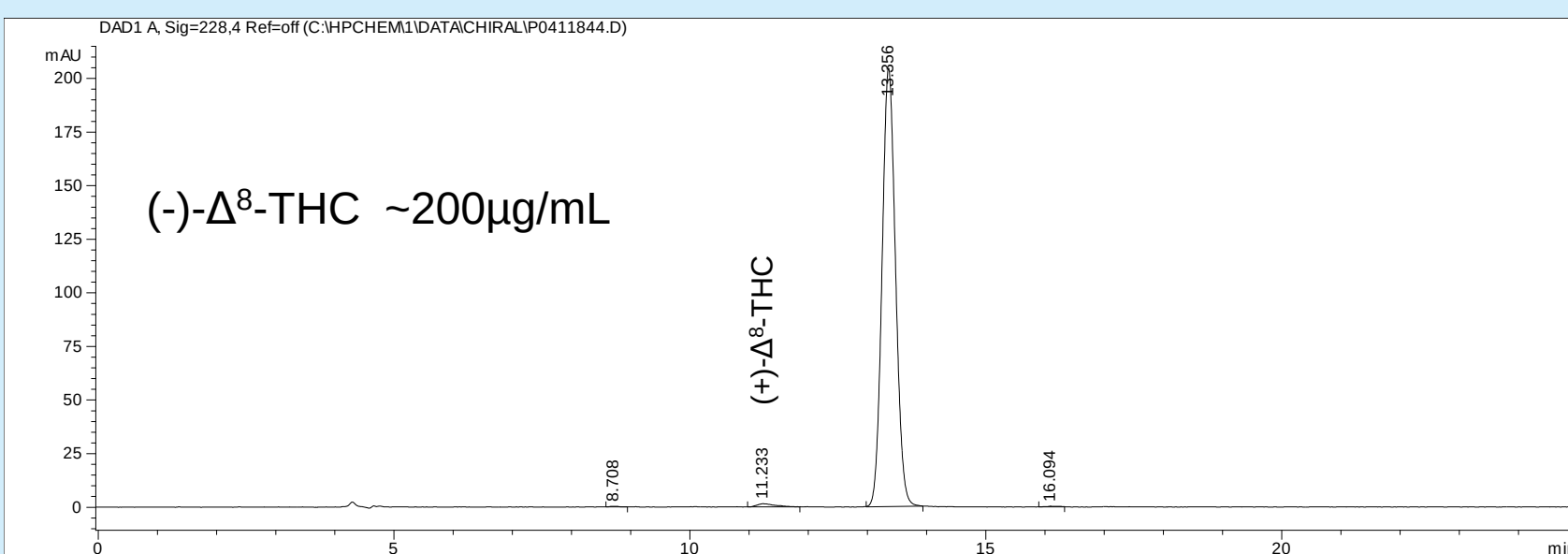
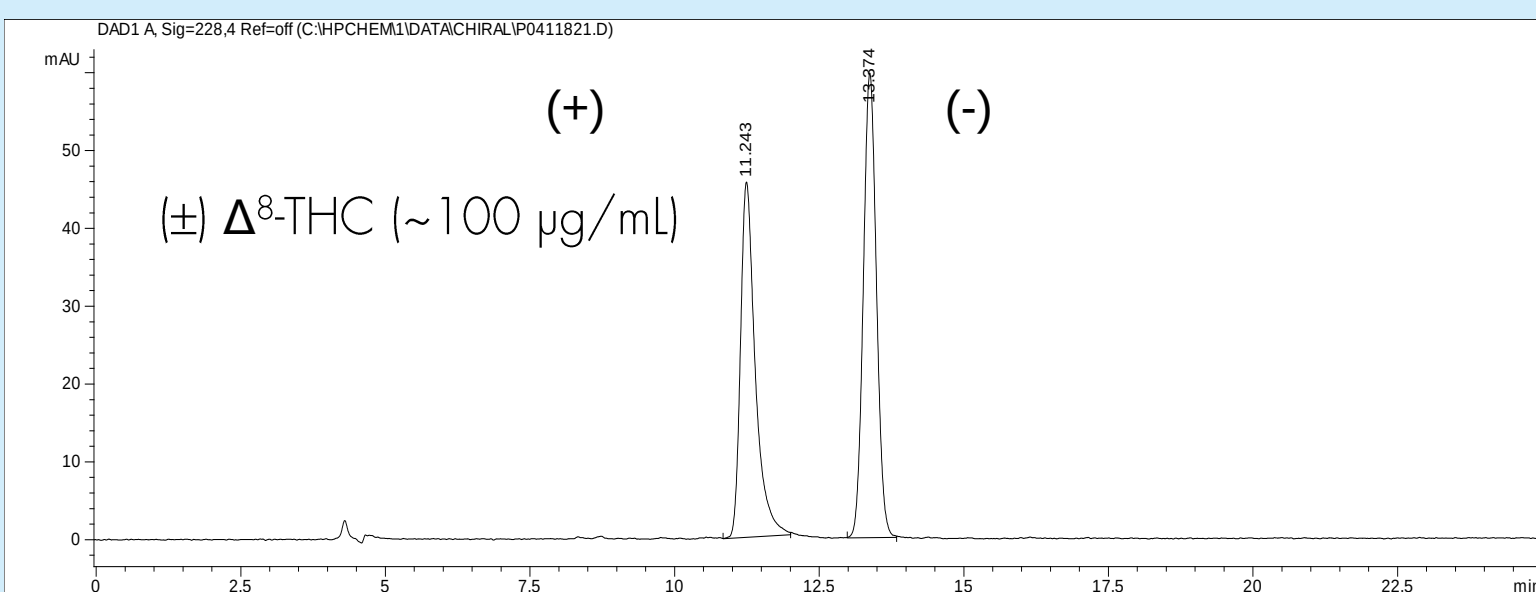
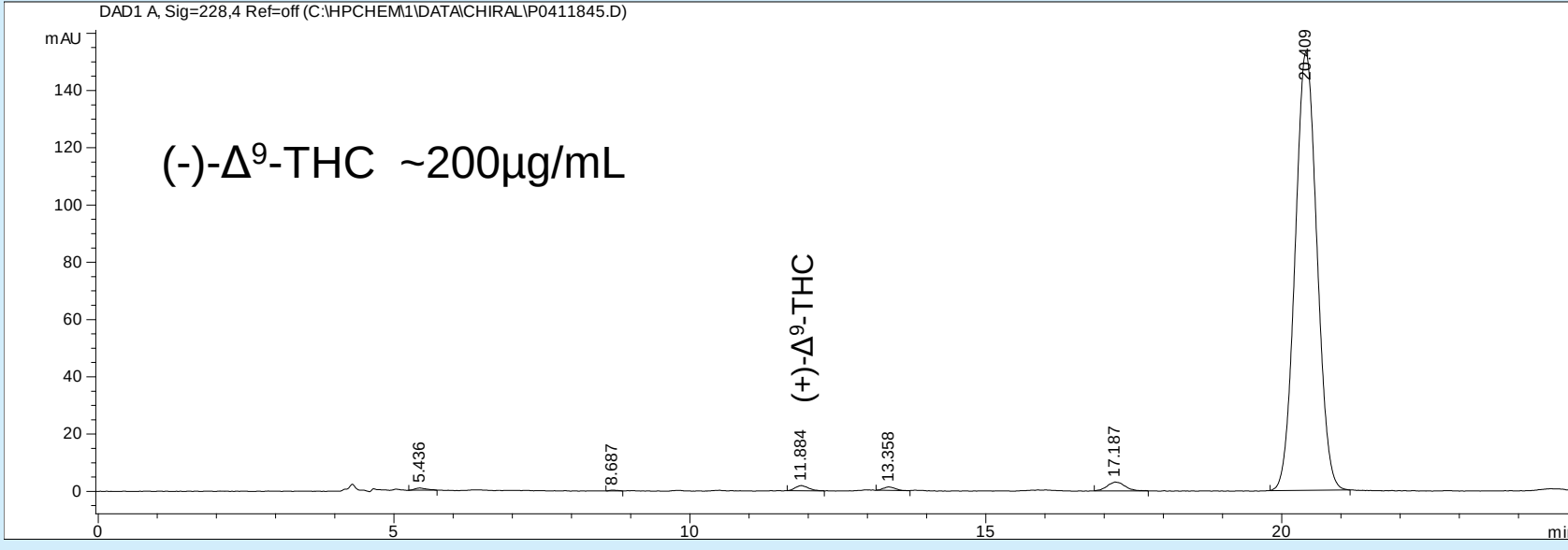
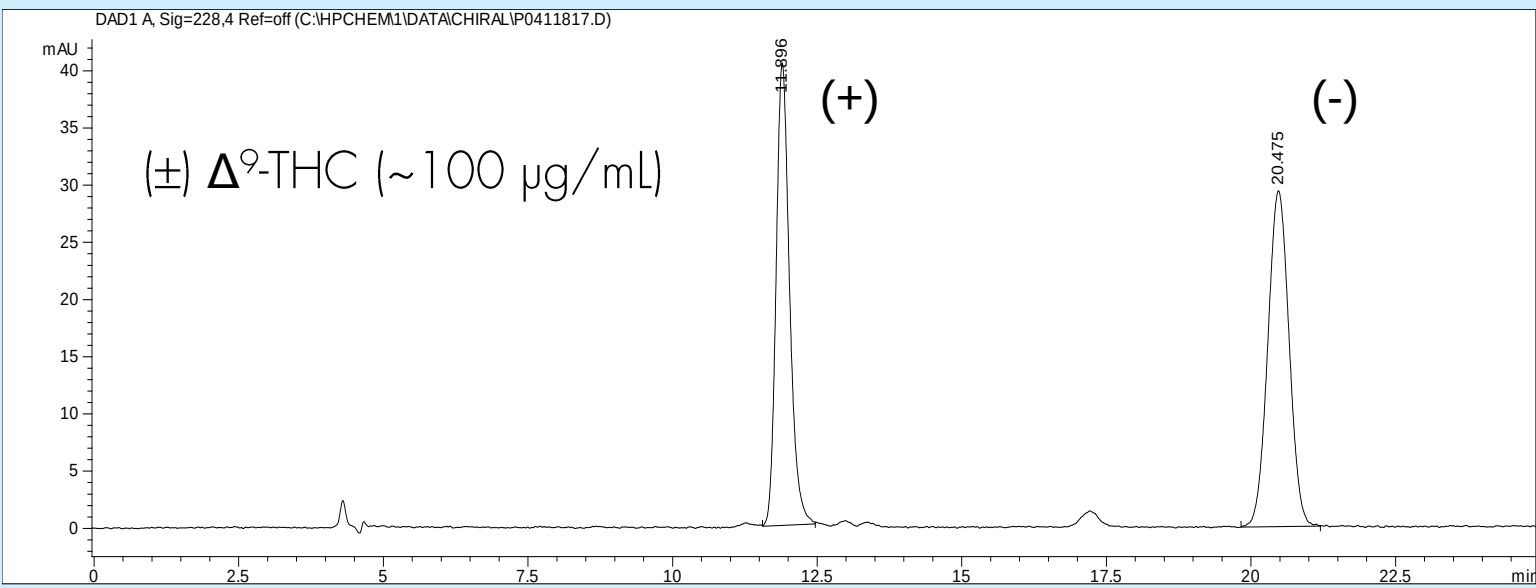
System Suitability

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

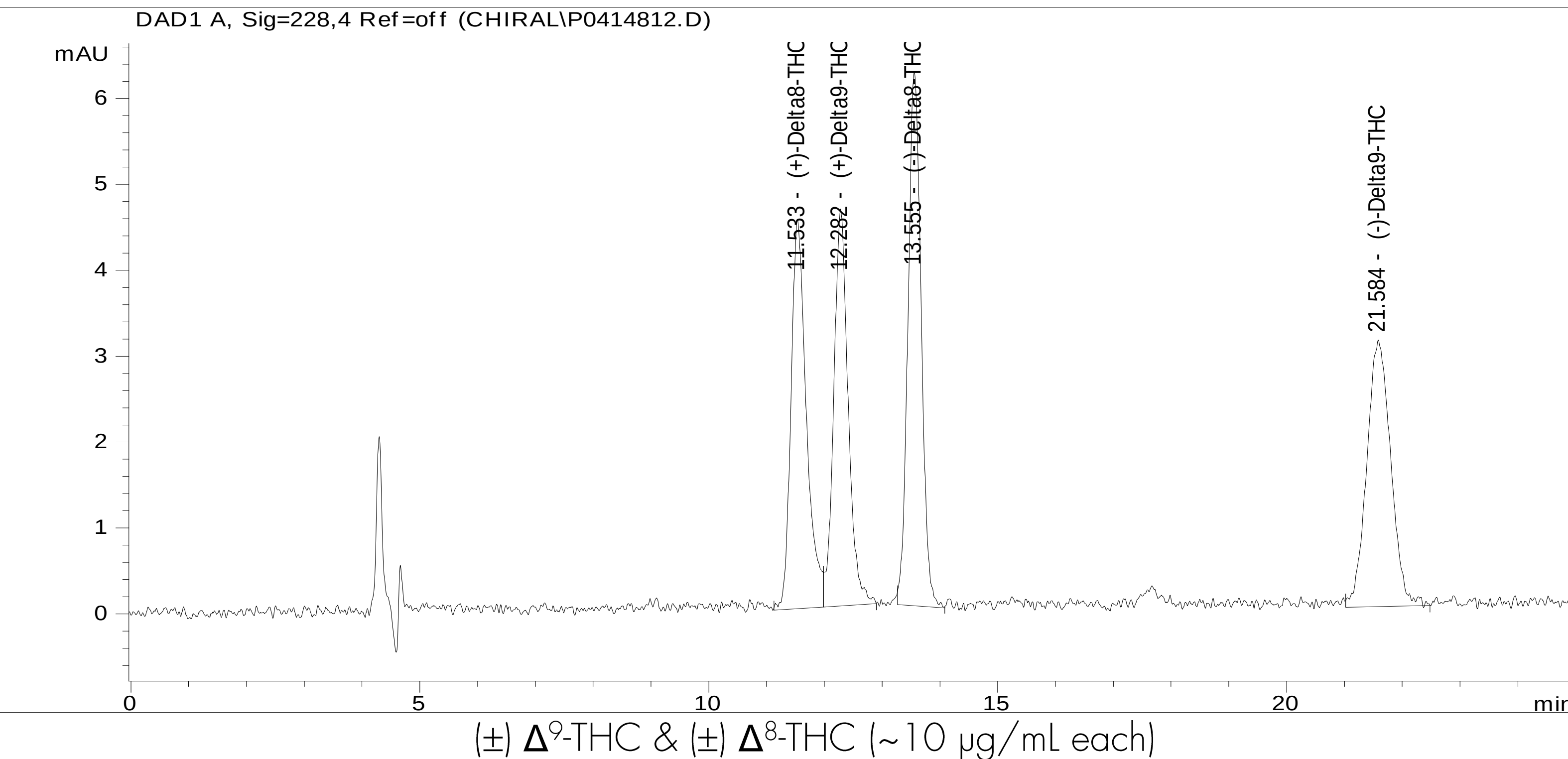
	Acceptance Criteria		Results (average)
Resolution	(+)-Δ ⁹ -THC, (-)-Δ ⁹ -THC	≥12.0	14.4
	(+)-Δ ⁸ -THC, (-)-Δ ⁸ -THC	≥3.0	5.1
	(+)-Δ ⁸ -THC, (+)-Δ ⁹ -THC	≥1.2	1.2
	(+)-Δ ⁹ -THC, (-)-Δ ⁸ -THC	≥1.8	4.0
Tailing	(+)-Δ ⁹ -THC	≤2.0	1.3
	(+)-Δ ⁸ -THC	≤2.0	1.6



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Racemic Resolution Standard



Linearity and Range

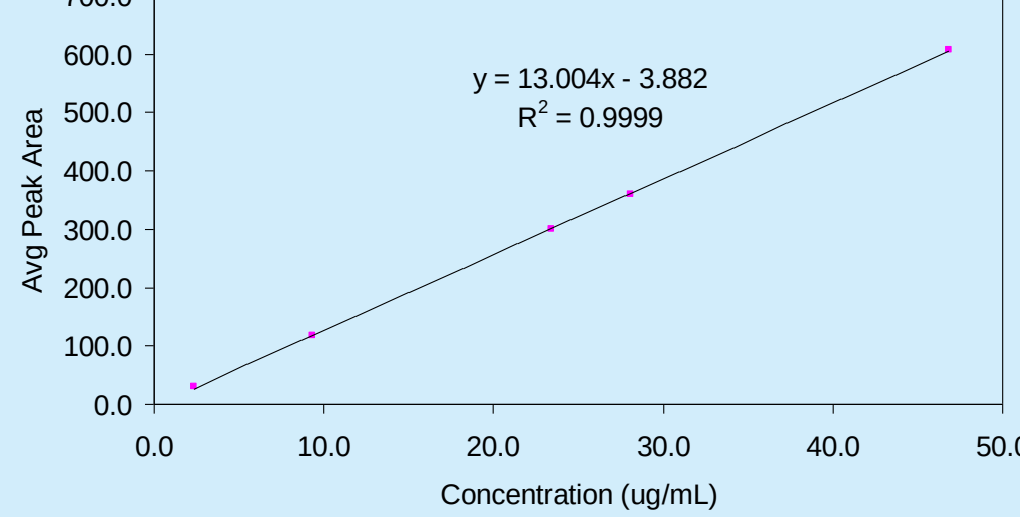
Method's ability to produce results that are directly proportional to the concentration of the analyte in the sample within a given range.

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

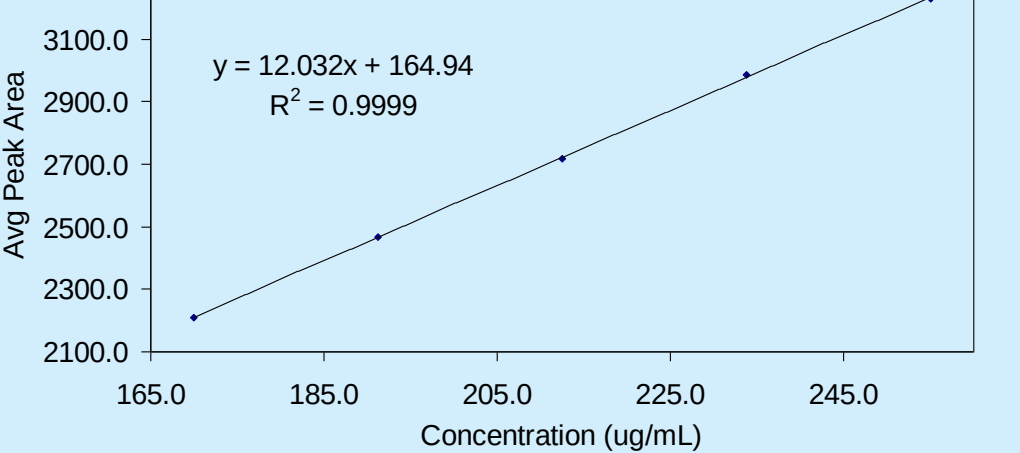
Summary of Data for Δ⁸-THC Linearity, LOD and LOQ

(+)-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	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	1971.594	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

Linearity of (+)-Δ⁸-THC
10-200% Nominal Concentration



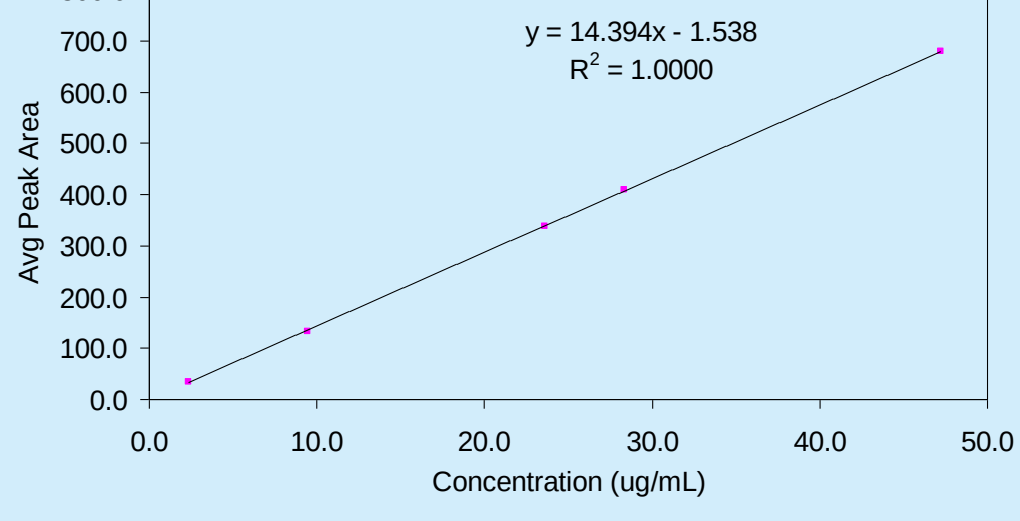
Linearity of (-)-Δ⁸-THC
80-120% Nominal Concentration



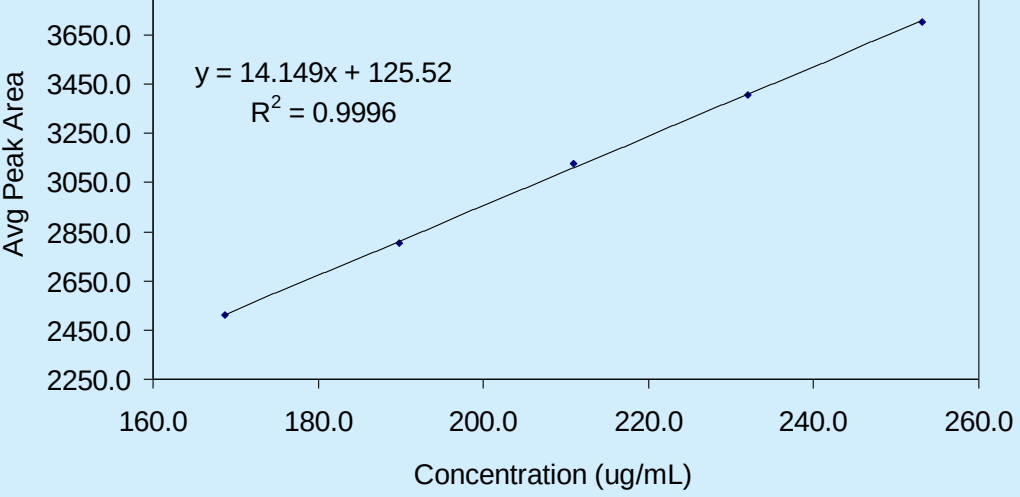
Summary of Data for Δ⁹-THC Linearity, LOD and LOQ

(+)-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	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%	14.5	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

Linearity of (+)-Δ⁹-THC
10-200% Nominal Concentration



Linearity of (-)-Δ⁹-THC
80-120% Nominal Concentration



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 (-)-Δ⁸-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 (-)-Δ⁸-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 (+)-Δ⁸-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 (+)-Δ⁸-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 (-)-Δ⁹-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 (-)-Δ⁹-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 (+)-Δ⁹-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 (+)-Δ⁹-THC (n=3)

Level	Peak Area		Observed % ee		Theoretical % ee	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.