



## APPLICATION NOTE

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# HPLC Method to Differentiate Four THC Stereoisomers: (6a*R*,9*R*)- $\Delta^{10}$ -THC, (6a*R*,9*S*)- $\Delta^{10}$ -THC, 9(*R*)- $\Delta^{6a,10a}$ -THC, and 9(*S*)- $\Delta^{6a,10a}$ -THC

Jeffrey B. Williams, MS, Kathleen B. Calati, Kirk W. Hering, Ph.D., Roxanne E. Franckowski, MS, and Donna M. Iula, Ph.D.

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## Key Features

- $\Delta^{10}$ -THC and  $\Delta^{6a,10a}$ -THC isomers produced from  $\Delta^9$ -THC degradation have stereochemical similarities that are challenging to differentiate.
  - Traditional reversed-phase (C18) HPLC analysis is not suitable for accurate determination of these isomers.
  - A method using a chiral HPLC column under normal-phase conditions offers reliable and robust identification of the four stereoisomers.
  - Use of a chiral HPLC stationary phase for characterization of these phytocannabinoid degradants is an essential tool for the determination of potency and safety.
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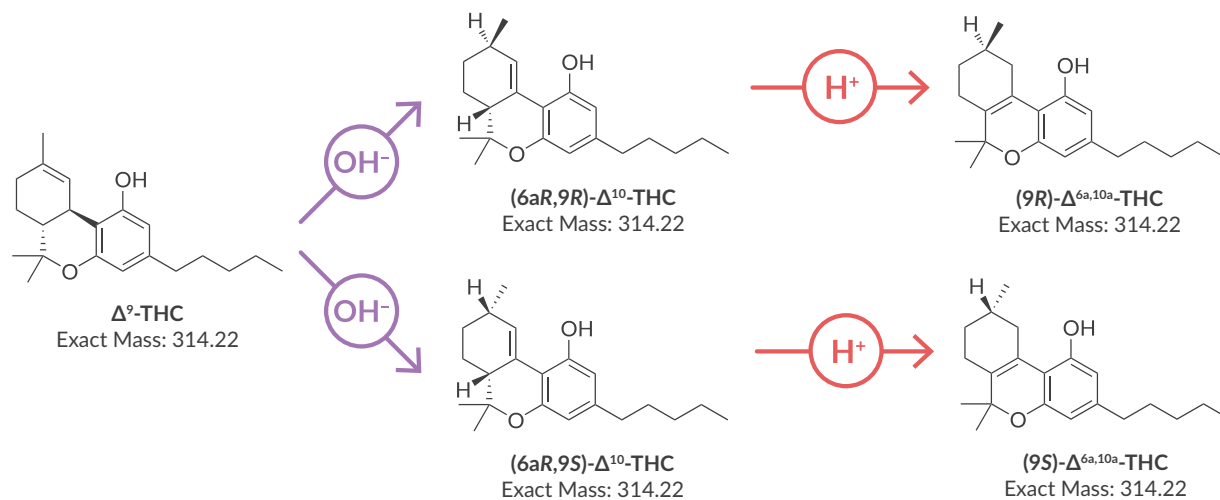
## Introduction

As the primary phytocannabinoid associated with psychoactive properties,  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) is crucial to analytical *Cannabis* testing. Degradation of  $\Delta^9$ -THC, from the processing of inflorescences for *Cannabis* isolates or other products, may lead to isomers that can be misidentified and provide invalid potency claims (**Figure 1**).  $\Delta^{10}$ -THC\* and  $\Delta^{6a,10a}$ -THC† isomers are of particular interest because of their stereochemical similarities, which makes traditional reversed-phase liquid chromatography (RPLC) analysis unreliable.

Under strongly alkaline conditions  $\Delta^9$ -THC is known to isomerize to two diastereomers, (6a*R*,9*S*)- $\Delta^{10}$ -THC and (6a*R*,9*R*)- $\Delta^{10}$ -THC.‡ These two distinct stereoisomers may undergo additional isomerization, even under mildly acidic conditions to form a pair of enantiomers, with the (6a*R*,9*S*)- $\Delta^{10}$ -THC providing the 9(*S*)- $\Delta^{6a,10a}$ -THC, and (6a*R*,9*R*)- $\Delta^{10}$ -THC providing the opposite enantiomer, 9(*R*)- $\Delta^{6a,10a}$ -THC. Only the 9(*S*)- $\Delta^{6a,10a}$ -THC isomer has been found to have psychoactive effects in animals, similar to  $\Delta^9$ -THC.§

The two diastereomers of  $\Delta^{10}$ -THC should be separable from each other and from the  $\Delta^{6a,10a}$ -THC enantiomers using reversed-phase HPLC conditions. However, their structural similarities make full resolution of these isomers difficult. Additionally, the enantiomers of  $\Delta^{6a,10a}$ -THC are not separable by HPLC without the use of chiral stationary phases.

This application note describes a method for the separation of the  $\Delta^{10}$ -THC and  $\Delta^{6a,10a}$ -THC isomers using a chiral HPLC stationary phase. The use of a chiral column under normal-phase liquid chromatography (NPLC) conditions provides an analytical method to fully separate these THC isomers for identification and accurate determination of potency.



**Figure 1.** Isomerization of  $\Delta^9$ -THC under acidic ( $\text{H}^+$ ) or basic ( $\text{OH}^-$ ) conditions to structurally similar isomers.

\*  $\Delta^2$ -THC in the monoterpene numbering system.

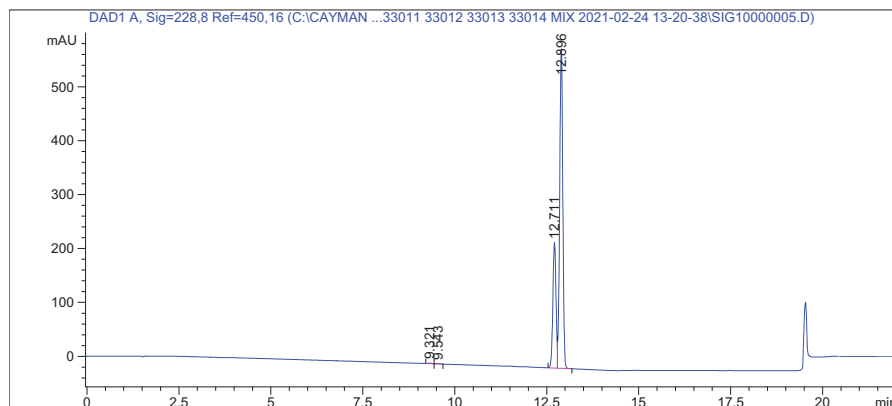
†  $\Delta^3$ -THC in the monoterpene numbering system.

‡ Srebnik, M., Lander, N., Breuer, A., *et al.* Base-catalysed double-bond isomerizations of cannabinoids: Structural and stereochemical aspects. *J. Chem. Soc. Perkin Trans. 1*, 2881-2886 (1984).

§ Järbe, T.U.C., Hiltunen, A.J., Mechoulam, R., *et al.* Separation of the discriminative stimulus effects of stereoisomers of  $\Delta^2$ - and  $\Delta^3$ -tetrahydrocannabinols in pigeons. *Eur. J. Pharmacol.* **156**(3), 361-366 (1988).

## Reversed-Phase HPLC

A typical RPLC method does not resolve these THC isomers. **Figure 2** shows a mix of all four isomers analyzed with a Gemini C18 column. The aqueous mobile phase (A) was prepared by adding 1 ml of trifluoroacetic acid (TFA) to 1,000 ml of deionized water to give 0.1% (v/v) TFA in water. The organic mobile phase (B) was acetonitrile. The mobile phase gradient is detailed in **Table 1**. Very similar results are seen with a variety of columns and buffer systems.



**Figure 2.** Typical resolution of the four isomers on reversed-phase chromatography.

**Table 1** - RPLC Conditions

Instrument	Agilent 1100 Series	
Column	Gemini C18 (150 x 4.6 mm, 5 µm)	
Mobile Phase	A: 0.1% (v/v) TFA in water B: Acetonitrile	
Gradient	Time (min)	%B
	0 – 12	50 – 90%
	12 – 20	90%
	20.1 – 25	50%
Flow Rate	1.0 ml/min	
Column Temp.	30°C	
Wavelength	UV monitored at 228 nm	

## Normal-Phase HPLC

The search for an RPLC method was extensive and, ultimately, an NPLC method using a chiral column was most successful at assessing the purity of each THC isomer (**Figure 3, Table 2**). An Agilent HPLC (1100 series) was set up for NPLC by switching the seals and thoroughly rinsing the lines to ensure all aqueous solvent had been removed. Chiral column CHIRALPAK® IB N-3 (250 x 4.6 mm, 3 µm) controlled at 30°C was used. Elution was accomplished with mobile phase 95:5 Hexane:Isopropyl Alcohol (IPA) at 0.85 ml/min for 15 minutes. A 1 µl injection of a 1 mg/ml solution in IPA was monitored at 228 nm.

The neat materials were formulated into certified reference material (CRM) solutions. To provide CRMs with optimal stability, each of the solutions were prepared as a 1 mg/ml solution in acetonitrile. Although acetonitrile and hexane are immiscible, the inclusion of IPA to the mobile phase maintains chromatography including baseline resolution of the analytes despite the diluent.

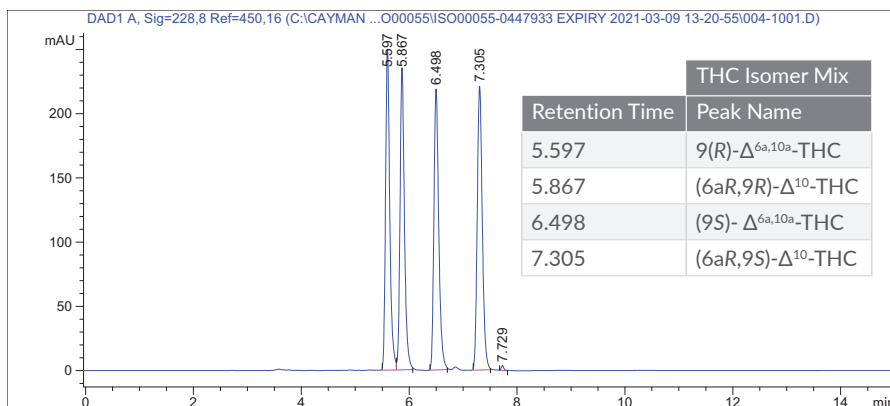


Figure 3. A co-injection of all four isomers in acetonitrile with the NPLC method.

Table 2 - NPLC Conditions

Instrument	Agilent 1100 Series
Column	CHIRALPAK® IB N-3 (250 x 4.6 mm, 3 $\mu$ m)
Mobile Phase	A: 95:5 Hexane:Isopropyl Alcohol
Flow Rate	0.85 ml/min
Column Temp.	30°C
Wavelength	UV monitored at 228 nm

## Conclusion

Determination of potency is essential for extracts, edibles, and other *Cannabis*-derived products. It is important to recognize that the additional processing required for providing these *Cannabis* products may result in their degradation. The four stereoisomers of  $\Delta^{10}$ -THC and  $\Delta^{6a,10a}$ -THC are products from one path of  $\Delta^9$ -THC degradation. Their stereochemical similarity makes optimal resolution of the four isomers unattainable with RPLC. Utilization of an immobilized cellulose chiral column under NPLC conditions provides baseline resolution of the four isomers to ensure an accurate qualification of phytocannabinoid components. This application note will aid in the development of testing methods to visualize and accurately quantify ingredients in *Cannabis* products.

## Cayman products used in the application

Item No.	Product Name
33011	(6aR,9R)- $\Delta^{10}$ -THC (CRM)
33012	(6aR,9S)- $\Delta^{10}$ -THC (CRM)
33013	9(R)- $\Delta^{6a,10a}$ -THC (CRM)
33014	9(S)- $\Delta^{6a,10a}$ -THC (CRM)

**Note:** All the items mentioned in this paper are manufactured in an ISO 17034 accredited facility.

Cayman's Phytocannabinoid Mixture 11 (CRM) can be used to identify more phytocannabinoids that coelute in a sample. It is provided as a DEA exempt preparation and is available at three different concentrations. Included are  $\Delta^9$ -THC,  $\Delta^8$ -THC, tetrahydrocannabinolic acid A, cannabinol, cannabidiol, cannabidiolic acid, ( $\pm$ )-cannabichromene, cannabigerol, cannabigerolic acid, cannabidivarin, and tetrahydrocannabivarin.

Item No.	Product Name
21306	Phytocannabinoid Mixture 11 (CRM) (1 ml, 250 $\mu$ g/ml)
32841	Phytocannabinoid Mixture 11 (CRM) (0.5 ml, 1 mg/ml)
32842	Phytocannabinoid Mixture 11 (CRM) (1 ml, 100 $\mu$ g/ml)



Learn about identifying unknown impurities in *Cannabis* products.  
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