Application Note

# GC Separation for Identification of *iso*-THC Contaminants and Accurate Quantification of $\Delta^8$-THC and $\Delta^9$-THC in *Cannabis* Samples

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## Key Features

- Synthetic conversion of cannabidiol (CBD) to the tetrahydrocannabinols (THCs) $\Delta^9$-THC and $\Delta^8$-THC produces measurable quantities of the *iso*-THC products $\Delta^8$-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC.
- Co-elution of THCs and *iso*-THCs makes C18 reversed phase-HPLC (RP-HPLC) methods unsuitable for the quantification of THCs. Quantities of THCs are distorted if the testing methods are unable to resolve the *iso*-THCs in analyzed samples.
- Gas chromatography (GC) offers a reliable and robust method for quantification of $\Delta^9$-THC, $\Delta^8$-THC, $\Delta^8$-*iso*-THC, and $\Delta^{4(8)}$-*iso*-THC. Additionally, this method represents a method for identifying THC derived from CBD conversion.

# Introduction

Analytical testing of *Cannabis* products for identification and accurate quantification of psychoactive components such as $\Delta^9$-THC and $\Delta^8$-THC is a regulatory requirement for safety and distribution. However, quantification of THCs may be complicated by the introduction of the closely eluting side products $\Delta^8$-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC.[1-3] These constitutional isomers of THC form during synthetic conversion of CBD under acidic conditions (**Figure 1**).

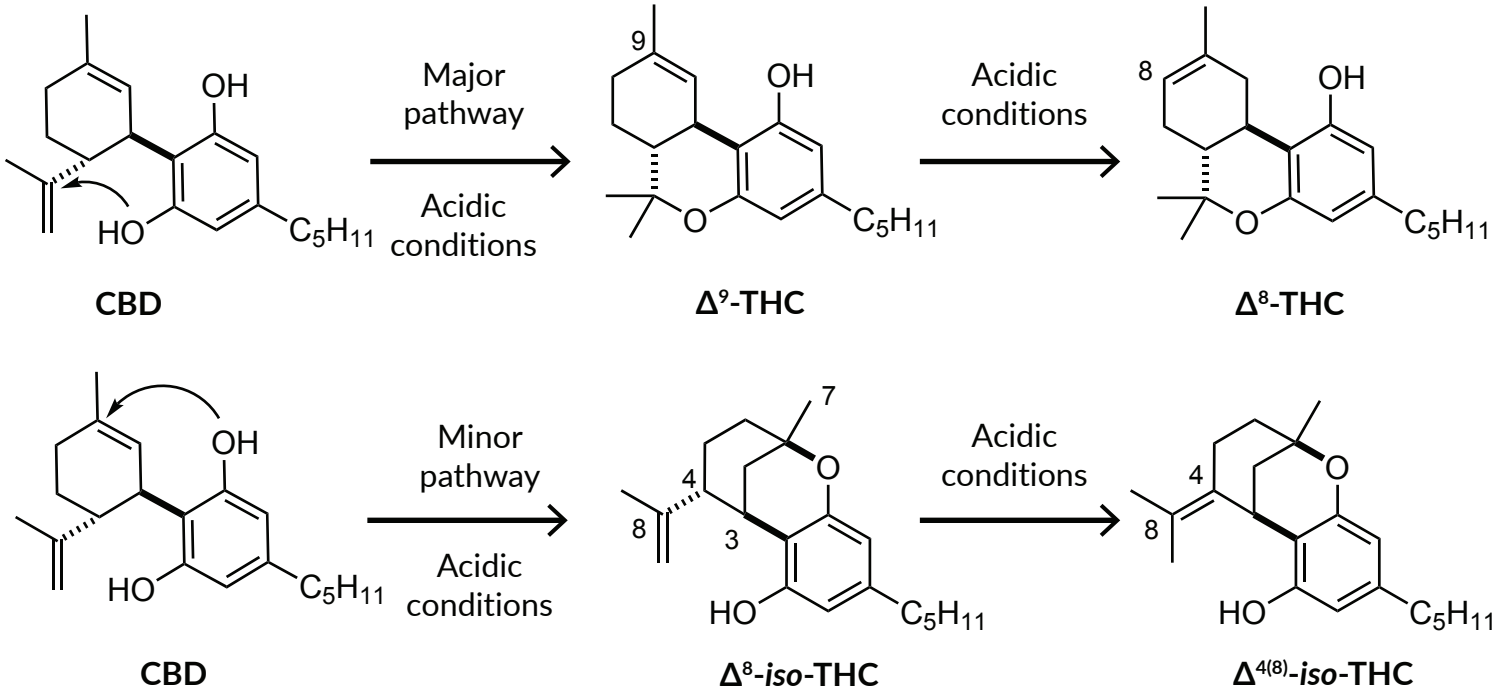


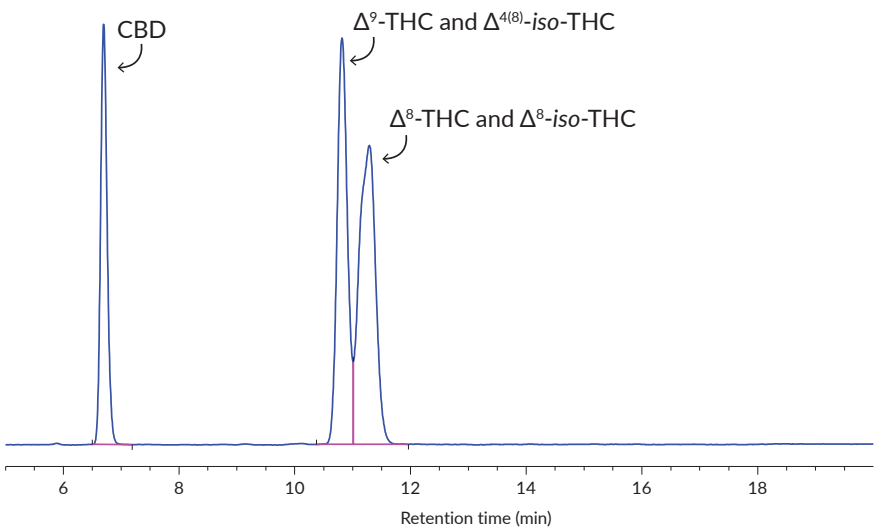

**Figure 1.** Acid-catalyzed cyclization of CBD to form THCs and *iso*-THCs.

The *iso*-THCs were first identified by Mechoulam's research group in a 1968 paper where CBD treated with the Lewis acid boron-trifluoride diethyl etherate led to formation of THC with 13% of $\Delta^8$-*iso*-THC isolated as a side product. Mechoulam also identified $\Delta^{4(8)}$-*iso*-THC in this same paper and demonstrated that it could be formed by boiling $\Delta^8$-*iso*-THC in a mixture of sulfuric acid and methanol.[4] A 2020 *Journal of Natural Products* publication by the Passarella group also highlights the formation of these two isomers under a number of acidic conditions and provides thorough NMR characterization.[5] To date, the *iso*-THCs have not been tested for pharmacological activity or safety in any published results. Since the *iso*-THCs have not been found naturally in any *Cannabis sativa L.*, their presence in *Cannabis* products serves as a useful marker to identify THC derived from the synthetic conversion of CBD.

This application note highlights the co-elution observed between THCs and *iso*-THCs by RP-HPLC and presents a GC method that provides for baseline separation and quantification of these species.

# RP-HPLC Methods

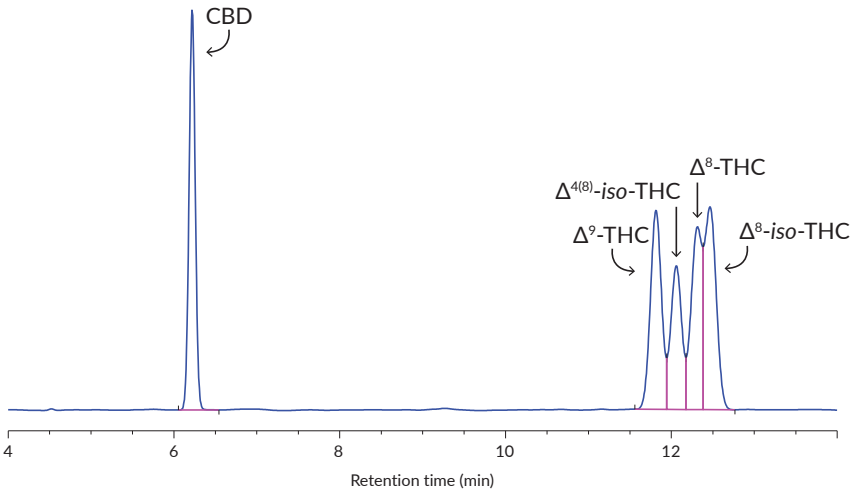
A mixture of equal parts CBD, Δ⁹-THC, Δ⁸-THC, Δ⁸-*iso*-THC, and Δ⁴(⁸)-*iso*-THC was injected on a Gemini® C18 column using isocratic RP conditions, resulting in significant co-elution of the THCs and *iso*-THCs (**Figure 2**). In conventional samples of CBD-derived THCs, the amount of *iso*-THCs present is likely to be a much smaller percentage relative to the THCs and would almost certainly be hidden underneath the THC peaks with similar chromatographic conditions. Consequently, the presence of these side products would likely be presented as a false enhancement of the co-eluting THC peak.




| Analyte | Retention time (min) | Item No. |
|---|---|---|
| CBD | 6.62 | ISO60156 |
| Δ⁹-THC | 10.828 | ISO60157 |
| Δ⁴(⁸)-*iso*-THC | 10.828 | 33863 |
| Δ⁸-THC | 11.115 | ISO60158 |
| Δ⁸-*iso*-THC | 11.287 | 33864 |

**Figure 2.** Co-injection of CBD, Δ⁹-THC, Δ⁴(⁸)-*iso*-THC, Δ⁸-THC, and Δ⁸-*iso*-THC on a Gemini® C18 column under RP conditions. Data was acquired on an Agilent 1100 Series HPLC with a Gemini® C18 column (250 mm x 4.6 mm, 5 µm) using a 20:80 water:acetonitrile with 0.1% acetic acid mobile phase, 1 ml/min flow rate, 40°C column temperature, and monitoring UV at 228 nm.

Efforts to find a more optimized RP-HPLC method involved screening several columns and conditions. Improved results were observed using a Raptor® ARC-C18 HPLC column under isocratic RP conditions (**Figure 3**). However, this method still results in significant overlap of the *iso*-THC and THC analytes and a lack of baseline resolution. As such, GC was explored next as another possible way to fully resolve these isomers.

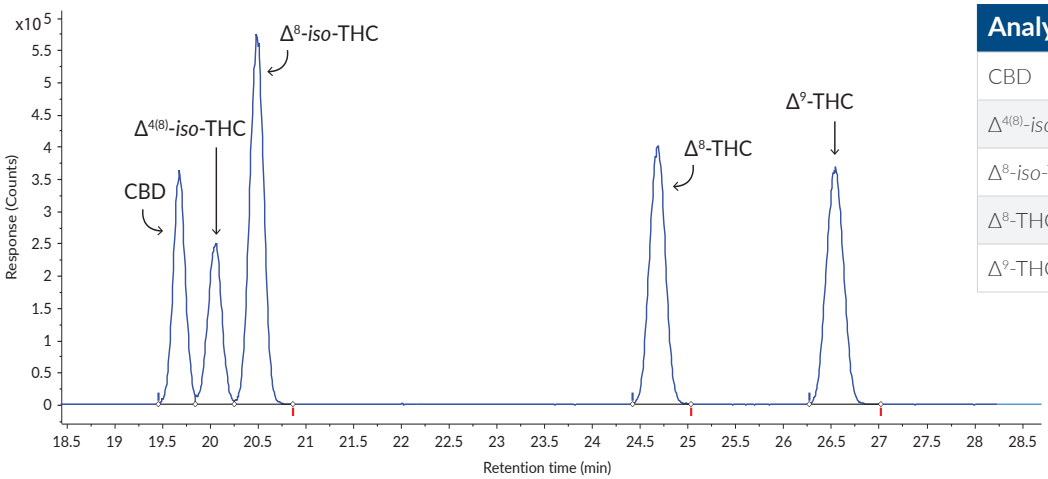



| Analyte | Retention time (min) | Item No. |
|---|---|---|
| CBD | 6.221 | ISO60156 |
| Δ⁹-THC | 11.816 | ISO60157 |
| Δ⁴(⁸)-*iso*-THC | 12.062 | 33863 |
| Δ⁸-THC | 12.317 | ISO60158 |
| Δ⁸-*iso*-THC | 12.469 | 33864 |

**Figure 3.** Co-injection of CBD, Δ⁹-THC, Δ⁴(⁸)-*iso*-THC, Δ⁸-THC, and Δ⁸-*iso*-THC on a Raptor® ARC-C18 column under RP conditions. Data was acquired on an Agilent 1100 Series HPLC with a Raptor® ARC-C18 column (150 mm x 4.6 mm, 2.7 µm) using a 30:70 water:acetonitrile with 0.1% acetic acid mobile phase, 1 ml/min flow rate, 40°C column temperature, and monitoring UV at 228 nm.

# GC Method

An optimized GC method for separating a mixture of CBD, Δ⁹-THC, Δ⁸-THC, Δ⁸-*iso*-THC, and Δ⁴⁽⁸⁾-*iso*-THC resulted in baseline resolution of all the peaks and is shown in **Figure 4**. One of the highlights of this method is that the *iso*-THCs elute closer to CBD and have greater than four minutes separation in retention time from the THCs. Hence, this provides an excellent means to determine the quantity of *iso*-THCs present in a sample with complete resolution from the THCs. While this method was demonstrated on a GC-MS instrument, the conditions may be amended for GC-FID instruments.




| Analyte | Retention time (min) | Item No. |
|---|---|---|
| CBD | 19.675 | ISO60156 |
| Δ⁴⁽⁸⁾-*iso*-THC | 20.066 | 33863 |
| Δ⁸-*iso*-THC | 20.482 | 33864 |
| Δ⁸-THC | 24.683 | ISO60158 |
| Δ⁹-THC | 26.546 | ISO60157 |

**Figure 4.** Co-injection of CBD, Δ⁴⁽⁸⁾-*iso*-THC, Δ⁸-*iso*-THC, Δ⁸-THC, and Δ⁹-THC on GC method.
Data was acquired on an Agilent 8890 GC equipped with a 5977B MS detector, using a Restek® Rtx-5MS capillary column (30 m x 320 µm x 0.5 µm). The injector temperature was set at 300°C, and the oven temperature was programmed to start at 50°C and increase 40°C/min to 210°C, hold 20 min, then increase 40°C/min to 300°C and hold 8.75 min. The total run time was 35 min. The injection mode was set to split with a ratio of 60:1. Helium was used as the carrier gas at a constant flow of 2 ml/min.

# Conclusion

Quantification of Δ⁹-THC and Δ⁸-THC content in extracts, edibles, and other *Cannabis*-derived products has become increasingly important as these materials are legalized for medicinal and recreational use. Both synthetic production and chemical conversion of plant-derived materials may result in the formation of *iso*-THCs, closely eluting side products of THCs. While most RP-HPLC methods may not fully resolve the *iso*-THCs from Δ⁹-THC and Δ⁸-THC, we present here a GC method that can be used to fully resolve these compounds, aiding in the development of testing methods to identify and accurately quantify THCs and *iso*-THCs in *Cannabis* products.


## Acknowledgements

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## References


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