

# Chromatographic Resolution of Naturally Occurring Enantiomeric Cannabinoids Using SFC-MS

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## Introduction

Membrane proteins are frequently the targets for binding ligands, including small drug molecules. The intrinsic chirality of protein drug receptors often governs specific pharmacological response to ligands with particular stereochemical geometries. Characterization of the potency, efficacy, and safety of individual enantiomeric ligands is therefore important.

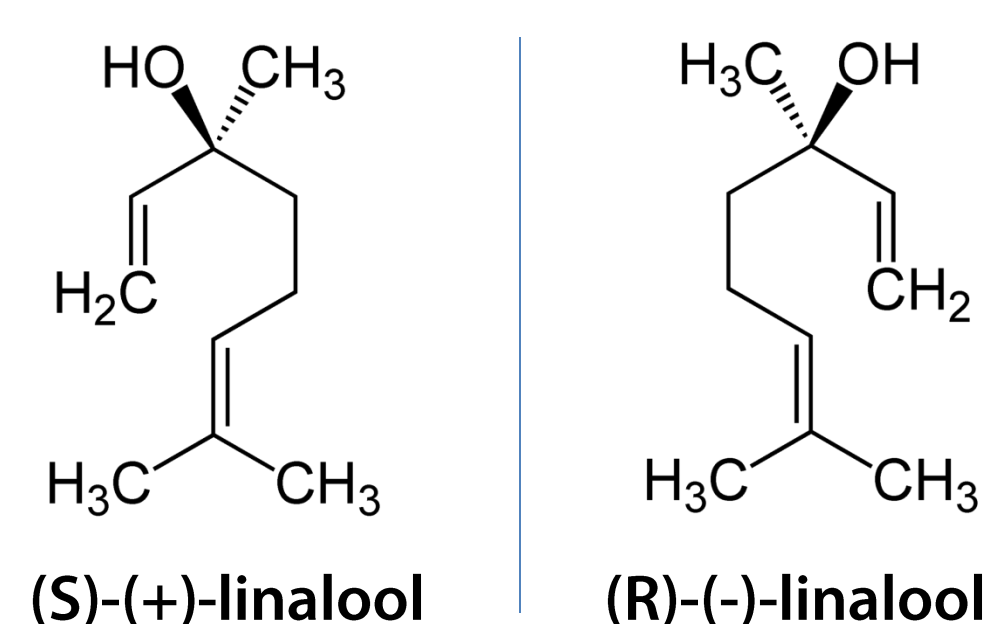
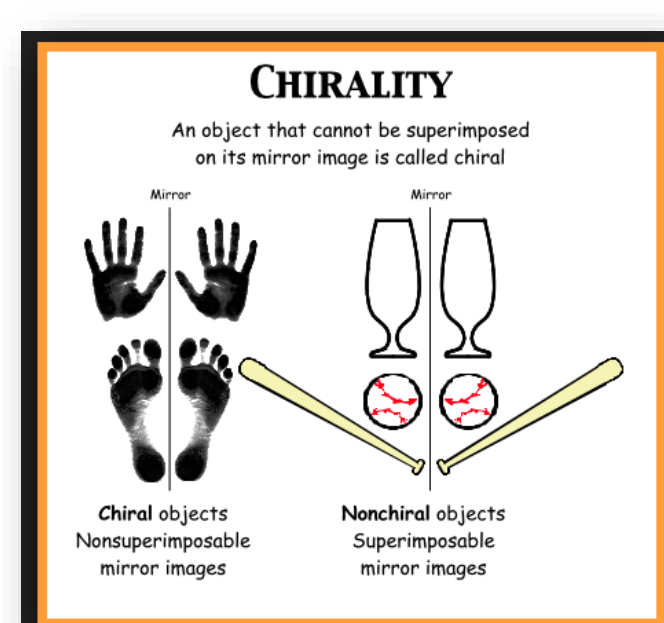
Naturally occurring cannabinoids often contain chiral molecules, and therefore stereoselective analysis of phytocannabinoids may prove essential for studying efficacy, phenotype determination, and quality control and stability testing of medicinal cannabis. Chiral analysis is even more critical for synthetically-produced cannabinoids, as creating stereoisomers in the synthesis process is often unavoidable.

Herein, we demonstrate chromatographic resolution of the natural racemic cannabinoid, cannabichromene (CBC), in plant extract, along with the synthetic racemic (+/-)- $\Delta^9$ -trans-tetrahydrocannabinol using supercritical fluid chromatography (SFC) coupled with mass spectrometry (MS).

## What is Chirality?

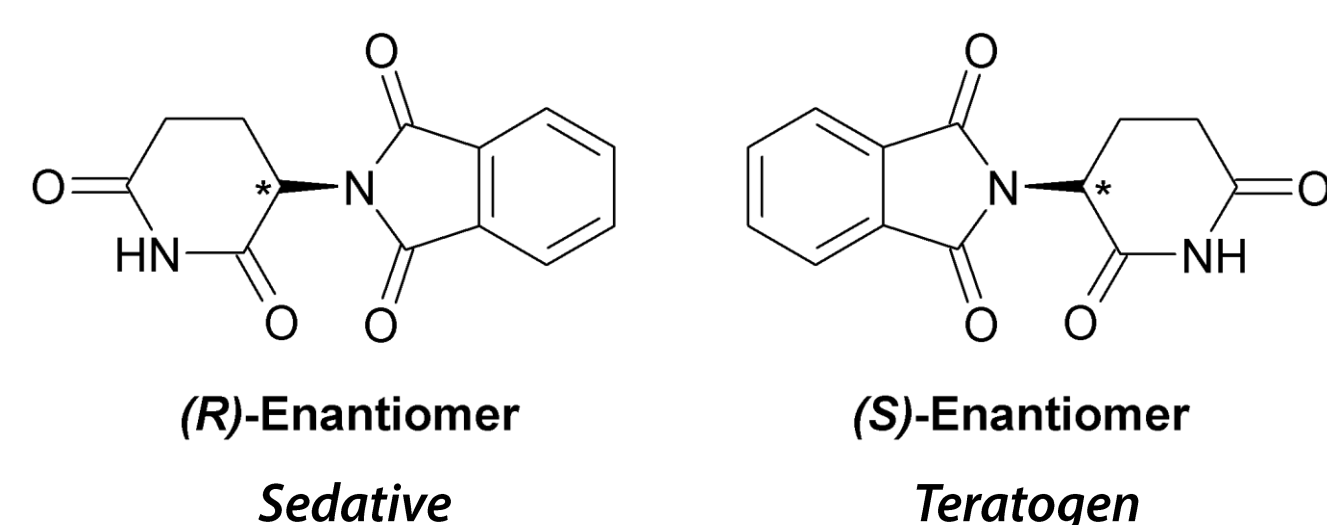
Chirality, the synonym for “handedness”, from the Greek word for hand, refers to an object that is nonsuperimposable on its mirror image (e.g., the left and right hand). Chiral pairs of molecules (i.e. enantiomers) are physically and chemically indistinguishable in achiral environments. They exhibit identical melting points, boiling points, densities, solubilities, etc.

Enantiomers are denoted by the configuration of the functional groups around a chiral center. The Cahn-Ingold-Prelog rules assign priority to the functional groups, and the ordering of those groups establishes the “R” or “S” designation of the chiral center.



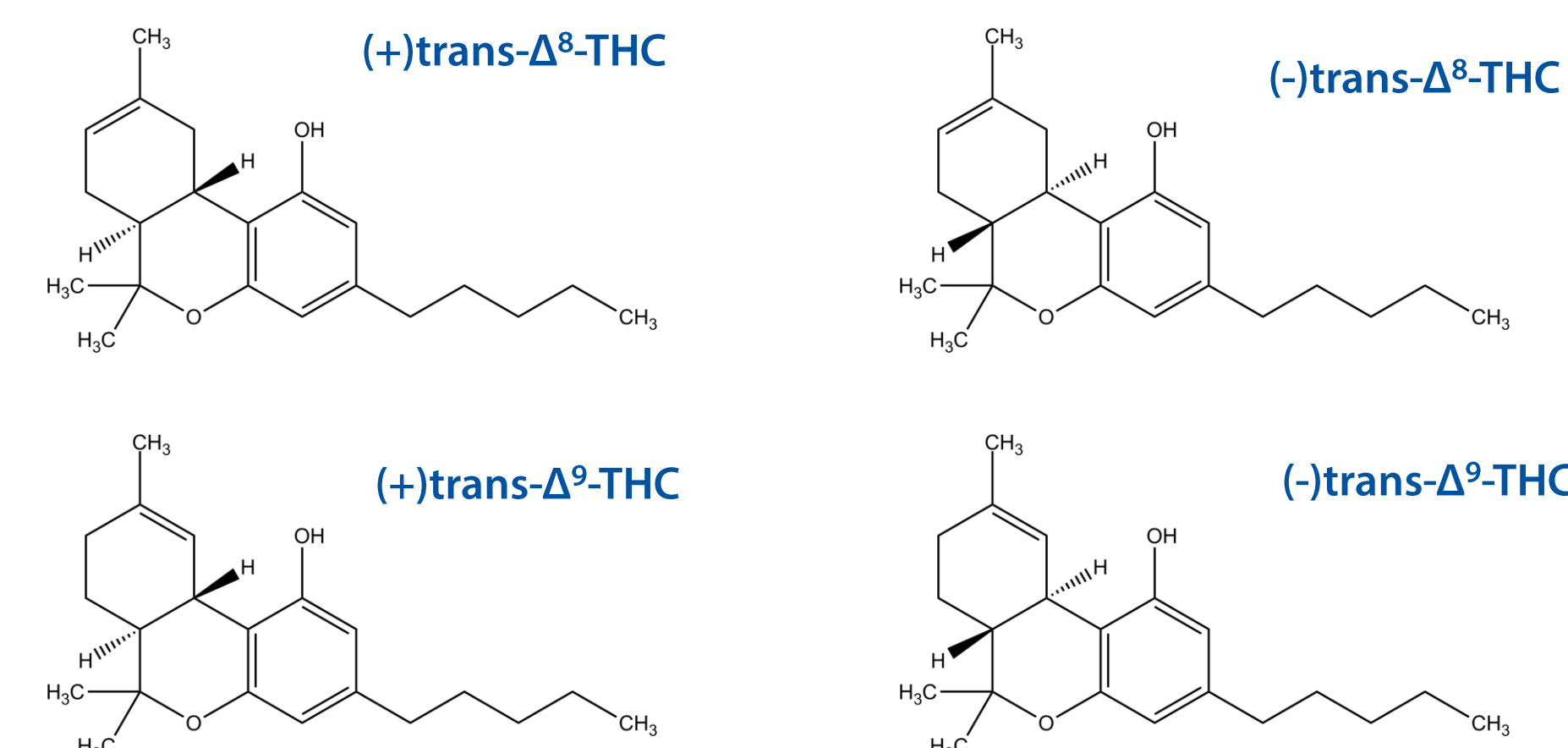
## Why is Chirality important?

Oftentimes, one enantiomer may bind to the receptor more readily and be responsible for the desired effect against a disease target. The other enantiomer may reduce potency and/or efficacy by binding to the receptor, but not causing the desired pharmacological effect. In the worst of cases, one enantiomer may cause toxic side effects. In the late 1950's/early 1960's, thalidomide was prescribed as a sedative and for treating morning sickness in pregnant women. The drug interfered with the babies' normal development, causing many of them to be born with shortened, absent, or flipper-like limbs. It was later learned that the S-configuration of the racemic thalidomide caused the observed birth defects, while the R-configuration caused the desired drug effect. The tragedy surrounding thalidomide prompted profound changes in FDA requirements for drug safety, and today, chirality be must considered early in the drug discovery process.



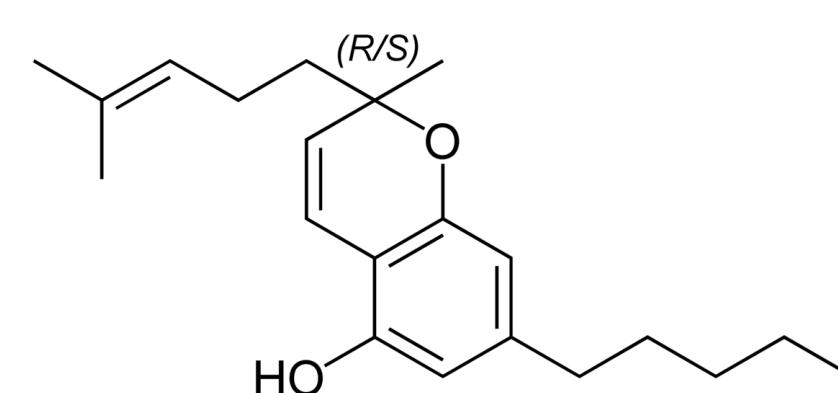
## What does Chirality have to do with Phytocannabinoids?

Depending on how they are derived, cannabinoids can exist in many isomeric forms. THC has four predominant forms and include (+)trans- $\Delta^8$ -THC, (-)trans- $\Delta^8$ -THC, (+)trans- $\Delta^9$ -THC, and (-)trans- $\Delta^9$ -THC. The main plant-derived stereoisomer is (-)trans- $\Delta^9$ -THC, while many synthetic preparations of THC produce the more stable  $\Delta^8$ -THC isomer or a mixture of positional and stereoisomers. Just as with other pharmaceutically active ingredients, the stereochemistry of THC affects pharmacological activity. As more research is done on the efficacy of THC and other cannabinoids against specific disease targets, it is important to have methods of separating stereoisomers to fully characterize potency, efficacy, and safety of individual enantiomeric ligands.



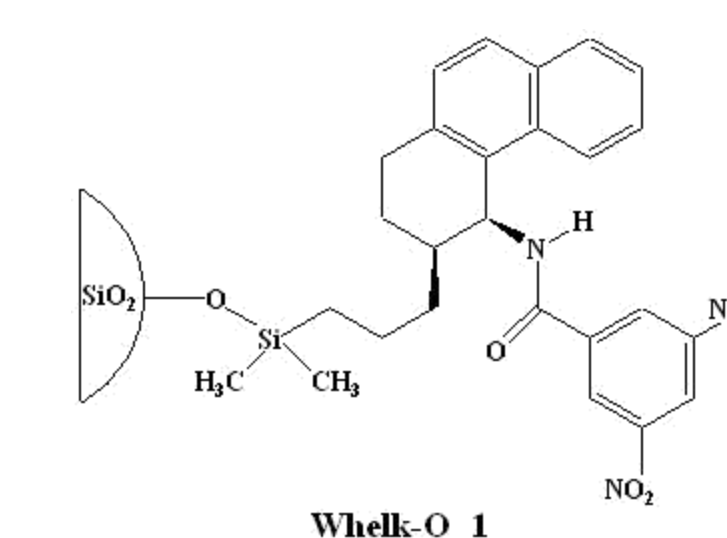
## Experimental

The presence of natural racemic compounds (*rac*-CBC) in plant extract was investigated using a Pirkle-type chiral stationary phase (CSP) with Supercritical Fluid Chromatography coupled with a mass spectrometer. All of the analyses presented here were performed using Whelk-O<sup>®</sup> 1 chiral columns packed with 1.8  $\mu$ m-sized particles. Method conditions are noted with resulting chromatograms.



## Pirkle-type Chiral Selectors

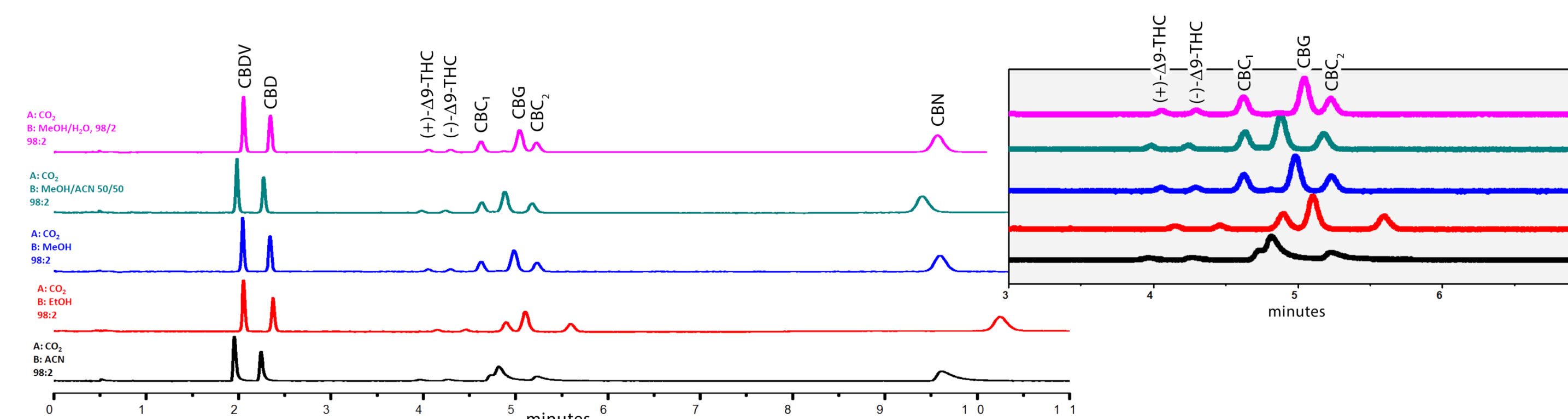
Chromatographic separations of enantiomers can only be performed in chiral environments. A chiral selector can be used to separate enantiomers based on the number and types of interactions between the selector and analytes. Pirkle-type chiral selectors are one of the most popular classes of chiral stationary phases used in HPLC and SFC. Whelk-O 1 is an n-electron acceptor/n-electron donor phase that has broad selectivity and resolution for a wide variety of racemates.



Since the phase is covalently bonded to silica, the column is compatible with all commonly used mobile phases and can be used for both normal phase and reversed-phase separations.

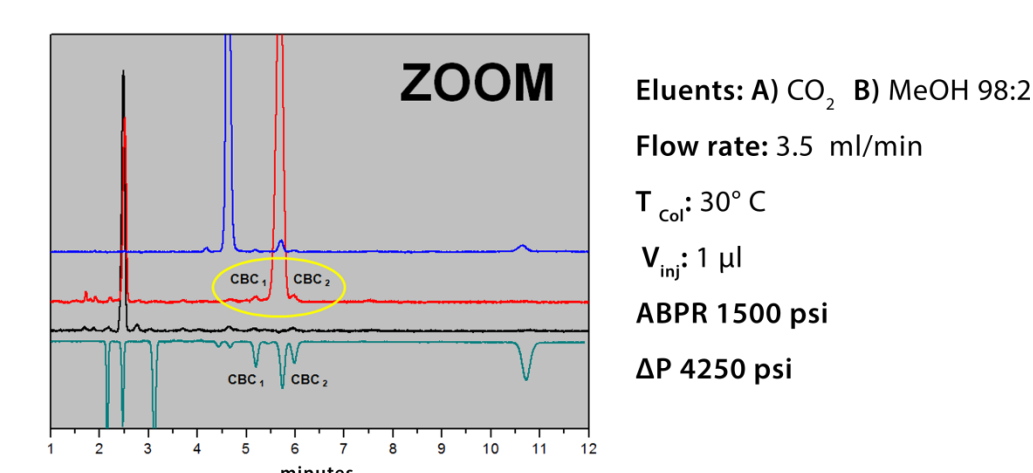
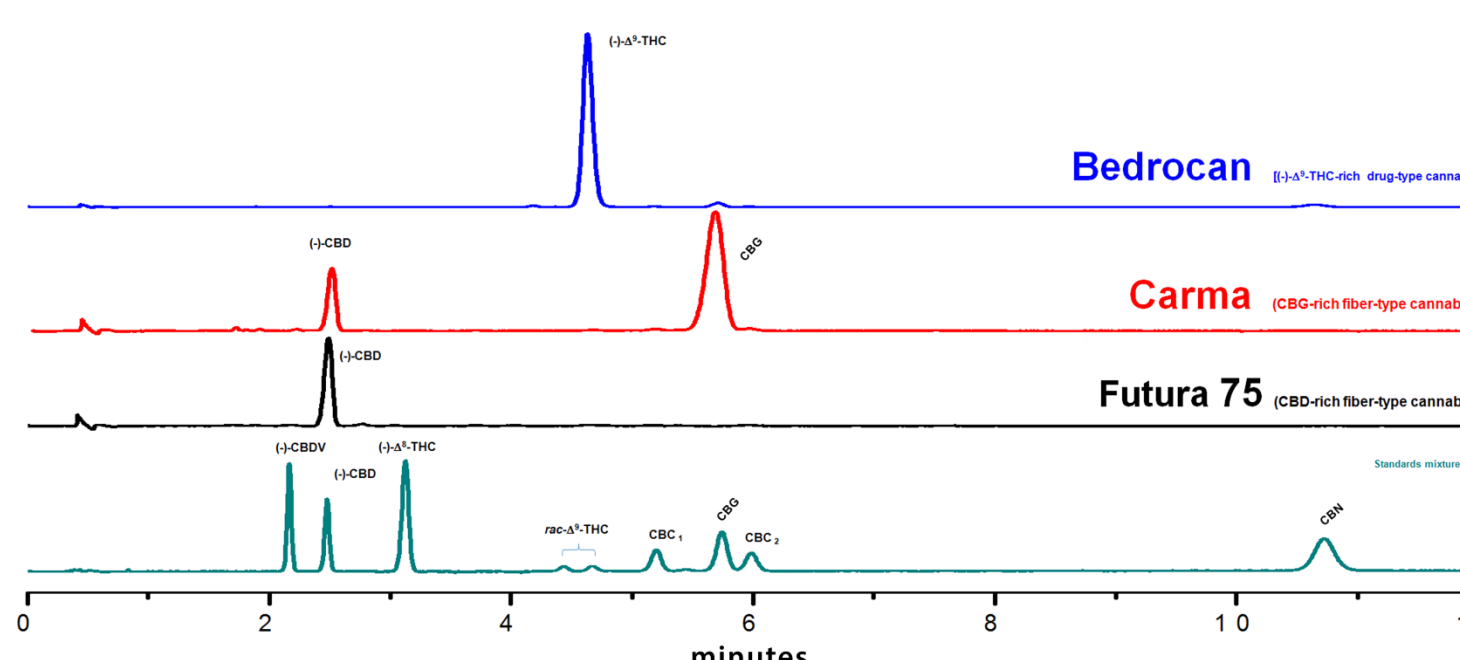
## Optimizing Separation of Cannabinoids and Stereoisomers

The (S,S) Whelk-O 1, 1.8  $\mu$ m, 100 x 4.6 mm column allowed complete resolution of cannabichromene's stereoisomers, the naturally occurring (-)- $\Delta^9$ -THC and the synthetic (+)- $\Delta^9$ -THC, and all other cannabinoids present in the mixture. Several organic modifiers were investigated using isocratic conditions to identify the one that produced the best separation, and methanol appeared to be the best.



## Application of Method to Decarboxylated Plant Extracts

We applied the method to analysis of several decarboxylated plant extracts derived from different chemotypes (chemical phenotypes) of cannabis. We obtained good separation, with high resolution for all cannabinoids, including the racemic isomers of cannabichromene (*rac*-CBC) found in the “Carma” CBG-rich extract. Extracts, such as “Bedrocan,” are valued for their high concentration of THC and show a high [total THC/total CBD] ratio ( $\gg 1.0$ ), and fiber-type plants (often referred to as “hemp”) exhibit a low [total THC/total CBD] ratio ( $\ll 1.0$ ).



## Conclusion

We obtained good separation, in terms of chemo- and enantio-selectivity, with high resolution for all cannabinoids in less than 12 minutes, using an (S,S) Whelk-O 1 column packed with 1.8  $\mu$ m particles. The combination of Whelk-O 1 chiral stationary phases and SFC represent an enabling approach to separate and isolate cannabinoids, including individual isomers, from plant extracts and synthetic preparations.

Chromatographic separation of enantiomers for both analytical and preparative purposes is an important tool to enable full characterization of potency, efficacy, and safety of individual enantiomeric ligands. This methodology can be used for qualitative and quantitative analysis of cannabinoids and their stereoisomers in natural and synthetic cannabinoid mixtures.