Purification of CBD Extracts using SFC and a Celeris™ 4-Ethyl Pyridine Column

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Introduction

In recent years, there has been a growing interest in the development of therapies and other consumer products derived from cannabis and/or hemp, including cannabidiol (CBD). With the passage of the US 2018 Farm Bill, hemp was removed from the Controlled Substances Act (CSA), which means that cannabis plants and derivatives that contain no more than 0.3 percent Tetrahydrocannibinol (THC) on a dry weight basis are no longer controlled substances under federal law. The small amount of THC found naturally in hemp plants can sometimes exceed the legal limit of 0.3% when extracted and concentrated along with the CBD. Therefore, it is important to have a means of removing the THC from CBD extracts in order to comply with Federal regulations.

Chromatography has been used successfully in many industries as a means for purifying compounds. Supercritical fluid chromatography (SFC) is a form of normal phase chromatography that uses a supercritical fluid such as carbon dioxide as the mobile phase. SFC excels at separating and purifying compounds derived from natural sources because it is faster, uses much less solvent, and, overall, is a less expensive and greener method compared to high pressure liquid chromatography (HPLC).

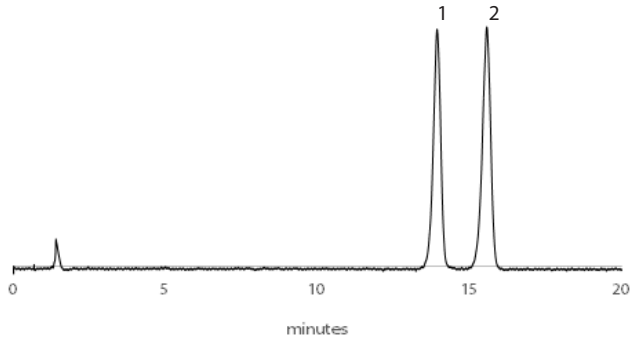
The chromatographic conditions described in this application use methanol as an organic modifier, and the conditions can be scaled up to preparative scale. The Celeris™ 4-EP achiral stationary phase is stable under SFC conditions and is available in 5- or 10 µm particle sizes as well as in bulk-to-pack large diameter columns.

Results and Discussion

Elution Order

It may be desirable to control the elution order of closely eluting analytes in an SFC analysis or purification, especially in cases where one component is much more abundant than the other. For instance, depending on the nature of the retention mechanisms and adsorption isotherm, an overloaded component may exhibit either peak fronting or tailing. As shown here using a Celeris 4EP column, CBD can be made to elute either before or after Δ9-THC by changing the proportion of methanol modifier in the mobile phase.

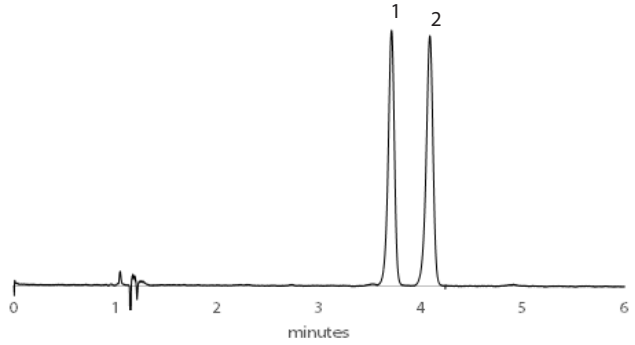
Elute CBD before THC



Sample: CBD and Δ9-THC
Column: Celeris 4EP, 5 µm, 25 cm x 4.6 mm
Mobile Phase: (98.5/1.5) CO_2/CH_3OH
Flow Rate: 5.0 mL/min
Temperature: 30°C
Pressure: 150 bar
Detection: UV 212 nm

Column Catalog #: 1-790501-300
Peaks:
1. Cannabidiol (CBD)
2. Δ9-Tetrahydrocannabinol (Δ9-THC)

Elute THC before CBD



Sample: Δ9-THC and CBD
Column: Celeris 4EP, 5 µm, 25 cm x 4.6 mm
Mobile Phase: (90/10) CO_2/CH_3OH
Flow Rate: 3.0 mL/min
Temperature: 30°C
Pressure: 150 bar
Detection: UV 228 nm

Column Catalog #: 1-790501-300
Peaks:
1. Δ9-Tetrahydrocannabinol (Δ9-THC)
2. Cannabidiol (CBD)

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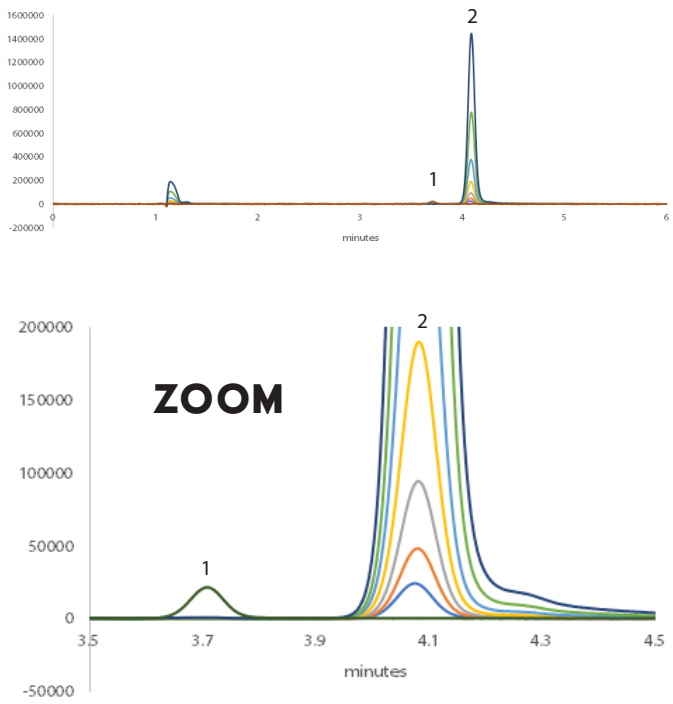
Removing THC from CBD Extracts

When CBD is isolated from cannabis/hemp, THC levels are required to be less than 0.3%. Shown here, SFC with Celeris 4EP allow for the minor Δ9-THC peak to be well-resolved from the major CBD peak. 5 µL injections of serially diluted CBD oil with concentration ranges between 50 µg/mL and 3.3 mg/mL were made and the resultant chromatograms are overlaid with a 5 µL injection of 50 µg/mL Δ9-THC.

Separate THC from CBD Oil



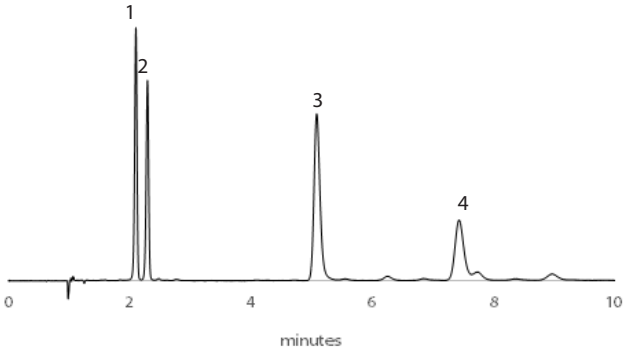
Sample: CBD oil spiked with Δ9-THC
Column: Celeris 4EP, 5 µm,
25 cm x 4.6 mm
Mobile Phase: (90/10) CO_2/CH_3OH
Flow Rate: 3.0 mL/min
Temperature: 30°C
Pressure: 150 bar
Detection: UV 228 nm

Column Catalog #: 1-790501-300
Peaks:
1. Δ9-Tetrahydrocannabinol (Δ9-THC)
2. Cannabidiol (CBD)

Measuring Inactive Acid Forms

The method can be expanded to measure the active forms of THC and CBD as well as the inactive, acid forms, THC-A and CBD-A. These four peaks are well-resolved using isocratic SFC and a Celeris 4EP column.

Separate THC and CBD from their acid forms



Sample: Δ9-THC, CBD, THCA-A, CBDA
Column: Celeris 4EP, 5 µm,
25 cm x 4.6 mm
Mobile Phase: (80/20) CO_2/CH_3OH
Flow Rate: 3.0 mL/min
Temperature: 30°C
Pressure: 150 bar
Detection: UV 228 nm

Column Catalog #: 1-790501-300
Peaks:
1. Δ9-Tetrahydrocannabinol (Δ9-THC)
2. Cannabidiol (CBD)
3. Tetrahydrocannabinolic acid A (THCA-A)
4. Cannabidiolic acid (CBDA)

Conclusion

SFC, combined with a Celeris 4EP column, allows for efficient isolation and removal of THC from CBD extracts to meet federal regulatory requirements for sale of CBD products.

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