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Purification of Cannabidiol from Hemp Oil Using the Prep150 LC System

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APPLICATION BENEFITS

- A preparative HPLC system that provides high performance with intuitive, easy-to-use software.
- With ChromScope™ Software, total software control of all system components.

WATERS SOLUTIONS

Prep 150 LC System

ChromScope Version 1.4.1 Software

ACQUITY UPLC® H-Class System

Empower® 3 Software

SunFire® Columns

KEY WORDS

Preparative chromatography, hemp, cannabidiol, Prep 150 LC, SunFire

INTRODUCTION

Extraction of a potentially active medicinal compound from plant materials can be achieved in many ways. Regardless of the extraction method, one is left with a raw extract that contains the active compound along with many other non-active constituents. Following extraction, purification or isolation of the compound is required to provide sufficient amounts of a high-purity compound to be used for a wide variety of purposes including preparation of standards, clinical trials, and bioassays. Cannabidiolic acid (CBDA) is produced in large abundance in some therapeutic hemp cultivars.[1] Cannabidiol (CBD, Figure 1), the heat-induced decarboxylation product of CBDA, is non-psychoactive and thought to have a wide scope of potential medicinal benefits including anti-inflammatory, anti-convulsant, anti-psychotic, anti-oxidant, neuroprotective, and immunomodulatory effects.[2] This application note will describe the purification of CBD from a CBD-rich paste using the Waters® Prep 150 LC System.

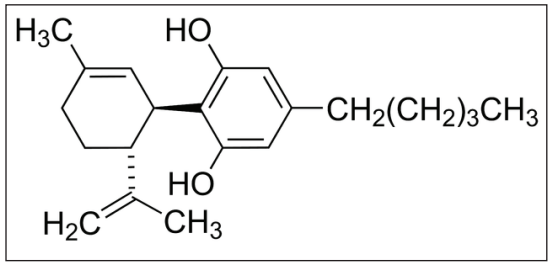

Figure 1. Chemical structure of Cannabidiol.

Sample preparation

A sample of high-CBD content paste was purchased from an internet supplier. According to the supplier, the oil was derived from stem and stalks of selectively grown industrial hemp plants high in cannabidiol content. As received, the sample had a mass of approximately 3 g, was unfiltered, and decarboxylated. The label indicated that the 3 g of paste contained 540 mg of CBD. A 500-mg portion of the paste sample was sonicated in 10 mL of methanol for 30 minutes and mixed well. Prior to injection, the sample was filtered through a 1.0-µm glass fiber filter.

EXPERIMENTAL

Separations

Preparative chromatographic separations were carried out using a Waters® Prep 150 LC System, which consisted of the following components:

Pump:	2545 Binary Gradient Module
Detector:	2489 UV/Visible(UV/Vis) Detector with Semi-Prep (3-mm path length) TaperSlit Flow Cell
Injector:	Preparative Injector configured with a 2-mL loop
Collector:	Waters Fraction Collector III (WFC III)
Software:	ChromScope Version 1.4.1

Analytical chromatographic separations (for HPLC method development and UPLC® assay method) were carried out using a Waters ACQUITY UPLC H-Class System equipped with an ACQUITY UPLC PDA Detector controlled by Empower 3 Software. Two initial analytical-scale separations were developed with the conditions described below.

Method conditions

Analytical UPLC gradient conditions (for assay and purity checks):

System:	ACQUITY UPLC H-Class
Column temp.:	50 °C
Flow rate:	1.0 mL/min
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in acetonitrile
Gradient:	60% to 73% B over 2.5 minutes
Detection:	UV @ 228 nm
Column:	ACQUITY UPLC BEH C_{18}, 130Å, 1.7 µm, 2.1 x 50 mm

Analytical HPLC gradient conditions (for prep scale-up)

System:	ACQUITY UPLC H-Class
Column temp.:	30 °C
Flow rate:	0.75 mL/min
Mobile phase A:	Water
Mobile phase B:	Methanol
Gradient:	85% to 100% B over 2.5 minutes, hold at 100% B for 2 minutes
Detection:	UV @ 228 nm
Column:	SunFire C_{18}, 100Å, 5 µm, 3 x 100 mm

Preparative HPLC Conditions

System:	Prep 150 LC
Column temp.:	Ambient
Flow rate:	30.0 mL/min
Mobile phase A:	Water
Mobile phase B:	Methanol
Gradient:	85% to 100% B over 2.5 minutes, hold at 100% B for 2 minutes
Detection:	UV @ 228 nm
Column:	SunFire C_{18} OBD™ Prep, 100Å, 5 µm, 19 x 100 mm

RESULTS AND DISCUSSION

An initial analysis of the prepared sample was performed on the ACQUITY UPLC H-Class System (Figure 2) using the UPLC conditions described in Table 1. Calculated amount of CBD was found to be 8.7 mg/mL. This value was consistent with the original hemp paste label claim.

Prior to preparative chromatography, an analytical HPLC method (Figure 3) was developed using the ACQUITY UPLC H-Class System on the column chemistry (SunFire C_{18} Column, 100Å, 5 µm) intended to be used for the preparative separations. Using this strategy, analysts can conserve sample and minimize solvent consumption while fine-tuning their separation methodology. Based on the analytical method, the flow rate for the preparative separation was scaled to a 19-mm I.D. column using the Analytical to Prep Gradient Calculator built into the ChromScope Software. This easy-to-use tool aids in analytical-to-preparative scaling calculations providing both scaled flow rate and gradient information. Scaled preparative chromatography will be very similar to the analytical chromatography, increasing confidence in the ability of the preparative method to collect peaks of interest. A previously published application note[3] describes in greater detail these scaling calculations.

The analytical HPLC method was also used to establish a reasonable sample loading for the preparative runs while maintaining resolution between the peak of interest and other material present in the sample (Figure 4). For this method, the maximum injection volume was 8 µL on a 3.0-mm I.D. column, which scales to approximately 320 µL on a 19-mm I.D. column. Based on the assay, each 320-µL prep injection could yield up to 2.8 mg per injection.

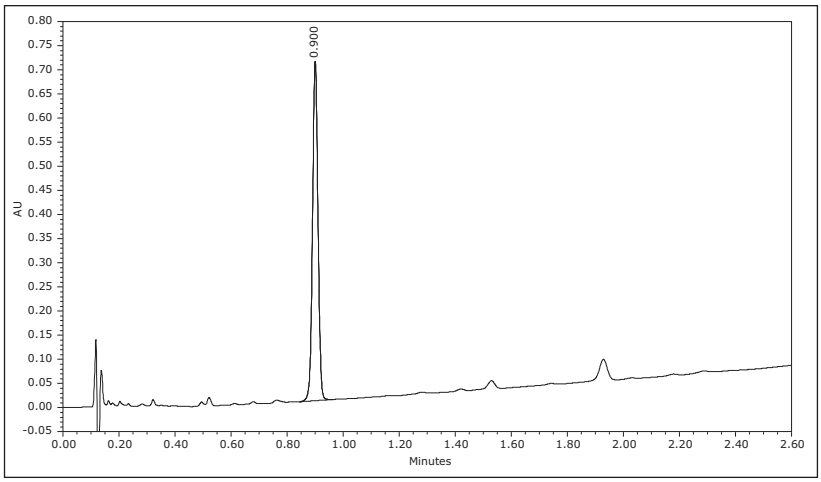

Figure 2. UPLC assay for CBD.

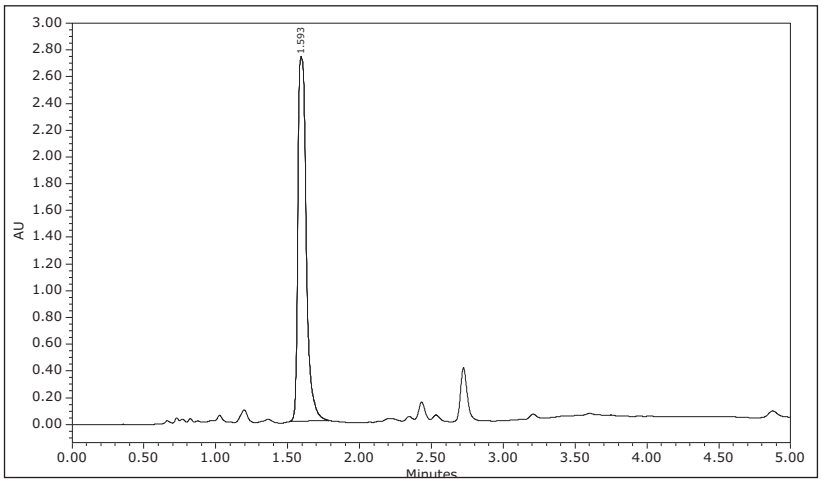

Figure 3. Analytical HPLC method for CBD.

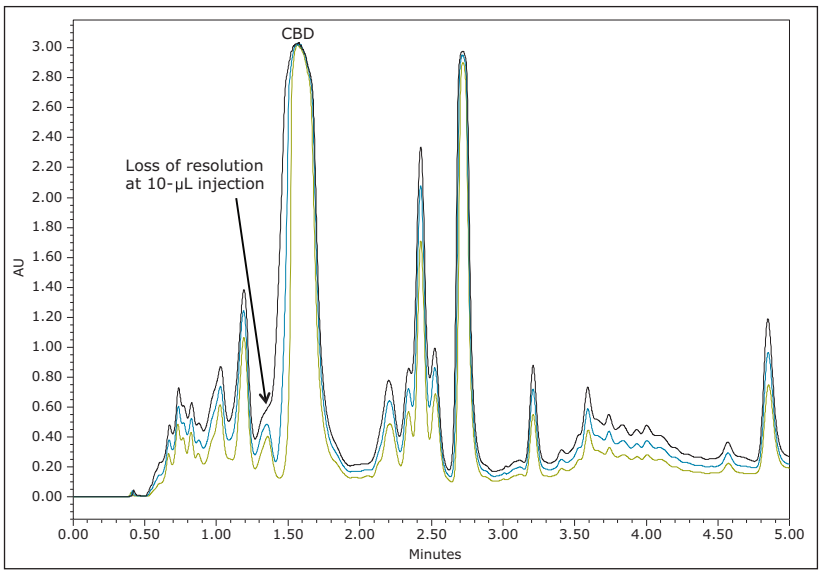

Figure 4. Loading Study, 6-µL (green), 8-µL (blue), and 10-µL (black) injections on the 3.0 x 100 mm analytical column. At a 10-µL injection volume, resolution between the main peak of interest (CBD) and shoulder peak begins to degrade.

Preparative HPLC runs were made with the Prep 150 LC System using the methodology described in Table 1. As expected, the scaled preparative runs (Figure 5) were similar to those achieved at the analytical scale. The collection parameters for the preparative runs were based on peak threshold within a window; collections were triggered when the peak intensity was greater than 1000 mAU in a time window of 1 to 2 minutes. Conversely, collection stopped when the threshold reached 500 mAU within that same time window.

The ChromScope v1.4.1 Method Editor (Figure 6) makes the setting up of this collection method very easy. A simulated collection method can be viewed using any previously collected chromatogram making it easy to fine tune collections as needed.

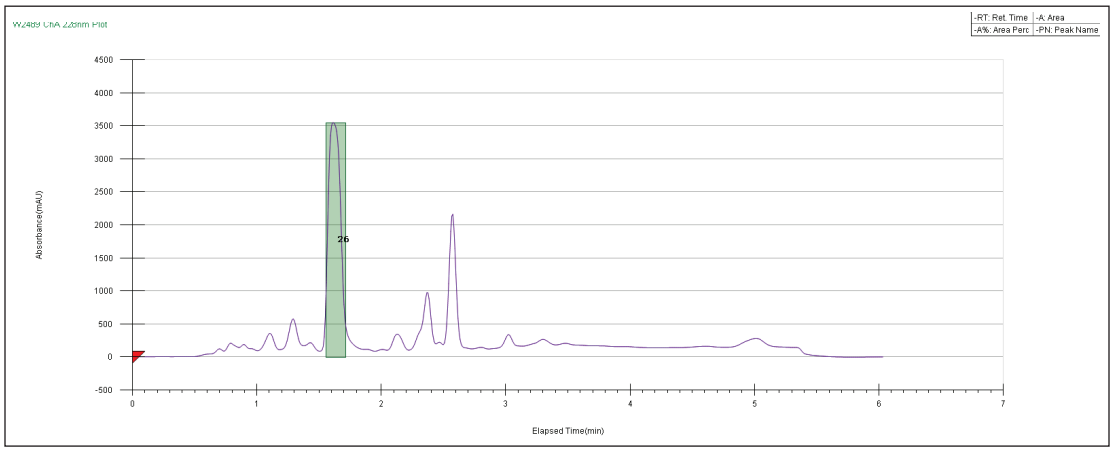

Figure 5. ChromScope preparative HPLC chromatogram showing collection of CBD from a 500-µL injection.

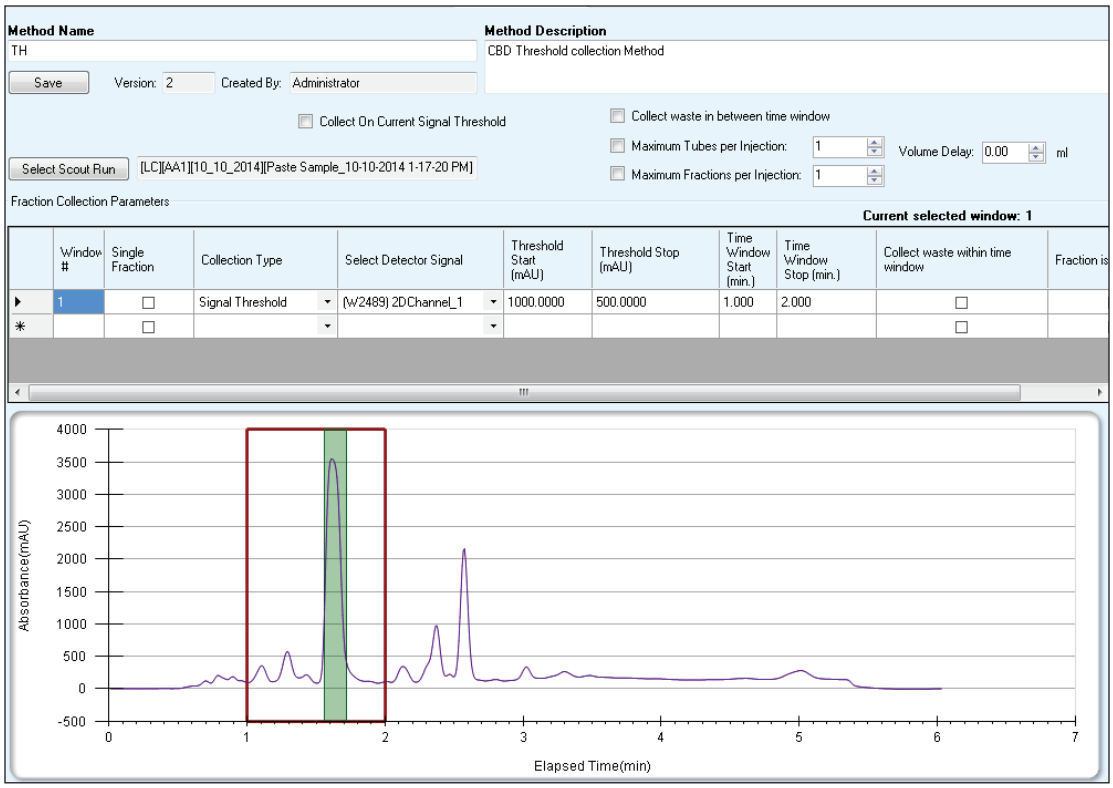

Figure 6. Chromscope Collection Method Editor showing collection simulation.

Assay of a collected fraction showed a CBD concentration of 0.56 mg/mL in a ~ 5 mL fraction volume. This value calculates to about 2.8 mg per injection of CBD collected.

In this simple example, a series of five prep injections were made, and collected fractions were pooled and dried down to liberate a total of ~12.3 mg of CBD.

CONCLUSIONS

A simple purification workflow, starting with a high-speed assay method using UPLC, followed by analytical scale HPLC method development, scale-up to prep, and finally preparative scale injections and collections were shown. Starting from a concentrated hemp paste, five preparative scale injections produced 15 mg of high purity CDB. Chromscope Software allowed for easy collection set-up, along with simple operation of the Prep 150 LC System. The ACQUITY UPLC H-Class System provided the flexibility to run high-speed (<3 minutes) UPLC assays, as well as analytical HPLC method development, which was scalable to the Prep 150 LC System.

References

1. American Herbal Pharmacopoeia, *Cannabis Inflorescence*, 2013, Roy Upton editor.
2. Antidepressant-like effect of Δ9-tetrahydrocannabinol and other cannabinoids isolated from Cannabis sativa L. Abir T. El-Alfy *et al*. *Pharmacol Biochem Behav.* Jun 2010; 95(4): 434–442.
3. Analytical HPLC to Preparative HPLC: Scale Up Techniques using a Natural Product Extract. Andrew Aubin and Ronan Cleary. Waters Application Note 720003120en, 2009.

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