Effective Cannabinoid Purification by Flash Chromatography

John R. Bickler and Elizabeth Denton, PhD
Biotage, 10430 Harris Oaks Blvd., Suite C, Charlotte North Carolina 28269, USA

Introduction

With the de-criminalization of recreational cannabis, containing the hallucinogen THC, and other cannabinoids with purported medicinal value, e.g. CBD (Figure 1), in several states a need for higher purity "products" has become a necessity. Current technology uses extraction with supercritical fluids or other non-supercritical solvents to remove the products of interest from other endogenous species such as lipids, terpenes, and chlorophylls as well as pesticides.

These techniques help clean up raw extracts and isolate cannabinoids with higher-purity but not to the levels desired by many producers so there is a developing need for a secondary purification step.

For some producers, reversed-phase chromatography has become the analytical tool of choice for determining the extract profile and for purification as well. However, the extracts still contain many other compounds which reduce load capacity and recovered purity of the product(s) of interest.

In this poster we will show the results of an orthogonal flash purification approach that first used normal-phase followed by reversed-phase flash chromatography of a cannabis extract.

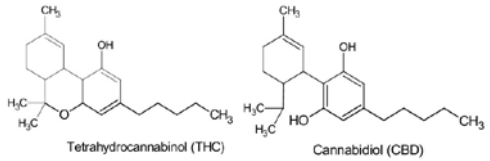

Figure 1. *Structures of two cannabinoids of interest*[1].

Experimental protocol

Reagents and Materials

Reagents used in study included: DMSO (Fisher), methanol, hexanes, ethyl ether (all from Reagents, Inc.), and house deionized water.

- A Biotage® SNAP Ultra C18 (12 g) flash cartridge and 10 g SNAP Ultra silica cartridges and Samplets were used for this study.
- A Biotage Isolera™ Spektra 1SV system was used for purification.
- A Biotage Isolera™ Dalton system was used to identify fraction masses.
- A Biotage® V-10 Touch was used for fraction evaporation.

Reversed-phase Flash Chromatography

A water/methanol gradient (80 to 90%) was used with a 12 g Biotage® SNAP Ultra C18 cartridge to purify the crude extract. The extract was dissolved in DMSO with a concentration of 0.5 g/mL; the injection volume was 0.1 mL.

The same method was used to purify the primary normal-phase flash fractions.

Normal-phase Flash Chromatography

The extract was dissolved in hexanes (~0.5 g /mL) and analyzed by silica TLC using a hexanes/ethyl ether (9:1) solvent system[2]. From this data a normal-phase flash chromatography method was created (2-20% ether in hexanes) using a 10 g SNAP Ultra cartridge and Samplet.

Select fractions isolated from the normal-phase purification were dried on the V-10, re-dissolved in 0.2 mL of DMSO, and purified using the reversed-phase gradient method.

Results and Discussion

Reversed-phase chromatography of the cannabis extract shows a moderately complex profile eluting one major peak and several minor peaks, Figure 2.

Based on HPLC results reported in literature, we believed the blue peak to be CBD, the yellow peak THC, and the pink peak THC-A[1]. Though this separation is good the load was quite low (50 mg) and none of the fractions were pure. Some probable contaminants are terpenes, chlorophylls, and pesticides.

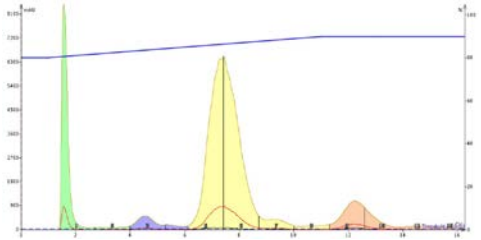

Figure 2. *Reversed-phase flash chromatography of a cannabis extract shows a moderately sample. The green peak is DMSO and the yellow, blue, and pink peaks likely cannabinoids.*

To see if the extract could be cleaned prior to reversed-phase flash the crude extract (0.5 g/mL in hexanes) was purified on a 10g silica cartridge (250 mg load). The thinking behind this is that potential interferences such as terpenes and chlorophylls found in the reversed-phase fractions can be removed by normal-phase (terpenes eluting near the solvent front and chlorophylls being retained).

The results from normal-phase chromatography with a hexane/ether gradient show a good separation with one major peak and a few minor peaks, Figure 3. Some un-identified terpenes are believed to be found in the first two fractions with the cannabinoid acids eluting late.

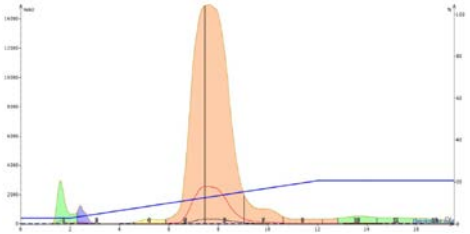

Figure 3. *Normal-phase flash chromatography of a cannabis extract.*

To determine how well the silica cartridge purified the crude, reversed-phase flash chromatography was performed on each of the fractions comprising the major peak (5, 6, and 7), Figure 4.

After evaporation, each normal-phase fraction was dissolved in 0.2 mL of DMSO, which was then injected on the reversed-phase flash cartridge.

The reversed-phase purification of normal-phase fraction 5 shows one major peak and a few minor peaks, a much cleaner profile than that in Figure 2. The MW for the major peak was determined by the Isolera Dalton to be 314, which is the MW for THC. The early eluting peak (blue) in figure 2, has been removed as has the majority of the pink peak.

Reversed-phase chromatography of normal-phase fraction 6 shows two major and a few minor peaks, also a cleaner profile than the crude. The large yellow peak also had a MW of 314 while the pink peak has a MW of 358 which matches the MW for THC-A. The blue peak is still missing.

Normal-phase fraction 7 purification by reversed-phase shows us that there are two compounds which make up the large yellow peak in fraction 6. Both have the same MW (314) and may be THC isomers, one being Δ9 and the other Δ8, but this has not been confirmed. Also now present is the blue peak which was believed to be CBD (MW 314).

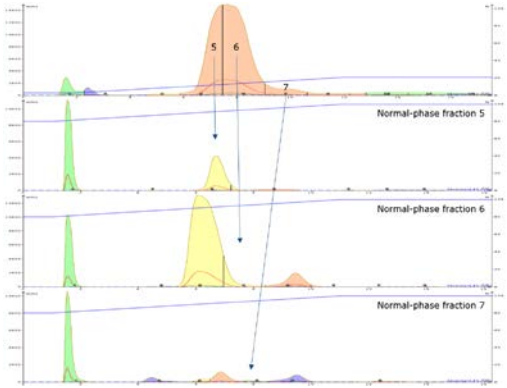

Figure 4. *Reversed-phase flash chromatography of fractions 5, 6, and 7 (top to bottom) from Figure 3. The reversed-phase data show that the two major normal-phase fractions (pink, labeled 5, 6, 7; top chromatogram) each contain only a few compounds, an indication the crude extract has been cleaned-up using normal-phase flash chromatography. Fraction 7 (bottom) shows that within the two main normal-phase fractions (fractions 5 and 6 of the top chromatogram) there are two compounds, likely the two versions of THC, Δ9 and Δ8. The green peak is DMSO, the blue peak at ~4.5 CV is thought to be CBD and the blue peak at ~9.5 CV is THC-A.*

These results show that crude extract clean-up using normal-phase flash chromatography improved subsequent reversed-phase purification of the collected fractions at an increased load (~2X).

Conclusions

Flash chromatography can be used to further purify previously extracted cannabis raw materials. However, taking an orthogonal approach with flash purification starting with normal-phase to remove potential co-eluting impurities followed by reversed-phase purification of collected normal-phase fractions dramatically improves separation efficiency, loading capacity, and compound purity.

[1] Andrew Aubin, What's in it & how much; Analysis of cannabinoids in plants and extracts, Waters Corp. BioBotanical Workshop, 2015.

[2] N. Galand, et. al., Separation and Identification of Cannabis Components by Different Planar Chromatography Techniques (TLC, AMD, OPLC), J. Chromatographic Science, April 2004.

This poster was first presented at ACS, Philadelphia, 2016
Part Number: P149