Flash Chromatography Methods for Isolating Cannabinoids

White Paper

Introduction

Research into cannabis for medical research has increased now that many countries have, at minimum, legalized CBD (cannabidiol) for therapeutic uses. While most in this market are focused on CBD (THC-free), others seek to differentiate themselves isolating some of the minor, naturally occurring, non-psychoactive phytocannabinoids such as CBG, CBC, CBN, and CBDV.

The Workflow

The workflow for isolating pure cannabinoids includes plant extraction (flower and/or whole plant) with a solvent (supercritical CO_2, ethanol, butane or propane), extract dissolution in ethanol, winterization (chilling at very low temperatures to remove insoluble fats, waxes, and some other components), and, if necessary, distillation (if the cannabinoid acids are not desired).

The extraction process removes not only cannabinoids but also many other compounds that are soluble in the extraction solvent. Some of these co-extracted compounds include pesticides, chlorophylls, carotenoids, terpenes/terpenoids, and phenolic compounds. Depending on plant varietal, the growing/harvest geographic region and methods (outdoor/hothouse);co-extractables can vary significantly. In fact, over 483 different compounds have been identified in cannabis leading to a highly complex extract (Brenneisen, 2007). Of these 483 compounds, 104 are classified as cannabinoids (ElSohly, 2014).

While winterization removes a few of these, distillation removes some of the others, including terpenes and terpenoids, since their boiling points are lower than the cannabinoids, and even some lower boiling cannabinoids. Distillation also converts acidic cannabinoids into neutral cannabinoids through a chemical process known as decarboxylation and can help degrade THC to CBN as well creating even more complexity.

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Purification of Cannabinoids

Isolating individual cannabinoids from this complex extract requires purification. A relatively simple process called flash chromatography, commonly utilized by the pharmaceutical industry, is successfully used by many processors in the hemp/cannabis space.

Other separation techniques are also used depending on the product desired. Cannabidiol (CBD) and other cannabinoids, for instance, can be isolated through precipitation/crystallization using heated pentane (Demski, 2018). This technique, however, removes any remaining essential oils and terpenes, often desired for their “entourage effect” .

Flash chromatography, however, separates individual cannabinoids from themselves, and other co-extractants including many pesticides. Extract purification with this technology removes many of the unwanted components while preserving some of the terpenes without the use of highly volatile and dangerous chemicals such as pentane.

For more than 30 years, Biotage has been at the forefront of flash chromatography technology development and application. During the past several years, Biotage has developed methods for THC remediation and minor cannabinoid purification as well as removal of some pesticides.

THC Remediation

Hemp is a primary source of CBD and its extracts typically contain 60% or more CBD with THC concentrations typically between 2% and 5%. For CBD to meet US federal purity and legality guidelines, THC content must be reduced to below 0.3%.

Distillation struggles to remove THC from CBD so flash chromatography has become the purification technique of choice. For this process, a mixture of ethanol and water is used to separate and elute the dissolved compounds from a column filled with a waxy media known as C18. This process is called reversed-phase purification. An ultraviolet detector (UV) detects the separated compounds and the detected compounds collected into either test tubes or bottles, depending on purification scale.

This purification technology can purify up to 28 kg/24 hr, and depending on the equipment used - with CBD eluting first and THC eluting later, (Figure 1), it is relatively simple to isolate THC from a variety of cannabinoids using this process.

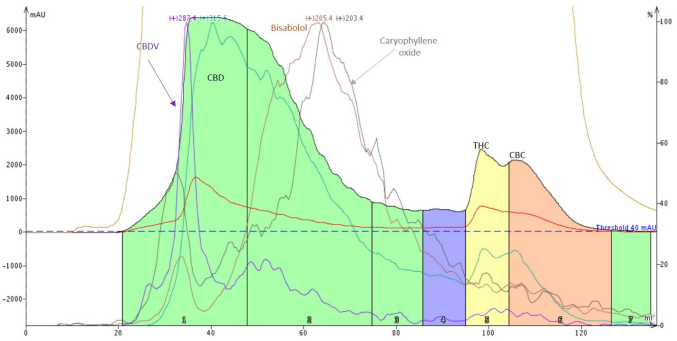

Figure 1. Hemp extract (800 mg) containing ~2.5% THC and ~75% CBD purified using a 12-gram Biotage® Sfär C18 flash chromatography column and the Biotage® Isolera Dalton 2000 system. The green peak is CBD, the yellow peak is THC and the pink peak is CBC. Also separated from THC are CBDV (purple trace) and two terpenoids, bisabolol (rust trace) and caryophyllene oxide (grey trace). Along with UV, an inline mass detector was used to verify compound ID. A 12-gram Sfär C18 column was used to purify 800 mg.

Other Minor Cannabinoid Purification

Interest in the other cannabinoids expressed by hemp and cannabis varietals is also growing (excuse the pun). While the typical hemp plant produces CBD in abundance and cannabis sativa creates mostly THC, there are varieties that produce some of the other cannabinoids of interest including CBN, CBG, CBC, CBL, CBCT, and others.

The same purification approach is used to separate and purify most of these minor cannabinoids, Figure 2. The same extract purified above was purified using a slightly different method to selectively elute CBN, CBC, and CBCT.

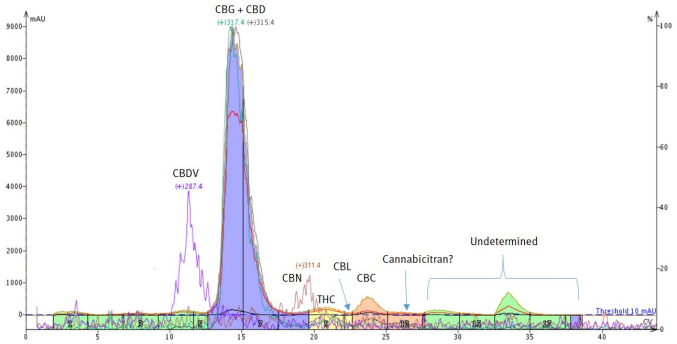

Figure 2. Flash chromatography separation and purification of a hemp extract. Separated compounds in this hemp extract included CBN, CBL, CBC, and possibly cannabicitran (CBCT).

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CBG Purification

However, CBG does not separate from CBD using this methodology and thus requires a different flash chromatography strategy. For CBG, a methodology called normal-phase is very helpful. This technique uses organic solvents (typically hexane and ethyl acetate) and a flash chromatography column filled with activated silica, Figure 3.

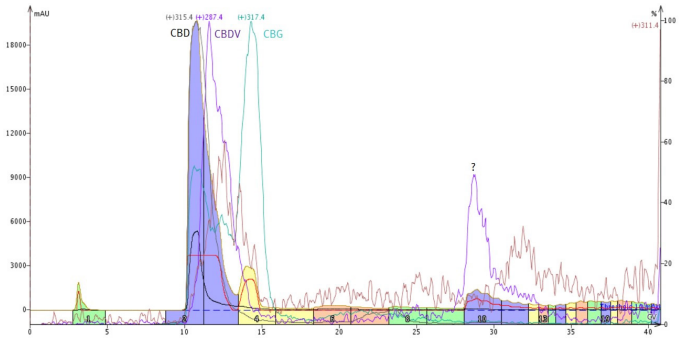

Figure 3. CBG purification from hemp using normal-phase flash chromatography. CBG has more polarity than most other cannabinoids and is easily separated.

Because of exploitable polarity differences between CBG and the other cannabinoids, this separation is possible. Though the solvents needed are not water compatible, they are easily evaporated allowing for quick recovery.

Summary

Whether needing to reduce THC to acceptable levels for therapeutic CBD applications or purifying individual cannabinoids, flash chromatography using pre-packed cartridges can provide a robust and process efficient means to accomplish both of these goals.

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