A Guide to Flash Chromatography Methods for Isolating Cannabinoids

Biotage®

Due to significant therapeutic interest, the cannabis industry is rapidly growing and new medicinal applications of cannabidiol (CBD) and tetrahydrocannabinol (THC), isolates, broad spectrum oils, tinctures are actively being developed. etc. While most of the science in this market is focused on CBD (THC-free), there are applications in isolating some of the minor, naturally occurring, phytocannabinoids such as cannabigerol (CBG), cannabichromene (CBC), and cannabinol (CBN).

The workflow for isolating individual cannabinoids typically includes

1. Biomass extraction with a solvent (supercritical CO_2, ethanol, ethyl acetate, butane, or propane)
2. Extract dissolution in ethanol (sometimes super cooled to eliminate winterization)
3. Winterization (chilling at very low temperatures to remove insoluble fats, waxes, and some other components)
4. Distillation (if the cannabinoid acids are not desired)

The extraction processes remove not only cannabinoids but also many other compounds that are soluble in the solvent. Some of these co-extracted compounds include pesticides, chlorophylls, carotenoids, terpenes/terpenoids, and phenolic compounds. Depending on the plant varietal, the growing/harvest geographic region, and methods (outdoor/hothouse), the 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 many terpenes and terpenoids and even some lower boiling cannabinoids. Distillation also converts thermally labile acidic cannabinoids (their natural plant form) into chemically neutral cannabinoids through a chemical process known as decarboxylation. The heat used for distillation can also degrade THC to CBN via oxidative processes, increasing the distillates complexity.

Isolating individual cannabinoids from these complex extracts requires purification. A relatively simple process called flash chromatography (commonly utilized by the pharmaceutical industry), is often used by processors in the medicinal hemp and cannabis industries to further purify cannabinoid mixtures.

Other separation techniques can be used depending on the product desired. Cannabidiol (CBD) and some other

cannabinoids, for instance, can be isolated through precipitation/crystallization using heated pentane (Demski, 2018). This technique, however, effectively removes all the remaining essential oils and terpenes, often desired for their "entourage effect" (an effect that is known and accepted in the formulation of therapeutic mixtures, essentially that the combination of active components can often be more effective that the action of the individual components themselves).

Flash chromatography has the advantage of supporting isolating of individual cannabinoids from a cannabinoid spectrum mixture, as well as being able to separate other co-extractants like pesticides. Extract purification with flash chromatography removes many unwanted components while preserving some terpenes and without the use of highly volatile and toxic chemicals such as pentane.

For more than 25 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 15%. THC is often regulated, with different tolerable thresholds of concentration, depending on the geographic location. In the US for example, for CBD to meet US federal purity and legality guidelines, THC content in the biomass and final products must be at levels below 0.3%.

Distillation is not highly effective at removing THC from CBD so flash chromatography has become the purification technique of choice. For this process, a mixture of ethanol (or methanol) and water is used to separate and elute the dissolved compounds using a column filled with a solid, waxy media known as C18. The media is known as a stationary phase and this process is called reverse-phase chromatography and incorporates an ultraviolet detector (UV) to detect and collect the separated compounds into either test tubes or bottles, depending on purification scale.

Depending on the equipment and methods used, and the initial cleanliness of the extracts, this chromatographic technology can purify over 20 kg of oil/24 hr using reversed-phase flash chromatography, CBD elutes first while THC, and most other cannabinoids, elute later. In Figure 1, the solvents used were water and ethanol and the distillate load on the 12-gram Biotage® Sfär C18 column was 800 mg (6.6% load by weight).

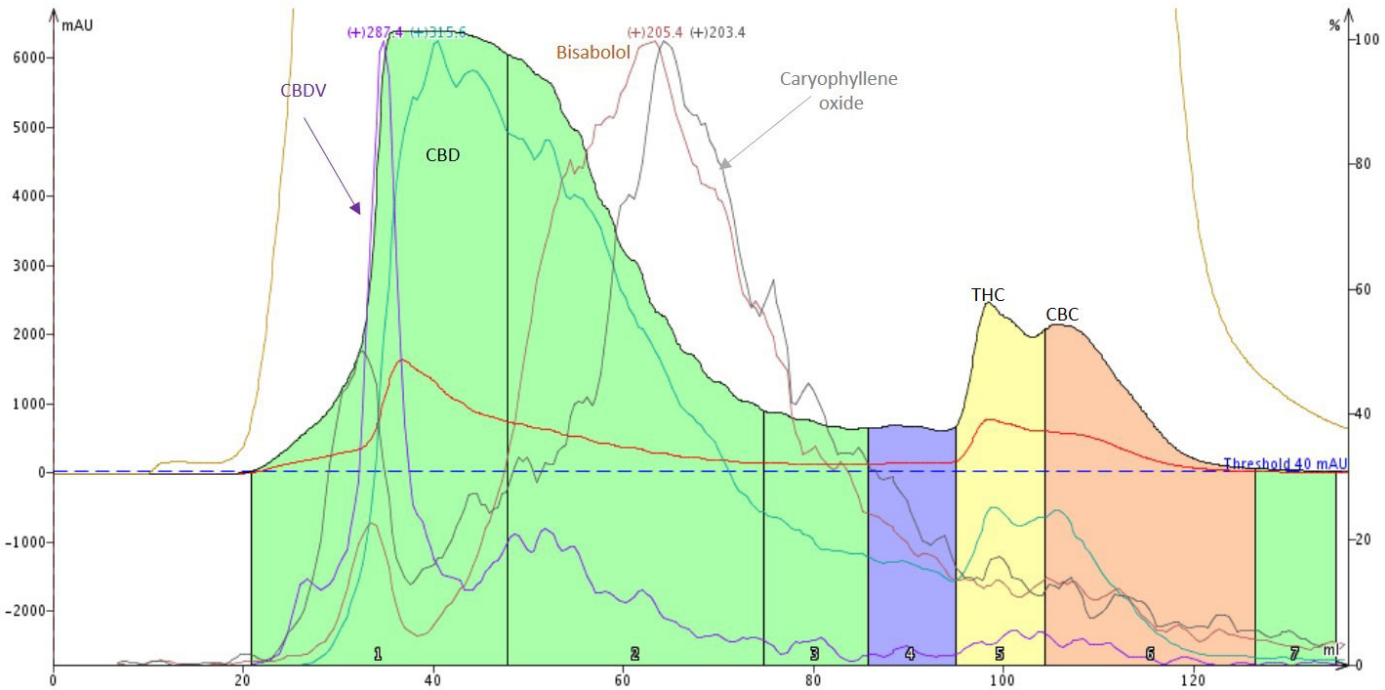

Figure 1. Hemp extract (800 mg load) containing ~2.5% THC and ~75% CBD purified using a 12-gram Biotage® Sfär C18 flash chromatography column and a Biotage® Isolera Dalton 2000 system. The green peak is CBD, the yellow peak is THC and the orange peak is CBC. Also removed from THC are CBDV (purple trace)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

The same methodology works for cannabinoid acid purification, producing an an identical elution pattern, CDBA first with THCA and CBCA later (Figure 2). In this example, 12 grams of winterized, but not distilled, extract was purified using a 120-gram Biotage® Sfär C18 column (10% load) using water and methanol as the purification solvents.

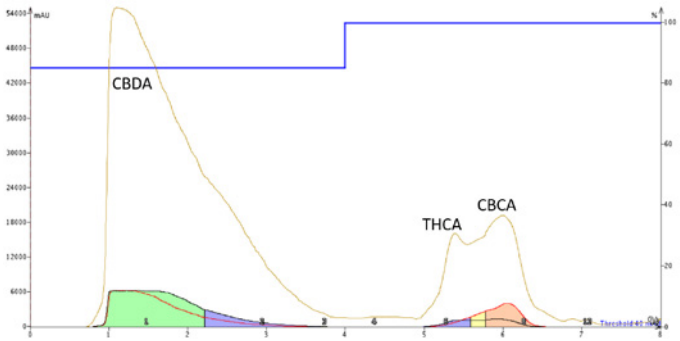

Figure 2. Reversed-phase flash purification of non-winterized hemp extract containing CBDA, THCA, and CBCA among other minor lipophilic compounds. A 120 g Sfär C18 column purified 12 g of winterized, but not distilled, extract.

Minor Cannabinoid Purification

Interest in the other cannabinoids expressed by hemp and other cannabis varietals is also growing. While the typical hemp plant produces CBD in abundance with smaller amounts of other cannabinoids, some cannabis varietals create mostly THC, and in some cases, CBG. Most other minor cannabinoids, CBC, CBL, and CBCT, as well as the short chain variants (CBDV, THCV, etc.), are produced in low abundance in the plants.

The minor cannabinoid CBN, however, is a natural oxidative degradation product of THC which can be also be synthesized from both THC and CBD (Pollastro, 2018). Again, the same reversed-phase flash purification approach, with different methods, can separate and purify most of these minor cannabinoids (CBN, CBC, CBL, CBCT, etc.), Figure 3.

CBN Purification

As seen in Figure 3, CBN elutes between CBD and THC and is present in a very low quantity. Synthetic CBN, however, can be created in significantly higher yields from either THC or CBD. Those reaction mixtures generate several impurities which need remediation from CBN. Using a similar methanol/water method as used for THC remediation, CBN can be efficiently purified, Figure 4.

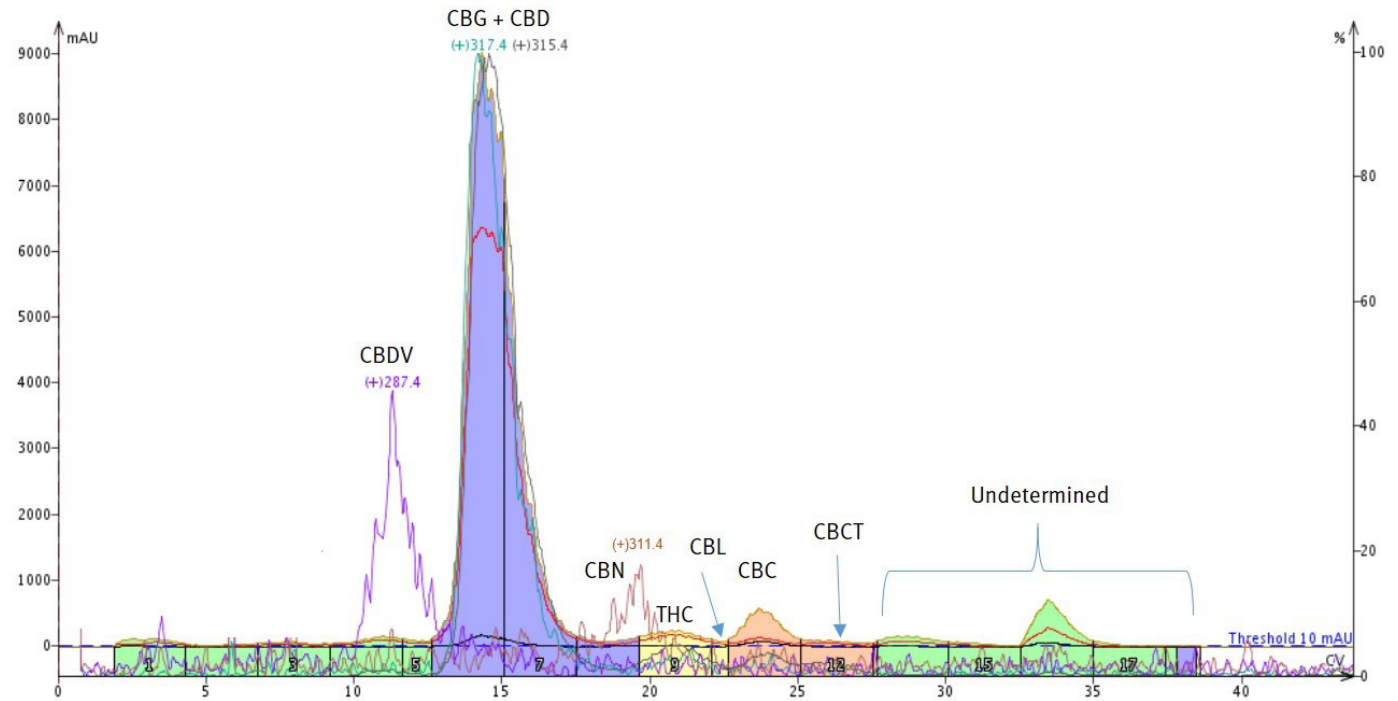

Figure 3. Flash chromatography separation and purification of a hemp extract. Separated compounds in this hemp extract included CBN, CBL, CBC, and cannabicitran (CBCT).

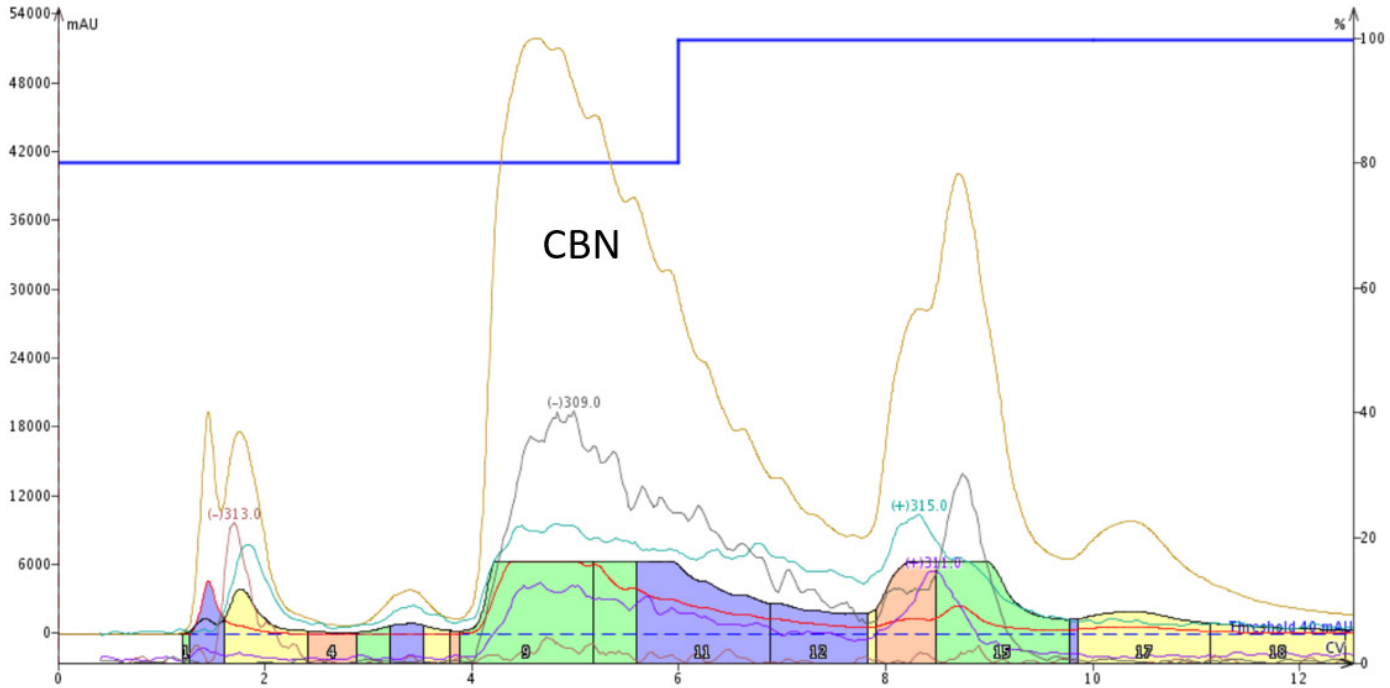

Figure 4. CBN purification using reversed-phase flash chromatography. CBN is easily separated from this reaction mixture's by-products. The optional in-line mass detector confirmed CBN's identity with a detected mass/charge ratio of 309.

Cannabinol is a unique cannabinoid in comparison to most others. Its molecular weight (310) is not shared by any typically found cannabinoid and it has a very distinct ultraviolet (UV) spectrum, Figure 5.

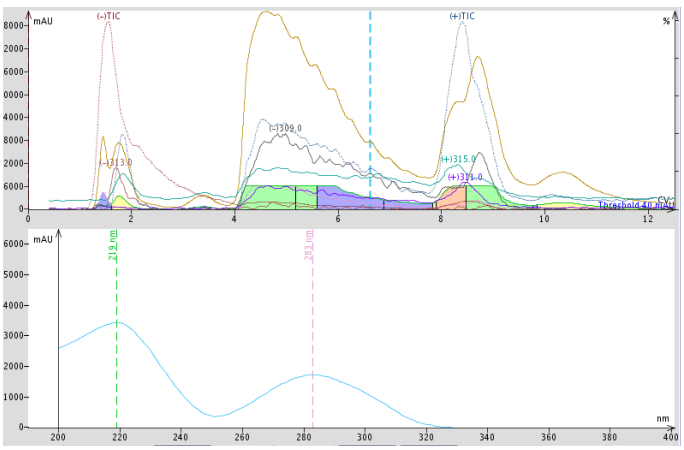

Figure 5. CBN UV spectrum shows maxima at 219 and 283 nm.

Every cannabinoid has its own UV spectrum. The spectra can be used to identify individual cannabinoids separated and detected during the chromatography process. In the case of CBN, its distinctive UV absorbance spectrum show "maxima" at 219 nm and 283 nm. No other cannabinoid shares this UV spectrum.

Like CBD, pure CBN is crystalline. Using the method in Figure 5, pure, crystalline CBN was obtained, Figure 6.

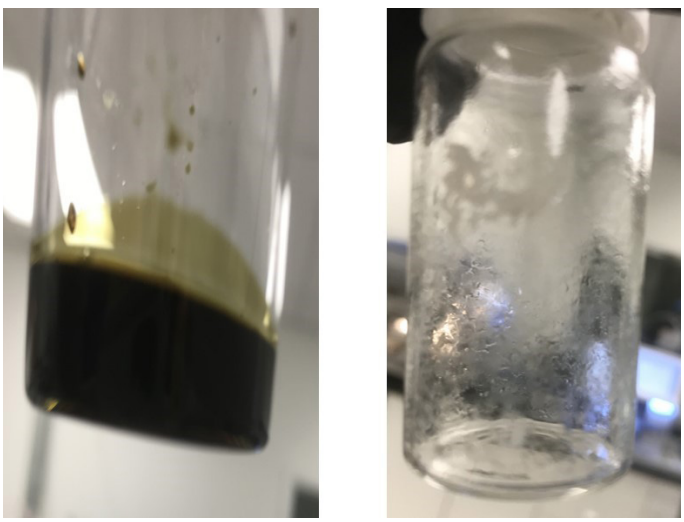

Figure 6. Before and after flash chromatography photos shows the crude CBN reaction mixture (left) and purified CBN (right).

CBC Purification

Cannabichromene, along with CBD and THC, are the cannabinoids typically expressed in the highest amounts. In some hemp varietals, CBC is expressed in similar amounts as THC.

The separation of CBC from THC, however, is not trivial. Although CBC is slightly more hydrophobic than THC the differences are not as large as THC and CBD, which adds much more complexity to any purification strategy. The separation of CBC from THC requires a moderately complex chromatographic method consisting of multiple, discrete steps to tease these chemically similar compounds away from each other, Figure 7.

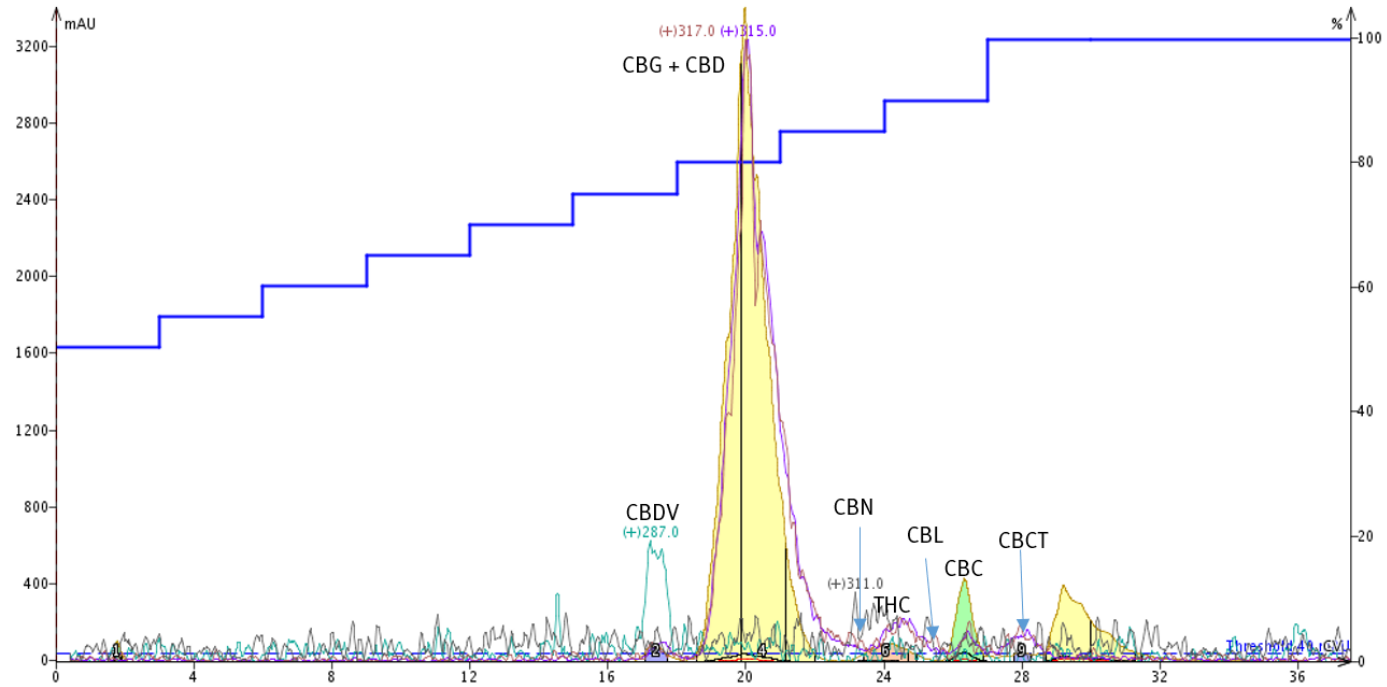

Figure 7. Reversed-phase flash chromatography separation and isolation of CBC from a hemp extract using a multi-step elution gradient. The green peak is CBC.

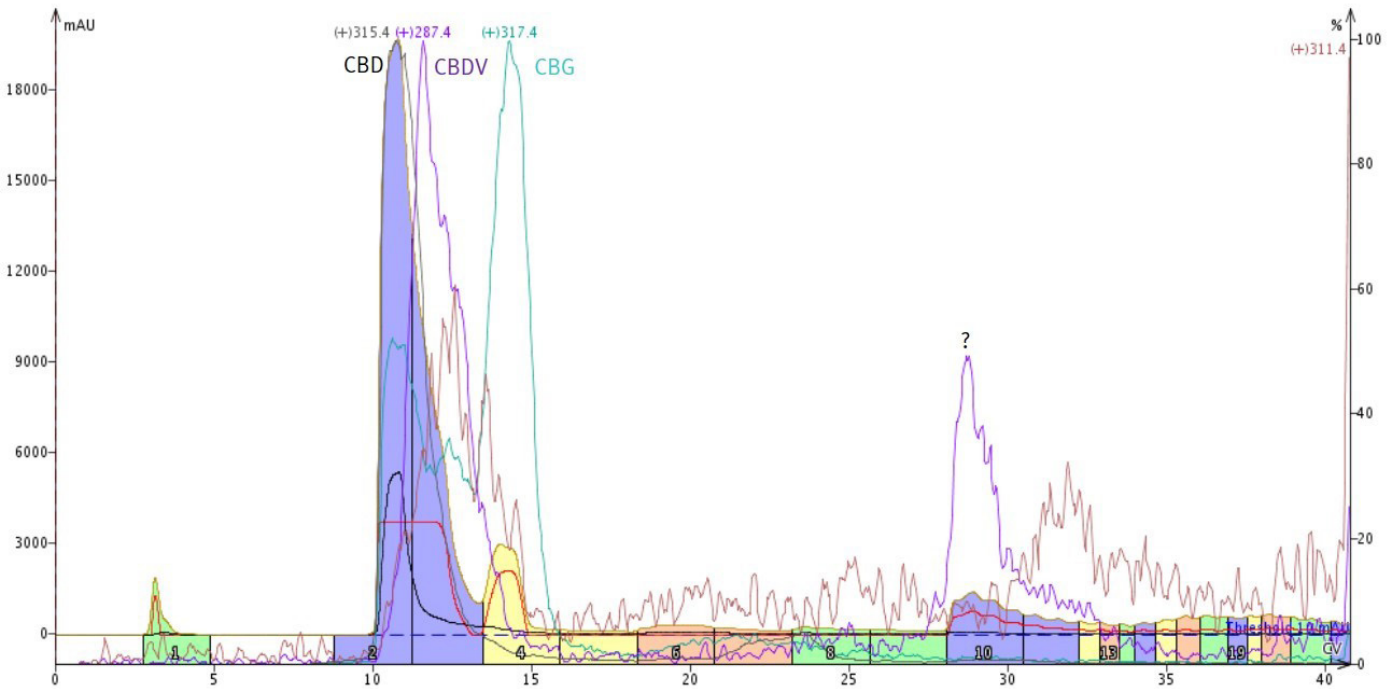

Figure 8. CBG purification from hemp using normal-phase flash chromatography. CBG has more polarity than most other cannabinoids and is easily separated (yellow peak). An optional mass detector used in this purification confirmed its identity with an ionized mass of 317.4 (green trace over the yellow peak.

Our investigative research optimizing CBC purification is currently in progress.

CBG Purification

Cannabigerol purification requires a totally different approach as it does not separate from CBD using reversed-phase flash chromatography. For CBG, normal-phase flash chromatography is very helpful. This technique uses organic solvents (typically hexane and ethyl acetate) and a flash chromatography column filled with activated normal phase silica. Because of exploitable polarity differences between CBG and the other cannabinoids, it is better retained on silica than most other cannabinoids, so can be separated.

Cannabigerol is also unique as it has a molecular weight of 316 which is different from all other cannabinoids. (Tetrahydrocannabinol, CBD, CBC, CBL, CBCT, and some other cannabinoids share the same molecular weight (314)). This difference is helpful when analyzing purified fractions by mass spectroscopy, Figure 8.

Like CBN, CBG has a distinctly different ultraviolet (UV) spectrum compared to most other cannabinoids. Flash chromatography systems with diode array UV detectors can be used to identify several different cannabinoids, Figure 9.

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 commercial CBD sales or purifying individual cannabinoids such as CBG or CBN, flash chromatography can provide an effective way to accomplish these goals.

Refences

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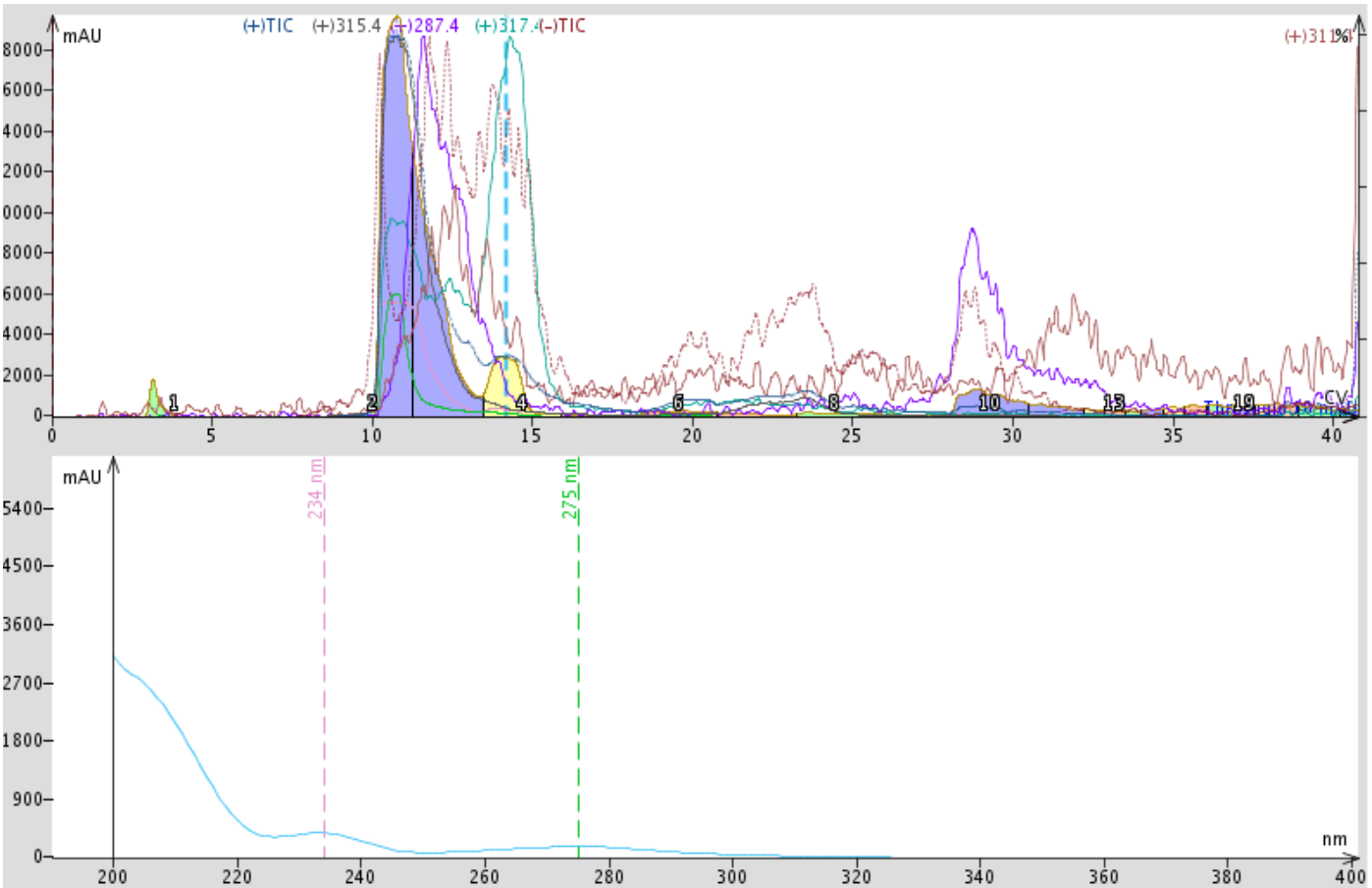

Figure 9. CBG UV spectrum shows maxima at 200, 234, and 275 nm.

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