Cannabinol (CBN) Purification Using Flash Chromatography

Summary

Most of the early interest in medicinal cannabis applications focused on cannabidiol (CBD), and this is now especially true with various introductions and revisions to global regulatory and legislative standards on this topic. For example, in the US, with the passage of the 2018 Farm bill, some cannabis products were de-scheduled from the Controlled Substances Act. There has also been concurrent and increasing scientific interest in medical applications involving the so-called minor cannabinoids such as CBN, CBG, and CBC. This application note focusses on CBN.

Introduction

CBN is a degradation product of THC and is expressed naturally only in small quantities in cannabis plants. Through oxidative processes (light/heat/air[1] or bio-enzymatically[2]), THC oxidizes to CBN. Conversion of CBD to CBN is also possible using iodine (Federica Pollastro, 2018).

Whether formed by natural degradation/oxidation or by chemical/enzymatic reaction, there is always contamination with other cannabinoids, reagents, and by-products all which need removal in order to isolate a pure product.

Methods - Synthetic CBN

Flash system
Biotage® Isolera Dalton 2000 (APCI)

Column
Biotage® Sfär C18, 12-gram

Solvent
A: Water

B: Methanol

Equilibration
80%B for 2 CV

Gradient
80%B for 6 CV

100%B for 6 CV

Flow Rate
25 mL/min

Detection
UV @ 283 nm, 218 nm (collect)

UV 200–400 nm (monitor)

Mass monitor m/z -309, -313, +311, +315

Evaporation
Biotage® V-10 Touch

Methods - Natural CBN

Biotage® Isolera Dalton 2000 (APCI)

Column
Biotage® Sfär C18, 12-gram

Solvent
A: Water

B: Methanol

Equilibration
80%B for 2 CV

Gradient
80%B for 20 CV

Flow Rate
25 mL/min

Detection
UV @ 283 nm, 254 nm (monitor)

UV 200–350 nm (collect)

Mass monitor m/z +311, +315, +287, +317

Protocol

Synthetic CBN

- Transfer 5 mL of crude CBN reaction mixture into a tared 20-mL scintillation vial
- Evaporate solvent using a hi-boil method with V-10 Touch
- Add methanol, QS to 5 mL (concentration 670 mg/mL)
- Create flash chromatography method
- Operate method
  - Load 1 mL (670 mg) onto column when prompted

Natural CBN

- To 100 mg of a 5-year old cannabis extract add 1 mL of methanol and dissolve completely
- Using the same column as above, inject 0.1 mL

Results and Discussion

Synthetic CBN

The ability to scale purification is important in a production environment so chromatographic methods need to be simple, robust and efficient. The method utilized in this application is a simple step gradient that separates CBN from its reaction mixture impurities, Figure 1.

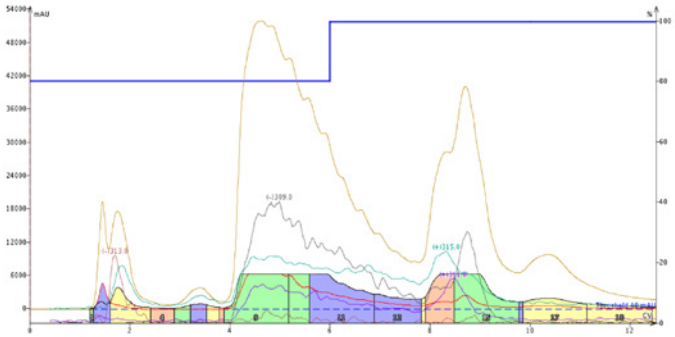

Figure 1. Simple step gradient purification method for synthetic CBN using both UV and mass detection. CBN is the big peak in the middle with a detected negative ionization mass of -309.

Both UV and mass detection were employed but only the UV signal was used to trigger fractionation (283 nm) lending to method simplicity. Mass detection was used to identify CBN as it has a unique molecular mass (310), which ionizes in the mass detector by adding a hydrogen (positive ionization) to create a detectable mass of +311 and subtracting a hydrogen (negative ionization) creating a detectable mass of -309. Most other cannabinoids have a molecular weight of 314.

Another useful technique to identify CBN is photo diode array UV (PDA), available on Biotage Isolera and Selekt systems. With PDA all the wavelengths emitted by the UV lamp are focused through the column effluent and the amount of light absorption at each wavelength is recorded and displayed in real time. The data can be analyzed post-run to evaluate UV peak purity and identify an individual compound, especially powerful when the compound's spectral footprint is known.

In the case of CBN, a literature search uncovered a paper showing both UV and mass spectra for various cannabinoids (Hazekamp, 2005). In this article, the CBN UV absorption spectra shows strong UV maxima at both 220 nm and 285 nm. By comparison, most other cannabinoids have strong UV maxima around 205 nm, medium absorbance near 230 nm, and minor absorption in the 270-280 nm range[3].

For the synthetic CBN purification, the UV spectra shows the same UV maxima as shown in the Hazekamp article. Because of the high CBN concentration, the UV spectra was evaluated at the beginning and end of the peak, Figure 2. Because the UV maxima are the same at the front and rear, this suggests the collected fractions are pure CBN.

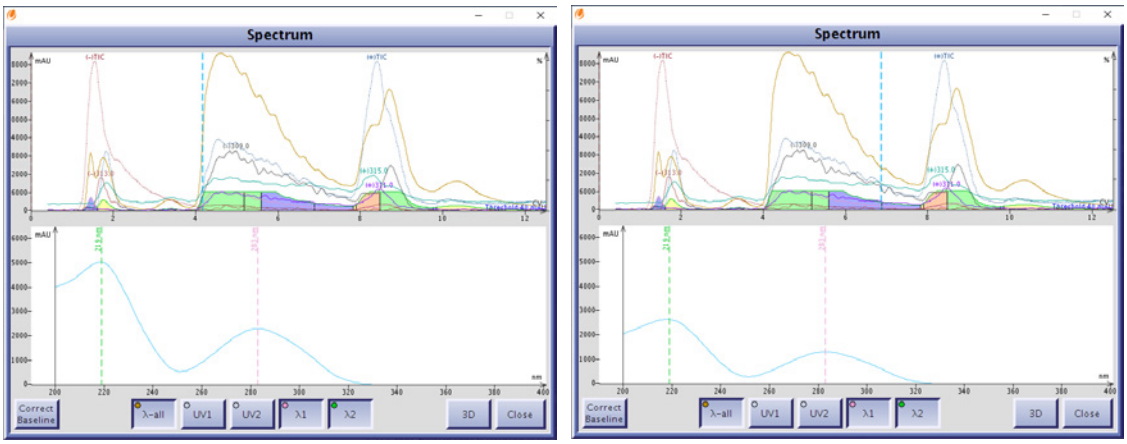

Figure 2. UV spectral analysis of purified CBN at the beginning of the peak (left) and at the end of the peak (right) shows the collected CBN fractions are pure. The amplitudes differ based on CBN concentration at the measurement point.

Post purification, the CBN fractions were evaporated using the V-10 Touch and a clear, semi crystalline product obtained, Figure 3.

Figure 3. Purified CBN from a synthetic reaction mixture.

Natural CBN

Because CBN is an oxidative degradation product of THC, its purification is a bit more challenging as the two cannabinoids elute closely to each other. To best facilitate this separation a simple isocratic method was employed using UV for fractionation and mass for identification. This method elutes CBN in 6 minutes with a detectable mass of +311; THC closely follows at ~7 minutes with a detectable mass of +315, Figure 4.

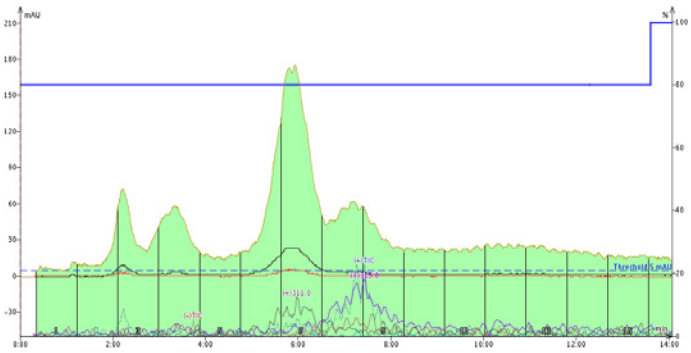

Figure 4. Isocratic purification of natural CBN created through THC oxidation. CBN is the large peak at 5 minutes with a detected mass of +311. THC elutes just after CBN and has a detected mass of +315.

As with the synthetic CBN, the UV spectra is a confirmatory tool that verified the compound identification, Figure 5.

Conclusion

Reversed-phase flash chromatography with diode-array UV and/or mass detection has been shown to both purify and identify CBN from synthetic and natural sources.

References

1. Reddit.com
2. US Patent US10538790B2
3. Hazekamp, A; Peltenburg, A; Verpoorte, R. Journal of Liquid Chromatography & Related Technologies®, 2005, 28, 2361–2382.

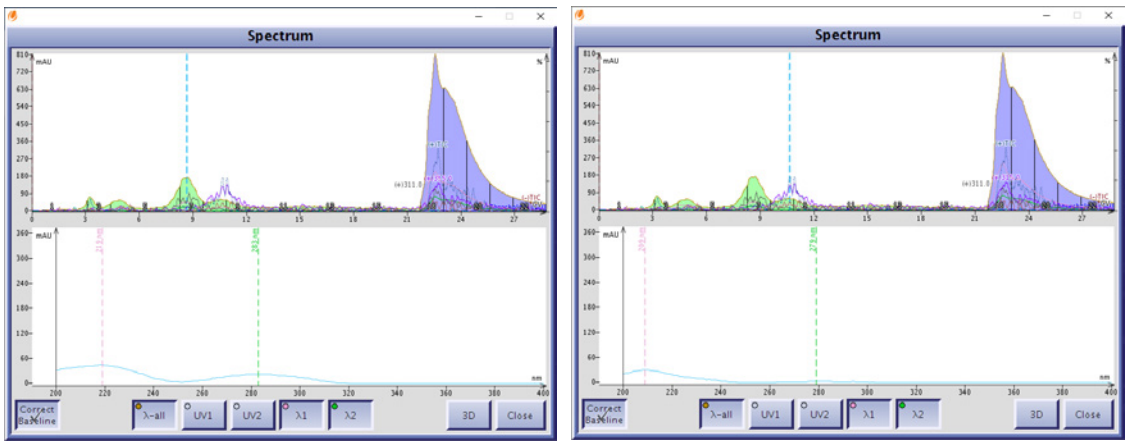

Figure 5. Purified degraded THC extract UV spectra identifies the tall green peak as CBN with UV maxima at 218 nm and 283 nm (left) and the adjacent peak to the right as THC with UV maxima of 209 nm and 278 nm (right).

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