Application Note: 21-001

Purification of Cannabinoids by preparative HPLC using ZEOsphere Silica Gel

Introduction

Cannabinoids are of growing interest to the Health and Pharmaceutical industries, with more than 100 different compounds already identified. The exact concentration can vary significantly depending on the type of plant, growing and processing conditions. Traditionally, attention focused on analysis and purification of the most famous cannabis derivative CBD, well known for its medical benefits. Only recently scientific interest started shifting to the other less known cannabinoids such as CBG and CBC. In addition to CBG & CBC, further investigation of the biosynthetic pathways of cannabinoids in raw plant tissues have shown that cannabinoids are essentially biosynthesized in their acidic (carboxylated) form.[1]
Cannabidiolic acid, or CBDA is the natural precursor to CBD. CBDA undergoes a transformation to its decarboxylated analog CBD when cannabis is thermally processed (Figure 1.)

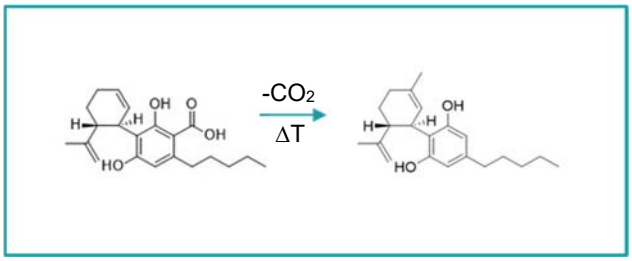

Figure 1. Conversion of cannabidiolic acid (CBDA), to cannabidiol (CBD)

Due to this thermal instability CBDA is a relatively recent discovery and was not even isolated until 1996. Lately, the huge potential of CBDA in therapeutic proposes has been discovered.[2] Recent studies find CBDA more effective than CBD in treating nausea or epilepsy.[3] Furthermore, it has been discovered that CBDA has the same binding mechanism as most common anti-inflammatory drugs such as ibuprofen and aspirin.

All that makes CBDA interesting to the Pharmaceutical and health Care industry. The cost-effective methods and materials to isolate and purify this cannabidiolic acid are being researched and developed.

Most of the standard methods used for analysis of cannabinoids are based on heating of the sample, and that has left the world in the dark in terms of the benefits of their acidic precursors. However, chromatographic purification offers the possibility to purify and determine the original composition (acidic forms) of plant cannabinoids, thus giving access to a more potent version of CBD to the fast-developing world of Cannabinoid therapeutics.

In this application letter, firstly we analyzed the cannabinoids in the hemp crude and showed the possibility to efficiently identify and purify multiple cannabinoids by using preparative HPLC. In the second part we analyzed hemp plant spiked with CBDA and showed excellent selectivity of our stationary C18 phase for separating CBDA from the other cannabinoids present in the raw plant sample.

Material and Method

Column

As stationary phase Zeochem's column, filled with spherically shaped, fully end-capped derivatized silica gel, ZEOsphere 100 C18 / 10 µm / 4.6*250mm (article number 300323) is used.

HPLC method

Oven temperature: 30°C
Flow: 1.6ml/min
Mobile phase A: 0.1% Phosphoric acid in water
Mobile Phase B: 0.1% Phosphoric acid in Acetonitrile
Mobile phase composition: 30% mobile phase B, 70% mobile phase
Gradient: none, isocratic
Detector: UV at 220 nm
Injection volume: 1 µl
Runtime: 30 minutes

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Results

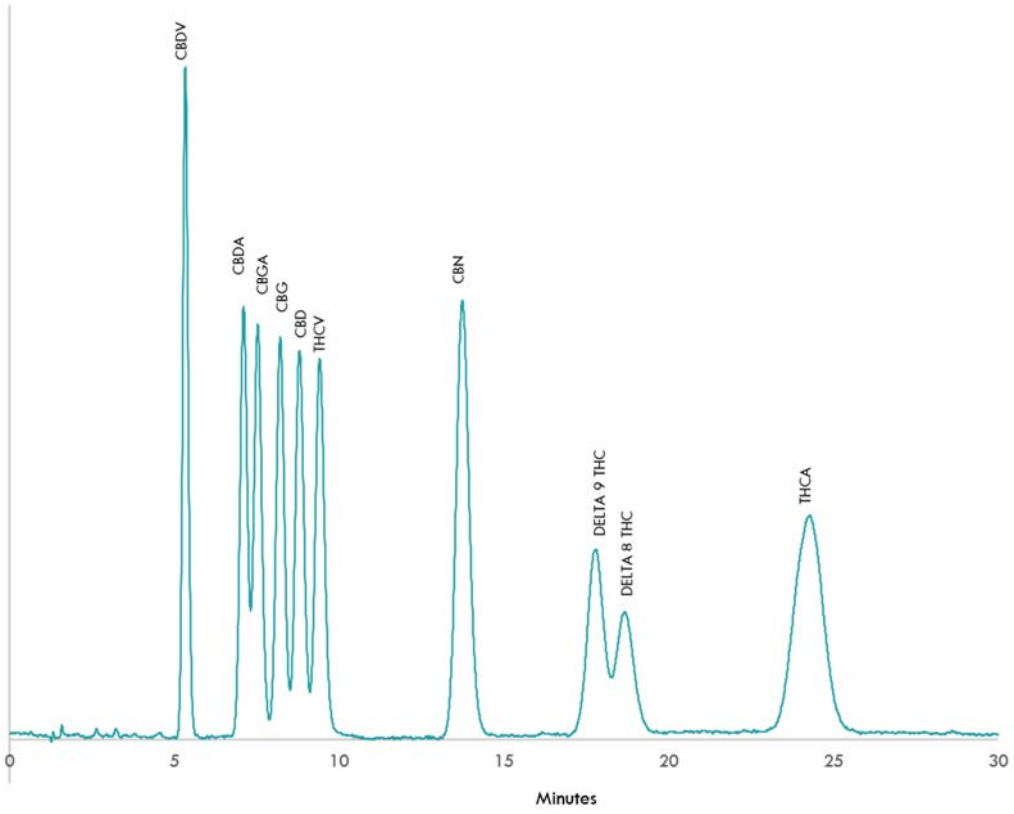

Figure 2. Isocratic separation of 10 cannabinoids in 25 minutes using ZEOsphere 100 C18

Peak #	Cannabinoid	Cannabinoid abbreviation	Retention time [min]	Resolution
1	Cannabidivarin	CBDV	5.4	-
2	Cannabidiolic acid	CBDA	7.3	4.88
3	Cannabigerolic acid	CBGA	7.8	1.05
4	Cannabigerol	CBG	8.5	1.52
5	Cannabidiol	CBD	9.1	1.20
6	Tetrahydrocannabivarin	THCV	9.7	1.15
7	Cannabinol	CBN	14.2	6.95
8	Delta-9-tetrahydrocannabinol	d9-THC	18.4	4.74
9	Delta-8-tetrahydrocannabinol	d8-THC	19.3	0.91
10	Delta 9 -tetrahydrocannabinolic acid	THCA	25.2	3.98

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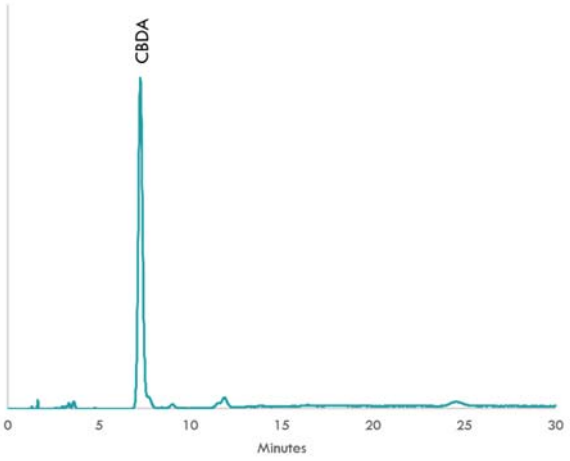

Figure 3. Hemp crude spiked with CBDA

Conclusion

ZEOsphere 100 C18 stationary phase demonstrated the possibility to purify 10 different cannabinoids from cannabis crude in 25 minutes using preparative HPLC method. Considering the particle size of 10 µm the ZEOsphere 100 C18 stationary phase offers the potential to be easily scaled up and used for industrial scale purifications.

CBDA is a hot topic in the scientific community since its pharmaceutical and nutraceutical potential is still far from being achieved. High content of this compound can be found in hemp varieties, especially those cultivated for food purposes, as well as in hemp wastes, such as pollen. Consequently, the recovery of pollen from industrial hemp could represent an abundant source of CBDA.

Solvent chromatography is a highly effective tool for achieving high yield purification of CBDA. In this application note, the isolation and purification of CBDA was carried out, on an extract enriched with CBDA by adopting the "relatively" simple, fast, technique of preparative scale reverse phase chromatography using a 10µm scalable silica C18 stationary phase.

Solvent chromatography at room temperature presents as an ideal candidate not only for CBDA purification but also for the yet much unexplored family of caboxylated cannabinoids present in Cannabis plant tissues.

Literature

(1) Wang, Y.-H.; Avula, B.; ElSohly, M. A.; Radwan, M. M.; Wang, M.; Wanas, A. S.; Mehmedic, Z.; Khan, I. A. Quantitative Determination of Δ9-THC, CBG, CBD, Their Acid Precursors and Five Other Neutral Cannabinoids by UHPLC-UV-MS. *Planta Med.* **2018**, *84* (04), 260–266. https://doi.org/10.1055/s-0043-124873.

(2) Formato, M.; Crescente, G.; Scognamiglio, M.; Fiorentino, A.; Pecoraro, M. T.; Piccolella, S.; Catauro, M.; Pacifico, S. (–)-Cannabidiolic Acid, a Still Overlooked Bioactive Compound: An Introductory Review and Preliminary Research. *Molecules* **2020**, *25* (11). https://doi.org/10.3390/molecules25112638.

(3) Bolognini, D.; Rock, E.; Cluny, N.; Cascio, M.; Limebeer, C.; Duncan, M.; Stott, C.; Javid, F.; Parker, L.; Pertwee, R. Cannabidiolic Acid Prevents Vomiting in Suncus Murinus and Nausea-Induced Behaviour in Rats by Enhancing 5-HT1A Receptor Activation. *Br. J. Pharmacol.* **2013**, *168* (6), 1456–1470. https://doi.org/10.1111/bph.12043.

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