Separation of CBDA and THCA from Cannabis and its relevance to biotechnological cannabinoid production

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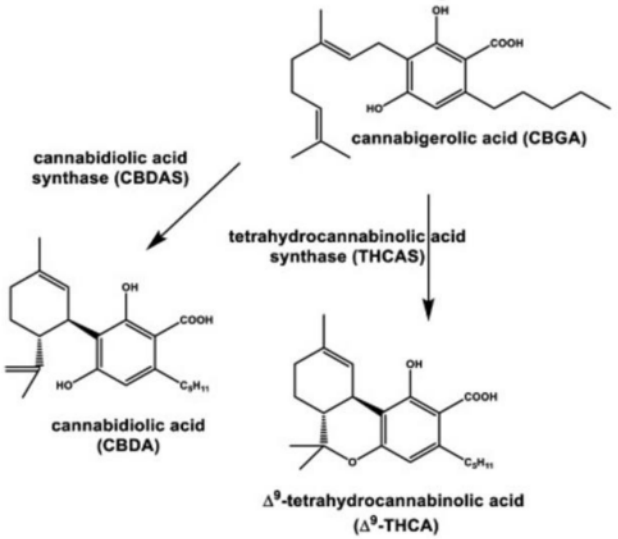
Chromatography Application Note ANSS-002



The plant *Cannabis sativa L*. has been utilized for millennia as stem fiber (hemp) and as a drug. In recent years, its secondary natural products cannabinoids have been extensively studied for their impressive pharmaceutical effects. Cannabinoids are predominantly found in oil compartments of trichomes of the plant *Cannabis sativa L*.

Two major cannabinoids that are naturally produced by their respective enzymes in the plant are acidic cannabinoids: Δ^9-tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA).

With cannabigerolic acid (CBGA) as the precursor, the enzyme Δ^9-tetrahydrocannabinolic acid synthase (THCAS) is responsible for the production of Δ^9-tetrahydrocannabinolic acid. The other enzyme cannabidiolic acid synthase (CBDAS) converts CBGA to cannabidiolic acid (CBDA).

In the work by B. Zirpel, O. Kayser, and F. Stechle[1], they showed, in comparison to the CBDAS wild-type, variant C_S116A has 2.8-fold increased to produce CBDA. Variant C_A414V displayed about 3.3-fold increased activity for CBDA and with about 19-fold increased activity for THCA."

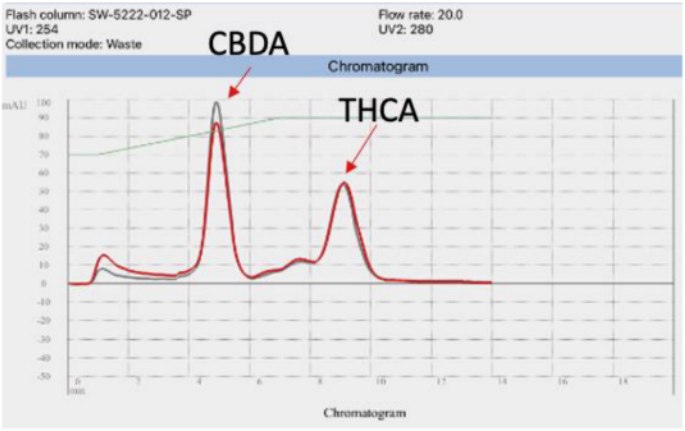
Cannabidiolic acid (CBDA, $C_{22}H_{30}O_4$) and Δ^9-trans-tetrahydrocannabinolic acid (THCA, $C_{22}H_{30}O_4$) are isomers, they both have the same chemical formula. Both can be heated to undergo decarboxylation which converts the acids to their neutral forms of CBD and THC.

We have developed an assay with very simple workup to isolate the CBDA and THCA from commercial cannabis buds from SQDC (Société québécoise du cannabis), after grinding the cannabis buds, the cannabinoids were extracted with 95% EtOH, after winterization at -20°C for 4 hours, the crude cannabinoids liquid sample was obtained after filtered through 0.22 μm membrane. The cannabinoids liquid sample was

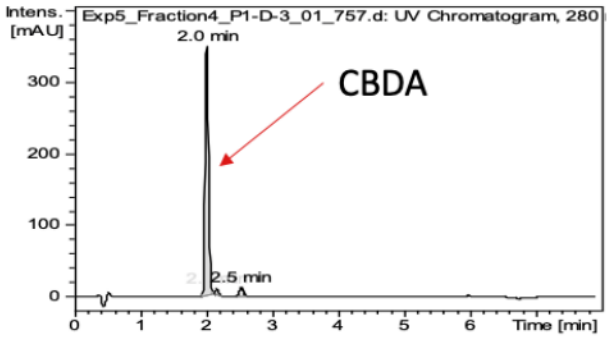
[1] B. Zirpel, O. Kayser, and F. Stechle, Journal of Biotechnology, Vol 284, 2018, 17-26.

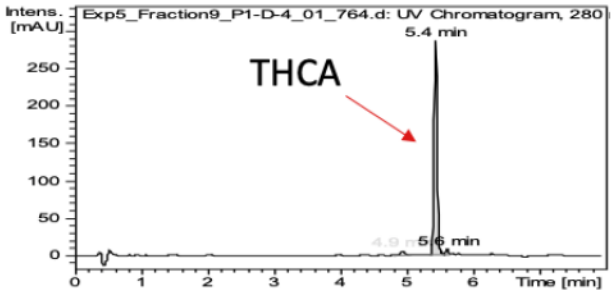
injected into our SepaBean machine 2 equipped with SepaFlash cartridge (High-efficiency spherical C18, 20-45 μm, 100 Å, carbon content 17%, end-capping, surface area 320 ㎡/g).

As shown in the graph below, there are excellent baseline separation between the CBDA and THCA .



The CBDA (fraction 4) is eluted around 5 mins, and the THCA (fraction 9) is eluted at 9 mins. Eluent A is water, eluent B is EtOH, flow rate is set to be 20 ml/min, UV 254 nm, solvent gradient ranges 70%~100%. CBDA (fraction 4) and THC A (fraction 9) were injected to LC-UV-MS for further analysis.





As demonstrated here, our SepaBean™ machine 2, an automatic flash chromatography system is an excellent and efficient instrument to purify CBDA and THCA from Cannabis. It enables the scientists to quickly assess the production of the two important target compounds in Cannabis. Our SepaBean costs only a fraction of a LC-MS or a HPLC. Biotechnological scientist or plant scientist can quick and cheaply get information on the plant production of those two compounds, therefore they can develop their genetic engineering assay or use the right plant signaling compound accordingly.

Acknowledgment. We appreciate our academia partners from the Chemistry Department, University of Montreal, Quebec, Canada (Alexandra Furtos, PhD. And Karine Gilbert, B.Sc.) for their invaluable contributions to this project. Their expertise in cannabis analysis was an indispensable part of this work.

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