Extraction Method Development for Cannabidiolic Acid from *Cannabis sativa L.* Using SepaBean™ Flash Chromatography Systems

Santai Science Inc.

Chromatography Application Note ANSS-005

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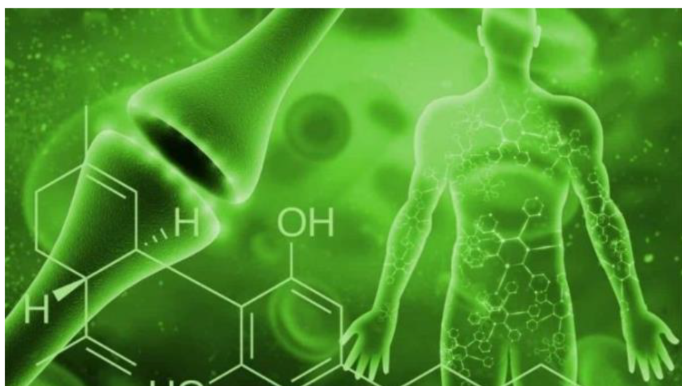
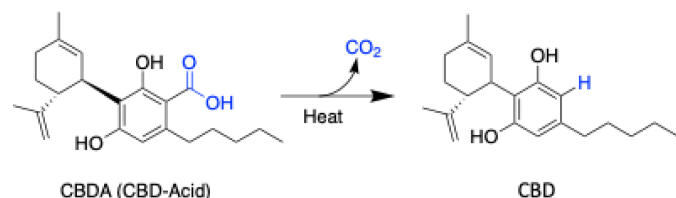


Cannabidiol (CBD) has been reported to possess a large variety of biological and medicinal activities, such as anti-tumor effects, anti-inflammatory, analgesic. CBD is usually obtained by non-enzymatic decarboxylation of its acidic precursor, cannabidiolic acid (CBDA) which is naturally produced by cannabis plants. This acidic cannabinoid is thermally unstable and can be decarboxylated to CBD when exposed to light or heat via smoking, baking, or refluxing[1].

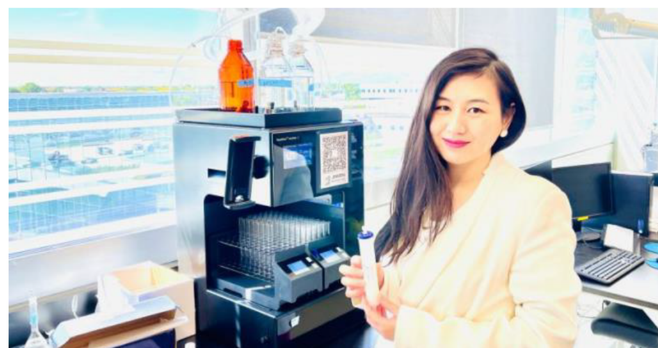
Therefore, a method that can rapidly isolate CBDA is useful for cannabis cultivation and medicinal research communities. Isolated CBDA is also important for the decarboxylation method development to maximize the yield of CBD.



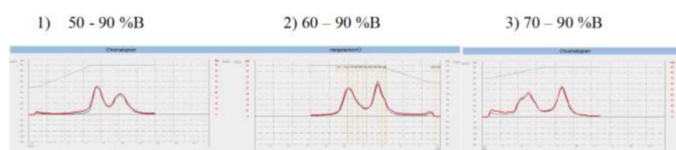
Extraction of CBDA from the plants poses the same challenges as any isolation of natural products. Here we have developed a flash chromatography method for the rapid purification of CBDA. Automated flash chromatography systems such as Santai SepaBean™ are composed of parts normally found in HPLC systems such as gradient pumps, injection systems, UV detectors, and fraction collectors.

Cannabis samples were purchased directly from SQDC (Société Québécoise du Cannabis). Prior to the flash chromatography, the cannabinoids were extracted from dry cannabis by the following method:

- 500mg dry cannabis
- Add 5 mL EtOH
- Sonicate 10min
- Vortex 10 min
- Winterize for minimum 4h at -20°C
- Centrifuge at 3500rpm for 10 minutes
- Filter through 0.22µm membrane



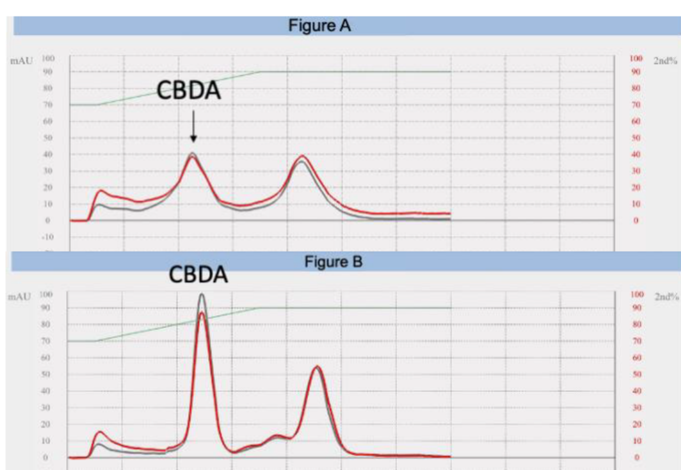
One of the challenges of the extraction of CBDA ($C_{22}H_{30}O_4$) is the presence of its structural isomer THCA ($C_{22}H_{30}O_4$). Those two have very similar chemical structure and therefore very similar physicochemical properties. In our initial normal phase experiments on SepaBean™ with regular silica columns, the two co-eluted. However, reversed-phase chromatography with C18 silica as the stationary phase showed base-line separation. Harmless water (solvent A) and 95% ethanol (solvent B) were chosen as solvents. On our workhorse irregular C18 column (SW-8201-012-IR), three different solvent gradients were tested: 50-90%B (**1**), 60-90%B (**2**) and 70-90%B (**3**). The best resolution, as illustrated below, is obtained with the 70-90%B gradient.



[1] Wang, M. et al. Cannabis and Cannabinoid Research V1 N1 (**2016**) 262.

The performance of irregular C18 silica vs spherical C18 silica was evaluated using this optimal gradient. SepaFlash™ cartridge irregular C18 (SW-5201-012-IR, 40-63 μm, 60 Å carbon content 17%, end-capped, surface area 500 m^2/g) and spherical C18 (SW-5222-012-SP, 20-45 μm, 100 Å carbon content 17%, end-capped, surface area 320 m^2/g) were used side-by-side as comparison.

The spherical C18 column (**Figure A**) generated sharper peaks and yielded better baseline resolution than the irregular C18 column (**Figure B)**



	Column number	Silica type	Silica size	Column size	Sample size
Figure A	SW-5201-012-IR	Irregular C18	40-63 μm	23 g	50 mg
Figure B	SW-5222-012-SP	Spherical C18	20-45 μm	20 g	50 mg

The identity of the CBDA peak was confirmed by mass spectrometry on a LC-UV-microTOF instrument.

The recovery rate of the CBDA was evaluated by measuring the UV absorbance at 280nm of a solution with and without flash chromatography separation. A 0.5mL aliquot (50 mg) of the cannabis extract was injected on the flash instrument. The CBDA fraction was collected, transferred to a 100 mL volumetric flask and completed to level with ethanol. An aliquot of 5 μL of this solution was injected into the LC-UV-microTOF instrument. A control solution was prepared with 0.5mL (50 mg) of the same cannabis extract in a 100mL volumetric flask, also in ethanol and 5 μL were injected into the LC-UV-microTOF system. The corresponding peak areas of flash chromatography collected and the control are shown in the table below. The calculated recovery of CBDA is 83.5%.

UV absorption Peak area of CBDA λ = 280 nm		Recovery rate
Before flash (standard)	221.2	83.5%
Combined flash fractions	184.8	

A reverse phase flash chromatography method was developed to extract CBDA rapidly and reliably from a cannabis extract. Spherical reverse-phase C18 with a solvent mixture of water and ethanol yielded an excellent separation with a recovery rate of 83.5%.

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