Rapid Isolation Procedure for Δ^9-tetrahydrocannabinolic acid A (THCA) From *Cannabis sativa L.* Using SepaBean™ Flash Chromatography Systems

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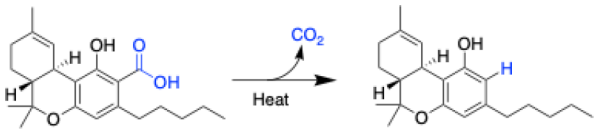
Δ^9-Tetrahydrocannabinolic acid A (THCA), predominantly found in the trichomes of the *Cannabis sativa L.* plant, is the precursor of the psychoactive Δ^9-THC. THCA is thermally unstable and can be decarboxylated to Δ^9-THC when exposed to light or heat via smoking, baking, or refluxing[1].



Δ^9–THCA (THC-Acid) Δ^9–THC

Reports also show that Δ^9-THC itself readily oxidizes to cannabinol (CBN) with oxygen and light during the decarboxylation process[2]. Therefore, a method that can rapidly isolate Δ^9-THCA is very important for cannabis cultivation and research communities. Isolated Δ^9-THCA is also very valuable for the decarboxylation method development to maximize the yield of the desired compound such as Δ^9-THC while avoiding the decomposing or unwanted conversion.

Flash chromatography is a powerful tool for the rapid separation of chemical mixtures. Automated flash chromatography system such as Santai SepaBean™ is composed of parts normally found in HPLC systems such as gradient pumps, injection ports, UV detectors, and fraction collection features.

In the pharmaceutical industry, flash chromatography is used to purify peptides, antibiotics, APIs, carbohydrates, and drug intermediates. It is also extremely useful for the extraction of natural products, such as tocopherols, alkaloids, xanthones, flavonoids, as well as cannabinoids.

Cannabis samples were purchased directly from SQDC (Société Québécoise du Cannabis). Prior to the flash chromatography, the cannabinoids were extracted by the following extraction 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 a 0.22μm membrane

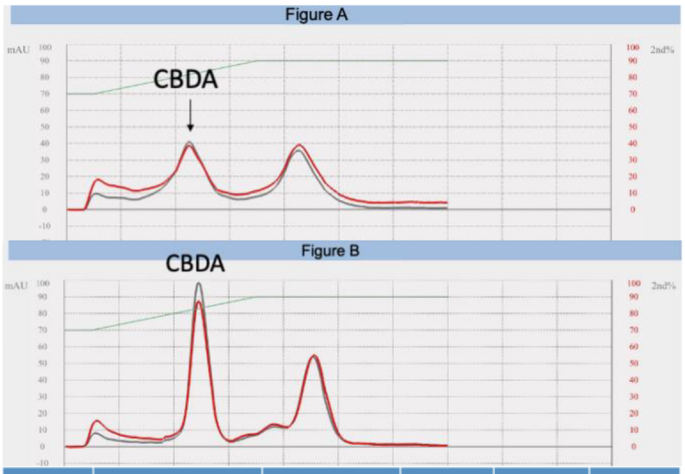
Several aliquots of the cannabis extract were injected into the SepaBean™ flash system. Reversed-phase chromatography with C18 silica as the stationary phase was preferred to normal phase for its environment-friendly nature *i.e.* it can be reused 40~60 times. Moreover, harmless water (solvent A) and 95% ethanol (solvent B) were

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

[2] Dussy, FE, et al. Forensic Sci. Int. V149, N1(**2005**), 1

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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