Textbook Separation of CBD and THC by SepaFlash™ Spherical C18 Columns

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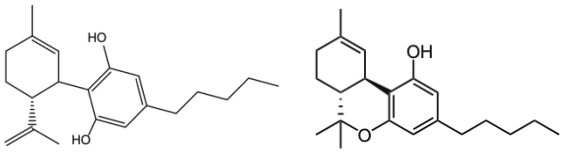
Chromatography Application Note

ANSS-006



Cannabidiol (CBD) 1 (Δ^9-THC-C_5)

There more than 400 compounds have been identified in the Cannabis plant, however most studies have focused on the effects of the cannabinoids, in particular Δ^9-(trans)-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD).

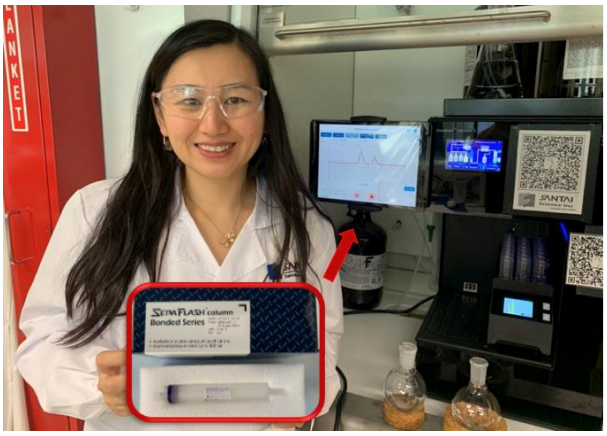
The isolation of CBD and THC are important for the research community and the production industrial. **Santai Science** (Montreal, Canada) has collaborated with **Synthèse AptoChem Inc** on the development of separation of CBD and THC mixture by flash chromatography.

These two cannabinoids interact with the endocannabinoid system (ECS). ECS is a complex cell-signaling system identified in humans and animals that plays role in regulating a range of functions and processes, such as sleep, mood, appetite, memory, and reproduction. Although Δ^9-THC and CBD have the same chemical formula, their function are quite different. CBD is a rather simple compound, it is found to have the capacity to affect basic physiological mechanisms rather than just a specific site, and its biological effects are widely spread and yet it is essentially non-toxic.

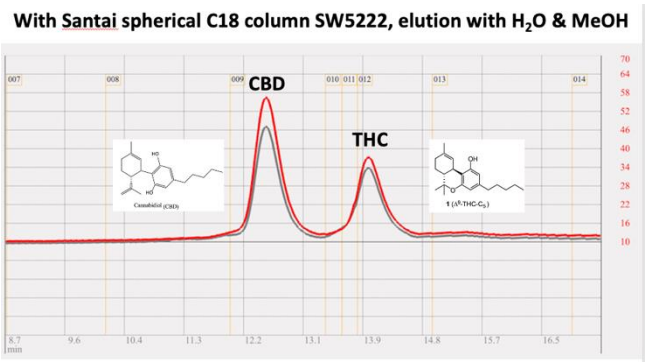
Early studies by the "father of cannabis"- Mechoulam in the 1980s has shown that THC activity is stereospecific.[1] It interacts strong with G protein-coupled receptor (CB1 and CB2) in mammalian brain. Although THC has been legalized in several country for recreational use, it still has adverse effect. For example, Taffe[2] has shown that in monkeys, THC impairs spatial working (short-term) memory.

Cannabidiol (CBD, $C_{21}H_{30}O_2$) and Δ^9-trans-tetrahydrocannabinol (Δ^9-THC, $C_{21}H_{30}O_2$) have the same chemical formula. Several attempt of the separation of those two on normal phase flash chromatography was unsuccessful.

Reverse-phase C18 flash chromatography was employed here, with water and methanol as solvent mixture. About 1:1 mixture of 400 mg of CBD and THC was dissolved in methanol; 10 drop of water was added into solution to obtain similar solvent combination as the starting elution gradient. The cannabinoids aliquot was injected into our SepaBean™ machine equipped with SepaFlash cartridge **SW-5222-025-SP** (UltraPure spherical C18, 20-45 µm, 100 Å, loading capacity 0.1~2%).



As shown in the graph below, excellent baseline separation was achieved, and both peaks are in good Gaussian distribution. Eluent A is water, eluent B is MeOH, flow rate is set to be 25 ml/min, solvent gradient ranges 70%~100% of the solvent B .

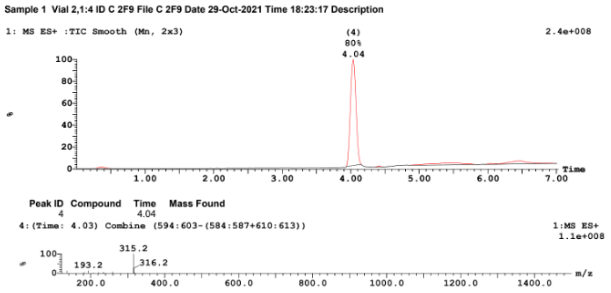


[1] Mechoulam, R., Feigenbaum, J.J., Lander, N. *et al. . Experientia* ***44,*** 762 (1988).

[2] Gullone, E., & Taffe, J. *Psychological Assessment,* ***24***, 409. (2012)

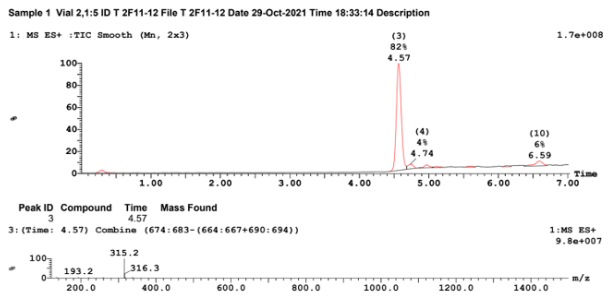
The identity of each peak was verified by LC-MS. In the LC-MS spectra of the CBD fraction below, it shows the main CBD peak at 4.04 mins, with molecular ion $M+H^+$ (m/z. 315.2).

LC-MS of CBD fraction



In the LC-MS of the THC fraction, the main THC peak appears at 4.57 mins, with molecular ion $M+H^+$ (m/z. 315.2).

LC-MS of THC fraction



Conclusion, excellent separation of CBD and THC can be achieved with Santai Spherical reverse-phase C18 silica column. Since C18 columns are reusable at least 30 times with proper storage, this is an effective and economical method for the isolation of CBD and THC.

High-efficiency spherical C18, 20-45 µm, 100 Å

(carbon content 17%, end-capping, surface area 320 m^2/g, loading capacity 0.1–2%)

Item Number	Column Size	Sample Size	Units/Box	Flow Rate (mL/min)
SW-5222-004-SP	5.4 g	5.4 mg–108 mg	2	5–15
SW-5222-012-SP	20 g	20 mg–0.40 g	1	10–25
SW-5222-025-SP	33 g	33 mg–0.66 g	1	10–25
SW-5222-040-SP	48 g	48 mg–0.96 g	1	15–30
SW-5222-080-SP	105 g	105 mg–2.1 g	1	20–50
SW-5222-120-SP	155 g	155 mg–3.1 g	1	30–60
SW-5222-220-SP	300 g	300 mg–6.0 g	1	40–80
SW-5222-330-SP	420 g	420 mg–8.4 g	1	40–80

※ Compatible with all flash chromatography systems on the market.

Synthèse AptoChem Inc (*7171 Rue Frederick Banting, Saint-Laurent, QC H4S 1Z9, 514-745-7575*) is authorized to conduct analytical testing on Cannabis and to process Cannabis under the Canadian Cannabis Act.

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