

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

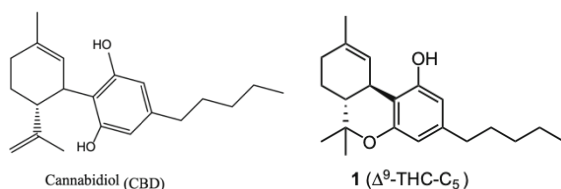
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Santai Science Inc.

Chromatography Application Note  
ANSS-006



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  $\Delta^9$ -(trans)-tetrahydrocannabinol ( $\Delta^9$ -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.

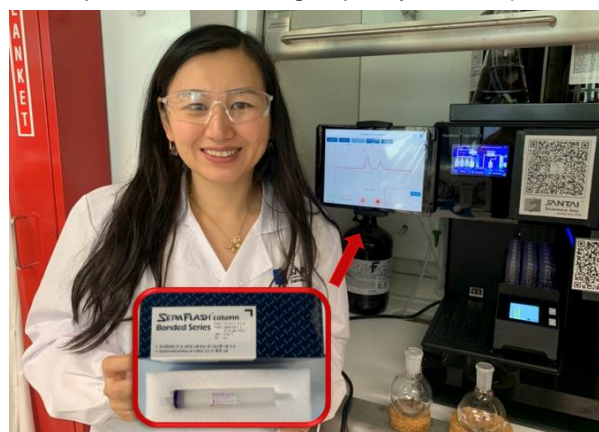
These two cannabinoids interact with the endocannabinoid system (ECS). ECS is a complex cell-signaling system identified in humans and animals that plays role in regulating a range of functions and processes, such as sleep, mood, appetite, memory, and reproduction. Although  $\Delta^9$ -THC and CBD have 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.<sup>1</sup> 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<sup>2</sup> has shown that in monkeys, THC impairs spatial working (short-term) memory.

Cannabidiol (CBD, C<sub>21</sub>H<sub>30</sub>O<sub>2</sub>) and  $\Delta^9$ -trans-tetrahydrocannabinol ( $\Delta^9$ -THC, C<sub>21</sub>H<sub>30</sub>O<sub>2</sub>) have the same chemical formula. Several attempt of the separation of those two on normal phase flash

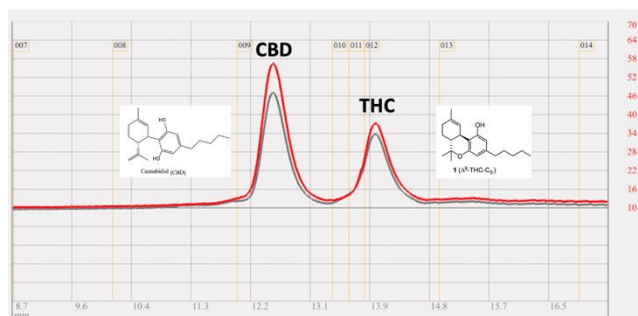
chromatography was unsuccessful.

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  $\mu$ m, 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 .

With Santai spherical C18 column SW5222, elution with H<sub>2</sub>O & MeOH

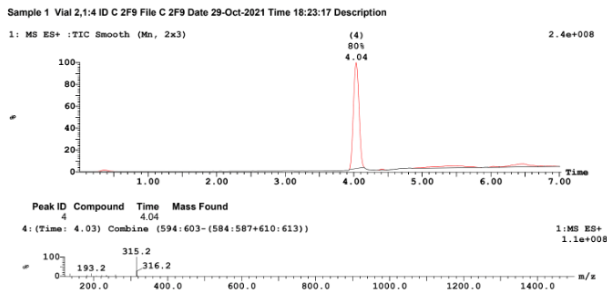


<sup>1</sup> Mechoulam, R., Feigenbaum, J.J., Lander, N. *et al.* . *Experientia* **44**, 762 (1988).

<sup>2</sup> Gullone, E., & Taffe, J. *Psychological Assessment*, **24**, 409. (2012)

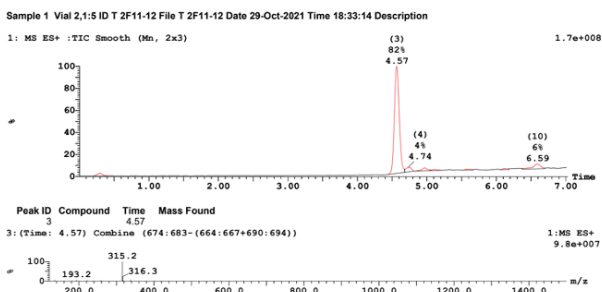
The identity of each peak was verified by LC-MS. In the LC-MS spectra of the CBD fraction below, it shows the main CBD peak at 4.04 mins, with molecular ion M+H<sup>+</sup> (m/z. 315.2).

### 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<sup>+</sup> (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<sup>2</sup>/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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