Application Note
No. 401/2020

Separation of cannabinoids and scaling-up with Flash-chromatography

1. Introduction

The compounds produced by the cannabis plant are famous for both recreational and medical purposes. The potency of the so-called cannabinoids in pharmaceutical applications, to ensure pain relief or to ease psychological burden, promoted the development of the cannabis industry. In many cases the challenge in this field is to purify the medically used substances from illegal psychoactive compounds.
There are more than one hundred of cannabinoids identified, some of them having similar chemical structure. This feature complicates the separation of these compounds according to their chemical properties. Chromatography is the technique of choice for separation of multicomponents samples but when used at industrial scale, compromise has to be found between its separating power and the amount that can be purified. Larger capacity and loading are usually detrimental to the separation, i.e. they lower the resolution between the eluting substances.
Flash-chromatography can be used to separate the most important components of cannabis extract despite its lower resolution. Depending on the criteria to isolate a given peak in the chromatogram, a high purity can be obtained without the need for baseline resolution. The material used for flash-chromatography also allows to increase the loading of sample to be purified, i.e. scale-up. Since scalling-up usually decreases the efficiency of separation, the process in chromatography is done by first optimizing the parameters for separation at a small-scale, before adaptation to the given size.

2. Equipment

- Pure C-850
- Sepacore pump C-605 / control module C-615
- T-valve and backpressure regulators from Pure sampling pump
- FlashPure Ecoflex C18 cartridges 12g, 120g
- PrepPure C18 10µm 150x20 mm
- Ultrasonic bath

3. Chemicals and Materials

Chemicals:

- Acetone, technical grade (ECSA)
- Deionized water.
- Ethanol, technical grade
- Cannabis extract (winterized and concentrated)

Potential Health Effects: Consult respective material safety data sheets.
Protection: Wear appropriate protective equipment.

4. Experimental

4.1 Optimisation of flash-chromatography

10 g of cannabis extract are weighted, dissolved in 10 g of pure ethanol (12.5 mL), sonicated for 15 min then filtered through 0.2 µm pore-size filters. The parameters for flash purification of CBD from other cannabinoids were optimized on a 12 g C18 cartridge (see *Table 1*). For each run, 0.25 mL (~200 mg sample) were introduced in the chromatography system with a syringe and purified with the parameters reported. Results of the separation are displayed in Figure 1.

Table 1: chromatography parameters.

Parameter			
Eluent A	Water		
Eluent B	Ethanol		
Column	12 g FP Ecoflex C18		
Flow rate	30 mL/min		
Gradient	**Time (min)**	**Type**	**%B**
	0 - 6.4	Gradient	70 - 90
	6.4	Step	100
	6.4 - 16	Hold	100
UV sensitivity	Low		
ELSD sensitivity	Low		
UV Wavelengths	220 nm 254 nm 276 nm 320 nm		
UV threshold	0.3 A.U.		
ELSD threshold	20 mV		

4.2 Scale-up

After the separation of cannabinoids reached a satisfying result for flash-chromatography on a small scale, the amount of sample to be purified in a single run was increased. For this purpose, a bigger cartridge (120 g) was used and the parameters of the run were updated automatically by the Pure software. 8 mL of cannabis extract (~6.6 g of sample) were injected by the use of a Sepacore pump module (C-605 and C-615). Six fractions (Figure 2) were collected from the separation and used for preparative separation.

4.3 Secondary separation with Prep-chromatography

The fractions collected from the scaled-up experiment were concentrated by evaporation on a rotavapor R-300, then redissolved in 2 mL ethanol. The Pure system was used in prep-mode with a PrepPure column (C18 10µm 150x20 mm) and 0.2 mL of fraction 1, 2, 3, 4 and 5 were injected for each run. The parameters used were similar to those of the 12 g flash-cartridge, but the gradient was slightly slower for early eluting fraction 1 and 2 (70-90% in 10 min), and steeper for late eluting fractions 4 and 5 (70-90% in 5 min).

5. Results and discussion

From observation of the chromatogram in Figure 1 a separation of the different cannabinoids is visible despite the absence of baseline resolution. Assignment of the different peaks was done by comparison with elution data from litterature[1]. CBD was eluted between 2-3 min, THC between 4-6 min and other cannabinoids or terpenes, less polar, after 8 min.

The separation achieved on a 120 g cartridge is displayed in Figure 2. Six different fractions were collected (underlined in green). Due to increased loading and efficient adaptation of the gradient, the chromatogram showed a better separation than on the 12 g cartridge. Notably, the 3rd and 5th fraction (12-16 and 25-30 min respectively) displayed more peaks despite the lack of resolution.

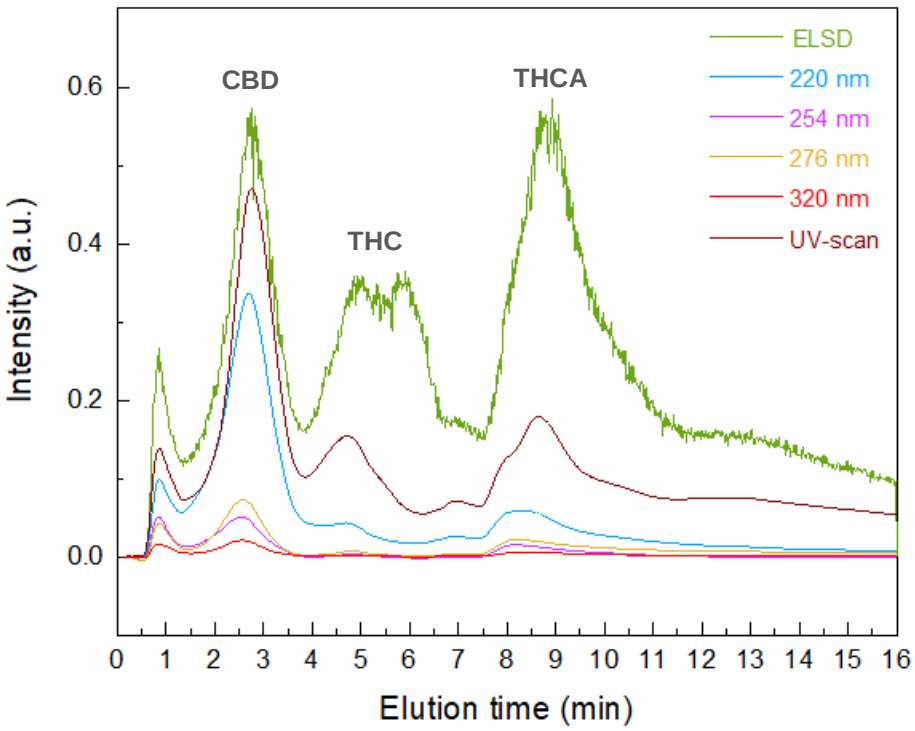

Figure 1. Chromatogram of the purification of cannabis extract on a 12g C18 FP Ecoflex.

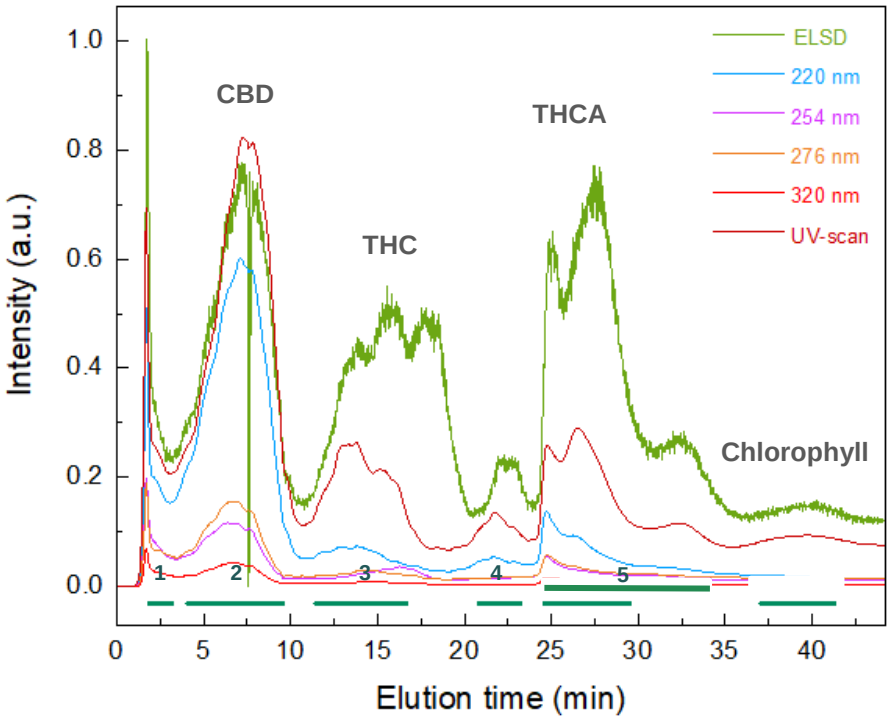

Figure 2. Chromatogram of the purification of cannabis extract on a 120 g C18 FP Ecoflex. Collected fractions are represented by the green underlines.

All fractions were concentrated by evaporation of the solvent on a R-300. After further dissolution in ethanol, fractions 2 and 3 started to crystalize due to the high concentration and saturation. These solutions were heated and sonicated to dissolve the material before separation in preparative mode. The chromatograms of fraction 1, 2, 3 and 5 are shown in Figure 3, 4, 5 and 6 respectively. Fraction 6 was not subjected to a second purification and was identified as chlorophyll after concentration. Assignment of the peaks was done by comparison with reference elution data and absorption spectra[1,2], using the UV-scan feature.

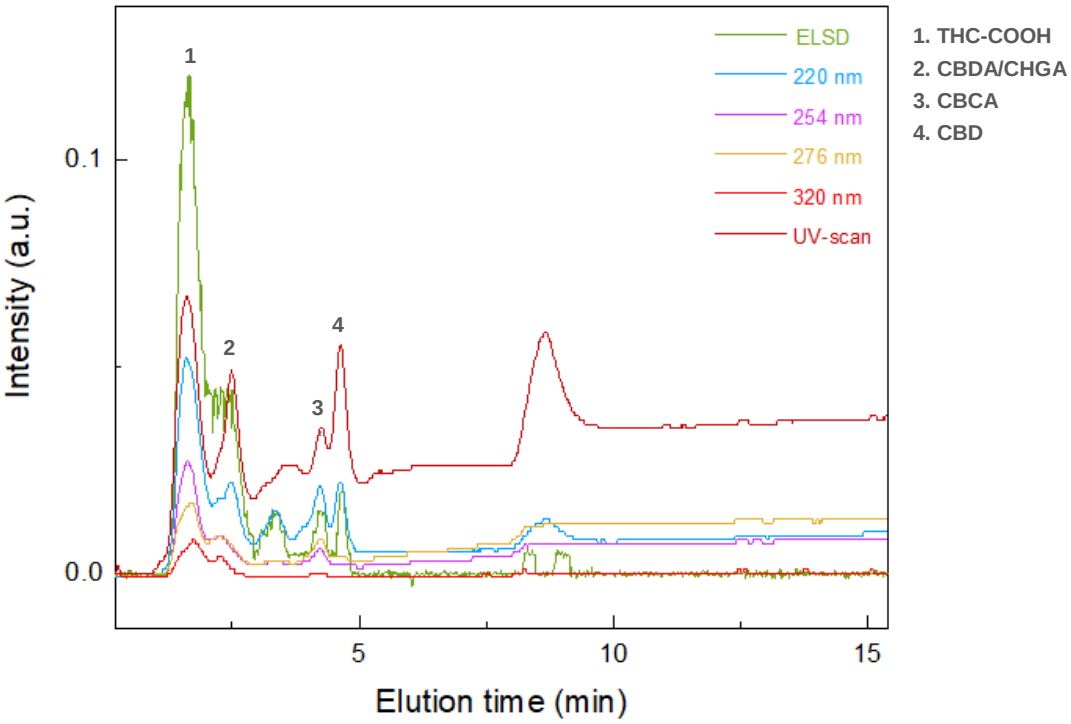

Figure 3. Chromatogram of the purification of fraction 1 with preparative column.

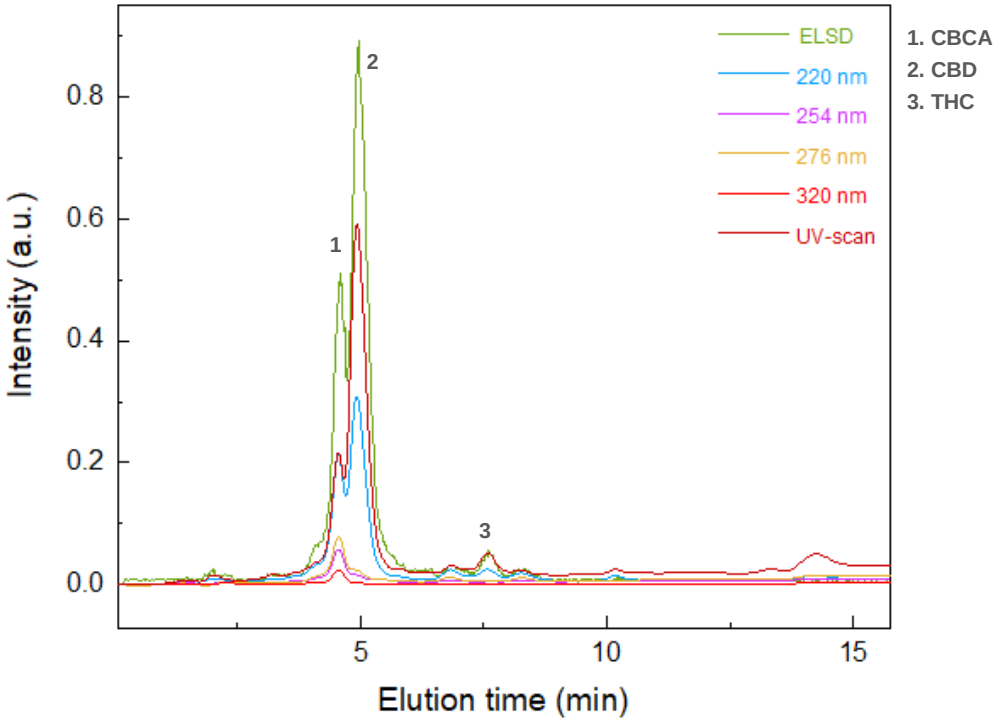

Figure 4. Chromatogram of the purification of fraction 2 with preparative column.

From elution and absorption references, the very first peak in Figure 3 is identified as THC-COOH while the second peak might correspond to either CBDA or CHGA. In Figure 3, 4 and 5, the two peaks eluting shortly before 5 min are assigned to CBCA first and CBD second, as the main component of the band. In Figure 4, a small impurity identified as THC eluted afterwards. However, this contamination could be avoided by discarding the part of fraction 2 where the peaks overlapped.

Figure 5 was concentrated on the hypothesized THC fraction. It still showed the two peaks of the CBD part and revealed at least three different substances between 7.5 and 10 min of elution. It is assumed that the middle peak with the highest absorbance at 220 nm is CBN, while the two other peaks are Δ^8-THC and Δ^9-THC. Fraction 4 (cfr. Figure 6) showed no further separation when applied on a preparative column and could come from CBC based on the elution order, although the concentration would be unexpectedly high. Fraction 5 on the other hand showed several peaks with the second purification (cfr. Figure 7). These multiple peaks are attributed to THCA derivatives.

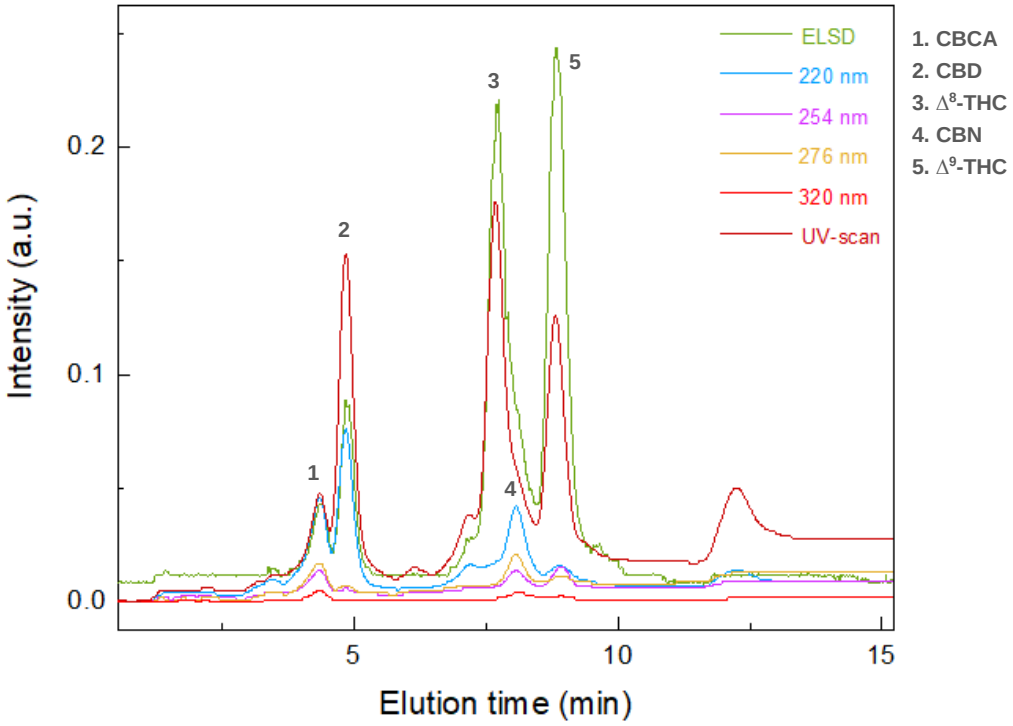

Figure 5. Chromatogram of the purification of fraction 3 with preparative column.

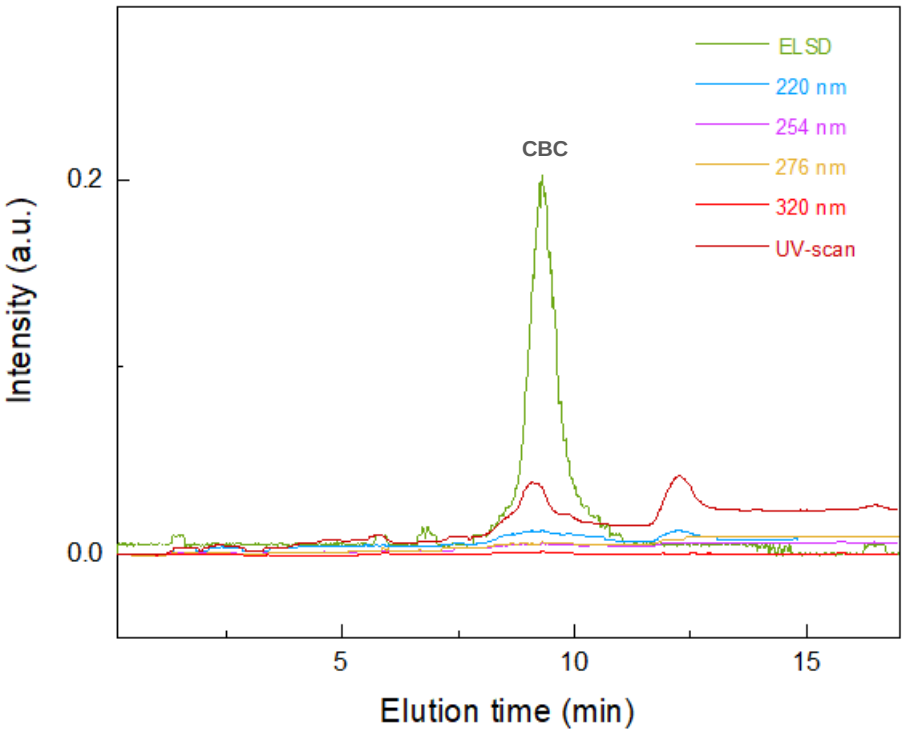

Figure 6. Chromatogram of the purification of fraction 4 with preparative column.

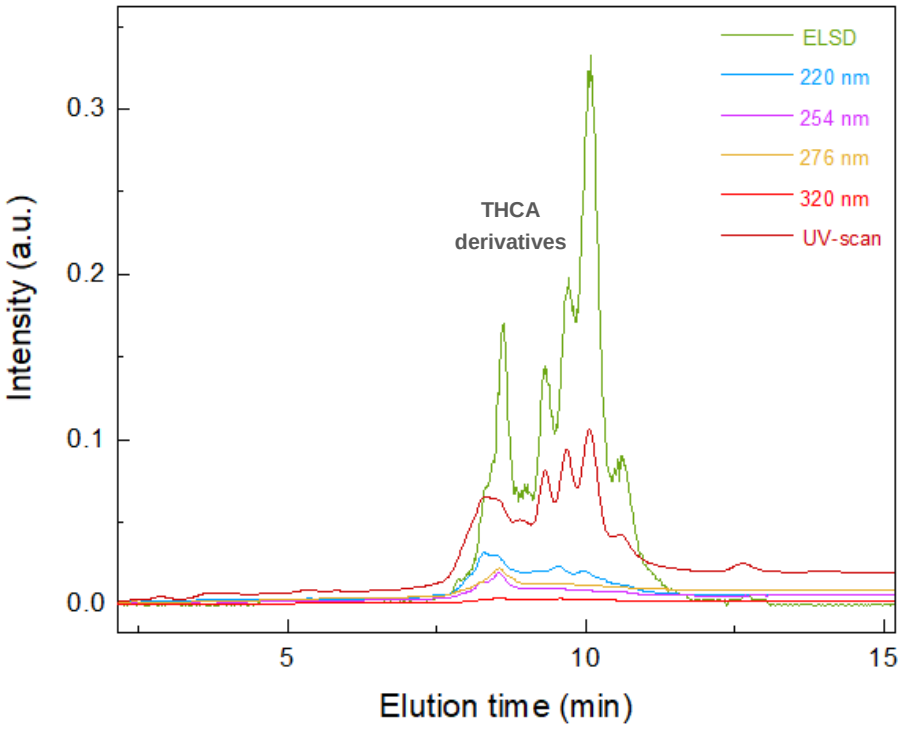

Figure 7. Chromatogram of the purification of fraction 5 with preparative column.

6. Conclusion

Here we showed how the separation of cannabinoids could be achieved on a small 12 g flash-cartridge. The conditions for the separation were adapted for scaling-up simply by using the embedded features of the Pure software which adapts protocols between cartridges of different sizes. A 30-fold scale-up was performed by using a 120 g flash-cartridge in overloaded conditions. The separated fractions were collected and separated further by preparative chromatography.
The successive flash and prep chromatography allowed to separate and identify at least 9 different cannabinoids and proved that a high purity could be achieved for CBD with flash chromatography, by collecting the appropriate part of the CBD peak in the chromatogram.

7. References

[1] A. Hazekamp, A. Peltenburg, and R. Verpoorte (2005*). Chromatographic and Spectroscopic Data of Cannabinoids from Cannabis sativa L.* Journal of Liquid Chromatography & Related Technologies, 28: 2361–2382.

[2] S. Zivovinovic et al. (2018*). Determination of cannabinoids in Cannabis sativa L. samples for recreational, medical, and forensic purposes by reversed-phase liquid chromatography-ultraviolet detection.* Journal of Analytical Science and Technology.