Fast Centrifugal Partition Chromatography (FCPC™): An effective way to isolate cannabinoids from *Cannabis sativa* extracts in production scale

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Introduction

The use of cannabinoids from *Cannabis sativa L.* in therapeutics is known since a long time in history. It involves various ways of administration and forms such as raw herbal material, cannabis extracts, natural cannabinoid-based medicines and synthetic cannabinoids as APIs. These materials are used especially in epilepsy, cancer palliation and primary treatment, chronic pain, Parkinson disease, metatraumatic stress, multiple sclerosis, anxiety, irritable bowel syndrome, neurodegenerative and other diseases [1,2]. However, the laws and regulations are substantially different between countries: Both the European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA) do not approve the use of herbal cannabis or its extracts. The FDA approved several cannabinoid-based medicines, so did 23 European countries and Canada [3]. Thus, there is an increasing need for pure cannabinoids, to be used in clinical trials as well as APIS, that are produced through effective and high-throughput industrial scale technologies. Up-to-date few such technologies are implemented in the relevant industry that is either very traditional, or used to operate in the borders of legality. Fast Centrifugal Partition Chromatography (FCPC®) is reputed as being one of the most efficient, high-throughput and selective chromatographic technologies existing in large scale, especially for cannabinoid purification. The Cannabis-related Academia has evaluated CPC as an efficient technology, for cannabinoid purification, especially for Cannabidiol (CBD) and Δ-9 Tetrahydrocannabinol (Δ-9 THC), from *Cannabis* extracts [4]. Nevertheless, there is a strong need for developing methods and processes in order to provide the industry with optimized, high-throughput, regulation and GMP-compliant Standard Operating Procedures.

Materials and methods

Plant material of *Cannabis sativa L.* (organically grown hemp) was provided as flower tops. Heptane extraction was performed in a thermostated bath at -42°C for 30min. Liquid/liquid countercurrent centrifugal extraction process was performed using BXP040 extractor (Rousselet Centrifugation, Annonay-France). Preparative CPC purifications were carried out on a Kromaton FCPC A (Rousselet Centrifugation, Annonay-France) with a 200ml rotor mounted and by using Gilson Spot-Prep peripheral system including pump, injection valve, 50ml injection loop, UV-detector 200-600nm (PDA) and fraction collector. Quantitative analyses were performed in HPLC-UV using the United Nations method for Cannabinoid analysis (250x4mm RP-8 (5 µm);, 30°C, Acetonitrile : water (8:2 v/v), isocratic, stop time 8 min., flow rate1 ml/min, Photodiode array (PDA), 220 nm and 240 nm.

Process Development Strategy

Cannabinoids are currently being used in various types of products such as medicines, supplements and food, while Δ-9 THC is considered as a Schedule I drug substance in various jurisdictions and CBD in a few of them. Thus, quality requirements and compliances for production methods of cannabinoids may vary significantly but generally those are rather demanding. Process development for the efficient purification has to meet several needs from an applicability, efficacy and regulation-meeting point of view. High-throughput and productivity, variable purities, low solvent consumption, high efficiency, respect of GMP-compliant SOPs for various sectors are the main parameters that need to be considered. For instance, as far as organic and other solvents are concerned, the less toxic ones that are used the higher penetration and applicability the SOPs will have in different markets and jurisdictions. Thus, an effort to include only ICH (International Council for Harmonization) guidelines Class III solvents is meaningful, although very challenging because not a single biphasic solvent system family composed exclusively by these solvents has ever been introduced in CPC and generally in liquid/liquid chromatography. On an efficiency and productivity perspective, processes that scale-up satisfactorily and can be characterized as semi-continuous, are demanded by the industry, while solvents need to be easily removable from the pure fractions. High purities (>95%) of the isolated substances are required, characterizing generally APIs.

References

[1] Cannabis Pharmacology: The Usual Suspects and a Few Promising Leads, Ethan B. Russo, Jahan Marcu, Advances in Pharmacology, vol. 80, 2017

[2] The therapeutic effects of Cannabis and cannabinoids: An update from the National Academies of Sciences, Engineering and Medicine report, Donald Abrahams, European Journal of Internal Medicine, vol. 49, 2018

[3] Medical use of cannabis and cannabinoids containing products – Regulations in Europe and North America, Ran AbuhasiraLiat Shbiro, Yuval Landschaft, European Journal of Internal Medicine, vol. 49, 2018

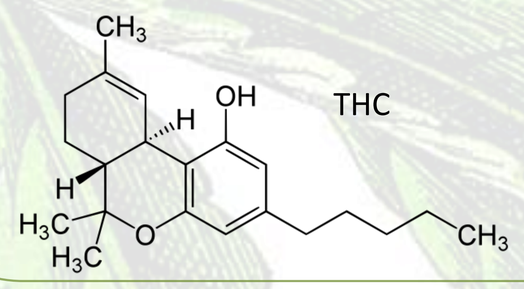
[4] Preparative Isolation of Cannabinoids from Cannabis sativa by Centrifugal Partition Chromatography, Hazekamp et al., Journal of liquid chromatography and related technologies, vol. 27, 2004

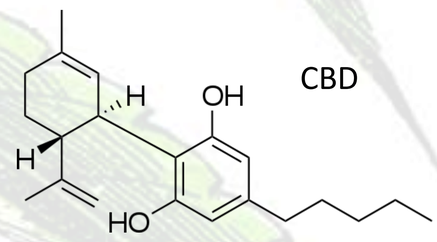
Extraction and development of concentration and purification process

Cannabis sativa L. Heptane extract was produced using dried flower tops (1Kg) in -40°C using a ratio of solvent/ground plant material 10:1. The whole process duration was 30min. The initial plant material contained 8.7% CBDA, 0.4% CBD, 0.3% THCA and 0.02% THC. The produced extract had a yield of 9.1%, containing 58.2% CBDA, 4.1% CBD , 1.5% THCA and 0.4% THC. This was used for the development of liquid/liquid separation method and purification process in FCPC™. For the liquid/liquid countercurrent centrifugal extraction process, a pH-swing protocol was implemented, where the heptane extract was fed into the BXP040, while through the other inlet water alkalized with sodium hydroxide (pH11) was fed counter currently. The aqueous effluent was collected into a vessel and it was brought back to pH7 by using water acidified with hydrochloric acid (pH3). A resinous precipitate was thus formed, with a yield of 8.5%. This was filtered out and, after HPLC analysis it was shown to contain 62.9% CBDA, 0.3% CBD, 2.0 % THCA and 0.1% THC. From the overall analytical profile, this enriched fraction seemed to contain much less neutral compounds, such as cannabinoids and terpenoids, in comparison to the heptane extract. Thus, it represented a better, more clean, material for subsequent FCPC purification. For the FCPC purification, an original quaternary biphasic solvent system has been developed, composed exclusively by four of the following (ICH class III) solvents:

Acetic acid	Ethanol	3-Methyl-1-butanol
Acetone	Ethyl acetate	Methylethyl ketone
Anisole	Ethyl ether	Methylisobutyl ketone
1-Butanol	Ethyl formate	2-Methyl-1-propanol
2-Butanol	Formic acid	Pentane
Butyl acetate	Heptane	1-Pentanol
tert-Butylmethyl ether	Isobutyl acetate	1-Propanol
Cumene	Isopropyl acetate	2-Propanol
Dimethyl sulfoxide	Methyl acetate	Propyl acetate

An experimental set of 35 shake-flask tests for the calculation of partition coefficients (*K*) and separation factors (α) and of 55 CPC runs was implemented for the development of the most performing solvent system, by adjusting simultaneously the most important of the operational parameters, flow rate, rotation speed and injection mass. The runs were all monitored by UV-detector (DAD) for the online detection of eluting cannabinoids and other compounds (220, 254, 365 and 200-600nm full scan). For injection in FCPC, the enriched fraction was always solubilized in the stationary phase and filtered through a 0.45µm nylon filter. Injection mass and pattern remained the same all through the runs: 3g of extract dissolved in 20ml of stationary phase, injected after equilibration of the column. Rotation speed varied among 1200 and 1800 rpm and flow rate between 10 and 20 ml/min, based on previous experience for optimal elution in FCPC A200ml rotor. Stationary phase retention (Sf) was calculated in the equilibrium.Fraction collection was set at 15ml per fraction. Elution was followed by extrusion of the stationary phase which was also collected in 15ml fractions. The entire operation was monitored and recorded by the Kromaflash peripheral through the Glider chromatography software. Preparative chromatography was followed by fraction analysis in HPLC-UV, fraction pooling (for fractions with similar composition), solvent evaporation to dryness for these fraction pools and, finally, quantitative analysis, again by HPLC-UV. Unfortunately, no more details can be shared on the exact composition of the solvent system or on the optimal operational parameters, as this remains confidential industrial property. The purities that were achieved after a run of 50 min. were: Fraction Pool 1: 95.6% CBDA and 1.2% CBD. Fraction pool 2: 95.1% THCA, and 2.1% THC.





Conclusions

Overall, the experimental set that was implemented allowed:

- the investigation of an extraction protocol that yields an enriched cannabinoids extract to be used as chromatography input.
- the thorough development of more than one exclusively ICH class III, biphasic solvent systems for the separation of the four target cannabinoids by implementing FCPC. Operational parameters have been adjusted accordingly to these solvent systems and compounds of interest, in order to ensure optimal chromatographic performance.

Intellectual Property

The Intellectual Property (IP) rights and eventual inventorship occurring from the above research project are attributed to Dr. Nikos Xynos and Kromaton.