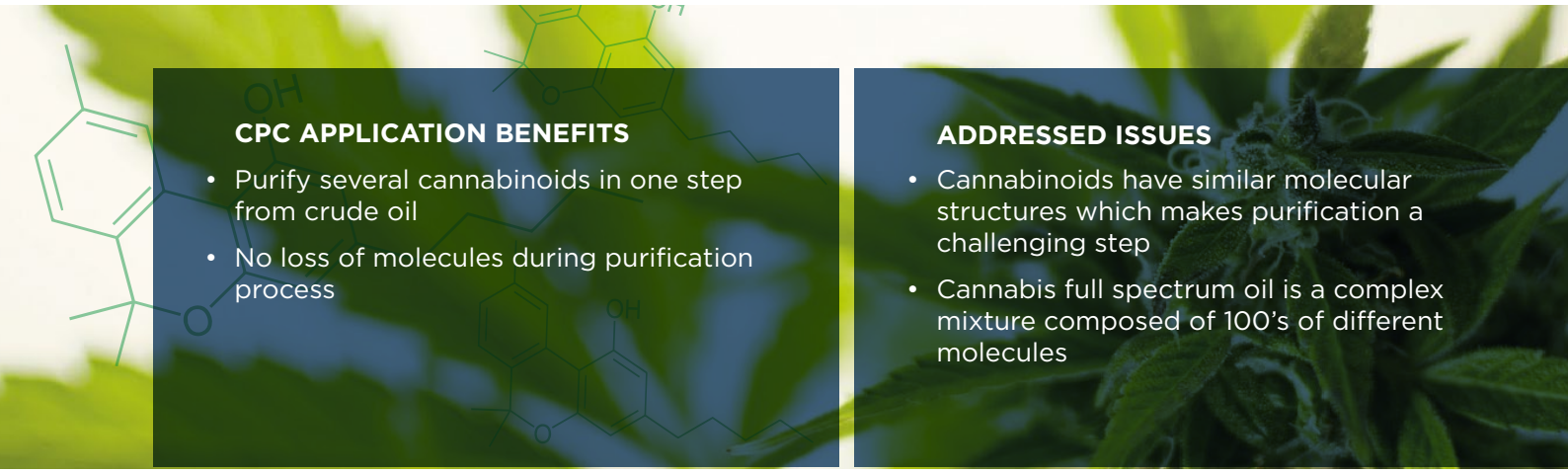
SINGLE-STEP PURIFICATION OF THC, CBD, CBN, CBG AND MINOR CANNABINOIDS BY CENTRIFUGAL PARTITION CHROMATOGRAPHY (CPC)

APPLICATION NOTE AN1036



CPC APPLICATION BENEFITS

- Purify several cannabinoids in one step from crude oil
- No loss of molecules during purification process

ADDRESSED ISSUES

- Cannabinoids have similar molecular structures which makes purification a challenging step
- Cannabis full spectrum oil is a complex mixture composed of 100's of different molecules

G.AUDO, C.LEQUEMENER | GILSON PURIFICATION, SAINT AVÉ, FRANCE
R.PERRY FEIGENBAUM, Y.NIR | CANNASOUL, CAESAREA, ISRAEL

INTRODUCTION

Purification of individual cannabinoids from a cannabis extract is often challenging as cannabis full spectrum oil is a complex mixture, composed of 100's of different molecules and cannabinoids that share very similar structures.[1]

When performed with classical liquid chromatographic techniques, such as FLASH or preparative HPLC, these separations can be both costly and time consuming due to the use of a solid support, such as silica. These techniques also have a significant environmental impact as they utilize large volumes of solvent, and silica must be recycled.

Centrifugal Partition Chromatography (CPC) exhibits many competitive advantages for natural product purification.[2] CPC is recognized to be the technology of choice for the purification of high quality/purity Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) for the pharmaceutical and food supplement markets.[3]

As the cannabinoid market develops, it is now focusing on other cannabinoids such as Cannabigerol (CBG), Cannabinol (CBN), Cannabichromene (CBC), Cannabichromevarin (CBCV), and Cannabidivarin (CBDV).

The objective of this study was to test the high selectivity of CPC and assess its capability to purify a set of key compounds (THC, CBD, CBN and CBG) and enrich several minor cannabinoids (CBDV, CBCV and CBC).

MATERIALS AND METHODS

Systems: A Gilson VERITY® CPC Lab System consists of two components, a CPC 250 and a PLC 2050 and are configured with a 50 mL/min quaternary gradient pump, UV/VIS detector, fraction collector, and Gilson Glider CPC control software was used for the purification step. (Figure 1)

Analytical HPLC was performed on a UHPLC – Thermo Fisher System configured with a photodiode array detector (PDA) (200—800 nm). All organic solvents were analytical or high-performance liquid chromatography (HPLC) reagent grade.

Sample: 100 mg of crude extract in solution was prepared from dried Cannabis sativa L. plant material and injected into the CPC 250.

Method: The elution flow rate was 12 mL/min, extrusion flow rate 50 mL/min, and rotation speed was 2000 rpm, with extrusion beginning after 35 minutes of elution. Detection was at 220 nm, 263 nm, 280nm and scan 200-400 nm.

Figure 1

Gilson VERITY® CPC Lab System

RESULTS AND DISCUSSION

CPC separation of the crude extract resulted in a single-step global fractionation of the sample, comprised of three distinct groups 1, 2 and 3 (Figure 2). The solvent system used in the CPC 250 determined the compounds, or family of molecules, targeted for this separation. In this study, it was selected to focus on cannabinoid compounds.

Group 1 is a mix of polar compounds.

Group 2 is divided into sub-fractions of pure cannabinoids: THC, CBN, CBD and a fraction of mixed cannabinoids.

Group 3 is a mix of non-polar molecules.

Analytical HPLC was performed on the sub-fractions from Group 2 to determine the purities of the separated cannabinoids. Analytical results confirmed that milligrams of CBD, THC, CBN, and CBG were separated, with purities of 100.0%, 98.9%, 90.0% and 53.9%, respectively (table 1).

Additional minor cannabinoids present in the crude were enriched in the same run. Subsequently, a second step purification of fractions F3, F6, F11, and F15 by CPC could be done to obtain highest purity of those specific minor cannabinoids (CBG, CBDV, CBCV and CBC).

Further investigations would have allowed the identification of the contents of Groups 1 and 3 (potentially some acid forms of cannabinoids, terpens, and phenols).

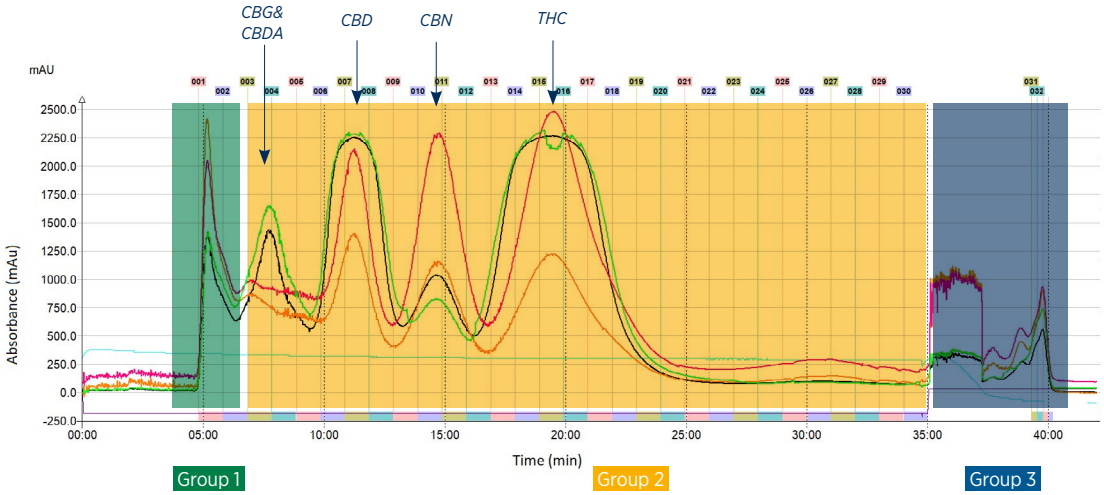


Figure 2

CPC Chromatogram of a 100 mg Injection of a Crude Cannabis Oil (220 nm, 263 nm, 280nm and scan 200-400 nm)

Table 1

HPLC Analysis of the Subfractions from CPC Group 2 (HPLC purity, w/w %)

Fractions	F3	F6	F7	F11	F15
Cannabinoids	**CBG** / CBDA	**CBD** / CBDV	**CBD**	**CBN** / CBCV	**THC** / CBC
HPLC purity	**53.9%** / 3.09%	**95,3%** / 3,1%	**100%**	**90%** / 1,2%	**98,9%** / 0,4%

CONCLUSIONS AND BENEFITS

The main goal of this study was to purify multiple cannabinoids in one step from a complex crude cannabis extract.

This study demonstrates the capability of Centrifugal Partition Chromatography (CPC) to perform, in one step, the purification of three cannabinoids at 90% to 100% purity (CBD, THC, CBN) with simultaneous enrichment of others (CBG, CBDV, CBCV and CBC). This was possible thanks to the unique selectivity of this technology to purify very similar molecules from hundreds of other molecules.

As a silica-free liquid-liquid chromatography technology, there is no irreversible adsorption of the sample to the matrix, and therefore no sample loss or denaturation. These features enable maximum recovery of all fractions from CPC purifications. In this study, groups 1 and 3 could also be valorized with further analytical investigation.

By using a larger scale CPC system such as the VERITY® CPC Process, this feasibility study could be easily scaled up for multi kg to tons production per year.

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ACKNOWLEDGEMENTS

About Cannasoul

Founded by world-renowned cannabis researcher Professor Dedi Meiri, from the Technion Israel Institute of Technology, CannaSoul Analytics is a global leader in cannabis research and development. Dedicated to developing scientific intellectual property, medical products and technologies, CannaSoul utilizes proprietary analytical expertise based on our accumulated clinical data and Professor Meiri's pioneering research.