Centrifugal Partition Chromatography for Purification of Cannabidiol from *Cannabis sativa*

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ABSTRACT

The *Cannabis sativa* plant is rapidly gaining in popularity because of the medicinal application of the non-psychotropic component, cannabidiol (CBD), which can aid in treatment of conditions including: pain, inflammation, epilepsy, and cancer. Interest in the purification, formulation, and detection of CBD has increased rapidly due to the recent changes in the legal status of cannabis compounds for medicinal use. As a result, Gilson has developed a rapid and reproducible method for large-scale purification of CBD using the liquid-liquid chromatography technique, centrifugal partition chromatography (CPC). A liquid stationary phase is retained inside the CPC column by a centrifugal field while a non-miscible liquid is pumped through as the eluent. The separation of components in the sample is dependent on the phase volume ratio inside the column and the partition coefficient of the solutes in both phases. The CPC can be operated in one of two modes for a particular run: ascending or descending, in which the CPC column is operating in normal or reversed phase. Following elution of compounds in the mobile phase, the solvents on the CPC column are replaced during the extrusion step in which stationary phase is loaded onto the CPC column, displacing remaining solvent and separated compounds, which can be optionally collected as fractions. A CPC 250 PRO column was controlled by a PLC 2250 Purification System equipped with a quaternary gradient pump, UV/VIS detector, fraction collector, and Gilson Glider CPC software. 5 g of crude *C. sativa* extract were subjected to purification by CPC in which 205 mg of CBD were purified to over 95% as shown by HPLC analysis. For each 5 g of sample, 1 L of solvent was consumed for every 10 minutes of separation. Purification parameters can be adjusted to target specified cannabinoids of interest and the method can be adapted from milligram to multi-kilogram scale providing reliable purifications for all industries.

INTRODUCTION

Cannabidiol (Figure 1) is a major component of the *Cannabis sativa* plant. CBD is of special interest because it is non-psychotropic, and studies suggest that it has therapeutic medicinal properties for the treatment of conditions, including: pain, inflammation, epilepsy, and cancer.[1,2] Recent changes in the legal status of cannabis compounds for medicinal use, as well as the decriminalization of marijuana in some locations, has led to increased interest in purification, formulation, and detection of CBD. Although CBD is still classified as a Schedule I drug in the United States, the U.S. Food and Drug Administration has authorized clinical trials to evaluate the use of CBD to treat children with rare forms of epilepsy.[3]

Cannabinoids are concentrated in a sticky resin found within the glandular trichomes, hairlike structures on the surface of the plant (Figure 2). Although most cannabinoids are nearly insoluble in water, they can typically be dissolved in oils, alcohols, and other non-polar solvents. To ensure consumer safety, it is critical to develop standardized CBD products that are free of tetrahydrocannabinol (THC) and other contaminants.

Gilson has developed a rapid and reproducible method for large-scale purification of CBD using centrifugal partition chromatography (CPC). The method can be adapted from milligram to multi-kilogram scale, requires little solvent, and recovers close to 100% of the CBD from a complex crude extract.

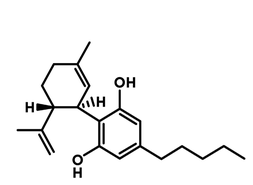

Figure 1
Chemical Structure of Cannabidiol.

Figure 2
Close-up View of Glandular Trichomes on the Surface of a Cannabis Plant.

CPC PRINCIPLE

CPC is a liquid-liquid chromatography technique that does not require a solid support, such as silica.

- Liquid stationary phase retained inside the column by a centrifugal field
- Non-miscible mobile phase is pumped through as the eluent

Retention of compounds depends on the phase volume ratio inside the column and the partition coefficient of the solute in both phases (Figure 3) where:

- Vm = V mobile phase inside the column
- Vs = V stationary phase inside the column
- Vc = V column = Vm + Vs
- Veq = Vm = V of equilibrium inside the column
- Kd = partition coefficient
- Vr = Vm + Kd * Vs
- R = [(Vc – Veq)/ Vc]*100
- Kd (DSC mode) = [C]stat/[C]mob
- Kd (ASC mode) = [C]mob/[C]stat

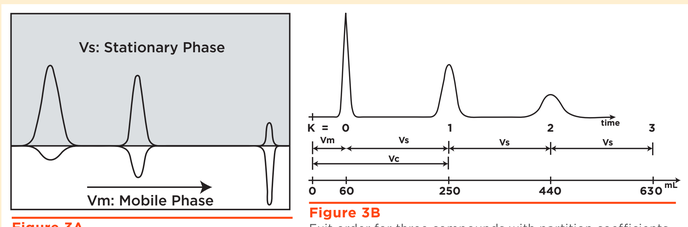

Figure 3A
Partition Behavior of Three Molecules in the CPC Column

Figure 3B
Exit order for three compounds with partition coefficients equal to 0, 1, and 2.
Conditions: 250 mL Column Capacity, 76% Stationary Phase Retention

Figure 3
Partition Behavior of Three Molecules in the CPC Column

STEPS OF CPC INJECTION

CPC runs by elution/extrusion are performed in six steps after solvent system preparation:

1. Choice of elution mode (Figure 4)
   b. Descending mode (DSC): upper organic stationary phase, lower aqueous mobile phase
   c. Ascending mode (ASC): lower aqueous stationary phase, upper organic mobile phase
2. Loading of the column with stationary phase
3. Pumping of mobile phase until equilibrium and measure of retained stationary phase volume (R)
4. Sample injection
5. Elution with mobile phase, detection and fraction collection
6. Extrusion with stationary phase (1.2 x Vc) while optionally collecting fractions

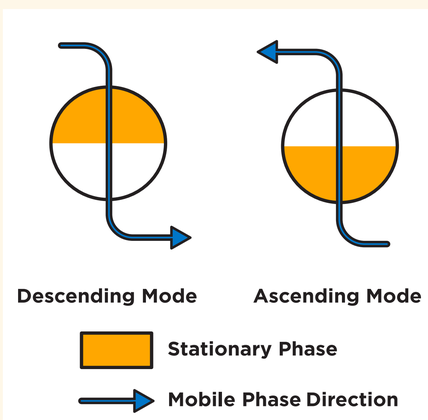

Figure 4
Choice of Elution Mode

MATERIALS & METHODS

A CPC 250 PRO column was controlled by a PLC 2250 Purification System equipped with a 250 mL/min quaternary gradient pump, UV/VIS detector, fraction collector, and Gilson Glider CPC software (Figure 5). The purification run was operated under the conditions in Table 1.

Table 1: CPC Purification Conditions

Column Volume	250 mL
Elution Flow Rate	70 mL/min
Extrusion Flow Rate	70 mL/min
Rotation Speed	3000 rpm
Sample	5 g *C. sativa* flower crude extract

Crude extract was prepared from dried *Cannabis sativa L.* plant material and was filtered before being subjected to CPC. All organic solvents were analytical or high performance liquid chromatography reagent grade.

RESULTS & DISCUSSION

In this study, 5 g of crude extract of *C. sativa* flowers were subjected to CPC. Using this one-step method resulted in clean separation of CBD from THC and other compounds (Figure 6). 205 mg of CBD was purified from 5 g of crude extract, and the final product had a purity of over 95% as shown by HPLC analysis. For each 5 g sample, 1 L of solvent was consumed for every 10 minutes of separation.

Figure 5
VERITY Compact CPC System

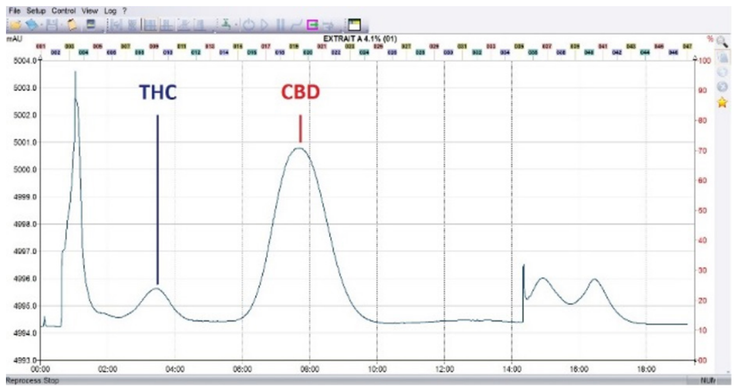

Figure 6
Chromatogram of CBD Separated from THC using CPC

CONCLUSION

CPC technology employs a silica-free liquid-liquid chromatography technique that can be used to purify CBD from crude extracts of cannabis in just one step.

Purification parameters can be adjusted to target specified cannabinoids of interest to achieve the desired purity for THC-free extracts, pure cannabinoids, pharmaceutical grade products, or standard molecules intended for use as reference materials or for clinical evaluation.

The methodology is adaptable from laboratory to industrial scale. There is no irreversible adsorption of the sample to the stationary matrix as the methodology does not employ solid silica resin, resulting in no sample loss.

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