Cost-effective Approach to the Purification of Cannabinoids using CPC

INTRODUCTION

Chromatographic purification of natural compounds presents many challenges to scientists because of the complex nature of the starting matrices that are used in the process. These starting materials can damage traditional columns and cartridges, decreasing the length of their usage and increasing costs; that is, if the particular system can even accommodate the starting material. Centrifugal partition chromatography (CPC), which uses both liquid stationary and mobile phases, can handle heavily contaminated, complex starting materials, such as direct extracts from many biological sources, and has been shown useful for the isolation of piperine from *Piper nigrum*[1], gingerol from *ginger*[2] and hundreds of other natural compounds from plants. Additionally, by relying on a liquid stationary phase, CPC columns do not need to be replaced like traditional columns and cartridges used by preparative HPLC and flash chromatography methods.

This article will discuss the basic principles behind CPC and explore the use and benefits of CPC in the purification of cannabinoids from crude cannabis oil.

WHAT IS CPC?

Centrifugal partition chromatography can be performed on pilot, preparative and industrial scales. Whereas both preparative and flash chromatography rely on a solid silica stationary phase, CPC is silica-free, using two immiscible liquids as stationary and mobile phases. Similar to both preparative HPLC and flash chromatography methods, the separation of the target molecule is based on its respective affinity to the liquid phases as expressed by the partition coefficient,

K_D, much as if you used a glass separatory funnel. With CPC, one phase is made stationary by centrifugal force while the other phase is pumped through the column. Molecules with greater affinity for the mobile phase will pass through faster and elute first, while molecules with greater affinity for the stationary phase will pass through slower and elute later. The CPC systems can work in both ascending and descending modes, which determines whether the lighter or heavier phase acts as the stationary phase on the column, respectively. These operational modes are comparable to normal phase typically used for flash chromatography and reversed phase commonly used for preparative HPLC. Traditional chromatography requires a column change to perform normal or reversed phase separations, whereas, with CPC, switching between ascending and descending modes performs this switch automatically on the column.

Using the separatory funnel further as an example, CPC purifications are like performing a liquid-liquid separation using hundreds of connected separatory funnels. This allows the separation to repeat many hundreds of times, increasing the efficiency of the purification (**Figure 1**). In addition, by changing the solvents used, it is possible to purify different compounds based on their individual partition coefficients, allowing one to achieve highly selective and efficient separations

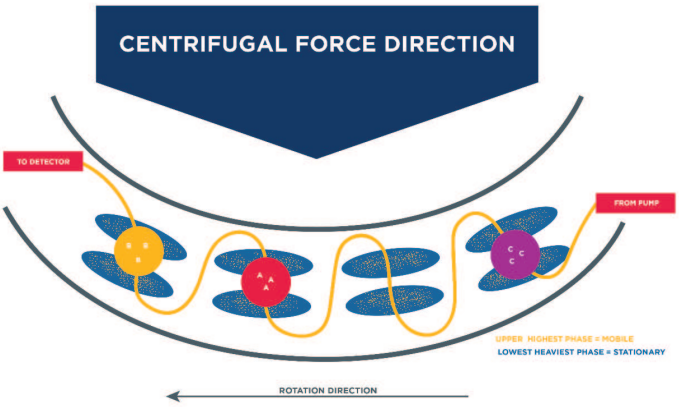

Figure 1. Principle behind CPC

The CPC column design is much like having a series of hundreds of separatory funnels connected end to end. In this case, three molecules with differing affinities to the two phases are introduced with the lighter mobile phase into the first funnel and shaken. After the phases settle, the heavier, lower phase is moved to the next funnel. This process is repeated until the last funnel. A CPC column mimics these hundreds of liquid-liquid separations with the two phases in this manner when operating in descending mode

The actual CPC column or rotor is composed of a stacked series of stainless steel discs that rotates on an axis, providing the centrifugal force to hold the stationary phase on the column (**Figure 2**). Each disc is engraved with hundreds of twin cells. These twin cells act similarly to the separatory funnel example, where the heavier mobile phase passes out one set of cells and is pumped into the next when operating in descending mode, repeating the separation hundreds of times. Molecules with greater affinity for the heavier mobile phase will move faster through the CPC column and elute earlier. This design provides better retention of the stationary phase, while allowing the use of higher flow rates for faster separations and increased productivity

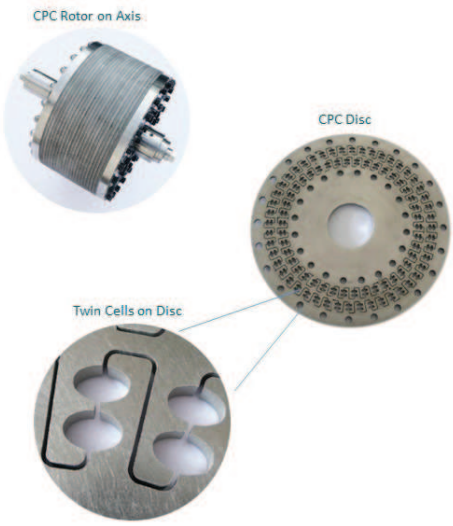

Figure 2. CPC column design

The CPC column rotates on an axis and is designed to be resistant to high pressures. The column is composed of numerous stacked discs, each of which are engraved with hundreds of twin cells. This design provides better retention of the stationary phase, allowing for higher flow rates for faster separations..

A variety of CPC columns exist, offering different options in flow rates and injection volumes. **Table 1** provides an example of various Gilson CPC columns and their characteristics. In addition to the column, the CPC system requires a preparative-scale pump, injector, and optional detector and collector to perform the purification just like the setup for preparative HPLC or flash chromatography. A CPC column can be attached to many existing preparative HPLC systems, replacing the traditional column. Software such as the Gilson Glider CPC software allows researchers to program automatic injections to stack runs, decreasing hands-on time and increasing productivity.

One of the key advantages with CPC is the ability to use increased flow rates, which can lead to faster run times. In addition, CPC offers numerous benefits over preparative HPLC and flash chromatography (**Table 2**). Unlike preparative HPLC, CPC can be used on complex mixtures. CPC also uses significantly less solvent and does not require replacing columns or cartridges as does preparative HPLC and flash chromatography systems, resulting in lower consumable costs. With CPC, the same solvent system can be used from run to run, replacing the stationary and mobile phases as necessary.

Table 1. Gilson CPC Column Parameters

Model	Maximum injection Capacity (g)	Maxiumum Elution Flow Rate (mL/min)	Maximum Pressure (bar)
CPC 100	1	15	100
CPC 250	6	15	100
CPC 1000	30	50	80
CPC 250 PRO	30	80	100
CPC 1000 PRO	100	350	80

Table 2. Comparison CPC, Flash Chromatography and Preparative HPLC Methods

Column Features	CPC	Preparative HPLC	Flash Chromatography
Silica	No	Yes	Yes
Sample	Simple to complex	Simple	Simple to complex
Solvent Consumption	5 times less	Significant	Significant
Efficiency	Medium	High	Low
Cost	No column consumables	Prep HPLC column	Cartridge
Flexibility	No column change necessary	Column change necessary	Column change necessary
Scale-up	Easy	Non-linear	Non-linear

Rather than purchasing a new column or cartridge for a new application, the CPC can be loaded with different solvents to create the column needed. CPC is also very easy to scale up for processing milligrams to kilograms of product efficiently, whereas preparative HPLC and flash chromatography may require substantial changes to optimize the purification methodology as it moves to a larger scale.

USE OF CPC IN CANNABINOID PURIFICATION

Cannabidiol (CBD), a non-psychotropic cannabinoid from Cannabis sativa, has been identified as a possible treatment for a variety of conditions including pain, inflammation, epilepsy and cancer[3,4]. The cannabinoids are found in the sticky resin of trichomes found on the plant's surface. However, for the safe use of CBD in possible treatments, it must be purified from other contaminants in a cannabis extract such as tetrahydrocannabinol (THC).

In general, the CBD purification begins with the production of a crude extract that is made by extracting dried marijuana with a solvent, such as ethanol, hexane or supercritical CO_2. This crude extract is then diluted and injected into the CPC system. The appropriate fractions are collected and solvent is removed, resulting in purified target cannabinoids.

Using this procedure with the Gilson CPC 250 PRO and PLC 2250 Purification System, 5 g of crude cannabis oil was injected and 600 mg of CBD with greater than 90% purity and 120 mg of THC with greater than 90% purity were isolated (**Figure 3**). The entire separation took approximately 20 minutes and used only 600 mL of solvent.

The use of CPC in the purification of cannabinoids from marijuana provides additional opportunities to improve productivity and cost effectiveness. Consider the purification results described above. If one were to automate injections using the Gilson Glider CPC software for continuous 20-minute runs, more runs could be performed within a given time period, increasing the amount of starting material processed. **Table 3** presents the potential amounts of starting material that could be purified using the Gilson CPC system described in the purification.

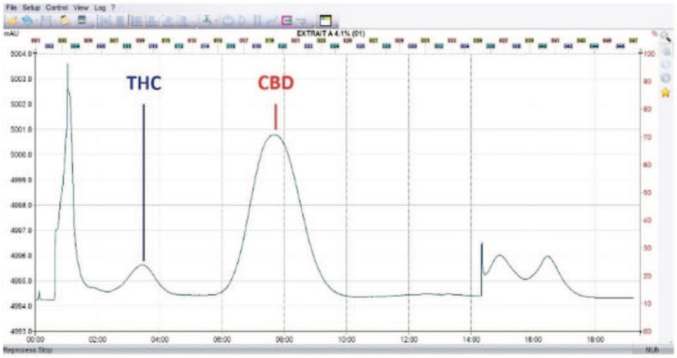

Figure 3. CPC purification of CBD and THC from cannabis oil using a Gilson CPC 250 PRO and PLC 2250 Purification System

Starting with 5 g of crude oil, 600 mg of CBD and 120 mg of THC were purified in 20 minutes. Purity determined by HPLC was over 90% for both compounds.

The CBD application demonstrates some of the main cost-effective benefits of using CPC for the purification of natural products. First, because CPC uses two immiscible liquid phases, there is no column to replace or silica to recycle, eliminating these costs. Also, the CPC purification method uses significantly less solvent than flash or preparative HPLC methods, saving money on disposal and solvent costs. Lastly, CPC accommodates high injection capacities, from milligrams to kilograms of sample, and faster run times, both of which can increase the amount of final purified cannabinoid components in a specified period of time over more traditional chromatography solutions.

SUMMARY

In summary, the use of CPC in the purification of natural products can provide a cost-effective solution to some of the problems facing scientists developing these methods. Because CPC employs both a liquid stationary and mobile phase, expensive preparative HPLC column or flash cartridge replacement and silica recycling are no longer necessary, eliminating those costs. In addition, the design of the CPC system uses less solvent than with preparative HPLC or flash chromatography, decreasing the costs associated with solvent use. Most importantly, CPC can accommodate complex samples and use faster flow rates, improving the productivity of the separation particularly in scenarios where multiple runs are automated to occur.

Table 3. Faster run times provide opportunity to increase the amount of starting material processed in a specified period of time*

Model	Run time (mins)	Amount injected/run (g)	Amount injected/hr (g)	Amount injected/8 hrs (g)
CPC 250 PRO	< 20	5	15	120
CPC 1000 PRO	< 20	30	90	720

***NOTE:** Injection mass and run time can vary depending on the crude oil used and the final goal (e.g., CBD content, THC content, etc). The productivity is based on the amount of crude oil treated.

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