Cannabinoid Isolation Models Utilizing Immobilized Chiral Stationary Phases and SFC



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INTRODUCTION

In the cannabis and hemp industry, chromatography is often talked about as a potential method for the purification and isolation of desirable cannabinoids contained within the plant. But because of the cost of equipment and technical skill required to operate, it's rarely utilized.

The exception to this is the rise in liquid flash chromatography (FC) usage over the past few years. The reasons are simple: the cost of equipment is relatively low, and it promises high quality results with minimal effort. However, the purchaser quickly realizes that a large volume of flammable solvent is needed to run the system, and additional equipment is required to evaporate the solvent (which is often mixed with water) making evaporation a long and energy consuming process.

What will be shown in this poster is how supercritical fluid chromatography (SFC) can be used as both an analytical technique for quantitating cannabinoids, and a preparative technique for either isolating Cannabidiol (CBD) or removing Tetrahydrocannabinol (THC).

OBJECTIVES

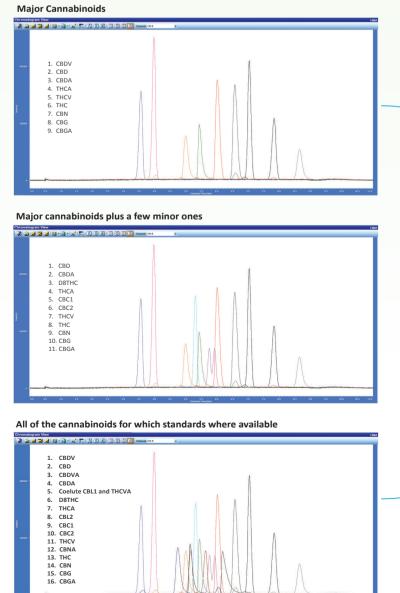
- First, an analytical method utilizing's immobilized chiral stationary phase (CSP) CHIRALPAK[®] IB N-5, from DAICEL Corporation, will be presented to show its performance in resolving the current lot of cannabinoid standards
- Second, an additional analytical method utilizing DCpak® P4VP, an achiral stationary phase (SP) from DAICEL Corporation, will be presented, to demonstrate its performance in resolving the lot of cannabinoid standards
- Third and final, the two stationary phases were used in a preparative method, and purification results of real-world sample injections will be presented along with scale up calculations. These results provide an alternative to flash chromatography, and present a more efficient and greener solution

CHIRALPAK[®] IB N-5 Analytical SFC Method Development

There are two types of CSPs: coated and immobilized. Coated phases are weakly attached to their silica substrates, and when subjected to improper conditions, this phase can be stripped away. This will cause the retention and peak shape of the cannabinoids to degrade to the point of unresolved peaks and difficult quantitation.

It is for this reason that the DAICEL catalog of immobilized CSPs was screened. DAICEL CHIRALPAK[®] IB N-5 ultimately yielded the best combination of selectivity, peak shape, and resolution of the key cannabinoids.

Below, the three chromatograms show:

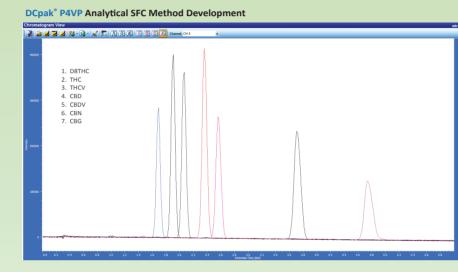


DAICEL DCpak® P4VP Analytical SFC Development

DAICEL's DCpak[®] P4VP is an achiral column containing an immobilized SP, similar to CHIRALPAK® IB N-5. When separated using this SP, the neutral cannabinoids show well resolved peaks with good shape in an advantageously short run time. The column used for this presentation contained 5µm particles, but with a 3µm particle size available, the separation could be completed even quicker if desired.

One important thing to note is the proclivity of the SP to retain the more acidic cannabinoids. This can be overcome using a gradient method with a steep rise after all of the neutral cannabinoids have eluted. This helps push the acidic cannabinoids off of the column, yielding better peak shape and resolution. The addition of an acidic modifier could also help in this instance.

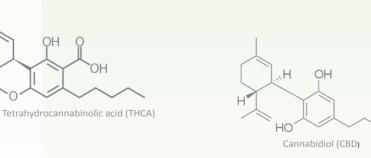
Another advantage of the **DCpak**^{*} **P4VP** is the orthogonal selectivity versus the CHIRALPAK[®] IB N-5. In this way it's a good choice to perform a traditional Pharma impurity assessment and act as an alternative prep method.



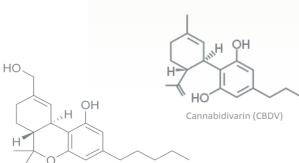
Comparison of Purification Methods Using SFC and Flash Chromatography

Currently, the predominate prep chromatography method utilizes Flash LC. As previously stated, this is due in part to the low cost to purchase a system. However, if the performance of a C18 Liquid FC method for CBD isolation is compared to a similar CHIRALPAK[®] IB N-5 or DCpak[®] P4VP SFC method there are two things that stand out:

- First, the SFC methods produce 1.5-2x more CBD per 24 hours than the Liquid FC method
- Second, solvent use is nearly halved for both SFC methods because CO₂ is the primary solvent. This results in a more cost effective production by avoiding evaporation and solvent purchasing
- And third, the cosolvent for the SFC methods is methanol. Unlike the aqueous/organic mixtures produced from liquid FC, which can be difficult to process, the methanol can be easily recycled and reused if desired.

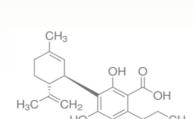


- a. CBC and CBL show two peaks each, indicating the standards were a mixture of stereoisomers
- The acids tail slightly, which could be corrected with the addition of acidic additives
- This work was done on a 5µm column, but a 3µm column could be used to shorten the run time, and sharpen the peaks





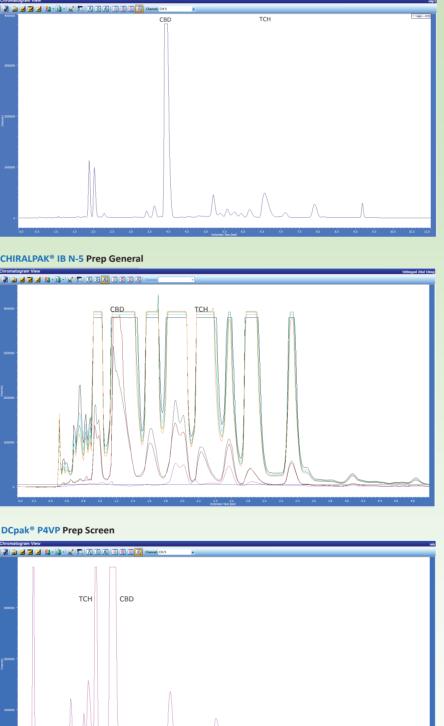
Note

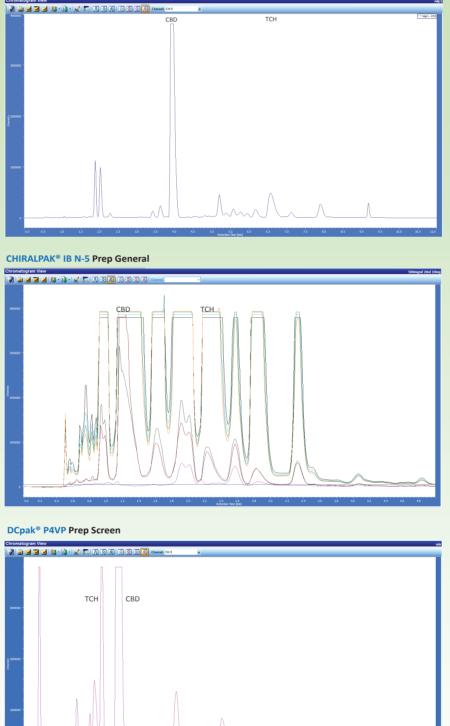


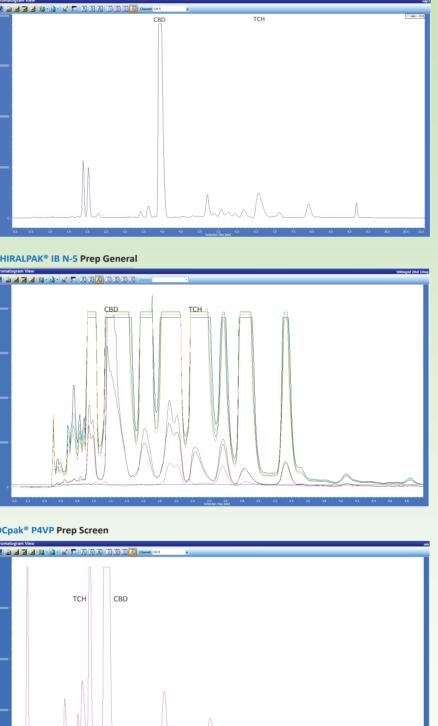
Cannabigerol (CBG)

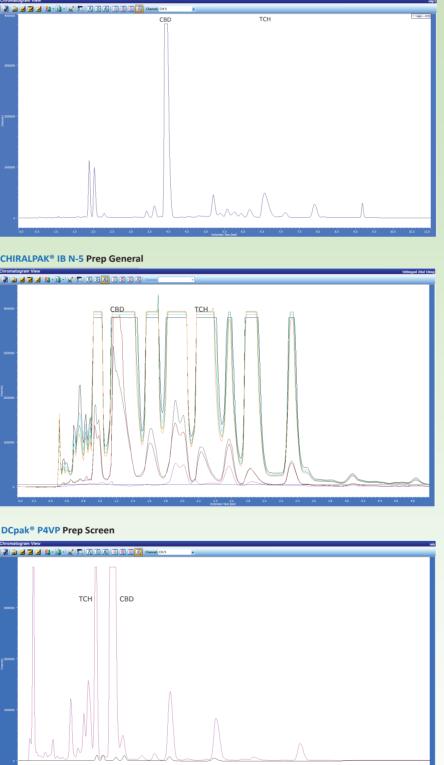
Cannabidivarinic acid (CBDVA

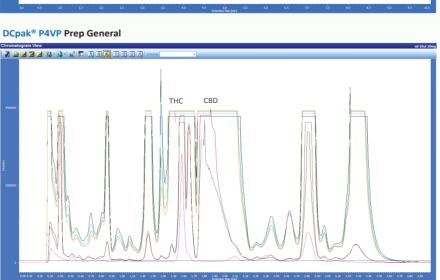
CHIRALPAK® IB N-5 Prep Screen













SFC Prep Development

Below are a few chromatographic examples from prep screening runs for the previously developed CHIRALPAK® IB N-5 and DCpak® P4VP analytical methods. The chromatograms labelled prep screen were obtained from the same hemp oil used in the analytical section, but at a higher concentration. This was done to get an initial sense of scalability and resolution. The next set of chromatograms labelled prep general, are tuned to be a general prep method. These methods demonstrate three things:

- If desired, only CBD can be isolated
- THC could be removed, leaving a THC free full spectrum hemp oil

• The other minor cannabinoids could be isolated from the major cannabinoids for research or product development



Prep Method Productivity Figures

CHIRALPAK IB N-5				
Cartridge	4.6	20	50	
Amount of stationary phase (g)	2.5	48	300	
Scale up factor	1	18.9	118	
Flow Rate ml/min (~14.5% MeOH)	6	113.4	708	
Loading (g)	0.02	0.378	2.36	
Cycle Time (min)	5	5	5	
Runs per 24hr	288	288	288	
Kg of oil in 24hr	0.00576	0.10886	0.67968	
CBD peak width (min)	0.4	0.4	0.4	
Vol. of CBD fraction per run (L)	0.00024	0.004536	0.02832	
Total Vol. of CBD fraction in 24hr (L)	0.0691	1.3064	8.1562	
Conc. of CBD fraction (g/L)	42	42	42	
Vol. of solvent used per 24hr (L)	1.25	23.68	147.83	
CBD Isolated in 24hr (g)	2.88	54.43	339.84	
Productivity (Kg CBD/Kg SP/Day)	1.13	1.13	1.13	

DCpak P4VP				
Cartridge	4.6	20	50	
Amount of stationary phase (g)	2.5	48	300	
Scale up factor	1	18.9	118	
Flow Rate ml/min (~12% MeOH)	8	151.2	944	
Loading (grams)	0.02	0.378	2.36	
Cycle Time (min)	4	4	4	
Runs per 24hr	360	360	360	
Kg of oil in 24hr	0.0072	0.1361	0.8496	
CBD peak width (min)	0.3	0.3	0.3	
Vol. of CBD fraction per run (L)	0.00024	0.004536	0.02832	
Total Vol. of CBD fraction in 24hr (L)	0.036	0.6804	4.248	
Conc. of CBD fraction (g/L)	42	42	42	
Vol. of solvent used per 24hr (L)	1.38	26.13	163.12	
CBD Isolated in 24hr (g)	3.6	68.0	424.8	
Productivity (Kg CBD/Kg SP/Day)	1.41	1.41	1.41	

Daily Production Parameters					
IB-N SFC	P4VP SFC	C18 Flash			
1.13	1.41	0.77			
433	383	740			
392 L	272 L	961 L			
	IB-N SFC 1.13 433	IB-N SFC P4VP SFC 1.13 1.41 433 383			

CONCLUSIONS

In recent years, liquid FC has been used for the separation and purification of cannabinoids. Due to high solvent usage and need for separate equipment to remove said solvent, it's notthe greenest or most efficient method for this separation.

The work in this presentation has demonstrated two new SFC-based separations utilizing two different SP from the DAICEL Corporation.

These SFC methods have been shown effective for both small scale analytical separations as well as larger preparative separations. Compared to liquid FC, these new SFC methods not only use less solvent, they also produce more CBD per kilogram of SP, leading to a more productive and efficient process. Moving forward, the cost saving benefits in applying these methods will become quickly apparent to the user, and should serve as an efficient, sustainable process for separation.