The SFC Isolation and Purification of Cannabinoids using Application Specific Stationary Phases Under Optimised Conditions

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Cannabis sativa is comprised of hundreds of individual compounds that can be classified in many chemical families, such as terpenes, amino acids, fatty acids, hydrocarbons, flavonoids, sugars and cannabinoids [1,2]. Cannabinoids represents a class of chemicals that are classified as terpenophenolic compounds. There are about 70 terpenophenolic compounds in the cannabinoid class. These are only found in cannabis plants [3]. Of the 70 cannabinoids found in *Cannabis* there are several cannabinoids that are of human physiological and medicinal interest [4]. These include the psychoactive Δ-9-tetrahydrocannabinol (THC), non-psychoactive cannabidiol (CBD) and the non-psychoactive tetrahydrocannabivarin (THCV) (5-8). THC, THCV and CBD are neutral forms of cannabinoids, obtained after a non-enzymatic decarboxylation of delta 9-tetrahydrocannabinolic acid (THCA), tetrahydrocannabivarinic acid (THCVA) and cannabidiolic acid (CBDA). It is the focus of this manuscript to utilise SFC chromatographic stationary phases that have been specifically developed for the isolation and purification of THCA, CBDA, THC, CBD and THCV. These specific cannabinoids require the use of several different stationary phases for optimised separation and purification of them individually.

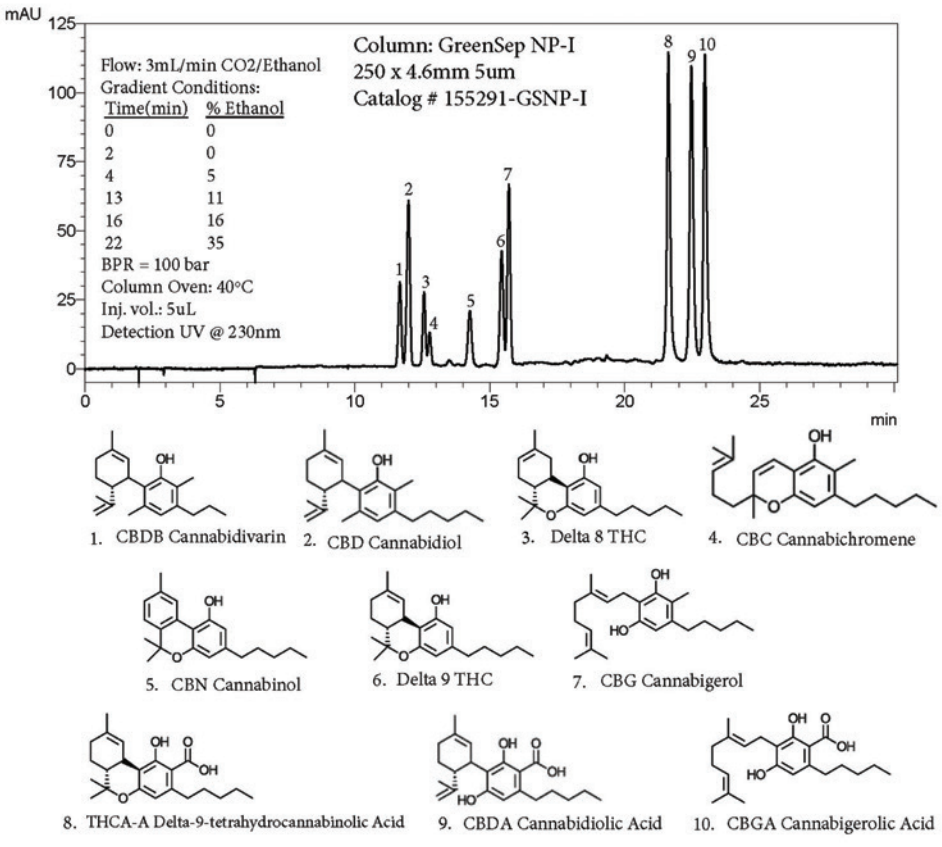

Figure 1: Separation of 10 cannabinoids chromatographed on GreenSep NP-I, a coated polysaccharide stationary phase.

Supercritical fluid chromatography (SFC) is a powerful chromatographic technique for the separation and isolation of complex mixtures from natural products. It has been useful in the area of preparative chromatography [9-11]. Virtually all current practitioners of SFC use carbon dioxide (CO_2) which offers several advantages when compared to preparative liquid chromatography [12]. The use of carbon dioxide (CO_2) as the primary component of the mobile phase is one of the key features that benefits preparative SFC chromatography since the CO_2 used for SFC is considered a 'Green' solvent. It is miscible with a wide range of organic solvents, nonflammable, has low UV absorbance at short wavelengths [13-15]. CO_2 SFC is particularly well suited in the area of preparative chromatography where it can be easily removed enabling the rapid recovery of isolated compounds. In addition, any residual amounts of CO_2 in isolated products are considered to be non-toxic [16]. Another advantage of SFC as a technique is that the diffusion coefficient of solutes in the SFC mobile phases have been shown to be 3-10 times higher than in normal liquids potentially allowing for very rapid separations. In addition the viscosity of SFC mobile phases are significantly lower than LC mobile phases hence producing a much lower pressure drop across the column

allowing the use of much higher mobile phase flow rates producing rapid preparative separations [17]. Given these attributes SFC chromatography is ideally suited to isolation and purification of cannabis extracts. In addition, super critical CO_2 extraction (SFE) of cannabis is routinely performed to produce a cannabis oil [18,19].

SCOPE

The preparative separation of cannabis mixtures to isolate specific components can be challenging. Traditional preparative liquid chromatography can be used to separate and isolate specific cannabis components. However, preparative liquid chromatography has several draw backs including the limits on flow rates and ultimately production throughput due to the relatively high viscosity of the mobile phase used. In addition, considerable amounts of ethanol and water are required for the liquid chromatographic separation of cannabis. In order to isolate the components, the ethanol/water mixture has to be removed or reduced in volume. This removal process is time consuming. The mixtures of CO_2/ethanol mobile phase are very low viscosity which can be used at very high flow rates to encourage higher production levels. In addition, CO_2 is rapidly released during component isolation and ethanol amounts are low and quickly removed.

One of the key factors for a successful SFC preparative separation and isolation of cannabinoids is stationary phase selection. There are a several of attributes that are necessary for the optimal stationary phase including:

1. The stationary phase should be designed to deliver the desired separation at the lowest level of organic modifier possible (in the case of Cannabis ethanol would be the organic modifier).

2. The stationary phase should be robust and easily scalable for preparative applications.

3. The stationary phase should not be expensive to manufacture.

Preliminary Investigations

Preliminary investigations for the SFC separations of cannabinoids employed modified polysaccharide phases coated phases for the SFC separations of natural products (NP) since they can be useful for the separation of structurally similar compounds. The GreenSep NP-I has been specifically optimised for the separation of 10 different cannabinoids. The chromatogram shown in Figure 1 is an example of the peak shape, performance and separation capacity obtainable with the GreenSep NP-I column with SFC for a high-resolution separation of a mixture of cannabinoids. Unfortunately, these polysaccharide phases whether coated or immobilised are expensive to manufacture, making these types of columns a major contributor to isolation costs.

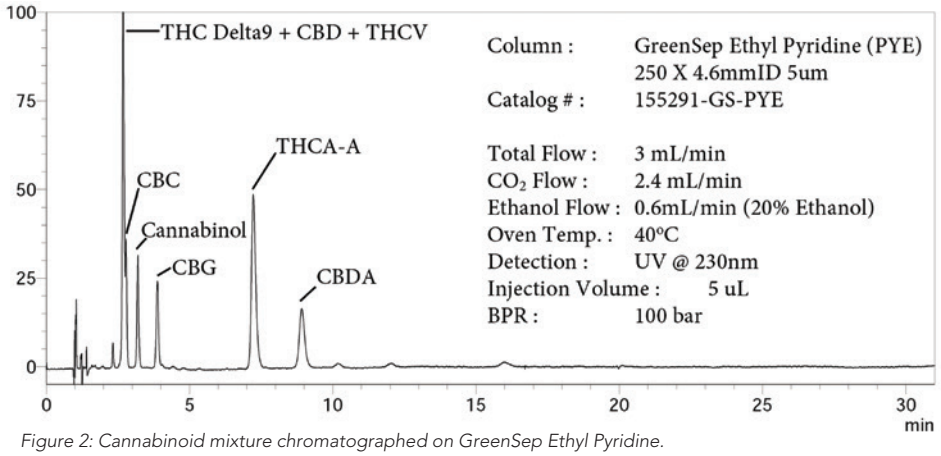

Figure 2: Cannabinoid mixture chromatographed on GreenSep Ethyl Pyridine.

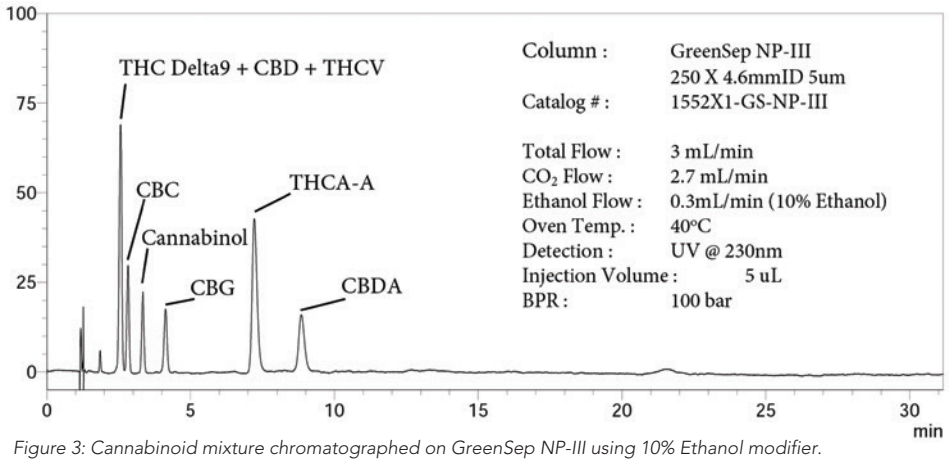

Figure 3: Cannabinoid mixture chromatographed on GreenSep NP-III using 10% Ethanol modifier.

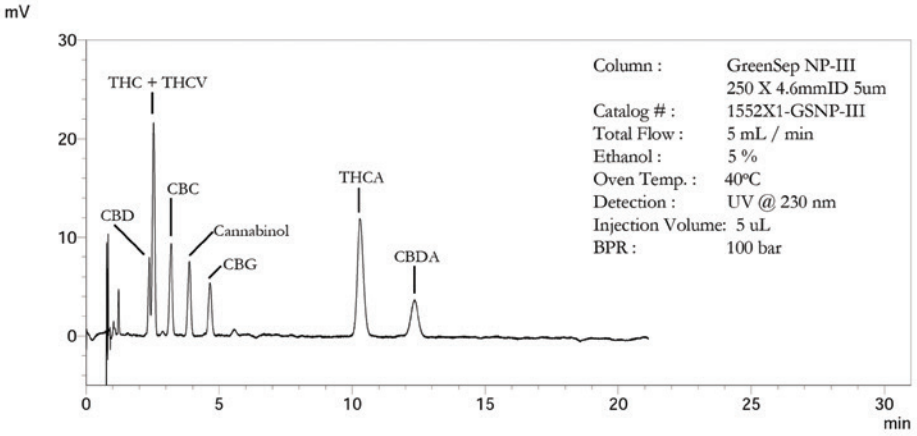

Figure 4: Cannabinoid mixture chromatographed on GreenSep NP-III using 5% Ethanol modifier.

Isolation of THCA and CBDA

Preparative SFC separations of cannabinoids have been performed using a column with 2-Ethyl pyridine bonded to silica as a stationary phase with ethanol used as co-solvent since it is less toxic compared to methanol or other organic solvents. This is of vital importance if the resulting isolate is for human consumption as no toxic solvent residues are present. A chromatogram showing the separation of mixture of cannabinoids is shown in Figure 2. CBDA and THCA are both well separated from the other cannabinoids, however, to elute these two components in less than 10 minutes 20% ethanol co-solvent is required.

GreenSep NP- III permits both CBDA and THCA to elute in less 10 minutes with only 10% ethanol (chromatogram shown in Figure 3), half as much when compared to 2-ethyl pyridine.

GreenSep NP-III can be used at higher total flow rates requiring only 5% ethanol to elute both THCA and CBDA in less than 13 minutes, while still maintaining good chromatographic resolution (chromatogram shown in Figure 4).

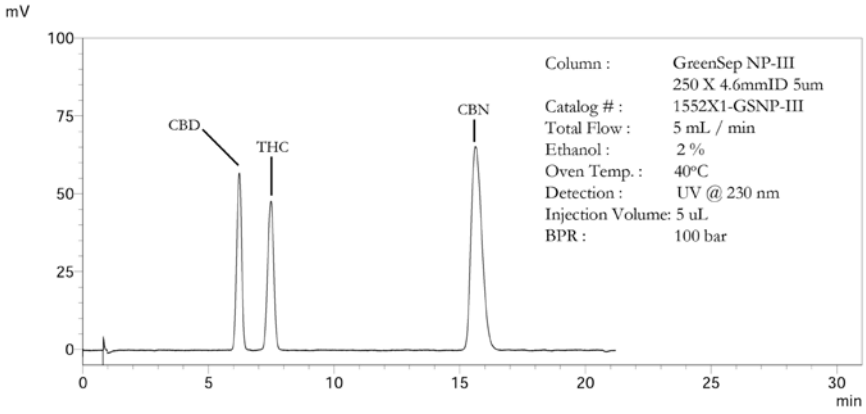

Figure 5: Separation of CBD, THC and CBN on GreenSep III using 2% ethanol.

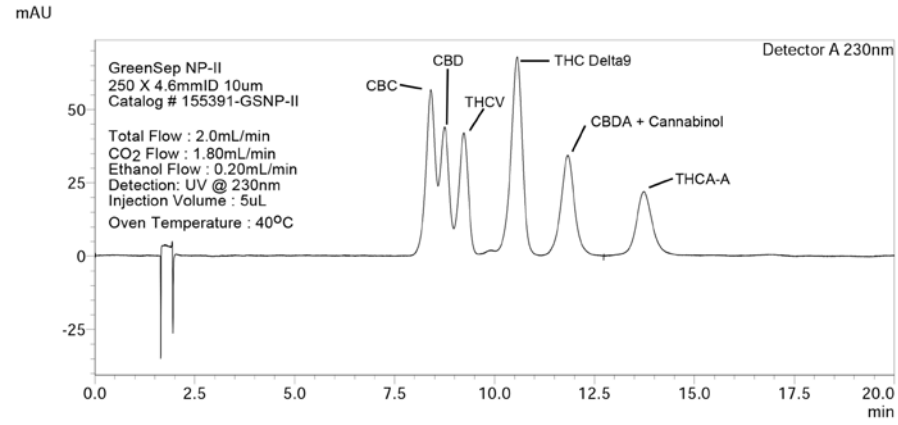

Figure 6: Cannabinoid mixture chromatographed on GreenSep NP-II using 10% ethanol.

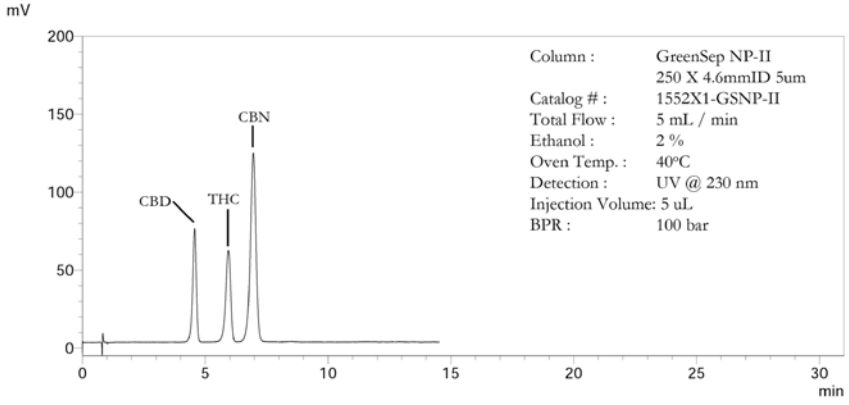

Figure 7: Separation of CBD, THC and CBN on GreenSep III using 2% ethanol.

Isolation of CBD and THC

During our investigation we discovered that GreenSep NP-III could be used for the SFC preparative separation of CBD and THC as shown in Figure 5 where both CBD and THC are eluted with only 2% ethanol.

The separation of CDB and THC on GreenSep NP-III provided the motivation to develop other new products specifically designed for optimised SFC preparative separation of cannabinoids. A chromatogram showing the separation of a cannabinoids mixture chromatographed on a GreenSep NP-II is shown in Figure 6 where THC can easily be removed from a cannabis extract and CBDA and THCA are still eluted in less than 15 minutes.

In some cases, it would be desirable to isolate full spectrum CBD without THC. Full spectrum CBD contains cannabinoids without THC and THCA. This full spectrum CBD may have additional therapeutic benefits when compared to pure CBD. Figure 7 shows the separation of CBD and THC. Based upon this chromatography THC and CBN can be removed from an extract to produce full spectrum CBD.

The separation of CBD from THC was further improved using another new stationary phase GreenSep NP-9. The chromatogram with enhanced separation between CBD and THC is shown in Figure 8. Using GreenSep NP-9 provides separation factor to effectively remove THC from a complex mixture of cannabinoids.

Isolation of CBD, THCV and THC

The cannabinoid THCV is another cannabinoid that has some medicinal interest. However it is difficult to separate THCV from THC and CBD by SFC. However, the SFC isolation of THCV from CBD and THC was achieved on GreenSep NP-12. A chromatogram of this separation is shown in Figure 9.

Conclusion

Several new stationary phases have been developed (GreenSep NP-III, GreenSep NP-II, GreenSep NP-9 and GreenSep NP-12) optimised for the preparative SFC separation and isolation of cannabinoids. GreenSep NP-III is optimised for the rapid separation of CDBA and THCA. GreenSep NP-II is useful for THC and THCA removal with a quick cycle time. GreenSep NP-9 is optimised to deliver the maximum separation alpha between CBD and THC and is best for the removal of THC. GreenSep NP-12 is designed to separate CBD, THCV and THC with maximum alpha value. The recommended use for each of these stationary phases are shown in Table 1. Loading studies are currently being conducted to define preparative loading and output for these cannabinoid isolates. These stationary phases separate the desired components and are designed to deliver the desired separation at the lowest level of liquid ethanol modifier possible. Ethanol minimisation is important since it is more expensive than CO_2 and more difficult to remove than CO_2. In addition, these stationary phases are robust, cost effective and designed for preparative SFC separations.

Table 1: Recommended Cannabis Component Isolation for the New GreenSep NP Stationary Phases.

GreenSep Column	Recommended Use
NP-II	THC and THCA removal with a quick cycle time
NP-III	Rapid isolation of CBDA and THCA
NP-9	Optimal THC removal to produce full spectrum CBD
NP-12	Optimal separation of THCV

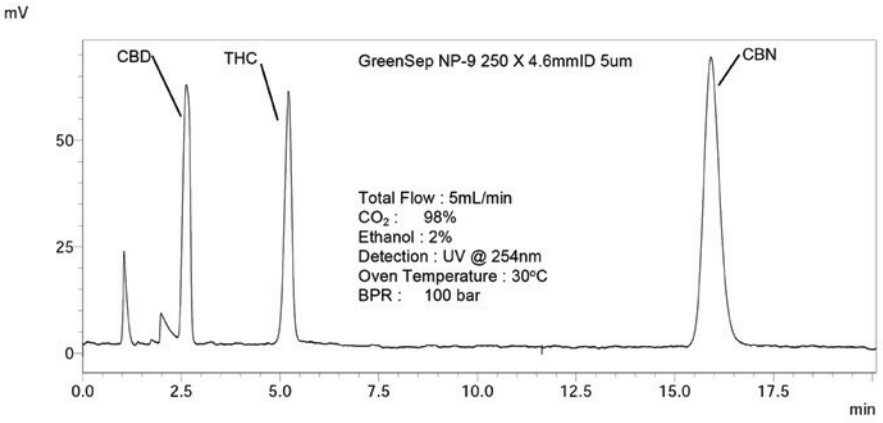

Figure 8: Separation of CBD, THC and CBN on GreenSep 9 using 2% ethanol.

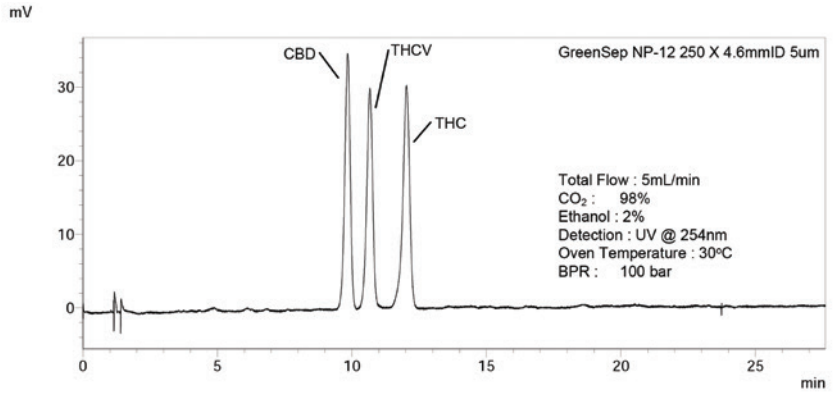

Figure 9: Separation of CBD, THCV and THC on GreenSep 12 using 2% ethanol.

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