Application Note: 21-003

Reversed phase chromatography for cannabinoid purification

Comparison of different stationary phases at preparative process conditions

Introduction

Cannabinoids are of growing interest to the Health and Pharmaceutical industries, with more than 100 different compounds already identified. The exact concentration of the various cannabinoids can vary significantly depending on the type of plant, growing and processing conditions.

Of the Cannabinoids so far identified, Tetrahydrocannabinol (THC) is most known for its psychotropic effects due to its binding directly with receptors in the brain. Consequently the maximum legal level of THC in Hemp is generally 0,3% (although this is often a case for debate in many Countries and States).
It is therefore critical for producers to ensure that any cannabinoid extract is sufficiently purified to meet this <0.3% legal requirement.

Cannabinoids are hydrophobic in nature and therefore purification is most effective using reverse phase chromatography.

However, not all Silicas are the same and this application note investigates the performance of different phases in relation to commercial scale purification of Cannabinoids.

Material and Method

Cannabinoid Crude

The cannabinoid oil and preparative HPLC-setup was kindly provided by AiFame AG. The crude is particularly rich in THC as it is the concentrated residue of a first CBD purification run. It was diluted 1:1 with pure ethanol before injection and doped with uracil (0.15mg/mL) as deadtime marker.

Column

Three different silica based, end-capped C18 and two different C8 phases are used as stationary phases, all having 100Å pore size and 10µm particle size. The column is a standard format HPLC column with the dimensions of 4.6x250 mm.

Preparative HPLC method

Column temperature: 30°C
Flow: 1 ml/min
Mobile phase A: 96% Ethanol
Mobile phase B: Millipore Water
Gradient: none, isocratic at 70% A, no buffer, no additive
Detector: UV at 284 nm (254nm for uracil)
Injection volume: 5µL
Runtime: 30 minutes

Performance parameters

As a parameter for the separation efficiency we determined the selectivity between THC and CBD ($\propto_{CBD-THC}$). From previous studies, we have proven that using Reverse Phase Chromatography there is a correlation between Cannabinoid selectivities. For example, if the selectivity between CBD and Δ9-THC is high, the selectivity between CBC and CBD will also be high. Therefore, the selectivity α between CBD and THC together with the retention factor k'_{CBD} are ideal parameters to track when carrying out method development and stationary phase comparisons.

$$k'CBD = \frac{t_{CBD} - t_{Uracil}}{t_{Uracil}} \quad (1)$$

$$\propto_{CBD-THC} = \frac{k'(CBD)}{k'(THC)} \quad (2)$$

Formulas 1 and 2 (above) are used to calculate the retention factor (k') and the selectivity respectively.
Both factors are based on the retention time of the target (CBD) and the dead time marker (Uracil) which is not retained on the stationary phase.

ZEOCHEM®

A Company of
CPH Chemie + Papier Holding AG

Zeochem AG
Joweid 5 · 8630 Rüti
Switzerland
Phone +41 44 922 93 93
info@zeochem.com · www.zeochem.com

Results

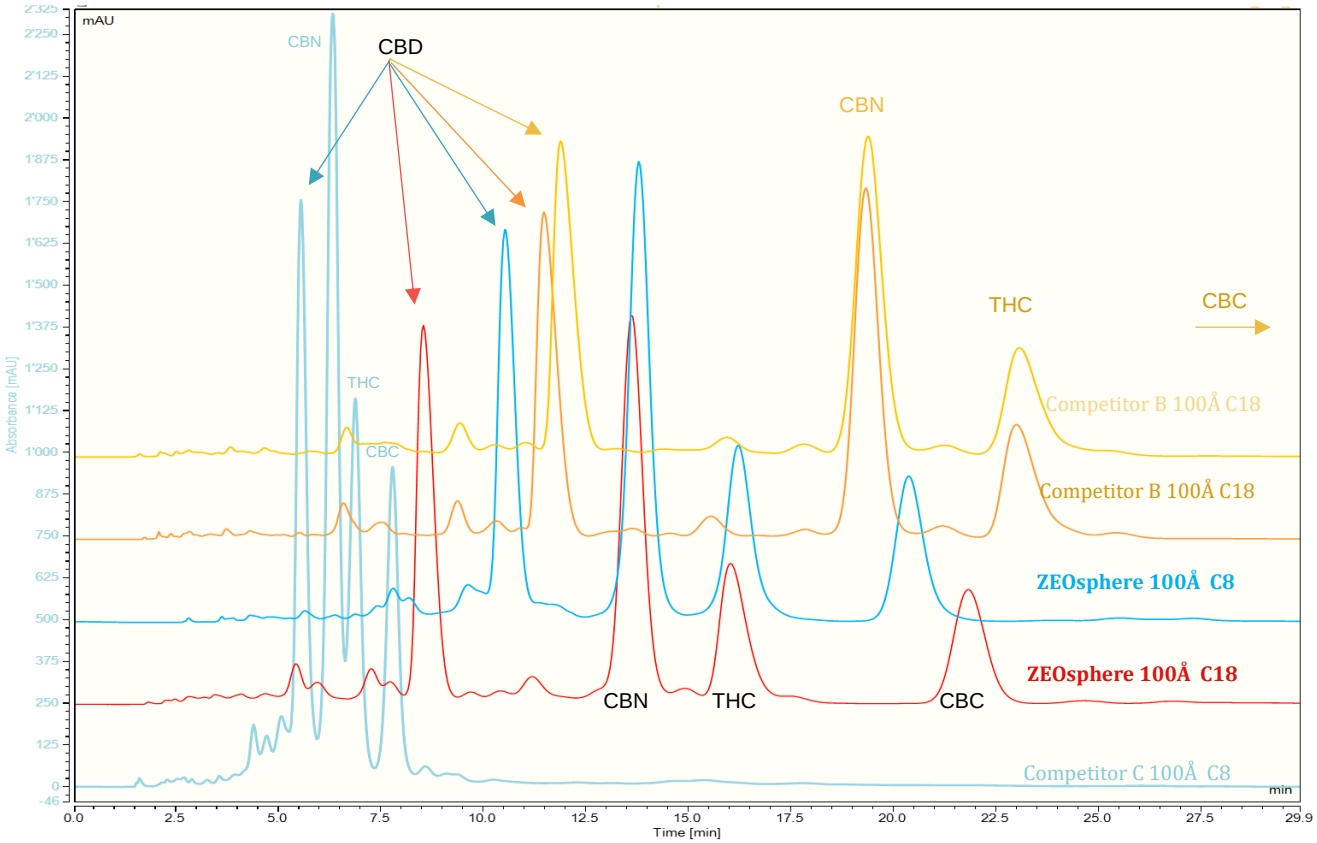

Figure 1. chromatograms of CBD crude purification with different C8 and C18 phases. Elution order is CBD, CBN, THC and CBC in all cchromatograms.

Figure 1 shows the separation of the cannabinoid crude using 5 different C18 and C8 phases. These stationary phases varied in hydrophobicity, shape selectivity and other reversed phase surface characteristics determined with standard Tanaka testing,(1) while silica base structures remain constant: 100Å pore size and 10µm particle size.

About Tanaka Testing: (1)

Hydrophobicity in Tanaka tests is defined as the retention factor ratio (selectivity) between pentylbenzene and butylbenzene, which reflects the ability of the phase to separate compounds that differ by only a single methylene group. Shape selectivity describes the driscimination between planar compounds (triphenyl-ene) and more special molecule (o-terphenyl).
Other characteristics include hydrogen bonding capacity of caffeine vs phenol on the stationary phase surface. Hydrogen bonding occurs between free silanol groups of the silica surface with the analyte. The PQRI database further characterizes hydrogen bonding capacity into hydrogen-bond acidity (A), the ability for non-ionized silanols to interact with bases and hydrogen bond basicity (B), the ability for surface and bonded-phase species to further interact with acidic analyte features.

Selectivity and Retention factor

Figure 2 shows the retention factors and selectivity of the different reversed phases. The most important parameter is the **selectivity**, whereby selectivities greater than 1.5 implies baseline separation and good loadability later in preparative runs. It is immediately apparent that the C8 phases do not show as good selectivity as the C18 phases. The selectivities with a C8 phase are < 2 and even < 1.5 in one case. This less hydrophobic stationary phase (Competitor C C8, light blue Figure 1) also does not show baseline separation of the peaks, even at low injection amounts.

While the selecitivites of CBD and THC on C18 stationary phases are all greater 2 and have much potential for higher sample loading and scaleup. This corroborates with what can be found in existing literature, and is due to the high hydrophobicity of cannabinoids.(2)

The **retention factor** is first of all used to compare performance of different separations with variations in mobile phase and or stationary phase, as it is the case now. It is additionally a measurement of process robustness and any k' above 2 implies a stable chromatographic separation.

ZEOCHEM®
A Company of
CPH Chemie + Papier Holding AG
Zeochem AG
Joweid 5 · 8630 Rüti
Switzerland
Phone +41 44 922 93 93
info@zeochem.com · www.zeochem.com

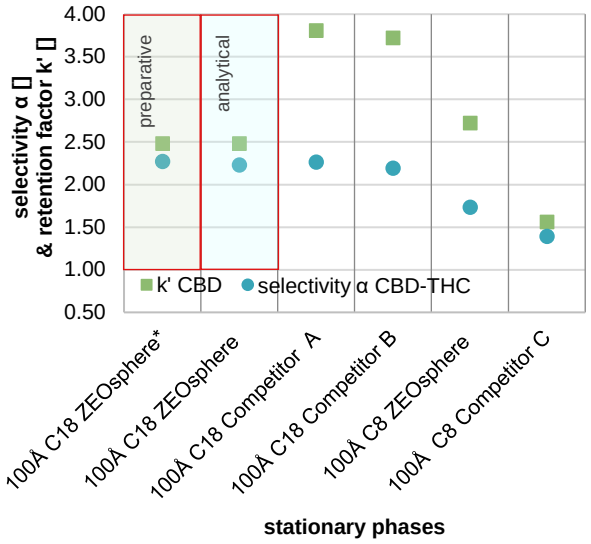

Figure 2. selectivity and retention factor of various stationary phases using a scalable HPLC method without buffers or additives and pure ethanol (70%).
100Å C18 ZEOsphere* *is the same stationary phase in preparative scale (column size 80x500mm).*

Therefore, all C18 phases are showing satisfactory results. A short retention factor (whilst still >2), however, will deliver more economical beneficial, as the run times will be shorter, therefore saving solvent usage and disposal costs.
ZEOsphere 100Å C18/10µm having shown the most promising purification at analytical scale was then packed in an 80 x 250mm preparative column.

The advantage of using ZEOsphere 100Å C18 / 10µm material in this case is its scalability: at analytical scale (4.6x250mm) and a flowrate of 1 mL/min all selectivities and retention factors are well comparable to this large scale (80x500mm) results at 100mL/ min, as demonstrated in fig 2.
Therefore, optimization can be performed cost effectively at analytical scale and then transferred easily to the preferred preparative scale[3,4]

Literature

(1) Tanaka et al., J Chrom Sci, Vol 27, 1989, 721-728

(2) J. Jakowieci et al. *J Chem Inf Model*. 2016 Dec 27;56(12):2457-2466

(3) JJ. van Deemter et al., *Chem. Eng. Sci.*, Vol 5(6), 1956, 271-289.

(4) O. Kaltenbrunner et al. *Progess in Biotechnology*, Vol 16, 2000, 201-206