

## Application Note: 21-002

Purification of  $\Delta^9$ -THC by preparative HPLC using ZEOsphere Silica Gel

## Introduction

Cannabinoids are of growing interest to the Health and Pharmaceutical industries, with more than 100 different compounds already identified. The exact concentration can vary significantly depending on the type of plant, growing and processing conditions. Furthermore, the various isomers of THC may have different side-effects in pharmaceutical applications. Thus, the purer the product the less trouble with regulatory concerns.<sup>(8)</sup>

In this application letter, firstly, we analyzed the cannabinoids in the hemp crude and showed the possibility to efficiently identify and purify multiple cannabinoids including  $\Delta^9$ -THC and  $\Delta^8$ -THC by using preparative HPLC. Robust and quantitative methods are well documented for the analytical separation of cannabinoids.<sup>(1,2)</sup> They usually use methanol and addition of acids, small particle sizes (< 5 $\mu$ m) of the stationary phase and expensive, but highly selective phases<sup>(3)</sup>.

However, an effective large-scale purification that is robust, scalable and still yields pure cannabinoid fractions, is still missing. In this note we show an effective purification method that is robust, scalable and within large-scale purification process constraints.

## THC stereoisomers

Tetrahydrocannabinol exists in many isomeric forms of which four are most common:

Trans- $\Delta^9$ -THC; cis- $\Delta^9$ -THC; trans- $\Delta^8$ -THC and cis- $\Delta^8$ -THC. The cis/trans refers to the position of the hydrogen at C6a and 10a.  $\Delta^8/\Delta^9$  describes the position of the double-bond in the ring. The stereoisomers can be found below. There are additionally the stereoisomers of each one in +/-, which depend on the direction of the hydrogen.

We show only one example of +/- isomers, but theoretically there are 8 different isomers of THC we know of.

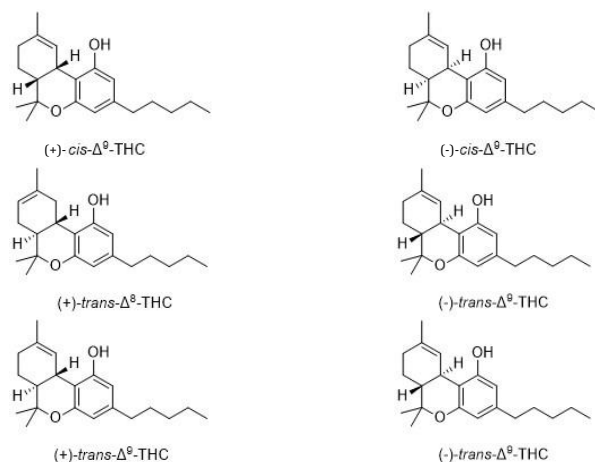


Figure 1. various THC stereoisomers.

Good separation of these isomers is crucial since their potency and their effect on the body and health vary significantly<sup>(4)</sup>: In general, the cis-isomers of THC are not psychoactive. The  $\Delta^8$ -THC isomers are much less psychoactive than the  $\Delta^9$ -THC. Of the  $\Delta^9$ -trans-THC isomers the (-)-trans- $\Delta^9$ -THC is regarded 100 times more potent than its (+)-trans- $\Delta^9$ -THC isomer.

## Material and Method

## Cannabinoid Crude

AiFame AG kindly provided the cannabinoid Oil and Preparative setup. 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.

## Column

The preparative column (dimensions 80 x 500mm) is filled with spherically shaped, fully end-capped derivatized silica gel: ZEOsphere 100 C18 / 10  $\mu$ m.

## Preparative HPLC method

Performed at room temperature 25°C – no column oven  
Flowrate: 100 ml/min; Injection volume 2ml CBD crude;  
Mobile phases: A=96% ethanol; B=water  
Gradient: none, isocratic at 70% A, no buffer, no additive  
Detector: UV at 284 nm

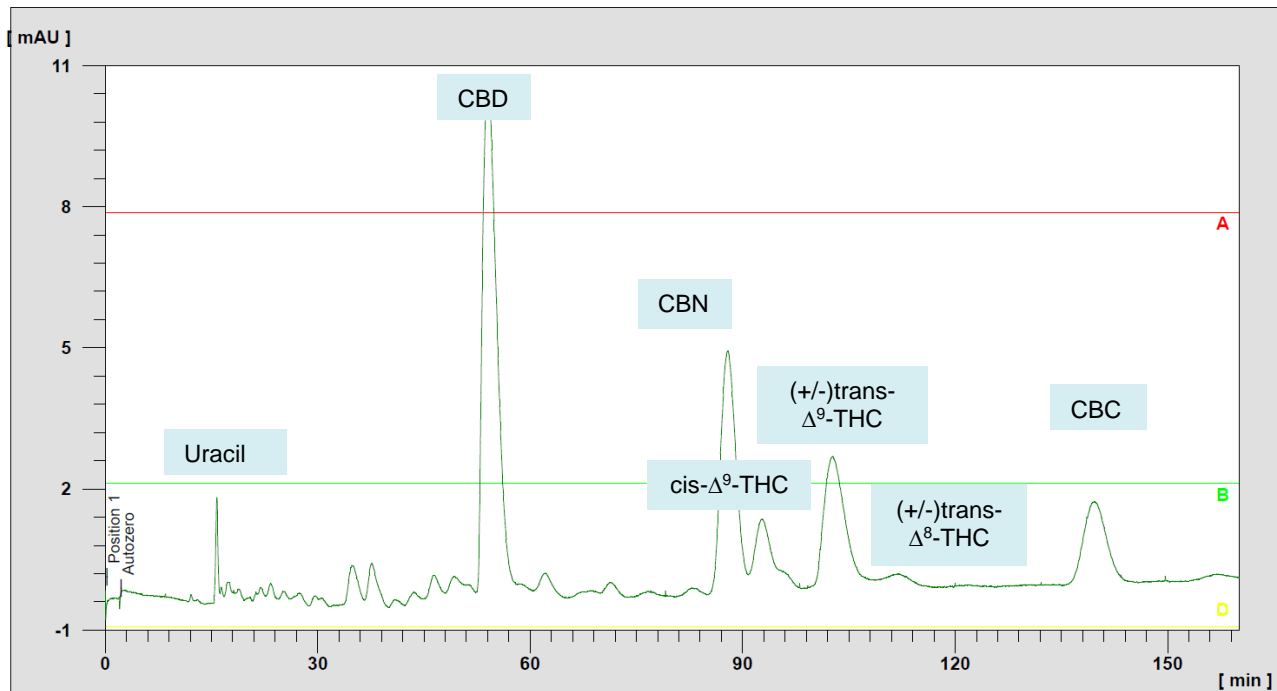


Figure 2. chromatogram of preparative separation of cannabinoids with C18 fully endcapped silica. Baseline separation of  $\text{trans-}\Delta^9\text{-THC}$  and  $\text{trans-}\Delta^8\text{-THC}$  is possible under these conditions.

A second run with higher loading (50 mL) and fraction collection was performed to determine purity of the underlying peaks.

## Results & Discussion

Figure 1 shows the separation of the cannabinoid crude, which is rich in THC. Uracil is a commonly used dead-time marker. Uracil was added to the crude to determine the separation conditions with highest selectivity and have comparability with analytical tests.<sup>(5)</sup>

The chromatographic conditions are capable of **baseline separation  $\text{trans-}\Delta^9\text{-THC}$  and  $\text{trans-}\Delta^8\text{-THC}$  and  $\text{cis-}\Delta^9\text{-THC}$  isomers.**

In a second experiment, the crude loading was increased and 50mL crude was injected onto the column, while the fractions were collected (100mL each): This higher loading resulted in 3 out of 5 fractions (60% yield) with >98%  $\Delta^9\text{-THC}$  and no known impurities. We only claim >98% purity because in presence of an unknown impurity, response factors are not known. The same run produces pure CBD with a yield of > 80%, pure CBN with a yield of 40% and pure CBC at a yield of 100%.

The separation conditions chosen are ideal for scale-up and large scale applications, because no additive, no gradient and only ethanol is used as mobile phase. To increase production rates, the ethanol concentration can be varied, but with ZEOsphere 100 C18, the selectivity does not change significantly. Additionally, the flow rates can be adjusted to the injection amount to increase productivity of the chromatographic separation of cannabinoids and THC-derivates.<sup>(6,7)</sup> The separation of

$\pm$  trans  $\Delta^9\text{-THC}$  is not possible in this case. There are only specialized, analytical methods available for the separation of these stereoisomers.<sup>(3)</sup> These methods use gradients and modifiers, which can be scaled up, but generates high costs and extra process steps that remove these components.

## Scalability

The advantage of using ZEOsphere 100 C18 / 10 $\mu\text{m}$  material is its scalability: at analytical scale (flowrate of 1 mL/min), all selectivity's and retention factors are well comparable to these large-scale results at 100mL/min.

Retention factors only vary by maximum 10% and selectivity's on by 3 % between analytical (1mL/min) and preparative scale. Therefore, optimization can be performed cost effective at analytical scale and then transferred to the preferred preparative scale.

## Literature

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- (6) JJ. van Deemter et al., *Chem. Eng. Sci.*, Vol 5(6), 1956, 271-289.
- (7) O. Kaltenbrunner et al. *Progress in Biotechnology*, Vol 16, 2000, 201-206
- (8) B.E. Erikson; Delta-8-THC craze concerns chemists, *C&EN* 30.08.2021.

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