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Analyzing cannabis flowers according to the German Pharmacopeia - monograph 2018

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SUMMARY

Since the change in German narcotics law in 2017 (§1 Abs. 1, Betäubungsmittelgesetz; BtMG) the need for reliable and robust HPLC methods for quality control has drastically increased[1]. Six common cannabinoids of high medicinal interest Cannabidiol (CBD), Cannabidiolic acid (CBDA), Cannabinol (CBN), Δ^8-Tetrahydrocannabinol (Δ^8-THC), Δ^9-Tetrahydrocannabinol (Δ^9-THC) and Δ^9-Tetrahydrocannabinolic acid (Δ^9-THCA) were quantified on the KNAUER AZURA® HPLC plus system according to the monograph of German Pharmacopeia 2018 (Deutsches Arzneibuch; DAB)[2]. The method was verified using an authorized medicinal cannabis flower available on the market. The assignment of the analytes was made with chemical reference standards.

INTRODUCTION

Cannabis sativa L. is one of the oldest agricultural and medicinal plants which produces a variety of compounds such as terpenoids, flavonoids and cannabinoids[3]. The interaction of cannabinoids with the body's own cannabinoid receptors, which occur in a variety of brain cells for coordination, memory processing and spatial orientation, provides new pharmacological and psychological treatment options[4]. Probably the most psychoactive cannabinoid of the four different isomers of Δ^9-THC is the (-)-Δ^9-*trans*-tetrahydrocannabinol, also known as dronabinol. In Germany, Δ^9-THC is controlled by the narcotics law, due to its psychoactive properties. Since March 2017, the regulation has changed due to the amendment of Article 1 BtMG. The amendment of annexes II and III of the BtMG now allows the marketing and prescription of cannabis such as marijuana plants and plant parts. Therefore they are authorised for medical purposes as ready-to-use medicinal products[1]. Production of cannabis products must be conducted and monitored in accordance with Good Manufacturing Practice guidelines (GMP). To guarantee accurate labelling of medicinal products, food and cosmetics, the demand for standardized methods for the quantitative and qualitative determination of ingredients is increasing, especially for cannabinoids[5]. The quality assurance of the plants may be ensured by employing the German Pharmacopeia method DAB[2]. In this work, the HPLC method for cannabis flowers according to the DAB monograph was carried out with the KNAUER AZURA® HPLC plus system.

Additional Information

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RESULTS

The measured 10 µg/mL standard mix from the six different cannabinoids results in the chromatogram in **Fig. 1**. The measurement of the cannabis flower bediol (Bedrocan, Veendam, Netherlands) with a dilution of 1 to 10 with ethanol is shown in **Fig. 2**. The repeatability for five cannabinoids was confirmed with a value of under 1% relative standard deviation over a six-time repetition of the bediol sample shown in **Tab. 1**. The relative retention compared to Δ⁹-THC is shown in **Tab. 2** with the given specifications.

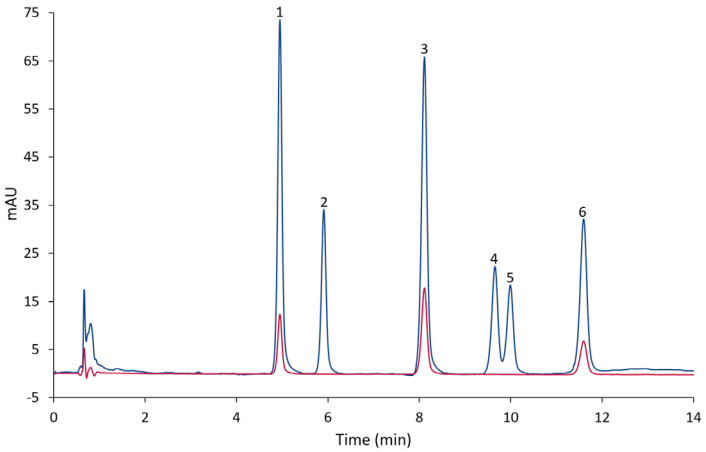

Fig. 1 Chromatogram of a 10 µg/mL standard mix measured with the DAB method; R=1.3 between Δ⁸-THC and Δ⁹-THC; blue - 225 nm, red – 306 nm, 1 - CBDA, 2 - CBD, 3 - CBN, 4 - Δ⁹-THC, 5 - Δ⁸-THC, 6 - Δ⁹-THCA.

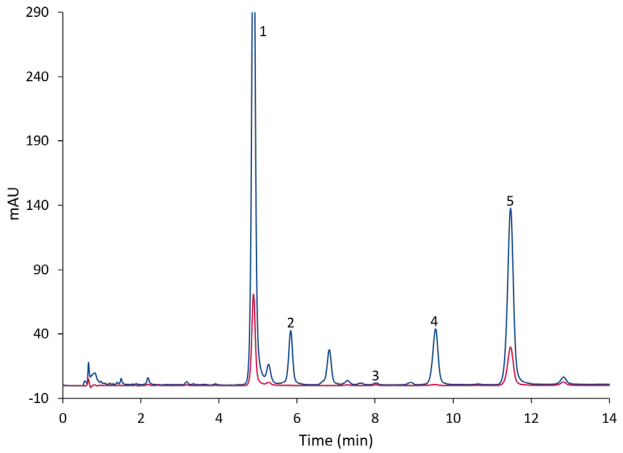

Fig. 2 Chromatogram of a 1 mg/mL bediol sample in ethanol; blue - 225 nm, red – 306 nm, 1 - CBDA, 2 - CBD, 3 - CBN, 4 - Δ⁹-THC, 5 - Δ⁹-THCA.

Tab. 1 Results repeatability

Analyte	CBDA	CBD	CBN	Δ⁹-THC	Δ⁸-THC	Δ⁹-THCA
Relative standard deviation [%]	0.26	0.89	0.84	0.34	-	0.14

Tab. 2 Results relative retention to Δ⁹-THC (9,56 min)

Analyte	CBDA	CBD	CBN	Δ⁸-THC	Δ⁹-THCA
Relative retention measured	0.51	0.61	0.84	1.05	1.20
Relative retention DAB	0.48	0.57	0.83	1.04	1.24

SAMPLE PREPARATIONS

A 5 g portion of bediol was grinded with a flower grinder. The difference between grinded and original bediol is shown in **Fig. 3**. The sample preparation was performed according to DAB, where 500 mg substance was extracted three times with 15 mL ethanol on a laboratory shaker for 15 minutes with a following centrifugation at 5,000 rpm for 1 minute. Mixing all the extracts within 50 mL measuring flask, a 1:10 dilution was carried out and measured with the HPLC system after filtrating over a 0.45 µm RC filter.

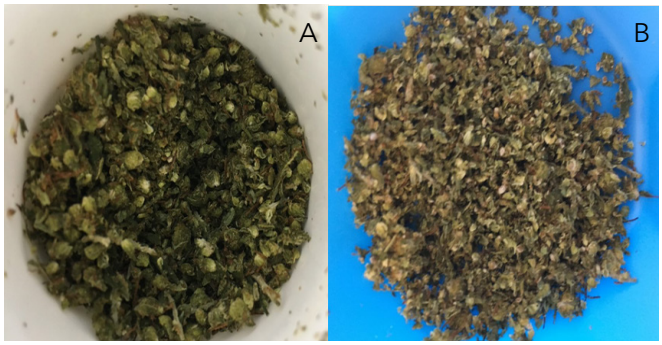

Fig. 3 Bediol sample: original (A) and grinded (B).

CONCLUSION

As shown in previous work for the DAB monograph 2017, the column Eurospher II C18P 100-3, 150x4.6 mm was determined as the most robust one while separating six different cannabinoids[6]. In the new monograph 2018 the change of the column dimensions results in an Eurospher II C18P 100-3, 150x3 mm. The additional modification of the gradient program compared to the 2017th monograph makes each chromatographic separation over 20 minutes faster. The measured chromatograms in this work show a sufficient separation of the six given cannabinoids. The specification of the DAB with a critical resolution of R>1.2 for the critical analyte pair Δ^8-THC and Δ^9-THC is confirmed with R=1.3 within the measurements. The sample measurement of bediol shows a sufficient assignment of the signals, whereas the not identified signals can be assumed as matrix or not categorized cannabinoids. Additionally, the use of two different wavelengths shows a differentiation between the acid and neutral form of the cannabinoids. CBN as well shows an absorption towards 306 nm due to the increased amount of conjugated systems compared to Δ^8-THC and Δ^9-THC.

REFERENCES

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MATERIALS AND METHODS

Tab. 3 Used standards and solvents

Analyte	CAS	Purity / Concentration
Cannabidiol (CBD)	13956-29-1	1,000 mg/mL (MeOH)
Cannabidiolic acid (CBDA)	1244-58-2	1,000 mg/mL (ACN)
Cannabinol (CBN)	521-35-7	1,000 mg/mL (MeOH)
Δ^8-Tetrahydrocannabinol (Δ^8-THC)	5957-75-5	1,000 mg/mL (MeOH)
Δ^9-Tetrahydrocannabinol (Δ^9-THC)	1972-08-3	1,001 mg/mL (MeOH)
Δ^9-Tetrahydrocannabinolic acid (Δ^9-THCA)	23978-85-0	1,000 mg/mL (ACN)
Solvent	**CAS**	**Purity / Concentration**
Acetonitril	75-05-8	Gradient grade
Ethanol	64-17-5	Gradient grade
H_3PO_4	7664-38-2	AnalaR 85% NORMAPUR

Tab. 6 System configuration

Instrument	Description	Article No.
Pump	P 6.1 L	APH34EA
Detector	MWD 2.1 L	ADB01
Flow cell	10 mm, 10 µL, Pressure proof	AMC38
Autosampler	AS 6.1 L	AAA00AA
Column thermostat	CT 2.1	A05852
Column	Eurospher II 100-3 C18 P, 150 x 3 mm	15XE182E2G
Software	Clarity Chrom 8.1	A1670

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Tab. 4 Method

Column temperature	40 °C
Injection volume	10 µL
Injection mode	Full loop
Detection	UV 225 nm / 306 nm
Data rate	10 Hz

Tab. 5 Gradient

Eluent (A)	Water, HPLC grade (H_3PO_4 85% 8,64 g/L)		
Eluent (B)	Acetonitrile, Gradient grade		
Flow rate	1,0 mL/min		
	Time [min]	**(A) %**	**(B) %**
Pump program	0	36	64
	16	18	82
	17	36	64
	20	36	64