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Application News

No. SCA_190_044

High Performance Liquid Chromatography

Cannabinoid Potency Testing by HPLC according to DAB Monography for Cannabis Flower

Werner Dreckmann, Dr. Stefan Vosskötter, SDG

Introduction

In the German Pharmacopoeia 2018 (DAB 2018) the Federal Institute for Drugs and Medical Devices (BfArM) publishes a revised monograph for cannabis flower (Cannabis flos) [1]. Since there is no equivalent monograph in the European Pharmacopeia (EP), currently the method described in the DAB 2018 depicts the obligatory procedure for potency testing of cannabinoids in cannabis flower in the EU [2].

The monograph constitutes the official pharmaceutical regulation according to the law on drugs (AMG) and will be considered for decisions made by the responsible authority.

Analytical Conditions

Separation of relevant cannabinoids according to the DAB monograph

- Cannabidiolic acid (CBDA)
- Cannabidiol (CBD)
- Cannabinol (CBN)
- Δ9-Tetrahydrocannabinol (THC)
- Δ9 Tetrahydrocannabinolic acid (THCA)

is achieved on a Shim-pack Velox SP-C18 column using the Nexera-i compact UHPLC system. Method parameters are listed in table 1.

Table 1: Method parameters

System:	Nexera-i 3D plus
Column:	Shim-pack Velox SP-C18; 2.7 µm; 150 x 3.0
Guard column:	Shim-pack Velox SP-C18; 2.7 µm; 5 x 3.0 (G)
Mobile Phase:	A: Water + Phosphoric acid 85% (8.64 g/l) B: Acetonitrile
Gradient:	0 min 64 %B, 16 min 82 %B, 17 – 20 min 64 %B
Detection:	UV 225 nm (CBD, CBN, THC) UV 306 nm (CBDA, THCA)
Flow rate:	1 ml/min
Oven temp.:	40 °C
Inj. Volume:	10 µL
Run time:	20 min

Potency Testing

Standard and sample solution are prepared according to the procedure described in the monograph:

0.5 g of pulverized flower are shaken with 20 ml of ethanol (96 %) for 15 min followed by centrifugation. The clear supernatant is transferred into a 50 ml measuring cylinder. The residue is extracted two more times with 12.5 ml of ethanol (96 %) each time. The organic fractions are combined in the measuring cylinder and made up to the 50.0 ml mark with ethanol (96 %).

The solution is filtered using a 0.45 µm membrane filter and 1.0 ml of the filtrate are measured into a 10 ml volumetric flask and made up to mark with ethanol (96 %) [1].

As reference standard solutions, single standards of the five compounds are dissolved in methanol. For calibration at least 6 calibration standard solutions for each compound are prepared, where one defined concentration for each analyte is mandatory (table 2).

Table 2: Standard concentrations

Analyte	reference [mg/100ml]	calibration [µg/ml]	requisite [µg/ml]
CBDA	15	0.5 – 100	50
CBD	10	0.5 – 75	10
CBN	10	0.1 – 10	1
THC	10	0.5 – 75	10
THCA	30	0.5 - 250	50

Performance Test

A reference solution of Δ9-Tetrahydrocannabinol and Δ8-Tetrahydrocannabinol is prepared for a performance test. Resolution between the two peaks has to be ≥ 1.2. Area reproducibility of the THCA reference solution, determined by six successive injections, has to result in %RSD < 1.

Calibration

Calibration is carried out by measurement of calibration standards as described in table 2. Area is plotted against concentration to obtain a linear calibration curve (Figure 1).

Sample measurement

Cannabis flower from all 3 groups classified in the monograph (table 3) were prepared and analysed according to the describe method (figure 2 - 4). For quantification of CBD, CBN and THC the UV trace at 225 nm was used, CBDA and THCA were measured at 306 nm.

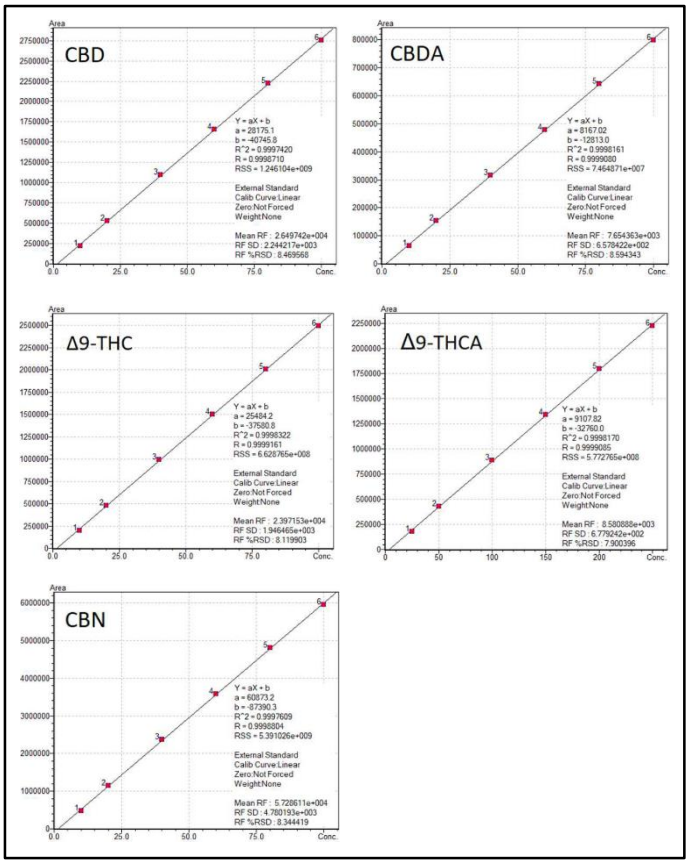

Figure 1: Linearity of cannabinoid measurement

Table 3: Classification of product groups

Group	Content
I	Δ9-Tetrahydrocannabinol >> Cannabidiol
II	Δ9-Tetrahydrocannabinol ≈ Cannabidiol
III	Δ9-Tetrahydrocannabinol << Cannabidiol

Results

Cannabis flowers are classified according to the ratio of THC and CDB concentration as described in table 3, where also the amount of acidic precursors are added to give the total cannabinoid content, referring to dry weight (table 4).

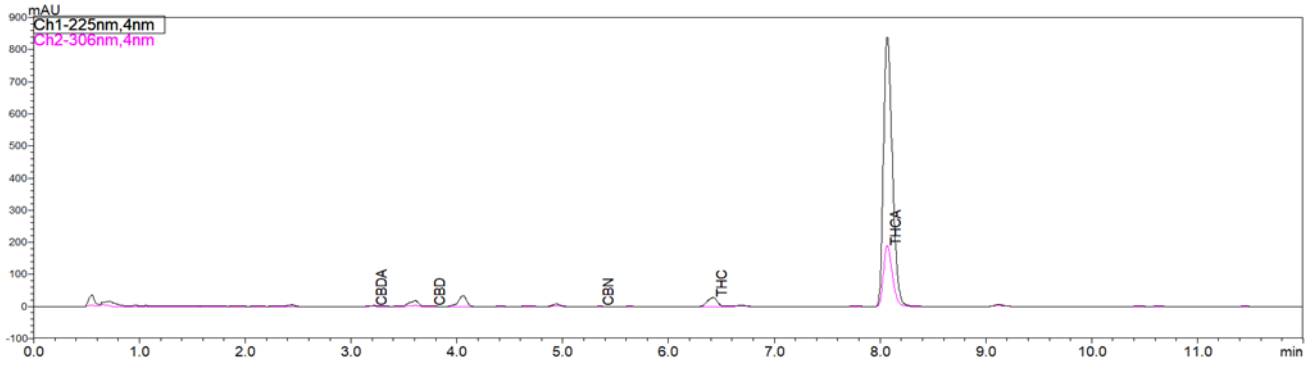

Figure 2: Chromatogram of cannabis flower from sample group I

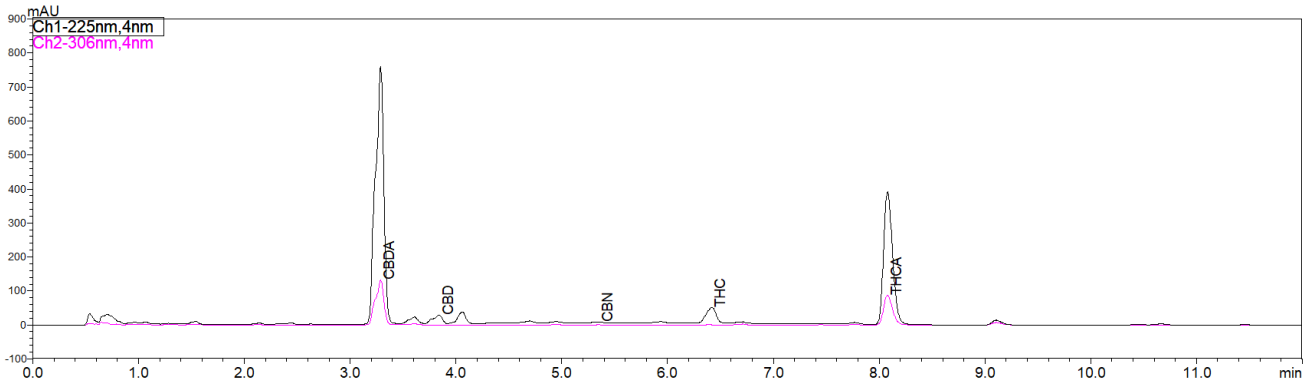

Figure 3: Chromatogram of cannabis flower from sample group II

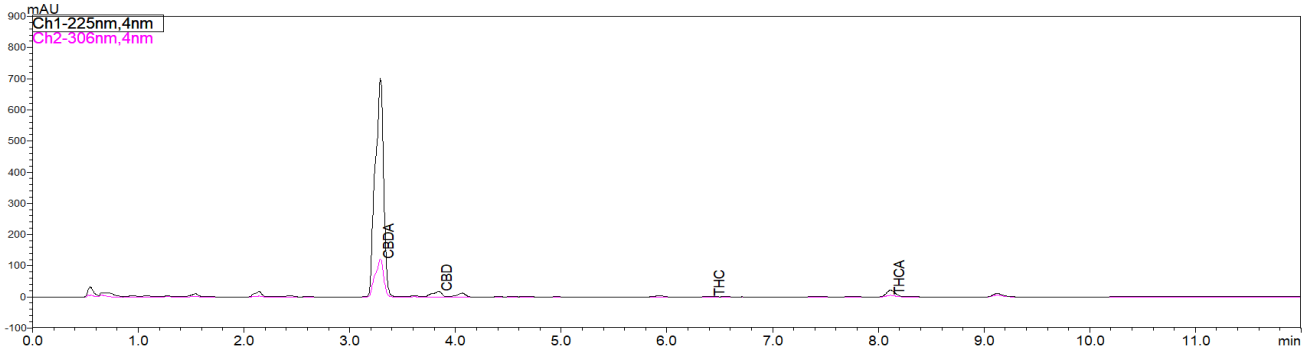

Figure 4: Chromatogram of cannabis flower from sample group III

Table 4: Results of potentcy testing in cannabis from product group I – III (content refers to dry weight)

Group I			Group II			Group III		
Analyte	RT [min]	Content	Analyte	RT [min]	Content	Analyte	RT [min]	Content
CBDA	3.209	0.24 %	CBDA	3.288	6.94 %	CBDA	3.291	7.79 %
CBD	3.762	0.18 %	CBD	3.842	0.6 %	CBD	3.843	0.53 %
CBN	5.353	0.18 %	CBN	5.349	0.14 %	CBN	-.--	-.--
THC	6.422	0.99 %	THC	6.42	1.16 %	THC	6.417	0.22 %
THCA	8.068	12.54 %	THCA	8.08	4.66 %	THCA	8.117	0.71 %
CBD-Total		0.42 %	CBD-Total		7.54 %	CBD-Total	8.32 %	8.32 %
THC-Total		13.53 %	THC-Total		5.82 %	THC-Total	0.93 %	0.93 %

Summary

The analytical method described in the DAB monograph cannabis flower was successfully tested using the Nexera-i compact UHPLC system. 3 samples from different product groups were analysed and reliably evaluated. Peakshape could be improved by using the automated co-injection function to dilute the strong sample solvent [3].

Acknowledgement

All measurements were carried out in the laboratory of the Federal Institute for Drugs and Medical Devices (BfArM) in Bonn, Germany.

References

[1] German Pharmacopoeia 2018 (DAB 2018)
[2] EU-Guideline 2001/83 consolidated Version, Annex I EG
[3] Shimadzu Application Note: Optimized Cannabinoid Potency Testing by HPLC according to DAB Monography for Cannabis Flower with use of Co-Injection function

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Flow rate:	1 ml/min
Oven temp.:	40 °C
Inj. Volume:	10 µL
Run time:	20 min

Standard and sample solutions are prepared and measured as described in the monograph [2, 3]. Reference and standard compounds are dissolved in methanol, while samples are extracted using ethanol. This discrepancy leads to differences in peak shape for cannabinoids in standard and sample solutions as ethanolic sample extraction results in peak dispersion on the column as shown in figure 1.

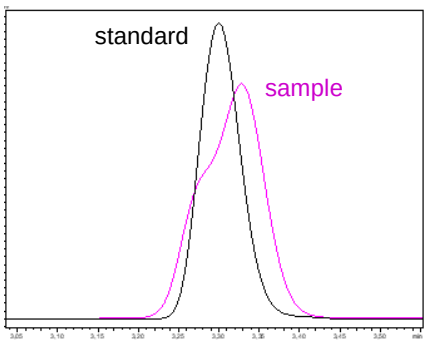

Figure 1: CBDA peak in sample and standard solution analyzed according DAB monograph.

As the monograph describes an obligatory procedure, pharmaceutical quality control can't deviate from the given procedure for standard and sample preparation. An acceptable way to comply with the method and still improve the chromatographic performance is the co-injection function, where a small amount of water can be injected together with the standard or sample solution.

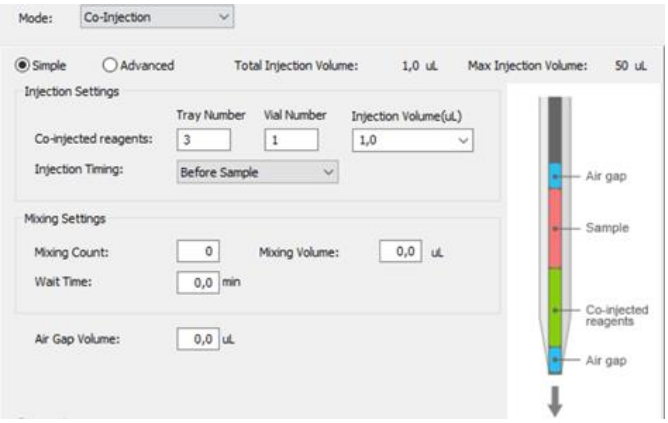

Figure 2: User interface in LabSolutions software to program co-injection for Nexera-i Plus system

The co-injection of water dilutes the strong organic solvent, rendering it a weaker eluent at the time of injection. The sample is focused on top of the stationary phase, resulting in sharper, symmetrical peakshape, as can be seen in the chromatograms in figure 3.

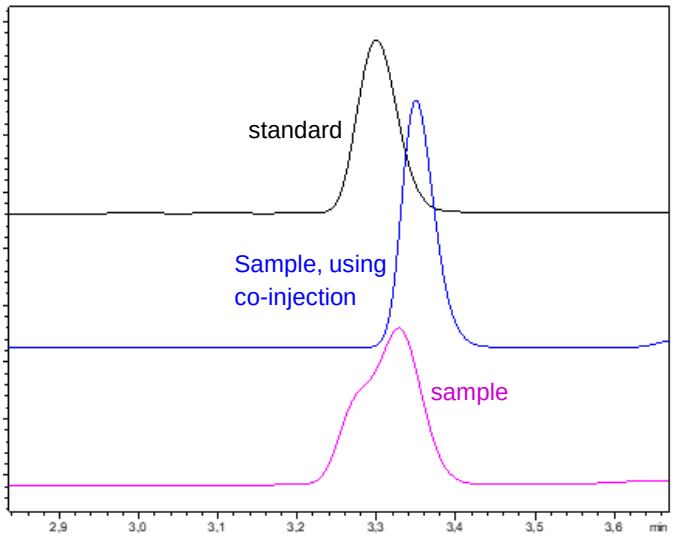

Figure 3: Comparison of conventional injection of sample and standard solution with co-injection with water of the sample solution

The co-injection function is a standard feature in the i-series Plus systems when using LabSolutions control software.

Analysis

A mixed standard solution containing the 5 compounds of interest was measured using the optimized method (figure 4). In the example shown 10 µl water was added to the 10 µl sample volume. To compare co-injection and standard method according to DAB monograph a real cannabis flower sample (Group II) was measured using the two different approaches (figure 5).

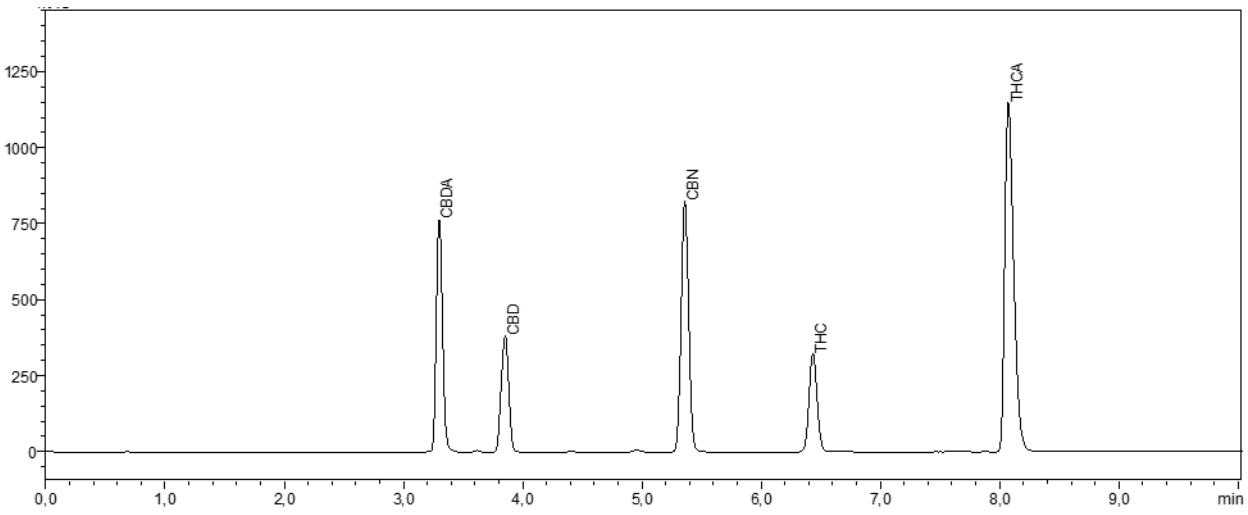

Figure 4: Chromatogram of mixed standard solution of CBDA, CBD, CBN, THC and THCA

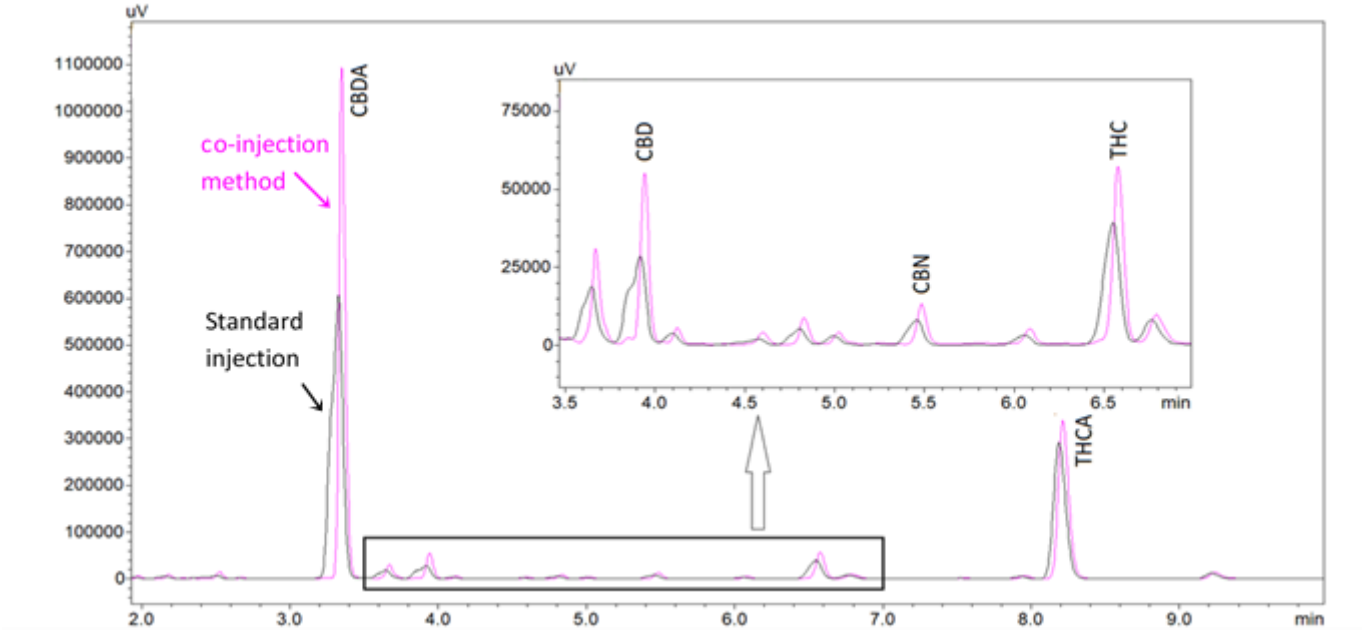

Figure 5: Chromatogramm of cannabis flower sample using standard co-injection method

Table 2: Comparison of peak width using standard and co-injection method

Analyte	Standard Method Width at half height [s]	Co-injection method Width at half height [s]	Reduction in peak width
CBDA	5.70	2.70	52.6 %
CBD	6.84	3.00	56.1 %
CBN	6.12	3.66	40.2 %
THC	6.06	3.96	34.7 %
THCA	5.46	4.62	15.4 %

Summary

Due to the use of different, strong organic solvents for standard dilution and extraction the analytical method for potency testing in cannabis flower according to DAB 2018 results in peak dispersion and different chromatographic retention for sample and standard solutions. Co-injection of water reduces these differences, and significantly improves peak shape and therefore resolution. The i-series Plus compact (U)HPLC system offers the automated co-injection function.

Acknowledgement

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References

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