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Application News

No. SCA_190_045

High Performance Liquid Chromatography

Cannabinoid Potency Testing by HPLC according to DAB Monography for Cannabis Flower with use of Co-Injection function

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 Raptor ARC-18 column (Restek) using the Nexera-i compact UHPLC system. Method parameters are listed in table 1.

Table 1: Method parameters

System:	Nexera-i 3D plus
Column:	Raptor ARC-18 2.7 µm, 150 x 3.0 mm
Guard column:	Raptor ARC-18 2.7 µm, 5 x 3.0 mm
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
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.

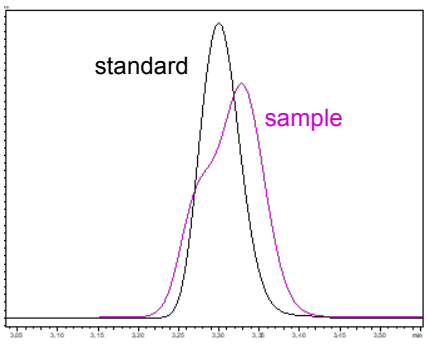

Figure 1: CBDA peak in sample and standard solution analyzed according DAB monograph.

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.

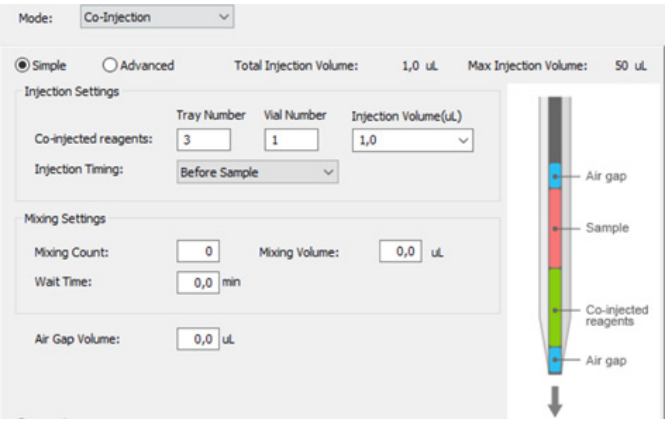

Figure 2: User interface in LabSolutions software to program co-injection for Nexera-i Plus system

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.

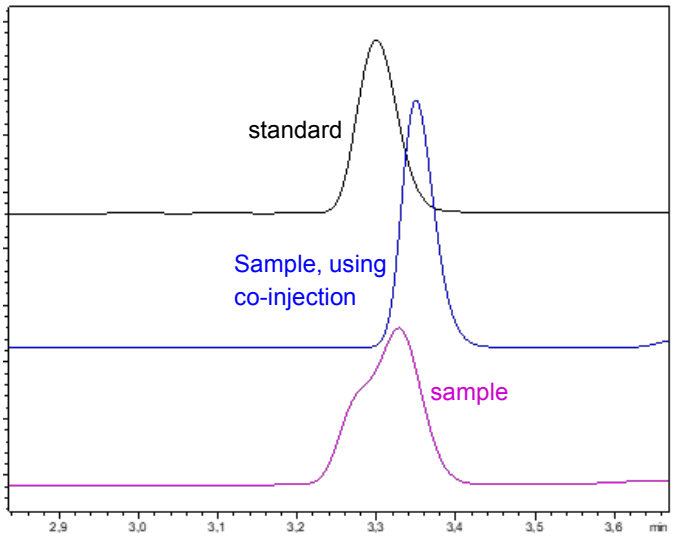

Figure 3: Comparison of conventional injection of sample and standard solution with co-injection with water of the sample solution

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).

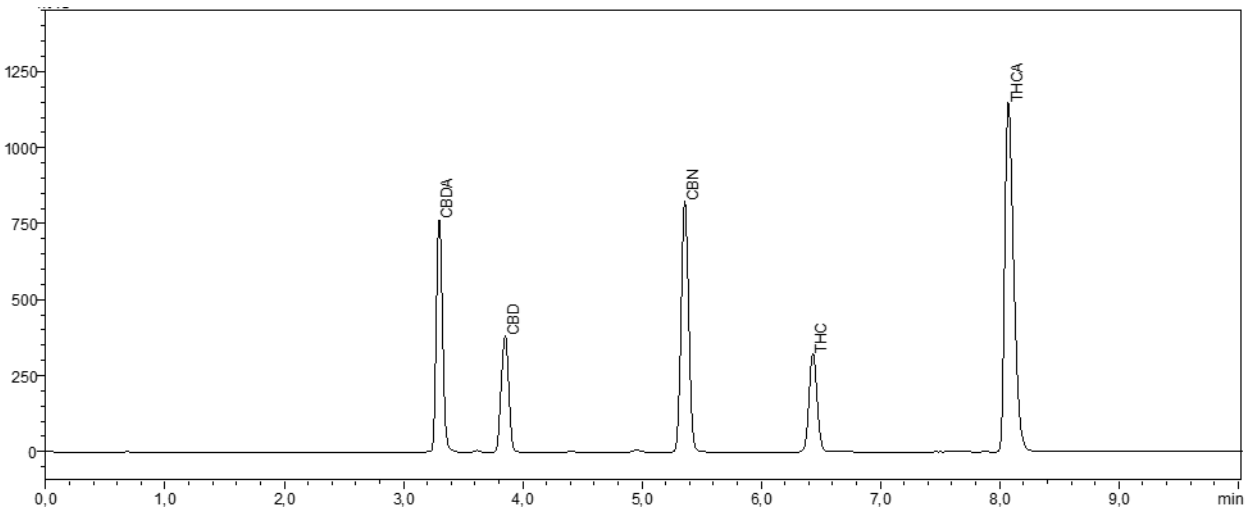

Figure 4: Chromatogram of mixed standard solution of CBDA, CBD, CBN, THC and THCA

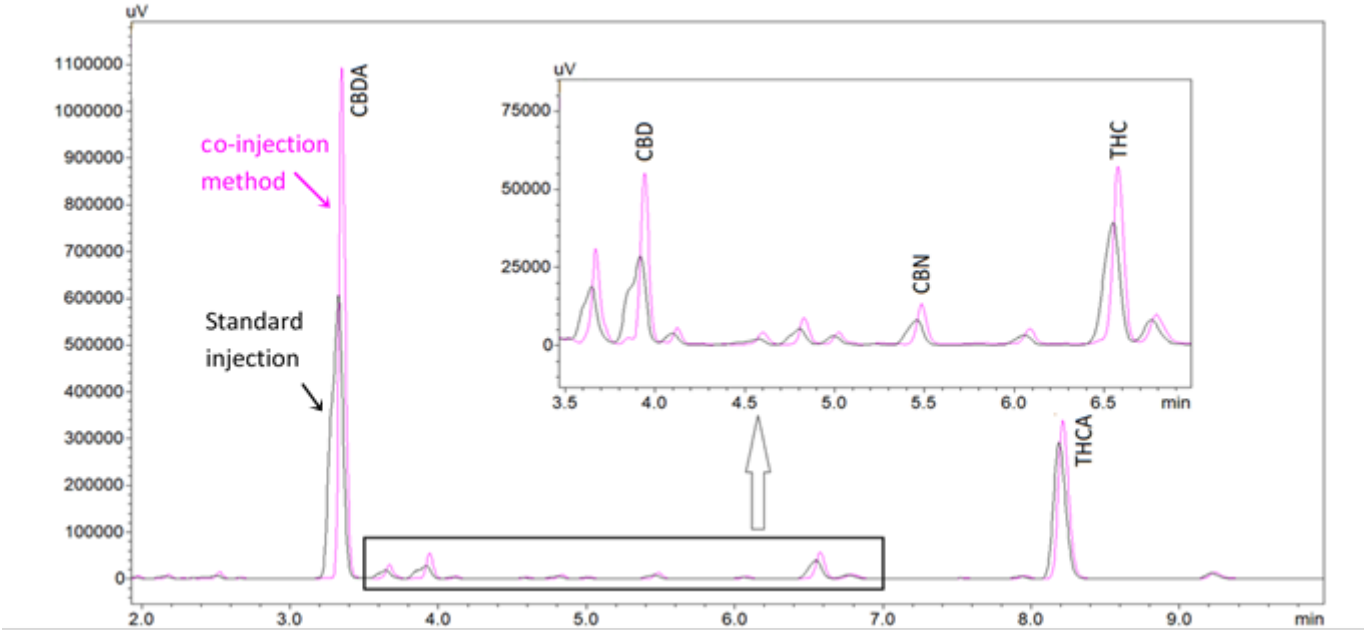

Figure 5: Chromatogramm of cannabis flower sample using standard co-injection method

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

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

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