MACHEREY-NAGEL
Chromatography application note

MN application note 03/2020

Potency testing in cannabis extracts using a LC–UV approach with additional MS/MS identification

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Abstract

This application note describes the determination of cannabinoids in Cannabis sativa using a LC–UV approach. Cannabis sativa was extracted according to DAC/NRF monograph and analyzed by HPLC–UV on a NUCLEOSHELL® RP 18 column. Identification of contained cannabinoids was performed by HPLC–UV–MS/MS.

Introduction

With the legalization of medical and recreational use of cannabis and cannabis-based products (e.g. concentrated oils, soda, candy and other edible forms) the need of quality control methodologies has grown year by year [1]. There is an increasing supply of various cannabis and cannabis-based products with adjusted potency. To verify the potency classification, the determination of cannabinoids in marijuana has become an important methodology of quality control for producers and distributors. Additionally, the cannabinoid profile in combination with the terpene profile is an important information that helps to determine the plant variety. Sample preparation has been carried out according to DAC/NRF regulations [2].

New quality control methods have to be developed to ensure product safety and to treat patients with the right amount of drug. These methods have to be quick, easy and cost-efficient. Using NUCLEOSHELL® core-shell particle technology, highest column efficiency and resolution at a short run time with much lower back pressure compared to fully porous particles could be achieved with common HPLC systems.

This work presents a quick, easy and cost-efficient LC–UV method for the simultaneous analysis of cannabinoids from Cannabis sativa. In addition, cannabinoids were identified by LC–MS/MS.

Sample pretreatment

Sample material

Three varieties of Cannabis sativa of different potency classes were purchased:

I: Δ^9-tetrahydrocannabinol ≫ cannabidiol

II: Δ^9-tetrahydrocannabinol ≈ cannabidiol

III: Δ^9-tetrahydrocannabinol ≪ cannabidiol

Extraction

- Weigh out 0.5 g of homogenized sample (milled in a grinder) into a 50 mL centrifuge tube (REF 730223)
- Add 20 mL ethanol and shake for 15 min
- Centrifuge the mixture at 4500 rpm, for 5 min at 4 °C
- Fill the supernatant in a 50 mL flask
- Repeat extraction of the residue twice with 12.5 mL of ethanol and combine the extracts
- Fill up the flask to 50 mL with ethanol
- Filter 1 ml of the extract through a syringe filter with regenerated cellulose (membrane pore size 0.45 µm, REF 729231) into a 10 mL flask
- Fill up with ethanol to 10 mL
- Use this mixture for HPLC analysis (REF 702293, REF 702107)

LC Method Parameters

Chromatographic conditions

Column:	EC NUCLEOSHELL® RP 18, 50 x 4 mm, 2.7 µm (REF 763152.40)
Eluent A:	0.1 % formic acid in water
Eluent B:	0.1 % formic acid in acetonitrile
Gradient:	in 5 min from 60 % to 95 % B, hold for 5.0 min, in 0.1 min to 60 % B, hold 60 % B for 4.9 min
Flow rate:	0.7 mL/min
Temperature:	40 °C
Injection volume:	1 µL
Detection:	UV @ 225 nm, 306 nm

MS conditions for peak identification

AB Sciex API 3200

Acquisition mode:	SRM
Interface:	ESI
Polarity:	positive/negative
Curtain gas:	20 psig
CAD:	3.0 psig
Ion spray voltage, ESI positive:	4500 V
Ion spray voltage, ESI negative:	– 4500 V
Temperature:	500 °C
Ion source gas 1:	45 psig
Ion source gas 2:	45 psig
Detection window:	90 s
Injection volume:	5 µL

Sample solution from LC–UV was diluted ten times with methanol for identification with positive polarity and 100 times with methanol for identification with negative polarity because of high analyte concentration of cannabinoids in cannabis extracts.

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Potency testing in cannabis extracts using LC–UV approach

MRM transitions

Analyte	Abbr.	$[M+H]^+$	Q_1	Q_2
Cannabigerol	CBG	317.2	193.1	123.1
Cannabidiol	CBD	315.1	193.1	259.1
Cannabinol	CBN	311.1	223.2	241.0
Δ^9-tetrahydrocannabinol	THC	315.1	193.1	259.1
Cannabichromene	CBC	315.1	193.1	81.0

Table 1: MRM transitions of cannabinoids from Cannabis sativa (positive polarity). (Abbr. = Abbreviation, Q_1 = Quantifier, Q_2 = Qualifier)

Analyte	Abbr.	$[M-H]^-$	Q_1	Q_2
Cannabidiolic acid	CBDA	357.1	313.1	245.0
Cannabigerolic acid	CBGA	359.1	341.2	315.2
Δ^9-tetrahydrocannabinolic acid	THCA	357.1	313.1	191.0

Table 2: MRM transitions of cannabinoids from Cannabis sativa (negative polarity). (Abbr. = Abbreviation, Q_1 = Quantifier, Q_2 = Qualifier)

Batch-to-batch reproducibility of NUCLEOSHELL® RP 18 columns

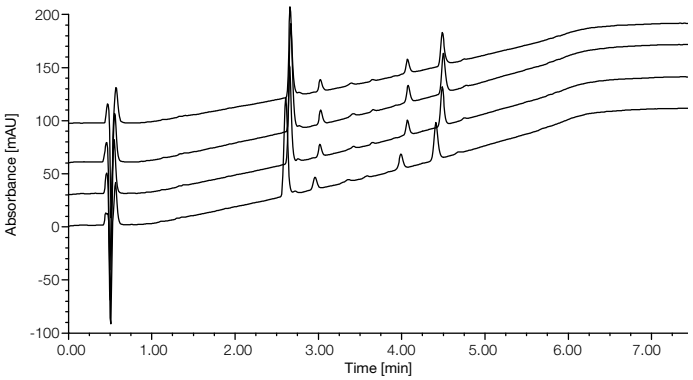

Figure 1: Comparison of chromatograms from an extract of Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol ≈ cannabidiol) on four different batches of NUCLEOSHELL® RP 18, UV-VIS 1 = 225 nm.

Figure 2: Marijuana plant leaves.

Representative chromatograms

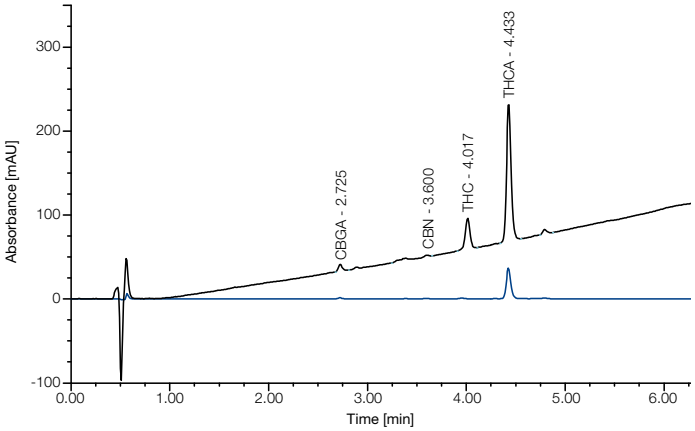

Figure 3: Chromatogram of an extract from Cannabis sativa (variety class I: Δ^9-tetrahydrocannabinol ≫ cannabidiol), UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm.

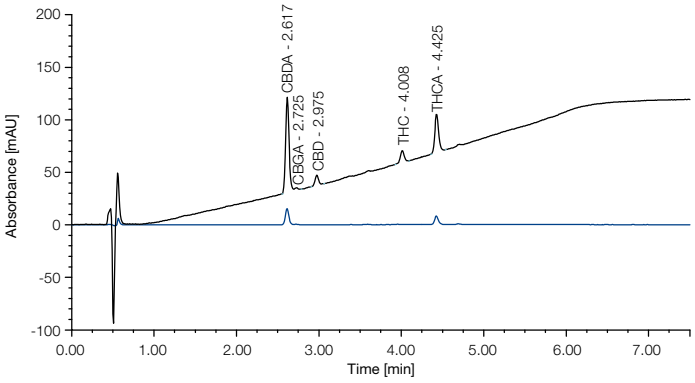

Figure 4: Chromatogram of an extract from Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol ≈ cannabidiol), UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm.

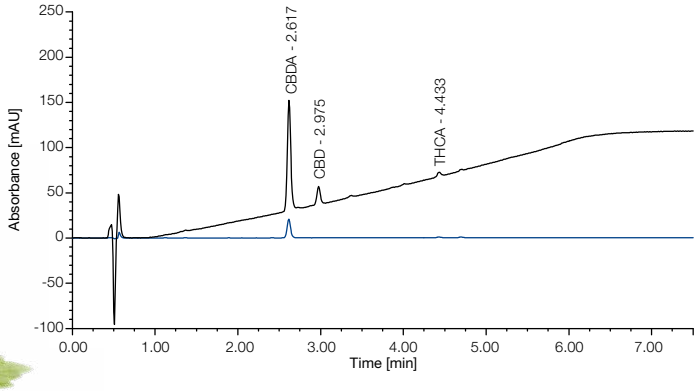

Figure 5: Chromatogram of an extract from Cannabis sativa (variety class III: Δ^9-tetrahydrocannabinol ≪ cannabidiol), UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm.

Potency testing in cannabis extracts using LC–UV approach

Batch-to-batch reproducibility of Cannabis sativa samples

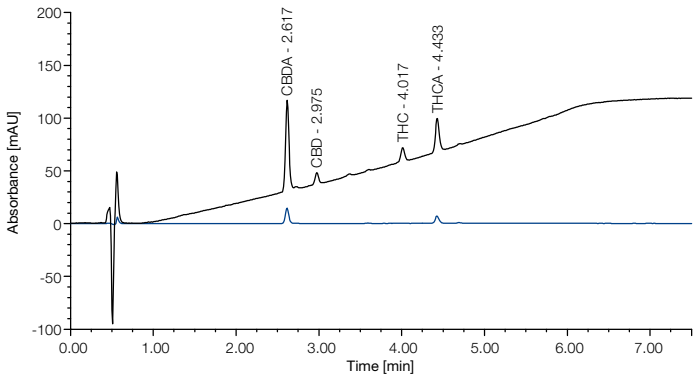

Figure 6: Comparison of chromatograms from an extract of Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol ≈ cannabidiol) on three different batches, UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm, batch 1.

Figure 7: Marijuana (Cannabis sativa).

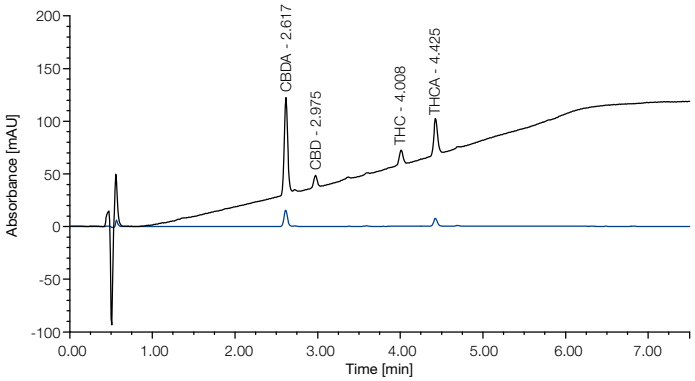

Figure 8: Comparison of chromatograms from an extract of Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol ≈ cannabidiol) on three different batches, UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm, batch 2.

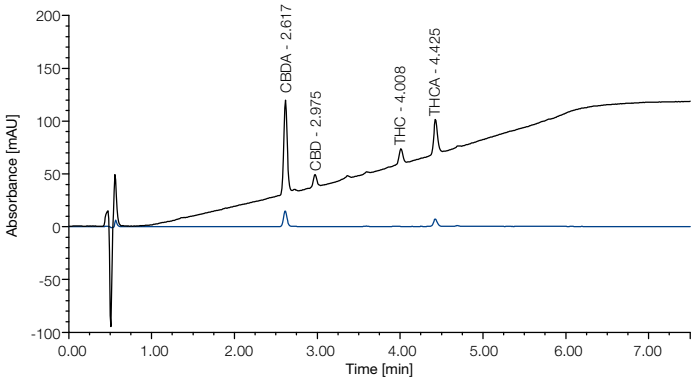

Figure 9: Comparison of chromatograms from an extract of Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol ≈ cannabidiol) on three different batches, UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm, batch 3.

Peak number	Peak name	Ret. time [min]	Area batch 1	Area batch 2	Area batch 3	Peak ratio batch 1	Peak ratio batch 2	Peak ratio batch 3
1	CBDA	2.62	4.463	4.295	4.019	0.582	0.578	0.575
4	CBD	2.98	0.545	0.568	0.528	0.071	0.076	0.076
6	THC	4.01	0.779	0.791	0.717	0.102	0.106	0.103
7	THCA	4.43	1.879	1.780	1.721	0.245	0.239	0.246

Peak number	Peak name	Ret. time [min]	Area batch 1	Area batch 2	Area batch 3	Peak ratio batch 1	Peak ratio batch 2	Peak ratio batch 3
1	CBDA	2.62	0.727	0.706	0.683	0.639	0.645	0.641
2	THCA	4.43	0.412	0.388	0.381	0.361	0.355	0.359

Table 3: Comparison of peak area and peak ratio from an extract of Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol ≈ cannabidiol) on three different batches, UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm, batch 1–3.

Potency testing in cannabis extracts using LC–UV approach

MS/MS identification of cannabinoids

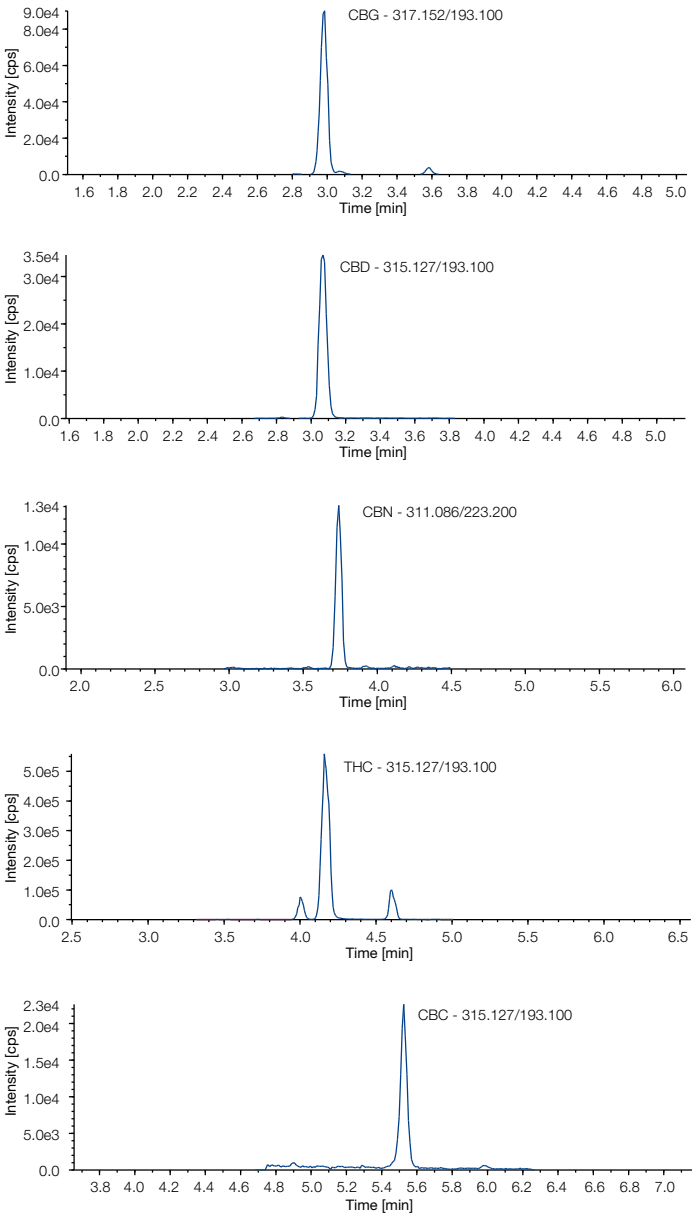

Figure 10: Chromatogram of an extract from Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol ≈ cannabidiol) (positive polarity).

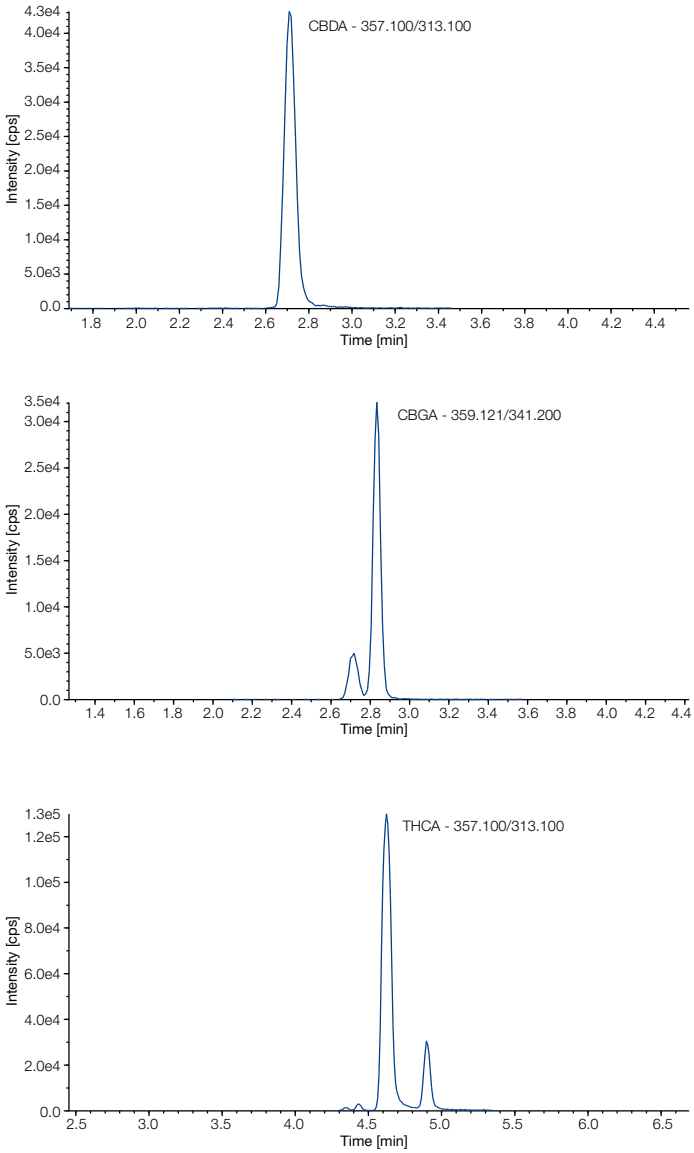

Figure 11: Chromatogram of an extract from Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol ≈ cannabidiol) (negative polarity).

Conclusion

This application note shows a simple chromatographic separation of major cannabinoids from marijuana samples in less than ten minutes. A good batch-to-batch reproducibility of NUCLEOSHELL® RP 18 can be seen in figure 1. In this work, the cannabinoid profile of three different cannabis varieties could be shown and sample materials could be successfully assigned to their described potency class. The figures 6, 8, and 9 show a batch-to-batch reproducibility of three different marijuana samples of the same variety.

The separation was achieved with sufficient resolution for major cannabinoids and is suitable for qualification and quantification. In addition, the identification of cannabinoids by mass spectrometry was successfully performed with presented chromatographic conditions on a NUCLEOSHELL® RP 18 column.

References

[1] R. L. Pacula, R. Smart Annu. Rev. Clin. Psychol. 2017 May 8; 13: 397–419.

[2] DAC/NRF 2016/1, C-053, Cannabisblüten (Cannabis flos).

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 763152.40, EC 50/4 NUCLEOSHELL® RP 18, 2.7 µm

REF 730223, CHROMABOND® centrifuge tubes with screw cap, 50 mL

REF 729231, CHROMAFIL Xtra RC-45/25 syringe filters, labeled, 25 mm, 0.45 µm

REF 702293, Screw neck vials N 9, 1.5 mL

REF 702107, N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red