MACHEREY-NAGEL
Chromatography application note

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Fast and simple High Performance Liquid Chromatography – Ultraviolet assay for the determination of cannabinoids in hemp products with additional MS/MS identification

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Abstract

This application note describes the determination of cannabinoids in hemp products using a LC–UV assay. Hemp products like tea, flour and buds were extracted according to DAC/NRF monograph and analyzed by HPLC–UV on a NUCLEOSHELL® RP 18 column. Hemp oil was diluted with methanol, filtered and analyzed like plant extracts. Identification of contained cannabinoids was additionally performed by HPLC–UV–MS/MS.

Introduction

Hemp products are often used because of their nutraceutical, cosmetic and pharmaceutical properties and are becoming more popular. Hemp seeds and flowers can be consumed or used to produce a variety of food products including hemp milk, hemp oil, hemp cheese substitutes, hemp flower tea, hemp flower flour or other plant powders. Hemp is commonly confused with marijuana. It belongs to the same family, but the two plants are very different. Marijuana is grown to contain high amounts of tetrahydrocannabinol (THC), the chemical that is responsible for its psychoactive properties. Hemp describes the edible plant parts like seeds or flowers and only contains a trace amount of THC.

From the perspective of medical law, hemp extracts and hemp products in most countries may only be sold to consumers in Europe if they were obtained exclusively from hemp and their THC content is ≤ 0.2 % [1]. The limit of THC content leads to a need of fast and simple methods for potency testing by HPLC–UV. The producers have to ensure that the products contain less than 0.2 % THC to comply with European law.

Sample preparation of hemp flower products was carried out according to DAC/NRF regulations [2]. For sample matrices that are complex or heavily processed this work shows a SPE method for cannabinoids that allows an effective enrichment of concentrated compounds.

The cannabinoids are determined by HPLC–UV using NUCLEOSHELL® core-shell particle technology. The well known advantages of these columns are highest column efficiency and resolution at a short run time with much lower back pressure compared to fully porous particles.

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

Sample pretreatment

Sample material

- Hemp flower tea
- Hemp flower flour
- Hemp flower buds
- Hemp oil
- Hemp CO_2 extract

Extraction procedure for tea, flour and buds

- Weigh out 0.5 g of homogenized sample (milled in a grinder) into a 50 mL centrifuge tube (REF 730223)
- Add 20 mL of 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 flasks to 50 mL with ethanol
- Filter 1 mL of extract through a syringe filter with regenerated cellulose (membrane pore size 0.45 µm, REF 729231) into a vial for HPLC analysis

Extraction procedure for oil and CO_2 extract

- Weigh out 0.1 g of homogenized oil into a 25 mL flask
- Add 1 mL isopropyl alcohol and shake for 1 min
- Fill up the flask to 25 mL with methanol
- Filter 1 mL of extract through a syringe filter with regenerated cellulose (membrane pore size 0.45 µm) into a 10 mL flask
- Fill up with methanol to 10 mL
- Use this mixture for HPLC analysis (REF 702293, REF 702107)

Figure 1: Hemp products (tea, flour, oils).

Determination of cannabinoids in hemp products

LC Method parameters

Chromatographic conditions

Column: EC NUCLEOSHELL® RP 18, 50 x 4.0 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: 2 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

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
Δ^9- tetrahydrocannabivarin	THCV	287.1	165.0	77.1
Cannabidivarin	CBDV	287.1	123.2	77.1

Table 1: MRM transitions of cannabinoids from hemp products (positive polarity). (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 hemp products (negative polarity). (Q_1 = Quantifier, Q_2 = Qualifier)

Representative chromatograms for hemp products

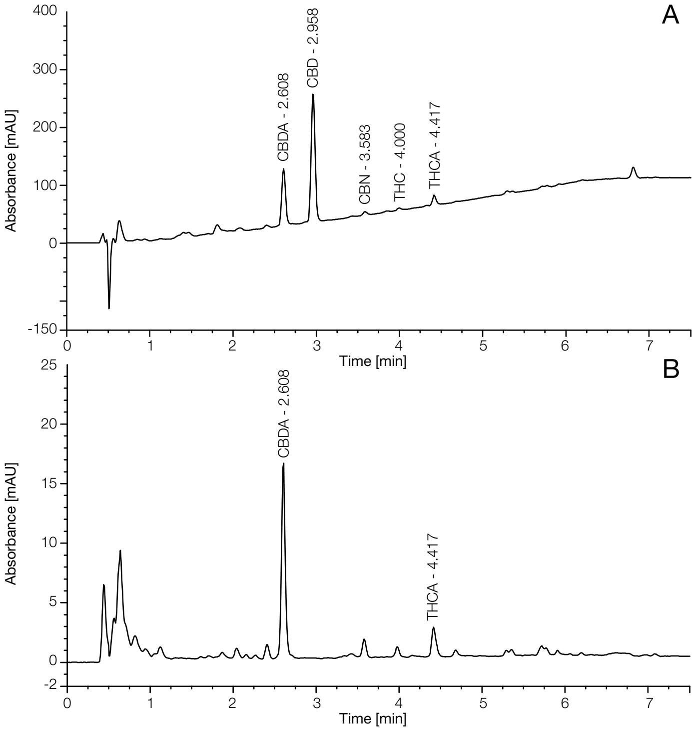

Figure 2: Chromatograms of an extract from hemp flower tea, @ 225 nm (A), 306 nm (B).

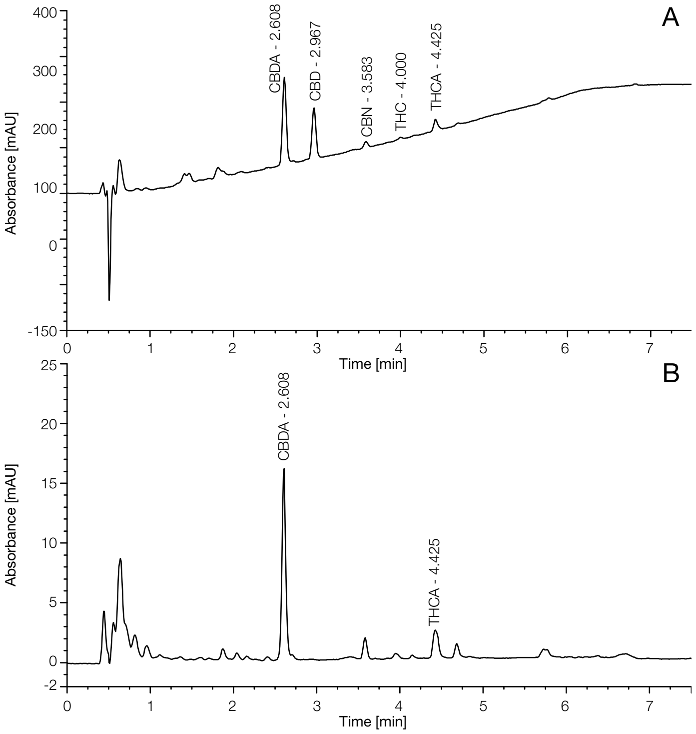

Figure 3: Chromatograms of an extract from hemp flower flour, @ 225 nm (A), 306 nm (B).

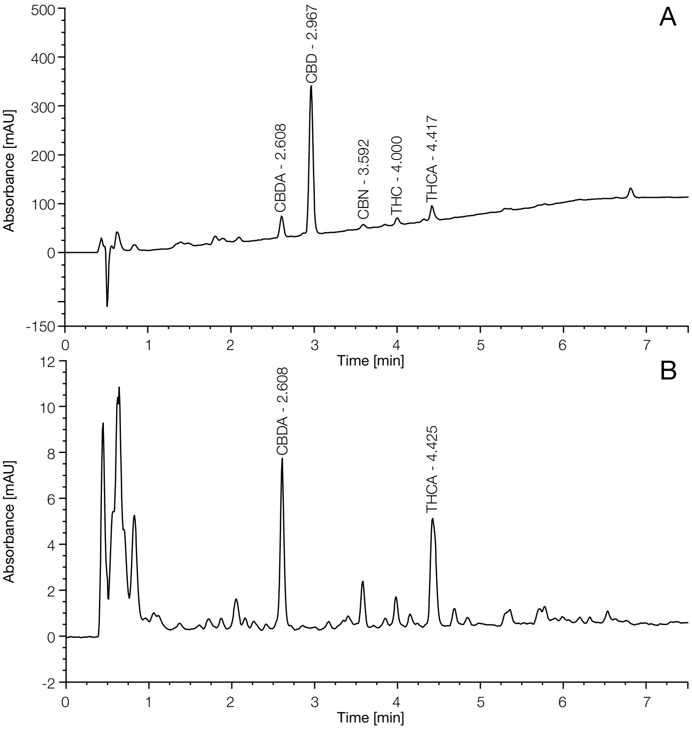

Figure 4: Chromatograms of an extract from hemp flower buds, @ 225 nm (A), 306 nm (B).

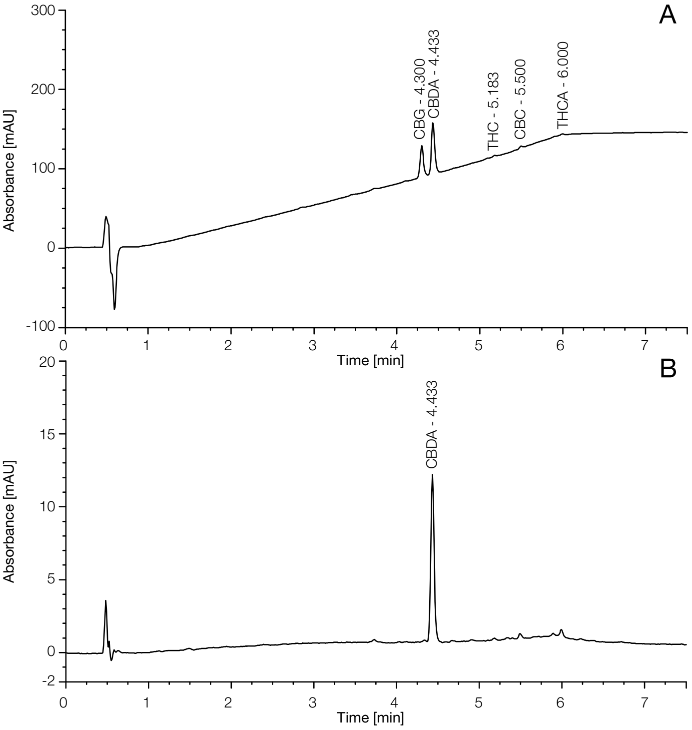

Figure 5: Chromatograms of an extract from hemp oil, @ 225 nm (A), 306 nm (B).

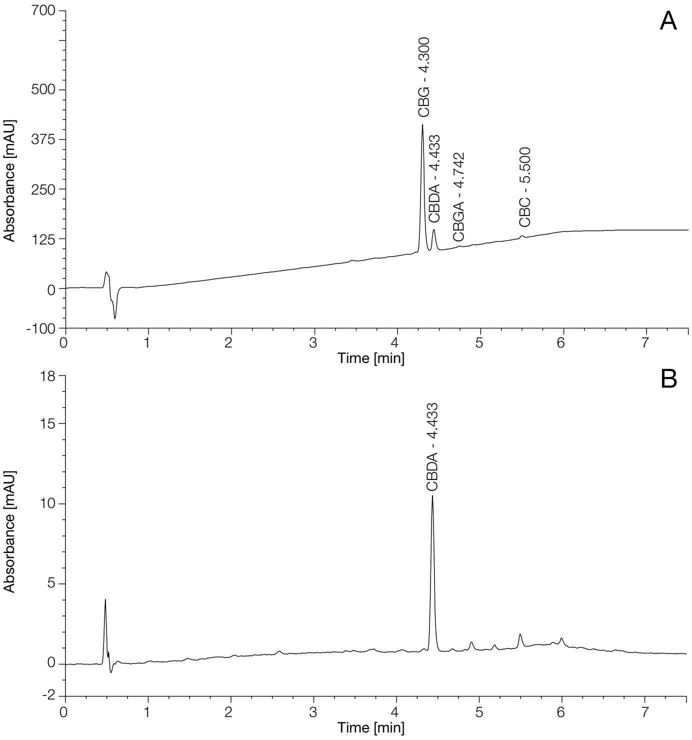

Figure 6: Chromatograms of an extract from hemp CO_2 extract, @ 225 nm (A), 306 nm (B).

Figure 7: Hemp flower buds, hemp flour and hemp tea (from left to right).

MS/MS identification of cannabinoids

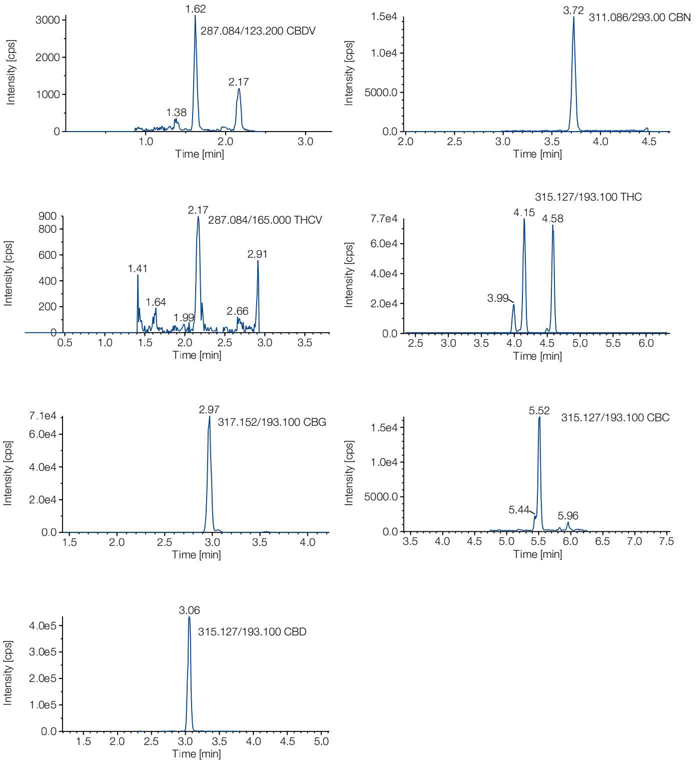

Figure 8: Chromatogram of an extract from hemp flower tea (positive polarity).

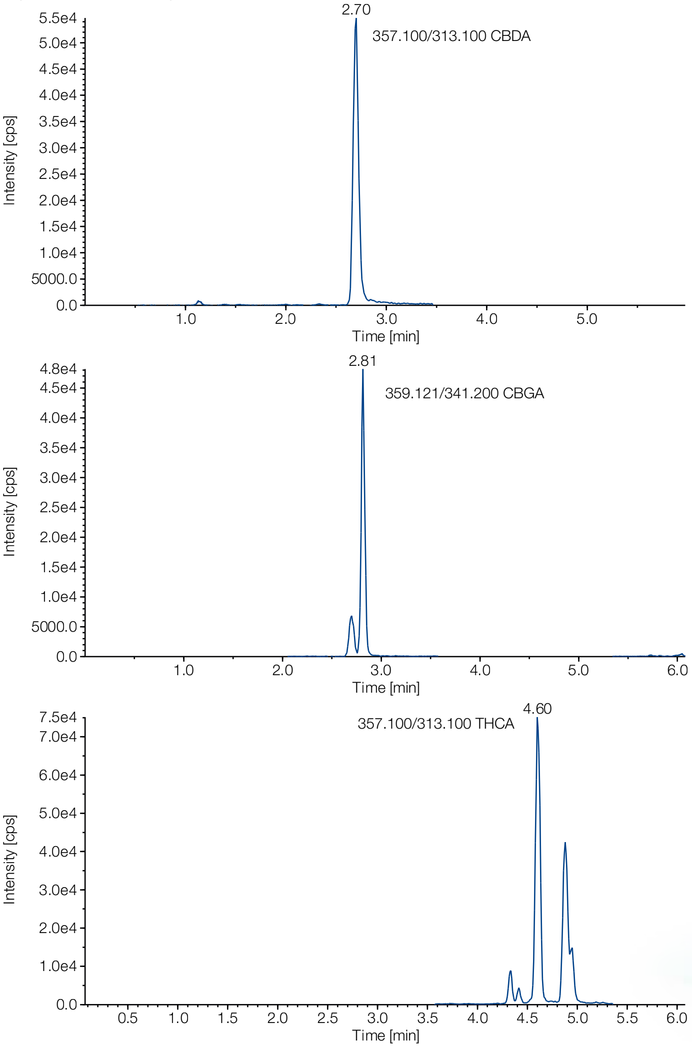

Figure 9: Chromatogram of an extract from hemp flower tea (negative polarity).

Calibration curves of CBD, CBDA, THC

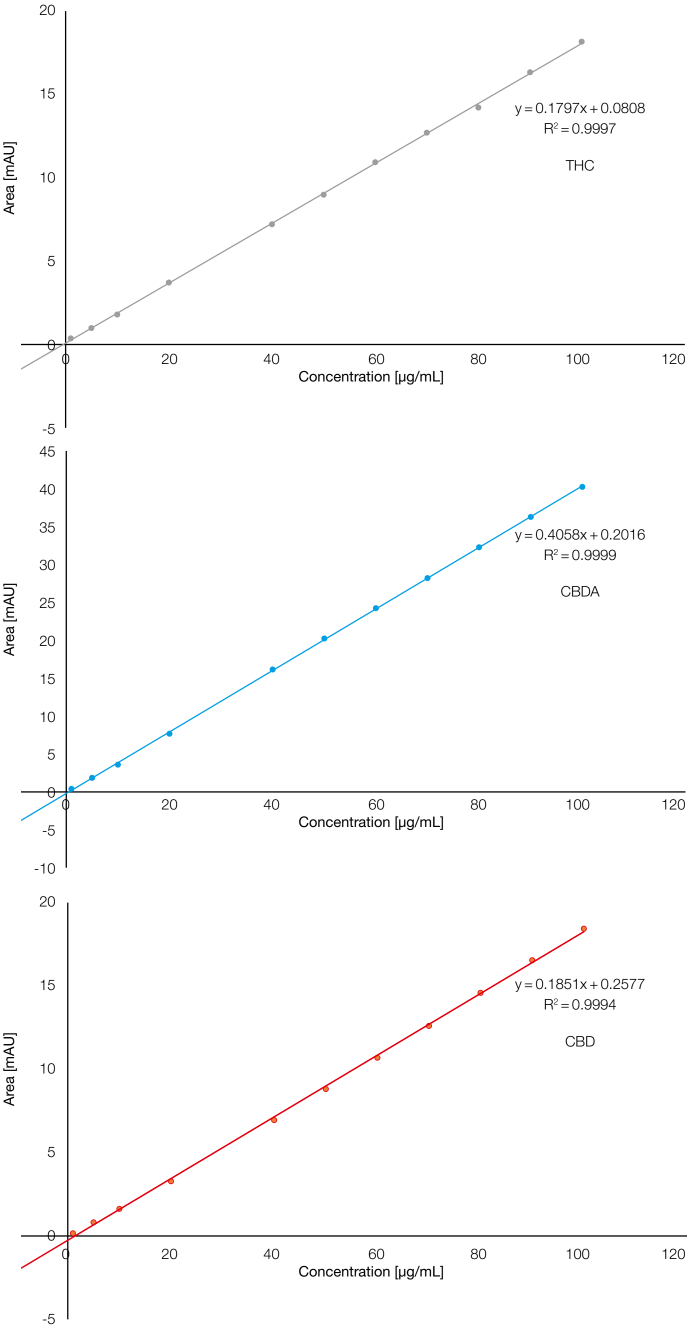

Figure 10: Calibration curves of Cannabidiol, Cannabidiolic acid, Δ^9-tetrahydrocannabinol.

Figure 11: Hemp oil and CO_2 extract.

Solid phase extraction for processed hemp products

Sample extract of hemp products was diluted 1:1 with water.

Column:	CHROMABOND® HR-X, 85 µm, 6 mL/200 mg (REF 730938)
Conditioning:	5 mL methanol, 5 mL 50 % methanol in water
Sample appl.:	20 mL sample mixture with a flow rate of 3 mL/min
Washing:	5 mL of 50 % methanol in water with a flow rate of 3 mL/min
Drying:	2 min with vacuum
Elution:	5 mL methanol
Eluent exchange:	Evaporate eluate to dryness at 40 °C under a stream of nitrogen and fill up to 1.0 mL with methanol.

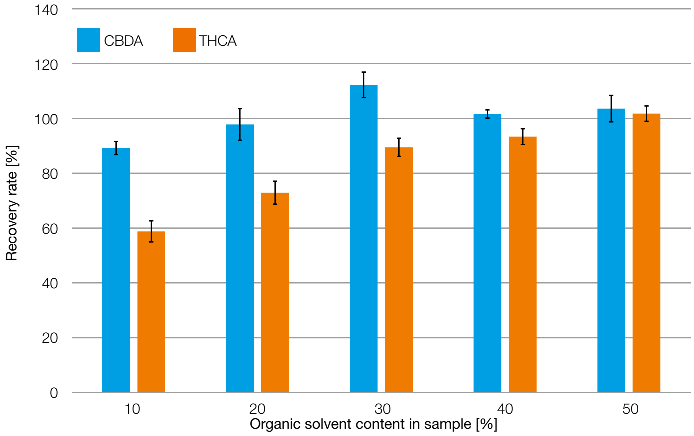

Figure 12: Recovery rates of CBD and THC, detection @ 225 nm.

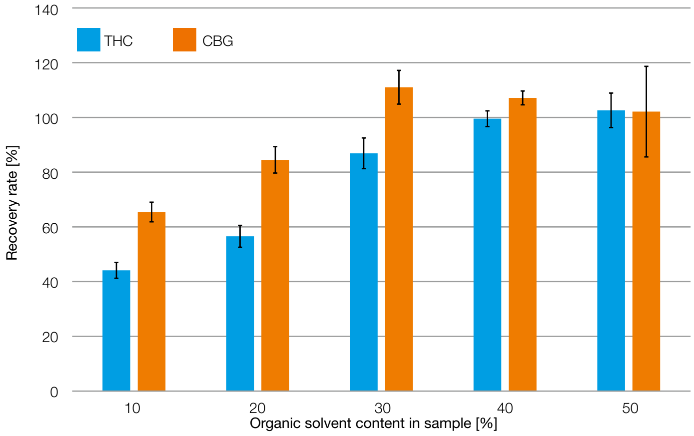

Figure 13: Recovery rates of CBDA and THCA, detection @ 306 nm.

Representative chromatograms for hemp products spiked with Cannabis sativa

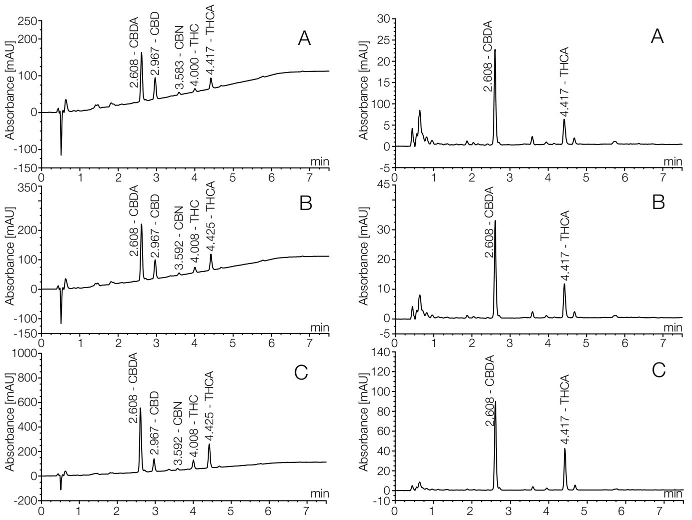

Figure 14: Chromatograms of extracts with a mixture of hemp flower tea and Cannabis sativa, spiking level A 1 %, spiking level B 2.5 %, spiking level C 10 %.

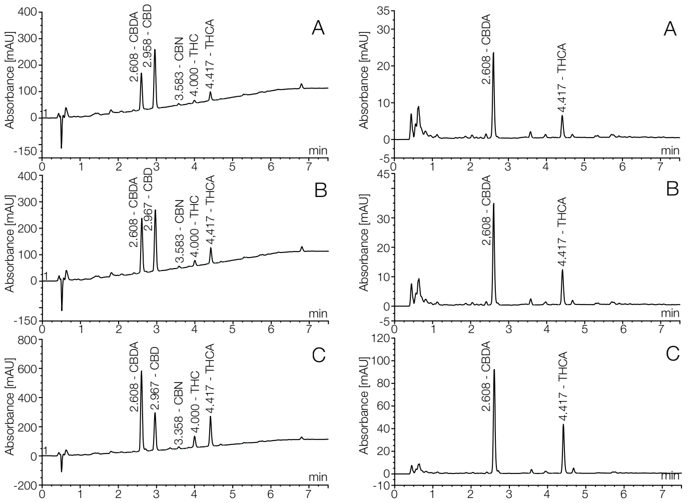

Figure 15: Chromatograms of extracts with a mixture of hemp flower flour and Cannabis sativa, spiking level A 1 %, spiking level B 2.5 %, spiking level C 10 %.

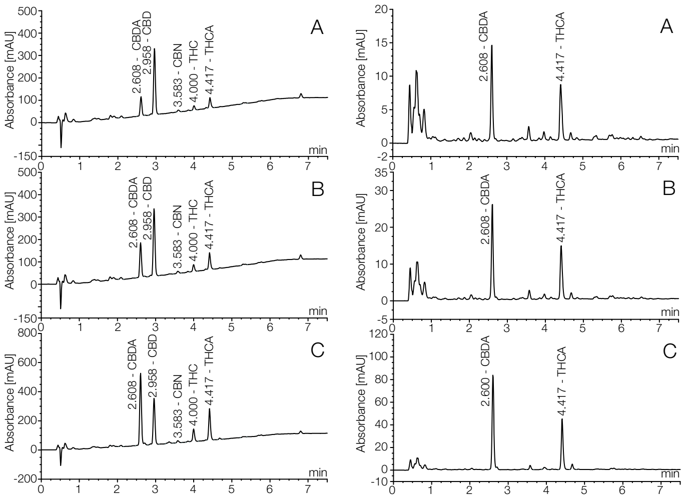

Figure 16: Chromatograms of extracts with a mixture of hemp flower buds and Cannabis sativa, spiking level A 1 %, spiking level B 2.5 %, spiking level C 10 %.

Determination of cannabinoids in hemp products

Conclusion

This application note shows a simple chromatographic separation of the major cannabinoids from several hemp product samples in less than ten minutes, that can be used for identification and quantification of small analyte amounts. This work presents an effective extraction method and achieves the goal to develop a SPE method for the enrichment of cannabinoids from difficult sample matrices with high recovery rates. The figures 10 and 11 elucidate that the organic content of the sample mixture plays an important role to get good results for cannabinoid enrichment. The addition of Cannabis sativa in hemp flower products is shown in the figures 12, 13 and 14.

In addition, the identification of cannabinoids by mass spectrometry was successfully performed with presented chromatographic conditions on a NUCLEOSHELL® RP 18 column.

References

[1] Drug policy of Germany.

[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 100/4 NUCLEOSHELL® RP 18, 2.7 µm

REF 730938, CHROMABOND® HR-X, 85 µm, 6 mL/200 mg

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

REF 729231, Syringe filters, labeled, CHROMAFIL® Xtra RC, 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