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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 $\gg$ cannabidiol
II: Δ^9-tetrahydrocannabinol $\approx$ cannabidiol
III: Δ^9-tetrahydrocannabinol $\ll$ 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.

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

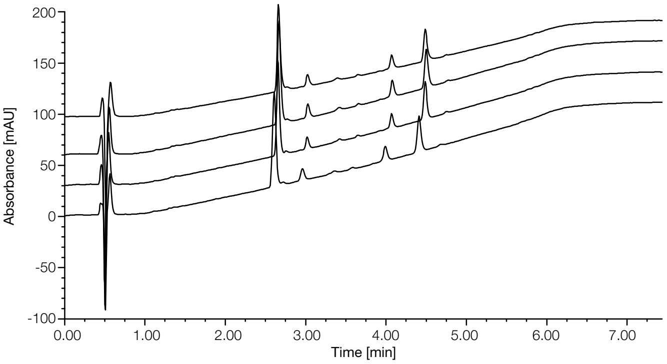

Figure 1: Comparison of chromatograms from an extract of Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol ≈ cannabidiol) on four different batches of NUCLEOSHELL® RP 18, UV-VIS 1 = 225 nm.

Figure 2: Marijuana plant leaves.

Representative chromatograms

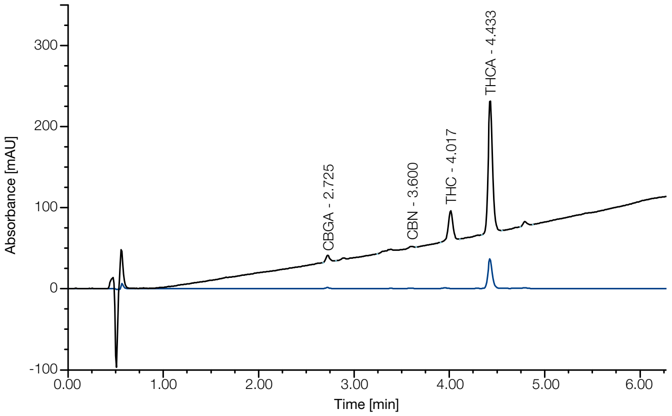

Figure 3: Chromatogram of an extract from Cannabis sativa (variety class I: Δ^9-tetrahydrocannabinol ≫ cannabidiol), UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm.

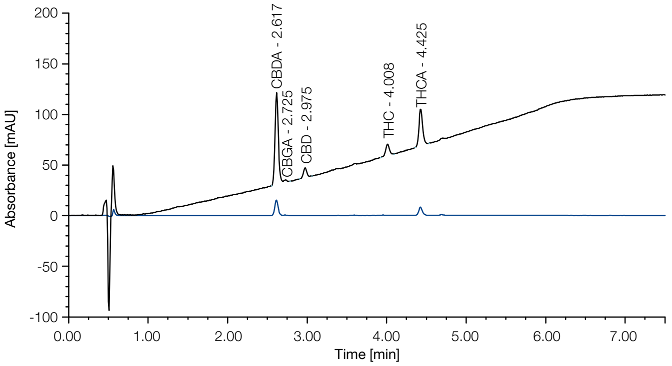

Figure 4: Chromatogram of an extract from Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol ≈ cannabidiol), UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm.

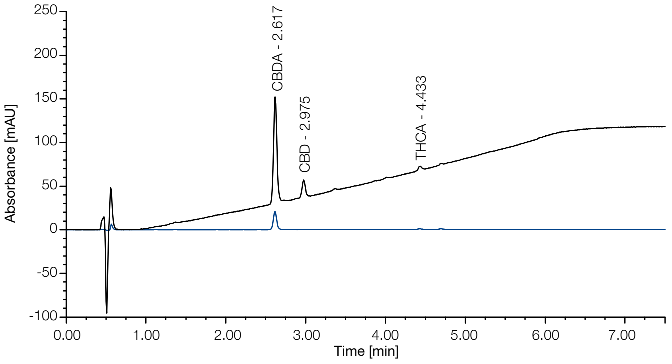

Figure 5: Chromatogram of an extract from Cannabis sativa (variety class III: Δ^9-tetrahydrocannabinol ≪ cannabidiol), UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm.

Potency testing in cannabis extracts using LC–UV approach

Batch-to-batch reproducibility of Cannabis sativa samples

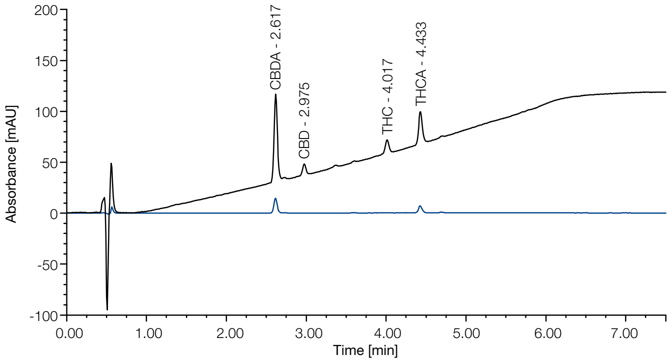

Figure 6: Comparison of chromatograms from an extract of Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol $\approx$ cannabidiol) on three different batches, UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm, batch 1.

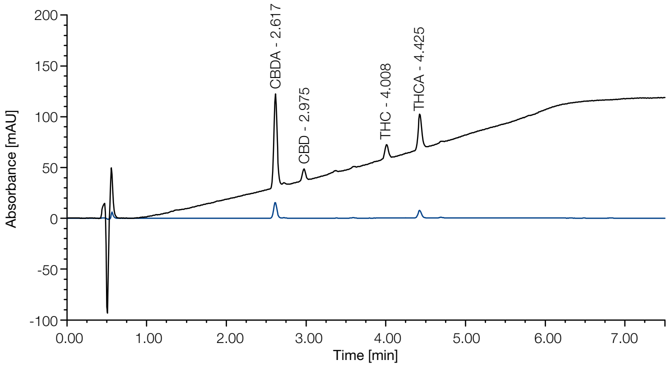

Figure 8: Comparison of chromatograms from an extract of Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol $\approx$ cannabidiol) on three different batches, UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm, batch 2.

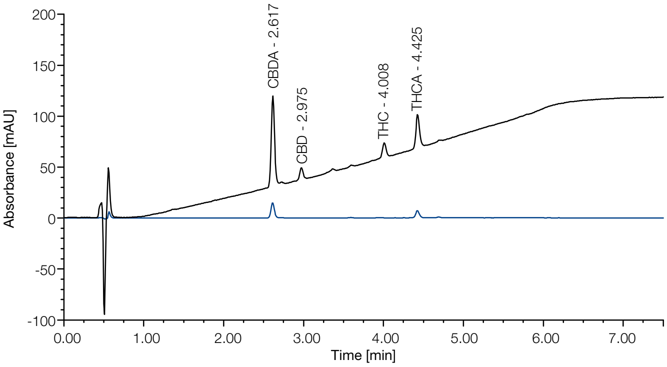

Figure 9: Comparison of chromatograms from an extract of Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol $\approx$ cannabidiol) on three different batches, UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm, batch 3.

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 $\approx$ cannabidiol) on three different batches, UV-VIS 1 = 225 nm, UV-VIS 2 = 306 nm, batch 1–3.

MS/MS identification of cannabinoids

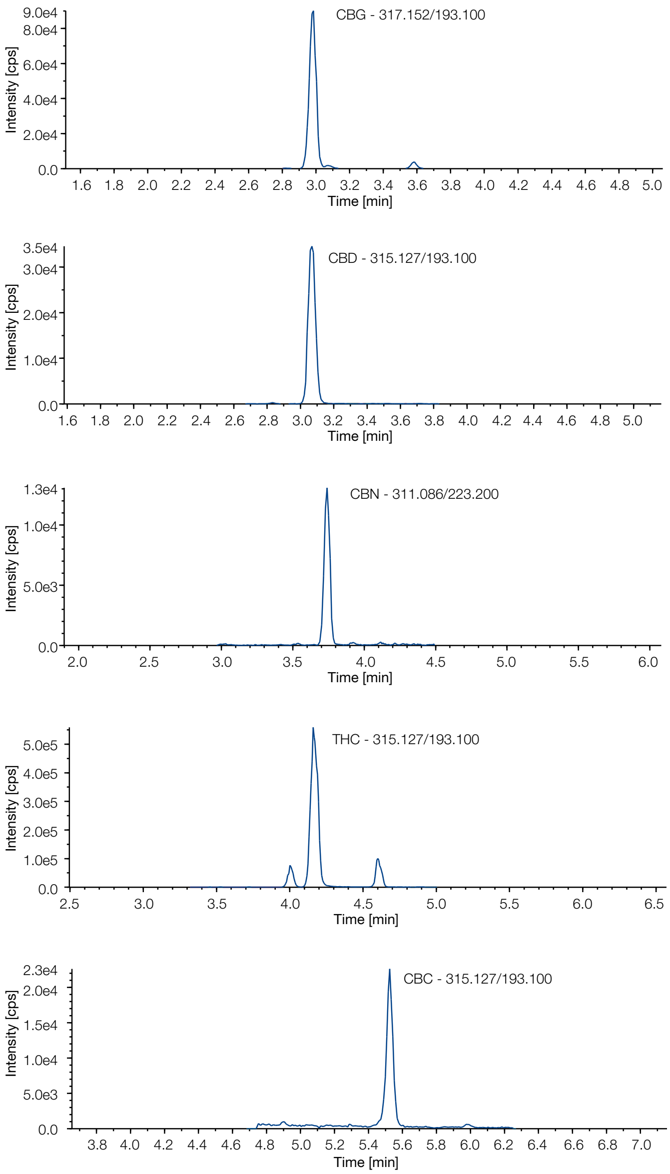

Figure 10: Chromatogram of an extract from Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol ≈ cannabidiol) (positive polarity).

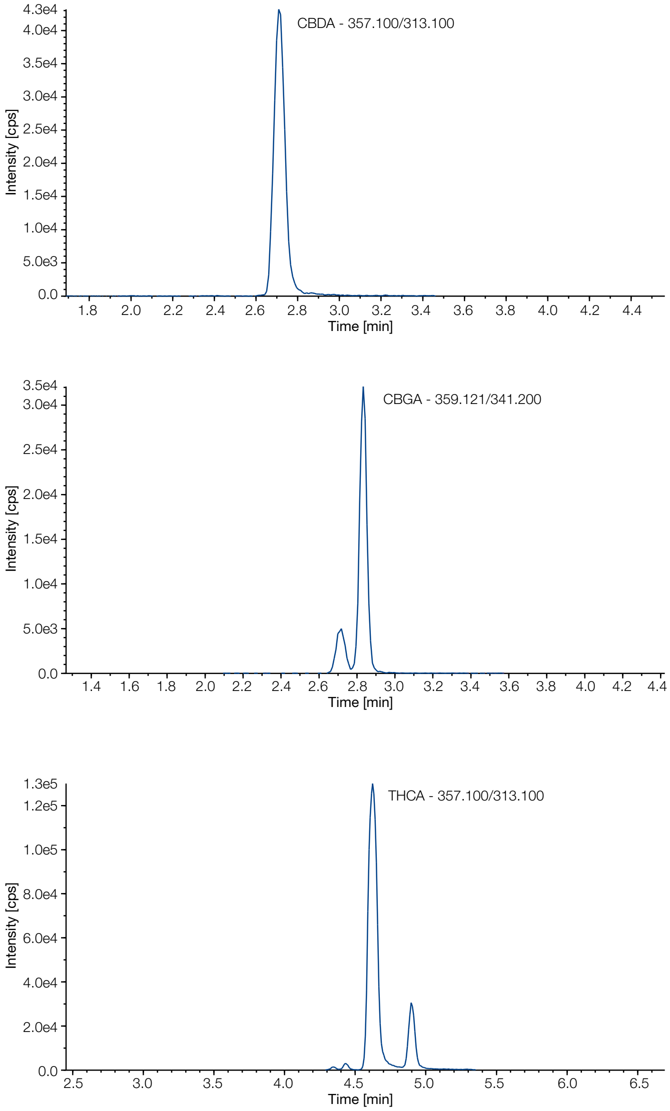

Figure 11: Chromatogram of an extract from Cannabis sativa (variety class II: Δ^9-tetrahydrocannabinol ≈ cannabidiol) (negative polarity).

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).

Determination of pesticide residues in Cannabis sativa using an optimized QuEChERS method with a low amount of carbon in the clean-up mix

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Abstract

This application note describes the determination of pesticide residues in Cannabis sativa using a QuEChERS method for sample clean-up. Interfering substances (like e.g., lipids and pigments), which were also extracted with the organic layer, are removed by optimized amounts of GCB (graphitized carbon black) and CHROMABOND® C_{18} ec adsorbents. The organic extracts are finally analyzed by HPLC-MS/MS.

Introduction

There is an increasing interest in the determination of pesticide residues in marijuana. While medical and recreational use of cannabis are legalized in more and more U.S. territories, the markets for cannabis and cannabis-based products (e.g. concentrated oils, soda, candy and other drinkable or edible forms) have grown year by year in North America and also in other countries [1]. To saturate the huge demand for marijuana the cultivation of hemp gets professional to improve growth yields. The use of pesticides is a common tool for this monocultural plant production.

New quality control methods have to be developed to ensure pro-duct safety and to reduce health risks by chronic exposure to pesticides. These methods have to be quick, easy, cheap, effective, rugged and safe like the QuEChERS extraction approach [2]. Interfering substances (like e.g., lipids and chlorophyll), which were also extracted with the organic layer, are removed by optimized clean-up mixes. On the other side, the composition of the clean-up mix has to ensure high recovery rates for pesticides, as well as adequate matrix removal properties. Therefor the respective mix needs to contain a sufficient amount of carbon adsorbent.

This work presents a QuEChERS method for the simultaneous analysis of more than 160 pesticides from Cannabis sativa. The organic extracts are finally analyzed by HPLC-MS/MS.

Figure 1: Marijuana (Cannabis sativa).

Dispersive solid phase extraction (dSPE)

Products for clean-up from competitors

Competitor 1:

QuEChERS dispersive kit, 15 mL centrifuge tube, 1200 mg $MgSO_4$, 400 mg PSA, 400 mg C_{18} ec, 45 mg GCB

Competitor 2:

QuEChERS SPE Q-sep, 15 mL centrifuge tube, 900 mg $MgSO_4$, 300 mg PSA, 300 mg C_{18}, 45 mg GCB

Extraction

- Weigh out 1 g of homogenized sample (milled in a grinder) into a 50 mL centrifuge tube (REF 730223)
- Add 100 µL of standard solution ($\beta = 1$ µg/mL for each analyte in acetonitrile) for determining recovery rates
- Add 10 mL water and shake
- Add 10 mL 1 % acetic acid in acetonitrile and shake for 30 min
- Add the CHROMABOND® QuEChERS extraction mix I (REF 730970)
- Shake vigorously for 2 min and cool down the mixture in an ice bath
- Centrifuge the mixture at 4500 rpm, for 5 min at 4 °C

Clean-up

- Add 6 mL of acetonitrile supernatant to the CHROMABOND® QuEChERS clean-up mix XLIX (REF 7300000)
- Shake vigorously for 60 s
- Centrifuge the mixture at 4500 rpm, for 5 min at 4 °C
- Take acetonitrile extract for injection

Subsequent analysis: HPLC-MS / MS

Chromatographic conditions

Column: EC NUCLEOSHELL® Bluebird RP 18, 50 x 4.6 mm, 2.7 µm (REF 763432.46)

Eluent A: 0.1 % formic acid in water

Eluent B: 0.1 % formic acid in acetonitrile

Gradient: in 5 min from 5 % to 100 % B, hold for 1.0 min, in 0.1 min to 5 % B, hold 5 % B for 3.9 min

Flow rate: 0.7 mL/min

Temperature: 30 °C

Injection volume: 2 µL

Pesticide residues in cannabis

MS conditions

AB Sciex QTRAP 5500

Acquisition mode: SRM
Interface: ESI
Polarity: positive
Curtain gas: 35 psig
Collision gas: medium
Ion spray voltage: 5000 V
Temperature: 450 °C
Ion source gas 1: 45 psig
Ion source gas 2: 45 psig
Detection window: 60 s

MRM transitions

Analyte	RT [min]	Q_1 Mass [Da]	Q_3 Mass [Da]
Cyromazine.1	0.88	167.1	85.1
Cyromazine.2	0.88	167.1	125.1
Propamocarb.1	1.45	189.2	102.1
Propamocarb.2	1.46	189.2	74.0
Aminocarb.1	1.49	209.2	137.2
Aminocarb.2	1.49	209.2	152.2
Formetanate HCl.1	1.52	222.3	165.0
Formetanate HCl.2	1.52	222.3	120.1
Pymetrozine.1	1.56	218.1	105.0
Pymetrozine.2	1.56	218.1	78.0
Acephate.1	1.61	184.1	143.0
Acephate.2	1.62	184.1	49.0
Omethoate.1	1.83	214.1	183.0
Omethoate.2	1.84	214.1	125.1
Nitenpyram.1	1.88	271.1	224.9
Nitenpyram.2	1.88	271.1	126.1
Aldicarb sulfoxide.1	1.98	207.1	132.1
Aldicarb sulfoxide.2	1.98	207.1	89.1
Carbendazim.1	1.98	192.1	160.1
Carbendazim.2	1.98	192.1	132.1
Dinotefuran.1	2.03	203.0	129.0
Dinotefuran.2	2.03	203.0	157.0
Mexacarbate.1	2.08	223.1	166.1
Mexacarbate.2	2.08	223.1	151.1
Aldicarb sulfone.1	2.10	240.0	86.0
Aldicarb sulfone.2	2.10	240.0	148.0
Oxamyl.1	2.22	237.2	90.0
Oxamyl.2	2.22	237.2	72.1
Flonicamid.1	2.36	230.2	203.1
Flonicamid.2	2.36	230.2	174.0
Methomyl.1	2.39	163.1	106.0
Methomyl.2	2.39	163.1	88.1
Thiamethoxam.1	2.52	292.2	181.2
Thiamethoxam.2	2.52	292.2	211.0
Monocrotophos.1	2.56	224.1	127.1
Monocrotophos.2	2.56	224.1	98.1
Ethirimol.1	2.68	210.2	140.2
Ethirimol.2	2.68	210.2	98.1
Dicrotophos.1	2.70	238.1	112.1
Dicrotophos.2	2.70	238.1	193.0
Pirimicarb.1	2.75	239.2	72.1
Pirimicarb.2	2.75	239.2	182.0
Trichlorfon.1	2.89	257.0	109.0
Imidacloprid.1	2.90	256.2	175.0
Imidacloprid.2	2.90	256.2	209.0
Clothianidin.1	2.91	250.2	169.1
Clothianidin.2	2.91	250.2	132.1
Fenuron.1	2.95	165.1	46.0
Fenuron.2	2.95	165.1	72.0
Vamidothion.1	2.98	288.1	146.1
Vamidothion.2	2.98	288.1	118.1
3-Hydroxycarbofuran.1	3.00	238.2	181.0
3-Hydroxycarbofuran.2	3.00	238.2	163.1
Dimethoate.1	3.05	230.1	198.8
Dimethoate.2	3.05	230.1	125.0
Acetamiprid.1	3.14	223.1	126.1
Acetamiprid.2	3.14	223.1	99.0
Cymoxanil.1	3.17	199.2	128.1
Cymoxanil.2	3.17	199.2	111.0
Simetryn.1	3.27	214.2	124.2
Simetryn.2	3.27	214.2	144.1
Prometon.1	3.31	226.2	142.1
Prometon.2	3.31	226.2	86.0
Terbumeton.1	3.32	226.2	170.0
Terbumeton.2	3.32	226.2	100.0
Secbumeton.1	3.33	226.2	170.0
Secbumeton.2	3.33	226.2	100.0
Mevinphos isomer 2.1	3.34	225.1	127.0
Mevinphos isomer 2.2	3.34	225.1	192.9
Mevinphos isomer 1.1	3.35	225.1	127.0
Mevinphos isomer 1.2	3.35	225.1	192.9
Butocarboxim.1	3.40	213.0	75.0
Imazalil.1	3.40	297.1	201.0
Imazalil.2	3.40	297.1	159.0
Thiacloprid.1	3.40	253.1	126.0
Thiacloprid.2	3.40	253.1	99.1
Butocarboxim.1	3.43	213.0	75.0
Butocarboxim.2	3.43	213.0	116.0
Aldicarb.1	3.44	208.1	116.0
Aldicarb.2	3.44	208.1	89.0
Tricyclazole.1	3.52	190.1	163.0
Tricyclazole.2	3.52	190.1	136.0
Mesotrione.1	3.56	340.1	228.0
Mesotrione.2	3.56	340.1	104.1
Oxadixyl.1	3.58	279.1	219.1

Analyte	RT [min]	Q_1 Mass [Da]	Q_3 Mass [Da]
Oxadixyl.2	3.58	279.1	132.1
Carbetamide.1	3.62	237.1	192.1
Carbetamide.2	3.62	237.1	118.2
Ametryn.1	3.66	228.1	186.0
Ametryn.2	3.66	228.1	96.0
Fenpropimorph.1	3.70	304.3	147.0
Fenpropimorph.2	3.70	304.3	117.2
Methoprotryne.1	3.76	272.2	198.0
Methoprotryne.2	3.76	272.2	240.0
Propoxur.1	3.76	210.1	168.1
Propoxur.2	3.76	210.1	111.0
Thiophanate-methyl.1	3.77	343.2	151.0
Thiophanate-methyl.2	3.77	343.2	311.1
Metribuzin.1	3.78	215.2	187.0
Metribuzin.2	3.78	215.2	84.1
Spiroxamine isomer 1.1	3.79	298.2	144.2
Spiroxamine isomer 1.2	3.79	298.2	100.1
Spiroxamine isomer 2.1	3.79	298.2	144.2
Spiroxamine isomer 2.2	3.79	298.2	100.1
Carbofuran.1	3.81	222.0	165.0
Carbofuran.2	3.81	222.0	123.0
Bendiocarb.1	3.83	224.1	167.1
Bendiocarb.2	3.83	224.1	109.0
Thidiazuron.1	3.84	221.1	102.1
Thidiazuron.2	3.84	221.1	127.9
Sulfentrazone.1	3.85	387.0	307.0
Sulfentrazone.2	3.85	387.0	146.0
Tebuthiuron.1	3.87	229.2	172.1
Tebuthiuron.2	3.87	229.2	116.0
Carbaryl.1	3.98	202.1	145.2
Carbaryl.2	3.98	202.1	127.1
Prometryne.1	3.99	242.2	158.1
Prometryne.2	3.99	242.2	200.1
Carboxin.1	4.00	236.1	143.1
Carboxin.2	4.00	236.1	87.1
Terbutryn.1	4.02	242.2	186.0
Terbutryn.2	4.00	242.2	68.1
Pyrimethanil.1	4.02	200.2	107.0
Pyrimethanil.2	4.02	200.2	82.2
Ethiofencarb.1	4.04	226.2	107.1
Ethiofencarb.2	4.03	226.2	164.0
Monolinuron.1	4.05	215.1	126.1
Monolinuron.2	4.05	215.1	99.0
Fluometuron.1	4.08	233.0	72.0
Flutriafol.1	4.14	302.2	70.1
Flutriafol.2	4.15	302.2	123.0
Chlorotoluron.1	4.15	213.1	72.2
Chlorotoluron.2	4.15	213.1	46.0
Propham.1	4.15	180.1	138.1
Propham.2	4.16	180.1	120.1
Isoprocarb.1	4.16	194.1	95.1
Isoprocarb.2	4.16	194.1	137.0
Metobromuron.1	4.17	259.1	169.9
Metobromuron.2	4.18	259.1	148.2
Metalaxyl.1	4.21	280.2	220.3
Metalaxyl.2	4.21	280.2	192.0
Isoproturon.1	4.23	207.1	72.1
Isoproturon.2	4.23	207.1	46.1
Methabenzthiazuron.1	4.25	222.1	165.0
Methabenzthiazuron.2	4.25	222.1	150.0
Butoxycarboxim.1	4.26	223.1	106.0
Butoxycarboxim.2	4.26	223.1	166.0
Cycluron.1	4.29	199.2	89.0
Cycluron.2	4.29	199.2	89.1
Forchlorfenuron.1	4.29	248.1	129.0
Forchlorfenuron.2	4.29	248.1	93.0
Desmedipham.1	4.33	318.2	154.0
Desmedipham.2	4.33	318.2	182.0
Diuron.1	4.33	233.1	72.2
Diuron.2	4.33	235.2	72.1
Chlorantraniliprole.1	4.36	484.0	453.0
Chlorantraniliprole.2	4.36	484.0	285.8
Spinosad (Spinosyn A).1	4.36	732.5	142.2
Spinosad (Spinosyn A).2	4.36	732.5	98.1
Bupirimate.1	4.37	317.2	166.1
Bupirimate.2	4.37	317.2	108.0
Phenmedipham.1	4.37	301.2	168.0
Phenmedipham.2	4.37	301.2	107.9
Fenobucarb.1	4.44	208.0	95.0
Diethofencarb.1	4.45	268.1	226.1
Diethofencarb.2	4.45	268.1	124.0
Ethiprole.1	4.45	397.2	350.9
Ethiprole.2	4.45	397.2	255.0
Furalaxyl.1	4.47	302.2	242.0
Furalaxyl.2	4.47	302.2	95.1
Spinosad (Spinosyn D).1	4.48	746.4	142.3
Spinosad (Spinosyn D).2	4.47	746.4	98.0
Hydramethylnon.1	4.48	495.2	323.2
Hydramethylnon.2	4.48	495.2	151.1
Spinetoram.1	4.48	748.4	142.3
Spinetoram.2	4.48	748.4	98.0
Cyprodinil.1	4.49	226.2	93.0
Cyprodinil.2	4.49	226.2	76.9
Siduron.1	4.52	233.1	137.1
Siduron.2	4.51	233.1	94.1
Fenamidone.1	4.52	312.1	92.0
Fenamidone.2	4.52	312.1	236.1
Azoxystrobin.1	4.53	404.1	372.1
Azoxystrobin.2	4.53	404.1	344.0
Linuron.1	4.53	249.1	160.0

Analyte	RT [min]	Q_1 Mass [Da]	Q_3 Mass [Da]
Linuron.2	4.53	249.1	182.1
Methiocarb.1	4.54	226.2	169.0
Methiocarb.2	4.54	226.2	121.2
Dimethomorph isomer 1.1	4.55	388.2	300.9
Dimethomorph isomer 1.2	4.55	388.2	164.9
Paclobutrazol.1	4.55	294.3	70.0
Paclobutrazol.2	4.55	294.3	125.0
Boscalid.1	4.56	343.0	307.0
Boscalid.2	4.56	343.0	140.0
Carfentrazone-ethyl.1	4.56	412.0	328.0
Carfentrazone-ethyl.2	4.56	412.0	356.0
Promecarb.1	4.57	208.1	151.0
Promecarb.2	4.57	208.1	109.2
Flutolanil.1	4.58	324.1	262.0
Flutolanil.2	4.58	324.1	241.9
Triadimenol.1	4.59	296.2	70.1
Triadimenol.2	4.59	296.2	227.1
Dimethomorph isomer 2.1	4.61	388.2	300.9
Dimethomorph isomer 2.2	4.61	388.2	164.9
Myclobutanil.1	4.61	289.1	70.0
Myclobutanil.2	4.61	289.1	125.0
Mepronil.1	4.62	270.2	119.1
Mepronil.2	4.62	270.2	227.9
Triadimefon.1	4.62	294.0	197.1
Triadimefon.2	4.62	294.0	225.0
Methoxyfenozide.1	4.65	369.2	149.1
Methoxyfenozide.2	4.65	369.2	313.2
Cyproconazole isomer 1.1	4.66	292.2	70.1
Cyproconazole isomer 1.2	4.66	292.2	125.1
Emamectin-benzoate B$_1$a.1	4.66	886.5	158.2
Emamectin-benzoate B$_1$a.2	4.66	886.5	82.2
Fenhexamid.1	4.68	302.0	97.0
Fenhexamid.2	4.68	302.0	55.0
Triticonazole.1	4.69	318.2	70.0
Triticonazole.2	4.68	318.2	125.0
Bifenazate.1	4.69	301.2	198.0
Bifenazate.2	4.69	301.2	170.0
Butafenacil.1	4.69	492.2	330.9
Butafenacil.2	4.69	492.2	348.9
Iprovalicarb isomer 1.1	4.70	321.2	119.2
Iprovalicarb isomer 1.2	4.70	321.2	203.1
Iprovalicarb isomer 2.1	4.70	321.2	119.2
Iprovalicarb isomer 2.2	4.70	321.2	203.1
Tetraconazole.1	4.70	372.2	158.9
Tetraconazole.2	4.70	372.2	70.1
Spirotetramat.1	4.71	374.2	330.2
Spirotetramat.2	4.71	374.2	302.2
Acibenzolar-S-methyl.1	4.72	211.1	136.1
Acibenzolar-S-methyl.2	4.72	211.1	91.0
Chloroxuron.1	4.72	291.1	72.1

Analyte	RT [min]	Q_1 Mass [Da]	Q_3 Mass [Da]
Chloroxuron.2	4.72	291.1	218.1
Flufenacet.1	4.72	364.2	152.2
Flufenacet.2	4.72	364.2	193.9
Mefenacet.1	4.72	299.2	148.0
Mefenacet.2	4.72	299.2	120.2
Fluquinconazole.1	4.73	376.0	349.0
Fluquinconazole.2	4.73	376.0	307.0
Prochloraz.1	4.75	376.1	307.9
Prochloraz.2	4.75	376.1	70.1
Fluoxastrobin.1	4.76	459.1	427.0
Fluoxastrobin.2	4.76	459.1	188.1
Fenarimol.1	4.77	331.0	268.0
Fenarimol.2	4.77	331.0	81.0
Fipronil.1	4.78	437.2	368.0
Fipronil.2	4.78	437.2	290.0
Etaconazole isomer 1.1	4.79	328.0	159.0
Etaconazole isomer 1.2	4.79	328.1	205.0
Fenbuconazole.1	4.80	337.1	125.1
Fenbuconazole.2	4.80	337.1	70.0
Cyazofamid.1	4.81	325.2	108.0
Cyazofamid.2	4.81	325.2	261.2
Epoxiconazole.1	4.81	330.1	121.1
Epoxiconazole.2	4.81	330.1	101.2
Mepanipyrim.1	4.81	224.1	77.0
Mepanipyrim.2	4.81	224.1	106.1
Flusilazole.1	4.83	316.2	247.0
Flusilazole.2	4.83	316.2	165.0
Tebufenozide.1	4.83	353.2	133.0
Tebufenozide.2	4.84	353.2	297.2
Flubendiamide.1	4.84	683.1	408.0
Flubendiamide.2	4.84	683.1	274.1
Diflubenzuron.1	4.85	311.2	158.2
Diflubenzuron.2	4.85	311.2	141.1
Diclobutrazol.1	4.87	328.0	70.0
Diclobutrazol.2	4.87	328.2	59.1
Fenoxycarb.1	4.87	302.3	88.0
Fenoxycarb.2	4.87	302.3	116.1
Mandipropamid.1	4.89	412.0	346.0
Mandipropamid.2	4.89	412.0	366.0
Neburon.1	4.89	275.1	88.0
Neburon.2	4.89	275.1	114.0
Tebuconazole.1	4.89	308.2	70.1
Tebuconazole.2	4.89	308.2	125.0
Picoxystrobin.1	4.90	368.2	145.1
Picoxystrobin.2	4.90	368.2	204.9
Bromucanozole isomer 1.1	4.91	378.1	70.0
Bromucanozole isomer 1.2	4.91	378.1	159.0
Dimoxystrobin.1	4.91	327.1	205.0
Dimoxystrobin.2	4.91	327.1	116.0
Penconazole.1	4.92	284.2	70.0

Analyte	RT [min]	Q_1 Mass [Da]	Q_3 Mass [Da]
Penconazole.2	4.92	284.2	158.9
Kresoxim-methyl.1	4.96	314.2	116.1
Kresoxim-methyl.2	4.96	314.2	131.1
Famoxadone.1	4.97	392.0	331.0
Famoxadone.2	4.97	392.0	238.0
Hexaconazole.1	4.97	331.0	105.0
Hexaconazole.2	4.97	314.2	159.0
Propiconazole isomer 1.1	4.97	342.1	159.0
Propiconazole isomer 1.2	4.97	342.1	69.0
Zoxamide.1	4.97	336.1	186.9
Zoxamide.2	4.97	336.1	158.9
Metconazole.1	4.99	320.2	70.1
Metconazole.2	4.99	320.2	125.1
Triflumuron.1	4.99	359.2	156.2
Triflumuron.2	4.99	359.2	139.1
Amitraz.1	5.00	294.2	148.3
Amitraz.2	5.00	294.2	91.2
Benalaxyl.1	5.00	326.3	148.2
Benalaxyl.2	5.00	326.3	294.2
Bitertanol.1	5.02	338.0	70.0
Bitertanol.2	5.02	338.0	99.0
Pyraclostrobin.1	5.05	388.2	194.0
Pyraclostrobin.2	5.05	388.2	163.0
Diniconazole.1	5.10	326.3	159.0
Diniconazole.2	5.10	326.3	70.0
Indoxacarb.1	5.10	528.3	202.9
Thiobencarb.1	5.10	258.3	125.0
Thiobencarb.2	5.10	258.3	89.1
Indoxacarb.2	5.11	528.3	218.0
Difenoconazole isomer 1.1	5.13	408.2	253.1
Difenoconazole isomer 1.2	5.13	406.0	251.0
Benzoximate.1	5.14	364.2	198.9
Benzoximate.2	5.14	364.2	105.0
Ipconazole isomer 2.1	5.14	334.5	70.0
Ipconazole isomer 2.2	5.14	334.5	125.1
Trifloxystrobin.1	5.15	409.0	186.0
Trifloxystrobin.2	5.15	409.0	145.0
Buprofezin.1	5.16	306.2	201.0
Buprofezin.2	5.16	306.2	116.1
Clofentezine.1	5.17	303.2	138.1
Clofentezine.2	5.17	303.2	102.1
Clethodim isomer 1.1	5.19	360.3	164.0
Clethodim isomer 1.2	5.19	360.3	268.1
Metaflumizone.1	5.22	507.3	177.9
Metaflumizone.2	5.22	507.3	287.1
Tebufenpyrad.1	5.26	334.2	145.0
Tebufenpyrad.2	5.26	334.2	117.1
Furathiocarb.1	5.27	383.2	195.1
Furathiocarb.2	5.27	383.2	252.0
Piperonyl butoxide.1	5.34	356.2	177.1
Piperonyl butoxide.2	5.34	356.2	119.2
Pyriproxyfen.1	5.39	322.1	96.0
Pyriproxyfen.2	5.39	322.1	184.9
Hexythiazox.1	5.40	353.2	228.0
Hexythiazox.2	5.40	353.2	168.1
Quinoxyfen.1	5.40	308.1	196.9
Quinoxyfen.2	5.40	308.1	161.9
Etoxazole.1	5.45	360.3	141.1
Etoxazole.2	5.45	360.3	57.0
Chlorfluazuron.1	5.48	540.0	383.0
Chlorfluazuron.2	5.48	540.0	158.0
Fenpyroximate.1	5.57	422.2	366.2
Fenpyroximate.2	5.57	422.2	134.9

Table 1: MRM transitions and retention times of pesticides. (.1 = first transition, .2 = second transition; Q_1 = Qualifier 1, Q_3 = Qualifier 3)

Pesticide residues in cannabis

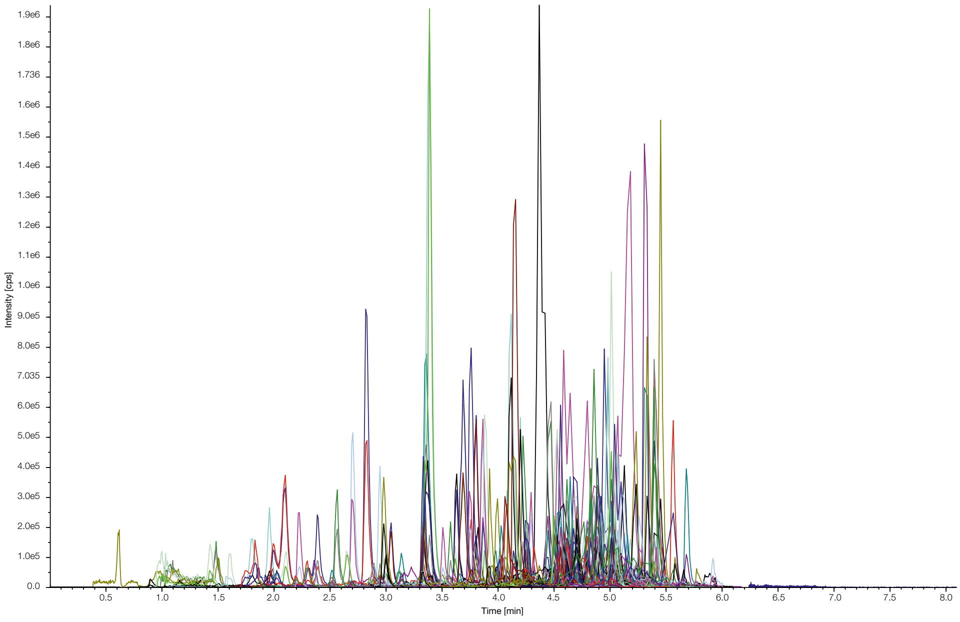

Figure 2: Separation of pesticides on NUCLEOSHELL® Bluebird RP 18 column (QuEChERS extract of Cannabis sativa spiked with β = 100 µg/g).

Matrix reduction

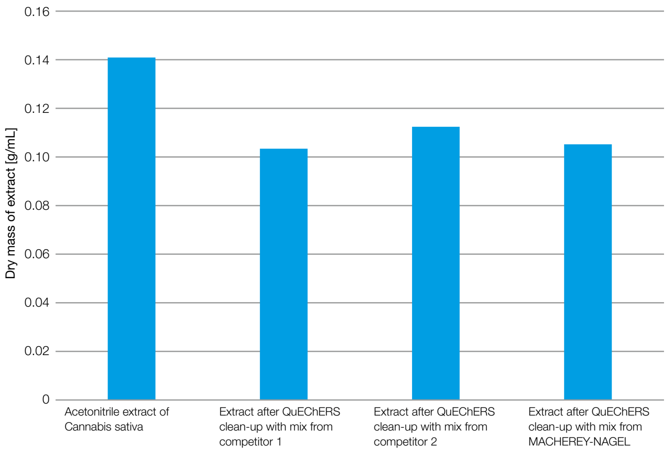

Figure 3: Comparison of drying residue of extracts before and after using clean-up.

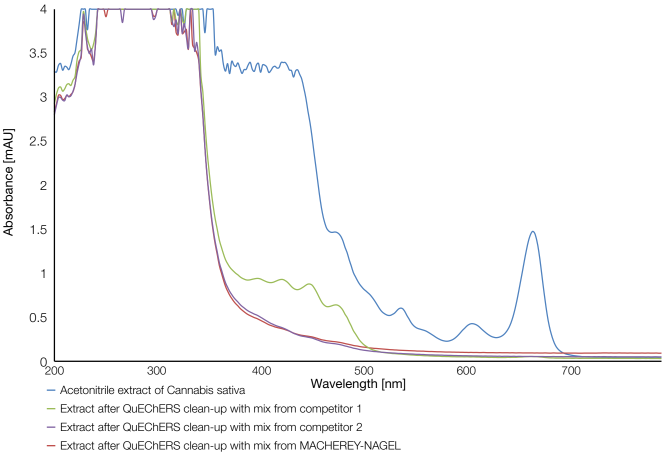

Figure 4: Comparison of spectra of extracts before and after using clean-up.

Recovery rates

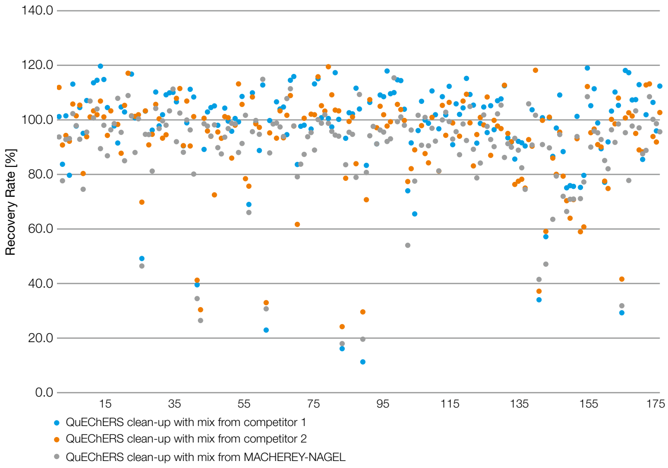

Figure 5: Comparison of recovery rates between MACHEREY-NAGEL and different competitors.

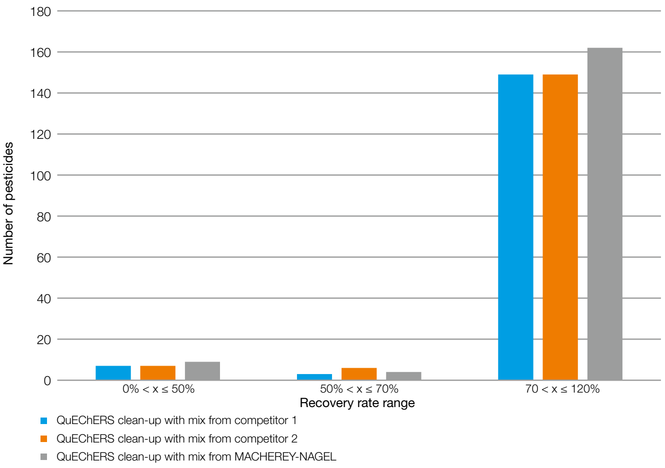

Figure 6: Comparison of distribution of recovery rates between MACHEREY-NAGEL and different competitors.

Pesticide residues in cannabis

	QuEChERS clean-up mix from MN	QuEChERS clean-up mix from comp. 1	QuEChERS clean-up mix from comp. 2
Analyte	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]
3-Hydroxycarbofuran	93.7 ± 15.0	101.2 ± 12.8	111.9 ± 20.3
Acephate	77.7 ± 14.3	83.7 ± 11.8	90.8 ± 17.4
Acetamiprid	92.8 ± 3.8	101.5 ± 6.7	94.3 ± 10.0
Acibenzolar-S-methyl	93.2 ± 24.7	79.7 ± 21.0	92.2 ± 17.6
Aldicarb sulfone	102.3 ± 10.3	113.1 ± 10.7	105.8 ± 16.5
Aldicarb sulfoxide	97.9 ± 8.0	97.8 ± 15.9	101.5 ± 15.2
Aldicarb	92.9 ± 8.4	104.5 ± 6.8	105.4 ± 17.5
Ametryn	74.6 ± 14.3	95.1 ± 9.4	80.4 ± 5.1
Aminocarb	95.5 ± 12.9	107.1 ± 6.5	93.8 ± 6.5
Amitraz	106.9 ± 18.6	out of specification*	101.1 ± 16.4
Azoxystrobin	100.7 ± 4.8	113.6 ± 7.3	103.3 ± 4.0
Benalaxyl	104.0 ± 6.3	114.5 ± 9.9	101.9 ± 8.2
Bendiocarb	89.5 ± 11.2	119.7 ± 10.7	106.9 ± 13.1
Benzoximate	98.0 ± 13.6	114.8 ± 21.0	101.1 ± 13.8
Bifenazate	86.8 ± 11.8	104.5 ± 13.6	94.3 ± 8.9
Bitertanol	96.4 ± 4.1	out of specification*	103.3 ± 9.7
Boscalid	98.6 ± 17.6	97.8 ± 17.5	out of specification*
Bromucanozole isomer 1	107.9 ± 12.4	91.5 ± 8.3	98.4 ± 16.3
Bupirimate	95.4 ± 8.3	104.7 ± 8.2	87.8 ± 8.3
Buprofezin	85.0 ± 1.0	102.9 ± 10.7	105.3 ± 10.6
Butafenacil	108.9 ± 10.0	out of specification*	117.1 ± 8.9
Butocarboxim	101.4 ± 9.4	116.8 ± 12.7	101.5 ± 15.0
Butoxycarboxim	88.1 ± 7.3	100.7 ± 17.8	101.3 ± 14.8
Carbaryl	100.4 ± 10.9	out of specification*	101.9 ± 12.0
Carbendazim	46.4 ± 21.4	49.2 ± 16.6	69.8 ± 13.0
Carbetamide	94.7 ± 6.0	103.2 ± 4.1	103.3 ± 3.2
Carbofuran	94.6 ± 7.4	out of specification*	90.8 ± 8.7
Carboxin	81.2 ± 6.1	96.2 ± 8.5	94.7 ± 7.9
Carfentrazone-ethyl	104.2 ± 4.5	110.2 ± 4.1	105.7 ± 12.4
Chlorantraniliprole	96.8 ± 6.9	97.8 ± 12.2	100.4 ± 16.2
Chlorotoluron	99.6 ± 7.2	101.9 ± 10.5	93.3 ± 10.8
Chloroxuron	98.0 ± 7.2	109.2 ± 10.0	94.6 ± 4.5
Clethodim isomer 1	103.3 ± 13.8	109.9 ± 23.2	out of specification*
Clofentezine	111.3 ± 14.1	110.1 ± 9.2	out of specification*
Clothianidin	92.0 ± 13.2	106.6 ± 11.2	107.9 ± 6.7
Cyazofamid	102.5 ± 9.9	out of specification*	111.5 ± 9.3
Cycluron	88.1 ± 11.9	90.5 ± 5.3	90.5 ± 8.6
Cymoxanil	100.0 ± 15.5	98.9 ± 24.4	106.9 ± 23.3
Cyproconazole isomer 1	96.3 ± 7.0	111.2 ± 8.4	90.4 ± 10.9
Cyproconazole isomer 2	80.1 ± 8.0	108.4 ± 22.8	101.2 ± 10.1
Cyprodinil	34.5 ± 7.6	39.5 ± 4.5	41.2 ± 18.7
Cyromazine	26.5 ± 11.0	out of specification*	30.4 ± 20.1
Desmedipham	98.6 ± 22.5	89.2 ± 11.5	100.6 ± 21.6
Diclobutrazol	97.7 ± 7.0	102.9 ± 7.1	95.9 ± 10.0
Dicrotophos-1	94.1 ± 9.0	104.5 ± 12.5	94.0 ± 11.1
Diethofencarb	98.2 ± 7.4	105.1 ± 12.0	72.5 ± 9.0
Difenoconazole isomer 1	89.8 ± 7.8	98.0 ± 3.5	95.6 ± 7.7
Diflubenzuron	87.5 ± 24.7	out of specification*	93.3 ± 18.7
Dimethoate	93.7 ± 12.8	104.3 ± 6.4	101.2 ± 10.9

	QuEChERS clean-up mix from MN	QuEChERS clean-up mix from comp. 1	QuEChERS clean-up mix from comp. 2
Analyte	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]
Dimethomorph isomer 1	96.8 ± 8.0	99.6 ± 4.8	100.8 ± 15.7
Dimethomorph isomer 2	98.7 ± 9.6	95.8 ± 5.6	86.0 ± 8.3
Dimoxystrobin	98.5 ± 4.0	100.5 ± 9.3	98.1 ± 9.8
Diniconazole	88.0 ± 19.6	99.3 ± 12.1	113.2 ± 19.8
Dinotefuran	91.4 ± 8.5	108.6 ± 8.7	105.7 ± 12.2
Diuron	101.7 ± 16.4	out of specification*	78.4 ± 19.1
Emamectin-benzoate B_1a	66.0 ± 15.9	69.0 ± 10.4	75.7 ± 6.4
Epoxiconazole	95.7 ± 8.0	109.9 ± 8.7	108.4 ± 10.2
Etaconazole isomer 1	99.8 ± 8.6	98.9 ± 5.7	98.5 ± 6.8
Ethiofencarb	94.7 ± 6.6	88.8 ± 2.0	97.2 ± 3.0
Ethiprole	114.9 ± 15.0	112.8 ± 6.7	out of specification*
Ethirimol	30.7 ± 11.5	23.0 ± 11.9	33.0 ± 12.4
Etoxazole	87.9 ± 14.0	99.7 ± 9.9	95.2 ± 15.6
Famoxadone	98.5 ± 20.5	out of specification*	out of specification*
Fenamidone	98.8 ± 5.1	106.6 ± 7.6	93.3 ± 5.9
Fenarimol	97.4 ± 4.9	104.0 ± 13.9	103.3 ± 12.1
Fenbuconazole	93.9 ± 16.0	104.7 ± 18.1	93.4 ± 11.7
Fenhexamid	107.8 ± 9.6	94.6 ± 16.1	101.7 ± 19.2
Fenobucarb	111.4 ± 18.2	114.5 ± 22.0	108.9 ± 21.2
Fenoxycarb	97.8 ± 11.2	115.9 ± 8.1	97.7 ± 10.2
Fenpropimorph	79.1 ± 7.5	83.7 ± 6.3	61.7 ± 9.9
Fenpyroximate	83.8 ± 21.5	97.7 ± 18.0	out of specification*
Fenuron	87.4 ± 5.7	98.1 ± 12.8	100.6 ± 17.2
Fipronil	93.8 ± 22.2	out of specification*	out of specification*
Flonicamid	95.4 ± 8.9	96.7 ± 8.8	102.1 ± 10.9
Flubendiamide	89.0 ± 8.4	113.2 ± 13.9	101.9 ± 7.0
Flufenacet	100.1 ± 7.7	115.1 ± 8.5	115.8 ± 10.0
Fluometuron	98.8 ± 6.7	100.8 ± 10.6	105.2 ± 14.2
Fluoxastrobin	101.9 ± 8.6	out of specification*	103.2 ± 8.0
Fluquinconazole	98.7 ± 14.0	100.5 ± 19.5	119.5 ± 9.0
Flusilazole	95.8 ± 10.5	97.4 ± 11.9	109.2 ± 8.5
Flutolanil	94.4 ± 4.8	117.3 ± 6.0	103.6 ± 5.5
Flutriafol	94.0 ± 3.9	100.2 ± 4.4	103.3 ± 6.8
Forchlorfenuron	18.0 ± 12.9	16.1 ± 8.5	24.2 ± 10.2
Formetanate HCl	83.6 ± 10.3	93.3 ± 11.3	78.6 ± 6.5
Furalaxyl	95.5 ± 4.2	102.6 ± 8.2	99.4 ± 10.1
Furathiocarb	94.7 ± 4.8	102.2 ± 9.4	101.1 ± 19.5
Hexaconazole	78.9 ± 22.3	111.6 ± 9.8	83.9 ± 14.2
Hexythiazox	109.3 ± 23.4	104.0 ± 17.9	out of specification*
Hydramethylnon	19.6 ± 9.2	11.3 ± 7.2	29.6 ± 5.5
Imazalil	80.8 ± 8.2	83.3 ± 4.1	70.7 ± 14.6
Imidacloprid	94.2 ± 10.7	106.4 ± 11.6	107.4 ± 7.5
Indoxacarb	97.6 ± 12.8	out of specification*	out of specification*
Ipconazole isomer 2	91.2 ± 16.3	91.2 ± 17.4	97.1 ± 8.3
Iprovalicarb isomer 1	96.3 ± 21.5	109.1 ± 12.0	105.0 ± 13.8
Iprovalicarb isomer 2	95.5 ± 5.3	108.5 ± 7.2	101.9 ± 9.0
Isoprocarb	92.1 ± 10.1	117.9 ± 13.1	97.7 ± 17.5
Isoproturon	93.1 ± 6.1	109.6 ± 10.9	99.3 ± 8.1
Kresoxim-methyl	115.4 ± 15.8	110.0 ± 13.5	out of specification*

Pesticide residues in cannabis

	QuEChERS clean-up mix from MN	QuEChERS clean-up mix from comp. 1	QuEChERS clean-up mix from comp. 2
Analyte	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]
Linuron	99.3 ± 8.6	114.7 ± 17.9	105.7 ± 7.6
Mandipropamid	101.1 ± 14.0	114.4 ± 8.3	103.8 ± 13.6
Mefenacet	98.0 ± 15.4	103.9 ± 12.9	99.8 ± 8.4
Mepanipyrim	54.0 ± 8.3	74.0 ± 13.8	77.4 ± 6.5
Mepronil	96.3 ± 3.6	91.6 ± 8.2	82.1 ± 14.4
Mesotrione	77.6 ± 13.3	65.5 ± 10.1	89.0 ± 3.5
Metaflumizone	101.7 ± 19.3	96.1 ± 18.8	out of specification*
Metalaxyl	90.7 ± 2.9	106.8 ± 3.3	97.9 ± 4.1
Metconazole	100.9 ± 9.7	99.9 ± 8.6	87.7 ± 4.9
Methabenzthiazuron	90.5 ± 6.7	98.7 ± 5.7	84.3 ± 11.4
Methiocarb	96.9 ± 2.8	110.6 ± 10.9	100.9 ± 8.2
Methomyl	92.2 ± 3.6	101.9 ± 17.9	out of specification*
Methoprotryne	81.3 ± 10.9	96.7 ± 9.8	81.2 ± 7.0
Methoxyfenozide	100.0 ± 6.7	108.5 ± 8.5	105.3 ± 3.7
Metobromuron	102.8 ± 10.8	101.8 ± 11.0	94.3 ± 16.9
Metribuzin	99.4 ± 9.8	112.3 ± 19.7	106.4 ± 8.2
Mevinphos isomer 1	93.0 ± 16.7	90.9 ± 12.9	98.9 ± 16.0
Mevinphos isomer 2	95.7 ± 15.0	105.9 ± 11.8	out of specification*
Mexacarbate	87.2 ± 7.3	102.0 ± 3.2	94.0 ± 8.8
Monocrotophos	98.5 ± 9.7	104.4 ± 7.6	106.9 ± 5.1
Monolinuron	92.4 ± 10.9	115.2 ± 7.0	109.4 ± 7.4
Myclobutanil	104.9 ± 4.6	109.3 ± 8.6	98.9 ± 5.3
Neburon	89.2 ± 9.5	105.4 ± 14.6	83.1 ± 13.8
Nitenpyram	78.7 ± 4.1	91.5 ± 8.1	94.6 ± 12.7
Omethoate	84.2 ± 8.9	98.7 ± 12.6	100.9 ± 20.7
Oxadixyl	101.8 ± 6.5	104.7 ± 12.3	98.1 ± 6.2
Oxamyl	97.6 ± 9.5	95.3 ± 9.0	108.4 ± 13.0
Paclobutrazol	92.8 ± 3.3	105.2 ± 5.1	87.0 ± 10.5
Penconazole	90.2 ± 7.2	96.4 ± 4.5	99.8 ± 7.8
Phenmedipham	102.2 ± 10.4	107.0 ± 9.7	97.8 ± 9.9
Picoxystrobin	105.4 ± 15.8	107.7 ± 10.2	96.8 ± 7.0
Piperonyl butoxide	86.2 ± 11.7	112.5 ± 6.8	112.7 ± 18.7
Pirimicarb	91.3 ± 8.0	93.4 ± 8.1	95.0 ± 5.9
Prochloraz	90.0 ± 10.0	91.8 ± 9.2	92.0 ± 15.2
Promecarb	93.3 ± 22.6	85.6 ± 8.5	76.3 ± 14.2
Prometon	88.9 ± 12.4	92.1 ± 13.0	77.5 ± 10.6
Prometryne	82.5 ± 3.5	91.6 ± 4.3	78.2 ± 12.2
Propamocarb	74.5 ± 6.8	90.4 ± 15.4	75.0 ± 7.1
Propham	105.9 ± 18.5	out of specification*	92.8 ± 9.0
Propiconazole isomer 1	90.8 ± 11.6	103.7 ± 12.3	90.4 ± 9.2
Propoxur	101.7 ± 10.2	out of specification*	118.2 ± 14.0
Pymetrozine	41.5 ± 14.1	34.0 ± 14.8	37.2 ± 16.8
Pyraclostrobin	90.9 ± 6.0	100.8 ± 12.9	99.8 ± 5.0
Pyrimethanil	47.1 ± 15.2	57.2 ± 5.0	59.1 ± 21.1
Pyriproxyfen	89.7 ± 11.2	100.7 ± 4.8	101.0 ± 16.5
Quinoxyfen	63.5 ± 20.7	86.6 ± 8.0	86.0 ± 20.3
Secbumeton	79.4 ± 8.1	97.0 ± 7.8	80.1 ± 9.3
Siduron	94.6 ± 10.0	109.1 ± 7.7	95.5 ± 6.8
Simetryn	72.0 ± 5.9	88.4 ± 7.3	79.4 ± 12.5

	QuEChERS clean-up mix from MN	QuEChERS clean-up mix from comp. 1	QuEChERS clean-up mix from comp. 2
Analyte	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]
Spinetoram	66.4 ± 11.8	75.1 ± 12.8	70.4 ± 10.6
Spinosad (Spinosyn A)	70.9 ± 6.4	75.9 ± 12.2	64.0 ± 21.5
Spinosad (Spinosyn D)	71.0 ± 7.6	75.6 ± 15.6	70.8 ± 21.8
Spirotetramat	93.9 ± 11.0	101.3 ± 6.4	93.1 ± 8.7
Spiroxamine isomer 1	71.1 ± 6.5	75.2 ± 7.8	59.0 ± 6.8
Spiroxamine isomer 2	77.2 ± 7.8	79.7 ± 3.2	60.8 ± 14.7
Sulfentrazone	108.5 ± 13.2	119.0 ± 9.0	112.2 ± 12.7
Tebuconazole	90.0 ± 10.0	105.2 ± 13.7	95.3 ± 6.1
Tebufenozide	95.9 ± 12.6	111.4 ± 11.7	96.7 ± 7.8
Tebufenpyrad	95.3 ± 10.3	98.9 ± 7.0	90.9 ± 16.4
Tebuthiuron	92.8 ± 14.0	89.4 ± 17.3	90.1 ± 19.2
Terbumeton	85.1 ± 7.7	77.1 ± 13.7	77.6 ± 4.4
Terbutryn	82.1 ± 7.9	91.9 ± 4.5	74.9 ± 12.3
Tetraconazole	96.4 ± 12.1	103.2 ± 5.7	100.2 ± 10.1
Thiacloprid	91.8 ± 15.5	110.2 ± 13.8	98.6 ± 12.6
Thiamethoxam	98.5 ± 8.9	105.3 ± 7.1	108.0 ± 13.3
Thidiazuron	31.9 ± 8.7	29.3 ± 13.3	41.6 ± 11.4
Thiobencarb	95.3 ± 22.0	118.1 ± 16.7	100.7 ± 9.1
Thiophanate-methyl	77.8 ± 14.1	117.3 ± 6.3	102.6 ± 15.8
Triadimefon	97.7 ± 11.1	107.3 ± 7.6	101.4 ± 11.0
Triadimenol	103.3 ± 7.9	107.4 ± 6.5	95.0 ± 9.6
Trichlorfon	97.1 ± 12.1	112.9 ± 21.0	89.0 ± 11.2
Tricyclazole	87.5 ± 9.7	85.5 ± 20.1	88.3 ± 18.5
Trifloxystrobin	88.8 ± 10.0	101.9 ± 9.1	112.8 ± 5.6
Triflumuron	108.5 ± 9.9	out of specification*	113.2 ± 12.7
Triticonazole	100.2 ± 8.8	106.4 ± 8.3	93.9 ± 8.9
Vamidothion	98.6 ± 12.1	96.0 ± 12.0	91.9 ± 8.7
Zoxamide	95.6 ± 9.8	112.4 ± 8.4	102.7 ± 13.1

Table 2: Recovery rates for presented QuEChERS clean-up mixes. The recovery rates are calculated by matrix matched standard calibration.
*specification = 0 – 120 % ± 25 %

Conclusion

This application note shows the reliable and successful determination of pesticide residues from marijuana samples with an optimized QuEChERS method. The optimized composition of QuEChERS salt clean-up mixes leads to high reduction of matrix components and high recovery rates for pesticides. The presented QuEChERS method resulted in an average recovery rate of 90.2 % for 175 pesticides. Most of the pesticides (162 analytes) show recovery rates between 70 % to 120 %. On the other side, high matrix reduction yields are achieved by using the presented clean-up mix. The amount of dry substance after clean-up is reduced to less than 40 % of raw acetonitrile extracts. With this clean-up approach interfering substances (like e.g., lipids and pigments) are successfully removed due to optimized amounts of GCB and CHROMABOND® C_{18} ec adsorbents.

The chromatographic separation of pesticides was performed using core-shell particles that are well known for fast and high-efficient separations combined with a reasonably low backpressure. In this work, a subsequent analytics was developed on a NUCLEOSHELL® Bluebird RP 18 column.

References

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Determination of pesticide residues in Cannabis sativa using an optimized QuEChERS method with high matrix reduction

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Abstract

This application note describes the determination of pesticide residues in Cannabis sativa using a QuEChERS method for most effective sample clean-up. Interfering substances (like e.g., lipids and pigments), which were also extracted with the organic layer, are almost completely removed with clean-up salt mixes with high amounts of GCB (graphitized carbon black) and CHROMABOND® C_{18} ec adsorbents. The organic extracts are finally analyzed by HPLC-MS/MS.

Introduction

While medical and recreational use of cannabis are legalized in more and more U.S. territories the markets for cannabis and cannabis-based products (e.g. concentrated oils, soda, candy and other edible forms) have grown year by year in North America and also in other countries[1]. A huge demand for marijuana has lead to professional cultivation forms of hemp to improve growth yields. The use of pesticides is a common tool for this monoculture plant production. Therefor an increasing interest in the determination of pesticide residues in marijuana is given.

New quality control methods have to be developed to ensure product safety and to reduce health risks by chronic exposure to pesticides. These methods have to be quick, easy, cheap, effective, rugged and safe like the QuEChERS extraction approach[2]. Interfering substances (like e.g., lipids and chlorophyll), which were also extracted with the organic layer, are nearly completely removed by using clean-up salt mixes with high amounts of GCB and of CHROMABOND® C_{18} ec adsorbents. On the other side, the composition of the clean-up salt mixes also has to ensure sufficient recovery rates for these pesticides. This work presents a QuEChERS method for the simultaneous analysis of more than 160 pesticides from Cannabis sativa. The organic extracts are finally analyzed by HPLC-MS/MS.

Figure 1: Marijuana (Cannabis sativa).

Dispersive solid phase extraction (dSPE)

Products from competitors

Competitor 1:

Supel™ QuE PSA/C_{18}/ENVI-Carb tube, 15 mL centrifuge tubes, 1200 mg $MgSO_4$, 400 mg Supleclean PSA, 400 mg Discovery DSC-18 und 400 mg ENVI-Carb

Competitor 2:

roQ™ QuEChERS dSPE kit, 15 mL centrifuge tubes, 1200 mg $MgSO_4$, 400 mg PSA, 400 mg C_{18} ec, 400 mg GCB

Competitor 3:

QuEChERS SPE Resprep Q352, 15 mL centrifuge tubes, 1200 mg $MgSO_4$, 400 mg PSA, 400 mg C_{18}, 400 mg Carbon

Extraction

- Weigh out 1 g of homogenized sample (milled in a grinder) into a 50 mL centrifuge tube (REF 730223)
- Add 100 µL of standard solution ($\beta = 1$ µg/mL for each analyte in acetonitrile) for determining recovery rates
- Add 10 mL water and shake
- Add 10 mL 1 % acetic acid in acetonitrile and shake for 30 min
- Add the CHROMABOND® QuEChERS extraction mix I (REF 730970)
- Shake vigorously for 2 min and cool down the mixture in an ice bath
- Centrifuge the mixture at 4500 rpm, for 5 min at 4 °C

Clean-up

- Add 6 mL of acetonitrile supernatant to the CHROMABOND® QuEChERS clean-up mix XLVII (REF 730845)
- Shake vigorously for 60 s
- Centrifuge the mixture at 4500 rpm, for 5 min at 4 °C
- Take acetonitrile extract for injection (REF 702293, REF 702107)

Pesticide residues in cannabis with high matrix reduction

Subsequent analysis: HPLC-MS / MS

Chromatographic conditions

Column: EC NUCLEOSHELL® Bluebird RP 18, 50 x 4.6 mm, 2.7 µm (REF 763432.46)

Eluent A: 0.1 % formic acid in water

Eluent B: 0.1 % formic acid in acetonitrile

Gradient: in 5 min from 5 % to 100 % B, hold for 1.0 min, in 0.1 min to 5 % B, hold 5 % B for 3.9 min

Flow rate: 0.7 mL/min

Temperature: 30 °C

Injection volume: 2 µL

MS conditions

AB Sciex QTRAP 5500

Acquisition mode: SRM

Interface: ESI

Polarity: positive

Curtain gas: 35 psig

Collision gas: medium

Ion spray voltage: 5000 V

Temperature: 450 °C

Ion source gas 1: 45 psig

Ion source gas 2: 45 psig

Detection window: 60 s

MRM transitions

Analyte	RT [min]	Q_1 Mass [Da]	Q_3 Mass [Da]
Propamocarb.1	1.45	189.2	102.1
Propamocarb.2	1.46	189.2	74.0
Aminocarb.1	1.49	209.2	137.2
Aminocarb.2	1.49	209.2	152.2
Formetanate HCl.1	1.52	222.3	165.0
Formetanate HCl.2	1.52	222.3	120.1
Pymetrozine.1	1.56	218.1	105.0
Pymetrozine.2	1.56	218.1	78.0
Acephate.1	1.61	184.1	143.0
Acephate.2	1.62	184.1	49.0
Omethoate.1	1.83	214.1	183.0
Omethoate.2	1.84	214.1	125.1
Nitenpyram.1	1.88	271.1	224.9
Nitenpyram.2	1.88	271.1	126.1
Aldicarb sulfoxide.1	1.98	207.1	132.1
Aldicarb sulfoxide.2	1.98	207.1	89.1
Dinotefuran.1	2.03	203.0	129.0
Dinotefuran.2	2.03	203.0	157.0
Mexacarbate.1	2.08	223.1	166.1
Mexacarbate.2	2.08	223.1	151.1
Flonicamid.1	2.36	230.2	203.1
Flonicamid.2	2.36	230.2	174.0
Fuberidazole.1	2.41	185.1	157.1
Fuberidazole.2	2.41	185.1	65.0
Thiamethoxam.1	2.52	292.2	181.2
Thiamethoxam.2	2.52	292.2	211.0
Monocrotophos.1	2.56	224.1	127.1
Monocrotophos.2	2.56	224.1	98.1
Dicrotophos-1	2.70	238.1	112.1
Dicrotophos-2	2.70	238.1	193.0
Pirimicarb.1	2.74	239.2	72.1
Pirimicarb.2	2.75	239.2	182.0
Trichlorfon.1	2.89	257.0	109.0
Imidacloprid.1	2.90	256.2	175.0
Imidacloprid.2	2.90	256.2	209.0
Clothianidin.1	2.91	250.2	169.1
Clothianidin.2	2.91	250.2	132.1
Fenuron.1	2.95	165.1	46.0
Fenuron.2	2.95	165.1	72.0
Vamidothion.1	2.98	288.1	146.1
Vamidothion.2	2.98	288.1	118.1
3-Hydroxycarbofuran.1	3.00	238.2	181.0
3-Hydroxycarbofuran.2	3.00	238.2	163.1
Dimethoate.1	3.05	230.1	198.8
Dimethoate.2	3.05	230.1	125.0
Acetamiprid.1	3.14	223.1	126.1
Acetamiprid.2	3.14	223.1	99.0
Prometon.1	3.31	226.2	142.1
Prometon.2	3.31	226.2	86.0
Terbumeton.1	3.32	226.2	170.0
Secbumeton.2	3.32	226.2	100.0
Secbumeton.1	3.33	226.2	170.0
Mevinphos isomer 1.1	3.35	225.1	127.0
Mevinphos isomer 1.2	3.35	225.1	192.9
Butocarboxim.1	3.40	213.0	75.0
Butocarboxim.2	3.40	213.0	116.0
Imazalil.1	3.40	297.1	201.0
Imazalil.2	3.40	297.1	159.0
Aldicarb.1	3.44	208.1	116.0
Aldicarb.2	3.44	208.1	89.0
Tricyclazole.1	3.52	190.1	163.0
Tricyclazole.2	3.52	190.1	136.0
Mesotrione.1	3.56	340.1	228.0
Mesotrione.2	3.56	340.1	104.1
Carbetamide.1	3.62	237.1	192.1
Carbetamide.2	3.62	237.1	118.2
Ametryn.1	3.66	228.1	186.0
Ametryn.2	3.66	228.1	96.0
Fenpropimorph.1	3.70	304.3	147.0

Analyte	RT [min]	Q_1 Mass [Da]	Q_3 Mass [Da]
Fenpropimorph.2	3.70	304.3	117.2
Methoprotryne.1	3.76	272.2	198.0
Methoprotryne.2	3.76	272.2	240.0
Thiophanate-methyl.1	3.77	343.2	151.0
Thiophanate-methyl.2	3.77	343.2	311.1
Metribuzin.1	3.78	215.2	187.0
Metribuzin.2	3.78	215.2	84.1
Spiroxamine isomer 1.1	3.79	298.2	144.2
Spiroxamine isomer 1.2	3.79	298.2	100.1
Spiroxamine isomer 2.1	3.79	298.2	144.2
Spiroxamine isomer 2.2	3.79	298.2	100.1
Carbofuran.1	3.81	222.0	165.0
Carbofuran.2	3.81	222.0	123.0
Sulfentrazone.1	3.85	387.0	307.0
Sulfentrazone.2	3.85	387.0	146.0
Tebuthiuron.1	3.87	229.2	172.1
Tebuthiuron.2	3.87	229.2	116.0
Carbaryl.1	3.98	202.1	145.2
Carbaryl.2	3.98	202.1	127.1
Prometryne.1	3.99	242.2	158.1
Prometryne.2	3.99	242.2	200.1
Carboxin.1	4.00	236.1	143.1
Carboxin.2	4.00	236.1	87.1
Pyrimethanil.1	4.02	200.2	107.0
Pyrimethanil.2	4.02	200.2	82.2
Terbutryn.1	4.02	242.2	186.0
Terbutryn.2	4.02	242.2	68.1
Ethiofencarb.1	4.03	226.2	107.1
Ethiofencarb.2	4.03	226.2	164.0
Monolinuron.1	4.05	215.1	126.1
Monolinuron.2	4.05	215.1	99.0
Fluometuron.1	4.08	233.0	72.0
Flutriafol.1	4.14	302.2	70.1
Flutriafol.2	4.14	302.2	123.0
Chlorotoluron.1	4.15	213.1	72.2
Chlorotoluron.2	4.15	213.1	46.0
Isoprocarb.1	4.16	194.1	95.1
Isoprocarb.2	4.16	194.1	137.0
Metobromuron.1	4.17	259.1	169.9
Metobromuron.2	4.18	259.1	148.2
Metalaxyl.1	4.21	280.2	220.3
Metalaxyl.2	4.21	280.2	192.0
Isoproturon.1	4.23	207.1	72.1
Isoproturon.2	4.23	207.1	46.1
Methabenzthiazuron.1	4.25	222.1	165.0
Methabenzthiazuron.2	4.25	222.1	150.0
Butoxycarboxim.1	4.26	223.1	106.0
Butoxycarboxim.2	4.26	223.1	166.0
Cycluron.1	4.29	199.2	89.0
Cycluron.2	4.29	199.2	89.1
Desmedipham.1	4.33	318.2	154.0
Desmedipham.2	4.33	318.2	182.0
Diuron.1	4.33	233.1	72.2
Diuron.2	4.33	235.2	72.1
Chlorantraniliprole.1	4.36	484.0	453.0
Chlorantraniliprole.2	4.36	484.0	285.8
Spinosad (Spinosyn A).1	4.36	732.5	142.2
Spinosad (Spinosyn A).2	4.36	732.5	98.1
Bupirimate.1	4.37	317.2	166.1
Bupirimate.2	4.37	317.2	108.0
Phenmedipham.1	4.37	301.2	168.0
Phenmedipham.2	4.37	301.2	107.9
Fenobucarb.1	4.44	208.0	95.0
Diethofencarb.1	4.45	268.1	226.1
Diethofencarb.2	4.45	268.1	124.0
Ethiprole.1	4.45	397.2	350.9
Ethiprole.2	4.45	397.2	255.0
Furalaxyl.1	4.47	302.2	242.0
Furalaxyl.2	4.47	302.2	95.1
Spinosad (Spinosyn D).1	4.48	746.4	142.3
Spinosad (Spinosyn D).2	4.47	746.4	98.0
Hydramethylnon.1	4.48	495.2	323.2
Hydramethylnon.2	4.48	495.2	151.1
Spinetoram.1	4.48	748.4	142.3
Spinetoram.2	4.48	748.4	98.0
Halofenozide.1	4.5	331.2	105.0
Halofenozide.2	4.5	331.0	275.0
Nuarimol.1	4.5	315.0	252.0
Nuarimol.2	4.5	315.0	81.0
Siduron.1	4.52	233.1	137.1
Siduron.2	4.51	233.1	94.1
Fenamidone.1	4.52	312.1	92.0
Fenamidone.2	4.52	312.1	236.1
Azoxystrobin.1	4.53	404.1	372.1
Azoxystrobin.2	4.53	404.1	344.0
Linuron.1	4.53	249.1	160.0
Linuron.2	4.53	249.1	182.1
Methiocarb.1	4.54	226.2	169.0
Methiocarb.2	4.54	226.2	121.2
Paclobutrazol.1	4.55	294.3	70.0
Paclobutrazol.2	4.55	294.3	125.0
Boscalid.1	4.56	343.0	307.0
Boscalid.2	4.56	343.0	140.0
Carfentrazone-ethyl.1	4.56	412.0	328.0

Analyte	RT [min]	Q_1 Mass [Da]	Q_3 Mass [Da]
Carfentrazone-ethyl.2	4.56	412.0	356.0
Fenarimol.1	4.56	331.0	268.0
Fenarimol.2	4.56	331.0	81.0
Promecarb.1	4.57	208.1	151.0
Promecarb.2	4.57	208.1	109.2
Flutolanil.1	4.58	324.1	262.0
Flutolanil.2	4.58	324.1	241.9
Triadimenol.1	4.59	296.2	70.1
Triadimenol.2	4.59	296.2	227.1
Dimethomorph isomer 1.1	4.60	388.2	300.9
Dimethomorph isomer 1.2	4.60	388.2	164.9
Myclobutanil.1	4.61	289.1	70.0
Myclobutanil.2	4.61	289.1	125.0
Mepronil.1	4.62	270.2	119.1
Mepronil.2	4.62	270.2	227.9
Triadimefon.1	4.62	294.0	197.1
Triadimefon.2	4.62	294.0	225.0
Methoxyfenozide.1	4.65	369.2	149.1
Methoxyfenozide.2	4.65	369.2	313.2
Cyproconazole isomer 1.1	4.66	292.2	70.1
Cyproconazole isomer 1.2	4.66	292.2	125.1
Fenhexamid.1	4.68	302.0	97.0
Fenhexamid.2	4.68	302.0	55.0
Triticonazole.1	4.69	318.2	70.0
Triticonazole.2	4.69	318.2	125.0
Bifenazate.1	4.69	301.2	198.0
Bifenazate.2	4.69	301.2	170.0
Butafenacil.1	4.69	492.2	330.9
Butafenacil.2	4.69	492.2	348.9
Iprovalicarb isomer 1.1	4.70	321.2	119.2
Iprovalicarb isomer 1.2	4.70	321.2	203.1
Iprovalicarb isomer 2.1	4.70	321.2	119.2
Iprovalicarb isomer 2.2	4.70	321.2	203.1
Tetraconazole.1	4.70	372.2	158.9
Tetraconazole.2	4.70	372.2	70.1
Chloroxuron.1	4.71	291.1	72.1
Chloroxuron.2	4.72	291.1	218.1
Spirotetramat.1	4.71	374.2	330.2
Spirotetramat.2	4.71	374.2	302.2
Flufenacet.1	4.72	364.2	152.2
Flufenacet.2	4.72	364.2	193.9
Mefenacet.1	4.72	299.2	148.0
Mefenacet.2	4.72	299.2	120.2
Fluquinconazole.1	4.73	376.0	349.0
Fluquinconazole.2	4.73	376.0	307.0
Prochloraz.1	4.75	376.1	307.9
Prochloraz.2	4.75	376.1	70.1

Analyte	RT [min]	Q_1 Mass [Da]	Q_3 Mass [Da]
Triflumizole.1	4.76	346.0	278.0
Triflumizole.2	4.76	346.0	73.0
Fluoxastrobin.1	4.76	459.1	427.0
Fluoxastrobin.2	4.76	459.1	188.1
Fenarimol.1	4.77	331.0	268.0
Fipronil.1	4.77	437.2	368.0
Fipronil.2	4.78	437.2	290.0
Etaconazole isomer 1.1	4.79	328.0	159.0
Etaconazole isomer 1.2	4.78	328.1	205.0
Fenbuconazole.1	4.80	337.1	125.1
Fenbuconazole.2	4.80	337.1	70.0
Cyazofamid.1	4.81	325.2	108.0
Cyazofamid.2	4.81	325.2	261.2
Epoxiconazole.1	4.81	330.1	121.1
Epoxiconazole.2	4.81	330.1	101.2
Mepanipyrim.1	4.81	224.1	77.0
Mepanipyrim.2	4.81	224.1	106.1
Flusilazole.1	4.83	316.2	247.0
Flusilazole.2	4.83	316.2	165.0
Tebufenozide.1	4.83	353.2	133.0
Tebufenozide.2	4.84	353.2	297.2
Flubendiamide.1	4.84	683.1	408.0
Flubendiamide.2	4.84	683.1	274.1
Diclobutrazol.1	4.87	328.0	70.0
Diclobutrazol.2	4.87	328.2	59.1
Fenoxycarb.1	4.87	302.3	88.0
Fenoxycarb.2	4.87	302.3	116.1
Mandipropamid.1	4.89	412.0	346.0
Mandipropamid.2	4.89	412.0	366.0
Neburon.1	4.89	275.1	88.0
Neburon.2	4.89	275.1	114.0
Tebuconazole.1	4.89	308.2	70.1
Tebuconazole.2	4.89	308.2	125.0
Picoxystrobin.1	4.90	368.2	145.1
Picoxystrobin.2	4.90	368.2	204.9
Bromucanozole isomer 1.1	4.91	378.1	70.0
Bromucanozole isomer 1.2	4.91	378.1	159.0
Dimoxystrobin.1	4.91	327.1	205.0
Dimoxystrobin.2	4.91	327.1	116.0
Penconazole.1	4.92	284.2	70.0
Penconazole.2	4.92	284.2	158.9
Hexaconazole.1	4.97	331.0	105.0
Hexaconazole.2	4.97	314.2	159.0
Propiconazole isomer 1.1	4.97	342.1	159.0
Propiconazole isomer 1.2	4.97	342.1	69.0
Zoxamide.1	4.97	336.1	186.9
Zoxamide.2	4.97	336.1	158.9

Analyte	RT [min]	Q_1 Mass [Da]	Q_3 Mass [Da]
Prothioconazole.1	4.99	344.0	189.0
Prothioconazole.2	5.00	344.0	125.0
Metconazole.1	4.99	320.2	70.1
Metconazole.2	4.99	320.2	125.1
Triflumuron.1	4.99	359.2	156.2
Triflumuron.2	4.99	359.2	139.1
Amitraz.1	5.00	294.2	148.3
Amitraz.2	5.00	294.2	91.2
Benalaxyl.1	5.00	326.3	148.2
Benalaxyl.2	5.00	326.3	294.2
Bitertanol.1	5.02	338.0	70.0
Bitertanol.2	5.02	338.0	99.0
Pyraclostrobin.1	5.05	388.2	194.0
Pyraclostrobin.2	5.05	388.2	163.0
Diniconazole.1	5.10	326.3	159.0
Diniconazole.2	5.09	326.3	70.0
Thiobencarb.1	5.10	258.3	125.0
Thiobencarb.2	5.10	258.3	89.1
Difenoconazole isomer 1.1	5.13	408.2	253.1
Difenoconazole isomer 1.2	5.13	406.0	251.0
Benzoximate.1	5.14	364.2	198.9
Benzoximate.2	5.14	364.2	105.0
Trifloxystrobin.1	5.15	409.0	186.0
Trifloxystrobin.2	5.15	409.0	145.0
Buprofezin.1	5.16	306.2	201.0
Buprofezin.2	5.16	306.2	116.1
Clethodim isomer 1.1	5.19	360.3	164.0
Clethodim isomer 1.2	5.19	360.3	268.1
Tebufenpyrad.1	5.26	334.2	145.0

Analyte	RT [min]	Q_1 Mass [Da]	Q_3 Mass [Da]
Tebufenpyrad.2	5.26	334.2	117.1
Furathiocarb.1	5.27	383.2	195.1
Furathiocarb.2	5.27	383.2	252.0
Piperonyl butoxide.1	5.34	356.2	177.1
Piperonyl butoxide.2	5.34	356.2	119.2
Temephos.1	5.35	467.1	419.0
Temephos.2	5.35	467.1	404.9
Pyriproxyfen.1	5.39	322.1	96.0
Pyriproxyfen.2	5.39	322.1	184.9
Hexythiazox.1	5.40	353.2	228.0
Hexythiazox.2	5.40	353.2	168.1
Quinoxyfen.1	5.40	308.1	196.9
Quinoxyfen.2	5.40	308.1	161.9
Propargite.1	5.43	368.2	231.2
Propargite.2	5.43	368.2	175.1
Etoxazole.1	5.45	360.3	141.1
Etoxazole.2	5.45	360.3	57.0
Chlorfluazuron.1	5.48	540.0	383.0
Chlorfluazuron.2	5.49	540.0	158.0
Eprinomectin.1	5.52	914.5	185.9
Eprinomectin.2	5.52	914.5	154.0
Fenpyroximate.1	5.57	422.2	366.2
Fenpyroximate.2	5.57	422.2	134.9
Pyridaben.1	5.59	365.2	308.9
Pyridaben.2	5.59	365.2	147.2
Fenazaquin.1	5.67	307.0	161.0
Fenazaquin.2	5.67	307.0	147.0
Moxidectin.1	5.7	640.4	528.5
Moxidectin.2	5.7	640.4	498.5

Table 1: MRM transitions and retention times of pesticides. (.1 = first transition, .2 = second transition; Q_1 = Qualifier 1, Q_3 = Qualifier 3)

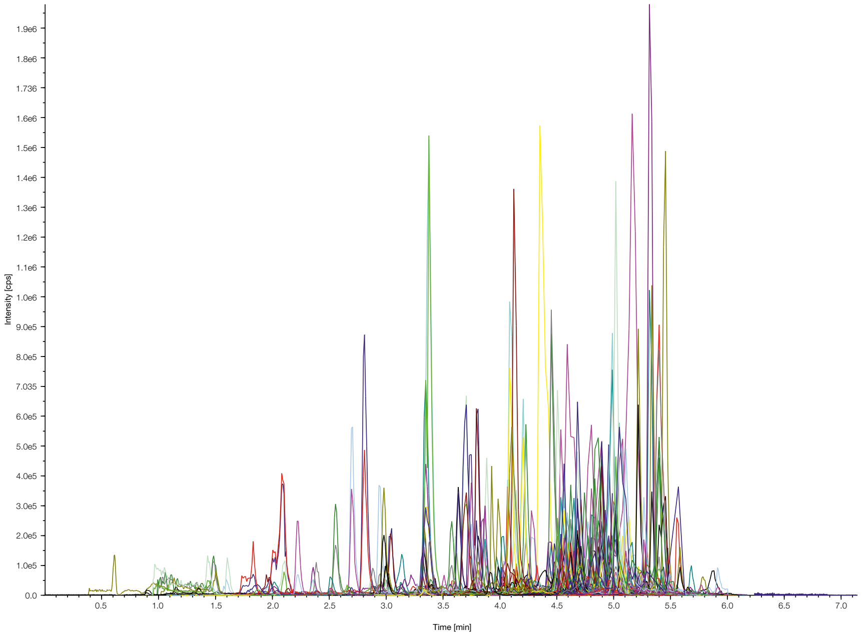

Figure 2: Separation of pesticides on NUCLEOSHELL® Bluebird RP 18 column (QuEChERS extract of Cannabis sativa spiked with β = 100 µg/g).

Pesticide residues in cannabis with high matrix reduction

Matrix reduction

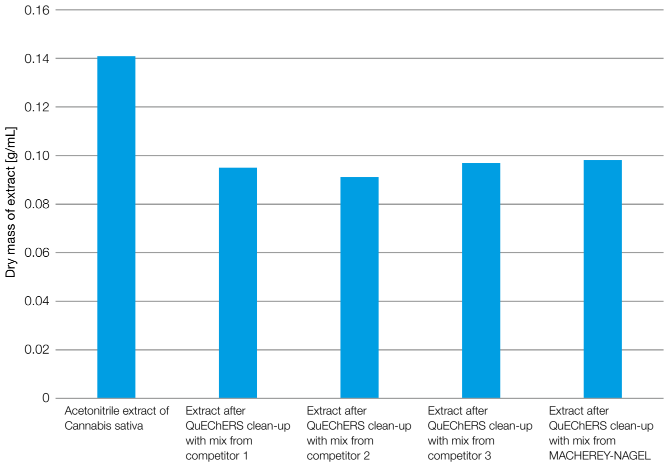

Figure 3: Comparison of drying residue of extracts before and after using clean-up.

Figure 4: Visible matrix reduction: cannabis extract, competitor 1, competitor 2, competitor 3, MACHEREY-NAGEL (from left to right).

Recovery rates

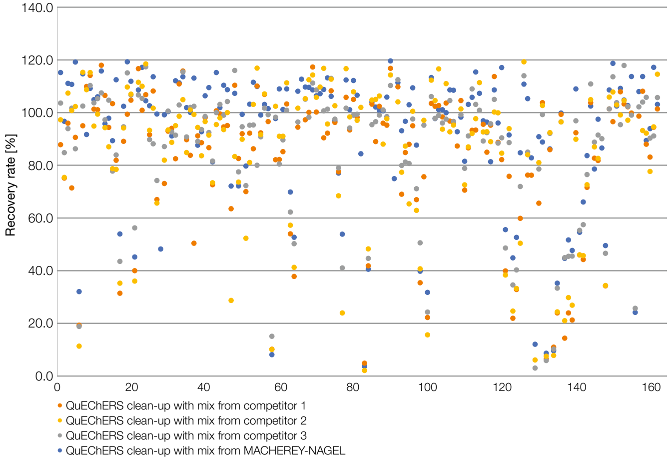

Figure 5: Comparison of recovery rates between MACHEREY-NAGEL and different competitors.

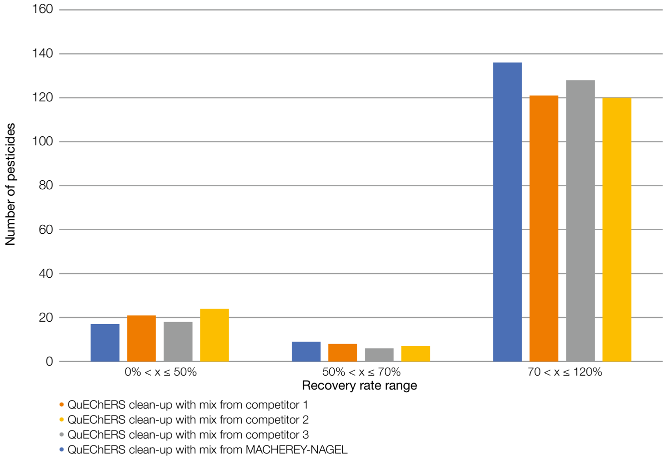

Figure 6: Comparison of distribution of recovery rates between MACHEREY-NAGEL and different competitors.

	QuEChERS clean-up mix from MACHEREY-NAGEL	QuEChERS clean-up mix from competitor 1	QuEChERS clean-up mix from competitor 2	QuEChERS clean-up mix from competitor 3
Analyte	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]
3-Hydroxycarbofuran	115.2 ± 7.8	87.8 ± 11.2	97.3 ± 5.8	103.6 ± 6.1
Acephate	96.7 ± 13.9	75.2 ± 13.1	75.4 ± 10.0	84.8 ± 21.8
Acetamiprid	111.1 ± 7.7	96.0 ± 3.6	107.4 ± 7.5	93.9 ± 6.5
Aldicarb sulfoxide	110.7 ± 8.3	71.4 ± 14.2	100.8 ± 14.1	101.7 ± 11.5
Aldicarb	119.2 ± 7.5	90.6 ± 6.9	102.3 ± 12.4	86.3 ± 9.1
Ametryn	32.1 ± 8.2	19.3 ± 5.3	11.3 ± 11.3	18.8 ± 15.5
Aminocarb	114.7 ± 10.0	95.0 ± 8.6	115.3 ± 14.8	102.5 ± 11.6
Amitraz	91.7 ± 11.2	109.9 ± 10.1	108.7 ± 14.1	out of specification*
Azoxystrobin	109.2 ± 9.8	114.1 ± 6.1	115.2 ± 6.2	110.3 ± 6.6
Benalaxyl	104.9 ± 10.7	101.3 ± 7.5	94.9 ± 7.2	103.7 ± 12.8
Benzoximate	106.0 ± 15.1	101.1 ± 10.1	99.4 ± 13.6	105.3 ± 15.7
Bifenazate	115.8 ± 11.6	118.0 ± 12.8	out of specification*	107.5 ± 13.5
Bitertanol	95.8 ± 18.4	106.5 ± 4.7	94.3 ± 10.2	97.3 ± 7.0
Boscalid	97.9 ± 15.0	94.4 ± 17.7	87.0 ± 17.8	104.7 ± 15.4
Bromucanozole isomer 1	89.3 ± 22.5	103.4 ± 3.2	78.7 ± 8.7	77.8 ± 20.9

Pesticide residues in cannabis with high matrix reduction

	QuEChERS clean-up mix from MACHEREY-NAGEL	QuEChERS clean-up mix from competitor 1	QuEChERS clean-up mix from competitor 2	QuEChERS clean-up mix from competitor 3
Analyte	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]
Bromucanozole isomer 2	112.5 ± 22.3	81.9 ± 19.0	78.5 ± 13.9	83.9 ± 13.0
Bupirimate	53.9 ± 6.3	31.4 ± 17.7	35.2 ± 2.6	43.5 ± 8.9
Buprofezin	102.4 ± 10.3	89.1 ± 11.1	94.2 ± 5.4	94.7 ± 7.4
Butafenacil	119.3 ± 9.2	99.4 ± 13.5	109.7 ± 2.6	115.1 ± 7.1
Butocarboxim	111.9 ± 5.7	104.7 ± 15.0	107.0 ± 8.1	106.7 ± 8.1
Butoxycarboxim	45.2 ± 15.1	40.0 ± 22.7	36.0 ± 10.4	56.2 ± 9.9
Carbaryl	108.6 ± 10.5	116.7 ± 17.2	111.5 ± 12.9	106.7 ± 10.9
Carbetamide	117.2 ± 5.4	100.9 ± 7.0	110.0 ± 5.2	105.4 ± 10.4
Carbofuran	104.5 ± 8.8	106.8 ± 11.1	118.5 ± 11.1	117.4 ± 6.0
Carboxin	102.6 ± 1.6	91.6 ± 3.4	93.3 ± 4.8	89.8 ± 8.4
Carfentrazone-ethyl	113.6 ± 11.2	108.2 ± 10.9	101.7 ± 3.2	88.2 ± 7.5
Chlorantraniliprole	99.5 ± 12.9	67.0 ± 13.7	65.6 ± 8.9	74.2 ± 13.9
Chlorfluazuron	48.2 ± 15.7	out of specification*	out of specification*	out of specification*
Chlorotoluron	99.2 ± 6.5	73.1 ± 8.1	81.9 ± 6.8	87.1 ± 3.1
Chloroxuron	100.4 ± 7.6	93.0 ± 7.2	93.4 ± 3.2	100.6 ± 12.8
Clethodim isomer 1	91.2 ± 19.0	94.0 ± 9.1	88.7 ± 13.7	92.6 ± 20.4
Clethodim isomer 2	112.1 ± 19.3	82.5 ± 10.3	95.1 ± 16.1	91.5 ± 14.5
Clothianidin	111.3 ± 15.1	110.9 ± 15.0	98.6 ± 7.8	102.9 ± 13.8
Cyazofamid	113.6 ± 10.1	115.8 ± 7.1	104.9 ± 12.7	115.4 ± 11.0
Cycluron	101.9 ± 8.0	89.7 ± 5.0	84.8 ± 9.9	90.8 ± 9.2
Cyproconazole isomer 1	100.1 ± 3.7	83.8 ± 14.0	101.0 ± 6.3	102.0 ± 3.2
Cyproconazole isomer 2	113.1 ± 9.3	50.4 ± 7.3	99.3 ± 9.1	102.4 ± 14.4
Desmedipham	87.6 ± 6.8	91.2 ± 2.5	94.4 ± 11.4	89.1 ± 9.1
Diclobutrazol	92.7 ± 10.6	88.6 ± 2.5	83.6 ± 12.4	93.6 ± 7.5
Dicrotophos-1	106.4 ± 5.3	98.1 ± 6.2	96.6 ± 7.7	98.5 ± 5.3
Diethofencarb	109.5 ± 19.1	86.7 ± 9.0	91.5 ± 15.7	92.4 ± 10.8
Difenoconazole isomer 1	81.3 ± 8.0	72.6 ± 9.1	73.4 ± 11.6	81.6 ± 7.9
Dimethoate	115.2 ± 9.5	100.7 ± 2.4	95.5 ± 8.7	100.0 ± 8.9
Dimethomorph isomer 1	101.9 ± 14.1	99.5 ± 8.4	98.0 ± 8.2	103.1 ± 9.9
Dimethomorph isomer 2	110.4 ± 6.1	94.6 ± 6.8	93.5 ± 2.5	99.0 ± 4.4
Dimoxystrobin	108.7 ± 15.1	95.1 ± 10.5	100.0 ± 6.9	104.2 ± 8.4
Diniconazole	72.1 ± 16.4	63.5 ± 18.0	28.7 ± 24.2	out of specification*
Dinotefuran	107.3 ± 14.9	110.4 ± 16.9	83.4 ± 22.3	116.0 ± 16.4
Diuron	72.2 ± 17.0	82.6 ± 18.9	73.6 ± 23.5	77.5 ± 16.4
Epoxiconazole	99.3 ± 11.8	92.0 ± 10.1	89.9 ± 9.9	94.6 ± 10.4
Eprinomectin	79.7 ± 24.0	70.0 ± 18.8	52.3 ± 23.1	72.3 ± 24.0
Etaconazole isomer 1	103.2 ± 14.1	92.2 ± 8.3	81.1 ± 9.5	95.2 ± 9.8
Ethiofencarb	111.5 ± 4.3	86.3 ± 5.2	103.1 ± 7.2	95.3 ± 2.4
Ethiprole	109.9 ± 13.5	110.1 ± 14.0	116.9 ± 6.9	80.0 ± 8.6
Etoxazole	99.1 ± 4.1	92.3 ± 4.5	91.7 ± 6.7	90.8 ± 12.6
Fenamidone	103.1 ± 7.2	106.9 ± 5.7	107.1 ± 5.9	101.7 ± 6.2
Fenarimol	101.5 ± 8.7	96.6 ± 9.0	out of specification*	99.2 ± 15.3
Fenazaquin	8.1 ± 22.2	10.2 ± 9.8	10.1 ± 19.0	15.1 ± 20.0
Fenbuconazole	97.8 ± 15.6	82.1 ± 19.6	102.4 ± 4.3	98.3 ± 11.7
Fenhexamid	109.0 ± 12.7	82.2 ± 12.5	91.0 ± 13.5	80.5 ± 8.0
Fenobucarb	101.2 ± 13.1	85.2 ± 18.2	91.0 ± 10.1	89.7 ± 11.0

Pesticide residues in cannabis with high matrix reduction

	QuEChERS clean-up mix from MACHEREY-NAGEL	QuEChERS clean-up mix from competitor 1	QuEChERS clean-up mix from competitor 2	QuEChERS clean-up mix from competitor 3
Analyte	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]
Fenoxycarb	109.0 ± 6.3	103.3 ± 13.3	111.3 ± 6.8	100.9 ± 13.2
Fenpropimorph	69.9 ± 10.8	54.0 ± 8.7	57.2 ± 5.0	62.3 ± 8.2
Fenpyroximate	52.7 ± 4.6	37.8 ± 6.7	41.3 ± 13.5	50.3 ± 21.5
Fenuron	108.1 ± 10.6	94.5 ± 7.0	101.7 ± 10.1	96.4 ± 11.5
Fipronil	112.7 ± 18.3	out of specification*	out of specification*	out of specification*
Flonicamid	109.7 ± 6.4	97.3 ± 6.0	105.3 ± 7.9	105.1 ± 4.2
Flubendiamide	109.6 ± 13.1	100.1 ± 7.1	112.5 ± 9.4	98.2 ± 12.8
Flufenacet	108.6 ± 2.0	117.3 ± 8.4	114.3 ± 5.1	111.2 ± 9.7
Fluometuron	106.4 ± 4.2	100.2 ± 12.0	105.8 ± 4.3	108.6 ± 7.6
Fluoxastrobin	107.2 ± 6.8	out of specification*	109.7 ± 8.5	out of specification*
Fluquinconazole	108.8 ± 18.0	90.4 ± 10.5	116.8 ± 20.4	100.7 ± 9.3
Flusilazole	113.2 ± 13.5	92.2 ± 12.5	95.7 ± 13.2	101.3 ± 9.2
Flutolanil	106.2 ± 9.9	108.3 ± 1.8	112.7 ± 7.1	113.0 ± 4.5
Flutriafol	96.8 ± 6.3	96.3 ± 2.7	102.6 ± 7.5	97.6 ± 6.8
Formetanate HCl	77.0 ± 5.1	77.5 ± 9.2	68.4 ± 8.9	79.0 ± 7.1
Fuberidazole	53.9 ± 17.9	out of specification*	24.0 ± 19.1	41.1 ± 10.3
Furalaxyl	112.4 ± 10.1	105.8 ± 6.5	116.7 ± 11.9	101.7 ± 7.2
Furathiocarb	101.1 ± 7.6	93.4 ± 6.1	98.4 ± 6.9	94.2 ± 15.8
Halofenozide	112.2 ± 16.9	105.0 ± 13.9	107.9 ± 14.9	99.0 ± 14.3
Hexaconazole	106.6 ± 14.2	98.2 ± 10.2	97.1 ± 15.2	99.4 ± 23.5
Hexythiazox	84.4 ± 13.0	out of specification*	102.0 ± 14.7	out of specification*
Hydramethylnon	3.7 ± 7.2	out of specification*	2.1 ± 5.3	out of specification*
Imazalil	40.5 ± 21.9	41.9 ± 19.1	48.3 ± 9.3	44.7 ± 16.7
Imidacloprid	104.0 ± 12.3	103.2 ± 10.1	104.8 ± 4.5	95.4 ± 9.0
Iprovalicarb isomer 1	102.1 ± 17.5	89.0 ± 16.6	105.0 ± 8.0	96.6 ± 5.1
Iprovalicarb isomer 2	100.8 ± 8.8	102.4 ± 5.4	106.3 ± 6.1	99.1 ± 5.7
Isoprocarb	100.7 ± 12.3	96.0 ± 23.6	88.0 ± 11.9	101.0 ± 14.5
Isoproturon	105.9 ± 4.6	95.2 ± 5.3	97.7 ± 4.5	98.5 ± 9.1
Linuron	119.6 ± 10.8	116.8 ± 15.5	114.3 ± 7.2	110.3 ± 14.5
Lufenuron	74.9 ± 15.5	out of specification*	out of specification*	out of specification*
Mandipropamid	111.4 ± 11.8	109.8 ± 9.8	104.1 ± 9.4	112.5 ± 9.1
Mefenacet	93.1 ± 9.1	69.0 ± 8.5	77.4 ± 14.4	79.9 ± 11.4
Mepronil	96.7 ± 8.5	84.8 ± 15.6	87.3 ± 9.1	81.2 ± 14.5
Mesotrione	103.0 ± 8.1	88.0 ± 5.9	65.4 ± 8.9	80.7 ± 3.6
Metalaxyl	109.4 ± 5.2	95.0 ± 7.6	100.5 ± 5.4	97.6 ± 5.0
Metconazole	87.7 ± 9.8	67.0 ± 16.4	62.9 ± 6.0	71.1 ± 8.1
Methabenzthiazuron	39.8 ± 8.6	35.4 ± 11.5	40.7 ± 6.9	50.6 ± 3.9
Methiocarb	110.7 ± 12.0	103.5 ± 7.3	100.6 ± 7.3	100.5 ± 1.8
Methoprotryne	31.8 ± 7.1	22.3 ± 6.1	15.7 ± 12.5	24.3 ± 7.2
Methoxyfenozide	113.3 ± 11.4	103.5 ± 3.7	112.3 ± 9.6	106.1 ± 8.7
Metobromuron	97.8 ± 8.6	102.4 ± 11.1	104.6 ± 6.7	97.8 ± 9.8
Metribuzin	101.1 ± 9.2	102.7 ± 8.0	116.6 ± 14.9	98.9 ± 9.2
Mevinphos isomer 1	101.1 ± 6.6	98.4 ± 22.1	93.7 ± 11.4	96.1 ± 13.6
Mexacarbate	100.0 ± 6.5	103.6 ± 10.1	111.0 ± 7.7	97.5 ± 5.7
Monocrotophos	108.6 ± 11.4	96.8 ± 9.8	92.7 ± 9.1	104.6 ± 11.6
Monolinuron	108.5 ± 5.9	96.0 ± 6.9	95.3 ± 8.3	96.7 ± 9.6

Pesticide residues in cannabis with high matrix reduction

	QuEChERS clean-up mix from MACHEREY-NAGEL	QuEChERS clean-up mix from competitor 1	QuEChERS clean-up mix from competitor 2	QuEChERS clean-up mix from competitor 3
Analyte	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]
Moxidectin	92.7 ± 15.5	87.3 ± 23.9	out of specification*	out of specification*
Myclobutanil	98.3 ± 5.9	104.6 ± 5.9	101.4 ± 8.7	99.2 ± 9.8
Neburon	81.5 ± 10.0	70.6 ± 16.3	72.6 ± 3.6	78.8 ± 14.1
Nitenpyram	103.1 ± 18.3	86.6 ± 6.2	87.0 ± 16.2	91.1 ± 11.3
Nuarimol	105.8 ± 16.1	93.3 ± 7.5	92.1 ± 7.5	109.0 ± 15.4
Omethoate	115.4 ± 12.7	93.4 ± 11.2	97.7 ± 16.2	103.1 ± 15.6
Paclobutrazol	96.9 ± 3.8	95.6 ± 3.2	92.7 ± 6.1	89.2 ± 4.7
Penconazole	96.4 ± 6.3	85.0 ± 9.1	92.7 ± 3.9	92.4 ± 5.7
Phenmedipham	81.4 ± 13.6	95.2 ± 13.7	89.0 ± 9.6	95.8 ± 10.1
Picoxystrobin	108.6 ± 6.1	113.7 ± 4.8	100.2 ± 4.8	105.0 ± 15.7
Piperonyl butoxide	96.1 ± 8.5	88.7 ± 8.9	99.9 ± 6.6	88.4 ± 9.7
Pirimicarb	117.1 ± 9.6	82.2 ± 5.6	84.6 ± 7.4	90.4 ± 6.1
Prochloraz	55.6 ± 10.2	39.9 ± 9.6	38.3 ± 8.7	48.6 ± 12.5
Promecarb	91.5 ± 16.5	75.8 ± 11.8	93.9 ± 13.2	88.6 ± 6.6
Prometon	44.9 ± 9.8	22.0 ± 10.5	24.7 ± 7.5	34.6 ± 9.2
Prometryne	52.6 ± 6.4	32.9 ± 6.2	33.2 ± 2.8	40.3 ± 5.1
Propamocarb	84.8 ± 7.1	59.8 ± 19.5	50.4 ± 6.6	71.9 ± 17.8
Propargite	110.8 ± 22.0	out of specification*	119.3 ± 13.9	114.0 ± 13.1
Propiconazole isomer 1	105.3 ± 17.6	76.3 ± 16.4	84.0 ± 14.4	85.0 ± 6.3
Prothioconazole	82.8 ± 15.1	76.3 ± 18.3	out of specification*	out of specification*
Pymetrozine	12.1 ± 10.7	out of specification*	6.1 ± 8.3	3.1 ± 24.6
Pyraclostrobin	90.9 ± 10.8	65.6 ± 10.1	81.0 ± 9.9	78.6 ± 6.7
Pyridaben	88.8 ± 11.1	103.8 ± 9.7	102.7 ± 16.0	99.3 ± 20.3
Pyrimethanil	8.7 ± 17.9	6.4 ± 10.6	7.5 ± 7.0	5.9 ± 15.0
Pyriproxyfen	86.2 ± 11.1	86.0 ± 7.6	92.4 ± 3.7	92.1 ± 6.6
Quinoxyfen	9.6 ± 7.3	11.0 ± 3.6	7.8 ± 10.0	10.2 ± 15.0
Secbumeton	35.2 ± 14.3	23.9 ± 6.2	24.4 ± 13.8	33.4 ± 9.9
Siduron	99.9 ± 8.4	99.3 ± 6.1	96.5 ± 3.5	96.5 ± 6.5
Spinetoram	44.7 ± 12.8	14.4 ± 13.7	21.0 ± 20.9	45.0 ± 16.7
Spinosad (Spinosyn A)	51.7 ± 14.0	23.9 ± 10.0	29.8 ± 18.8	45.5 ± 10.6
Spinosad (Spinosyn D)	47.7 ± 14.1	21.3 ± 12.3	26.9 ± 11.3	45.5 ± 6.1
Spirotetramat	109.0 ± 8.5	92.4 ± 9.1	89.9 ± 12.1	96.9 ± 10.8
Spiroxamine isomer 1	54.6 ± 22.5	46.0 ± 13.1	46.0 ± 4.2	55.3 ± 11.3
Spiroxamine isomer 2	66.1 ± 3.6	44.3 ± 8.5	45.7 ± 3.7	57.5 ± 10.6
Tebuconazole	83.7 ± 8.8	71.6 ± 6.6	72.6 ± 10.0	76.5 ± 11.8
Tebufenozide	102.5 ± 7.7	103.8 ± 12.7	104.9 ± 5.3	110.9 ± 8.6
Tebufenpyrad	78.6 ± 9.1	82.7 ± 11.4	87.0 ± 9.4	88.8 ± 7.7
Tebuthiuron	97.5 ± 5.8	81.8 ± 9.0	82.6 ± 6.8	91.5 ± 9.2
Temephos	86.6 ± 21.3	out of specification*	out of specification*	90.1 ± 20.9
Terbutryn	49.5 ± 4.9	34.2 ± 4.5	34.4 ± 2.1	46.6 ± 3.6
Tetraconazole	108.6 ± 16.0	101.4 ± 7.7	105.9 ± 11.0	101.4 ± 5.8
Thiamethoxam	118.7 ± 9.2	96.6 ± 4.7	107.8 ± 10.9	113.6 ± 6.1
Thiobencarb	102.5 ± 10.6	out of specification*	102.9 ± 10.4	103.8 ± 17.1
Thiophanate-methyl	109.2 ± 8.0	107.9 ± 9.8	99.1 ± 8.5	95.4 ± 13.8
Triadimefon	104.8 ± 9.2	103.3 ± 9.4	104.6 ± 4.6	117.9 ± 9.3
Triadimenol	102.7 ± 7.4	102.2 ± 4.0	97.1 ± 8.6	102.3 ± 3.0

	QuEChERS clean-up mix from MACHEREY-NAGEL	QuEChERS clean-up mix from competitor 1	QuEChERS clean-up mix from competitor 2	QuEChERS clean-up mix from competitor 3
Analyte	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]	Recovery rate [%]
Trichlorfon	113.7 ± 17.4	99.9 ± 21.6	out of specification*	99.1 ± 5.8
Tricyclazole	24.2 ± 15.7	out of specification*	out of specification*	25.7 ± 18.1
Trifloxystrobin	108.0 ± 17.4	98.7 ± 5.8	out of specification*	102.2 ± 10.7
Triflumizole	113.7 ± 10.1	108.1 ± 14.3	93.2 ± 6.5	104.0 ± 12.0
Triflumuron	89.4 ± 11.6	88.0 ± 17.0	92.2 ± 7.6	105.8 ± 9.5
Triticonazole	93.9 ± 7.6	83.1 ± 5.9	77.7 ± 6.2	90.3 ± 10.1
Vamidothion	117.2 ± 14.0	94.4 ± 11.5	94.6 ± 13.4	91.1 ± 4.5
Zoxamide	103.1 ± 12.1	101.4 ± 6.3	114.6 ± 10.4	105.7 ± 7.0

Table 2: Recovery rates for presented QuEChERS clean-up mixes. The recovery rates that are calculated by matrix matched standard calibration. *specification = 0 – 120 % ± 25 %

Conclusion

This application note shows the reliable and successful determination of pesticide residues from marijuana samples with an optimized QuEChERS method. The optimized composition of QuEChERS salt clean-up mixes leads to high reduction of matrix components and to high recovery rates for pesticides. The presented QuEChERS method leads to an average recovery rate for pesticides of 92.3 % for 162 pesticides. Most of the pesticides (138 analytes) show recovery rates between 70 % to 120 %. On the other side, high matrix reduction yields were also possible by using the presented clean-up mix. The amount of dry substance after clean-up is reduced to less than 70 % of raw acetonitrile extracts. With this clean-up approach interfering substances (like e.g., lipids and pigments) are successfully removed due to high amounts of GCB and CHROMABOND® C_{18} ec adsorbents.

The chromatographic separation of pesticides was performed by using core-shell particles that are well known for fast and high-efficient separations combined with a reasonably low back pressure. In this work, a subsequent analytics was developed on a NUCLEOSHELL® Bluebird RP 18 column.

References

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

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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

MACHEREY-NAGEL application department · Dr. H. R. Wollseifen, T. Kretschmer, S. Schneider

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, 729231) into a vial for HPLC analysis (REF 702293, REF 702107)

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 (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

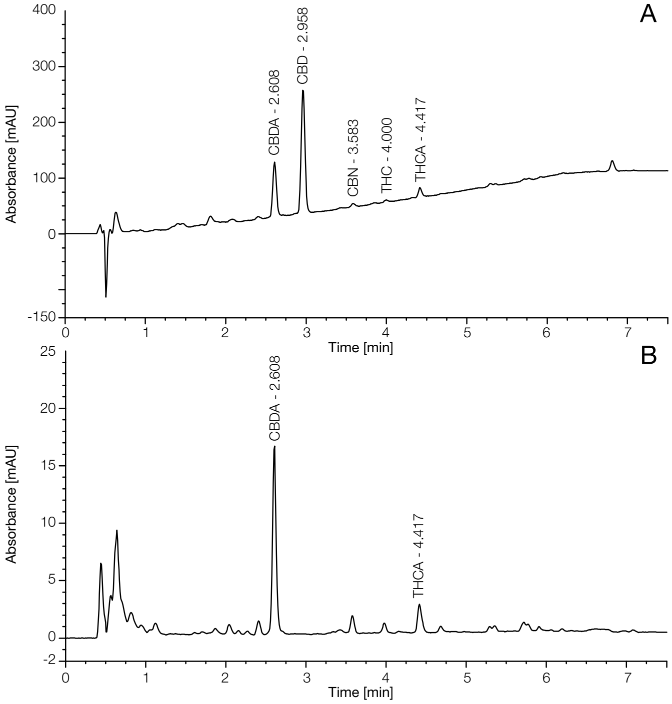

Figure 2: Chromatograms of an extract from hemp flower tea, @ 225 nm (A), 306 nm (B).

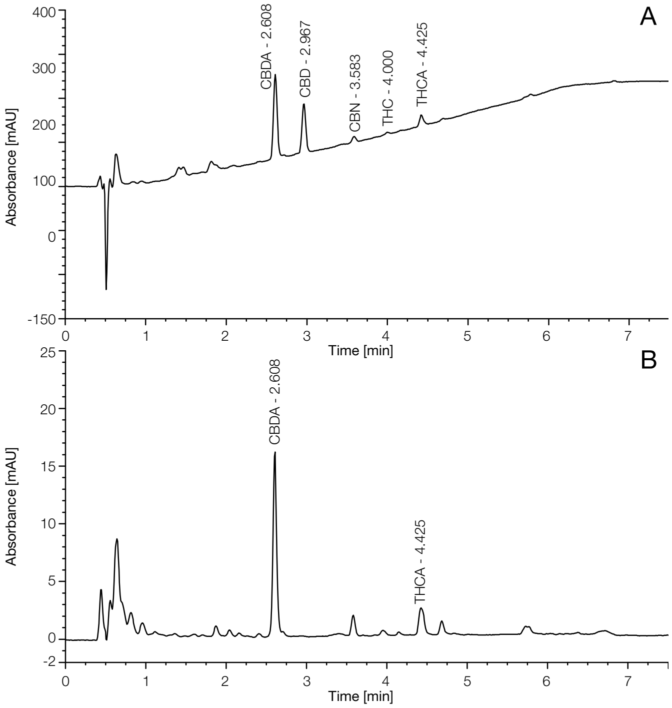

Figure 3: Chromatograms of an extract from hemp flower flour, @ 225 nm (A), 306 nm (B).

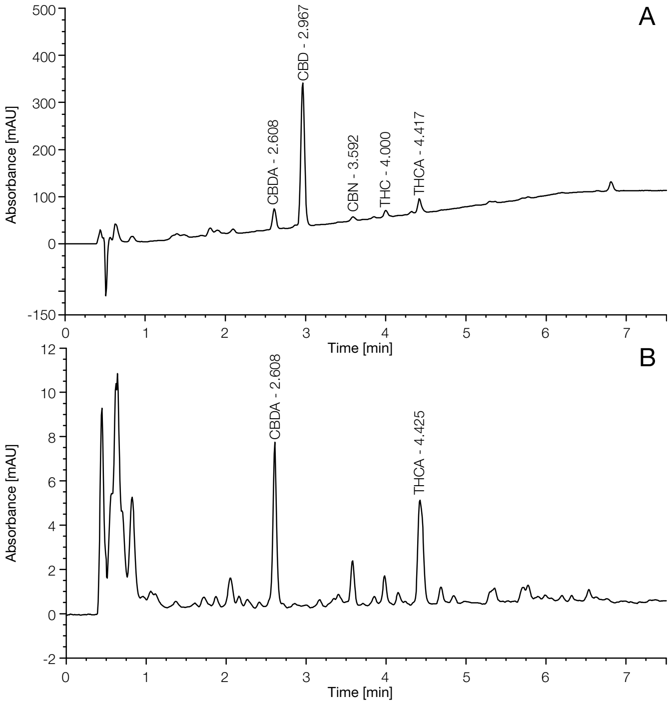

Figure 4: Chromatograms of an extract from hemp flower buds, @ 225 nm (A), 306 nm (B).

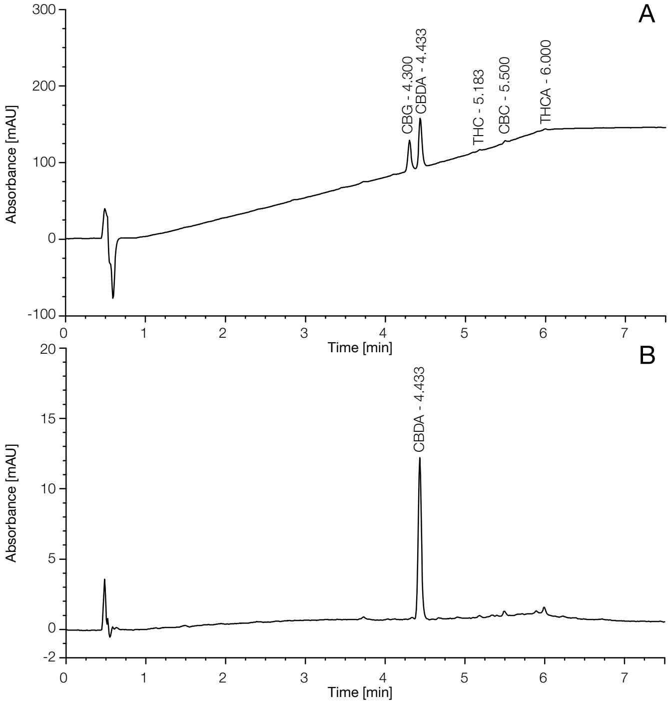

Figure 5: Chromatograms of an extract from hemp oil, @ 225 nm (A), 306 nm (B).

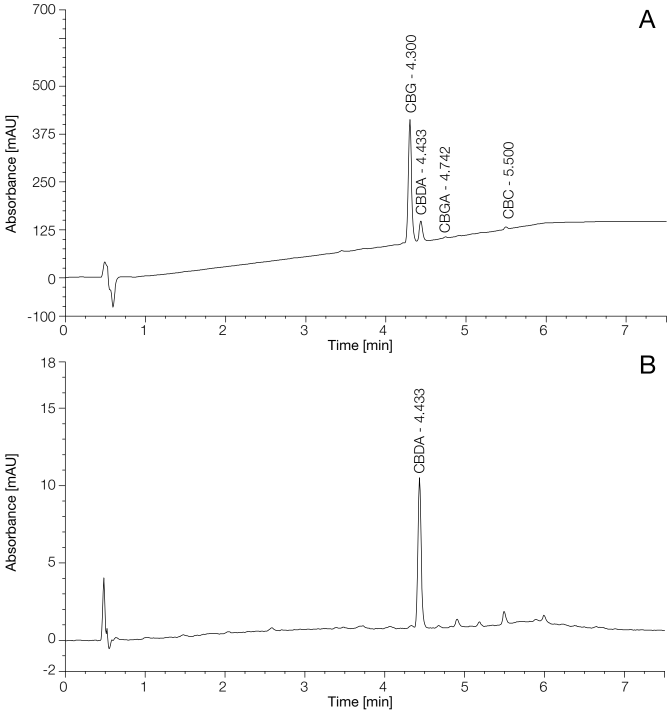

Figure 6: Chromatograms of an extract from hemp CO$_2$ extract, @ 225 nm (A), 306 nm (B).

Figure 10: Hemp flower buds, hemp flour and hemp tea (from left to right).

Determination of cannabinoids in hemp products

MS/MS identification of cannabinoids

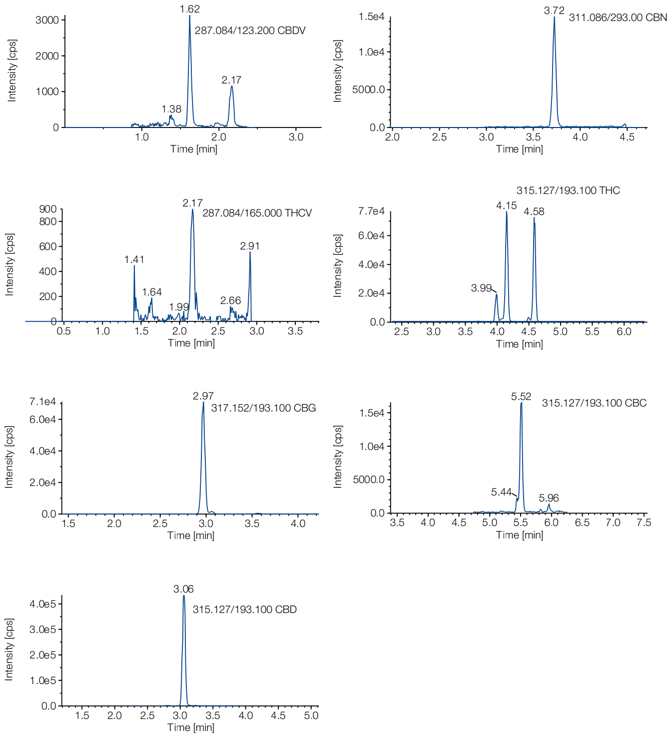

Figure 8: Chromatogram of an extract from hemp flower tea (positive polarity).

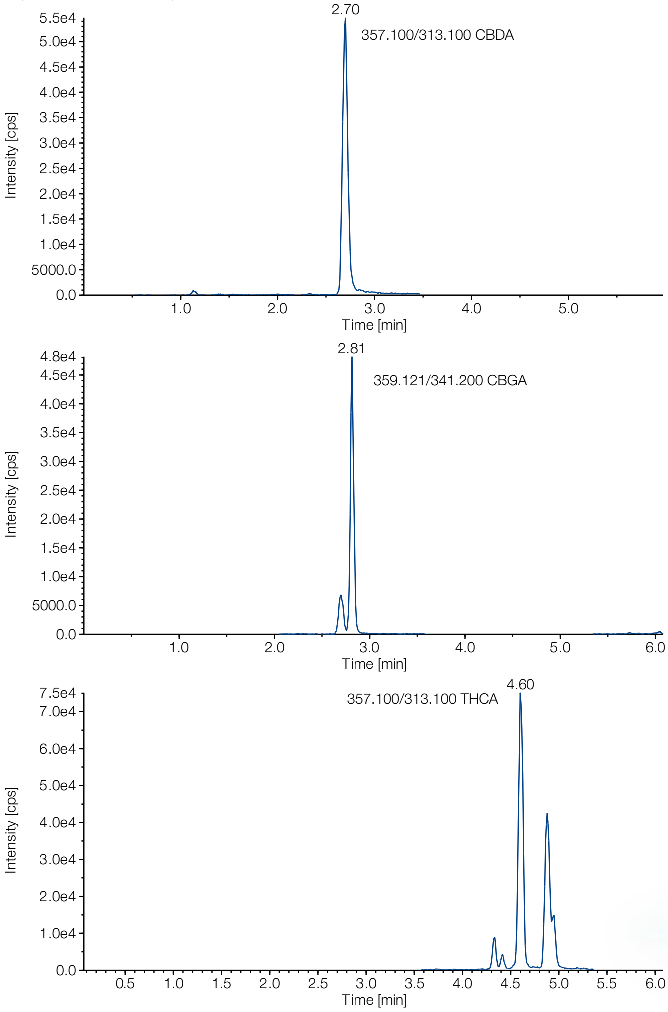

Figure 9: Chromatogram of an extract from hemp flower tea (negative polarity).

Calibration curves of CBD, CBDA, THC

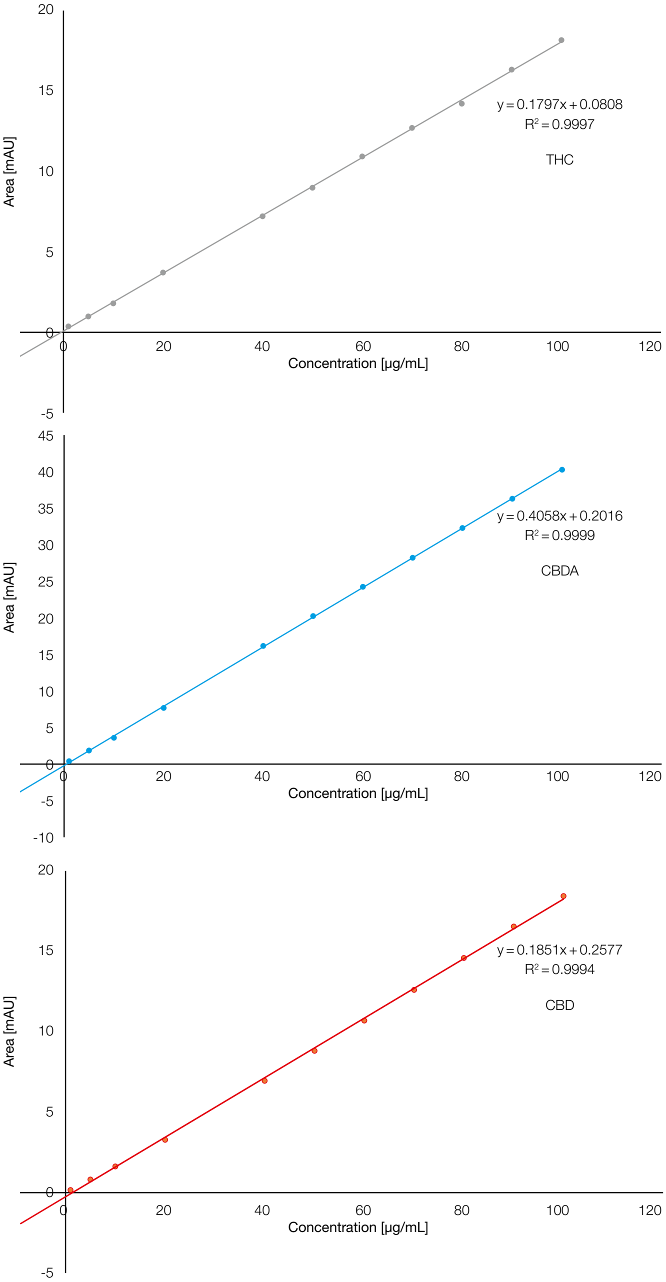

Figure 10: Calibration curves of Cannabidiol, Cannabidiolic acid, Δ^9-tetrahydrocannabinol.

Figure 7: 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.

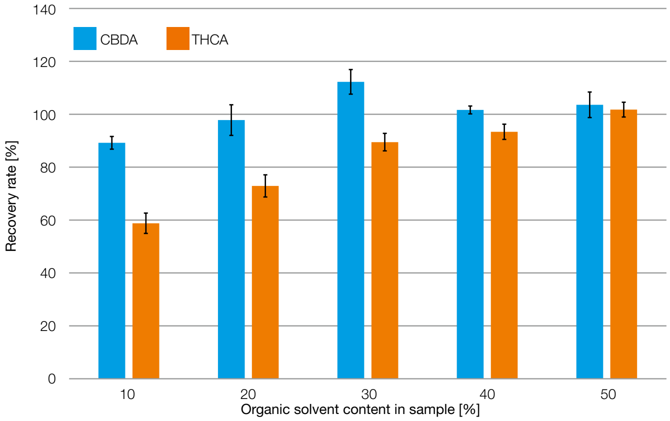

Figure 12: Recovery rates of CBD and THC, detection @ 225 nm.

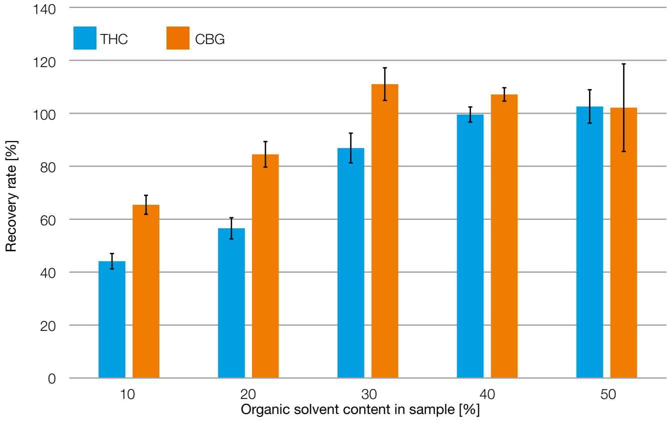

Figure 13: Recovery rates of CBDA and THCA, detection @ 306 nm.

Representative chromatograms for hemp products spiked with Cannabis sativa

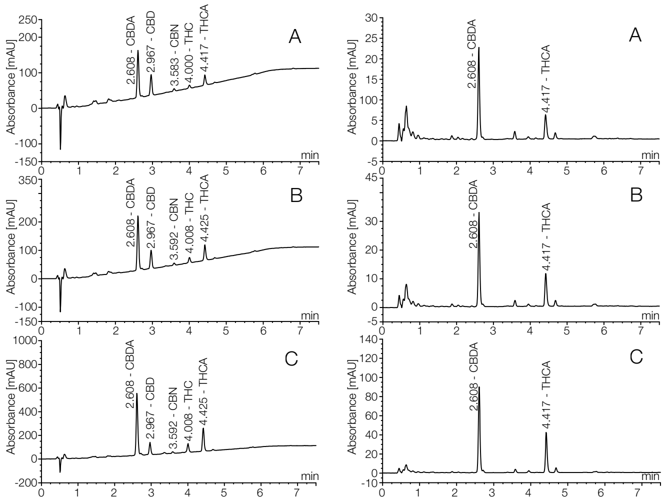

Figure 14: Chromatograms of extracts with a mixture of hemp flower tea and Cannabis sativa, spiking level A 1 %, spiking level B 2.5 %, spiking level C 10 %.

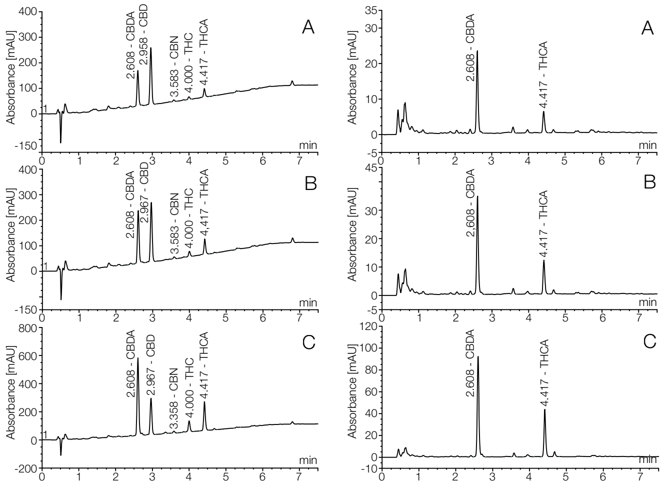

Figure 15: Chromatograms of extracts with a mixture of hemp flower flour and Cannabis sativa, spiking level A 1 %, spiking level B 2.5 %, spiking level C 10 %.

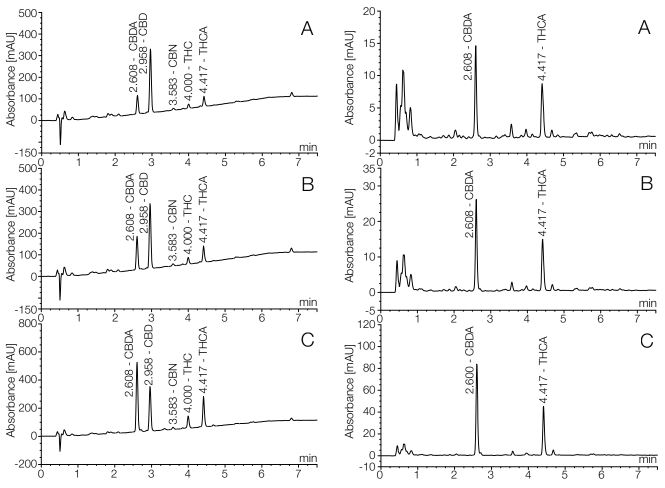

Figure 16: Chromatograms of extracts with a mixture of hemp flower buds and Cannabis sativa, spiking level A 1 %, spiking level B 2.5 %, spiking level C 10 %.

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.

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Determination of cannabinoids (THC) in human urine with LC-MS

MACHEREY-NAGEL application department · Dr. H. R. Wollseifen

Abstract

This application note describes the determination of THC and its metabolites (THC–OH and THC–COOH) from urine in the lower ng/mL range using manual solid phase extraction or the LCTech FreeStyle™ liquid handling system. Since the target analytes are secreted as glucuronide conjugates in human urine, it is necessary to cleave the glucuronides prior to solid phase extraction. This is done by basic hydrolysis. The pretreated sample solution is then subjected to a solid phase extraction (SPE) step and finally analyzed by HPLC.

Introduction

Cannabis, also known as Marihuana or Hashish, is the most widely consumed drug in the world. Its consumption leads to mood-altering behavior like euphoria, relaxation and altered-time perception. A routinely consumption can lead to dependence and tolerance [1,2]. In recent years there is also an increasing interest in therapeutic effects of cannabinoids and the development of potential cannabinoid medications. Therefore it is investigated for treatment of chronic pain, muscle spasticity, nausea and AIDS wasting disease, for instance [1,3,4]. This also leads to an increasing demand for the development of accurate and sensitive analytical methods for the quantification of cannabinoids in biological fluids [1]. The measurement of urine cannabinoids is necessary for pharmacokinetic studies, drug treatment, workplace drug testing and for drug impaired driving investigations. Detailed urine collection procedures with comprehensive chain-of-custody documentation have been developed for forensic applications [1].

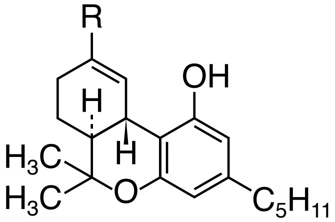

Figure 1: Compound of interest.

Analyte	R	Formula	Mass [g/mol]
THC	CH_3	$C_{21}H_{30}O_2$	314.5
THC–OH	CH_2OH	$C_{21}H_{30}O_3$	330.5
THC–COOH	COOH	$C_{21}H_{28}O_4$	344.4

Table 1: Overview of the analytes.

Sample pretreatment – alkaline hydrolysis

5 mL urine are spiked with a) 50 µL standard solution and b) 100 µL standard solution resulting in samples with a) 50 ng each of THC, THC–OH and THC–COOH and b) 100 ng each of the respective standards. The glucuronide is hydrolyzed by treating 5 mL of the spiked urine (a) c = 10 ng/mL b) c = 20 ng/mL) with 300 µL NaOH solution (10 mol/L) at 60 °C for 15 min in a heating block. The hydrolyzed solution is cooled and mixed with 200 µL glacial acetic acid. Then 2 mL ammonium acetate solution (50 mmol/L) are added and the mixture is adjusted to pH 6–7 with either acetic acid or diluted NaOH solution. The sample is transferred into a vial N 18. The hydrolysis tube is rinsed with 3 mL methanol which are combined with the sample solution.

Solid phase extraction (manual procedure)

(All steps with a flow of 1–2 mL / min)

Column type:

CHROMABOND® HR-X polypropylene columns (85 µm), 3 mL, 200 mg, (REF 730931)

Conditioning:

2 mL methanol, 2 mL water, 2 mL ammonium acetate buffer (50 mmol/L)

Sample application:

with a flow of 1–2 mL/min

Washing:

5 mL water – methanol (7:3, v/v), then drying for 10 min

Elution:

3 mL hexane – ethyl acetate – glacial acetic acid (75:25:1, v/v/v)

Determination of THC in human urine with LC-MS

Automated solid phase extraction (LCTech FreeStyle™ SPE module)

Step		Volume	Dispensing speed	Waiting time after dosage	Dispense into
Conditioning	with methanol	2 mL	2 mL/min	10 s	waste
	with water	2 mL	2 mL/min	10 s	waste
	with ammonium acetate buffer (50 mmol/L)	2 mL	2 mL/min	10 s	waste
Load	quantitative transfer over sample loop from vial type 1@16 mL	12 mL	1 mL/min	25 s	
	rinse vial 3 times with water – methanol (7:3, v/v) tube rinse volume: 1 mL	1 mL	0.3 mL/min		waste
Washing	water – methanol (7:3, v/v)	5 mL	2 mL/min	35 s	waste
Drying	with nitrogen (120 s)				waste
Elution	hexane – ethyl acetate – glacial acetic acid (75:25:1, v/v/v)	3 mL	5 mL/min	15 s	vial type 1@4 mL
Drying	with air	20 mL	100 mL/min		

Table 2: Automated procedure for solid phase extraction with LCTech Freestyle™ SPE module.

Eluent exchange:

Eluent exchange is performed manually. Eluates from the SPE are evaporated to dryness at 40 °C under a stream of nitrogen and then redissolved in an organic solvent suited for the subsequent analysis. For HPLC-MS/MS 1 mL methanol is used.

Subsequent analysis: HPLC-MS/MS

Chromatographic conditions

Column:

EC 50/2 NUCLEOSHELL® RP 18, 2.7 µm, (REF 763132.20)

Eluent A:

water (ultrapure) + 0.1 % formic acid

Eluent B:

acetonitrile + 0.1 % formic acid

Gradient:

50–100 % B in 2.5 min, 100 % B for 2.5 min, 100–50 % B in 0.1 min, 50 % B for 2.4 min

Flow rate:

0.3 mL/min

Temperature:

40 °C

Injection volume:

5 µL

MS conditions:

API 3200 (AB Sciex), ion source ESI, positive ionization mode, curtain gas 20 psig, ion spray voltage 5500 V, temperature 550 °C, nebulizer gas 20 psig, turbo gas 20 psig, CAD 6.0 psig

MRM transitions

Analyte	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
THC	315.2	193.2	123.1
THC–OH	331.2	313.3	43.1
THC–COOH	345.2	327.3	299.4

Table 3: MRM transitions for the analysis of THC and THC derivates.

Chromatograms

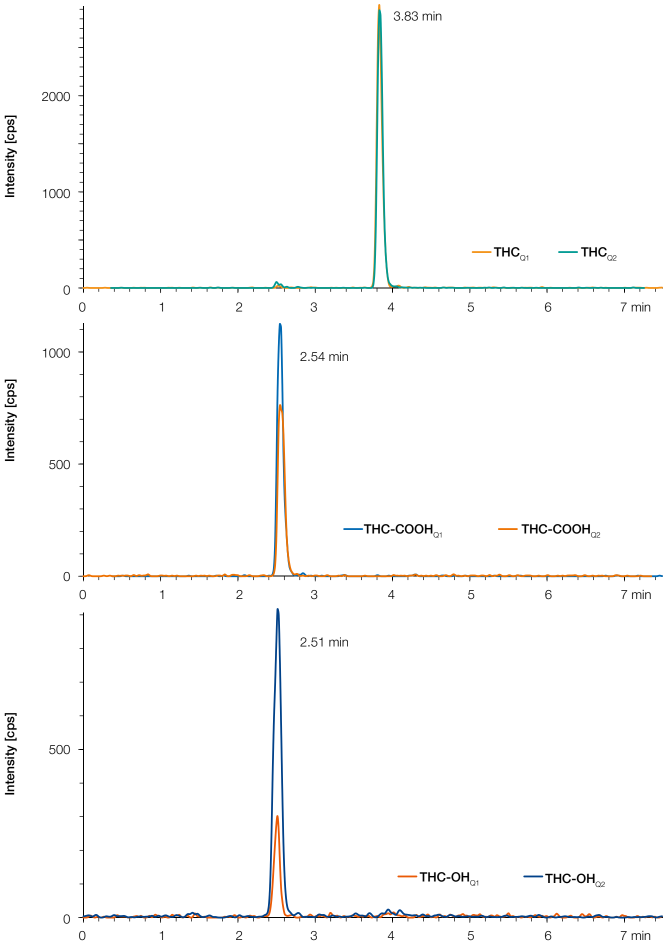

Figure 2: Chromatograms of HPLC-MS/MS analysis.

Determination of THC in human urine with LC-MS

Calibration curves

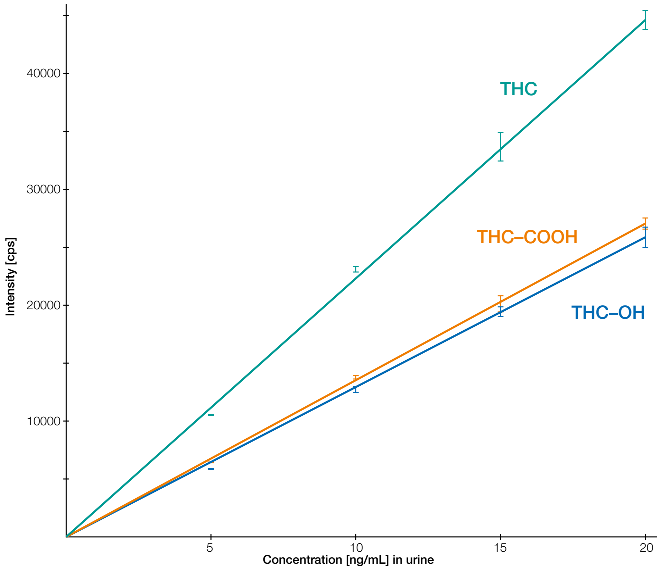

Figure 3: Calibration curves of THC and THC-derivates in urine matrix.

Recovery rates

Analyte	Recovery rate [in %]
Manual SPE	
THC	80 ± 3
THC–OH	77 ± 10
THC–COOH	98 ± 8
Automated SPE (LCTech FreeStyle™)	
THC	90 ± 9
THC–OH	80 ± 6
THC–COOH	93 ± 8

Table 4: Recovery rates for the manual and automated SPE methods (average from triple determination).

LOD/LOQ

	THC	THC–OH	THC–COOH
LOD (ng/mL urine)	0.12	0.75	0.33
LOQ (ng/mL urine)	0.17	1.29	0.75

Table 5: Limit of quantification (LOQ) and limit of detection (LOD), the concentration that provides a signal-to-noise ratio (S/N) of > 3 was considered as LOD and S/N > 10 was considered as LOQ.

Conclusion

Using the automated FreeStyle™ SPE sample preparation system in combination with CHROMABOND® HR-X columns and a subsequent LC-MS analysis using NUCLEOSHELL® RP 18 showed reliable results for the analysis of THC and its metabolites from human urine. Using the automated system even better recovery rates were found than using a manual SPE method.

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4. Blake, D. R., Robson, P., Ho, M., Jubb, R. W. McCabe, C. S., Preliminary assessment of the efficiacy, tolerability and safety of a cannabis-based medicine in the treatment of pain caused by rheumatoid arthritis, Rheumatology, 45 (1): 50–52 (2006).

Additional information

The following applications regarding “The Determination of THC in human urine” and further applications can be found on our online application database at *https://ChromaAppDB.mn-net.com*

SPE (manual procedure):	MN Appl. No. 305990
SPE (automated procedure):	MN Appl. No. 306000
HPLC:	MN Appl. No. 127380

Acknowledgment

We thank the company LCTech GmbH for the cooperation and for providing the robotic system, called FreeStyle® SPE module.

MACHEREY-NAGEL homepage

Website Relaunch 2020

Take a look at our new website!

Our brand new designed website is a webshop with a wide range of products.

- Intuitive – Find your product of interest in only 3 clicks!
- Easy – Clearly arranged product information
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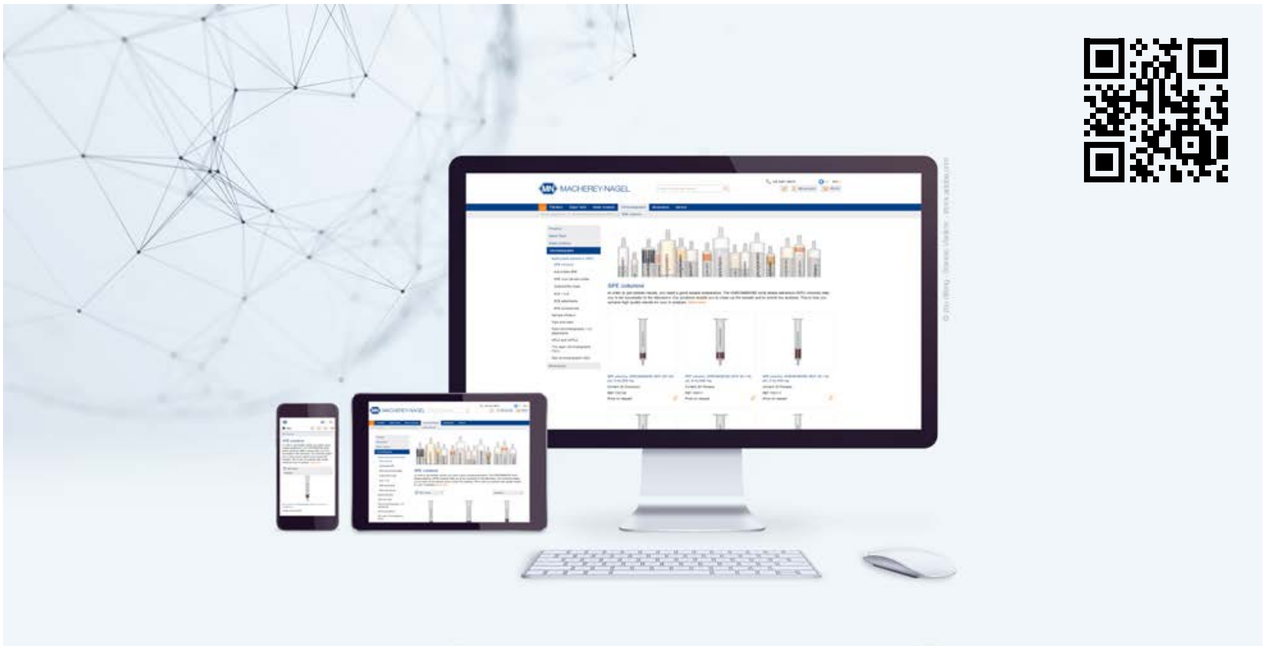

Determination of cannabinoids (THC) in urine samples with GC-MS

MACHEREY-NAGEL application department · Dr. H. R. Wollseifen, A. Mengus-Kaya, T. Kretschmer

Abstract

This application describes the determination of cannabinoids from urine matrix, prior to GC-MS analysis.

Introduction

There is an increasing interest in the determination of cannabinoids from different matrices like urine, hair and blood for pharmacokinetic studies, drug treatment, workplace drug testing, drug impaired driving investigations, and for evaluating the time of cannabis use. Fast and sensitive procedures capable of quantifying THC and its metabolites are necessary for all this application fields.

Delta-9-tetrahydrocannabinol (THC) is the major psychoactive component of marijuana and it will be quickly metabolized to hydroxylated and carboxylated forms (THC-OH, THC-COOH) after consumption [1]. THC, THC-OH and THC-COOH are conjugated to glucuronide conjugates in human body to enhance water solubility facilitating urinary excretion [2]. Total urine cannabinoid conjugates can only be quantified by gas chromatography–mass spectrometry (GC-MS) methods after hydrolysis prior to extraction of urine sample. After a liquid-liquid extraction procedure of hydrolyzed urine sample the extract was concentrated and derivatized with MSTFA [3].

Using deuterated internal standards by GC-MS facilitates the identification and quantitation of the focused analytes in sample extracts.

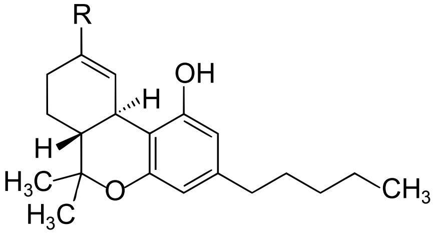

Figure 4: THC and its metabolites.

Analyte	R	Formula	M [g/mol]
THC	CH_3	$C_{21}H_{30}O_2$	314.5
THC-OH	CH_2OH	$C_{21}H_{30}O_3$	330.5
THC-COOH	COOH	$C_{21}H_{28}O_4$	344.4

Table 6: Compounds of interest.

Sample pretreatment

- Homogenize urine sample by stirring
- Fill 1 mL sample into a safe-lock tube (2 mL)
- Add standard solution, internal standard solution like described in table 2
- Add 50 µL of β-glucuronidase solution (β = 2500 units/mL) and 125 µL phosphate buffer with pH 6.8
- Shake mixture and incubate in a shaking bath at 37 °C over night (shaking speed 2000)
- Cool down samples at room temperature
- Add 125 µL sodium hydroxide solution (0.5 N) and 500 µL of a mixture (ethyl acetate – *n*-hexane (9+1, v+v))
- Shake vigorously for 30 sec
- Centrifuge at room temperature at 13400 rpm for 10 min
- Take up organic phase for derivatization in a vial
- Add 125 µL acetic acid (99 %) and 500 µL of a mixture (ethyl acetate – *n*-hexane (9+1, v+v)) to aqueous phase
- Shake vigorously for 30 min
- Centrifuge at room temperature at 13400 rpm for 10 min
- Add organic phase to the organic phase of first extraction procedure
- Concentrate organic phase at 40 °C under nitrogen stream to dryness
- Add 50 µL ethyl acetate and 50 µL MSTFA (REF 701270.201)
- Shake vigorously for 30 sec and incubate mixture at 80 °C for 30 min
- Take sample solution for GC-MS analysis

Spike level [ng/mL]	Volume internal standard mixture [µL]	Volume standard mixture [µL]
0	50	0 (β = 1 µg/mL)
25	50	50 (β = 1 µg/mL)
50	50	100 (β = 1 µg/mL)
100	50	40 (β = 5 µg/mL)
150	50	60 (β = 5 µg/mL)
200	50	80 (β = 5 µg/mL)
250	50	100 (β = 5 µg/mL)

Table 7: Pipette scheme for sample pretreatment.

Subsequent analysis: GC-MS

Chromatographic conditions

Column:
Optima® 5 HT, 0.25 µm, 30 m, 0.25 mm ID (REF 726106.30)

Injection volume:
1 µL

Injection Mode:
Splitless

Injection Temperature:
250 °C

Carrier Gas:
Helium

Column Flow:
1.31 mL/min

Oven Programm:
70 °C [2 min] → [20 °C/min] → 250 °C [4 min] → [20 °C/min] → 300 °C [17 min]

MS conditions:
GCMS-QP2010plus, Shimadzu, ion source EI, scan type SIM

Tune:
Autotune

Ion Source temperature:
200 °C

Interface temperature:
250 °C

Solvent delay:
4 min

Analyt	Retention time [min]	M/Z Registered in SIM mode
Tetrahydrocannabinol (THC)	13.465	303, 371, 386
Hydroxy Tetrahydrocannabinol (THC-OH)	16.055	371, 459, 474
Carboxy Tetrahydrocannabinol (THC-COOH)	17.180	371, 473, 488
D_3-Tetrahydrocannabinol (THC-D_3)	13.440	306, 374, 389
D_3-Hydroxy Tetrahydrocannabinol (THC-OH-D_3)	16.030	374, 462, 477
D_3-Carboxy Tetrahydrocannabinol (THC-COOH-D_3)	17.160	374, 476, 491

Table 8: M/Z Registered in SIM mode for cannabinoids.

Chromatograms

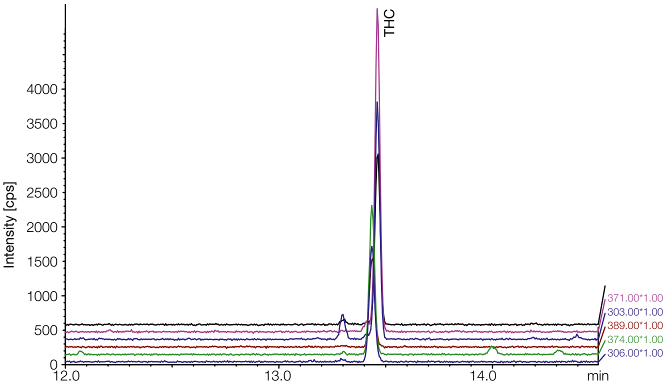

Figure 5: Chromatograms of THC and THC-D_3 from standard solution (β = 100 ng/mL).

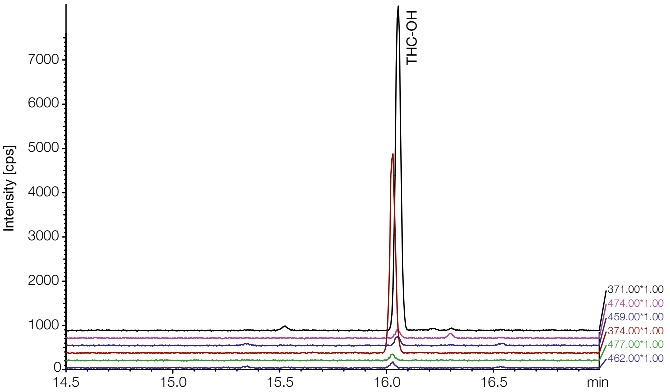

Figure 6: Chromatograms of THC-OH and THC-OH-D_3 from standard solution (β = 100 ng/mL).

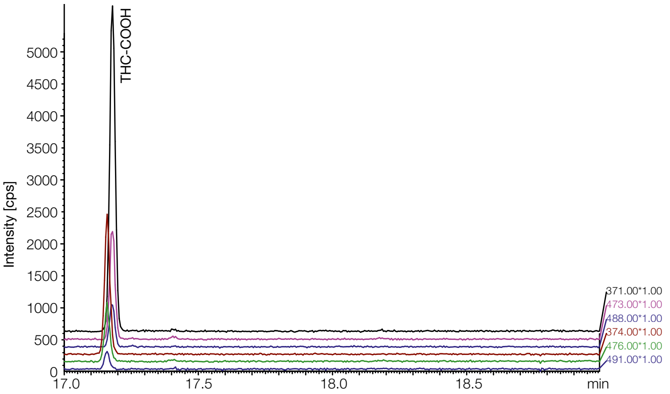

Figure 7: Chromatograms of THC-COOH and THC-COOH-D_3 from standard solution (β = 100 ng/mL).

Chromatograms (cont.)

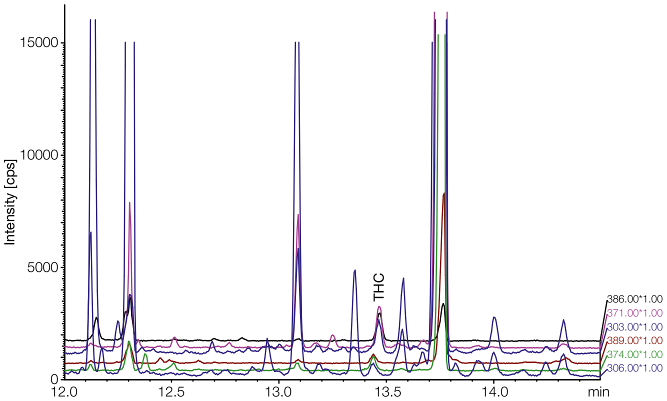

Figure 8: Chromatograms of THC and THC-D_3 from spiked urine sample ($\beta = 150$ ng/mL).

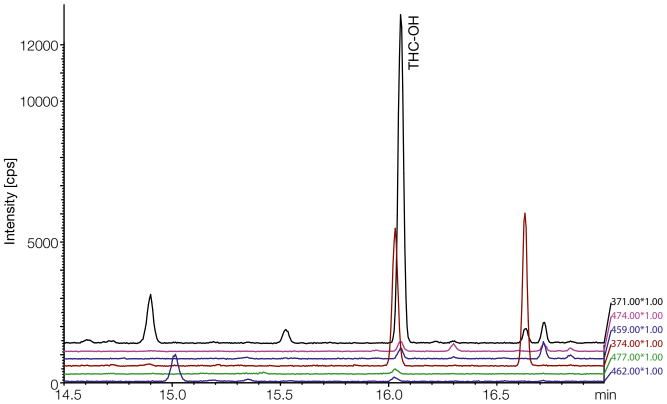

Figure 9: Chromatograms of THC-OH and THC-OH-D_3 from spiked urine sample ($\beta = 150$ ng/mL).

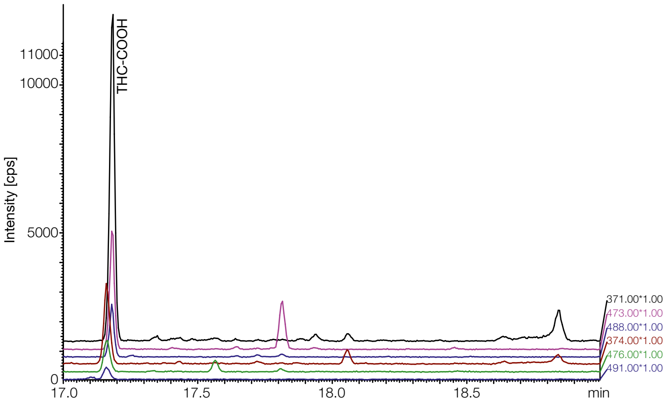

Figure 10: Chromatograms of THC-COOH and THC-COOH-D_3 from spiked urine sample ($\beta = 150$ ng/mL).

Calibration curves

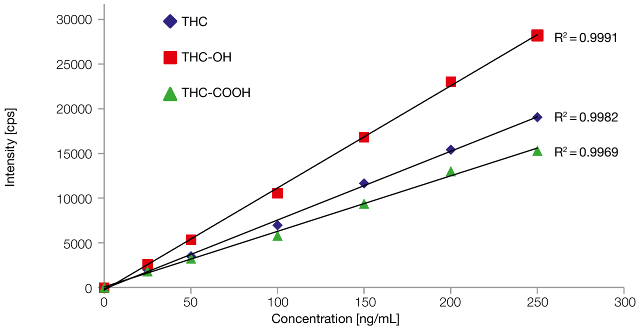

Figure 11: Calibration curves for cannabinoids in concentration range between 25 ng/mL and 250 µg/mL with an excellent coefficient of determination from standard solutions.

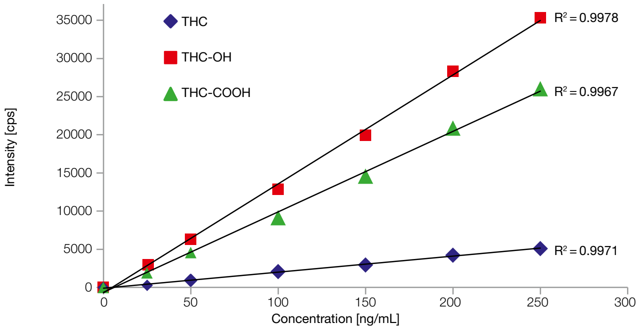

Figure 12: Calibration curves for cannabinoids in concentration range between 25 ng/mL and 250 µg/mL with an excellent coefficient of determination from urine samples.

Conclusion

This application note shows that the determination of cannabinoids from urine samples could be carried out successfully with all the tested products. The calibration curve from standard solution and urine samples indicate good linearity with excellent correlation coefficients. Sample preparation and derivatization procedure were simple and efficient for the determination of THC and its metabolites from urine sample matrix. By using a MS detector with higher sensitivity or a solid phase extraction method, it would be possible to determine cannabinoids from urine in lower concentration levels. In summary the presented application describes a quick and convenient method for the determination of cannabinoids from urine with a simple and efficient sample preparation procedure.

References

1. Constituents of Cannabis sativa. Georg Thieme Verlag Stuttgart · New York.
2. F. Grotenhermen, Journal of Cannabis Therapeutics, Vol. 3(1) 2003, Clinical Pharmacokinetics of Cannabinoids.
3. T. Nadulski, F. Sporkert, M. Schnelle, A.M.I Stadelmann, P. Roser, T. Schefter, F. Pragst, Journal of Analytical Toxicology, Volume 29, Issue 8, 1 November 2005, Pages 782–789.

Determination of THC in human urine with GC-MS

Additional information

The following application regarding “Determination of cannabinoids (THC) in urine samples with GC-MS” and further applications can be found on our online application database at *https://ChromaAppDB.mn-net.com*

GC: MN Appl. No. 215340

Determination of cannabinoids (THC) in plasma and serum samples with GC-MS

MACHEREY-NAGEL application department · Dr. H. R. Wollseifen, A. Mengus-Kaya, T. Kretschmer

Abstract

This application describes the determination of cannabinoids from plasma and serum matrix, prior to GC-MS analysis.

Introduction

There is an increasing interest in the determination of cannabinoids from different matrices like plasma or serum for pharmacokinetic studies, drug impaired driving investigations, and for evaluating the time of cannabis use. Fast and sensitive procedures capable of quantifying THC and its metabolites are necessary for all this application fields.

Delta-9-tetrahydrocannabinol (THC) is the major psychoactive component of marijuana and it will be quickly metabolized to hydroxylated and carboxylated forms (THC-OH, THC-COOH) after consumption [1, 2]. The ratio of THC-OH to the parent compound can be used to interpret the approximate time of use. Cannabis consumption can be proofed by gas chromatography–mass spectrometry (GC-MS) methods by analyzing THC and its metabolized hydroxylated and carboxylated forms (THC-OH, THC-COOH). After a liquid-liquid extraction procedure of biological sample fluid the extract was concentrated and derivatized with MSTFA [3].

Using deuterated internal standards by GC-MS facilitates the identification and quantitation of the focused analytes in sample extracts.

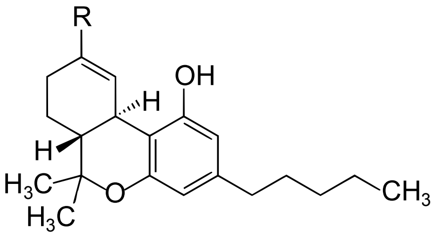

Figure 13: THC and its metabolites.

Analyte	R	Formula	M [g/mol]
THC	CH_3	$C_{21}H_{30}O_2$	314.5
THC-OH	CH_2OH	$C_{21}H_{30}O_3$	330.5
THC-COOH	COOH	$C_{21}H_{28}O_4$	344.4

Table 9: Compounds of interest.

Sample pretreatment

- Homogenize biological fluid sample by stirring
- Fill 0.5 mL sample into a safe-lock tube
- Add standard solution, internal standard solution as described in table 2
- Add 25 µL acetic acid (25 %) and 1000 µL of a mixture of ethyl acetate – *n*-hexane (9+1, v+v)
- Shake vigorously for 30 sec
- Centrifuge at room temperature at 13400 rpm for 10 min
- Take up 500 µL of the organic phase for derivatization in a vial
- Concentrate organic phase at 40 °C under nitrogen stream to dryness
- Add 50 µL ethyl acetate and 50 µL MSTFA (REF 701270.201)
- Shake vigorously for 30 sec and incubate mixture at 80 °C for 30 min
- Take sample solution for GC-MS analysis

Spike level [ng/mL]	Volume internal standard mixture [µL]	Volume standard mixture [µL]
0	50	0 (β = 1 µg/mL)
25	50	50 (β = 1 µg/mL)
50	50	100 (β = 1 µg/mL)
100	50	40 (β = 5 µg/mL)
150	50	60 (β = 5 µg/mL)
200	50	80 (β = 5 µg/mL)
250	50	100 (β = 5 µg/mL)

Table 10: Pipette scheme for sample pretreatment.

Determination of THC in plasma and serum with GC-MS

Subsequent analysis: GC-MS

Chromatographic conditions

Column:
Optima® 5 HT, 0.25 µm, 30 m, 0.25 mm ID (REF 726106.30)

Injection volume:
1 µL

Injection Mode:
Splitless

Injection Temperature:
250 °C

Carrier Gas:
Helium

Column Flow:
1.31 mL/min

Oven Programm:
70 °C [2 min] → [20 °C/min] → 250 °C [4 min] → [20 °C/min] → 300 °C [17 min]

MS conditions:
GCMS-QP2010plus, Shimadzu, ion source EI, scan type SIM

Tune:
Autotune

Ion Source temperature:
200 °C

Interface temperature:
250 °C

Solvent delay:
4 min

Analyt	Retention time [min]	M/Z Registered in SIM mode
Tetrahydrocannabinol (THC)	13.465	303, 371, 386
Hydroxy Tetrahydrocannabinol (THC-OH)	16.055	371, 459, 474
Carboxy Tetrahydrocannabinol (THC-COOH)	17.180	371, 473, 488
D_3-Tetrahydrocannabinol (THC-D_3)	13.440	306, 374, 389
D_3-Hydroxy Tetrahydrocannabinol (THC-OH-D_3)	16.030	374, 462, 477
D_3-Carboxy Tetrahydrocannabinol (THC-COOH-D_3)	17.160	374, 476, 491

Table 11: M/Z Registered in SIM mode for cannabinoids.

Chromatograms

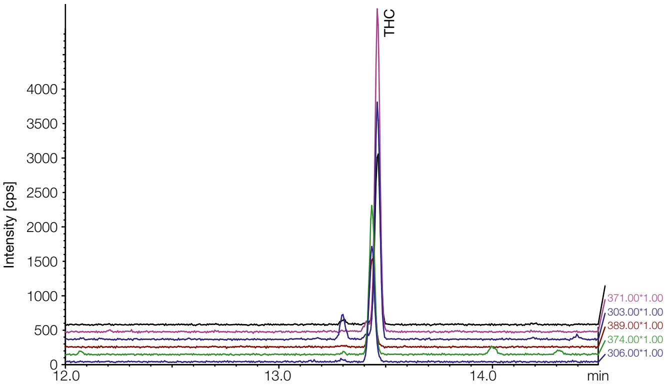

Figure 14: Chromatograms of THC and THC-D_3 from standard solution (β = 100 ng/mL).

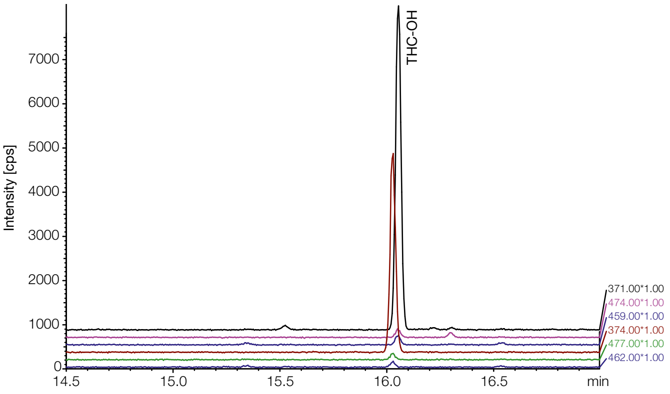

Figure 15: Chromatograms of THC-OH and THC-OH-D_3 from standard solution (β = 100 ng/mL).

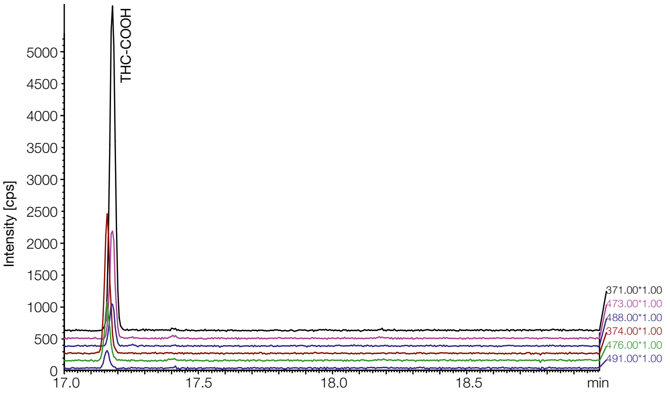

Figure 16: Chromatograms of THC-COOH and THC-COOH-D_3 from standard solution (β = 100 ng/mL).

Chromatograms (cont.)

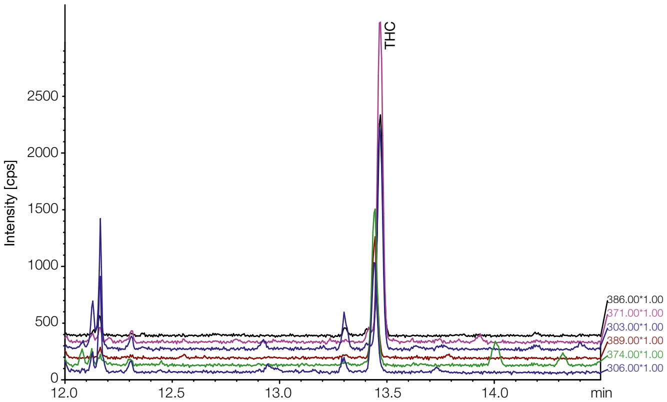

Figure 17: Chromatograms of THC and THC-D_3 from spiked plasma sample ($\beta = 100$ ng/mL).

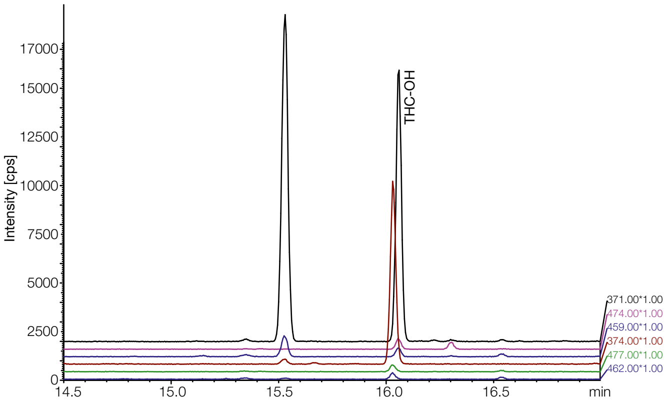

Figure 18: Chromatograms of THC-OH and THC-OH-D_3 from spiked plasma sample ($\beta = 100$ ng/mL).

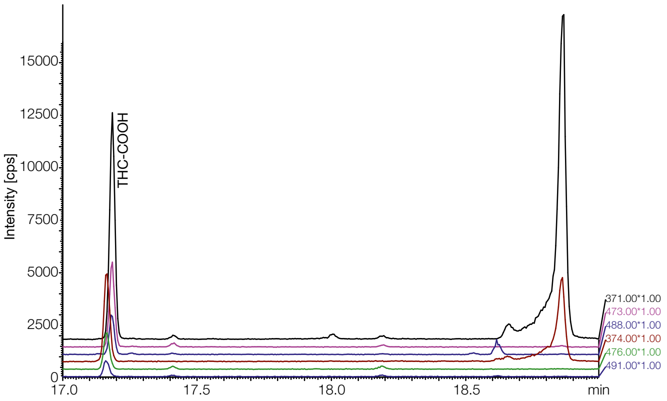

Figure 19: Chromatograms of THC-COOH and THC-COOH-D_3 from spiked plasma sample ($\beta = 100$ ng/mL).

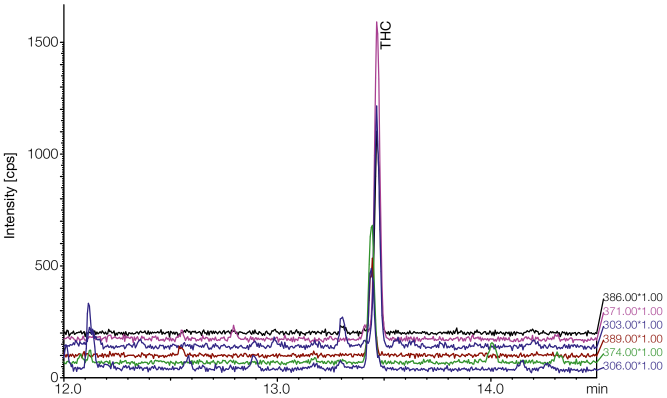

Figure 20: Chromatograms of THC and THC-D_3 from serum ($\beta = 100$ ng/mL).

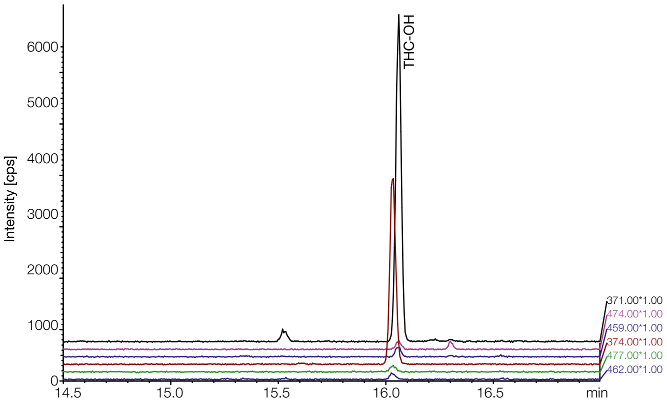

Figure 21: Chromatograms of THC-OH and THC-OH-D_3 from serum ($\beta = 100$ ng/mL).

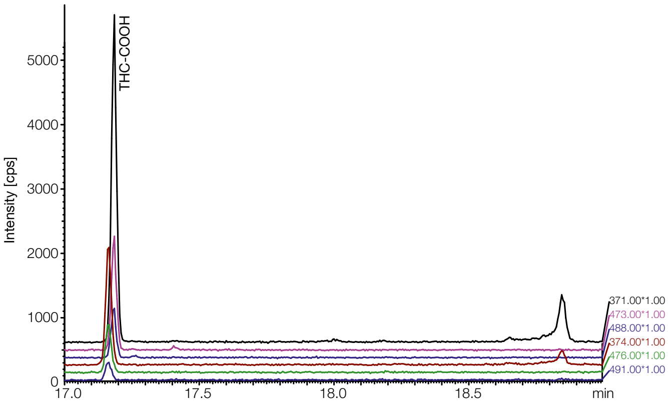

Figure 22: Chromatograms of THC-COOH and THC-COOH-D_3 from serum ($\beta = 100$ ng/mL).

Determination of THC in plasma and serum with GC-MS

Calibration curves

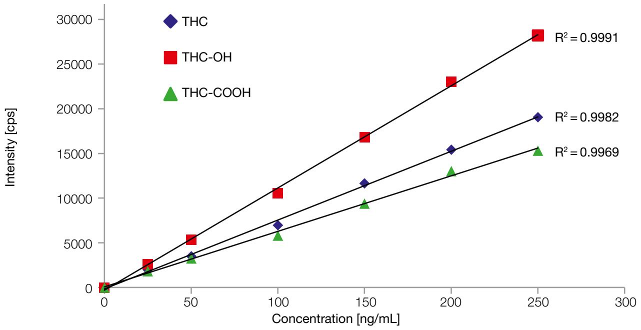

Figure 23: Calibration curves for cannabinoids in concentration range between 25 ng/mL and 250 µg/mL with an excellent coefficient of determination from standard solutions.

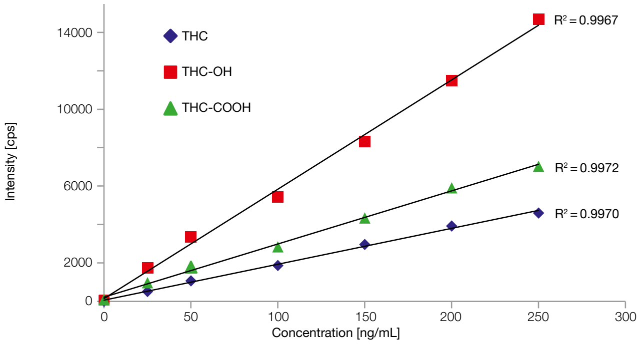

Figure 24: Calibration curves for cannabinoids in concentration range between 25 ng/mL and 250 µg/mL with an excellent coefficient of determination from plasma samples.

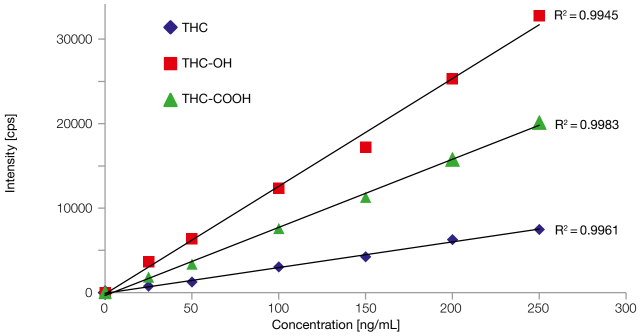

Figure 25: Calibration curves for cannabinoids in concentration range between 25 ng/mL and 250 µg/mL with an excellent coefficient of determination from serum samples.

Conclusion

This application note shows that the determination of cannabinoids from plasma and serum samples could be carried out successfully with all the tested products. The calibration curves from standard solution and biological samples indicate good linearity with good correlation coefficients. Sample preparation and derivatization procedure were simple and efficient for the determination of THC and its metabolites from plasma and serum sample matrix. By using a MS detector with higher sensitivity or a solid phase extraction method, it would be possible to determine cannabinoids from biological samples in lower concentration levels.

In summary the presented application describes a quick and convenient method for the determination of cannabinoids from plasma and serum samples with simple and efficient sample preparation procedure.

References

[1] Constituents of Cannabis sativa. Georg Thieme Verlag Stuttgart · New York.

[2] F. Grotenhermen, Journal of Cannabis Therapeutics, Vol. 3(1) 2003, Clinical Pharmacokinetics of Cannabinoids.

[3] T. Nadulski, F. Sporkert, M. Schnelle, A.M.I Stadelmann, P. Roser, T. Schefter, F. Pragst, Journal of Analytical Toxicology, Volume 29, Issue 8, 1 November 2005, Pages 782–789.

Additional information

The following application regarding “Determination of cannabinoids (THC) in plasma and serum samples with GC-MS” and further applications can be found on our online application database at *https://ChromaAppDB.mn-net.com*

GC: MN Appl. No. 215350

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EC 50/2 NUCLEOSHELL® RP 18, 2.7 µm	763132.20

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OPTIMA® 5 HT, 0.25 µm, 30 m, 0.25 mm ID	726106.30

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Article description	Article number
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CHROMABOND® HR-X, 85 µm, 6 mL/200 mg	730938
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CHROMABOND® QuEChERS extraction mix I	730970
CHROMABOND® QuEChERS clean-up mix XLVII	730845
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Article description	Article number
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N 18 Screw neck vials, 16.0 mL	702098
N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red	702107
N 13 PP Screw cap, black, colored top, silicone white / PTFE red	702052

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