

Supplementary Materials: A Quantitative ^1H NMR Method for Screening Cannabinoids in CBD Oils

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Automated quantification routine using PULCON principle

$$f_{\text{ERETIC}} \left[\frac{\text{ppm} \cdot \text{l}}{\text{mol}} \right] = \frac{1}{N} \sum_i^N \frac{(I_{\text{std}} \cdot k_{\text{sp}}) \cdot \text{SW} \cdot M_{\text{std}}}{\text{SI} \cdot \beta_{\text{std}} \cdot N_{\text{std}}^{\text{H}}} \quad (\text{S1})$$

with

f_{ERETIC} :	device-specific response factor
I_{std} :	absolute integral of the standard signal
SW :	spectral width [ppm]
M_{std} :	molecular weight of the standard [g/mol]
SI :	size of real spectrum
β_{std} :	mass concentration of the standard [mg/l]
$N_{\text{std}}^{\text{H}}$:	numbers of protons generating the selected signal
k_{sp} :	spectroscopic correction factor
	$k_{\text{sp}}(\text{TCNB}) = 1.30$; $k_{\text{sp}}(\text{EB}) = 1.22$

$$\beta_x \left[\frac{\text{g}}{\text{l}} \right] = \frac{I_x \cdot \text{SW} \cdot M_x}{\text{SI} \cdot f_{\text{ERETIC}} \cdot N_x^{\text{H}} \cdot f_{\text{dil}}} \cdot \left(\frac{P1_x}{P1_{\text{std}}} \cdot \frac{NS_{\text{std}}}{NS_x} \right) \quad (\text{S2})$$

with

β_x :	mass concentration of the analyte x [mg/l]
I_x :	absolute integral of the analyte x
SW :	spectral width in [ppm]
M_x :	molecular weight of the analyte x [g/mol]
SI :	size of real spectrum
f_{ERETIC} :	device-specific response factor, see equation (S1)
N_x^{H} :	numbers of protons generating the selected signal
f_{dil} :	dilution factor
$P1$:	pulse of sample (x) or standard (std) [μs]
NS :	number of scans, sample (x) or standard (std)

$$w_x \left[\frac{\text{mg}}{\text{kg}} \right] = \frac{\beta_x \cdot \left(\frac{W}{\rho} + V_s \right)}{100} \quad (\text{S3})$$

with

w_x :	mass fraction of cannabinoid content [mg/kg]
β_x :	mass concentration of the analyte [mg/l]
W :	weighing [mg]
ρ :	density of CBD oil [g/ml]
V_s :	volume of the solvent [μl]

Table S1. Integration limits used for the quantification of the cannabinoids.

Cannabinoids	Signal Label	Assignment in the Molecule (cf. Figure 1)	Number of Protons	Integration Limit [ppm]
cannabidiol	CBD 1	H-1	1	3.837–3.915
	CBD 2*	H-9 <i>cis</i>	1	4.506–4.568
	CBD 3	H-9 <i>trans</i>	1	4.597–4.673
cannabinol	CBN 1	H-2	1	7.019–7.074
	CBN 2	H-5	1	7.097–7.153
	CBN 3	H-4	1	8.173–8.250
Δ^9 -tetrahydrocannabinol	Δ^9 -THC*	H-3''	1	hemp oil: 6.135–6.148
				MCT oil: 6.151–6.186
Δ^8 -tetrahydrocannabinol	Δ^8 -THC*	H-3''	1	hemp oil: 6.10–6.138
				MCT oil: 6.1385–6.1524
ethylbenzene	EB	arom. H	3	7.0162–7.2341
tetrachloronitrobenzene	TCNB	arom. H	1	7.64–7.83

* integrated using line fitting algorithm

Composition of standards and solutions in validation studies

Table S2. Mixtures of the standard solutions used for the calibrations.

Standard Solution	Mixture
cannabidiol standard solution (CBD-S)	10.130 mg 5 mL CDCl ₃
cannabinol standard solution (CBN-S)	4.995 mg 2 mL CDCl ₃

Table S3. Cannabidiol calibration in the concentration range up to 1 mg/l. CBD-S, cannabidiol standard solution.

Calibration Point	Concentration [mg/L]	Mixture
Blank		103.07 mg hemp seed oil + 600 µl CDCl ₃
1	114.05	100.18 mg hemp seed oil + 40 µl ml CBD-S + 560 µl CDCl ₃
2	342.03	100.39 mg hemp seed oil + 120 µl CBD-S + 480 µl CDCl ₃
3	569.31	101.23 mg hemp seed oil + 200 µl CBD-S + 400 µl CDCl ₃
4	798.34	100.18 mg hemp seed oil + 280 µl CBD-S + 320 µl CDCl ₃
5	1024.72	101.26 mg hemp seed oil + 360 µl CBD-S + 240 µl CDCl ₃

Table S4. Cannabidiol calibration in the concentration range of 7.7–37 g/L.

Calibration Point	Concentration [mg/L]	Mixture
Blank		154.09 mg hemp seed oil 1 mL
1	7745	7.75 mg CBD + 153.13 mg hemp seed oil 1 mL CDCl ₃
2	14970	14.97 mg CBD + 153.73 mg hemp seed oil 1 mL CDCl ₃
3	22375	22.38 mg CBD + 153.91 mg hemp seed oil 1 mL CDCl ₃
4	31525	31.53 mg CBD + 155.24 mg hemp seed oil 1 mL CDCl ₃
5	36950	36.95 mg CBD + 153.59 mg hemp seed oil 1 mL CDCl ₃

Table S5. Cannabinol calibration. CBN-S, cannabinol standard solution.

Calibration Point	Concentration [mg/L]	Mixture
blank		99.90 mg CBD oil (15 %) + 600 µl CDCl ₃
1	140.36	99.90 mg CBD oil (15 %) + 40 µL CBN-S + 560 µL CDCl ₃
2	420.71	100.46 mg CBD oil (15 %) + 120 µL CBN-S + 480 µL CDCl ₃
3	702.23	99.61 mg CBD oil (15 %) + 200 µL CBN-S + 400 µL CDCl ₃
4	980.38	101.29 mg CBD oil (15 %) + 280 µL CBN-S + 320 µL CDCl ₃
5	1262.19	100.30 mg CBD oil (15 %) + 360 µL CBN-S + 240 µL CDCl ₃

*blank = CBD oil sample with declared content 15 % CBD based on hemp seed oil.

Table S6. Δ⁹-tetrahydrocannabinol calibration. Sol, solution.

Calibration Point	Concentration [mg/L]	Mixture
blank		101.94 mg CBD oil (10 %)
1	140.29	100 µL Δ ⁹ -THC-Sol + 100.87 mg CBD oil (10 %) + 600 µL CDCl ₃
2	418.94	300 µL Δ ⁹ -THC-Sol + 103.78 mg CBD oil (10 %) + 600 µL CDCl ₃
3	700.50	500 µL Δ ⁹ -THC-Sol + 101.71 mg CBD oil (10 %) + 600 µL CDCl ₃
4	980.83	700 µL Δ ⁹ -THC-Sol + 101.63 mg CBD oil (10 %) + 600 µL CDCl ₃
5	1259.68	900 µL Δ ⁹ -THC-Sol + 102.34 mg CBD oil (10 %) + 600 µL CDCl ₃

*blank = self-made CBD oil (10 %) based on hemp seed oil.

Table S7. Δ^8 -tetrahydrocannabinol calibration. Sol, solution.

Calibration Point	Concentration [mg/L]	Mixture
blank		100.19 mg CBD oil (10 %)+ 600 μ L CDCl_3
1	140.33	100 μ L Δ^8 -THC-Sol + 100.66 mg CBD oil (10 %) + 600 μ L CDCl_3
2	421.09	300 μ L Δ^8 -THC-Sol + 100.52 mg CBD oil (10 %) + 600 μ L CDCl_3
3	697.85	500 μ L Δ^8 -THC-Sol + 104.14 mg CBD oil (10 %) + 600 μ L CDCl_3
4	984.23	700 μ L Δ^8 -THC-Sol + 99.43 mg CBD oil (10 %) + 600 μ L CDCl_3
5	1259.62	900 μ L Δ^8 -THC-Sol + 102.37 mg CBD oil (10 %) + 600 μ L CDCl_3

*blank = self-made CBD oil (10 %) based on hemp seed oil.

Influence of NMR solvents

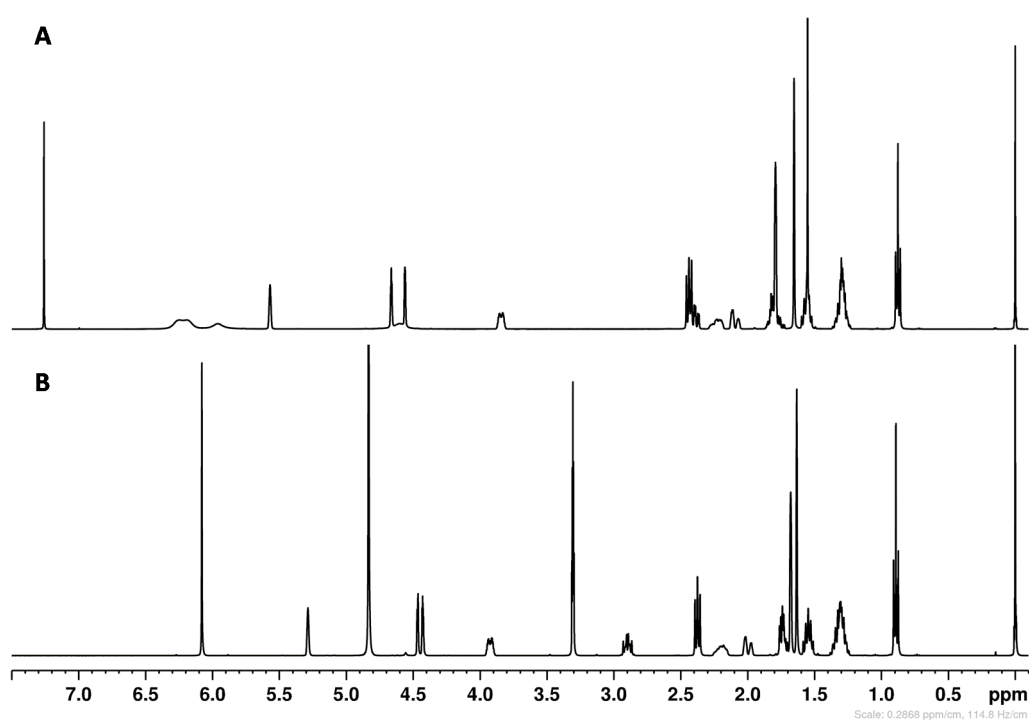


Figure S1. ^1H NMR spectra of cannabidiol in (A) deuterated chloroform (CDCl_3) and (B) deuterated methanol (CD_3OD).

Signal assignments of the cannabinoids

Table S8. Assignment of the ^1H NMR signals of cannabidiol and cannabidiolic acid. With s = singlet, d = doublet, t = triplet, q = quintet, m = multiplet, br = broad.

Pos.	Cannabidiol		Cannabidiolic Acid
	^1H NMR in CDCl_3^a	^1H NMR in CD_3OD^a	^1H NMR in CDCl_3^a
1	3.84 (1H, dm, 10.0 Hz)	3.93 (1H, dm, 9.9 Hz)	4.10 (1H, m)
2	5.57 (1H, s)	5.29 (1H, s)	5.56 (1H, s)
3			
4	2.09 (1H, m); 2.24 (1H, m)	1.99 (1H, dd, 9.2 Hz); 2.20 (1H, m)	---
5	1.82 (2H, m) ^g	1.74 (2H, m)	---
6	2.40 (1H, m) ^e	2.89 (1H, td, 16.2 Hz, 4.5 Hz)	---
7	1.79 (3H, s) ^g	1.67 (3H, s)	1.79 (s)
8			
9	4.67 (<i>trans</i> , 1H, m) ^c 4.60 (<i>cis</i> , 1H, m) ^c	4.47 (<i>trans</i> , 1H, m) 4.43 (<i>cis</i> , 1H, m)	4.54 (<i>trans</i> , 1H, m) 4.41 (<i>cis</i> , 1H, m)
10	1.65 (3H, s)	1.63 (3H, s)	1.71 (s)
1'			
2'			
3'	6.25 (1H, brs) ^f	6.08 (2H, s) ^h	
4'			
5'	6.19 (1H, brs) ^f	6.08 (2H, s) ^h	6.23 (1H, s)
6'			
1''	2.44 (2H, t, 7.6 Hz) ^e	2.38 (2H, t, 7.6 Hz)	2.94 (1H, m), 2.82 (1H, m)
2''	1.56 (2H, q, 15.2 Hz) ^b	1.54 (2H, q, 14.9 Hz)	1.56 (2H, m)
3''	1.30 (4H, m) ^d	1.31 (4H, m) ⁱ	1.33 (4H, m) ^j
4''	1.30 (4H, m) ^d	1.31 (4H, m) ⁱ	1.33 (4H, m) ^j
5''	0.89 (3H, t, 6.9 Hz)	0.89 (3H, t, 7.0 Hz)	0.87 (3H, t, 7.0 Hz)
2'-OH	5.95 (1H, brs)		9.16 (1H, brs)
6'-OH	4.60 (1H, brs)		6.52 (1H, s)
COOH			

^a spectrum recorded with 400 MHz at 300 K, chemical shift [ppm] referred to TMS; ^b signals overlapped with water residues; ^c overlap with OH group; ^{d-j} signals overlapped in the spectra; --- signal was not assigned.

Table S9. Assignment of the ^1H NMR signals of Δ^9 -tetrahydrocannabinol (Δ^9 -THC), Δ^9 -tetrahydrocannabinolic acid A (Δ^9 -THCA-A), Δ^8 -tetrahydrocannabinol (Δ^8 -THC) and Δ^9 -tetrahydrocannabivarin (THCV). With s = singlet, d = doublet, t = triplet, q = quintet, m = multiplet, br = broad.

Pos.	Δ^9 -THC ^1H NMR ^a	Δ^9 -THCA A ^1H NMR ^a	Δ^8 -THC ^1H NMR ^a	THCV ^1H NMR ^a
1	3.17 (1H, dm, 11 Hz)	3.23 (1H, dm, 5.6 Hz)	2.70 (1H, td, 11 Hz, 4.8 Hz)	3.20 (1H, m)
2	6.30 (1H, q)	6.39 (1H, brs)	3.19 (1H, dd, 16.2 Hz, 3.7 Hz) 1.85 (1H, m) ⁱ	6.30 (1H, q)
3				
4	2.17 (2H, m)	2.17 (2H, m)	5.43 (1H, brd, 4.7 Hz)	2.17 (2H, m)
5	1.91 (1H, m)	1.92 (1H, m)	2.14 (1H, m)	1.91 (1H, m)
	1.40 (1H, m) ^c	1.43 (1H, m) ^f	1.81 (1H, m) ⁱ	1.40 (1H, m) ^k
6	1.70 (1H, m) ^d	1.69 (m) ^g	1.79 (1H, m) ⁱ	1.69 (1H, m) ^l
7	1.69 (3H, s) ^d	1.68 (3H, s) ^g	1.70 (3H, s)	1.67 (3H, m) ^l
8				
9	1.40 (3H, s) ^c	1.44 (3H, s) ^f	1.37 (3H, s)	1.40 (3H, s) ^k
10	1.09 (3H, s)	1.11 (3H, s)	1.10 (3H, s)	1.09 (3H, s)
1'				
2'				
3'	6.14 (1H, d, 1.6 Hz)		6.10 (1H, d, 1.6 Hz)	6.14 (1H, d, 1.6 Hz)
4'				
5'	6.26 (1H, d, 1.5 Hz)	6.25 (1H, s)	6.27 (1H, d, 1.5 Hz)	6.26 (1H, d, 1.6 Hz)
6'				
1''	2.44 (2H, td, 7.6 Hz, 1.6 Hz)	2.94 (1H, m) 2.77 (1H, m)	2.44 (2H, td, 7.9 Hz, 3.1 Hz)	2.42 (2H, td, 7.5 Hz, 2.2 Hz)
2''	1.55 (q) ^b	1.57 (m) ^b	1.56 (m) ^b	1.57 (m) ^b
3''	1.30 (4H, m) ^e	1.31 (4H, m) ^h	1.30 (4H, m) ⁱ	0.91 (3H, t, 7.3 Hz)
4''	1.30 (4H, m) ^e	1.31 (4H, m) ^h	1.30 (4H, m) ⁱ	
5''	0.88 (3H, t, 7.0 Hz)	0.90 (3H, t, 7.0 Hz)	0.88 (1H, t, 7.1 Hz)	
2'-OH	4.69 (1H, s)	12.23 (1H, s)	4.62 (1H, s)	4.70 (1H, s)
COOH				

^a spectrum recorded with 400 MHz at 300 K, chemical shift [ppm] referred to TMS; ^b signals overlapped with water residues; ^{f-i} signals overlapped in the ^1H NMR spectra.

Table S10. Assignment of the ^1H NMR signals of cannabigerol and cannabinol. With s = singlet, d = doublet, t = triplet, q = quintet, m = multiplet, br = broad.

Pos.	Cannabigerol ^1H NMR ^a	Pos.	Cannabinol ^1H NMR ^a
1		1	
2		2	8.15 (1H, s)
3		3	
4	6.25 (2H, s) ^c	4	7.07 (1H, d, 8.0 Hz)
5		5	7.14 (1H, d, 7.8 Hz)
6	6.25 (2H, s) ^c	6	
1'	3.39 (2H, d, 7.1 Hz)	7	2.38 (3H, s)
2'	5.27 (1H, m)	8	
3'		9	1.59 (s) ^{b, g}
3'-Me	1.81 (3H, s)	10	1.59 (s) ^{b, g}
4'	2.08 (4H, m) ^d	1'	
5'	2.08 (4H, m) ^d	2'	
6'	5.05 (1H, m)	3'	6.30 (1H, d, 1.5 Hz)
7'		4'	
8'	1.59 (s) ^{b, e}	5'	6.44 (1H, d, 1.5 Hz)
9'	1.67 (3H, s)	6'	
1''	2.46 (2H, t, 7.6 Hz)	1''	2.50 (2H, t, 7.8 Hz)
2''	1.56 (q) ^{b, e}	2''	1.61 (m) ^{b, g}
3''	1.31 (4H, m) ^f	3''	1.32 (4H, m) ^h
4''	1.31 (4H, m) ^f	4''	1.32 (4H, m) ^h
5''	0.88 (3H, t, 6.8 Hz)	5''	0.89 (3H, t, 7.2 Hz)
OH	4.96 (2H, s)	2'-OH	5.12 (1H, s)

^a spectrum recorded with 400 MHz at 300 K, chemical shift [ppm] referred to TMS; ^b signals overlapped with water residues; ^{c-h} signals overlapped in the ^1H NMR spectra.

Optimization of NMR protocol receiver gain and signal to noise ratio

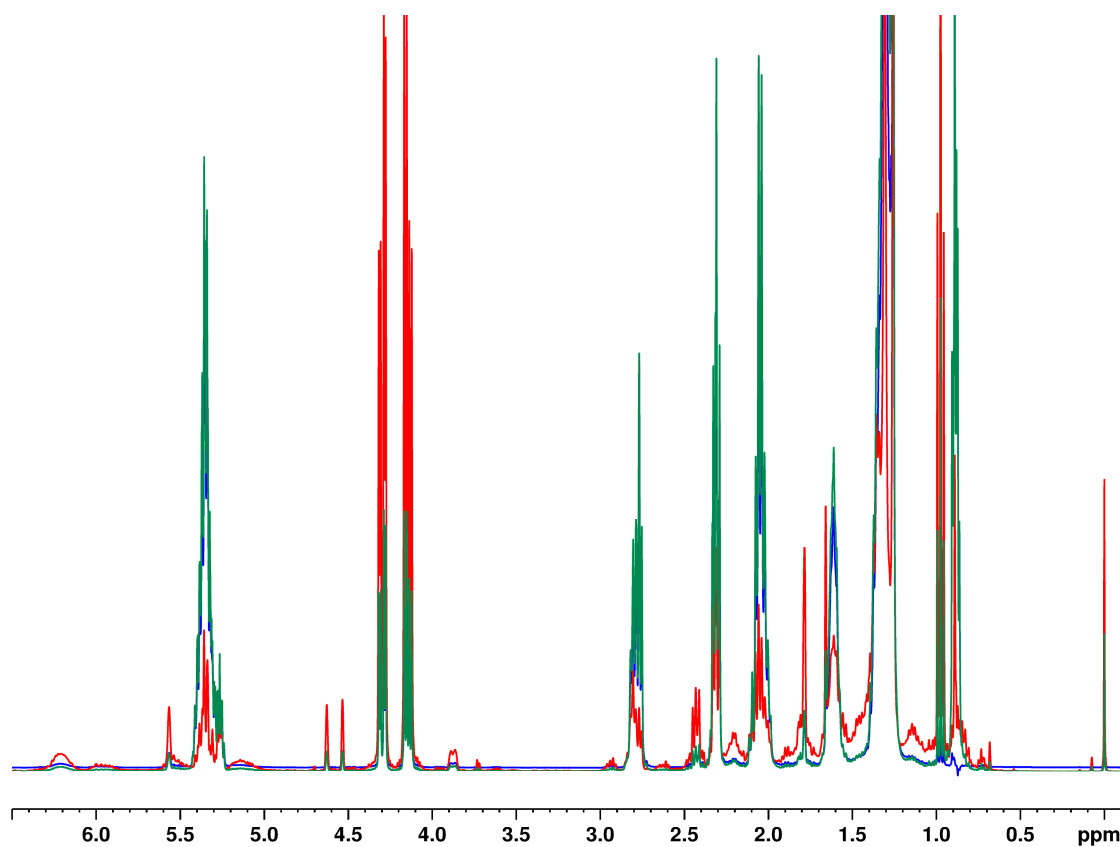


Figure S2. ¹H NMR spectra of a CBD oil in CDCl₃ recorded with different suppression programs. Blue, with suppression of the CH₃ lipid signal (RG: 8); Green, without suppression (RG: 5.6); Red, multiple suppression (RG: 16). The results were obtained with a sample solution of 100 mg oil dissolved in 600 μL CDCl₃.

Method validation: linearity

Table S11. Calibration data of the investigated cannabinoid signals. CBD, cannabidiol; Δ^9 -THC, Δ^9 -tetrahydrocannabinol; CBN, cannabinol; Δ^8 -THC, Δ^8 -tetrahydrocannabinol; THCA Δ^9 -tetrahydrocannabinolic acid A; c, concentration; δ , chemical shift; H signal, proton signal; R, correlation coefficient; s_{x0} , process standard deviation; v_{x0} , coefficient of variation.

Cannabinoid	C [mg/L]	H Signal	δ [ppm]	R	s_{x0} [mg/L]	v_{x0} [%]
CBD	114.05–1024.72	H-1	3.837–3.915	0.99932	15.34	2.69
		H-9 <i>cis</i>	4.506–4.568	0.9999	5.93	1.04
		H-9 <i>trans</i>	4.597–4.673	0.99946	13.62	2.39
CBD	7745–36950	H-1	3.837–3.915	0.99981	269.74	1.21
		H-9 <i>cis</i>	4.506–4.568	0.99985	237.52	1.06
		H-9 <i>trans</i>	4.597–4.673	0.99998	87.28	0.39
Δ^9 -THC	140.29–1259.68	H-3'	6.135–6.148	0.99862	26.92	3.85
CBN	140.36–1262.45	H-2	7.019–7.074	0.999	22.88	3.26
		H-5	7.097–7.153	0.99856	27.54	3.93
		H-4	8.173–8.250	0.99905	22.32	3.18
Δ^8 -THC	140.33–1259.62	H-3'	6.10–6.138	0.99977	11.08	1.58

Method validation: recovery

Table S12. Recoveries of the cannabinoids in [%]. CP, calibration point; CBD, cannabidiol; CBN, cannabinol; Δ^9 -THC, Δ^9 -tetrahydrocannabinol; Δ^8 -THC, Δ^8 -tetrahydrocannabinol.

Signal	CBD 1*	CBD 2*	CBD 3*	CBN 1	CBN 2	CBN 3	Δ^8 -THC	Δ^9 -THC
CP 1	120	117	117	117	125	120	57	35
CP 2	122	118	117	119	123	123	86	62
CP 3	119	114	115	119	122	124	93	80
CP 4	119	115	114	126	131	132	92	84
CP 5	117	113	114	129	135	134	93	84

*recovery of the calibration in the high concentration range; dark gray = concentration of the CP under the LOD; light gray = concentration of the CP under the LOQ.

Table S13. Recovery in [%] of cannabidiol (CBD) determined from calibration without matrix. CP, calibration point.

signal*	CBD 1 (H-1)	CBD 2 (H-9 <i>cis</i>)	CBD 3 (H-9 <i>trans</i>)	H-2	H-3'' + 5''	H-5''	H-10	H-1'' + 6
CP 1	128	133	77	121	124	11	114	124
CP 2	122	129	79	117	122	14	106	119
CP 3	122	128	79	117	122	10	107	119
CP 4	123	129	79	117	122	10	106	119
CP 5	121	127	79	117	124	10	104	117

* assignment of the cannabidiol signals according to Figure 1; dark gray = signals overlapped with OH-group; light gray = signal influenced by multiple suppression.

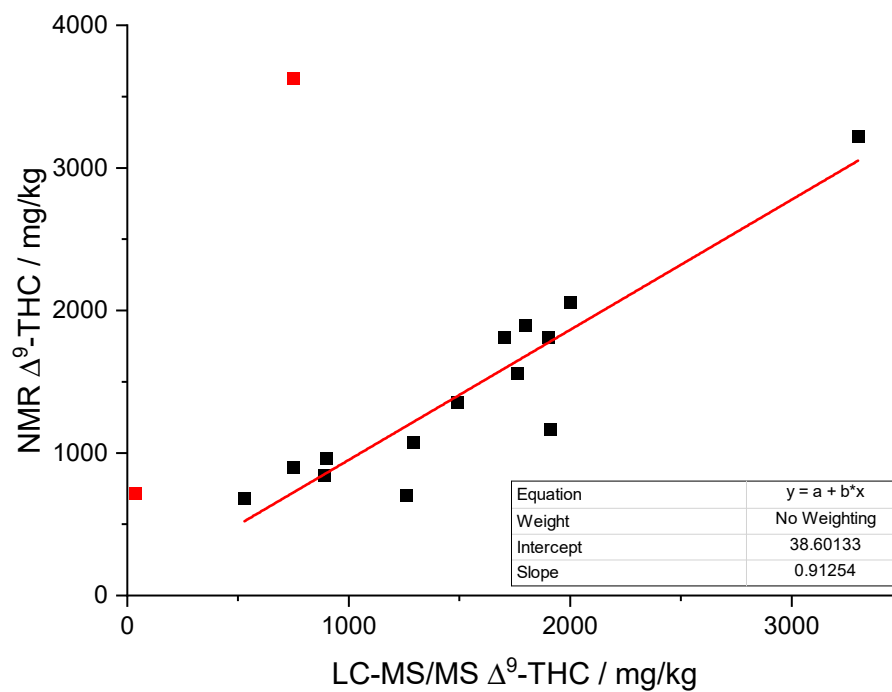
Comparison and fit of NMR and LC-MS/MS results for Δ^9 -THC

Figure S3. Linear fit of NMR and LC-MS/MS results of all samples with NMR results above the NMR(LOD).