

Pharmacokinetics of Cannabidiol in Rat Brain Tissue After Single-Dose Administration of Different Formulations

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Table S1. Recoveries (%) of various phytocannabinoids extracted from rat brain tissue evaluated using different solvent systems (acetonitrile, isopropyl alcohol, methanol and ethanol), modified acetonitrile-based extractions (with water ratios of 3:1 and 30:1) and a QuEChERS-based extraction method.

Extraction/Analytes	Recoveries (%)												
	CBD	Δ^9 -THC	CBDV	CBDVA	THCV	CBN	THCVA	CBNA	CBL	CBC	CBLA	CBDA	CBGA
Acetonitrile	110	94											
Isopropyl alcohol	24	97											
Methanol	70	111											
Ethanol	81	172											
Acetonitrile (3:1)	90	85	69	82	83	72	80	192	67	83	69	30	64
Acetonitrile (30:1)	110	82	94	77	82	69	82	114	54	40	88	53	69
QuEChERS	100	98	108	116	122	114	111	109	94	127	117	19	101

Table S2. Optimized parameters for the determination of phytocannabinoids and their metabolites by QqQ-MS.

Parameter Types	Start Value	End Value	Step Value
High Pressure RF (V)	70	210	20
Low Pressure RF (V)	40	160	20
Sheath Gas Temperature (°C)	200	400	50
Sheath Gas Flow (L/min)	10	12	1
Drying Gas Temperature (°C)	120	230	30
Drying Gas Flow (L/min)	11	20	2
Nebulizer Pressure (psi)	20	40	5
Capillary Voltage (V)	1500	4500	500
Nozzle Voltage (V)	0	2000	500

Table S3. Validation parameters.

Compounds	Rat brain tissue spiked with a mixed solution of all targeted analytes									LOQ (µg/kg) ¹	Linear range (µg/kg)
	8 µg/kg brain, n=5			80 µg/kg brain, n=5			160 µg/kg brain, n=5				
	Recoveries (%)	RSD (%)	SSE (%)	Recoveries (%)	RSD (%)	SSE (%)	Recoveries (%)	RSD (%)	SSE (%)		
Neutral phytocannabinoids											
CBGV	97	10	110	110	12	108	104	12	110	4	4 - 160
CBDV	94	7	110	97	5	125	89	6	98	1.6	1.6 - 400
CBD	108	7	152	95	5	107	99	1	83	4	4 - 400
CBG	94	5	103	96	4	116	87	12	102	1.6	1.6 - 400
THCV	86	7	109	88	5	133	82	6	106	1.6	1.6 - 400
CBN	94	1	114	92	1	114	95	1	83	1.6	1.6 - 400
Δ ⁹ -THC	82	3	107	85	2	114	90	2	85	1.6	1.6 - 400
Δ ⁸ -THC	90	4	99	96	7	115	85	7	96	1.6	1.6 - 400
CBL	77	4	93	84	9	111	71	7	95	1.6	1.6 - 400
CBC	81	5	157	86	7	160	77	2	146	4	4 - 160
Phytocannabinoid acids											
CBDVA	82	7	80	80	4	106	73	7	89	4	4 - 160
CBDA	71	3	87	79	5	94	80	13	103	1.6	1.6 - 160
CBGA	83	2	129	92	6	116	84	8	107	1.6	1.6 - 400
THCVA	81	3	100	77	4	115	73	4	102	4	4 - 400
CBNA	86	4	156	93	8	168	83	10	153	1.6	1.6 - 400
Δ ⁹ -THCA-A	85	5	102	72	6	284	73	4	290	1.6	1.6 - 160
CBLA	95	3	156	102	6	280	83	6	238	1.6	1.6 - 160
CBCA	69	7	186	86	14	261	67	7	217	4	4 - 160
Metabolites of phytocannabinoids											
7-COOH-CBD	112	6	127	84	6	107	88	7	97	8	8 - 160
7-OH-CBD	96	1	97	94	4	96	93	6	94	8	8 - 160
11-nor-9-COOH-Δ ⁹ -THC-gluc	61	9	80	70	20	68	61	10	59	4	4 - 160
11-OH-Δ ⁹ -THC	93	4	89	92	4	86	98	2	85	4	4 - 400
11-nor-9-COOH-Δ ⁹ -THC	83	2	86	80	8	98	84	7	109	8	8 - 400

¹In this study, the limit of detection (LOD) was determined to be one-third of the limit of quantification (LOQ)

Table S4. Mass transitions, collision energy and retention time of phytocannabinoid metabolites, neutrals and acids.

Compound Name	Molecular Formula	Ret Time (min)	Polarity	Precursor Ion (<i>m/z</i>)	Collision Energy (eV)	Product Ion (<i>m/z</i>) ¹
Neutral phytocannabinoids						
CBGV	C ₁₉ H ₂₈ O ₂	3.5	[M+H] ⁺	317.2	14/34/50	165.1/123.0/91.0
CBDV	C ₁₉ H ₂₆ O ₂	4.3	[M+H] ⁺	287.2	24/16/16	165.0/135.2/231.0
CBD	C ₂₁ H ₃₀ O ₂	4.9	[M+H] ⁺	315.2	28/40/20	193.0/123.0/135.1
CBD-D ₃	C ₂₁ H ₂₇ O ₂ D ₃	4.9	[M+H] ⁺	318.3	24/20/28	196.0/135.1/107.2
CBG	C ₂₁ H ₃₂ O ₂	5.0	[M+H] ⁺	317.2	20/40/36	193.0/123.0/137.0
CBG-D ₃	C ₂₁ H ₂₉ O ₂ D ₃	5.0	[M+H] ⁺	320.3	22/42/46	196.1/123.1/69.1
THCV	C ₁₉ H ₂₆ O ₂	5.3	[M+H] ⁺	287.2	24/20/40	165.0/135.1/123.1
CBN	C ₂₁ H ₂₆ O ₂	7.5	[M+H] ⁺	311.2	20/16/16	223.1/293.2/241.1
CBN-D ₃	C ₂₁ H ₂₃ O ₂ D ₃	7.5	[M+H] ⁺	314.2	26/18/22	223.1/296.2/241.1
Δ ⁹ -THC	C ₂₁ H ₃₀ O ₂	8.6	[M+H] ⁺	315.2	36/24/32	123.0/135.0/107.2
Δ ⁹ -THC-D ₃	C ₂₁ H ₂₇ O ₂ D ₃	8.6	[M+H] ⁺	318.3	24/40/24	196.0/123.0/135.1
Δ ⁸ -THC	C ₂₁ H ₃₀ O ₂	9.1	[M+H] ⁺	315.2	24/36/20	193.0/123.1/259.1
CBL	C ₂₁ H ₃₀ O ₂	9.4	[M+H] ⁺	315.2	20/44/28	235.2/81.1/165.1
CBC	C ₂₁ H ₃₀ O ₂	10.4	[M+H] ⁺	315.2	16/32/16	259.1/123.0/233.1
Phytocannabinoid acids						
CBDVA	C ₂₀ H ₂₆ O ₄	3.5	[M-H] ⁻	329.2	20/32/24	311.2/217.2/283.1
CBDA	C ₂₂ H ₃₀ O ₄	5.1	[M-H] ⁻	357.2	24/24/24	339.2/311.2/289.2
CBGA	C ₂₂ H ₃₂ O ₄	5.9	[M-H] ⁻	359.2	20/24/40	341.3/315.2/191.1
THCVA	C ₂₀ H ₂₆ O ₄	8.0	[M-H] ⁻	329.2	24/28/36	285.2/217.1/163.1
CBNA	C ₂₂ H ₂₆ O ₄	9.2	[M-H] ⁻	353.2	20/44/64	309.3/279.1/222.0
Δ ⁹ -THCA-A	C ₂₂ H ₃₀ O ₄	11.2	[M-H] ⁻	357.2	28/36/32	313.1/245.2/311.3
Δ ⁹ -THCA-A-D ₃	C ₂₂ H ₂₇ O ₄ D ₃	11.2	[M-H] ⁻	360.2	26/34/38	316.2/248.2/194.1
CBCA	C ₂₂ H ₃₀ O ₄	11.6	[M-H] ⁻	357.2	24/28/44	313.2/339.2/191.1
CBLA	C ₂₂ H ₃₀ O ₄	12.0	[M-H] ⁻	357.2	24/44/28	313.2/191.1/339.2
Metabolites of phytocannabinoids						
7-COOH-CBD	C ₂₁ H ₂₈ O ₄	2.0	[M+H] ⁺	345.2	18/18/30	327.2/299.2/193.1
7-COOH-CBD-D ₃	C ₂₁ H ₂₅ O ₄ D ₃	2.0	[M+H] ⁺	348.2	22/20/22	302.4/196.1/274.1
7-OH-CBD	C ₂₁ H ₃₀ O ₃	2.1	[M+H] ⁺	331.2	14/26/20	313.0/201.1/193.1
7-OH-CBD ₃	C ₂₁ H ₂₇ O ₃ D ₃	2.1	[M+H] ⁺	334.3	22/22/26	316.1/201.0/133.1
11-nor-9-COOH-Δ ⁹ -THC-glu	C ₂₇ H ₃₆ O ₁₀	2.5	[M+H] ⁺	521.2	16/26/52	345.2/327.2/193.1
11-nor-9-carbo-Δ ⁹ -THC-glu-D ₃	C ₂₇ H ₃₃ O ₁₀ D ₃	2.5	[M+H] ⁺	524.3	14/30/38	348.2/330.2/302.2
11-hydroxy-Δ ⁹ -THC	C ₂₁ H ₃₀ O ₃	4.1	[M+H] ⁺	331.2	18/20/32	313.1/193.1/133.1
11-hydroxy-Δ ⁹ -THC-D ₃	C ₂₁ H ₂₇ O ₃ D ₃	4.1	[M+H] ⁺	334.2	12/32/24	316.1/196.1/133.1
11-nor-9-carboxy-Δ ⁹ -THC	C ₂₁ H ₂₈ O ₄	4.6	[M+H] ⁺	345.2	14/22/30	327.2/299.2/193.1
11-nor-9-carboxy-Δ ⁹ -THC-D ₉	C ₂₁ H ₁₉ O ₄ D ₉	4.6	[M+H] ⁺	354.3	18/20/34	336.3/308.3/124.1

¹The product ions are listed according to their abundance in the MS/MS spectrum, with the first one listed being the quantifier ion and the others the qualifier ions.