Supercritical fluid chromatography method for the systematic toxicology analysis of cannabinoids and their metabolites

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Supporting information

Experimental S1

Instrumentation

All SFC experiments were carried out on an Agilent 1260 Infinity Analytical SFC System (Waldbronn, Germany). The system consists of the following modules: Agilent 1260 Infinity SFC Control Module for CO_2 pre- and post-conditioning; Agilent 1260 Infinity SFC Binary Pump; Agilent 1260 Infinity degasser and autosampler; Agilent 1260 Infinity Column Compartment and Agilent 1260 Infinity Diode Array Detector with high pressure SFC flow cell. The system was controlled by Agilent OpenLab software. The Zorbax Rx-SIL column (150 mm x 4.6 mm i.d.), with particle size of 5 µm was obtained from Agilent Technologies (Waldbronn, Germany). Centrifuge Centro-8 was purchased from Letslab (Barcelona, Spain).

Material and methods

The carbon dioxide (CO_2, 4.8 laser, used for SFC) was purchased from SIAD (Prague, Czech Republic). Acetonitrile (ACN, Chromasolv®, gradient grade, for HPLC, ≥ 99.9%), methanol (MeOH, LC-MS Ultra Chromasolv®, ≥ 99.9%), propane-2-ol (IPA, Chromasolv®, for HPLC, 99.9%) and β-glucuronidase (Type B-10, 10,000 units/mg) were supplied by Sigma-Aldrich (St. Louis, USA). Sodium acetate (CH_3COONa, 99%) and ammonium acetate (CH_3COONH_4, 98-100%) were purchased from Lachema (Brno, Czech Republic). Chloroform ($CHCl_3$, p.a.) was supplied by IPL (Uherský Brod, Czech Republic) and hydrochloric acid (HCl, 36%) was purchased from Analytika (Prague, Czech Republic). The deionized water was prepared by a water purification system 18 MΩ, Millipore (Molsheim, France). Solid-phase extraction cartridges, Supel™-Select SCX SPE Tube (1 mL x 30 mg), were supplied by Agilent Technologies (Waldbronn, Germany).

The analytes, AM-2201, AM-2201 M1, cannabidiol (CBD), JWH-018 6-hydroxyindole, JWH-018 *N*-(4-hydroxypentyl), JWH-018 *N*-pentanoic acid, JWH-019 5-hydroxyindole, JWH-019 *N*-(6-hydroxyhexyl), JWH-073, JWH-073 5-hydroxyindole, JWH-073 6-hydroxyindole, JWH-200, JWH-200 4-hydroxyindole, JWH-210 *N*-(5-carboxypentyl), JWH-250, RCS-4 *N*-4-hydroxypentyl and THC were acquired from LGC Standards (Teddington, United Kingdom) .

Separation conditions

The MPs composed of supercritical CO_2 containing ACN, MeOH or IPA as OMs were used for separation of the mixture of THC, CBD and a wide group of the SCs and their metabolites. The method optimization was performed to improve separation by fine tuning of the MP (the type and amount of the OM were considered) and by adjusting the flow rate, temperature and BP. The optimized conditions were as follows: Zorbax Rx-SIL column (150 mm x 4.6 mm i.d.), with particle size of 5 µm, MP composed of CO_2/ACN 93/7 (*v*/*v*), flow rate 2.5 mL/min, column temperature 40 °C, BP 95 bars and UV detection at 210 nm. The injection volume was 5 µL. All measurements were performed in triplicates. The void volume was determined using the solvent peak. The identification of analytes was carried out by spiking with standards and by comparison of the DAD spectra using the wavelengths in the range from 200 nm to 400 nm. The detection wavelength used was 210 nm because the majority of the studied analytes exhibited absorption maximum at the given wavelength.

Sample extraction

The salting out protocol was as follows: To 100 µL of spiked urine was added 10 µL of HCl (36%) and 200 µL of ACN. The mixture was mixed by vortexing for 10 s. Then 50 µL of CH_3COONH_4 solution, as salting-out reagent (concentration 10 M), was added and vortexed for 10 s. The samples were centrifuged at 10,000 rpm for 3 minutes in microcentrifuge tubes. From the upper organic layer, 200 µL were removed and transferred to a clean vial. The organic phase was evaporated by nitrogen stream. The dry residue was dissolved in 100 µL MeOH.

In the case of real sample, to 1.0 mL of urine was added 100 µL HCl (36%) and 2.0 mL ACN. The mixture was mixed by vortexing for 10 s. Then 500 µL of CH_3COONH_4 solution (10 M) was added and vortexed for 10 s. The sample was centrifuged at 10,000 rpm for 3 minutes. From the upper organic layer, 200 µL were removed and transferred to a clean vial. The organic phase was evaporated by nitrogen stream. The dry residue was dissolved in 20 µL MeOH.

Urine sample obtained from a patient after intoxication (smoking of herbal blend containing JWH-073) was enzymatically hydrolyzed by β-glucuronidase and extracted. 10 mL of urine (either blank urine or urine obtained from intoxicated patient), 6 mL of 2 M CH_3COONa solution, pH 4.50, and 2 mL of β-glucuronidase (about 10,000 units/mg) were mixed and heated at 60 °C for 1 hour. After hydrolysis the metabolites were extracted using the same extraction procedure as described above.

Standard and calibration samples preparation

Drug free human urines were obtained from 8 healthy volunteers (of different sex and age). The urine samples were centrifuged at 3000 rpm for 10 min and filtered through 0.45 µm nylon membrane. All these samples obtained from healthy volunteers were analyzed prior to their use for verification of drug-free status (negative urine).

Stock solutions of each analyte were prepared at a concentration of 1 mg/mL using MeOH as solvent and then diluted with MeOH to appropriate concentration. The samples for calibration were prepared in negative urine at the appropriate concentrations.

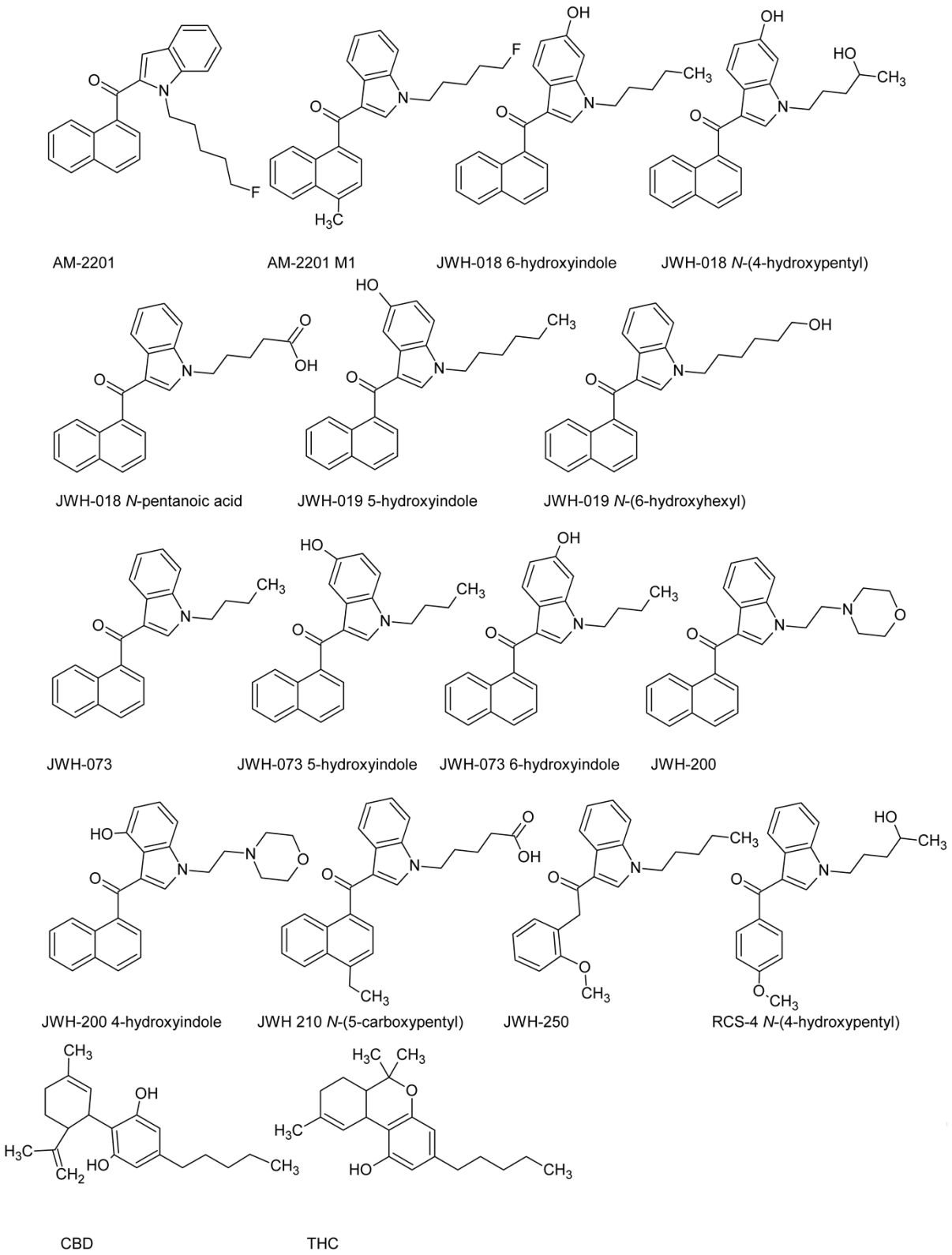

Figure S1. Chemical structures of the studied analytes.

Table S1. Precision data expressed as intra-day and inter-day repeatability RSD% of retention times (t_R) and peak areas (A).

Analytes	Intra-day RSD%						Inter-day (3 days) RSD%	
	t_R			A			t_R	A
	3.0 µg/mL	5.0 µg/mL	10.0 µg/mL	3.0 µg/mL	5.0 µg/mL	10.0 µg/mL	5.0 µg/mL	5.0 µg/mL
THC	0.01	0.01	0.01	0.65	1.61	2.84	0.01	2.19
CBD	0.01	0.01	0.01	0.91	1.31	1.67	0.01	1.69
JWH-250	0.01	0.01	0.01	0.24	1.57	2.09	0.02	3.39
JWH-073	0.02	0.03	0.01	0.33	2.18	2.94	0.02	3.95
AM-2201	0.02	0.02	0.01	0.93	3.82	2.62	0.02	4.26
JWH-019 5-hydroxyindole	0.02	0.01	0.01	0.86	3.15	3.11	0.01	3.99
JWH-073 5-hydroxyindole	0.01	0.01	0.01	0.40	3.41	1.86	0.01	4.59
JWH-018 6-hydroxyindole	0.03	0.02	0.01	0.61	1.40	3.57	0.04	1.94
JWH-073 6-hydroxyindole	0.02	0.02	0.01	0.42	0.95	2.56	0.03	3.14
JWH-210 *N*-(5-carboxypentyl)	0.02	0.02	0.01	0.17	1.19	2.36	0.03	1.39
JWH-018 *N*-pentanoic acid	0.02	0.02	0.01	3.17	1.46	3.83	0.07	2.06
AM-2201 M1	0.02	0.02	0.07	0.66	1.52	3.71	0.03	6.58
RCS-4 *N*-(4-hydroxypentyl)	0.02	0.02	0.01	0.12	4.65	3.04	0.04	5.98
JWH-018 *N*-(4-hydroxypentyl)	0.12	0.15	0.06	0.22	2.42	1.42	0.15	6.35
JWH-019 *N*-(6-hydroxyhexyl)	0.10	0.10	0.10	0.45	5.12	3.82	0.15	6.30
JWH-200	0.13	0.16	0.13	0.53	1.91	2.24	0.18	3.74
JWH-200 4-hydroxyindole	0.10	0.15	0.07	0.37	2.57	1.11	0.19	3.87

Table S2. Chromatographic data of blank urine sample spiked with 5.0 µg/mL working solution of analytes under optimized experimental conditions. MP composition: CO_2/ACN 93/7 (*v*/*v*); flow rate

2.5 mL/min; column temperature 40 °C; 95 bars as BP; UV detection at 210 nm, injection volume 5 µL, t_R; retention time, *k*; retention factor, $R_{1/2}$; resolution.

Analytes	t_R [min]	*k*	$R_{1/2}$
THC	1.80	1.22	
CBD	2.05	1.53	2.87
JWH-250	2.50	2.08	4.63
JWH-073	2.79	2.44	2.77
AM-2201	3.40	3.20	4.99
JWH-019 5-hydroxyindole	4.89	5.03	8.71
JWH-073 5-hydroxyindole	5.26	5.48	1.83
JWH-018 6-hydroxyindole	6.68	7.24	6.36
JWH-073 6-hydroxyindole	7.04	7.68	1.41
JWH-210 *N*-(5-carboxypentyl)	7.41	8.14	1.32
JWH-018 *N*-pentanoic acid	8.12	9.01	2.25
AM-2201 M1	8.98	10.08	2.77
RCS-4 *N*-(4-hydroxypentyl)	9.37	10.56	1.36
JWH-018 *N*-(4-hydroxypentyl)	10.31	11.72	3.13
JWH-019 *N*-(6-hydroxyhexyl)	11.19	12.79	2.45
JWH-200	11.82	13.57	1.65
JWH-200 4-hydroxyindole	12.47	14.38	1.60

Table S3. Linearity and sensitivity parameters (n = 3, six point calibration method).

Analytes	Regression line				Sensitivity	
	Linearity range [µg/mL]	Slope [mAU s mL /µg]	Intercept [mAU s]	Coefficient of determination, R^2	LOD [µg/mL]	LOQ [µg/mL]
THC	0.5-20.0	6.982	1.056	0.9997	0.15	0.50
CBD	0.5-20.0	6.124	1.075	0.9998	0.15	0.50
JWH-250	0.5-20.0	4.758	-0.056	0.9992	0.15	0.50
JWH-073	0.5-20.0	12.745	-0.165	0.9993	0.15	0.50
AM-2201	0.5-20.0	9.894	0.296	0.9995	0.15	0.50
JWH-019 5-hydroxyindole	1.0-20.0	7.957	0.311	0.9996	0.17	0.57
JWH-073 5-hydroxyindole	1.0-20.0	13.137	-0.726	0.9996	0.18	0.60
JWH-018 6-hydroxyindole	1.5-20.0	7.325	0.523	0.9997	0.35	1.17
JWH-073 6-hydroxyindole	1.0-20.0	7.985	0.277	0.9995	0.25	0.83
JWH-210 *N*-(5-carboxypentyl)	1.5-20.0	6.458	0.952	0.9995	0.33	1.10
JWH-018 *N*-pentanoic acid	1.5-20.0	9.428	0.034	0.9997	0.35	1.17
AM-2201 M1	1.0-20.0	11.485	0.498	0.9996	0.26	0.87
RCS-4 *N*-(4-hydroxypentyl)	2.0-20.0	4.812	2.058	0.9989	0.50	1.67
JWH-018 *N*-(4-hydroxypentyl)	1.5-20.0	13.973	-1.519	0.9997	0.31	1.03
JWH-019 *N*-(6-hydroxyhexyl)	2.0-20.0	8.258	0.489	0.9995	0.52	1.73
JWH-200	1.5-20.0	10.078	-0.989	0.9996	0.36	1.20
JWH-200 4-hydroxyindole	2.0-20.0	8.210	2.451	0.9980	0.52	1.73

Table S4. Mean recovery data.

Analytes	Recovery%		RSD%	
	5.0 µg/mL	10.0 µg/mL	5.0 µg/mL	10.0 µg/mL
THC	124.3	87.7	2.90	4.75
CBD	108.1	77.2	1.57	2.76
JWH-250	99.1	91.9	3.45	1.86
JWH-073	100.3	78.1	5.38	2.00
AM-2201	103.0	75.5	4.27	1.84
JWH-019 5-hydroxyindole	106.5	71.2	3.99	3.39
JWH-073 5-hydroxyindole	100.3	75.6	4.59	0.90
JWH-018 6-hydroxyindole	96.2	89.7	1.94	3.43
JWH-073 6-hydroxyindole	98.8	74.6	3.14	2.49
JWH-210 *N*-(5-carboxypentyl)	76.6	93.8	1.39	1.66
JWH-018 *N*-pentanoic acid	82.1	86.4	2.06	4.41
AM-2201 M1	97.1	85.4	6.66	3.44
RCS-4 *N*-(4-hydroxypentyl)	96.2	75.2	4.65	2.75
JWH-018 *N*-(4-hydroxypentyl)	111.4	92.6	6.35	4.41
JWH-019 *N*-(6-hydroxyhexyl)	108.4	96.9	2.03	4.94
JWH-200	74.2	80.9	3.74	4.43
JWH-200 4-hydroxyindole	91.3	89.5	2.57	4.29

The recoveries were calculated as the ratio of blank urine samples spiked with all analytes before and after the extraction step.