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Simultaneous analysis of several synthetic cannabinoids, THC, CBD, CBN in hair by ultra-high performance liquid chromatography tandem mass spectrometry. Method validation and application to real samples

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Abstract

A simple procedure for the quantitative detection of JWH-018, JWH-073, JWH 200, JWH-250, HU-210, Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) in hair has been developed and fully validated. After digestion with NaOH and liquid-liquid extraction, the separation was performed with an ultra-high performance liquid chromatography system coupled to a triple quadrupole mass spectrometer operating in the selected reaction monitoring mode. The absence of matrix interferents, together with excellent repeatability of both retention times and relative abundances of diagnostic transitions, allowed the correct identification of all analytes tested. The method was linear in two different intervals at low and high concentration, with correlation coefficient values between 0.9933 and 0.9991. Quantitation limits ranged from 0.07 for JWH-200 up to 18 pg/mg for CBN. The present method for the determination of several cannabinoids in hair proved to be simple, fast, specific and sensitive. The method was successfully applied to the analysis of 179 real samples collected from proven consumers of *Cannabis*, among which 14 were found positive to at least one synthetic cannabinoid.

Keywords

Hair; ultra-high performance liquid chromatography; synthetic cannabinoids; Spice; method validation

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Introduction

New synthetic drugs that mimic the psychotropic effects of tetrahydrocannabinol (THC) are proliferating across the world, taking advantage of the delay occurring between their introduction into the market and the legal ban. In the last few years, several herbal mixture declared as incense or air sweeteners became available in "smart shops" in various European countries and were also offered via Internet shops. The substance defined "JWH-018" (naphthalen-1-yl-(1-pentyl-1H-indol-3-yl)methanone) was the first cannabimimetic drug identified in 2008, as a psychoactive ingredient in different herbal blends referred to as “Spice” [1,2]. Soon afterwards, several European countries and the Drug Enforcement Administration of the United States undertook legal action to ban this substance together with some of its analogues, progressively synthesized and appearing on the market. Indeed, the efforts to develop new non-steroidal anti-inflammatory agents, possessing high affinity to cannabinoid receptors CB1 and CB2 led to the synthesis of a series of aminoalkylindoles, whose pharmacological activity was studied [2-5]. Recently, the new cannabimimetic drugs JWH-073, JWH-019, JWH-250, JWH-398, JWH-015, JWH-122, JWH-200 and AM-694 have been detected in the black market of different countries [6-9]. Therefore, analytical methods were developed in several forensic laboratories to enable the detection of the new psychoactive substances or their metabolites in either blood or urine [10-16]. A fully validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for the detection and quantitation of ten synthetic cannabinoids in human serum revealed high positivity rates (56.4%) for at least one aminoalkylindole drug among the 101 serum samples from 80 subjects studied [11]. On the other hand, the occurrence of extensive metabolic processes makes the content of the native drug in urine samples extremely low or totally absent [13,14]. Thus, detailed identification of their metabolites, excreted in urine and present also in blood proved to be necessary before any non-invasive screening of the new drugs could be proposed. The cytochrome P450 phase I metabolites of JWH-018 were investigated by in-vitro drug incubation with human liver microsomes, followed by LC-MS/MS analysis [13]. In a later study, the chemical synthesis of five potential JWH-018 in-vivo metabolites was reported and the quantification of these metabolites in authentic doping control urine samples was assessed [14]. Further studies, identified various metabolites as effective intake markers for a limited number of synthetic cannabinoids, [16-18]. The analysis of real urine samples proved that the illicit consumption of synthetic cannabimimetics can only be ascertained by GC–MS or LC–MS/MS detection of their urinary metabolites and not the original drugs.

Aim of our study was to develop a UHPLC-MS/MS method to detect JWH-018, JWH-073, JWH-200, JWH-250, HU-210, Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) in hair samples (Figure 1). The set of investigated substances was coherent with the range of cannabimimetics actually present in the black market during the investigated period of hair sampling. The method was fully validated and applied to the analyses of 179 real samples collected from previously proven *Cannabis* consumers.

Experimental

Reagents, standards and samples

HU-210 and the internal standard JWH-018-d_9 were obtained from Cayman Chemical (Ann Harbor, MI, USA), Δ^9-tetrahydrocannabinol (THC) cannabinol (CBN) and cannabidiol (CBD) were purchased from LGC Promochem (Milan, Italy). JWH-018, JWH-073, JWH-200 and JWH-250 were kindly provided by the 'Istituto Superiore della Sanità' - National Institute of Health (ISS, Rome, Italy). The purity of the analytical standards was at least ≥95%. Dichloromethane, methanol, formic acid, acetonitrile, n-hexane and ethylacetate were purchased from Sigma-Aldrich (Milan, Italy). Sodium hydroxide was obtained from Carlo Erba Reagents (Milan, Italy). Ultra-pure water was obtained using a Milli-Q® UF-Plus apparatus (Millipore, Bedford, MA, USA). Stock standard solution were stored at -20°C until used. Working solution were prepared at final concentration of 100 ng/mL by dilution with methanol.

Sample preparation

About 50 mg of hair sample was washed twice with dichloromethane (2 mL, vortex mixing for 3 min). After complete removal of the washing solvent, the hair aliquot was dried at room temperature with a gentle nitrogen flow and then cut into 1-2 mm segments. The resulting hair samples were fortified with 5 µL of internal standard (ISTD), to yield a final ISTD concentration of 100 pg/mg, before the addition of 3 mL NaOH 1N and subsequent incubation at 95 °C for 10 min. Immediately afterwards, the samples were extracted with 5 mL of n-hexane/ethylacetate 90:10 (v/v). The organic phase was separated, dried under a nitrogen flow at 70°C and reconstituted with 50 µL of methanol. An aliquot of 1 µL methanol solution was directly injected into the UHPLC-MS/MS system.

Instrumentation

Analyses were performed on a Shimadzu LC-20A Series system (Shimadzu, Duisburg, Germany) interfaced to an API 5500 triple quadrupole mass spectrometer (Applied Biosystem/Sciex, Darmstadt, Germany) with an electrospray Turbo Ion source operating in the positive ion mode. A Zorbax XDB-C18 column 30 mm × 2.1 mm, 1.8 μm (Agilent Technologies, Italy), protected by a C18 guard column, was used for the separation of the target analytes. The column oven was maintained at 50°C and the elution solvents were water/formic acid 5mM (solvent A) and acetonitrile (solvent B). After an initial isocratic condition at 75% A for 0.5 min, the mobile phase composition was varied by a linear gradient (a:b; v/v) from 75:25 to 17:83 in 3.5 min; then isocratic elution at 100% B was established for 1.0 min. The flow rate was 0.8 mL/min and the total run time was 7.5 min including re-equilibration at the initial conditions before each injection. MS/MS detection was executed in the selected reaction monitoring (SRM) mode. In order to establish appropriate SRM conditions, each analyte was individually infused into the electrospray ionization (ESI) capillary and the declustering potential (DP) was adjusted to maximize the intensity of the protonated molecular species $[M+H]^+$. The collision offset voltage (CE) was set so as to preserve approximately 10% of precursor ion and the cell exit potentials (CXP) were also optimized. The SRM transitions were monitored during a time window of ±12.5 s around the expected retention time, and the cycle time of the SRM program was 0.25 s. Optimal signals were obtained using a source block temperature of 500°C and an ion-spray voltage of 4000V. Nitrogen was employed as the collision gas (5×10^{-3} Pa). Both Q1 and Q3 were operated at unit resolution. Gas pressures were set as follows: curtain gas 27.0 psi, collision gas 9.0 psi, ion source gas (1) 30.0 psi and ion source gas (2) 30.0 psi. SRM transitions and potential settings for the analytes and the internal standard are presented in Table 1.

Method validation

The method was validated by investigating the following parameters: specificity, linearity, identification and quantitation limits (LOD and LOQ), precision, accuracy [19-21]. Carry-over and matrix effects were also evaluated. Blank hair specimens required for the experiments were obtained from five healthy volunteers (two females, three males).

Selectivity

The occurrence of possible interferences from endogenous substances was tested on the pool of five blank hair sample by monitoring for each investigated compound the signal-to noise ratio (S/N) of SRM chromatographic profiles at the expected retention time interval. Selectivity was positively ascertained when S/N values remained below 3.

Identification criteria and repeatability of diagnostic fragment ions relative abundances

Identification criteria for the analytes were established according to 2006 SOFT/AAFS guidelines [12]. The intra-day and inter-day repeatability of the relative SRM peak intensities for each analyte was determined from five spiked hair samples at a concentration of 50 pg/mg. The inter-day repeatability was verified for three consecutive days.

Linearity, limit of detection and limit of quantitation

Two standard calibration curves at low and high concentrations were prepared by fortifying blank hair samples in the range of, respectively, 1.0-25.0 pg/mg (20-500 pg/mg for THC, CBD, CBN and HU-210) and 20-500 pg/mg (100-1000 pg/mg for THC, CBD, CBN and HU-210). Two replicates for each concentration were analyzed and then averaged to build the curve. The linear calibration parameters were obtained using the least squares regression method. The squared correlation coefficient (R^2) was used to estimate linearity. Quantitative data resulting from area counts were corrected using the ISTD signal. The limit of detection (LOD) was estimated as the analyte concentration whose response provided a S/N value equal to 3, as determined from the least abundant among the qualifier ions; LOD was extrapolated from S/N values of the three lowest concentrations of the calibration curve. The calculated LODs were then experimentally confirmed by analyzing spiked samples at LODs concentration for all analytes. The limit of quantification (LOQ) was estimated based on the S/N ratio to be equal or greater than 10 [20].

Matrix effect

The matrix effect was calculated as the mean value obtained from five different hair sources. Hair samples were spiked after the extraction step at the final concentration of 25 pg/mg (500 pg/mg for THC, CBD, CBN and HU-210). For each analyte, the chromatographic peak area were compared with the peak area of standard solutions prepared in methanol, which is the reconstitution solvent. For each sample, analyses were repeated three times. Variability of matrix effect among different hair samples were expressed as CV%.

Precision and accuracy

For all analytes, intra-day precision (expressed as percent variation coefficient, CV%) and accuracy (%) were evaluated by extracting and analyzing ten replicates of blank hair samples spiked with the standard solutions at two concentration levels (2.5 and 25 pg/mg for JWH-018, JWH-073, JWH-200, JWH-250; 50 and 500 pg/mg for CBD, CBN, THC, HU-210) covering low and high concentrations of the calibration range. Inter-day precision and accuracy were evaluated by

preparing and analyzing for three consecutive days the same set of ten hair samples used for the intra-day tests. Standard criteria designated satisfactory assay precision when CV% values were below 25% for lower concentrations and below 15% for upper concentrations. Since the acceptance criteria for accuracy are not fixed by internationally standardized rules, we chose that satisfactory accuracy was achieved when the experimentally determined concentrations lied within ± 25% from the expected values. The parameters most commonly changing in everyday toxicological analysis, namely sample volume, reagent batch and operator, were deliberately varied to test if satisfactory accuracy was maintained.

Carry-over

The background chromatographic profiles for each analyte main transitions were monitored during the analysis of blank hair sample injected for five times after a chromatographic run where a blank hair sample was spiked with all analytes at the highest calibration level. To assure the absence of carry-over, for each transition, the signal to noise ratio (S/N) had to be lower than 3.

Results and Discussion

The optimized UHPLC-MS/MS method allowed the determination of five relevant synthetic cannabinoids plus THC, CBD and CBN in less than 4 min. The whole chromatographic run, comprehensive of the time required for column re-equilibration before the following injection, was completed in 7.5 min. Figure 2 shows a typical UHPLC–MS/MS chromatogram recorded from a hair sample spiked with a mixture of analytes at a concentration of 5 pg/mg (100 pg/mg for THC, CBD, CBN and HU-210).

Method validation

Identification criteria and selectivity

For each analyte, the characteristic SRM transitions and retention time are presented in Table 1. The three SRM transitions selected for each analyte provided at least 4 identification points, while the substantial stability of their relative abundances proved compliant for the unambiguous identification of all analytes included in the assay, in agreement with CE/2002/657 decision and 2006 SOFT/AAFS guidelines criteria. The SRM chromatograms of five blank hair samples showed no interfering signals (i.e. S/N value lower than 3) at the retention time interval when each analyte is expected to elute. This demonstrated that the method is selective for all tested compounds and free from positive interference from hair components and column bleeding.

Linearity, limit of detection and limit of quantitation

The SRM protocol described in Table 1 was used to build the calibration plots for the eight analytes from spiked blank hair samples. A good fit and linearity of the calibration curves was uniformly observed. Range of linearity, R^2 values, LOD and LOQ values are showed in Table 2. Positive detection (S/N >3) of analytes at the approximate LOD concentrations was confirmed experimentally. LOD values ranged from 0.02 pg/mg (JWH-200) to 0.18 pg/mg (JWH-018) for aminoalkylindoles and from 1.2 pg/mg (THC) to 5.4 pg/mg (CBD) for cannabinoids. LOQ values ranged correspondingly from 0.07 pg/mg to 18 pg/mg.

Matrix effect

The variability among different hair samples was acceptable (CV% <25%, as shown in Table 2), so we pooled together the five sources of hair to perform validation experiments such as precision and accuracy. The effect of the real hair matrix appeared to be significant for most of the analytes tested (see Table 2). Large ion suppression is evident for JWH-018 and JWH-250 (matrix effect <-25%), while ion enhancement is evident for CBD and HU-210 (matrix effect >+25%). In general, ion suppression was observed with the aminoalkylindoles, while ion enhancement occurred with cannabinoids. To compensate as much as possible for the matrix effect present in real hair samples analysis, all calibration and validation tests were conducted on a pool of human hair samples, spiked with the standard analytes solutions. On the other hand, the good linearity observed in the calibration plots demonstrated that the observed matrix effect is proportionally constant, i.e. does not depend on the analytes' concentrations.

Precision and accuracy

Intra- and inter-day data on precision and accuracy are reported in Table 3. At low concentration, the results showed satisfactory repeatability, as the percent variation coefficient (CV%) is lower than 15% for all the spiked analytes; in particular, intra-day precision exhibits CV% values ranging from 1.3% (JWH-200) up to 7.2% (CBD). At high concentration, the method precision proved also compliant for all analytes, with CV% values varying from 2.1% (JWH-200) to 4.8% (CBN).

Intra-day accuracy expressed as percent bias showed a general trend, with more positive biases observed at low concentrations. However, all the accuracy results were satisfactory, as all biases were below 15% and widely within the expected ±25% interval. In particular, at the lower concentration tested, bias values ranged from no-error (JWH-018) to +14.5% (CBN), whereas at the higher concentration the bias% ranged from –5.2% (JWH-073) to +2.8% (CBD).

At the same concentrations, also the inter-day precision and accuracy proved satisfactory, as the CV% values ranged from 2.7% for JWH-250 (at 25 pg/mg) to 11.5% for CBD (at 500 pg/mg), while the bias% ranged from −6.1% for JWH-073 and JWH-200 (at 25 pg/mg) to +17.4 for CBN (at 50 pg/mg).

Carry-over

The background chromatographic profiles for the main transitions of each analyte, which were monitored during the analysis of blank hair extracts injected after highly concentrated samples, did not show the presence of any significant signal (i.e., the S/N value was always lower than 3) at the retention time intervals of the tested analytes. Therefore, the presence of carry-over effect could be excluded.

Application to real samples

179 real samples (152 head hair, 27 pubic hair) were collected and analyzed in 2010 in our laboratory arising from subjects that proved to be frequent users of THC-containing products. These hair samples were analyzed once more in 2011 using the present UHPLC–MS/MS method for the potential detection of cannabimimetic drugs. All the samples considered in this study were randomly selected from two groups of subjects, namely driving re-licensing and drug abuse and withdrawal control subjects. Only the proximal 0-6 cm segment was analyzed whenever a longer head hair sample was collected. Shorter head hair, as well as pubic hair samples, were analyzed in their full length. Fourteen (7.82%) samples were found positive for at least one synthetic cannabinoid, two of which were pubic hair samples (see Table 4). All positive samples originated from male subjects aged 30.0 ± 9.74 years (mean ± SD%, range 18-48). The average values for THC, CBD and CBN were, respectively, 129.5 ± 154.2 pg/mg (range 50-553), 300.3 ± 552.7 (range 18-1862) and 66.2 ± 48.0 (range 31-205). The drug most frequently detected was JWH-018, which could be found in 9 samples at concentrations of 28.1 ± 28.8 pg/mg (0.60–70.5 pg/mg), followed by JWH-073 and JWH-250, each found in 8 samples. The concentration of JWH-073 was 116.9 ± 183.2 pg/mg (0.50–413.3 pg/mg), whereas the concentration of JWH-250 was 179.6 ± 242.2 pg/mg (1.50–729.4 pg/mg). Although the cut-off level of 50 pg/mg is internationally accepted for THC in hair, there are no enough data in current literature in order to draft similar conclusion for synthetic cannabinoids. Further studies should be performed in order to discriminate between chronic consumption and occasional use (or external contamination), particularly when the concentration detected are lower than 50 pg/mg. The other analytes included in the method (JWH-200 and HU-210) were not identified in any of the analyzed samples. Seven samples were positive for only one

drug, three samples contained two, and four samples contained three synthetic cannabinoids. Totally, 6 samples out of 14 were positive for at least one synthetic cannabinoid at level higher than 50 pg/mg. The chromatogram of a real sample positive to THC, CBD, CBN, JWH-018, JWH-073 and JWH-250 is shown in Figure 3. The samples that were positive for JWH-018 and JWH-073 were collected between February and December 2010, although the Italian Ministry of Health had included these two cannabinoids into the list of prohibited substances since June 2010. The first sample positive to JWH-250 was collected in June 2010 (length: 3.5 cm, result: 209 pg/mg), although this drug was banned in Italy only since May 2011. JWH-200 and HU-210 have not been specifically banned yet, but, in any case, all the hair samples tested in the present study proved negative to their presence. Apparently, these two substances were not yet present in the Italian black market during the year 2010.

Conclusions

The present UHPLC–MS/MS method proved suitable for the detection and quantitation of several synthetic cannabinoids in keratinic matrix, taking advantage from the fact that the original drugs, not their metabolites, are found in hair samples. The proposed method allows a fast determination of all target compounds in a single run of 4.0 minutes plus 3.5 min of column re-equilibration time. In general, the introduction of UHPLC-MS/MS drastically reduced the analysis time required for carrying out the toxicological procedures, hence achieving a drastic increase of the overall laboratory productivity without sacrificing chromatographic resolution, accuracy and precision. Nonetheless, the method appears to be sufficiently flexible to include new substances and more Internal Standards, for a continuously expanding set of analytes.

The present method was successfully applied to the analysis of 179 real samples from frequent *Cannabis* consumers with the aim of understanding the recent diffusion of cannabimimetics in the overall drug abuse. Plausibly, consumers of cannabimimetics have to be found chiefly among former or still active *Cannabis* consumers, whose incidence in the general population is, in turn, quite accurately estimated. To our knowledge, this is the first inference study on cannabimimetics consume in the Italian territory, which has to be repeated with a larger panel of analytes and clear reference to both a fixed period of time and a comparative population of casually sorted subjects. Despite the statistical limitations of the present approach, it was nevertheless demonstrated a noteworthy presence of positive samples in the investigated target population, showing that the consume of synthetic cannabinoids is being a health emergency, at least among *Cannabis* abusers since early 2010. Further in-depth examinations are needed to investigate this phenomenon, possibly including the new substances that are progressively introduced into the black market and

exploring other populations of potential consumers. Nonetheless, tentative cut-off levels should be proposed in order to discriminate between chronic consumption and occasional use (or external contamination), or to elucidate if the cut-off of 50 pg/mg used for THC in hair might be similarly accepted for synthetic cannabinoids.

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Table 1. SRM transitions and corresponding potentials for the target compounds and internal standard.

Compound	RT (min)	SRM transitions[a] (m/z)	DP (V)	CE (V)	CXP (V)
JWH-200	1.03	**385.2 → 155.1**	53	29	20
		385.2 → 114.0	53	32	18
		385.2 → 127.0	53	62	20
JWH-250	3.17	**336.2 → 121.0**	68	29	19
		336.2 → 91.0	68	62	15
		336.2 → 200.3	68	34	15
JWH-073	3.22	**328.1 → 155.0**	85	32	15
		328.1 → 200.2	85	31	17
		328.1 → 127.0	85	62	21
CBD	3.39	**315.0 → 193.2**	40	30	10
		315.0 → 123.0	40	43	19
		315.0 → 259.2	40	27	11
JWH-018	3.47	**342.1 → 155.1**	41	31	13
		342.1 → 214.2	41	32	9
		342.1 → 127.1	41	55	13
CBN	3.67	**311.1 → 223.2**	64	28	17
		311.1 → 195.1	64	36	8
		311.1 → 241.3	64	27	8
HU-210	3.67	**387.3 → 243.1**	110	25	10
		387.3 → 71.1	110	27	10
		387.3 → 261.1	110	24	11
THC	3.85	**315.0 → 193.0**	45	30	8
		315.0 → 123.1	45	44	12
		315.0 → 259.1	45	27	10
JWH-018-*d9*	3.46	**351.2 → 154.9**	70	35	21
		351.2 → 127.3	70	62	11

[a]Target transitions used for quantitation are marked in bold

Table 2. Range of calibration, linearity, LODs and LOQs values, and matrix effect for all analytes

Compound	Low levels		High levels		LOD (pg/mg)	LOQ[a] (pg/mg)	Matrix effect[b]	
	Linearity Range (pg/mg)	Correlation coefficient (R^2)	Linearity Range (pg/mg)	Correlation coefficient (R^2)			Mean (±%)	CV%
JWH-200	1-25	0.9941	20-200	0.9959	0.02	0.07	-7.6	19.5
JWH-250	1-25	0.9974	20-200	0.9997	0.04	0.13	–30.9	20.0
JWH-073	1-25	0.9991	20-200	0.9963	0.10	0.33	–19.9	19.7
CBD	20-500	0.9985	100-800	0.9957	5.4	18	+32.1	19.8
JWH-018	1-25	0.9985	20-500	0.9968	0.18	0.59	–59.1	10.3
CBN	20-500	0.9968	100-1000	0.9958	1.6	5.3	+11.8	12.6
HU-210	20-200	0.9933	100-1000	0.9943	3.0	9.9	+29.8	11.9
THC	20-500	0.9980	100-1000	0.9954	1.2	3.9	+8.9	21.9

[a] calculated LOQ
[b] Matrix effect was evaluated using five different sources of hair

Table 3. Intra/Inter-day precision (CV%) and accuracy (bias%) for each analyte tested.

Compound	Concentration (pg/mg)	Intraday (n=10)		Interday (n=30)	
		Precision (CV%)	Accuracy (Bias%)	Precision (CV%)	Accuracy (Bias%)
JWH-200	2.5	1.3	+6.1	4.4	+4.6
	25	2.1	–4.0	6.8	–6.1
JWH-250	2.5	2.3	+2.0	7.2	–4.8
	25	2.4	–4.5	2.7	–5.0
JWH-073	2.5	1.9	+8.6	8.2	–1.4
	25	3.4	–5.2	4.4	–6.1
CBD	50	7.2	+5.7	9.9	+1.4
	500	2.8	+2.8	11.5	–1.8
JWH-018	2.5	4.2	0.0	5.8	–4.3
	25	2.3	–3.0	4.2	–1.1
CBN	50	2.0	+14.5	8.8	+17.4
	500	4.8	+0.4	11.0	+1.5
HU-210	50	3.1	+13.6	7.9	+13.8
	500	2.3	+2.6	5.8	+0.9
THC	50	1.7	+13.4	6.4	+11.2
	500	2.2	–1.7	8.4	–1.9

Table 4. Synoptic summary of real samples positive to synthetic cannabinoids

Positive case	Age	Gender	Type of hair	THC (pg/mg)	CBD (pg/mg)	CBN (pg/mg)	JWH-018 (pg/mg)	JWH-073 (pg/mg)	JWH-250 (pg/mg)
1	29	male	head	73	42	64	70.5	413.3	-
2	29	male	pubic	68	57	67	1.5	-	-
3	18	male	head	553	1217	137	38.3	-	-
4	n/a	male	head	70	55	36	-	1.3	208.8
5	22	male	head	57	<LOQ	39	70.4	37.0	729.4
6	22	male	head	57	222	60	-	-	1.5
7	48	male	head	54	25	31	44.9	409.3	262.0
8	43	male	head	50	24	36	0.8	0.5	-
9	20	male	head	69	85	62	-	-	67.4
10	26	male	pubic	60	88	60	-	1.7	-
11	32	male	head	115	460	51	10.9	66.7	138.6
12	44	male	head	59	38	31	0.6	-	-
13	37	male	head	417	1862	205	14.8	5.2	2.9
14	20	male	head	112	18	47	-	-	26.0

n/a: not available

FIGURE CAPTIONS

Figure 1

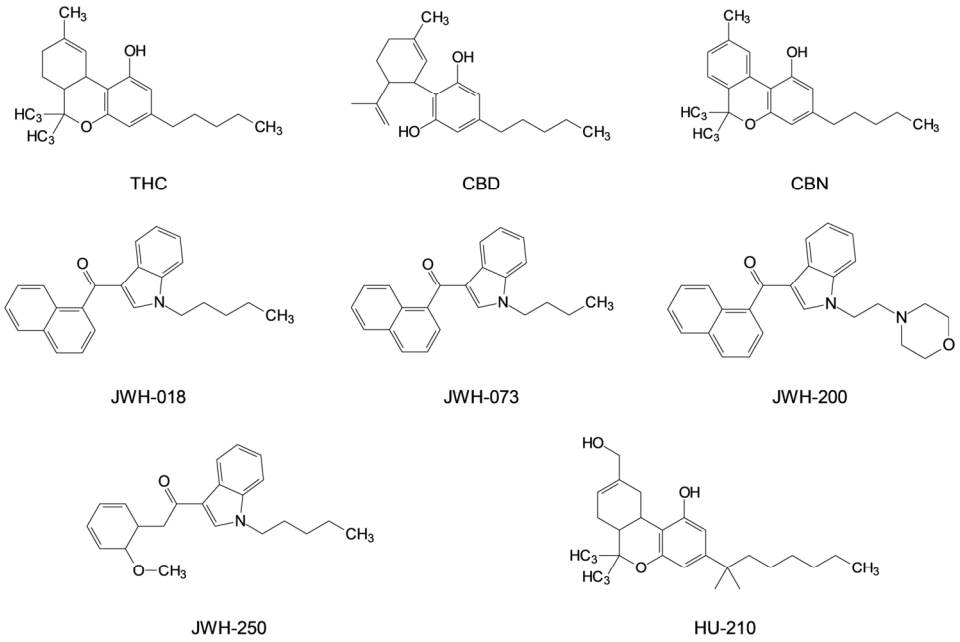
Molecular structures of the screened compounds

Figure 2

MRM chromatogram of a blank hair sample fortified with the target compounds at final concentration of 5 pg/mg for JWH-200, JWH-250, JWH-073, JWH-018 and 100 pg/mg for CBD, CBN, HU-210 and THC.

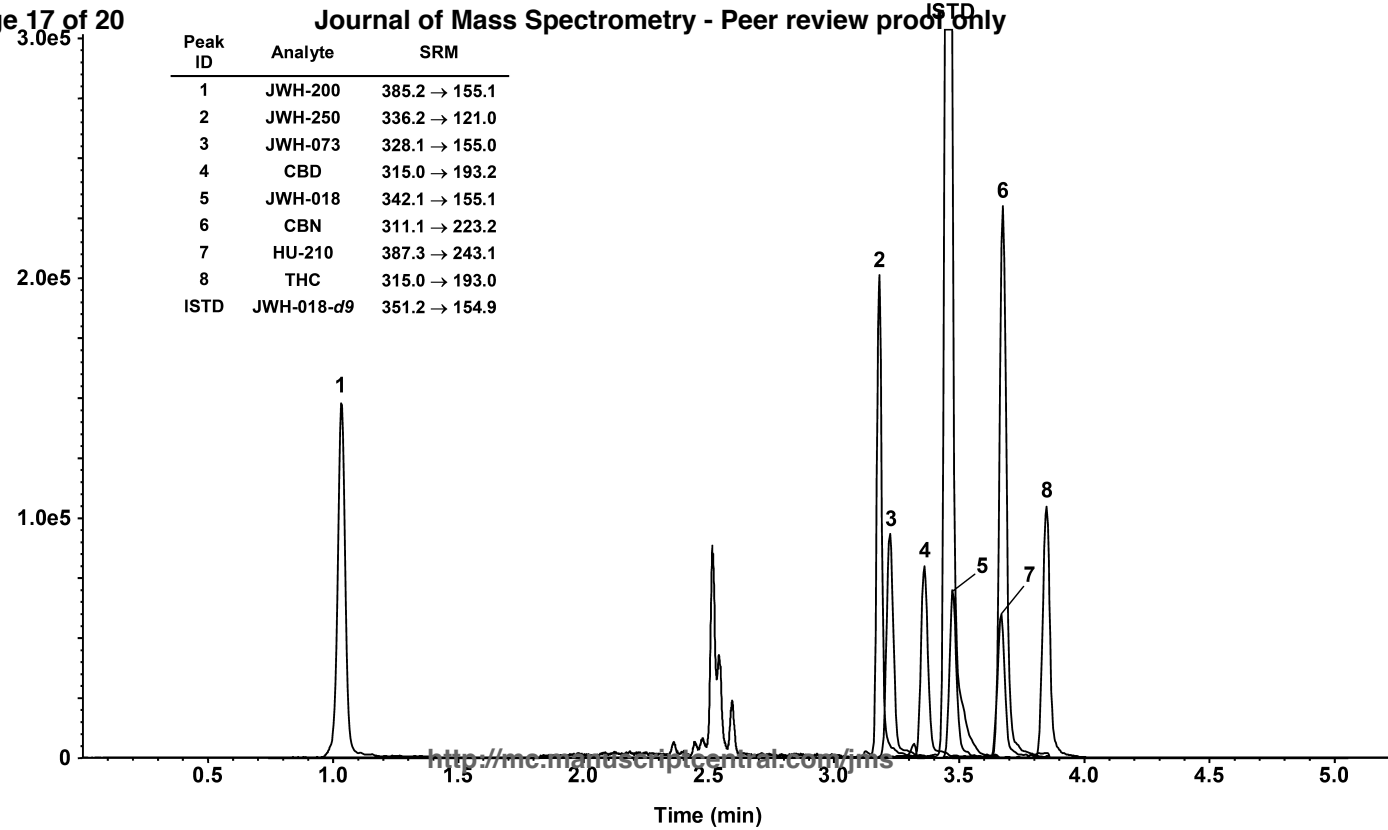
Figure 3

Analysis of a real sample positive to THC, CBD, CBN, JWH-018, JWH-073 and JWH-250.



75x50mm (600 x 600 DPI)

Peak ID	Analyte	SRM
1	JWH-200	385.2 → 155.1
2	JWH-250	336.2 → 121.0
3	JWH-073	328.1 → 155.0
4	CBD	315.0 → 193.2
5	JWH-018	342.1 → 155.1
6	CBN	311.1 → 223.2
7	HU-210	387.3 → 243.1
8	THC	315.0 → 193.0
ISTD	JWH-018-*d9*	351.2 → 154.9



Peak ID	Analyte	SRM	Concentration (pg/mg)
1	JWH-250	336.2 → 121.0	2.9
2	JWH-073	328.1 → 155.0	5.2
3	CBD	315.0 → 193.2	1862
4	JWH-018	342.1 → 155.1	14.8
5	CBN	311.1 → 223.2	205
6	THC	315.0 → 193.0	417
ISTD	JWH-018-*d9*	351.2 → 154.9	100