Journal of Chromatography B, 903 (2012) 95–101

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of 22 synthetic cannabinoids in human hair by liquid chromatography–tandem mass spectrometry

Melanie Hutter, Stefan Kneisel, Volker Auwärter, Merja A. Neukamm*

Institute of Legal Medicine, University Medical Center, Albertstrasse 9, D-79104 Freiburg im Breisgau, Germany

ARTICLE INFO

Article history:
Received 16 April 2012
Accepted 6 July 2012
Available online 17 July 2012

Keywords:
Spice
Hair analysis
LC–MS/MS
Cannabimimetic
Herbal mixtures

ABSTRACT

Herbal mixtures of the "Spice"-type contain a variety of synthetic cannabinoids. To prove the contact of a person with synthetic cannabinoids in a previous period of up to several months, hair testing is ideally suited. A rapid, simple and sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay was developed to determine 22 synthetic cannabinoids in human hair. The synthetic cannabinoids JWH-007, JWH-015, JWH-018, JWH-019, JWH-020, JWH-073, JWH-081, JWH-122, JWH-200, JWH-203, JWH-210, JWH-250, JWH-251, JWH-398, AM-694, AM–2201, methanandamide, RCS-4, RCS-4 ortho isomer, RCS-8, WIN 48,098 and WIN 55,212-2 were extracted from 50 mg hair by 3-h ultrasonification in ethanol. The extracts were analysed on a triple-quadrupole linear ion trap mass-spectrometer in scheduled multiple reaction monitoring mode (sMRM). The method was fully validated and proved to be accurate, precise, selective and specific with satisfactory linearity within the calibrated range and a lower limit of quantification of 0.5 pg/mg for 20 compounds. Authentic hair samples from chronic consumers showed the presence of two to six synthetic cannabinoids in the same segment. In the first segment, concentrations of up to 78 pg/mg JWH-081 were present. In segmented hair, the concentrations of most substances increased from the first (proximal) to the third segment. The highest concentration was ca. 1100 pg/mg JWH-081. The results of segmental hair analysis in chronic users suggest incorporation of the drugs in head hair via side-stream smoke condensation as a major route. In summary, the method can be used to prove the contact with herbal mixtures containing synthetic cannabinoids and thus contributes to an efficient abstinence control.

1. Introduction

Herbal mixtures of the "Spice"-type became popular around 2008. The products are labelled "not for human consumption" and are advertised e.g. as incense or plant growth regulator. These mixtures are declared to be purely herbal, but exhibit strong cannabimimetic effects after smoking. This is because they have been adulterated with synthetic cannabinoid receptor agonists [1]. Most of the synthetic cannabinoids identified in herbal mixtures, for instance the aminoalkylindole JWH-018, feature high binding affinity to the cannabinoid receptor type 1 [2–5], thus inducing similar effects as Δ^9-tetrahydrocannabinol (THC).

The legal status of the compounds varies in different countries. In Germany, the compounds JWH-018, CP-47,497 and its C6-, C8- and C9-homologues were banned in January 2009, shortly after their first identification in herbal mixtures. One year later, the synthetic cannabinoids JWH-019 and JWH-073 were banned and it has recently been announced that the synthetic cannabinoids JWH-007, JWH-015, JWH-081, JWH-122, JWH-200, JWH-203, JWH-210, JWH-250, JWH-251, 3-(1-adamantoyl)-1-pentylindole [6], AM-694 and RCS-4 are considered to be put under the controlled substances legislation. Similar legal measures on synthetic cannabinoids have been imposed in other countries.

In order to prove the consumption of synthetic cannabinoids, analytical methods for the detection of these compounds in blood or serum have been developed [7–9]. However, the range of analytes covered by these methods had to be expanded [10,11], as the number of different synthetic cannabinoids identified in herbal mixtures is continuously increasing [12–17]. The synthetic cannabinoids used in these products are mainly high-potency drugs [18–20]. Consumers stated as their motivation for smoking synthetic cannabinoids that the herbal mixtures appear to be a legal alternative to cannabis and can be purchased easily in so called "head shops" or via the Internet. Another stated motivation is that synthetic cannabinoids are not detected in common immunological drug tests [21], so that the consumption of synthetic cannabinoids seems to be particularly attractive in conditions involving regular urine drug screening, for instance in driver's licence recovery or in forensic psychiatry settings. Matrices like urine, oral fluid and hair are commonly used to avoid invasive sampling or to

* Corresponding author. Tel.: +49 761 203 6827; fax: +49 761 203 6858.
E-mail address: merja.neukamm@uniklinik-freiburg.de (M.A. Neukamm).

1570-0232/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2012.07.002

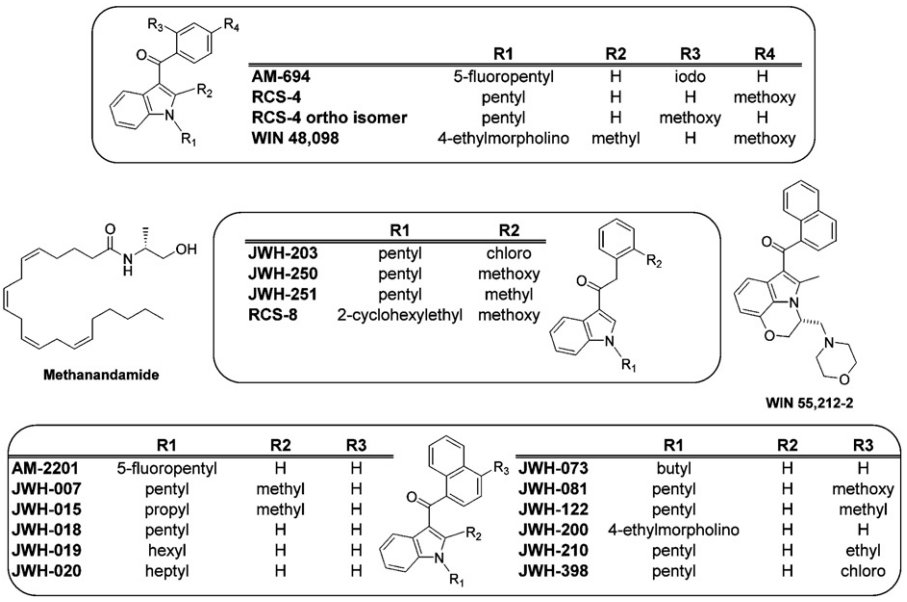

Fig. 1. Structures of the 22 synthetic cannabinoids covered by the method.

confirm earlier drug consumption. While in oral fluid and hair the parent compounds of synthetic cannabinoids are analysed [22], their metabolites are determined in urine analysis. Therefore, for analysis of synthetic cannabinoids in urine, the main metabolites of the parent compounds have to be identified prior to developing analytical methods [23–25]. As a consequence, hair analysis for synthetic cannabinoids can be adapted faster than urine analysis, because the detection of the parent compounds allows for rapidly upgrading the analytical method with newly available compounds.

Hair analysis is preferentially used to prove abstinence from drugs [26]. The process of drug incorporation into hair after smoking is currently discussed extensively [27]. To our knowledge, few data concerning the analysis of synthetic cannabinoids in hair have been published until today [28–30].

In this article, we present a validated method for the quantitative determination of the following 22 synthetic cannabinoids in human hair based on liquid chromatography–tandem mass spectrometry: JWH-007, JWH-015, JWH-018, JWH-019, JWH-020, JWH-073, JWH-081, JWH-122, JWH-200, JWH-203, JWH-210, JWH-250, JWH-251, JWH-398, AM-694, AM–2201, methanandamide, RCS-4, RCS-4 ortho isomer, RCS-8, WIN 48,098 and WIN 55,212-2 (structures are given in Fig. 1). The method was successfully applied to authentic hair samples obtained from forensic psychiatry patients.

2. Experimental

2.1. Chemicals and reagents

All solvents and substances were at least of analytical or HPLC grade. 2-Propanol was obtained from Merck (Darmstadt, Germany). Ethanol and ammonium formate were from Sigma–Aldrich (Steinheim, Germany). Formic acid and petroleum ether (40–60 °C) were purchased from Carl Roth GmbH (Karlsruhe, Germany). Methanol was from J.T. Baker (Deventer, Netherlands) and acetone from VWR International S.A.S. (Fontenay-sous-Bois, France). Deionized water was prepared with a cartridge deionizer from Memtech (Moorenweis, Germany).

JWH-018, JWH-073, JWH-018-d11 and JWH-073-d9 were obtained from Chiron AS (Trondheim, Norway). 11-Hydroxy-Δ^9-tetrahydrocannabinol-d3 (OH-THC-d3) and flunitrazepam-d7 were purchased from LGC Standards GmbH (Wesel, Germany). JWH-007, JWH-398, RCS-8, JWH-007-d9 and JWH-250-d5 were purchased from Cayman Chemical (Ann Arbor, MI, USA). Methanandamide was from Sigma–Aldrich (Steinheim, Germany). JWH-020 and WIN 55,212-2 were provided by the German Federal Criminal Police Office (Bundeskriminalamt, Wiesbaden, Germany). JWH-019 was provided by the Finnish customs. RCS-4 ortho isomer was provided by the State Bureau of Criminal Investigation (Landeskriminalamt) of Lower Saxony. JWH-015, JWH-081, JWH-122, JWH-200, JWH-203, JWH-210, JWH-250, JWH-251, AM-694, AM-2201, RCS-4 as well as WIN 48,098 were purchased as 'research chemicals' from different Internet providers. Identities and purities of all substances not obtained from professional suppliers were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (NMR), gas chromatography–mass spectrometry (GC–MS) as well as by thin layer chromatography (TLC) analysis. All substances showed purities greater than 98%.

2.2. Instrumentation and method

The hair samples were analysed using a liquid chromatography–tandem mass spectrometry (LC–MS/MS) system consisting of a QTrap 4000 triple-quadrupole linear ion trap mass spectrometer fitted with a TurboIonSpray interface (Applied Biosystems/Sciex, Darmstadt, Germany) and a Prominence HPLC system by Shimadzu (Duisburg, Germany) with 3 LC-20ADsp isocratic pumps, CTO-20AC column oven, SIL-20AC autosampler, DGU-20A3 degasser and a CBM-20A controller. Separation was achieved on a Luna Phenyl Hexyl column (50 mm × 2 mm, 5 μm particle size) with an equivalent guard column (4 mm × 2 mm) (Phenomenex, Aschaffenburg, Germany). Solvent A consisted of water with 0.2% formic acid and 2.0 mmol/L ammonium formate and solvent B was methanol. The gradient started with 50% solvent B at a 0.4 mL/min flow rate and increased within 7.0 min to 90% solvent B, which was kept for 1.5 min. Starting conditions were restored within 0.5 min and kept for 3.0 min, allowing the system to re-equilibrate. A post-column addition of 2-propanol at a 0.2 mL/min flow rate was used to enhance sensitivity. Injection volume was 20 μL. The mass spectrometer was operated in positive ionization mode. Ion-spray voltage was set to +2500 V. The gas settings were as follows: curtain gas 30 psi, collision gas 6 psi, ion

Table 1
MRM transitions, internal standards, retention times and corresponding voltages for the liquid chromatographic–mass spectrometric analysis.

Compound	Internal standard	Q1 (amu)	Q3 (amu)	*tR* (min)	DP (V)	EP (V)	CE (V)	CXP (V)
AM-694*	JWH-073-d9	436.3	231.0	5.5	80	5	40	16
AM-694		436.3	203.0	5.5	80	5	67	8
AM-2201*	JWH-073-d9	360.2	155.0	6.1	85	5	36	11
AM-2201		360.2	127.0	6.1	85	5	64	8
JWH-007*	JWH-007-d9	356.1	155.2	6.9	85	5	36	12
JWH-007		356.1	127.2	6.9	85	5	70	9
JWH-015*	JWH-073-d9	328.2	155.1	5.9	85	5	34	11
JWH-015		328.2	127.1	5.9	85	5	60	8
JWH-018*	JWH-018-d11	342.2	155.1	6.8	90	5	35	7
JWH-018		342.2	127.1	6.8	90	5	61	8
JWH-019*	JWH-018-d11	356.2	155.1	7.2	90	5	36	11
JWH-019		356.2	127.1	7.2	90	5	71	9
JWH-020*	JWH-007-d9	370.3	155.1	7.6	100	5	36	10
JWH-020		370.3	127.1	7.6	100	5	65	8
JWH-073*	JWH-073-d9	328.2	155.2	6.3	90	5	32	11
JWH-073		328.2	127.2	6.3	90	5	65	8
JWH-081*	JWH-018-d11	372.2	185.1	7.1	80	5	35	12
JWH-081		372.2	214.2	7.1	80	5	35	10
JWH-122*	JWH-018-d11	356.2	169.1	7.2	94	5	36	12
JWH-122		356.2	141.1	7.2	94	5	66	9
JWH-200*	Flunitrazepam-d7	385.2	155.1	2.3	80	5	33	11
JWH-200		385.2	114.1	2.3	80	5	37	8
JWH-203*	JWH-018-d11	340.1	125.0	6.4	80	5	36	8
JWH-203		340.1	188.2	6.4	80	5	28	13
JWH-210*	JWH-007-d9	370.2	183.1	7.5	80	5	35	8
JWH-210		370.2	214.2	7.5	80	5	35	10
JWH-250*	JWH-250-d5	336.3	121.1	6.1	75	5	30	8
JWH-250		336.3	91.1	6.1	75	5	63	5
JWH-251*	JWH-073-d9	320.2	105.1	6.3	80	5	34	6
JWH-251		320.2	214.1	6.3	80	5	34	9
JWH-398*	JWH-007-d9	376.2	189.2	7.5	85	5	37	14
JWH-398		376.2	161.2	7.5	85	5	62	12
Methanandamide*	JWH-018-d11	362.2	76.0	6.7	51	5	33	4
Methanandamide		362.2	91.0	6.7	51	5	66	5
RCS-4*	JWH-073-d9	322.2	135.1	5.9	80	5	34	10
RCS-4		322.2	77.1	5.9	80	5	77	4
RCS-4 ortho isomer*	JWH-073-d9	322.1	135.1	5.7	80	5	32	10
RCS-4 ortho isomer		322.1	77.1	5.7	80	5	74	4
RCS-8*	JWH-018-d11	376.2	121.0	7.2	60	5	34	8
RCS-8		376.2	91.0	7.2	60	5	74	5
WIN 48,098*	Flunitrazepam-d7	379.2	135.0	1.5	70	5	30	9
WIN 48,098		379.2	114.1	1.5	70	5	41	8
WIN 55,212-2*	OH-THC-d3	427.2	155.1	5.6	80	5	36	11
WIN 55,212-2		427.2	127.1	5.6	80	5	76	8
Flunitrazepam-d7		321.0	275.2	2.7	65	5	50	8
JWH-007-d9		365.1	155.1	6.9	90	5	36	11
JWH-018-d11		353.3	155.1	6.8	90	5	35	7
JWH-073-d9		337.3	155.1	6.3	90	5	32	11
JWH-250-d5		341.1	121.1	6.1	75	5	30	8
OH-THC-d3		334.3	316.3	5.5	65	5	20	8

Q1, mass-charge ratio of precursor; Q3, mass-to-charge ratio of fragment; amu, atomic mass unit; *tR*, retention time; DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, cell exit potential.
The transitions used for quantitation are marked with an asterisk.

source gas (1) 40 psi and ion gas (2) 50 psi. Source temperature was set to 600 °C.

The scheduled multiple reaction monitoring (sMRM) method contained two transitions for each analyte and one transition for each internal standard. The MRM transitions were analysed at a ±50 s time window around the expected retention time, and the total cycle time of the MRM mode was 1.2 s including a 5 ms pause time between the MRM transitions. Declustering potential (DP), entrance potential (EP), cell entrance potential (CEP), collision energy (CE) and cell exit potential (CXP) were optimized for each compound. For the internal standards OH-THC-d3 and flunitrazepam-d7, the MRM transition and suitable CE were selected using an in-house MS/MS library [31]. All compounds, the respective MRM transitions and the corresponding potentials and energies are shown in Table 1. The software Analyst 1.5 (ABSciex, Darmstadt, Germany) was used for LC–MS/MS operation and data analysis.

2.3. Calibration standards and controls

To prepare the stock solution, appropriate volumes of standard solutions were mixed and diluted with ethanol to a final concentration of 1.0 μg/mL. To prepare the working solutions (100 ng/mL, 10 ng/mL and 1.0 ng/mL), the stock solution was diluted with ethanol. The internal standard working solution consisted of 40 ng/mL for OH-THC-d3, 20 ng/mL for JWH-007-d9, flunitrazepam-d7 and 10 ng/mL for JWH-018-d11, JWH-073-d9 and JWH-250-d5 in ethanol. For the preparation of the internal standard working solution, appropriate volumes of the internal standard solutions were mixed and diluted with ethanol. The hair calibration standards (0.5, 1.0, 2.5, 5.0, 10, 25, 50 and 75 ng/mg) were prepared by adding adequate amounts of the working solutions and 25 μL of the internal standard working solution to 50 mg of drug-free hair–ethanol mixture prior to extraction. The quality control samples with concentrations of 2.0 pg/mg, 20 pg/mg and

60 pg/mg were prepared in the same manner using separate working solutions.

2.4. Hair sample preparation

Hair samples were washed under continuous shaking with water, acetone and petroleum ether for 4 min each. After drying and cutting the hair samples into pieces of 1–2 mm, the internal standard solution was added to 50 mg of each sample followed by extraction with 1.5 mL of ethanol in an ultrasonic bath for 3 h. Subsequently, 1 mL of the extraction solution was transferred to a glass vial and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The dry residue was reconstituted in 100 μL of liquid chromatography solvents A/B, 50/50 (v/v). As the analytes are highly soluble in ethanol and it provides good swelling properties [32], ethanol was chosen as extraction solvent.

2.5. Validation

The LC–MS/MS method was validated for the quantification of synthetic cannabinoids in human hair samples. Method validation was carried out according to the German Society of Toxicology and Forensic Chemistry (GTFCh) guidelines for method validation and quality control in hair analysis. For statistical analysis, the software Valistat 2.0 (Arvecon GmbH, Walldorf, Germany) and MS Excel 2003 (Microsoft Corporation, Redmond, WA, USA) were used. The validation samples were prepared by spiking the adquate amount of standard solutions into the hair–ethanol mixture prior to extraction unless stated otherwise. Selectivity and specificity were tested by analysis of six different drug-free hair samples without internal standard addition (blank samples) and two drug-free hair samples with internal standard addition (zero samples). Additionally, one drug-free hair sample was fortified with a mixture of the following drugs (1000 pg/mg each): Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA), methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine (EDDP), cocaine, benzoylecgonine, methylecgonine, tramadol, o-desmethyltramadol, tilidine, nortilidine, morphine, dihydromorphine, codeine, dihydrocodeine, oxymorphone, hydrocodone, oxycodone, normorphine, hydromorphone, norcodeine, norcocaine, cocaethylene, buprenorphine, norbuprenorphine, 7-aminoclonazepam, 7-aminoflunitrazepam, alprazolam, α-hydroxyalprazolam, bromazepam, brotizolam, camazepam, chlordiazepoxide, clobazam, clonazepam, clotiazepam, delorazepam, diazepam, estazolam, flunitrazepam, flurazepam, lorazepam, lormetazepam, medazepam, midazolam, nitrazepam, nordazepam, norflunitrazepam, oxazepam, temazepam, tetrazepam, triazolam, α-hydroxytriazolam, zaleplon, zopiclone, zolpidem. For the determination of carry-over, a blank sample was analysed directly after the injection of a sample fortified with all analytes to a concentration of 3000 pg/mg. The calibration model was evaluated by five replicate determinations of the calibration curve. Accuracy and precision experiments were carried out by analysing quality control samples at a high (60 pg/mg), middle (20 pg/mg) and low (2.0 pg/mg) concentration level regarding the calibration range. Two replicates of each level were analysed at five different days.

The lower limit of quantification was determined by five replicate determinations of samples fortified to 0.5 pg/mg (fortified to 5.0 pg/mg for JWH-398 and methanandamide). Bias and relative standard deviations had to be ≤20% at the limit of quantification. Evaluation of processed sample stability was carried out as follows: low concentration (2.0 pg/mg) and high concentration (60 pg/mg) samples were processed, pooled, aliquoted to ten autosampler vials each per concentration level and placed in the autosampler at 4 °C. The aliquots were injected regularly within 9 h.

Matrix effects, extraction recovery and overall process efficiency were determined according to the procedure suggested by Matuszewski et al. [33]. Three sample sets were prepared: set 1 consisted of dilutions of synthetic cannabinoids and internal standards in solvents A/B 50/50 (v/v). Set 2 consisted of extracts of five different drug-free hair samples fortified with synthetic cannabinoids and internal standards. Set 3 consisted of five different drug-free hair samples fortified with synthetic cannabinoids and internal standards prior to extraction. Matrix effects were determined by comparison of the peak areas of set 2 and set 1. Recoveries were determined by comparison of the peak areas of sets 2 and 3. Process efficiencies were determined by comparison of the peak areas of sets 3 and 1. All experiments regarding matrix effects, recovery and process efficiency were carried out at the low (2.0 pg/mg) and high concentration (60 pg/mg) level. In order to assess the influence of hair amount on quantification 10, 20, 30, 40 and 50 mg of a hair sample was fortified to a concentration of 20 pg/mg and the relative standard deviation of the measured concentrations was calculated. To determine the optimal extraction time, an authentic hair sample was subjected to a 3-h extraction and an overnight extraction.

2.6. Authentic samples

Hair samples were obtained from forensic psychiatry inpatients for drug testing in mid-February 2011. All patients had previously been tested positive for at least one synthetic cannabinoid in serum samples sent to our laboratory [34]. In an interview during hair sampling, all patients admitted chronic consumption of several different herbal mixtures in the last few months before sampling. The self-stated consumption ranged from three times in six weeks up to a daily consumption of half a package for seven months. The hair was cut according to the Society of Hair Testing (SoHT) guideline. A hair tuft from the posterior vertex region of 3–4 mm diameter was cut with scissors directly on the skin surface. The hair tuft was fixed with a string if the hair was longer than 4 cm. Alternative hair (arm and leg) was collected if head hair was unavailable. The hair colour, length and body site were noted. No hair sample showed obvious cosmetic treatment. The hair sample was wrapped in aluminium foil and stored at room temperature until analysis. Hair samples were segmented whenever hair length and amount allowed for segmentation.

3. Results and discussion

3.1. Calibration model, selectivity and specificity

For all compounds, a linear relationship between the response and the concentration in the range of the lower limit of quantification to the highest calibrator was confirmed by Mandel test (99% significance). To compensate for heteroscedasticity, a weighted least squares model with a weighting factor $1/x$ was applied for all compounds except for the following: a weighting factor $1/x^2$ was applied for JWH-020 and JWH-200, a weighting factor $1/x^3$ was applied for WIN 48,098 and AM-2201, and no weighting was necessary for JWH-398.

Blank and zero samples as well as the sample fortified with other relevant drugs and metabolites did not reveal any interference on the MRM transitions of the analytes or the internal standards. Carry-over after injection of a sample fortified to a concentration of 3000 pg/mg was below the limit of detection.

Table 2
Precision and accuracy data of the method. The relative standard deviations were below the allowed maximum of ≤15%.

Analyte	Intraday precision (RSD, %) (n = 10)			Interday precision (RSD, %) (n = 5)			Accuracy (Bias, %) (n = 5)		
	2 pg/mg	20 pg/mg	60 pg/mg	2 pg/mg	20 pg/mg	60 pg/mg	2 pg/mg	20 pg/mg	60 pg/mg
AM-694	7.8	4.1	3.1	7.8	4.1	5.9	−6.1	5.2	−0.7
AM-2201	4.8	2.4	2.4	10.3	5.7	4.6	−3.1	4.8	−3.0
JWH-007	3.0	3.7	4.4	8.4	3.7	5.4	−6.9	−1.8	−3.4
JWH-015	5.6	3.8	4.4	6.7	3.8	4.4	−0.1	0.0	1.3
JWH-018	6.7	1.8	5.5	8.2	4.1	5.5	−3.8	3.5	−0.8
JWH-019	4.5	4.8	5.3	6.5	6.4	5.3	−2.5	4.2	−2.6
JWH-020	11.1	4.5	9.1	11.1	8.0	9.1	−6.3	−0.8	−1.0
JWH-073	8.2	7.7	5.9	11.6	7.8	7.9	−4.6	1.9	−0.9
JWH-081	6.9	3.2	4.7	9.5	6.1	5.9	−3.0	4.0	−4.5
JWH-122	7.1	2.8	5.3	7.1	6.6	5.3	−4.2	1.8	−4.3
JWH-200	5.0	1.6	3.7	8.1	4.7	5.1	−6.9	−0.8	−0.5
JWH-203	3.6	4.3	2.8	5.1	8.1	3.6	−6.1	0.3	−2.5
JWH-210	8.9	3.7	8.0	9.5	8.3	8.0	−6.2	−0.1	−0.9
JWH-250	4.5	4.3	4.2	9.2	5.7	4.2	−0.1	6.1	−3.2
JWH-251	6.6	3.0	1.2	10.9	3.7	3.0	−4.1	−0.5	−4.3
JWH-398	8.2	3.1	1.4	9.8	8.8	10.5	−4.5	1.1	−1.0
Methanandamide	6.7	2.4	4.2	8.8	4.8	5.8	−4.1	−0.5	−6.3
RCS-4	6.3	3.1	3.4	8.7	4.5	5.1	1.2	5.2	−0.3
RCS-4 ortho isomer	4.4	2.6	7.1	6.8	2.6	3.5	−3.8	2.7	−1.2
RCS-8	8.6	2.9	3.9	8.7	4.5	4.1	−4.4	3.1	−0.2
WIN 48,098	7.8	1.8	3.9	9.9	5.2	4.7	−6.4	1.9	0.5
WIN 55,212-2	8.2	6.0	7.6	9.9	6.3	7.6	−3.8	3.9	0.3

3.2. Accuracy and precision

Precision and accuracy data are shown in Table 2. The intraday and interday precisions for each compound at all concentration levels were below 12% (RSD ≤ 12%). Bias was ≤6.9%.

3.3. Analytical limits

The lower limit of quantification was 5.0 pg/mg for methanandamide and JWH-398. The lower limit of quantification for all the other synthetic cannabinoids was 0.5 pg/mg. For all analytes, the

Table 3
Matrix effects, recoveries and process efficiencies for analytes and internal standards (not determined for WIN 55,212-2).

Analyte	Matrix effect [%]	Low concentration (2 pg/mg)			Process efficiency [%]	RSD (n = 5) [%]	Matrix effect [%]	High concentration (60 pg/mg)			Process efficiency [%]	RSD (n = 5) [%]
		RSD (n = 5) [%]	Recovery [%]	RSD (n = 5) [%]				RSD (n = 5) [%]	Recovery [%]	RSD (n = 5) [%]		
AM-694	60	13	84	8	50	7	61	4	96	7	60	7
AM-2201	46	18	85	11	38	9	56	3	97	7	55	8
JWH-007	30	25	81	14	24	14	36	6	96	14	26	15
JWH-015	57	15	83	8	47	8	67	3	97	6	65	6
JWH-018	34	25	83	12	28	14	38	5	97	13	38	14
JWH-019	22	20	82	13	18	11	26	4	98	16	26	17
JWH-020	21	15	79	12	17	11	22	6	99	18	21	16
JWH-073	21	17	83	9	17	13	31	7	98	15	31	16
JWH-081	22	16	85	9	18	11	21	5	99	16	22	18
JWH-122	18	18	82	16	15	9	25	3	97	16	24	16
JWH-200	89	15	86	9	76	6	79	4	95	6	77	5
JWH-203	20	15	83	11	17	13	28	8	99	16	29	17
JWH-210	50	10	95	11	47	11	24	7	97	17	23	17
JWH-250	49	19	84	8	41	12	59	5	96	7	58	6
JWH-251	20	19	81	12	16	13	28	6	98	13	29	15
JWH-398	17	22	80	15	13	12	17	8	100	22	17	20
Methanandamide	30	26	82	9	24	19	29	7	98	19	29	17
RCS-4	45	18	81	12	36	12	56	4	97	9	56	10
RCS-4 ortho isomer	58	13	83	8	48	7	70	2	98	5	69	6
RCS-8	18	19	80	11	14	12	27	4	97	15	26	17
WIN 48,098	98	15	87	9	85	8	87	4	95	4	83	3

Internal standard		Matrix effect [%]	RSD (n = 5) [%]	Recovery [%]	RSD (n = 5) [%]	Process efficiency [%]	RSD (n = 5) [%]
JWH-007-D9	c = 10 pg/mg	30	19	90	12	26	13
JWH-018-D11	c = 5 pg/mg	23	20	91	15	21	13
JWH-073-D9	c = 5 pg/mg	26	19	91	15	24	13
JWH-250-D5	c = 5 pg/mg	49	11	92	11	45	9
Flunitrazepam-D7	c = 10 pg/mg	87	9	96	13	83	8
OH-THC-d3	c = 20 pg/mg	45	18	88	16	39	17

Table 4
Concentrations of synthetic cannabinoids in pg/mg detected in the proximal segment of authentic hair samples, date of sampling: 16.02.2011.

Person	Sampling site	Gender	Age	Hair colour	Hair length (cm)	Reported last drug use	JWH-081	JWH-250	JWH-073	JWH-018	JWH-210
1	Head	Male	32	Black	4	07/2010	Negative	0.5	2.0	Negative	Negative
2	Head	Male	31	Brown	3.5	08/2010	Negative	Negative	2.1	Negative	Negative
3	Head	Male	26	Black	1	12/2010	10	2.9	Negative	Negative	Negative
4	Head	Male	20	Medium blond	3.5	11/2010	7.3	4.8	0.7	Negative	0.5
5	Head	Male	37	Medium blond	3.5	11/2010	31	11	21	5.1	2.7
6	Arm	Male	22	Brown	0.5	08/2010	6.1	14	Negative	Negative	Negative
6	Leg	Male	22	Brown	0.5	08/2010	5.1	17	0.7	Negative	Negative
7	Head	Male	29	Brown	0–3	08/2010	78	24	3.2	5.7	5.2
8	Head	Male	26	Brown	0–3	08/2010	12	1.2	0.7	Negative	Negative

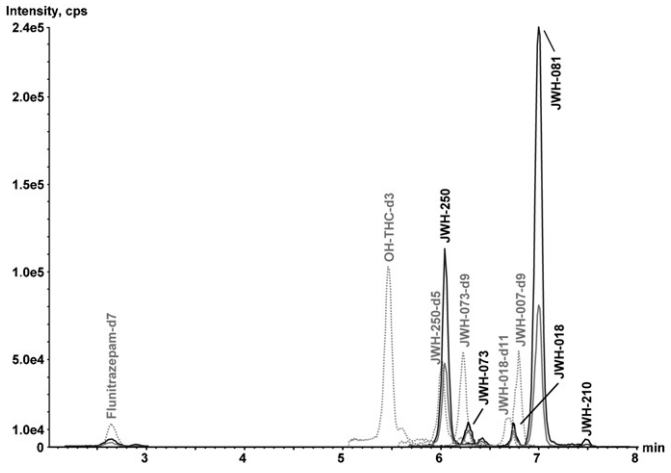

Fig. 2. Chromatogram of the proximal hair segment of patient 7. The synthetic cannabinoids JWH-018, JWH-073, JWH-081, JWH–210 and JWH-250 were present in the sample.

limit of detection was set to the level of the lower limit of quantification.

3.4. Processed sample stability

All analytes were stable in the autosampler for 9 h (degradation < 18% at the low concentration level and <13% at the high concentration level) except JWH-020, which showed degradation of 16% and 17% after 6 h at the high and the low concentration level, respectively, and degradation of 21% after 8 h at the low concentration level.

3.5. Matrix effects, recoveries and process efficiencies

Matrix effects, recoveries and process efficiencies are summarized in Table 3. Matrix effects at the 2.0 pg/mg concentration level ranged from 17% to 98% (RSD ≤ 26%). Matrix effects at the 60 pg/mg concentration level ranged from 17% to 87% (RSD ≤ 8%). Recoveries ranged from 79% to 95% at the 2.0 pg/mg concentration level (RSD ≤ 15%) and from 95% to 100% at the 60 pg/mg concentration level (RSD ≤ 22%), respectively. Process efficiencies ranged from 13 to 85% at the 2.0 pg/mg concentration level (RSD ≤ 19%) and from 17 to 83% at the 60 pg/mg concentration level (RSD ≤ 20%), respectively. Matrix effects, recoveries and process efficiencies of the internal standards are similar to those of the corresponding analytes. The relative standard deviation of quantitative results when using different amounts of hair samples (10, 20, 30, 40 and 50 mg) fortified to a concentration of 20 pg/mg was below 20%. Overnight extraction of an authentic sample revealed an increase of the JWH-210-concentration of ca. 40% compared to the 3-h extraction, but the overnight extraction had a zero to negative effect on the concentrations of JWH-018, JWH073, JWH-081, JWH-200 and JWH-250. Thus, the 3-h extraction was preferred to a longer extraction time.

3.6. Authentic samples

The concentrations of the detected synthetic cannabinoids in hair of eight patients are shown in Table 4. Two to six different

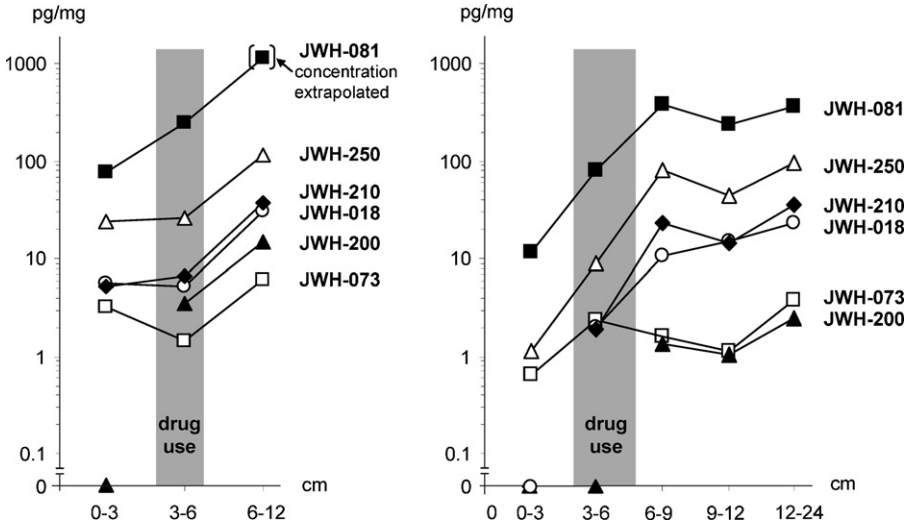

Fig. 3. Increase of synthetic cannabinoid concentrations from the first to the third segment of patient 7 (left) and patient 8 (right) (concentrations in logarithmic scale). The self-reported period of drug use is given in gray, based on a monthly hair growth rate of 10 mm.

compounds were present in a hair sample at the same time. A representative chromatogram is shown in Fig. 2. The synthetic cannabinoids were detected in a broad concentration range: JWH-210, JWH-073 and JWH-018 showed concentrations around the lower limit of quantification. The highest concentration in the first segment was noted for JWH-081 (78 pg/mg). The concentrations in arm and leg hair of the same person differed less than 20% (see Table 4 patient 6). This patient admitted daily consumption of one herbal mixture package in July and August 2010.

In segmented hair, the concentrations of most substances increased from the first (proximal) to the third segment (see Fig. 3). For the determination of concentrations exceeding the calibration range (75 pg/mg), only 10 mg of hair sample, and a three point calibration with the following concentrations was employed: 75, 200 and 500 pg/mg. The coefficient of determination (r^2) of the three point calibration was ≥0.994. The maximum measured concentration detected in the third segment of patient 7 exceeded even this calibration range, and was, therefore, extrapolated to ca. 1100 pg/mg. Remarkably, the highest concentrations of synthetic cannabinoids in hair were found in segments related to the time before the self-reported beginning of drug use (unless the subjects concealed an earlier consumption from the interviewer). An additional incorporation route of synthetic cannabinoids in hair by condensation of side stream smoke may contribute to reach these high concentrations.

Synthetic cannabinoids found in the hair samples were also positive in the corresponding washing solvents water and acetone. In petroleum ether, in some cases the corresponding synthetic cannabinoids could be found in traces.

4. Conclusions

The presented liquid chromatography–tandem mass spectrometry method proved to be suitable for the detection and quantification of the 22 synthetic cannabinoids JWH-007, JWH-015, JWH-018, JWH-019, JWH-020, JWH-073, JWH-081, JWH-122, JWH-200, JWH-203, JWH-210, JWH-250, JWH-251, JWH-398, AM-694, AM-2201, methanandamide, RCS-4, RCS-4 ortho isomer, RCS-8, WIN 48,098 and WIN 55,212-2 in human hair samples. The method is accurate, precise, selective and specific with satisfactory linearity within the calibrated range. Lower limits of quantification were in the low to sub pg/mg range. Evaluation of the analyte stability in processed samples suggests an analysis within 6 h. Authentic hair samples were successfully analysed with this method. The method can be expanded easily when new synthetic cannabinoids appear on the market.

With authentic hair samples, no relation between the self-stated consumption and the concentrations in the corresponding hair sample could be established. The results of segmental hair analysis in chronic users (see Fig. 3) suggest incorporation of the drugs in head hair via side-stream smoke condensation as a major route. Additional deposition of drugs via blood in the hair root and via sebum and sweat may also contribute. This finding is in good agreement with the results of Δ^9-tetrahydrocannabinol analysis in hair of cannabis consumers [35]. In summary, the method can be used to prove the contact with herbal mixtures containing synthetic cannabinoids and thus contributes to an efficient abstinence control.

References

[1] V. Auwärter, S. Dresen, W. Weinmann, M. Mueller, M. Puetz, N. Ferreiros, J. Mass Spectrom. 44 (2009) 832.
[2] J.L. Wiley, D.R. Compton, D. Dai, J.A. Lainton, M. Phillips, J.W. Huffman, B.R. Martin, J. Pharmacol. Exp. Ther. 285 (1998) 995.
[3] J.W. Huffman, L.W. Padgett, Curr. Med. Chem. 12 (2005) 1395.
[4] J.W. Huffman, P.V. Szklennik, A. Almond, K. Bushell, D.E. Selley, H. He, M.P. Cassidy, J.L. Wiley, B.R. Martin, Bioorg. Med. Chem. Lett. 15 (2005) 4110.
[5] L.S. Melvin, G.M. Milne, M.R. Johnson, B. Subramaniam, G.H. Wilken, A.C. Howlett, Mol. Pharmacol. 44 (1993) 1008.
[6] S. Kneisel, F. Westphal, P. Bisel, V. Brecht, S. Broecker, V. Auwärter, J. Mass Spectrom. 47 (2012) 195.
[7] M.A. Neukamm, T.E. Mürdter, H.D. Wehner, F. Wehner, D. Ratge, C. Knabbe, Toxichem. Krimtech. 76 (2008) 118.
[8] M. Neukamm, T. Muerdter, C. Knabbe, H.D. Wehner, F. Wehner, Blutalkohol 49 (2009) 373.
[9] J. Teske, J.P. Weller, A. Fieguth, T. Rothamel, Y. Schulz, H.D. Troger, J. Chromatogr. B 878 (2010) 2659.
[10] S.L. Kacinko, A. Xu, J.W. Homan, M.M. McMullin, D.M. Warrington, B.K. Logan, J. Anal. Toxicol. 35 (2011) 386.
[11] S. Dresen, S. Kneisel, W. Weinmann, R. Zimmermann, V. Auwärter, J. Mass Spectrom. 46 (2011) 163.
[12] R. Lindigkeit, A. Boehme, I. Eiserloh, M. Luebbecke, M. Wiggermann, L. Ernst, T. Beuerle, Forensic Sci. Int. 191 (2009) 58.
[13] S. Dresen, N. Ferreiros, M. Putz, F. Westphal, R. Zimmermann, V. Auwärter, J. Mass Spectrom. 45 (2011) 1186.
[14] S. Kneisel, F. Westphal, P. Rosner, V. Brecht, A. Ewald, B. Klein, M. Puetz, S. Thiemt, V. Auwärter, TIAFT Bull. 41 (2011) 38.
[15] S. Kneisel, F. Westphal, B. Moosmann, V. Brecht, P. Bisel, C. Vidal, A. Jacobsen-Bauer, W. Bork, V. Auwärter, TIAFT Bull. 41 (2011) 29.
[16] B. Moosmann, S. Kneisel, U. Girreser, V. Brecht, F. Westphal, V. Auwärter, Forensic Sci. Int. (2012).
[17] S. Kneisel, P. Bisel, V. Brecht, S. Broecker, M. Müller, V. Auwärter, Forensic Toxicol. (2012).
[18] S. Every-Palmer, Drug Alcohol Depend. 117 (2011) 152.
[19] U. Zimmermann, P. Winkelmann, M. Pilhatsch, J. Nees, R. Spanagel, K. Schulz, Deut. Ärzteblatt Int. 106 (2009) 464.
[20] S. Every-Palmer, Addiction 105 (2010) 1859.
[21] R. Vandrey, K.E. Dunn, J.A. Fry, E.R. Girling, Drug Alcohol Depend. 120 (2012) 238.
[22] C. Coulter, M. Garnier, C. Moore, J. Anal. Toxicol. 35 (2011) 424.
[23] T. Sobolevsky, I. Prasolov, G. Rodchenkov, Forensic Sci. Int. 200 (2010) 141.
[24] C.L. Moran, V.H. Le, K.C. Chimalakonda, A.L. Smedley, F.D. Lackey, S.N. Owen, P.D. Kennedy, G.W. Endres, F.L. Ciske, J.B. Kramer, A.M. Kornilov, L.D. Bratton, P.J. Dobrowolski, W.D. Wessinger, W.E. Fantegrossi, P.L. Prather, L.P. James, A. Radominska-Pandya, J.H. Moran, Anal. Chem. 83 (2011) 4228.
[25] M. Hutter, S. Broecker, S. Kneisel, V. Auwärter, J. Mass Spectrom. 47 (2012) 54.
[26] M.R. Moeller, H. Sachs, F. Pragst, in: P. Kintz (Ed.), Analytical and Practical Aspects of Drug Testing in Hair, CRC-Press, 2006, p. 273.
[27] V. Auwärter, A. Wohlfarth, J. Traber, D. Thieme, W. Weinmann, Forensic Sci. Int. 196 (2010) 10.
[28] M.A. Neukamm, T.E. Mürdter, H.D. Wehner, F. Wehner, M. Renger, D. Ratge, H. Sachs, C. Knabbe, Ann. Toxicol. Anal. 21 (2009) 21.
[29] M.A. Neukamm, H. Sachs, T.E. Mürdter, S. Fehn, T. Pöhlmann-Moore, H.D. Wehner, C. Knabbe, D. Ratge, Toxichem. Krimtech. (2011) 219.
[30] A. Salomone, E. Gerace, F. D'Urso, D. Di Corcia, M. Vincenti, J. Mass Spectrom. 47 (2012) 604.
[31] S. Dresen, M. Gergov, L. Politi, C. Halter, W. Weinmann, Anal. Bioanal. Chem. 395 (2009) 2521.
[32] M. Rothe, F. Pragst, J. Anal. Toxicol. 19 (1995) 236.
[33] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[34] S. Kneisel, V. Auwärter, J. Mass. Spectrom. (2012), http://dx.doi.org/10.1002/jms.3020.
[35] M. Uhl, H. Sachs, Forensic Sci. Int. 145 (2004) 143.