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ORIGINAL ARTICLE

Hair analysis for JWH-018, JWH-122, and JWH-210 after passive in vivo exposure to synthetic cannabinoid smoke

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Abstract Hair analysis is often used to confirm abstinence from drug use. However, interpretation of hair analysis results can be challenging, particularly with regard to smoked substances like synthetic cannabinoids, because hair can be contaminated by side-stream smoke. In this study, we measured the concentrations of synthetic cannabinoids in scalp hair after exposure to side-stream smoke from a cigarette containing the synthetic cannabinoids JWH-018, JWH-122, and JWH-210. Three participants exposed their hair to the side-stream smoke once each working day for 3 weeks to mimic realistic conditions experienced by consumers of these drugs. Two other participants exposed their hair once to the side-stream smoke of one cigarette. Scuba regulators with external air supply were used to avoid inhalation of smoke. Hair segments and wash solutions were analyzed by liquid chromatography–tandem mass spectrometry. The highest measured concentrations were 70 pg/mg of JWH-018, 260 pg/mg of JWH-122, and 950 pg/mg of JWH-210 in distal hair segments collected at the end of the exposure period. At 2–3 weeks after the end of the repeated exposure, all three synthetic cannabinoids were detected in the hair samples of both participants with longer hair. In these samples, the ratio of cannabinoid amount in acetone wash to that in hair was below 0.5 for all synthetic cannabinoids, which could be interpreted as evidence of consumption. However, with the nonconsumption of synthetic cannabinoids by our study participants being confirmed by urine testing, it is apparent that even high substance concentrations in hair samples do not prove consumption and can be explained by external contamination after contact with synthetic cannabinoids alone.

Keywords Synthetic cannabinoids · Hair analysis · Passive exposure · External contamination · JWH-018 · JWH-122

Introduction

Hair analysis is routinely applied to confirm abstinence from drug abuse, particularly in cases involving driving license restrictions or in drug rehabilitation programs. Positive findings are usually interpreted to indicate drug consumption and may result in serious consequences for the subject. Therefore, the analyst must be very careful when interpreting the results. In particular, the influence of possible external contamination has to be considered. Drugs of abuse like cannabis, cocaine, or heroin tend to contaminate the hair externally, particularly when the drugs are smoked or snorted [1]. Synthetic cannabinoids are usually consumed as cannabis substitutes in the form of herbal mixtures or pure substances smoked in a joint or a water pipe. During smoking, significant proportions of the active ingredients are released into the surroundings. Subsequently, exhaled smoke and side-stream smoke may condense on any surfaces present, including furniture and the skin, hair, and clothes of people present. Moosmann et al. [2] showed that large amounts of Δ^9-tetrahydrocannabinol (THC) were incorporated into scalp hair after daily in vivo exposure to marijuana smoke over a 3-week period,

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supporting the hypothesis that findings of THC in the hair of cannabis users mainly derive from external contamination [3, 4].

Several methods for analysis of synthetic cannabinoids in hair have been published and these have been applied to hair samples of suspected consumers [5–9]. In these hair samples, a wide range of synthetic cannabinoid concentrations was found. The highest concentration detected was 2,800 pg/mg for JWH-122 in the proximal segment [8]. In a previous study, we found increasing concentrations in segmented hair samples of alleged consumers from proximal to distal segments [5]. The highest concentrations were detected in hair segments corresponding to time periods before the self-reported start of consumption and before the particular substance was available [10]. These findings strongly suggest a permanent incorporation of synthetic cannabinoids into the hair matrix by external contamination via smoke or contaminated hands.

To minimize the risk of misinterpretation because of external contamination, the Society of Hair Testing recommends an appropriate washing procedure including both organic solvents and aqueous solutions [11]. Furthermore, metabolites of the substances of interest should be analyzed, and cutoff levels should be established. Kim et al. [7] reported on the detection of the *N*-(3-hydroxybutyl) metabolite and the butanoic acid metabolite of JWH-073 in the hair of rats after intraperitoneal administration of JWH-073. In another part of their study, the *N*-(5-hydroxypentyl) metabolite of JWH-018 was detected in hair samples of suspected consumers. However, in our case work with hair of alleged consumers, metabolites of synthetic cannabinoids were not detected in hair samples, even in cases with extremely high parent compound concentrations.

The setting of the present study was chosen to be as realistic as possible, considering the influence of sebum, moistening of the hair by sweat, changing temperatures, and hair movement on the incorporation of externally applied substances into the hair. These influences were not systematically considered in previous in vitro studies on the contamination of hair by marijuana smoke [12] or nicotine and metabolites [13]. In the present study, levels of the synthetic cannabinoids JWH-018, JWH-122, and JWH-210 were determined in hair. All three substances have been identified in herbal mixtures [14–16] and showed high prevalence in body fluids [17].

Materials and methods

Chemicals

All common solvents and substances were commercially available and were of analytical or HPLC grade. Deionized water was prepared with a cartridge deionizer from Memtech (Moorenweis, Germany). Blank hair for calibration was provided by volunteers and analyzed for the target cannabinoids prior to use. JWH-018, JWH-122, JWH-210, and JWH-018-d_{11} were obtained from LGC Standards (Wesel, Germany); JWH-007-d_9 was purchased from Cayman Chemical (Ann Arbor, MI, USA).

Calibration standards

To prepare the stock solution of synthetic cannabinoids, 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, 10, and 1.0 ng/ml), the stock solution was diluted with ethanol. The internal standard (IS) solution consisted of 20 ng/ml of JWH-007-d_9 and 10 ng/ml of JWH-018-d_{11} in ethanol.

Herbal mixtures

Authentic herbal mixtures with defined concentrations of synthetic cannabinoids (JWH-018, JWH-122, or JWH-210) were obtained from the German Federal Criminal Police Office (BKA, Wiesbaden, Germany).

Study design

This study procedure was not impacted by German regulations requiring approval by an ethics committee. Thus, the University of Freiburg ethics committee did not have any objection against the experiments and the evaluation of the results. Participants in the repeated exposure study included one man (participant 1) and two women (participants 2 and 3). Participants 2 and 3 had partially bleached long hair (26 and 42 cm, respectively); participant 1 had short brown hair (5 cm). All participants were laboratory staff and were in contact with materials containing synthetic cannabinoids prior to the study. Every weekday morning over a 3-week period, the heads of the participants were exposed to the smoke of 1 joint containing synthetic cannabinoids, leading to a total of 15 exposures. The joints were prepared from 500 mg of tobacco and 500 mg of herbal mixture that contained, on average, 4.8 mg/g JWH-018, 20 mg/g JWH-122, and 62 mg/g JWH-210. To avoid any contamination through hands, skin, or clothes, latex gloves and full-body protective suits were worn during the entire preparation and exposure period. The exposure took place in a room measuring 2.5 × 2 × 2.5 m and the joint was smoked using a water-jet vacuum pump. Long hair was tied back in a pony tail. Inhalation of the smoke was excluded by breathing compressed air through scuba regulators. The three participants sat in a small circle facing each other and the joint was held in front of the mouth of

one participant for 10–15 s, connected to the vacuum for one puff, and then passed on to the next participant. The whole procedure was repeated until the joint was completely burned down (15–20 min). The participants followed their daily routine until the next morning. The hair was washed every day or every second day using commercially available hair shampoo. The washing procedure and brand of shampoo were not standardized and reflect possible variations in hair care and use of hair cosmetics. To test the impact of a single exposure, two participants (participant 4, male, hair length 1.5 cm; participant 5, female, hair length 50 cm) were exposed to the side-stream smoke of 1 joint as described above.

Sampling

Prior to the exposure period, one blank hair sample was obtained from each participant. During the exposure period, one hair sample was obtained each week from each of participants 1–3. They provided one hair strand per week for 2–3 weeks following the exposure period. For participant 3, another strand was obtained about 1 year after the exposure period. One hair sample from the participants with a single exposure was collected 1 day after exposure and again 1 week later. All samples were collected as close to the scalp as possible from the posterior vertex region of the head (remaining hair length at the scalp about 1 mm). The proximal end of the hair was labeled and the samples were stored in aluminum foil in darkness at room temperature until analysis. In addition, weekly urine samples were obtained from the participants during the exposure period to exclude systemic uptake of the drugs or unreported drug use.

Hair sample preparation

For sample preparation, the hair strands of participants 2 and 3 were segmented. Due to the short hair length of participant 1, segmentation was carried out only for the sample collected 3 weeks after the end of exposure. For sample work-up, a previously published method was applied [5]. Briefly, hair samples were consecutively washed under continuous shaking with 4 ml of 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 approximately 20–50 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 extract was transferred into 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 mobile phase A/B (50:50, v/v). Solvent A consisted of 0.2 % formic acid and 2.0 mM ammonium formate in water and solvent B was methanol.

LC–MS–MS method

A fully validated liquid chromatography–tandem mass spectrometry (LC–MS–MS) method was applied for the quantification of synthetic cannabinoids in hair as described by Hutter et al. [5]. Briefly, the hair extracts were analyzed using an LC–MS–MS system consisting of a QTrap 4000 triple-quadrupole linear ion trap mass spectrometer fitted with a TurboIonSpray interface (AB Sciex, Darmstadt, Germany) and a Prominence HPLC system from Shimadzu (Duisburg, Germany) with three LC-20ADsp isocratic pumps, a CTO-20AC column oven, an SIL-20AC autosampler, a DGU-20A3 degasser, and a CBM-20A controller. Separation was achieved on a Luna Phenyl Hexyl column (50 × 2 mm i.d., 5 µm particle size) with a Phenyl Hexyl guard column (4 × 2 mm) (Phenomenex, Aschaffenburg, Germany). The limit of detection and the lower limit of quantitation for the three synthetic cannabinoids investigated were 0.5 pg/mg.

Analysis of wash solutions

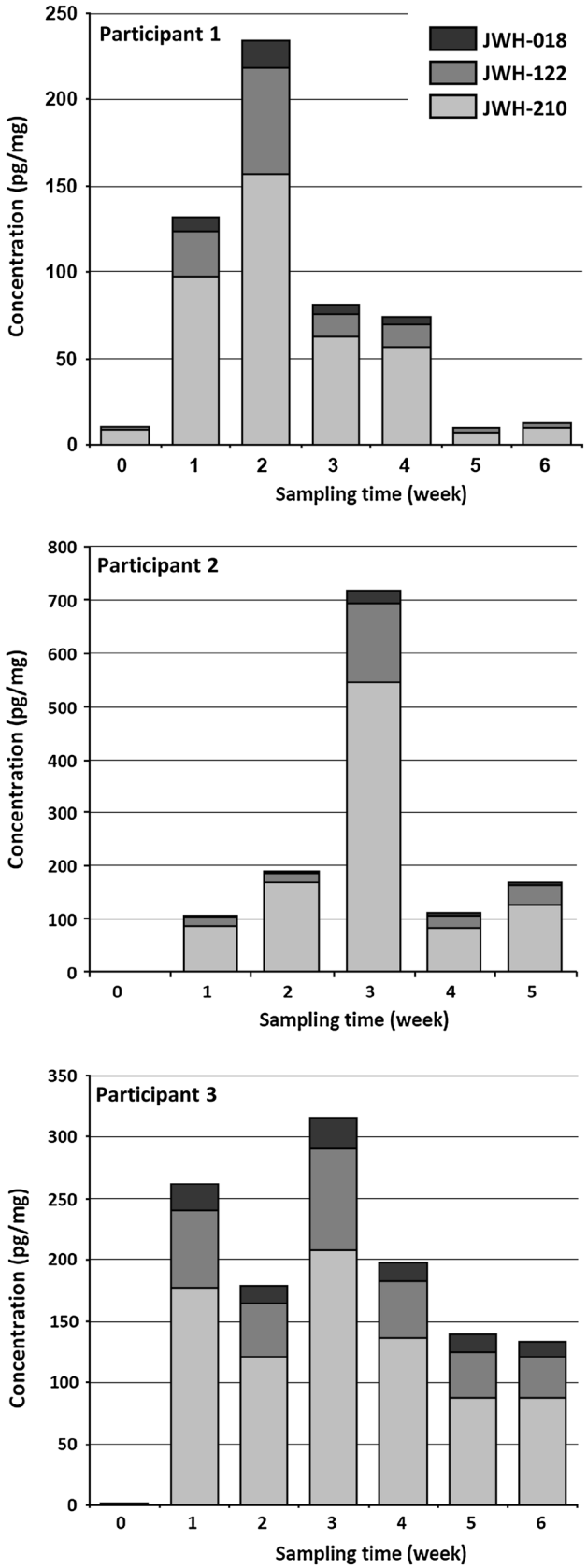
For estimation of the cannabinoid concentrations in the wash solutions (water, acetone, petroleum ether), drug-free wash solutions from blank hair samples were used for calibration. The calibration standards (2, 5, and 150 ng each of JWH-018, JWH-122, and JWH-210 per 4 ml of wash solution) were prepared by adding adequate amounts of the corresponding working solution and 5 µl of the IS solution to 100 µl of wash solution. The solvents were evaporated and the dry residues reconstituted in 100 µl of mobile phase A/B (50:50, v/v) [5]. Only the wash solutions for participant 3 were analyzed.

Analysis of urine samples

Urine samples were analyzed using a validated LC–MS–MS method [18] for detection of the major metabolites of 18 synthetic cannabinoids, including JWH-018, JWH-122, and JWH-210.

Results and discussion

Synthetic cannabinoid concentration profiles of participants 1–3 for unsegmented hair during the study period are depicted in Fig. 1. Concentrations rose up to 540 pg/mg for JWH-210 during the exposure period and fall in a similar range to those found in the hair of alleged consumers [5, 6, 8]. The maximum concentrations of all three synthetic



◀**Fig. 1** Concentrations of JWH-018, JWH-122, and JWH-210 in the whole hair length for three participants before, during (weeks 1–3), and after exposure (weeks 4–6) to side-stream smoke of joints containing the above synthetic cannabinoids

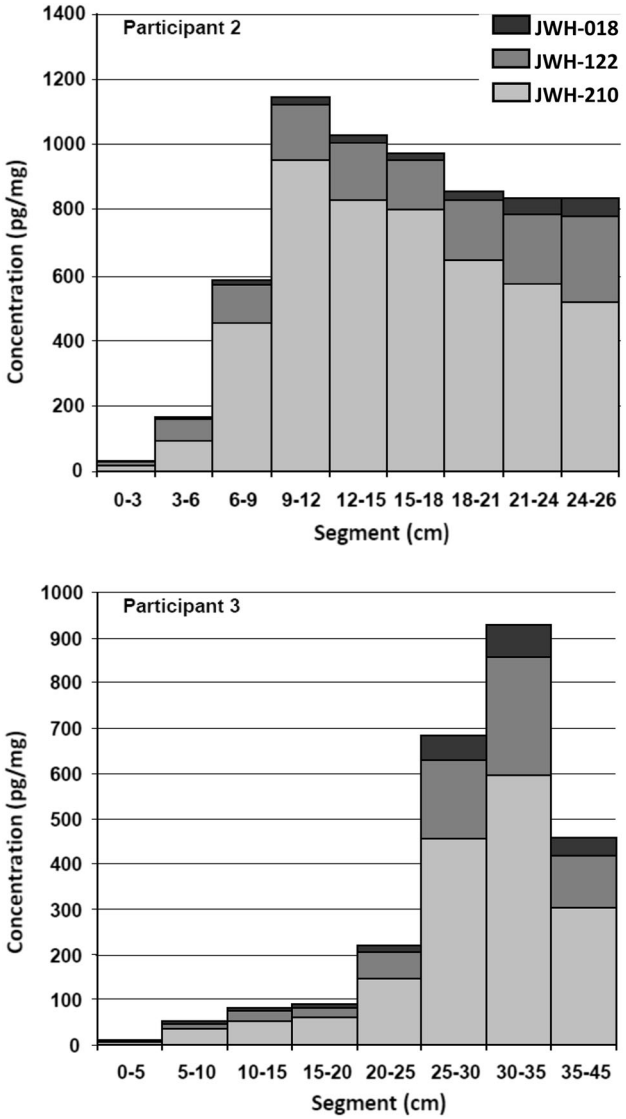


Fig. 2 Concentrations of JWH-018, JWH-122, and JWH-210 in hair segments obtained from participants 2 and 3, directly after the end of the exposure period (3 weeks)

cannabinoids were detected in the hair samples of both participants with longer hair (participants 2 and 3) for samples collected at the end of the exposure period (week 3). In contrast, a decrease of the concentrations in the hair samples of participant 1 (short hair) collected after the third week of exposure was observed. Furthermore, concentrations in the hair of participant 1 were generally lower than

Table 1 Concentrations of synthetic cannabinoids in head hair segments after one-time exposure

Participant 4	Hair length (cm)	JWH-018 (pg/mg)	JWH-122 (pg/mg)	JWH-210 (pg/mg)
Before the study	1.5	ND	ND	ND
1 day after exposure	1.5	13	46	50
1 week after exposure	1.5	1.8	6.3	4.5
Participant 5	Segment (cm)	JWH-018 (pg/mg)	JWH-122 (pg/mg)	JWH-210 (pg/mg)
Before the study	0–6	ND	ND	ND
	6–12	ND	ND	ND
	12–18	ND	ND	ND
	18–24	ND	ND	ND
	24–30	ND	ND	ND
	30–36	ND	ND	ND
	36–42	ND	ND	ND
	42–50	ND	ND	ND
1 day after exposure	0–3	ND	ND	ND
	3–6	ND	ND	ND
	6–9	ND	ND	ND
	9–12	ND	ND	ND
	12–15	ND	1.4	3.5
	15–18	ND	4.4	14
	18–21	ND	16	43
	21–24	ND	8.0	11
	24–27	2.3	8.0	17
	27–30	2.7	9.3	21
	30–33	4.6	8.6	22
	33–36	4.4	13	26
	36–39	3.3	13	35
	39–42	6.2	15	40
	42–45	1.5	8.4	21
	45–48	4.3	17	64
1 week after exposure	0–3	ND	ND	ND
	3–6	ND	ND	ND
	6–9	ND	ND	ND
	9–12	ND	ND	ND
	12–15	ND	ND	ND
	15–18	ND	4.9	ND
	18–21	ND	6.5	ND
	21–24	ND[a]	Positive[a]	Positive[a]
	24–27	ND	8.4	12
	27–30	ND	9.5	19
	30–33	ND	9.5	23
	33–36	3.6	14	28
	36–39	2.9	12	24
	39–44	2.4	9.7	20

ND not detected

[a] No quantification possible due to nondetection of internal standard

the concentrations in participants 2 and 3. This may be a result of more efficient removal of compounds from short hair during daily hair washing, combined with a more effective removal of sebum from the scalp, which counteracts incorporation of substances along the hair shaft.

The concentration order of the synthetic cannabinoids in the hair samples reflected the concentration order in the joint (JWH-018 < JWH-122 < JWH-210). In the samples collected 2–3 weeks after the end of the exposure, all three synthetic cannabinoids were still detected for the participants with longer hair (participants 2 and 3). This indicates that synthetic cannabinoids are only slowly removed from hair after diffusion into the hair matrix. In an unsegmented hair sample of participant 3 collected approximately 1 year after the end of the exposure (hair length 45 cm), no synthetic cannabinoids were detected.

Segmental hair analysis of participants 2 and 3 (longer hair) revealed lower concentrations in the proximal segments (Fig. 2). Because all hair samples were collected at the posterior vertex region, the segments closest to the scalp were covered by other hair strands and thus were less exposed to smoke during the experiments. This could explain the lower concentrations in the proximal segments. Very similar results regarding the distribution of concentrations along the hair shaft were observed when analyzing segmented hair samples of heavy users [5], strongly suggesting external contamination as the main route of incorporation. Moosmann et al. [2] reported a similar distribution of concentrations in a comparable study investigating external contamination of hair with marijuana smoke. The lowest concentrations of THC, Δ^9-tetrahydrocannabinolic acid A, and cannabinol were found in the proximal segments and the highest concentrations in the distal segments.

The highest overall concentrations were found in the distal hair segments with up to 70 pg/mg of JWH-018, 260 pg/mg of JWH-122, and 950 pg/mg of JWH-210. On the other hand, the distal segments showed a more pronounced decline of analyte concentrations after the end of the exposure period than the proximal segments (Fig. 2 and Supplemental material Table SM1). Both effects could be caused by the fact that in the more distal parts, especially in the hair tips, the cuticula tends to be more damaged than in other regions along the hair shaft, and, therefore, analytes may be more easily incorporated but also more easily washed out.

In the hair samples collected prior to the exposure period, 1.5 pg/mg of JWH-122 and 9.0 pg/mg of JWH-210 were detected for participant 1 and 1.4 pg/mg of JWH-210 for participant 3 (Table SM1). These findings can be explained by the work-related procedural handling of the substances and respective herbal mixtures in the laboratory by the participants [19]. No metabolites of synthetic cannabinoids were detected in the urine samples of all participants collected each week during the exposure period. Hence, inhalation of the smoke during the experiments or any other systemic uptake of the drugs was excluded. This finding shows that even exposure to drug material (without smoking) may lead to positive hair findings, even though the concentrations were significantly lower than after side-stream smoke exposure.

After one-time exposure of the hair of two participants to side-stream smoke from a joint containing synthetic cannabinoids, all three tested substances were detected (Table 1). In the hair sample of participant 5 (longer hair, 44–50 cm), no synthetic cannabinoids were detected in the proximal segments (up to 12 cm). In the segments containing synthetic cannabinoids, the maximum concentration was 64 pg/mg (JWH-210), which is lower than the maximum concentrations detected in the hair samples from the 3-week exposure study (Table 1 and Table SM1). Similar to the 3-week exposure study, the highest concentrations were detected in the distal segments. In addition, the decrease of the concentrations was more pronounced in the hair samples of participant 4 than in the samples from participant 5. This is most probably due to more efficient removal of substances by hair shampooing in shorter hair, which is similar to the observations made in the 3-week exposure study.

Comparison of the absolute amount of drug in a hair segment to the absolute amount in the corresponding wash solution can indicate an external contamination of the hair sample according to Tsanaclis and Wicks [1] and Pragst and Balikova [20]. In general, a ratio of wash solution content to hair content above 0.5 [1] or 1.0 [20] is regarded as an indicator for pronounced external contamination of the hair. In the present study, synthetic cannabinoids were found in none of the water wash solutions, probably because of the low solubility of the investigated synthetic cannabinoids in water. Low amounts of synthetic cannabinoids were found in petroleum ether (third washing step) in two segments (hair tips) only during the exposure period. However, synthetic cannabinoids were present in almost all of the acetone wash solutions. Given that all three synthetic cannabinoids are readily soluble in petroleum ether [21], it is likely that most contamination was removed in the second wash step with acetone. Figure 3 shows the absolute amounts of JWH-210 in every hair segment of participant 3 and in the corresponding acetone wash. In weeks 1, 2, and 4, the first two or four proximal segments showed a higher amount of JWH-210 in acetone than in hair, while in week 3 the amount of JWH-210 was higher in acetone in the most distal segment, thus suggesting pronounced external contamination of this segment [20]. In all other segments and weeks, the amount of JWH-210 in hair was higher than in the acetone wash. This pattern was also observed for

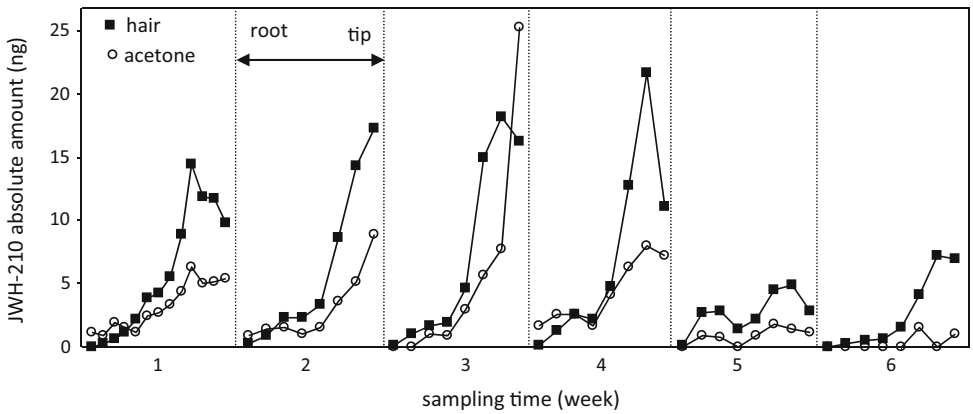


Fig. 3 Absolute amounts of JWH-210 in hair segments (50 mg) and in the corresponding acetone wash solutions for participant 3 (for segment length see Fig. 2) during exposure (weeks 1–3) and after exposure (weeks 4–6)

JWH-018 and JWH-122 in segments that contained these two analytes. The absolute amounts and the acetone-to-hair ratios for JWH-018, JWH-122, and JWH-210 for all segments are given in the supplementary material (Table SM2). The acetone-to-hair ratio ranged from 0 to 1.8 for JWH-018, from 0 to 2.4 for JWH-122, and from 0 to 20 for JWH-210. In week 6 (3 weeks after the last exposure), the acetone wash solutions of most hair segments were free of synthetic cannabinoids. In contrast, most of the hair extracts still returned positive results, falsely suggesting incorporation through the bloodstream. In all samples collected 2–3 weeks after the end of exposure, the ratio of substance amount in acetone wash to that in hair was below 0.5, and might therefore not indicate extensive external contamination [1]. The observed results show that synthetic cannabinoids remain incorporated into the hair structure for a long time after exposure to side-stream smoke.

Conclusions

The current study showed that synthetic cannabinoids remain incorporated into hair long time after exposure to side-stream smoke. Because these substances cannot be completely removed by routine washing procedures prior to analysis, and the concentration ratios of wash solutions to hair extracts do not necessarily reflect external contamination, the positive results caused by side-stream smoke exposure can lead to erroneous conclusions. Therefore, when using hair analysis to confirm abstinence from drug use or in other forensic/clinical applications, the results must be interpreted with the utmost care.

Comparison of the concentration distributions in segmental hair analysis observed in this study with those of known heavy users of synthetic cannabinoids suggests that the major part of analyte incorporation into hair occurred by external contamination in both cases. As a consequence, positive findings of synthetic cannabinoids in hair segments should not be correlated to time or duration of drug use.

Because detection of metabolites generally proves ingestion of xenobiotics, it is common practice to determine metabolites in hair samples. However, in our casework to date, no metabolites of synthetic cannabinoids have been detected in hair samples of heavy users. Further studies are needed to investigate whether metabolites of synthetic cannabinoids in hair can be used as valid markers for systemic uptake. Even if high concentrations of parent compounds are detected in hair samples, this should only be regarded as evidence of contact with drug material or pure substances, and does not prove consumption. Therefore, to prove drug consumption in forensic cases, simultaneous analysis of body fluids is strongly recommended.

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Conflict of interest There are no financial or other relations that could lead to a conflict of interest.

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