Metabolites of synthetic cannabinoids in hair – proof of consumption or false friends for interpretation?

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Introduction & Aims

Generally, the detection of drug metabolites in hair is considered as a proof of consumption.[1] For synthetic cannabinoids (SC), which comprise compounds carrying labile ester or amide bonds, not much is known about mechanisms of incorporation into hair. For a correct interpretation regarding hair findings of these compounds and their metabolites/degradation products it is necessary to be aware of the different potential routes of incorporation.
An authentic hair sample of a patient with a known history of heavy consumption of SC was analyzed in ten segments. To enable a valid interpretation of the distribution of the detected analytes and their metabolites along the hair shaft, experiments including intentional contamination of head hair as well as analysis of soaked blank hair and smoke condensates were performed.

Methods

Hair sample preparation:

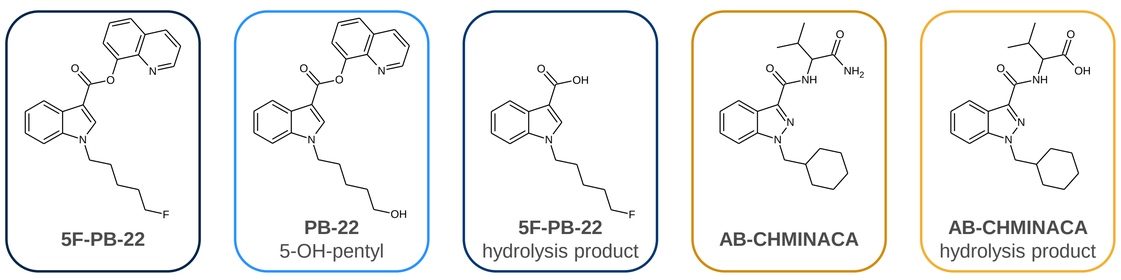
- Washing (water, acetone, petroleum ether)
- Homogenization (cutting with scissors)
- Methanolic extraction (3 h ultrasonication)
- Evaporation to dryness
- Reconstitution in mobile phase

Analytical methods:

- SCIEX QTRAP® 4000 + Shimadzu Prominence
  MRM(+) - 75 SC (at least 2 transitions per compound)
- SCIEX API™ 5000 + Dionex UltiMate® 3000RS
  MRM(+) – Metabolites of 43 SC (at least 2 transitions per compound)
  MRM(+) – 5F-PB-22 and metabolites (2 transitions per compound)

Experiments & Results

5F-PB-22 and AB-CHMINACA as well as their main metabolites 5F-PB-22 hydrolysis product, PB-22-5-OH-pentyl and AB-CHMINACA hydrolysis product were detected in all segments of the authentic hair sample (chemical structures see below).[2,3]



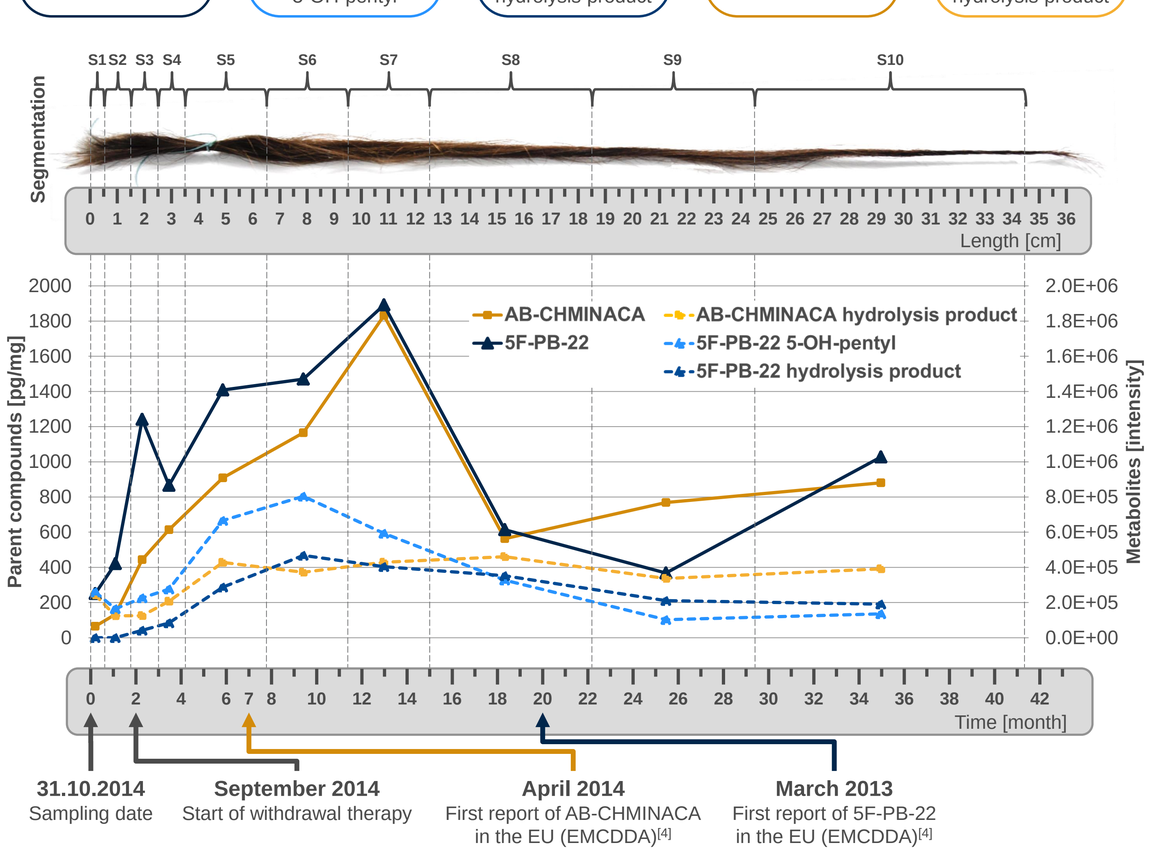


Fig. 1: Concentrations of the parent compounds and intensity of their metabolites detected in the ten hair segments. Sampling date, start of withdrawal therapy and the first report of the consumed substances to the EMCDDA are given assuming an average hair growth rate of 1.2 cm/month.

One test person rubbed AB-CHMINACA and 5F-PB-22 into his head hair to simulate external contamination. One week later, a head hair sample was taken, homogenized and washed. Aliquots of the homogenized sample were stored under different conditions (Fig. 2) for one week and then analyzed.

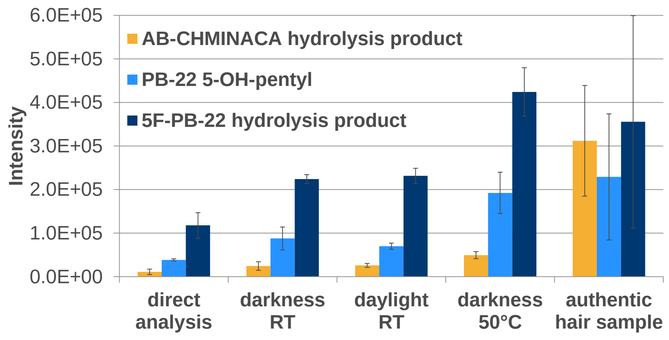


Fig. 2: Analysis results of the externally contaminated hair samples (triplicates) after storage under different conditions (darkness at room temperature, daylight exposure at room temperature and darkness at 50°C) for one week in comparison to directly analyzed hair samples and to the average intensity measured in the authentic hair sample.

5F-PB-22 hydrolysis product, PB-22-5-OH-pentyl and AB-CHMINACA hydrolysis product were detected in the externally contaminated, stored samples. Highest signals were received after storage at 50°C.

In a second experiment blank hair was soaked with a solution of 5F-PB-22 (1,000 pg/mg) for one week. After washing aliquots were stored under different conditions (Fig. 3) for two weeks prior to analysis.

Again, 5F-PB-22 hydrolysis product and PB-22-5-OH-pentyl were detected. After storage at 50°C the peak area of both degradation products were about ten times greater than after storage at room temperature.

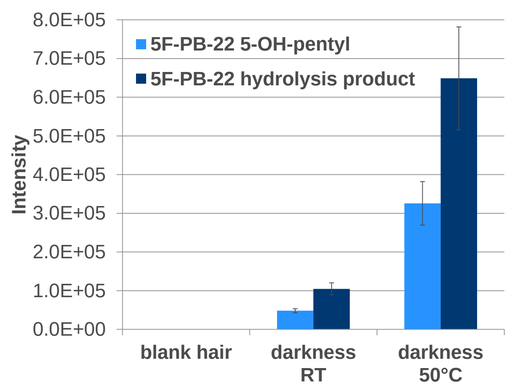


Fig. 3: Analysis results of the soaked (1,000 pg/mg 5F-PB-22 solution) blank hair samples (triplicates) after storage under different conditions (darkness at room temperature and darkness at 50°C) for two weeks.

In addition, joints containing 10 mg of AB-CHMINACA or 10 mg of 5F-PB-22, respectively, were burned down by using a water jet pump sucking the smoke through a wash bottle filled with methanol. The methanolic solutions containing smoke condensate were also tested positive for the two hydrolysis products.

Conclusion

Comparing the results of the authentic hair sample with the anamnestic data and the time of availability of 5F-PB-22 and AB-CHMINACA on the European market, it is evident that the findings in the hair segments do not correlate with the drug use in the time period in which the respective segments have grown. The following effects may explain these findings: The qualitative distribution of the detected compounds along the hair shaft appears typical for an incorporation via sebum and sweat. However, relevant amounts of the hydrolysis products of AB-CHMINACA and 5F-PB-22 could also be deposited by condensation on the hair after pyrolysis during smoking or by external contamination of the hair with the parent compounds and subsequent degradation. This mechanism can be compared to the known hydrolysis of cocaine to benzoylecgonine in hair.[1] Surprisingly, even hydrolytic defluorination of the chemically stable 5-fluoro-pentyl side chain of 5F-PB-22 was observed under different storage conditions, leading to relevant signal intensities of the respective 5-OH-pentyl metabolite in hair. For a better understanding of this process further investigations are required. As a consequence, interpretation of ‘metabolite’ findings of chemically labile compounds and defluorination products should be carried out with utmost care, taking into account the different mechanisms of formation and incorporation into hair.

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