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***In-vitro* metabolism of the synthetic cannabinoid JWH-018 and implementation of its major metabolites in human doping controls**

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Extended Abstract

Referred to as “Spice”, several new drugs, advertised as herbal blends, appeared on the market in the last years containing synthetic cannabinoids. As one major active ingredient the psychoactive substance JWH-018 (1-pentyl-3-(1-naphthoyl)indole), first synthesized by the group of John W. Huffman, was identified. Despite no obvious structural similarity to classical cannabinoids, it possesses an increased affinity to the cannabinoid receptors compared with Δ^9 -tetrahydrocannabinol and cannabis-like effects were described by some users. Therefore, synthetic cannabinoids are considered relevant to doping controls and were consequently prohibited in elite sport in-competition by the World Anti-Doping Agency under paragraph S8.

For the implementation of JWH-018 into sports drug testing programs, the knowledge of its metabolism in the body is of crucial importance as urine specimens are still the most frequently provided doping control samples. Therefore, after mass spectrometric characterization of JWH-018 by high-resolution/high-accuracy mass spectrometry, its phase-I metabolism was simulated under *in-vitro* conditions using human liver microsomal fractions. Employing the characteristic product ions, the presence of mono-, bis- and trishydroxylations was confirmed. Furthermore, dihydrodiol formations and dehydrogenations were observed, both also in combination with hydroxylations, as well as the *N*-dealkylated JWH-018, which could also be hydroxylated or oxidized at the arene residue leading to *N*-dealkylated dihydrodiols.

Based on these findings, a urine specimen of a healthy male person declaring to have smoked

a “Spice” enriched cigarette was screened for potential phase-I metabolites and except for the dehydrogenated and the *N*-dealkylated metabolite, all other *in-vitro* identified metabolites were observed. As the active compound JWH-018 was not detectable in the urine specimen, the most abundant metabolite, monohydroxylated at the alkyl side chain ($m/z = 358$), was chosen as target analyte and implemented into routine doping control screening assays based on enzymatic hydrolysis, liquid-liquid extraction and LC-MS/MS analysis employing multiple reaction monitoring. Validation was performed for the parent compound, due to the lack of metabolites, considering the parameters specificity, limit of detection (0.1 ng/mL), recovery (88%), intraday and interday precisions (3.7-18.2%), as well as ion suppression/enhancement effects (<10%) with JWH-015 as ISTD.

For further details please refer to

Wintermeyer A, Möller I, Thevis M, Jübner M, Beike J, Rothschild M A, Bender K. (2010) In vitro phase I-metabolism of the synthetic cannabimimetic JWH-018. *Anal Bioanal Chem* 398, 2141-2153.

Möller I, Wintermeyer A, Bender K, Jübner M, Thomas A, Krug O, Schänzer W, Thevis M. (2010) Screening for the synthetic cannabinoid JWH-018 and its major metabolites in human doping controls. *Drug Test Analysis*, DOI: 10.1002/dta.158.