Short communication

Drug Testing and Analysis

Received: 13 June 2011 Revised: 30 July 2011 Accepted: 30 July 2011 Published online in Wiley Online Library: 18 November 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.350

The detection of the urinary metabolites of 3-[(adamantan-1-yl)carbonyl]-1-pentylindole (AB-001), a novel cannabimimetic, by gas chromatography-mass spectrometry

Andrej Grigoryev,[a]* Pierce Kavanagh[b] and Aleksandra Melnik[a]

3-[(Adamantan-1-yl)carbonyl]-1-pentylindole (AB-001), a synthetic cannabimimetic, was identified in head shop products in Ireland in 2010. German authorities also reported it to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) *via* the Early Warning System (EWS) in 2011. As indole-derived cannabimimetics, such as JWH-018, JWH-073, and JWH-250, undergo extensive metabolism, it was expected that AB-001 would behave similarly. To include it in our toxicological screening protocols, we have identified its urinary metabolites in humans following oral administration. The major metabolites were found to be adamantane mono-hydroxylated and adamantane mono-hydroxylated/*N*-dealkylated products. No parent compound was found in urine, and metabolites were detectable for up to 160 h following administration. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: AB-001; cannabimimetic; herbal mixture; metabolite; GC-MS

Introduction

The use of herbal smoking mixtures containing synthetic cannabimimetics is well established among recreational drugs users.[1] Initially, the active ingredients in these products included compounds such as JWH-018, JWH-073, and (C8)-CP 47,497[2–5] but over the past several years a number of countries have introduced legislation to control the distribution, sale, and possession of these 'first-generation' compounds. This has forced manufacturers to seek new active ingredients to circumvent such laws.[6]

One such compound is 3-[(adamantan-1-yl)carbonyl]-1-pentylindole (AB-001) which was identified in Ireland in the 'herbal' smoking mixture *Atomic bomb*.[7] It was also reported by German authorities to the European monitoring Centre for Drugs and Drugs Addiction (EMCDDA) *via* the Early Warning System (EWS) in 2011.[8] AB-001, which is currently uncontrolled in Ireland and Russia, is the *N*-pentyl analogue of the cannabimimetic AM1248 ((1-((1-methylpiperidin-2-yl)methyl)-1*H*-indol-3-yl)(adamantyl)methanone).[9]

Although the effects of cannabimimetics are widely discussed on Internet drug forums, formal toxicological, metabolism, and pharmacological studies have not been widely undertaken. Published work is limited to earlier compounds such as JWH-018, JWH-073, and (C8)-CP 47,497.[2,10–14] Studies on the metabolism of the *N*-alkylindole derivatives JWH-018 and JWH-073,[10–14] JWH-250,[15] JWH-210, and JWH-251 (our unpublished results) have shown that mono- and di-hydroxylated side-chain products are the major urinary metabolites in humans. *N*-Dealkylated and hydroxylated/*N*-dealkylated metabolites have also been identified as minor metabolites. The parent compounds are generally absent in urine. In this work, we describe the identification of urinary metabolites of AB-001 following oral administration.

Experimental

Chemicals and reagents

3-[(Adamantan-1-yl)carbonyl]-1-pentylindole was synthesized and characterized in the Department of Pharmacology and Therapeutics, Trinity College, Dublin. N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1% TMCS), tetramethylammonium hydroxide (25% *w/w* in methanol) and methyl iodide were purchased from Acros Organics (Geel, Belgium).

Administration

Self-administration was conducted by two of the authors, a 47-year-old male and a 43-year-old female. Thirteen mg (male) and 26 mg (female) were taken orally (corresponding to 0.22 and 0.52 mg/kg body mass). No physiological effects were noticed. Urine samples were collected at intervals up to one week after administration. The samples were stored at −20 °C until analysis.

* *Correspondence to: Andrej Grigoryev, Bureau of Forensic-Medical Expertise, Forensic-Chemical Division, Belgorod, Russia. E-mail: chrzond4250@yandex.ru*

a *Bureau of Forensic-Medical Expertise, Forensic-Chemical Division, Belgorod, Russia*

b *Department of Pharmacology and Therapeutics, School of Medicine, Trinity College, Dublin, Ireland*

Copyright © 2011 John Wiley & Sons, Ltd.

Sample preparation

Hydrochloric acid (0.25 ml, 30%) was added to urine (2.5 ml) and the mixture was heated at 90–95 °C for 60 min. After cooling, the pH was adjusted to 8–9 with aqueous ammonium hydroxide solution (25%). Internal standard (50 μl, 50 μg/ml papaverine in methanol) was added. The mixture was extracted with chloroform (3 ml), centrifuged and the organic phase was blown to dryness with nitrogen at 45 °C or below.

Derivatization

Derivatization of the residues for the gas chromatography-mass spectrometry (GC-MS) analysis was performed by methylation (Me) and trimethylsilylation (TMS). For methylation, the dry residue was dissolved in mixture of dry dimethyl sulfoxide (100 μl) and of tetramethylammonium hydroxide in methanol (25%, 5 μl). After shaking for 2 min, methyl iodide (20 μl) was added and shaking was continued for 10 min. Hydrochloric acid (0.1 M, 200 μl) was added and the mixture was extracted with ethyl acetate (3 ml). The upper organic phase was washed with 3 ml of water and evaporated to dryness. The residue was dissolved in 50 μl of ethyl acetate and injected in chromatograph or trimethylsilylated (TMS). TMS of underivatized or methylated samples was carried out using a mixture of BSTFA and ethyl acetate (25 μl of each) at 60 °C for 30 min.

GC-MS analysis

GC-MS analysis was performed by Agilent 6890 chromatograph coupled with 5975VL mass spectrometer under the following conditions: EVDX-5 ms column (25 m × 0.20 mm × 0.33 μm) with helium as carrier gas (1 ml/min); oven temperature program was 50 °C (0.5 min), 99 °C/min (100 °C, 1 min), and 60 °C/min (320 °C, 15 min); 1 μl was injected in splitless mode. Mass spectra were recorded in electron ionisation (EI) mode (70 eV).

Results and discussion

Seven major metabolites (Figure 1) and number of minor metabolites were tentatively identified in the urine samples following the administration of AB-001. The major metabolites were found to be *N*-dealkylated/mono-hydroxylated adamantane (**M1–M3**), mono-hydroxylated adamantane (**M4–M6**) and mono-hydroxylated adamantane/mono-hydroxylated *N*-pentyl (**M7**) products. The parent drug was not detected. The mass spectra and retention indices of metabolites are shown in Figures 2b–2d and 3.

The elimination of methyl group from trimethylsilyl residue with formation of ions *m/z* $[M\text{-}15]^+$ was an initial fragmentation step for all of these compounds. The base peak, *m/z* 214, in the EI mass spectrum of AB-001 is formed by cleavage of the carbonyl-adamantane bond resulting in the *N*-pentylindole acylium ion (Figure 2a). Subsequent loss of pentylene results in the *m/z* 144 indole acylium ion. The adamantyl ion, *m/z* 135, is also observed in the mass spectrum. Similar fragmentation pathways would be expected in AB-001 metabolites where the *N*-pentylindole moiety remains intact.

The base peak, *m/z* 158, in metabolites **M1–M3** (*N*-Me and *O*-TMS derivatives) corresponding to an *N*-methylated indole residue and mass ion, *m/z* 223, corresponding to a monohydroxylated adamantane residue, indicated *N*-dealkyalted adamantane mono-hydroxylated products (Figures 2b–2d). The *N*-pentylindole acylium ion, *m/z* 214, is present in the EI mass spectra of metabolites **M4–M6**. A low intensity *m/z* 223 in metabolites **M4** and **M5** molecules indicated that they were mono-hydroxylated adamantane metabolites (Figures 3a–3c). The absence of *m/z* 223 in the EI mass spectrum of M6 suggests that the hydroxyl may in a tertiary position.[16]

For the di-hydroxylated metabolite **M7** (Figure 3d), the mass ions, *m/z* 223 and 302, indicate hydroxylation of the adamantane and *N*-pentylindole residues respectively. The presence of the low-intensity ions, *m/z* 144, corresponding unaltered indole ring

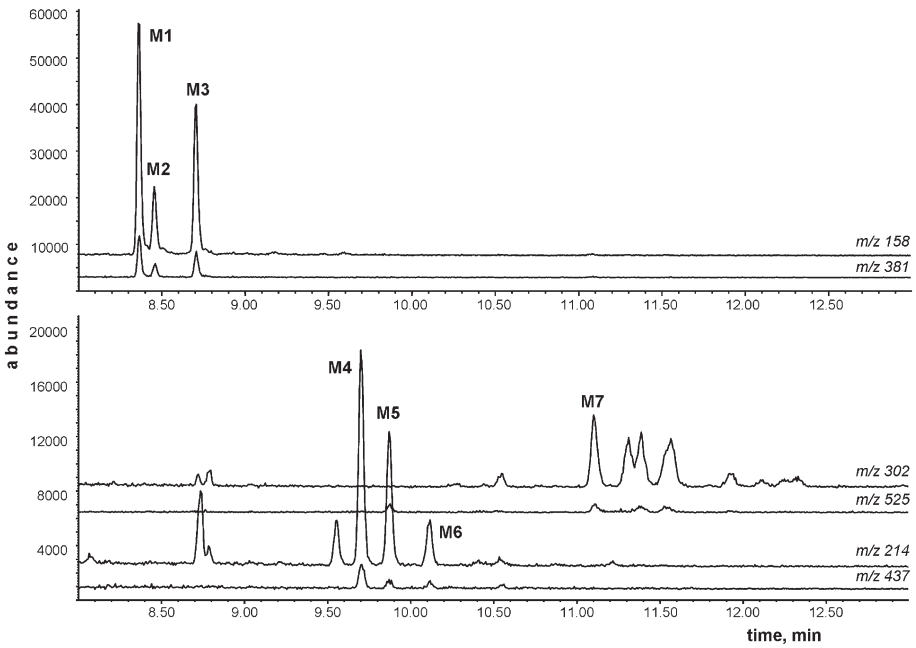

Figure 1. Extracted ion chromatograms for AB-001 metabolites (EI GC-MS).

Copyright © 2011 John Wiley & Sons, Ltd.

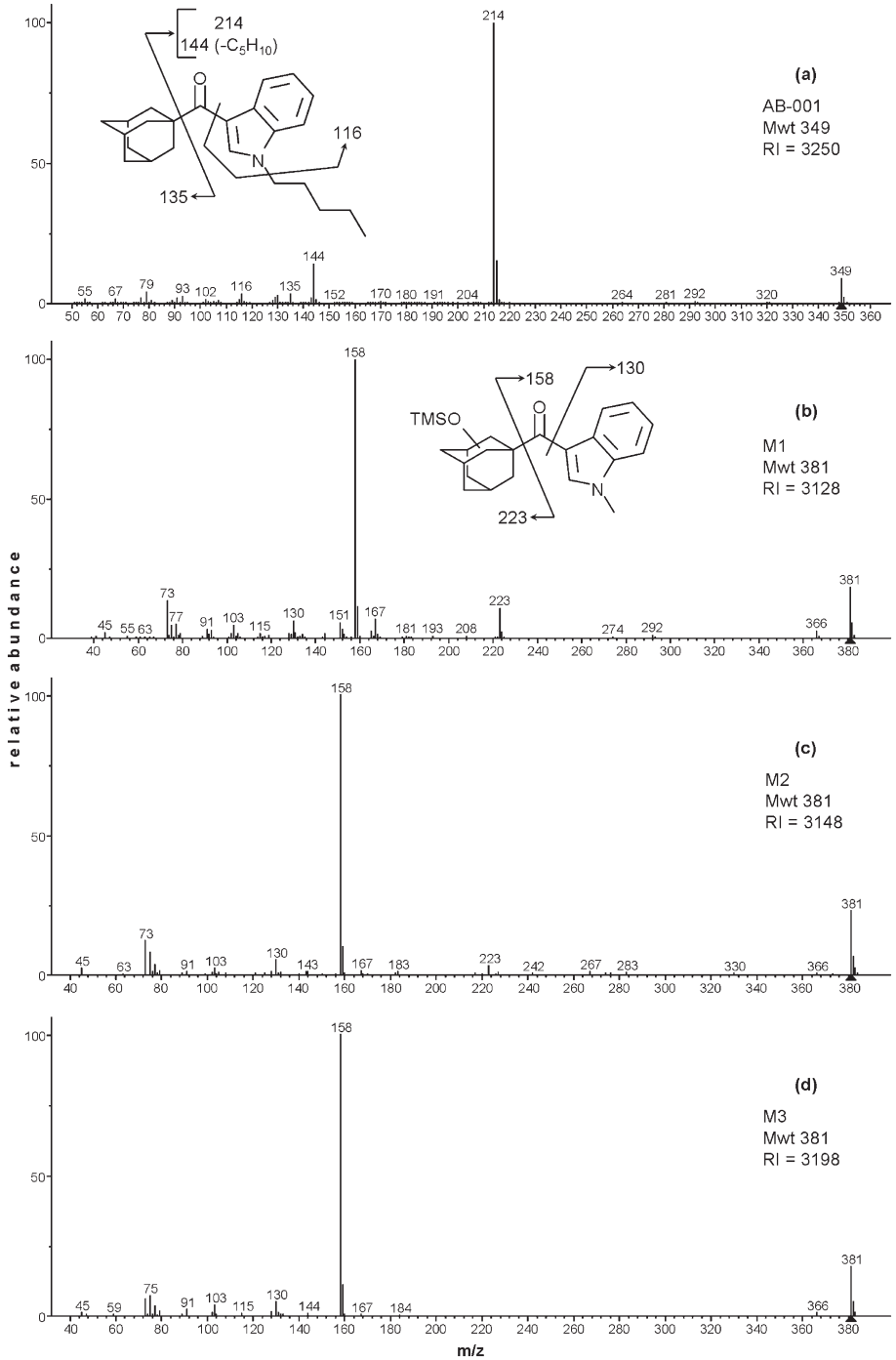

Figure 2. EI GC-MS data and retention indices for AB-001 and metabolites **M1–M3** (*N*-Me and *O*-TMS derivative).

structure, and *m/z* 212, formed by elimination of trimethylsilanol, suggests that the pentyl chain is hydroxylated.

The proposed metabolic pathways are shown in Figure 4. One difference between the metabolism of AB-001 and other *N*-alkylindole analogues is that the large aliphatic adamantane moiety is the major site for hydroxylation resulting in low levels of hydroxylated *N*-alkyl products. Highest concentrations of metabolites in urine samples were observed 5–7 h after AB-001 administration. Metabolites **M4**, **M5**, and **M7** were detectable in SIM mode (*m/z* 214, 302 for **M4** and **M5**) and (*m/z* 437, 525 for **M7**) up to 160 h after administration. The concentrations (SIM mode, arbitrary units) of **M4** and **M7** metabolites *vs* time are shown in Figure 5.

Oral administration was used for convenience and safety as the dose is easily controlled and undesirable physiological effects are minimized. Urinary metabolite profiles for indole cannabimimetics generally tend to be similar for administration by inhalation (smoking) or orally (our unpublished results) but doses are more unpredictable with the former, resulting in variable concentrations of metabolites.[14,15]

Copyright © 2011 John Wiley & Sons, Ltd.

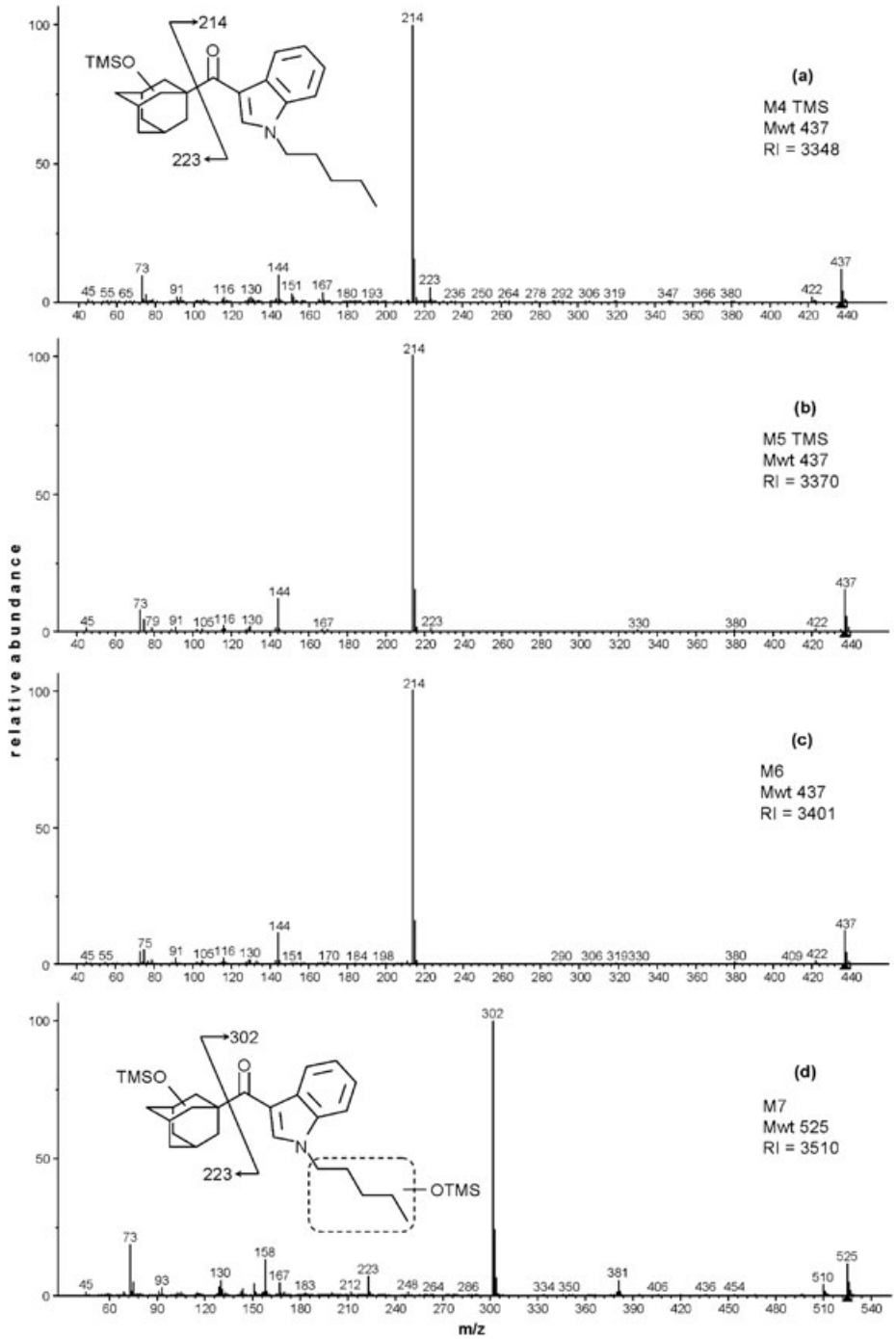

Figure 3. EI GC-MS data and retention indices for metabolites **M4–M7** (TMS derivative).

The use of authentic standards in forensic and toxicological analysis has always been considered a necessity. However, with the rapid appearance of previously unknown recreational drugs, low doses, time constraints on laboratories to generate results, the formation of multiple metabolites *in vivo*, and the absence of the parent compound in urine, it may be necessary to accept a post-administration urine sample from a controlled study as an 'authentic reference standard'. The protocol presented here is an example of this and it may be used to identify the ingestion of AB-001. The detection of the mono-hydroxylated metabolites **M4** and **M5**, and di-hydroxylated metabolite **M7** were found to be useful indicators for the compound's administration.

Conclusions

Seven major and number of minor metabolites of AB-001 were tentatively identified in human urine samples following oral administration. The seven major metabolites were three *N*-dealkylated/mono-hydroxylated adamantine; three hydroxylated adamantine; and one mono-hydroxylated adamantane/mono-

Copyright © 2011 John Wiley & Sons, Ltd.

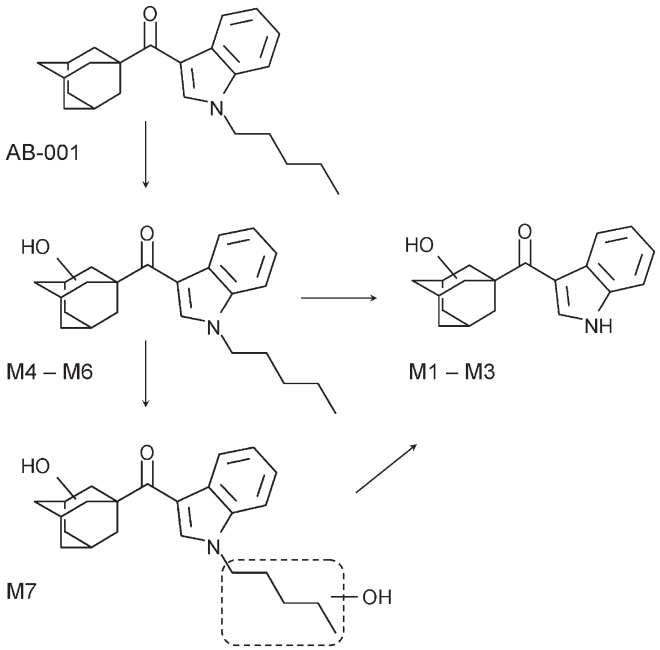

Figure 4. The proposed metabolic pathways for AB-001.

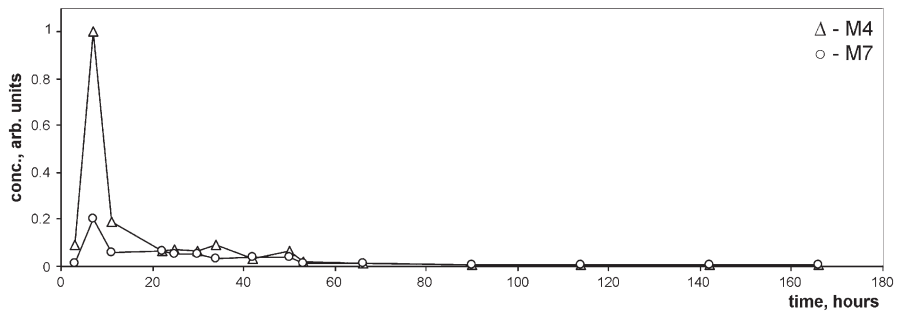

Figure 5. The excretion profiles of metabolites **M4** and **M7**.

hydroxylated *N*-pentyl products. The highest metabolite concentrations were observed 5–7 h after administration. No parent drug was detected in the urine samples. Two mono-hydroxylated and one di-hydroxylated metabolites were found to be useful target analytes.

References

[1] R. Sedefov, A. Gallegos, L. King, D. Lopez, V. Auwärter, B. Hughes, *et al.* Understanding the 'Spice' Phenomenon. EMCDDA Thematic paper, **2009**.

[2] V. Auwärter, S. Dresen, W. Weinmann, M. Müller, M. Pütz, N. Ferreirós. 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? *J. Mass Spectrom.* **2009**, *44*, 832.

[3] R. Lindigkeit, A. Boehme, I. Eiserloh, M. Lubbecke, M. Wiggermann, L. Ernst, *et al.* Spice: A never ending story? *Forensic Sci. Int.* **2009**, *191*, 58.

[4] N. Uchiyama, R. Kikura-Hanajiri, J. Ogata, Y. Goda. Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. *Forensic Sci. Int.* **2010**, *198*, 31.

[5] N. Uchiyama, R. Kikura-Hanajiri, N. Kawahara, Y. Goda. Identification of a cannabimimetic indole as a designer drug in a herbal product. *Forensic Toxicol.* **2009**, *27*, 61.

[6] S. Hudson, J. Ramsey. The emergence and analysis of synthetic cannabinoids. *Drug Test. Anal.* **2011**, DOI:10.1002/dta.268.

[7] P. Kavanagh, P. Spiers, J. O'Brien, S. McNamara, D. Angelov, D. Mullan, *et al.* Head Shop 'Legal Highs' Active Constituents Identification Chart, July-August **2010**, 714–823. Available at: http://www.drugsandalcohol.ie/13972/1/Head_shops_identification_July_Aug_2010.pdf [10 December 2010].

[8] BEWDS (Belgian Early Warning System on Drugs). Available at: http://ewsd.wiv-isp.be/New_psychoactive_substances_in_Europe_Registration/JWH-018%20adamantoyl%20derivative.aspx [10 June 2011].

[9] A. Makriyannis, H. Deng. Cannabimimetic Indole Derivatives. US Patent No. 208/0090871, **2008**.

[10] T. Kraemer, K. Y. Rust, M. R. Meyer, D. K. Wissenbach, D. Bregel, M. Hopf, *et al.* Studies on the metabolism of JWH-018 and of a homologue of CP 47,497, pharmacologically active ingredients of different misused incense 'Spice' using GC-MS and LCMSn techniques. *Ann. Toxicol. Anal.* **2009**, *21*(S1), O43.

[11] A. Wintermeyer, I. Möller, M. Thevis, M. Jübner, J. Beike, M. A. Rothschild, *et al.* In vitro phase I metabolism of the synthetic cannabimimetic JWH-018. *Anal. Bioanal. Chem.* **2010**, *398*, 214.

[12] T. Sobolevsky, I. Prasolov, G. Rodchenkov. Detection of JWH-018 metabolites in smokingmixture post-administration urine. *Forensic Sci. Int.* **2010**, *200*, 141.

[13] I. Möller, A. Wintermeyer, K. Bender, M. Jübner, A. Thomas, O. Krug, *et al.* Screening for the synthetic cannabinoid JWH-018 and its major metabolites in human doping controls. *Drug Test. Anal.* **2010**, DOI:10.1002/dta.158.

Copyright © 2011 John Wiley & Sons, Ltd.

[14] A. Grigoryev, S. Savchuk, A. Melnik, N. Moskaleva, J. Dzhurko, M. Ershov, *et al*. Chromatography–mass spectrometry studies on the metabolism of synthetic cannabinoids JWH-018 and JWH-073, psychoactive components of smoking mixtures. *J. Chromatogr. B* **2011**. *879*, 1126.
[15] A. Grigoryev, A. Melnik, S. Savchuk, A. Simonov, V. Rozhanets. Gas and liquid chromatography–mass spectrometry studies on the metabolism of synthetic phenylacetylindole cannabimimetic JWH-250, psychoactive components of smoking mixtures. *J. Chromatogr. B* **2011**, DOI: 10.1016/j.jchromb.2011.07.004.
[16] D. H. van de Kerkhof, D. de Boer, R. A. A. Maes. The position of the hydroxy group in the main bromantane metabolite, in Recent Advances in Doping Analysis (6), (Eds: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke). Sport und Buch Strauß, Köln, **1999**, pp. 361–374.

Copyright © 2011 John Wiley & Sons, Ltd.