Short communication

Drug Testing and Analysis

Received: 11 December 2011 Revised: 13 February 2012 Accepted: 13 February 2012 Published online in Wiley Online Library: 23 April 2012

(www.drugtestinganalysis.com) DOI 10.1002/dta.1336

The detection of the urinary metabolites of 1-[(5-fluoropentyl)-1H-indol-3-yl]-(2-iodophenyl) methanone (AM-694), a high affinity cannabimimetic, by gas chromatography – mass spectrometry

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AM-694 (1-[(5-fluoropentyl)-1*H*-indol-3-yl]-(2-iodophenyl)methanone), a synthetic indole-based cannabimimetic, was first reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) *via* the Early Warning System (EWS) by Irish authorities in 2010. Using gas chromatography–mass spectrometry (GC-MS), we have identified six AM-694 metabolites in post-ingestion samples. The metabolites were tentatively identified as products of (1) hydrolytic defluorination, (2) carboxylation, (3) monohydroxylation of *N*-alkyl chain, and (4) hydrolytic defluorination combined with monohydroxylation of *N*-alkyl chain. The parent compound was not detected. The excretion of major metabolites was observed up to 117 h following administration. One metabolite (a product of hydrolytic defluorination) was also identified in urine samples from two individuals admitted to hospital suffering from suspected drug overdoses. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: AM-694; herbal mixture; metabolite; cannabimimetic; GC-MS

Introduction

A wide range of synthetic cannabimimetics has been identified in 'herbal' smoking mixtures in recent years.[1–5] One such compound, AM-694, (1-[(5-fluoropentyl)-1*H*-indol-3-yl]-(2-iodophenyl) methanone), was first reported to the EMCDDA (European Monitoring Centre for Drugs and Drug Addiction) *via* the EWS (Early Warning System) by Ireland in 2010.[6] It was identified in a number of herbal smoking products such as 'Shamrock Extra Special', 'Smoke XXX', 'The Green Harp', 'Pulse Ultra', and 'Warrior Ultimate'.[6] AM-694 was first synthesized by Makriyannis and has a high affinity for the CB1 receptor ($K_i = 0.08$ nM).[7] According to reports on popular drugs forums, a 1–3 mg is sufficient to produce noticeable effects. However, little is known about the metabolism or toxicology of AM-694 in humans.

From previous studies it has been shown that *N*-alkyl-3-aroylindole cannabimimetics generally undergo extensive metabolism resulting in the absence of or low concentrations of the parent compound in urine thus making detection difficult.[8,11–13] Both *in vivo*[8–13] and *in vitro*[14,15] metabolism studies have shown that monohydroxylation of the N-alkyl chain is the major metabolic pathway. Polyhydroxylated and *N*-dealkylated metabolites have also been detected. In this paper, we report the identification of the human urinary AM-694 metabolites using gas chromatography–mass spectrometry (GC-MS); the excretion profiles of two major metabolites are also presented.

Experimental

Chemicals and reagents

AM-694 was isolated from an herbal smoking mixture and characterized in the Department of Pharmacology and Therapeutics, Trinity College, Dublin. *N,O*-Bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (BSTFA + 1% TMCS), tetramethylammonium hydroxide (25% *w/w* in methanol), methyl iodide and high performance liquid chromatography (HPLC) grade acetonitrile were purchased from Acros Organics (Geel, Belgium).

Administration

Two self-administration experiments were conducted, with a two-month interval, by one of the authors, a 47-year-old male. For the first experiment, AM-694 (10 mg) was taken orally. For the second experiment, a smoking mixture was prepared by adding a solution of AM-694 (1 mg in 1 ml of acetone) to 100 mg of tobacco and allowing the solvent to evaporate. The amounts ingested correspond to 167 (oral) and 16.7 (smoking) μg/kg body mass. No physiological effects were noted following ingestion. Urine samples were collected at intervals up to one week after administrations and were stored at −20 °C until analysis. Ethical approval is not required for self-administration studies of AM-694 in Russia.

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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%). Papaverine (50 µl, 50 µg/ml in methanol) was added as an internal standard, the mixture was extracted with chloroform (3 ml), centrifuged and the organic phase was blown to dryness with nitrogen at 45 °C.

Purification/concentration of metabolites by HPLC

A urine sample (50 ml) was hydrolyzed as described above, extracted with chloroform (50 ml), evaporated to dryness and the residue was dissolved in acetonitrile (3 ml). This solution was passed through an AccuBond II ODS-C18 SPE cartridge (200 mg, Agilent Technologies) to remove compounds strongly retained on the HPLC column. The cartridge was washed with acetonitrile (2 ml) and the combined eluates were evaporated to dryness. The residue was re-dissolved in mobile phase (0.5 ml) and fractionated by HPLC using an Agilent 1200 chromatograph equipped with a binary pump, diode-matrix detector and the following conditions: column (SB-18, 4.6 mm × 150 mm); mobile phase, acetonitrile/water (75/25); flow rate, 1 ml/min with one washing step (100 % of acetonitrile for 1 min) after 4 min. Eight fractions were collected (min): *1* (1.3–1.7), *2* (1.7–1.9), *3* (1.9–2.1), *4* (2.1–2.3), *5* (2.3–2.7), *6* (2.7–3.1), *7* (3.1–3.5), *8* (3.5–4). Each fraction was evaporated to one-third its volume and an equal volume of water was then added. The mixture was extracted with ethyl acetate. The extracts were evaporated to dryness with nitrogen and derivatized as described below.

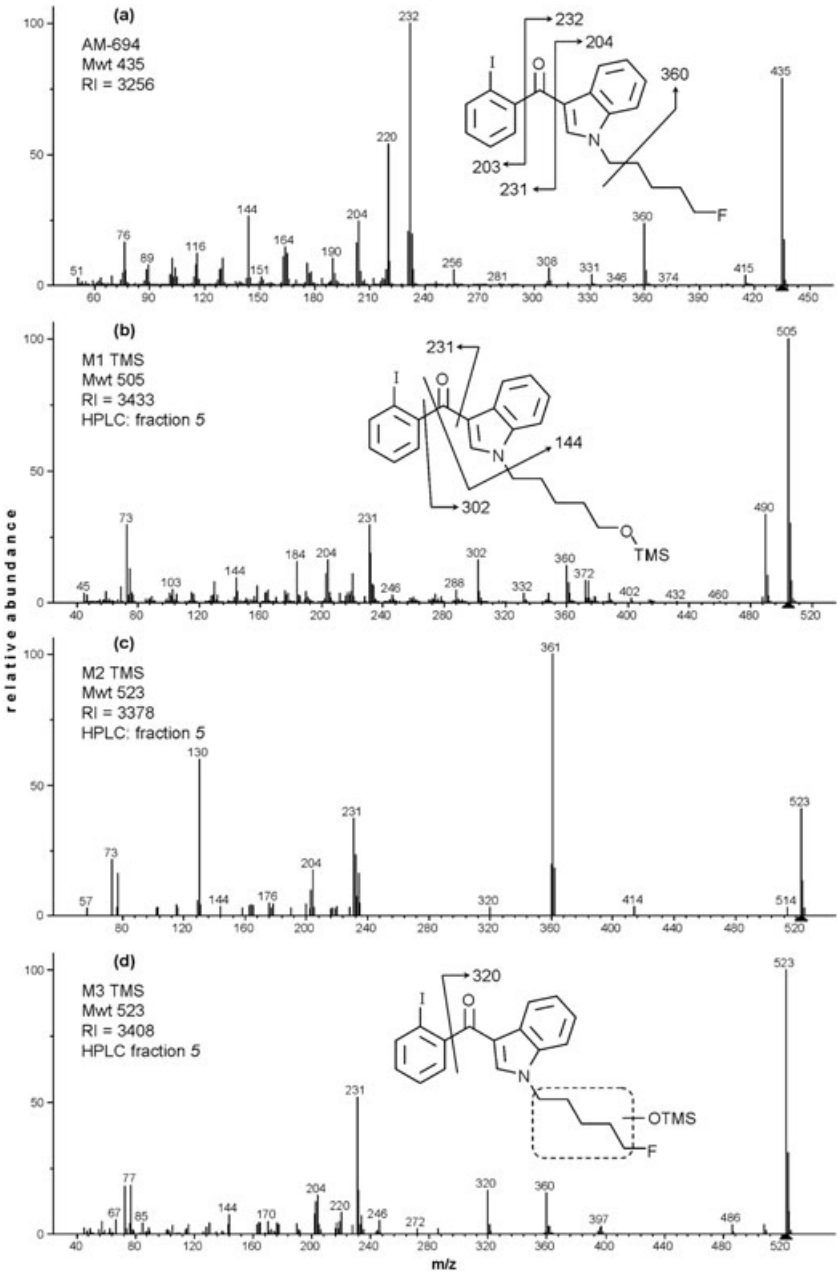

Figure 1. EI GC-MS data for AM-694 and metabolites **M1–M3** (TMS).

Derivatization

For methylation (Me), the dry residue was dissolved in a mixture of dry dimethyl sulfoxide (100 μl) and methanolic tetramethylammonium hydroxide (25%, 5 μl). The mixture was shaken for 2 min, methyl iodide (20 μl) was then added and shaking was continued for 10 min. Aqueous ammonium hydroxide (0.1 M, 2 ml) was added and the mixture was extracted with ethyl acetate (3 ml). The extract was washed with aqueous ammonium hydroxide (0.1 M, 2 ml), evaporated to dryness and the residue was dissolved in ethyl acetate (50 μl). Underivatized or methylated samples were trimethylsilylated (TMS) by heating the residue with a mixture of BSTFA and ethyl acetate (25 μl of each) at 60 °C for 30 min.

Gas chromatography–mass spectrometry

GC-MS analysis was performed on an Agilent 6890 chromatograph coupled to 5975VL MSD using the following conditions: column, VF-5 ms (30 m × 0.25 mm × 0.25 μm, Varian Inc.); carrier gas, helium (1 ml/min); oven temperature program, 50 °C (0.5 min), 99 °C/min (100 °C, 1 min) and 60 °C/min (320 °C, 15 min); injection volume, 1 μl (splitless mode); injector port temperature,

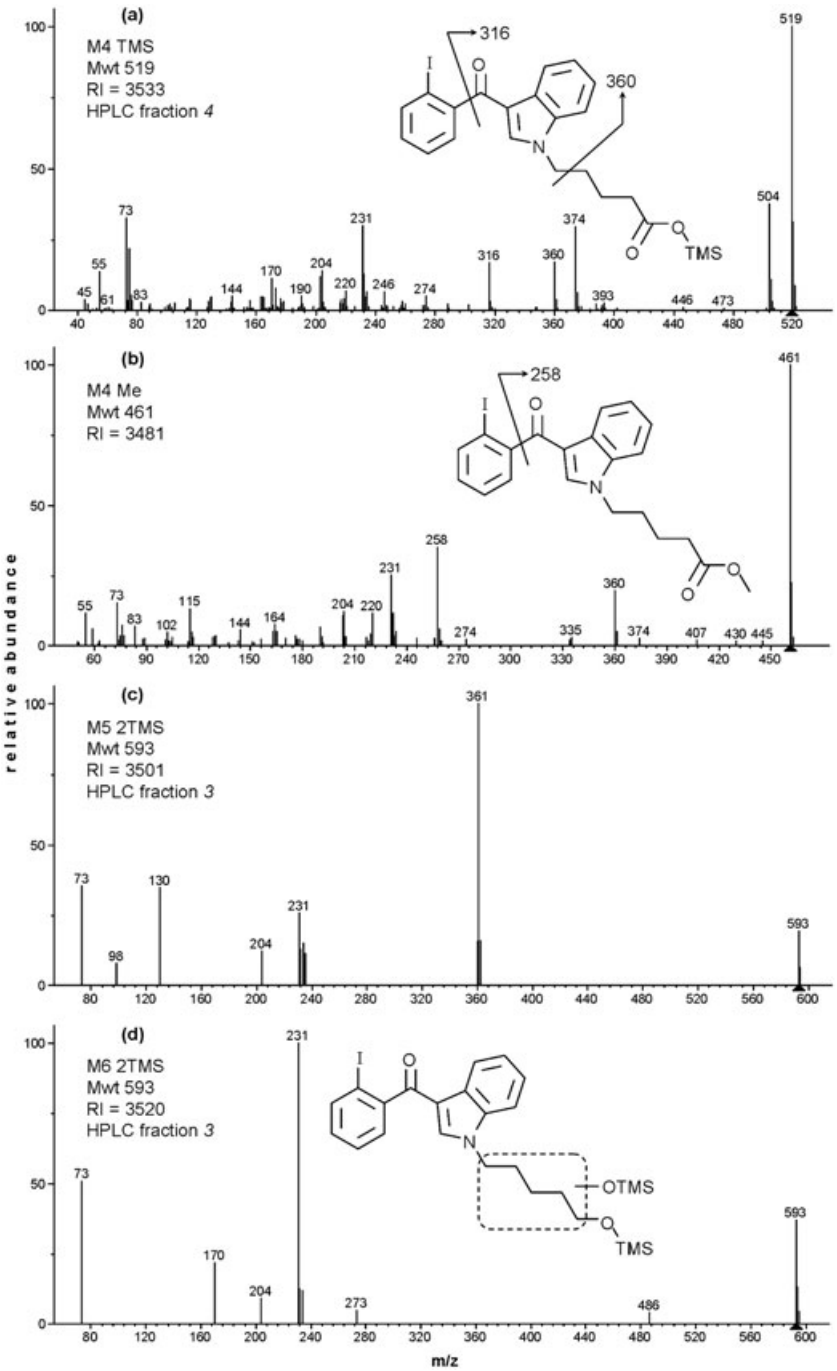

Figure 2. EI GC-MS data for metabolites **M4** (TMS and Me) and **M5**, **M6** (TMS).

270 °C. Mass spectra were recorded in electron ionisation (EI) mode (70 eV).

Results and discussion

Six metabolites (**M1–M6**), present only in the hydrolyzed fractions, were identified in the post-ingestion urine samples. The parent AM-694 was not detected. The EI mass spectra and retention indices of AM-694 and its metabolites are shown in Figures 1 and 2, respectively. The basic mass spectrometric fragmentation pathways for these metabolites were found to be similar to those of other 3-aroylindole cannabimimetics, the major ions being formed by cleavage of bonds between carbonyl group and aryl moieties.[1–5] In the mass spectrum of AM-694 (Figure 1a), the base peak, *m/z* 232, corresponds to *N*-fluoropentylindol-oxomethylium fragment with loss of the fluoropentyl group resulting in the formation of indol-oxomethylium ion, *m/z* 144. The mass ion, *m/z* 308, arises through loss of iodine from the molecular ion.

The presence of fragment ions, *m/z* 144 and 231, in the mass spectrum of metabolite **M1** (Figure 1b) show that the indole and iodophenyl moieties are unaltered while the mass ion, *m/z* 302, indicates hydrolytic defluorination. Metabolites **M2** and **M3** (Figures 1c and 1d) contain a hydroxyl group on the *N*-fluoropentyl chain, as shown with mass ions, *m/z* 144, 231 and 320, while ions, *m/z* 361 (**M2**) and 360 (**M3**), indicate unchanged aromatic moieties. For metabolite **M4** (Figures 2a and 2b), the mass ions, *m/z* 316 (TMS), 258 (Me) and 360 (TMS and Me), indicate that the hydroxyl

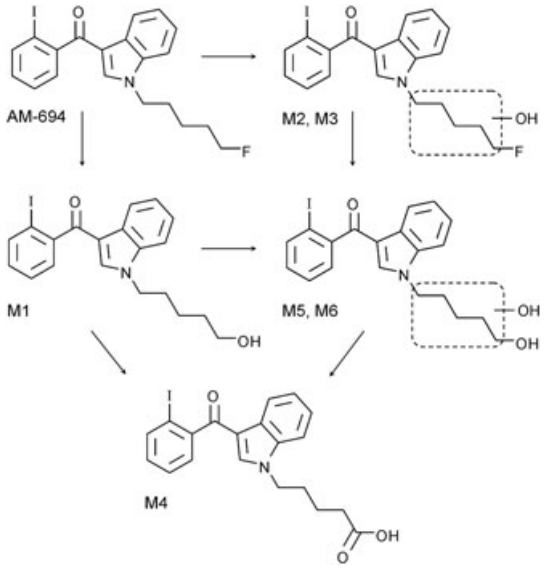

Figure 4. The proposed metabolic pathways for AM-694.

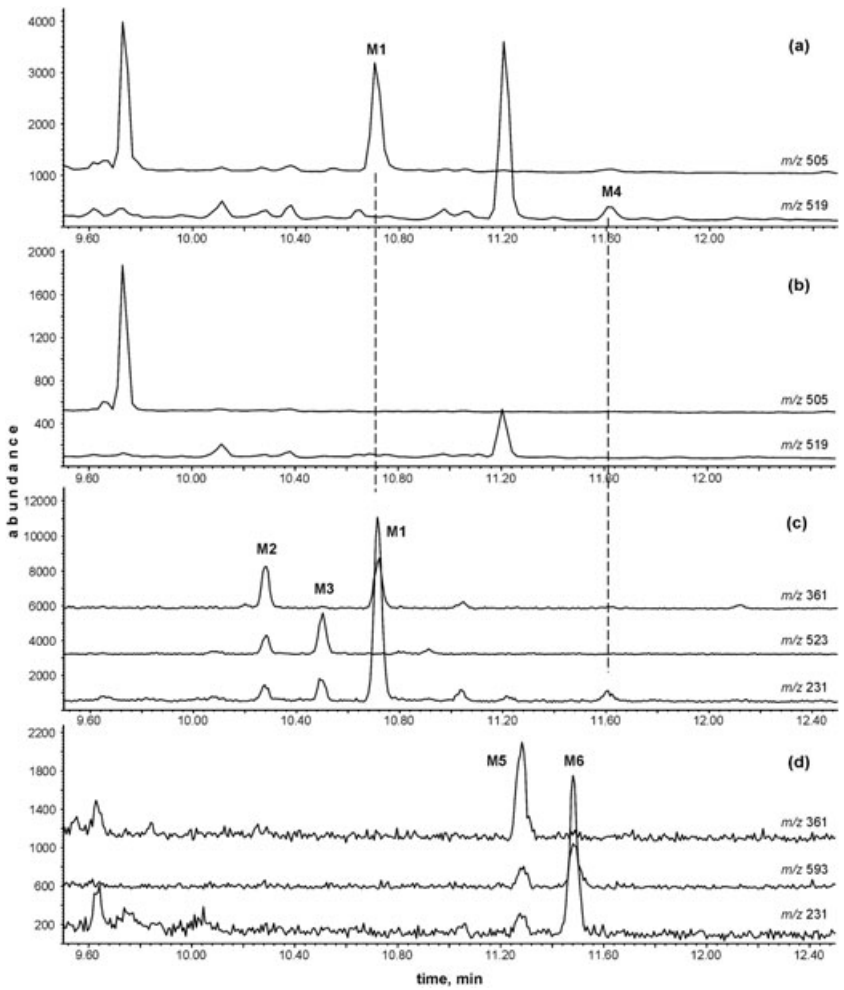

Figure 3. Extracted ion chromatograms of urine sample after AM-694 smoking (a), blank urine sample (b), HPLC fractions *5* (c), and *3* (d).

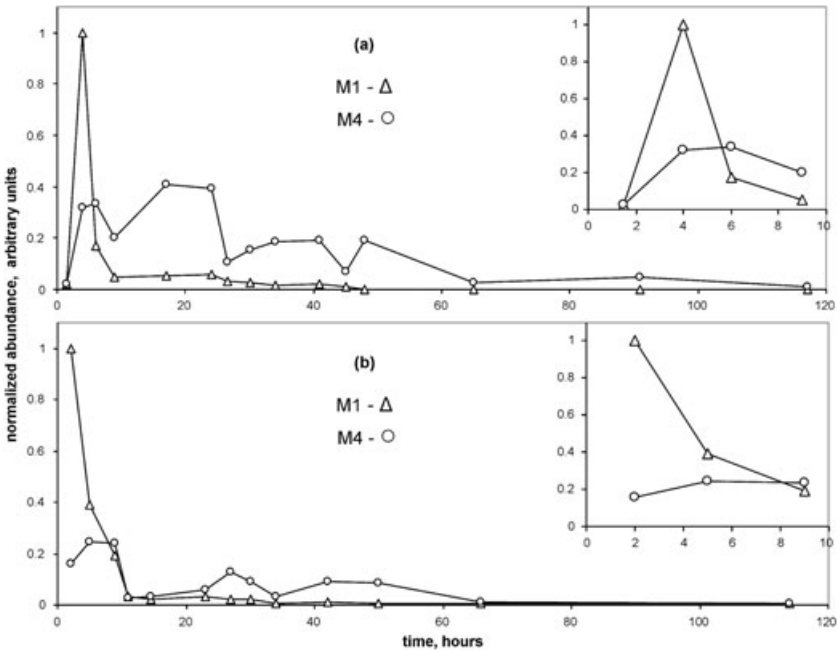

Figure 5. The excretion profiles of urinary metabolites **M1** and **M4** after administration by oral (a), and smoking (b).

and carbonyl groups are located on the alkyl side chain. This was confirmed by methylation and variation of the hydrolysis mode. Two minor metabolites (**M5** and **M6**, Figures 2c and 2d) were identified as products of combined hydrolytic defluorination and monohydroxylation of the *N*-fluoropentyl chain. The mass spectra of metabolites **M2** and **M5** have several fragment ions in common and the mass ion *m/z* 130 (N-methylindolylium, $C_9H_8N^+$) has been seen previously in the mass spectrum of a side chain monohydroxylated metabolite of JWH-073.[13]

The extracted ion chromatograms (EICs) for the metabolites and proposed metabolic pathways are shown in Figures 3 and 4, respectively. Two major metabolites (**M1** and **M4**) were directly detectable in the urine samples but the four minor metabolites (**M2**, **M3**, **M5**, and **M6**) required prior HPLC enrichment. The excretion profiles of metabolites **M1** and **M4** (SIM mode, using *m/z* of molecular ions) are shown in Figure 5. The highest concentrations of major metabolite **M1** were observed at 4 and 2 h following oral administration and inhalation (smoking), respectively. Metabolite **M1** was also found in urine samples from two patients who were admitted to hospital with suspected drug overdoses.

In the absence of reference standards and considering the fact that the relative signal responses of the metabolites compared to the parent drug may be quite different, we were not able to determine concentrations. It must be pointed out that some caution must be exercised when establishing AM-694 ingestion by described analytical method but we believe that with the rapid appearance of previously unknown recreational drugs, GC-MS data obtained from administration studies may have to be used in the absence of authentic reference standards.

Conclusions

Six metabolites of AM-694 were identified in urine samples following oral and inhalation administration. The major metabolites were hydrolytically defluorinated and carboxylated products. Neither the parent drug nor its *N*-defluoropentylated metabolites were detected. The highest concentration of the hydrolytically defluorinated metabolite was observed at 4 and 2 h following oral administration and inhalation, respectively, and this metabolite was also identified in urine samples from individuals admitted to hospital suffering from suspected drug overdoses.

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