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Metabolomic Characterization of Newest Designer Drugs

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1. Introduction

Designer drugs are structural or functional analogs of controlled substances which have been designed to mimic pharmacological effects of the original drugs [1]. A large group of designer drugs is synthetic cannabinoids (SCs), which are mainly sold online as 'herbal smoking mixtures' as a legal alternative of marijuana [2]. The consumption of SCs today is a serious problem, because they have significantly higher binding affinities to the CB1 and CB2 cannabinoid receptors than the well-known Δ^9-tetrahydrocannabinnol (THC) thank to their special pharmacodynamics properties [3].

In forensic and clinical practice the most commonly used techniques for quantitation of SCs in urine and blood samples are high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) [4]. Only detecting the parent compounds cannot justify the consumption of SCs due to their low concentration and rapid metabolism [5].

The present study aims to identify suitable marker metabolites by investigating of *phase I* metabolism of the chiral compound methyl (*S*)-3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido)butanoate (**MDMB-4en-PINACA**) and (1-(4-fluorobenzyl)-1H-indol-3-yl) (2,2,3,3-tetramethylcyclopropyl) methanone (**FUB-144**) as the newest SCs in the Hungarian drug market (***Figure 1***).

2. Materials and methods

The MDMB-4-en-PINACA and FUB-144 standards (purity 99.0 ± 2.2%) were kindly provided by the Drug Investigation Department of The Hungarian Institute for Forensic Sciences (HIFS).

The slightly modified human liver microsome (HLM) experiment was performed according to the described procedure by Franz et al. [6].

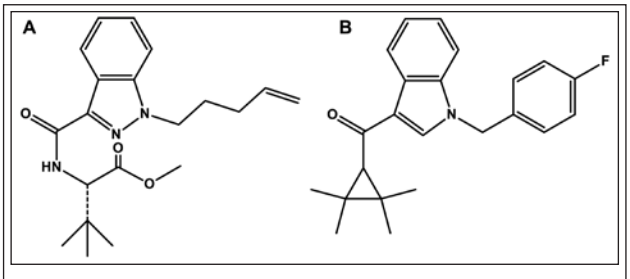

Figure 1*. Structures of MDMB-4en-PINACA (A) and FUB-144 (B)*

The analysis was performed on a Waters Acquity I-Class UPLC™ (Waters, Milford, MA, USA) coupled to a Q Exactive™ Plus hybrid Quadrupole-Orbitrap™ mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

The electrospray ionization was in positive ion mode. The obtained LC-MS raw data files were directly imported into Progenesis QI 2.1 (Nonlinear Dynamics, Newcastle, UK) software for deconvulation and statistical evaluation. The possible structure of candidate metabolites was determined by LC-MS/MS measurement.

3.1 Results of MDMB-4en-PINACA

MDMB-4en-PINACA is the first SC with an alkene functional group in the alkyl chain. *In vitro* studies resulted in the detection of 42 metabolites for HLM incubations of MDMB-4en-PINACA. ***Figure 2*** shows the fragmentation behaviours of ester hydroxylated and mono-hydroxylated metabolites of MDMB-4en-PINACA. Beside previously described metabolites of MDMB-4en-PINACA [7] we characterized new metabolities such as amide hydrolysis, amide hydrolysis with mono-hydroxylation and ester hydrolysis with dehydrogenation.

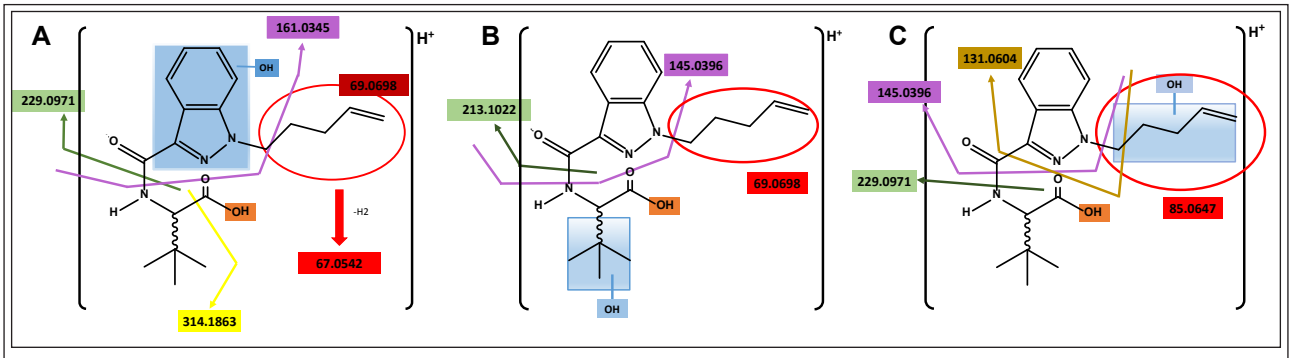

Figure 2 *Ester hydroxylated and mono-hydroxylated metabolites of MDMB-4en-PINACA with proposed fragmentation patterns. Mono-hydroxylation at the indazole moiety (A), at the linked group (B) and at the alkene chain (C)*

3.2 Result of FUB-144

FUB-144 is a SC with tetramethylcyclopropyl linked group. To the best of our knowledge, metabolites of FUB-144 have not been published in the literatures. We could identify 23 metabolites of FUB-144 *in vitro*, such as internal dehydration, mono- and dihydroxylations and mono-hydroxylation with hydrogenation.

4. Conclusions

The use of *in vitro* techniques can help to understand the metabolic pathway of designer drugs. In this study, a total of 42 metabolites of MDMB-4en-PINACA and 23 metabolites of FUB-144 were characterized. Nevertheless, for confirmation of the drug intake, *in vivo* metabolites from authentic urine or/and blood samples, are necessary.

5. Acknowledgements

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