IDENTIFICATION OF THE MAIN METABOLITES OF THREE SYNTHETIC CANNABINOIDS USING LC-MS/MS TECHNIQUE

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Abstract
The consumption of designer drugs today is a serious problem, especially among young people involvement. 'Herbal mixtures' containing synthetic cannabinoids (SCs) that mimic the effect of marijuana and there are easily available via the Internet. For analysis of urine samples, knowledge of the main metabolites is necessary as the mother compounds are usually not found in urine after using, due to their fast metabolism. The aims of this study were the in vitro identification of metabolites of ADB-FUBINACA, 5F-MDMB-PICA and CUMYL-PEGACLONE and to determine which analytical targets are excreted into urine. Metabolites identified after incubation of SCs with pooled human liver microsomes (HLM). The authentic urine samples were analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for investigation of the major in vivo metabolites. The main metabolites were the mono-hydroxylation of ADB-FUBINACA and CUMYL-PEGACLONE in positive urine specimens. We didn't have positive sample of 5F-MDMB-PICA.

Introduction
Synthetic cannabinoids are a group of designer drugs that mimic and magnify natural cannabinoids effect. The CB_1 and CB_2 cannabinoid receptor agonists SCs sold as 'herbal smoking mixtures' are promoted as legal alternative to marijuana, to circumvent drug scheduling legislation [1]. The SCs are highly potent and responsible for many acute intoxications and deaths [2, 3]. In forensic practice the SC consumption is detecting the parent molecules in urine and blood specimens. Due to their fast metabolism prior the renal extraction, in most cases the parent compounds are detectable in narrow time window in human urine. The present study aims to identify appropriate marker metabolites by investigating of *phase I* metabolism of ADB-FUBINACA as a most commonly used SC, 5F-MDMB-PICA and CUMYL-PEGACLONE as the newest SCs (Figure 1), using pooled human liver microsome (HLM), and to confirm the results in authentic human urine samples.

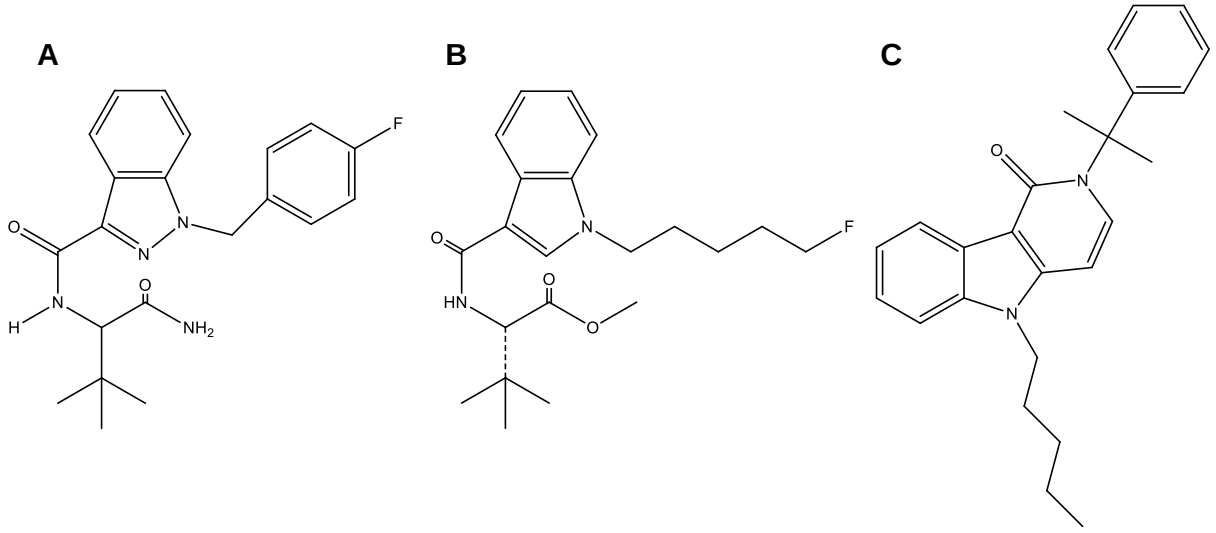

Figure 1 Chemical structure of ADB-FUBINACA (A), 5F-MDMB-PICA (B) and CUMYL-PEGACLONE (C) synthetic cannabinoids

Experimental

The LC-MS/MS method and the new sample preparation was developed for identification and analysis of metabolites in HLM and urine samples. The SCs was incubated with HLM at 37°C for 30 min. The urine samples were analysed after β-glucuronidase hydrolysis. The analysis was performed on a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) coupled with a Waters Acquity I-Class UPLC™ (Waters, Manchester, UK). Compound separation was achieved using a Kinetex C18 column (150 x 2.1 mm, 2.6 µm, Phenomenex, Torrance, CA, USA) combined with a guard column maintained at 50°C at a constant flow rate of 0.4 mL/min. Mobile phase A consisted of 0.1% formic acid, and mobile phase B was acetonitrile with 0.1% formic acid. The HLM incubates and urine samples were analyzed in positive electrospray ionization (ESI) mode. The mass spectrometer was operated in full scan and parallel reaction monitoring acquisition (PRM) modes.

Results and discussion

The developed analytical LC-MS/MS method provided the separation and characterization of numerous ADB-FUBINACA, 5F-MDMB-PICA and CUMYL-PEGACLONE *phase I* metabolites.

7 *phase I* metabolites of ADB-FUBINACA were detected in authentic urine sample (Table 1). The identified metabolites were assigned to 5 different biotransformations, including amide hydrolysis, dehydrogenation, monohydroxylation, formation of carbonyl derivatives and their isomers. The main metabolite of ADB-FUBINACA was the aliphatic mono-hydroxylated (M4) form [4].

Table 3 Identified metabolites of ADB-FUBINACA in HLM and authentic urine sample

Biotransformation		Formula	Retention time (min)	$[M+H]^+$ (m/z)	Fragment ions (m/z)	Identify in urine sample	Identify in HLM
	ADB-FUBINACA	$C_{21}H_{23}N_4O_2F$	17.24	383.1878	109, 253, 270, 338, 366	Yes	Yes
M1	Methylenefluorophenyl loss	$C_{14}H_{18}N_4O_2$	8.12	275.1503	145, 162, 230	No	Yes
M2	Dihydrodiol formation	$C_{21}H_{25}N_4O_4F$	9.22	417.1933	109, 241, 304, 372	No	Yes
M3	Amide hydrolysis + dehydrogenation	$C_{21}H_{20}N_3O_3F$	13.74	382.1561	109, 253, 324	Yes	Yes
M4	Aliphatic mono-hydroxylation	$C_{21}H_{23}N_4O_3F$	13.73	399.1827	109, 253, 354, 382	Yes	Yes
M5	Aliphatic hydroxylation + dehydrogenation	$C_{21}H_{21}N_4O_3F$	13.80	397.1671	109, 253, 270, 324	Yes	Yes
M6	Indazole mono-hydroxylation	$C_{21}H_{23}N_4O_3F$	14.32	399.1827	109, 269, 354	Yes	Yes
M7	Indazole mono-hydroxylation	$C_{21}H_{23}N_4O_3F$	14.84	399.1827	109, 145, 163, 269, 354	Yes	Yes
M8	Amide hydrolysis + aliphatic hydroxylation	$C_{21}H_{22}N_3O_4F$	14.89	400.1667	109, 253, 324, 382	Yes	No
M9	Indazole mono-hydroxylation	$C_{21}H_{23}N_4O_3F$	15.24	399.1827	109, 145, 269, 354	No	Yes
M10	Carbonylation	$C_{21}H_{19}N_4O_3F$	15.85	395.1514	109, 253, 270	Yes	Yes
M11	Amide hydrolysis	$C_{21}H_{22}N_3O_3F$	18.95	384.1718	109, 253, 338	No	Yes

For 5F-MDMB-PICA (Fig. 2), 13 *phase I* metabolites were identified by accurate m/z values and the fragmentation behaviour known from the literature [5].

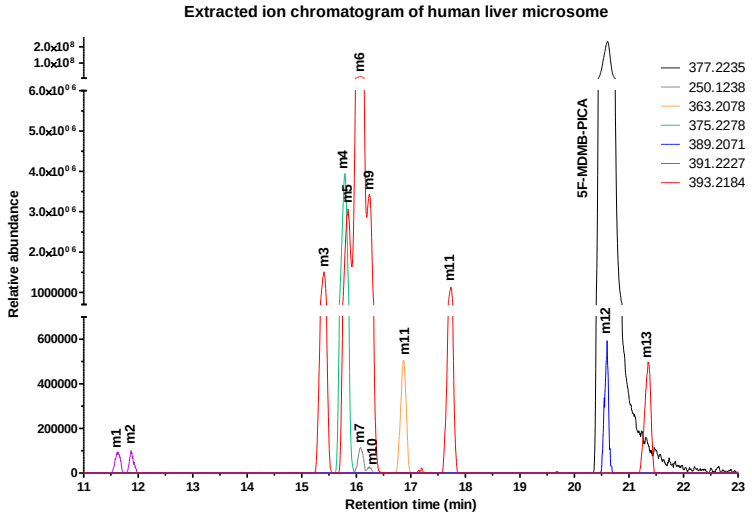

Figure 2 Extracted ion chromatogram of identified metabolits of 5F-MDMB-PICA in HLM

The new analytical method provided over 35 *phase I* metabolites of CUMYL-PEGACLONE in authentic urine specimens, such as formation of dehydrogenation, mono- and di-hydroxilation, dealkylation, carbonylation and carboxylation and their isomers. Fig. 3 shows

the MS/MS spectra of three di-hydroxilated metabolites of CUMYL-PEGACLONE. The biotransformation site on the structure of the molecule was determined by characteristic fragment ions. The mono-hydroxylated metabolite (M45) was identified as specific and sensitive urinary markers to proof consumption of CUMYL-PEGACLONE [6].

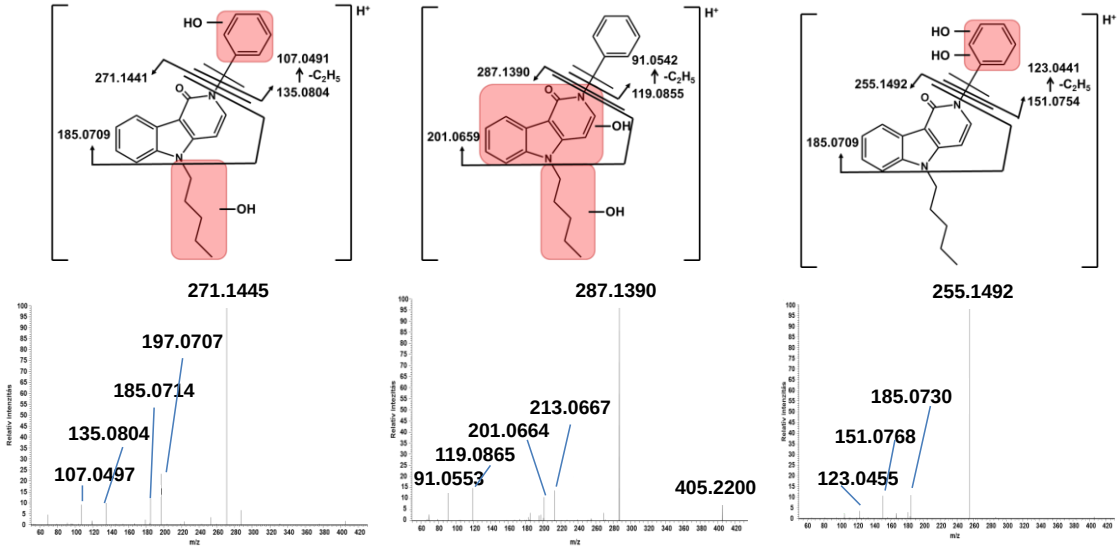

Figure 3 Di-hydroxylated metabolies of CUMYL-PEGACLONE and their MS/MS spectra in positive mode

Conclusion

The present study describes the identification of phase I metabolites of ADB-FUBINACA, 5F-MDMB-PICA and CUMYL-PEGACLONE after incubation with pooled human liver microsomes. The main metabolite of ADB-FUBINACA and CUMYL-PEGACLONE was formation of mono-hydroxylation in authentic urine specimens.

Acknowledgements

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