In vitro biotransformation of 3-methylmethcathinone (3-MMC) in human liver microsomes and correlation with the *in vivo* situation

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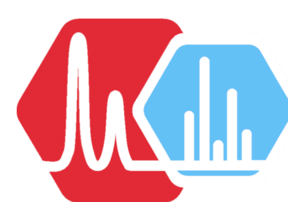

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INTRODUCTION

- Synthetic cathinones are the second largest group, after synthetic cannabinoids, of new psychoactive substances (NPS) monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).
- 3-methylmethcathinone (3-MMC) has recently been introduced into the market but has gained popularity rapidly.
- Due to the scarcity of information, biotransformation pathway information is crucial to understand the pharmacological, pharmacokinetic, and toxicological profile of 3-MMC.
- The identification of valuable human biomarkers is needed to develop analytical detection methods for screening of human matrices.

OBJECTIVES

- Gain more insight into the Phase I and Phase II metabolism of 3-MMC
- Combining suspect screening (suspect list based on literature and *in-silico* software) with (non-) targeted screening approaches to identify 3-MMC metabolites
- Confirm whether *in vitro* results match authentic human samples

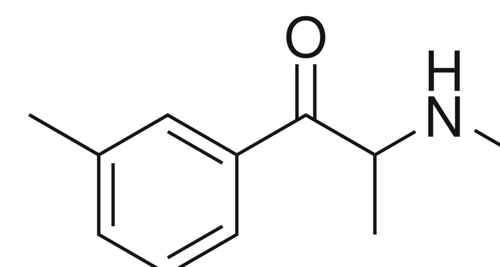

Figure 1: Structure of 3-methylmethcathinone (3-MMC)

METHODS

IN VITRO

HUMAN LIVER MICROSOMES (HLM)

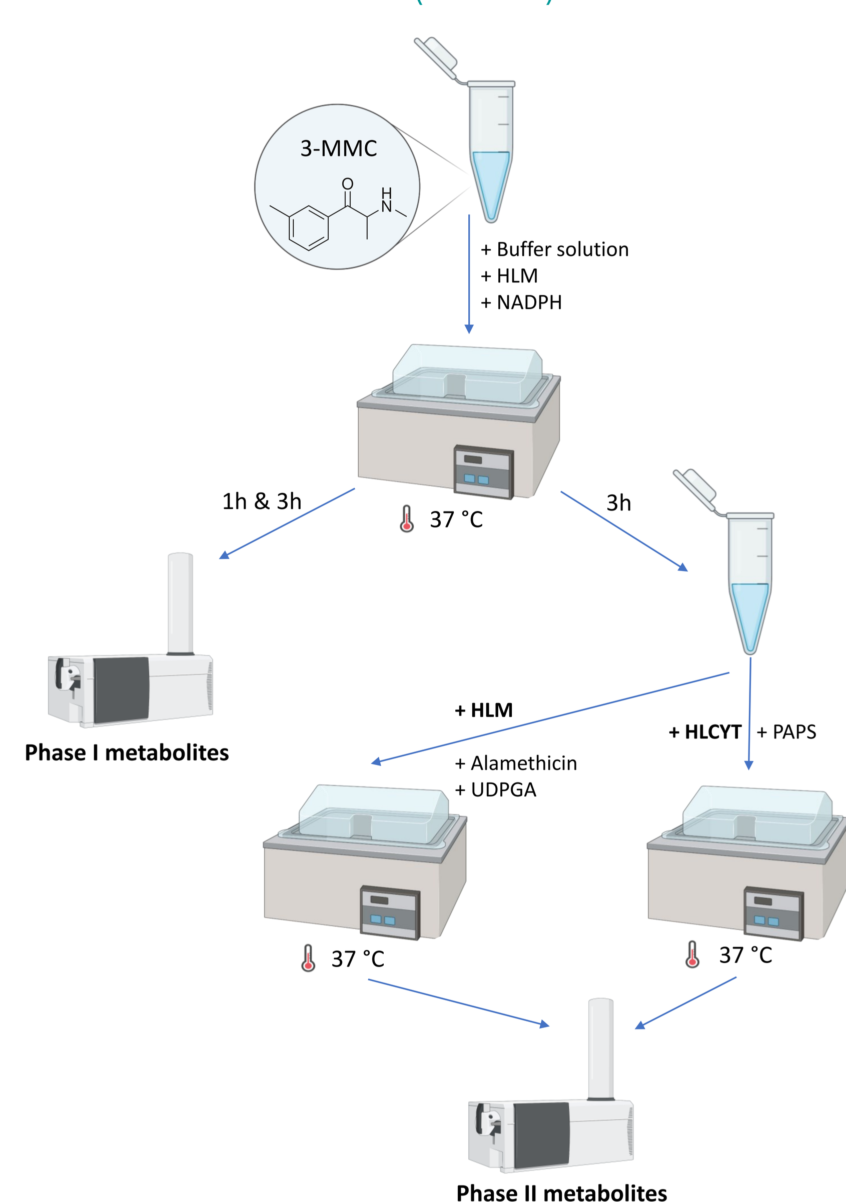

Figure 2: *Protocol for HLM analysis. Phencatin and 4-nitrophenol (4-NP) were used as positive controls for Phase I and Phase II, respectively. UDPGA = Uridine diphosphate glucuronic acid, PAPS = 3'-Phosphoadenosine-5'-phosphosulfate, HLCYT = Human Liver Cytosol*

IN VIVO

HAIR (n = 2)

- Pre-treatment: wash (H_2O, Acetone), grind (5 min, 30 Hz) and weight (20 mg)
- Extraction (0.5 mL : 25% ACN / 25% MeOH / 50% 2 mM AmmForm+ACN (pH 5.3):H2O (8:92, *v/v*), incubation (18h, 750 rpm), filtration (0.2 µm) and centrifugation (10 min, 12 500 rpm)

SALIVA (n = 1)

- Homogenisation (5 min, 750 rpm), filtration (0.2 µm) and centrifugation (10 min, 8000 rpm)

PLASMA (n = 1)

- 'Dilute and shoot' (MeOH, 1:3.5, *v/v*)
- Homogenisation (2.5 min, 2000 rpm), filtration (0.2 µm) and centrifugation (5 min, 10 000 rpm)

URINE (n = 18)

- 'Dilute and shoot' (MeOH, 1:4, *v/v*)
- Deconjugation was not applied
- Homogenisation (2.5 min, 2000 rpm), filtration (0.2 µm) and centrifugation (5 min, 10 000 rpm)

Analysis performed with Agilent 6530 LC-QTOF-MS and Agilent 6495 LC-QQQ-MS/MS

- Column: Phenomenex® Kinetex Biphenyl 100x2.1mm, 2.6 µm
- Mobile phase ESI + : A: H_20 + 0.1% formic acid ; B: MeOH:H_20 (9:1, *v/v*) + 0.1% formic acid
- Mobile phase ESI - : A: H_20:MeOH (98:2, *v/v*) + 5 mM NH_4Ac ; B: MeOH:H_2O (98:2, *v/v*) + 5 mM NH4Ac

RESULTS

Metabolite	Liver (HLM)	Hair	Saliva	Plasma	Urine
Metabolite 1: $C_{11}H_{15}NO_2$, hydroxylated derivate	yes	0/2	0/1	1/1	4/18
Metabolite 2: $C_{11}H_{17}NO$, keto-reduced derivate	yes	0/2	0/1	1/1	10/18
Metabolite 3: $C_{10}H_{13}NO$, N-desmethyl derivate	yes	1/2	0/1	1/1	8/18

- In total three Phase I metabolites were determined as *in vitro* metabolites of 3-MMC based on *in silico* predictions and *in vitro* experiments.
- Additionally, these metabolites were confirmed in real human samples.
- No Phase II metabolites were found.
- Similar metabolites were reported for 4-MMC (*Pozo et al.*, 2014 ; *Pedersen et al.*, 2012)
- 68% of the analysed human samples were positive for at least one of the three metabolites.
- Interestingly, two urine and one plasma sample were positive for all three metabolites.

CONCLUSIONS

- By identifying three metabolites in *in vitro* biotransformation tests and confirming them in real human samples, this research provides new insides into the 3-MMC metabolisation pathways.
- The applicability of using human liver microsomes as an *in vitro* alternative for *in vivo* metabolite screening is illustrated.
- Although *in vitro* results and prediction softwares have proven their usefulness, the importancy of real human matrix screening is demonstrated.

FUTURE PERSPECTIVES

- Increasing the number of analysed human samples is required to obtain a more representative image of the *in vivo* presence of these metabolites.
- Quantification of these proposed metabolites is imperative in the development of analytical detection methods for screening of human matrices.