Metabolite Profiling of Novel Psychoactive Substances in Human Liver Microsomal Incubation by Using LC/MS/MS

Sarah Wolf, B.S.[1], Amanda L.A. Mohr, M.S.F.S.[2], Karen Scott, Ph.D.[1], and Sean Yu, Ph.D.[3]
[1]MSFS Program, Arcadia University, Glenside, PA [2]The Center for Forensic Science Research and Education, Willow Grove, PA [3]RMI Laboratories, North Wales, PA

Introduction

Drug abuse of novel psychoactive substances (NPS) is becoming increasingly popular among young people, particularly within the Electronic Dance Music (EDM) scene. Often, newly popular drugs on the market may be minor modifications of more well-known drugs, allowing them to be developed, marketed, and distributed online while circumventing the law. These designer drugs are rapidly introduced to the market and are quickly modified, and the metabolic pathway of many of these remains largely unknown. This information is essential for detecting prior usage of drugs. Although there is research concerning the metabolism of certain NPS drugs of abuse, it remains a constantly changing market, and new drugs with unstudied metabolic pathways continue to be introduced and popularized as recreational drugs. The intent for this study was to use human liver microsomes to generate metabolites of two NPS compounds (α-PVP and dimethylone, shown in figures 1 and 2) and identify these metabolites by analysis with Ultra Performance Liquid Chromatography/ Quadrupole Time of Flight Mass Spectrometry (UPLC/Q-TOF).

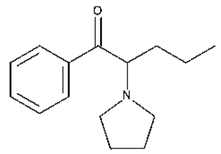

Figure 1. Structure of α-PVP

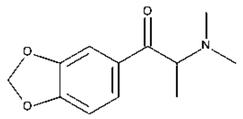

Figure 2. Structure of Dimethylone

Alpha -pyrrolidinovalerophenone, or α-PVP, is a stimulant related to methylenedioxypyrovalerone (MDPV) that has become recently available on illicit drug markets. Users have reported effects including stimulatory effects, euphoria, increased heart rate, decreased focus, and nausea, with hallucinations being associated only with very high doses. Alpha-PVP has been federally regulated as a Schedule I compound.

Beta-keto-3,4-methylenedioxydimethamphetamine (bk-MDDMA) is more commonly referred to as dimethylone, and is structurally similar to the more commonly abused methylone (the β-keto derivative of MDMA), differing only by a second methyl group on the amine. Dimethylone remains unscheduled, although its positional isomer butylone was scheduled as schedule I alongside α-PVP. Effects reported are similar to those of methylone and include euphoric, empathogenic, and stimulatory effects.

Methods

The method for in vitro metabolism by human liver microsomes was first optimized by incubation with diazepam. The variables optimized were the amount of drug, NADPH, and microsomes, and the time of incubation.

Based on this optimization, each reaction mixture contained:

- 5000 ng of substrate (drug)
- 50 µL of 10 mM NADPH solution
- 25 µL of human liver microsomes (20 mg/mL), and
- 520 µL phosphate buffer (100 mM, pH=7.4, with 10 mM magnesium chloride)

These mixtures were:

- Incubated for two hours at 37°C
- Stopped with 500 µL of acetonitrile
- Vortexed and centrifuged
- Partially dried down to remove excess acetonitrile and
- Filtered before transfer to autosampler vials

Analysis occurred by Waters Acquity UPLC® Iclass Waters Xevo® G2-S QTOF, using Water's Toxicology Screening Method, with data analysis using UNIFI™.

Alpha-PVP Incubation Results

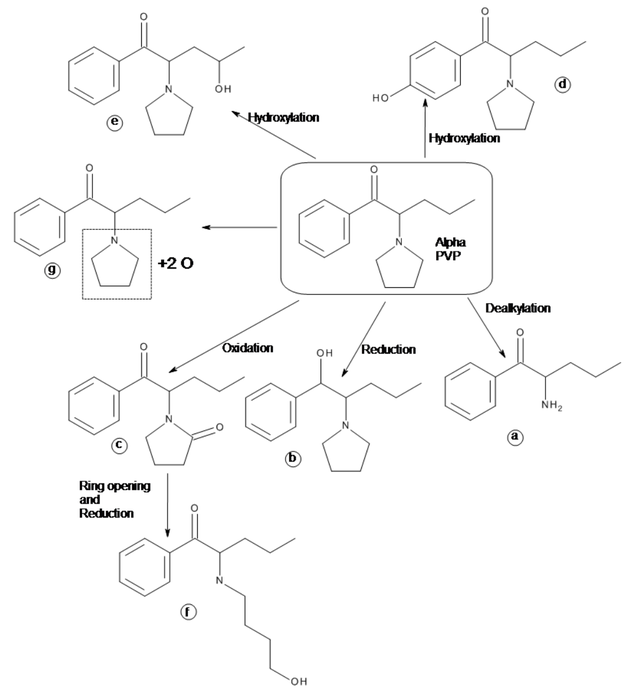

Figure 3. Proposed metabolic profile of α-PVP based on incubations with HLM. Alpha-PVP underwent extensive phase I metabolism, and eight phase I metabolites were identified.

Dimethylone Incubation Results

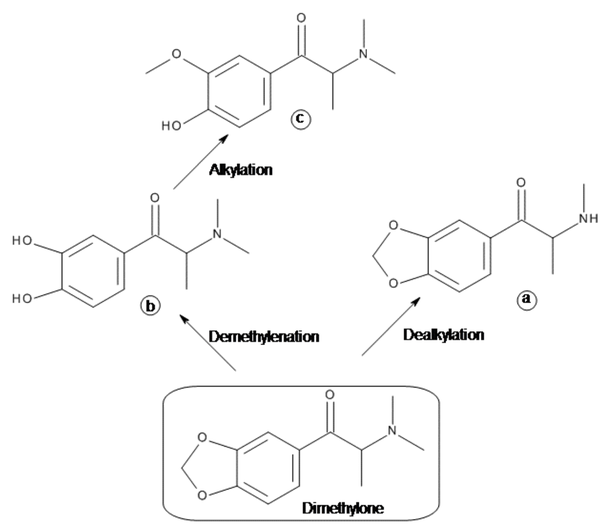

Figure 4. Proposed metabolic profile of Dimethylone based on incubations with HLM. Dimethylone metabolized by similar routes as those previously reported in methylone.

Discussion

Listed in Table 1 are the analytes observed in HLM incubations with α-PVP. One metabolite, whose structure could not be confirmed by its fragmentation, was also observed. Its mass was consistent with the addition of two oxygens to the parent structure, although the pathway leading to this metabolite has not been confirmed.

Table 1. Analytes in Alpha PVP Incubations

Metabolite	Retention time (min)	m/z	Relative Abundance
α-PVP (Parent compound)	4.24	232.1696	--
Amino-α-PVP (3a)	3.25	178.1227	+
5-OH-α-PVP (3b)	4.68	234.1857	+ +
2"-Oxo-α-PVP (3c)	9.60	246.1489	+ + +
OH-Phenyl-α-PVP (3d)	2.84	248.1645	+
OH-Alkyl-α-PVP 1 (3e)	3.35	248.1645	+ +
OH-Alkyl-α-PVP 2 (3e)	3.64	248.1645	+ +
Butylamino,OH-Alkyl-α-PVP (3f)	3.89	250.1805	+ + +
Proposed α-PVP metabolite (3g)	3.92	264.1594	+ + +

Dimethylone incubations were compared to the results from in vitro metabolism of methylone. The metabolites observed in the methylone incubations were not observed in the dimethylone incubations. Table 2 lists all analytes identified in these incubations and Figure 4 shows the proposed metabolic profile of dimethylone.

Table 2. Analytes in Dimethylone Incubations

Metabolite	Retention time (min)	m/z	Relative Abundance
Dimethylone (Parent compound)	2.07	222.1125	--
Methylone (4a)	1.86	208.0968	+ + +
3,4-Dimethoxy-N,N-dimethylcathinone (4b)	0.93	210.1125	+ + +
4-hydroxy-3-methoxy-N,N-dimethylcathinone (4c)	1.91	224.1281	+

Conclusions

The optimized method for in vitro metabolism was successfully used to metabolize α-PVP and dimethylone into metabolites, which were identified by UPLC/Q-TOF.
The metabolic pathways of alpha-PVP were hydroxylation, reduction of the ketone to an alcohol, degradation of the pyrrolidine ring to an amine, and oxidation of the pyrrolidine ring to a lactam followed by ring opening and reduction to the corresponding alcohol.
The observed metabolic pathways of dimethylone were demethylenation followed by methylation, and dealkylation into methylone. However, the metabolic products observed in the methylone incubation (dealkylation, demethylenation, and hydroxylation of methylone) were not seen as products of dimethylone metabolism. The metabolic profile of dimethylone has not been previously reported, and future work includes confirming these metabolites by analysis of authentic urine samples of users.

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