K54 4-Methoxy-A-PVP: *In Silico* Metabolite Prediction, Assessment of Metabolic Stability With Human Liver Microsomes, and Metabolite Identification After Human Hepatocyte Incubation With High Resolution-Mass Spectrometry

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After attending this presentation, attendees will better understand different approaches of investigating metabolic pathways and of selecting potential 4-methoxy-a-PVP metabolites to be targeted in human specimens.

This presentation will impact the forensic science community by demonstrating the applicability of utilizing *in silico* software predictions and *in vitro* metabolism experiments to elucidate the metabolic profiles of these emerging novel psychoactive substances.

Introduction: Synthetic cathinones emerged on the designer drug market as popular "legal" alternatives to illicit drugs during the late 2000s and are marketed as "legal highs" not for human consumption to avoid regulation. These novel psychoactive substances are continuously developed to circumvent legislative and regulatory efforts. As such, limited pharmacological and toxicological information is available. Recently, 4-methoxy-α-PVP was identified for the first time in illegal products purchased in Japan in 2013.[1] No metabolism studies have been performed on this compound. Complete metabolic profiles are needed for these novel psychoactive substances to enable identification of their intake and to link adverse effects and educate the public about the dangers of these "designer drugs."

Objectives: To evaluate *in silico* metabolism predictions, perform metabolic stability assessment with Human Liver Microsomes (HLM), and to identify metabolites after human hepatocyte incubation with High-Resolution Mass Spectrometry (HRMS) for 4-methoxy-α-PVP.

Methods: *In silico* predictions were performed with MetaSite software, which predicts metabolites based on enzyme-substrate recognition and site reactivity of the molecule. To investigate the kinetics of 4-methoxy-α-PVP, 1µM drug was incubated with 50-donor-pooled human liver microsomes at 0, 3, 8, 13, 20, 30, 45, and 60min. For the human hepatocyte experiments, 10µM drug was incubated at 37ºC with pooled cryopreserved human hepatocytes at 0, 1, and 3h based on HLM half-life. Diclofenac also was incubated as a control for human hepatocyte functional viability. Chromatographic separation of HLM samples, diluted 1:100 with mobile phase A (0.1% formic acid in water), was achieved with an Accucore™ C18 column (2.6µm, 100mm x 2.1mm) with mobile phase A and B (0.1% formic acid in acetonitrile) within 20min. Hepatocyte samples were diluted 1:5 with mobile phase A, and separated utilizing a Synergi 4 Hydro-RP column (80A, 150mm x 2mm) within 30min. Data from HLM and human hepatocyte samples were collected with a Thermo Scientific™ QExactive™ high-resolution mass spectrometer, and analyzed by WebMetabase software-assisted data mining. HLM and hepatocyte data were acquired using a high-resolution, full-scan, data-dependent mass spectrometry method. In addition, hepatocytes were acquired with and without an inclusion list of predicted metabolites generated by MetaSite, and with an All-Ion-Fragmentation (AIF) mass spectrometry method to identify potential unexpected metabolites. Scans were thoroughly data mined with different data processing algorithms utilizing WebMetabase.

Results: 4-methoxy-α-PVP exhibited a long half-life of 79.7min in HLM, with an intrinsic clearance of 8.7µL/min x mg. In addition, this compound is predicted to be a low-clearance drug with an estimated human hepatic clearance of 8.2mL/min/kg. Based on the structure of this synthetic cathinone and the results obtained, the following biotransformations were proposed: O-demethylation, aliphatic ring hydroxylation, ketone reduction, iminium formation, pyrrolidine ring hydroxylation followed by dehydrogenation of the corresponding lactam, pyrrolidine ring opening followed by oxidation to carboxylic acid, iminium formation and aliphatic hydroxylation, dihydroxylation, ketone reduction and O-demethylation, and ketone reduction and hydroxylation. The most dominant metabolite in the HLM and human hepatocyte samples was 4-hydroxy-α-PVP, also predicted *in silico*.

Conclusions: These are the first data identifying 4-methoxy-α-PVP metabolites that could document drug intake for forensic and clinical investigations. It is necessary to elucidate the metabolic pathways of these new psychoactive substances to enable linkage to adverse effects and document 4-hydroxy-α-PVP intake. This presentation will impact the forensic science community by demonstrating the applicability of utilizing *in silico* software predictions, and *in vitro* metabolism experiments to elucidate the metabolic profiles of these emerging novel psychoactive substances.

Supported by the National Institutes of Health, Intramural Research Program, NIDA.

Reference:

1. N. Uchiyama et al., *Forensic Toxicology*, 32, 105–115 (2014)

4-Methoxy-A-PVP, In Silico Prediction, In Vitro Metabolism