K55 Identification of Major Metabolites in Human Blood and Urine Associated With the Ingestion of Alpha-Pyrrolidinopentiophenone (Alpha PVP)

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After attending this presentation, attendees will be able to identify the metabolic profile associated with use of alpha-PVP and identify its major metabolites. Attendees will be able to describe the process of using human liver microsomal incubations to verify the identity of novel drug metabolites.

This presentation will impact the forensic science community by providing an example of the use of various laboratory-based analytical and *in vitro* tools for metabolite identification of Novel Psychoactive Substances (NPS).

The purpose of this project was to collect biological samples from volunteers attending Electronic Dance Music (EDM) festival and evaluate samples for emerging NPS. Blood samples screening positive for alpha-PVP were further investigated for the presence of potential metabolites that had been produced *in vitro* using Human Liver Microsomes (HLM).

Alpha-PVP is an emerging novel psychoactive substance of the pyrrolidinophenone family, which has been identified in forensic casework samples over the last three years. Limited data exists on the mechanisms of action of alpha-PVP; however, it is believed to produce similar effects to Methylenedioxypyrovalerone (MDPV), which acts as a norepinephrine-dopamine reuptake inhibitor, producing stimulant-like effects and euphoria. Its metabolic profile has been investigated in a rat model with analysis of the urine by Gas Chromatography/Mass Spectrometry (GC/MS); however, there have been no reports of its metabolism in humans.

The analysis of several urine specimens found to contain alpha-PVP also disclosed the presence of other compounds with similar mass spectral characteristics. This prompted the investigation of the identity of these compounds to verify their origin as alpha-PVP metabolites. Several tools including High Resolution Accurate Mass Spectrometry (HRAMS) on a Liquid Chromatograph/Quadrupole/Time-Of-Flight/Mass Spectrometer (LC/Q/TOF), Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), GC-MS, and comparison of the profile of authentic human urine specimens from drug users with analysis of the products of incubation of the parent compound with a human liver microsome preparation were used to identify the metabolites.

Metabolites of alpha-PVP were produced by incubating a solution of alpha-PVP with pooled HLM. Phosphate buffer (pH 7.4) was spiked with 5,000ng of alpha-PVP. NADPH, a co-factor for the enzymatic reaction, was added to the buffer solution and allowed to incubate for two hours at 37°C. Following filtration, the samples were analyzed using LC/Q/TOF to generate exact mass data. Incubated samples were compared with controls that were incubated without the essential NADPH co-factor to help identify products of metabolism.

Authentic human blood samples from cases in which paired urine samples had tested positive for alpha-PVP were extracted using a basic liquid-liquid extraction using 0.1M borate buffer (pH=10.4) into n-butyl chloride and ethyl acetate. The organic phase was evaporated to dryness and reconstituted in 90:10 5mM ammonium formate and 0.1% formic acid in acetonitrile and analyzed using LC/Q/TOF.

Human blood and urine samples that had screened positive for alpha-PVP by LC/Q/TOF were further examined using extracted ion chromatograms for the exact masses of potential metabolites produced using the HLM incubation procedure described above.

One of the major alpha-PVP metabolite candidates from the HLM experiments had a retention time and accurate mass (246.1645g/mol) equivalent to $C_{15}H_{21}NO_2$, which is consistent with methoxetamine, an unrelated NPS compound with dissociative anesthetic properties; however, the fragment ions in the high energy mass spectra were not consistent with those present in the methoxetamine standard.

The identity of the apparent alpha-PVP metabolite was established as resulting from hydroxylation of the side chain on alpha-PVP (Figure 1). Using structural elucidation tools with the metabolite structure, possible fragmentation patterns were suggested by the software, based upon the ions seen in the high energy mass spectra. Three of the fragments proposed from the software were found in the high energy mass spectra, confirming its identity as a hydroxylated metabolite of alpha–PVP.

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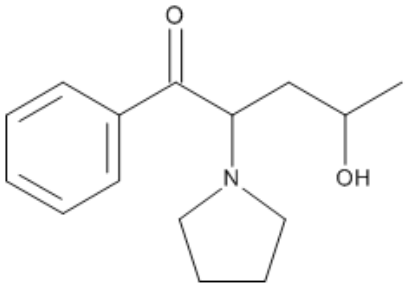

Figure 1

The combined results from analysis of mass spectrometric data, HLM incubations, and analysis of urine and blood from authentic cases of alpha-PVP ingestion allowed identification of several major metabolites in humans, several of which were consistent with results from published rat metabolic profiles; in addition other previously unknown metabolites were identified.

Alpha-PVP, Metabolism, Novel Psychoactive Substances

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