Metabolic Profile Determination of Novel Psychoactive Substances using Human Liver Microsomes

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Novel Psychoactive Substances have been increasingly abused, particularly within the Electronic Dance Music (EDM) scene. With the continued popularity of "designer drugs," the market is constantly changing, requiring continued research, including the metabolic pathways of these newly emerging drugs. The aim of this research is to identify the metabolites of several novel psychoactive substances that are commonly used within the EDM community, by in vitro metabolism with human liver microsomes.

A method for in vitro metabolism using pooled human liver microsomes was first optimized by incubating the microsomes with diazepam. The optimized incubation mixtures contained a phosphate buffer (pH= 7.4), Magnesium Chloride, 50 µL NADPH (10 mM), 5000 ng diazepam, and 25 µL of microsomes (20 mg/ml), with a final volume of 600 µL, and were incubated for two hours at 37°C. Once it was established that the microsomal incubation mixture was effectively producing metabolites as seen in vivo, this optimized method was used to produce the metabolites of the drugs of interest. Analysis occurred by Liquid Chromatography/ Quadrupole Time of Flight Mass Spectrometry (LC/Q-TOF). This allowed for exact mass data to confirm the molecular formula and structure of proposed metabolites. After incubation with alpha-PVP, several previously published metabolites were confirmed in human samples. The metabolic pathways observed were hydroxylation of the side chain or of two locations. Also seen was reduction of the ketone to an alcohol, oxidation of the pyrrolidine ring to a lactam, and degradation of the pyrrolidine ring to a primary amine. These metabolites confirmed previous publications. A previously unreported metabolite was observed resulting from ring opening in the lactam metabolite followed by reduction of the resulting aldehyde into an alcohol.

Work on this project is continuing with investigation of the metabolic profile of dimethylone. Initial investigation has indicated that dimethylone metabolizes primarily into methylone and also into a demethylenated product. The latter of these is a metabolite unique to dimethylone and could be used to indicate dimethylone (as opposed to methylone) use. Work is continuing to confirm these metabolites and investigate any other metabolites produced.