Metabolic Profile Determination of NBOMe Compounds Using Human Liver Microsomes

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After attending this presentation, attendees will be able to assess and review the proposed *in vitro* metabolic profile of members of the hallucinogenic NBOMe series, including 25I-NBOMe, 25H-NBOMe, 25C-NBOMe and 25B-NBOMe.

This presentation will impact the forensic science community by providing information on the identities of unique metabolites for the various NBOMe compounds that can be added to analytical protocols for biological markers of their ingestion and increase the likelihood of detection of their use.

In recent years, a new group of emerging psychoactive substances known as NBOMes have gained popularity with recreational drug users as an alternative to the classical hallucinogen lysergic acid diethylamide (LSD-25). NBOMes are derivatives of a group of substituted phenethylamines known as 2C compounds, which were first pioneered by Alexander Shulgin in the 1980s. These stimulant type drugs act on the 5-HT 2A serotonin receptors resulting in a constellation of psychedelic and hallucinogenic effects. The NBOMe name describes the N-methoxybenzyl substitution for corresponding 2C compounds, such as 25I-NBOMe (2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine) which is derived from 2C-I (2,5-Dimethoxy-4-iodophenethylamine). Other NBOMe substitutions of the various existing 2C compounds, such as 25B-NBOMe, 25H-NBOMe, and 25C-NBOMe, have also been available to recreational drug users by clandestine laboratories making and distributing such compounds as "research chemicals."

Administration of this substance comes in the forms of blotter paper dosages, powders, and liquid formulations. The NBOMe substitution is suggested to increase the potency and activity of these substances relative to the 2C compounds. This is problematic for users of NBOMes who often mistake the drug for LSD because recommended dosages for its desired effects are overestimated and can result in acute toxicity. NBOMes' adverse effects include bouts of violent tendencies, paranoia, episodes of intense psycho-stimulation, and fatal intoxications.

The goal of this project was to identify the main metabolites of various NBOMe compounds using *in vitro* incubation with human liver microsomes and ultimately to confirm these findings in authentic forensic specimens.

Aqueous standards containing the drugs of interest were incubated with human liver microsome preparations (20 mg/mL, 50-individual pool) and a NADPH generating system. The mixtures were incubated for 2 hours at 37°C then stopped with acetonitrile. Aliquots of the reaction mixture were extracted after centrifugation at 10,000 rpm, brief evaporation of the acetonitrile from the supernatant, and final centrifugation at 10,000 rpm with a centrifugal filter. Extracts

were then analyzed by liquid chromatography time-of-flight mass spectrometry using a Waters Acquity UPLC® Iclass Waters Xevo® G2-S QTof. Data analysis was performed using the Waters Forensic Toxicology Application Solution with UNIFI 1.7.

The UPLC separation was accomplished using an Acquity UPLC® BEH C18 column (2.1x150 mm, 1.8 μm) with a column temperature of 50^{o}C and a gradient elution method. Mobile phase A was 5 mM ammonium formation, pH 3, and mobile phase B was 0.1% formic acid in acetonitrile. The MS data was obtained using electrospray ionization in positive ion mode, with a scan range of 50-1000 m/z. Data analysis was done using UNIFI.

The primary metabolite identified for 25I-NBOMe following this incubation method was its N-demethoxybenzylated form, also known as 2C-I. Other phase I metabolites of 25I-NBOMe yielded O-demethylated and several demethoxylated isomers of the parent compound. 25H-NBOMe (or 2-(2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine) showed similar metabolic fate, including identification of its 2C analog, 2C-H (2-(2,5-dimethoxyphenyl)ethanamine), and a hydroxylated form. 25B-NBOMe (2-(4-bromo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine) showed at least 4 unique metabolites including a debrominated O-demethylated hydroxylated form. Other metabolic transformations observed included dehydrogenation, demethylation, and hydroxylation of the parent compound. The primary phase I metabolite changes to the NBOMe class include O-demethylation at the 3 methoxy groups, single and *bis*-hydroxylations, and cleavage at the amine (N-demethoxybenzylation).

In conclusion, successful *in vitro* metabolism of NBOMes with human liver microsomes and subsequent analysis of metabolites using a high mass accuracy method with LC/QTOF provided confident identification for the primary metabolites by CYP enzymes. The results of this project will positively impact the ongoing research and efforts to identify biological markers of use for NBOMes as they gain prevalence among recreational drug users and are seen with increasing prevalence within a forensic setting.