Application of Time-of-Flight/Mass Spectrometry (TOF/MS) With Three Different Fragmentation Modes to the Toxicological Screening of Urine Samples Collected From an Electronic Dance Music (EDM) Population

Helen Piper, BS, 3550 Bartram Road, #31, Willow Grove, PA 19190; Alexander L. Maggitti III, BS, 3701 Welsh Road, Willow Grove,PA 19090; Jared Castellani, BS, 150 Ridge Pike, #108-A, Lafayette Hill, PA 19444; Francis X. Diamond, BS, 3701 Welsh Road, Willow Grove, PA 19090; Matthew M. McMullin, MS, 3701 Welsh Road, Willow Grove, PA 19090; and Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will better understand the pattern of use of Novel Psychoactive Substances (NPS) drugs for a population at risk of abuse of the newest compounds on the illicit market. Attendees will also have a better understanding of current trends describing the compounds that are most prevalent and those emerging on the scene.

This presentation will impact the forensic science community by providing information that can be used for harm reduction, education, and increased certainty of detection following the use of the newest compounds on the market. Manufacturers of standards, law enforcement, emergency medical care professionals, and those involved with user education and drug treatment programs can all utilize this information.

A variety of analytical methodologies including immunoassay (Enzyme-Linked Immuno-Sorbent Assay (ELISA)), Gas Chromatography Mass Spectrometry (GC/MS), and Liquid Chromatography/Mass Spectrometry (LC/MS) screening were applied to samples collected from attendees at an electronic dance music festival where there was a high level of self-reported use of NPS drugs. Samples were additionally analyzed using three different modes of Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS).

The range of NPS drugs is constantly changing and many similar compounds, analogs, and isobaric compounds are being sold and distributed; the ability to distinguish between closely related compounds is critical for forensic toxicology testing. Considering High Resolution Accurate Mass Spectrometry (HRAMS) data of both the parent compound and fragment ions provides more specific structural information that can be used for identification of drugs and their metabolites, eliminating some of the false positives caused by artifacts, minor metabolites, degradation products, drug analogs, and isomers in complex forensic specimens. Incorrect presumptive identifications from a screening method may lead to unnecessary confirmatory testing and/or an excess of candidate compounds requiring thorough manual data evaluation for a simple presumptive screening identification.

An Agilent® 1290 HPLC/6530 Q/TOF mass spectrometer with Jet Stream® Technology was used to analyze samples; LC conditions were kept constant while evaluating three different ionization modes. The modes were conventional Quadrupole Time-of-Flight (Q-TOF) and two All-Ions ionization modes: Collision-Induced Dissociation in the Source (CIDS) and Collision-Induced Dissociation in the Collision Cell (CIDCC). The conventional Q-TOF mode uses targeted MS/MS analysis, while the CIDS and CIDCC All-Ions modes provide fragmentation data through the use of alternating fragmentor voltages in the source or

collision energies in the collision cell, respectively. CIDS mode also allows for the acquisition of Q-TOF-like data on a conventional TOF mass spectrometer. The elution profiles of each of the ions, parents, and fragments are correlated for use in compound identification.

Samples were analyzed against a database/library containing approximately 140 emerging drugs of abuse or NPS. Each entry was complete with molecular formula, providing the accurate mass up to four decimal places, and the retention time; MS/MS spectra was available for some compounds. Presumptive identifications were made based on retention time, mass accuracy, isotope ratios and spacing, abundance thresholds, the presence of fragment ions, and the comparison of ion ratios to the database reference entry.

Of the samples analyzed, the most common compounds identified and confirmed consisted of methylone, alpha-PVP, MDA, MDMA, amphetamine, and ethylone. O-desmethyltramadol has not been confirmed but was commonly found by all three modes and the additional screening methods. Some of the other compounds that have been confirmed in at least one sample were fluoroamphetamine, buprenorphine, butylone, dextromethorphan, methamphetamine, norketamine, and psilocin. Of the more prevalent compounds, ethylone was the most commonly missed compound by all three methods, with the Q-TOF not identifying it in any of the samples for which it was confirmed. MDMA was another relatively common false negative missed mostly by the Q-TOF mode; however, there was no Q-TOF reference spectra for MDMA in the database. Amphetamine and alpha-PVP yielded few false negatives. In addition to the false negatives observed, the Q-TOF mode produced the most false positive findings. Generally, the All Ions methods performed better than the Q-TOF method in terms of false positive and false negative findings.