K36 Fragmentation Pathways and Structural Characterization of Mitragynine and Its Metabolite Using Electrospray Ionization (ESI) and High Resolution Mass Spectrometry

Stephanie Basiliere, BS, 3019 Sam Houston Avenue, Apt G302-2, Huntsville, TX 77340; Sarah Kerrigan, PhD, Sam Houston State University, 1003 Bowers Boulevard, SHSU Box 2525, Huntsville, TX 77341; and Kelsie Bryand, MS, PO Box 2525, Huntsville, TX 77340*

After attending this presentation, attendees will be able to identify common fragmentation pathways associated with Mitragynine (MG), 7-Hydroxymitragynine (OH), and other corynanthe-type alkaloids.

This presentation will impact the forensic science community by highlighting the importance of structural identification during routine method development.

MG (9-methoxycorynantheidine, Kratom) is a naturally occurring corynanthe-type indole alkaloid that is present in the leaves of *Mitragyna speciosa*. This flowering plant of the *Rubiaceae* genus contains more than 20 alkaloids, of which MG is the principal pharmacologically active component. While OH is a minor constituent, it is considerably more potent and is also produced as a metabolite following MG use. Although MG is structurally related to yohimbine, its effects are notably different. Unlike yohimbine, which has pronounced adrenergic and serotonergic effects, MG behaves as a μ-opioid receptor agonist. While not federally regulated in the United States, Kratom's dual stimulant and opiate-like effects are somewhat unique, making it an ideal candidate for misuse among recreational drug users.

Although Gas Chromatography/Mass Spectrometry (GC/MS) is the most widely used technique in forensic toxicology laboratories, identification of MG-OH in biological samples presents a significant challenge in terms of analytical detection. Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) is a high resolution MS technique that offers high sensitivity and significant benefits in terms of mass accuracy and structural identification. Tandem Mass Spectrometry (MS/MS) spectra following optimization of ionization conditions can provide valuable structural information. Characterization of fragmentation pathways and subsequent structural identification of ions should play an important role in new assay development.

During the development of an analytical method to determine MG and MG-OH in urine using ESI/LC/qTOF/MS, the fragmentation pathways for MG, OH, and their deuterated analogs were investigated. MS/MS spectra were used to tentatively identify fragments and make mass assignments. Ultimately, this process plays an important role in the selection of highly specific precursor ion transitions. A total of three transitions were selected for each of the compounds (and their respective internal standards).

The most abundant product ions for both MG and MG-OH were associated with C-ring cleavage and the loss of the substituted piperidine (D-ring) between C2 and C5. The abundance and specificity ultimately led to this being selected for quantitation for both MG (399→174) and MG-OH (415→190). Variations of C-ring cleavage predominated for all other major product ions, as well as formation of intact substituted piperidine ions.

Chromatographic separation and mass spectral acquisition are particularly important analytical variables due to the potentially large number of structurally similar alkaloids and diastereoisomers found in *M. speciosa*. LC/qTOF/MS and other high resolution MS techniques are particularly useful for complex analytes such as these.

Mitragynine, 7-Hydroxymitragynine, Fragmentation

*Presenting Author