K13 The Identification of Five Kratom Alkaloids Using High Resolution Mass Spectrometry

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After attending this presentation, attendees will be able to identify and separate kratom alkaloids using Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS). The influence of mobile phase additives and adduct formation will be discussed and common fragmentation pathways of corynanthe-type alkaloids will be explored.

This presentation will impact the forensic science community by highlighting the importance of mobile phase selection, optimization of ionization conditions, and structural identification of fragment ions using high-resolution MS.

Mitragynine (MG) (9-methoxycorynantheidine, kratom) and 7-hydroxymitragynine (MG-OH) are naturally occurring corynanthe-type indole alkaloids present in the leaves of *Mitragyna speciosa*. This flowering plant of the *Rubiaceae* genus contains more than 20 alkaloids, of which mitragynine is the principal pharmacologically active component, with 7-hydroxymitragynine being a minor psychoactive constituent. Mitragynine and 7-hydroxymitragynine are μ-opioid agonists. Kratom also contains two diastereoisomers of mitragynine (speciociliatine and speciogynine) and paynantheine. Although these three compounds are not known to be psychoactive, their presence in biological specimens may indicate kratom use. Although not yet federally regulated, kratom's dual stimulant and opiate-like effects are somewhat unique, making it an ideal candidate for misuse among recreational drug users.

Separation and identification of MG, MG-OH, Speciociliatine (SC), Speciogynine (SG), and Paynantheine (PY) in biological samples presents a significant analytical challenge. LC/qTOF/MS is a high-resolution MS technique that offers high sensitivity and significant benefits in terms of mass accuracy and structural identification. Mobile phase composition and optimization of the ionization conditions is essential in order to achieve high sensitivity and adequate chromatographic separation. Tandem Mass Spectrometry (MS/MS) spectra can provide valuable structural information. Characterization of fragmentation pathways and identification of ions is important for new assay development.

During the development of an analytical method for MG, MG-OH, PY, SC, and SG in urine, a total of three mobile phase additives were evaluated in deionized water/acetonitrile: 0.1% formic acid; 10mM ammonium formate, and 5mM ammonium acetate. Chromatographic resolution, ionization efficiency, and the formation of adducts were investigated. Fragmentation pathways for MG, MG-OH, PY, SC, and SG were elucidated. MS/MS spectra were used to 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.

The most abundant product ions for all compounds 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 purposes for MG (399→174), MG-OH (415→190), SC (399→174), SG (399→174), and PY (397→174). 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.

Kratom, Fragmentation, LC/qTOF/MS