K61 Identification of Mitragyna Alkaloids and Metabolites as Biomarkers of Kratom Use in Postmortem Urine Samples

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Learning Overview: After attending this presentation, attendees will be familiar with the identification of mitragyna alkaloids in biological samples using Liquid Chromatography/quadrupole/Time Of Flight/Mass Spectrometry (LC/qTOF/MS).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by identifying additional alkaloids of *Mitragyna speciosa* as biomarkers of kratom use. In addition, the merits of enzymatic and chemical hydrolysis of phase II metabolites will be discussed.

Mitragyna speciosa (kratom) is a tree native to Southeast Asia that has been utilized as both a medicine and cultural tradition by the local populace. The plant is known to have dose-dependent effects—in low doses it produces a stimulant effect, and in high doses an opiate effect. Although not federally regulated, kratom's unique effects and unregulated status make it an ideal target for recreational drug use. The plant is known to contain more than 20 corynanthe-type indole alkaloids, of which the primary psychoactive components are Mitragynine (MG) and 7-Hydroxymitragynine (MG-OH). Other prominent compounds of kratom include two distereoisomers of mitragynine (Speciogynine (SG) and Speciociliatine (SC)) and another structurally similar alkaloid, Paynantheine (PY). These three alkaloids are not reported to be psychoactive. In addition, MG's metabolism has been studied and several prominent phase I metabolites have been identified including 16-Carboxymitragynine (16-COOH-MG), 9-O-Demethylmitragynine (9-O-DM-MG), and most notably, MG-OH. Following the administration of kratom, biological samples may contain a mixture of these structurally similar mitragyna alkaloids in addition to their metabolites. This can present a significant challenge in terms of analytical detection.

A previously validated LC/qTOF/MS assay was used for the quantitative and qualitative identification of four kratom alkaloids (MG, MG-OH, SG, and SC) in postmortem urine. A modified LC/qTOF/MS assay was also used to further investigate phase I and phase II metabolites of MG among these kratom users. Chemical and enzymatic hydrolysis of glucuronides and sulfates were evaluated. In the absence of reference standards, phase I metabolites were identified using high-resolution mass spectra and retention time matching. Recombinant cytochrome P450 enzymes were used to generate phase I metabolites *in vitro* for this purpose.

Using unhydrolyzed specimens, parent drug (MG) was identified in all samples tested over a concentration range of 26-1,987ng/mL. Other kratom alkaloids were also detectable in all 16 samples with concentrations ranging from 2–317ng/mL (PY), 59–1,684ng/mL (SG), and 23–3,309ng/mL (SC). Notably, among 12 of the 16 samples, concentrations of speciociliatine, speciogynine (or both alkaloids) exceeded that of MG. MG-OH, which is known to be unstable, was detected in nine samples. 9-O-DM-MG was identified in 12 of the 16 samples, but 16-COOH-MG was identified in only one specimen.

Deconjugation using recombinant enzymes proved more effective than either chemical hydrolysis or enzymatic hydrolysis using traditional glucuronidase and sulfatase preparations. Detectability of the carboxylated metabolite (16-COOH-MG) significantly increased following hydrolysis. However, the abundance of 9-O-DM-MG and MG-OH were only marginally improved by the additional sample preparation step, and stability issues were identified. Overall, deconjugation of phase II metabolites in urine proved to be of little benefit.

Given the increase of kratom usage in the United States and its reported use for the non-medically supervised treatment of opioid abstinence syndrome, identification strategies are needed. In addition to MG and MG-OH, several other alkaloids (SC, PY, SG) can be used to identify kratom use. In addition to these potential biomarkers, 9-O-DM-MG was the most abundant metabolite and was readily detected without deconjugation. However, until an analytical reference standard is available, it is unlikely to be incorporated into routine testing.

Kratom, Urine, Biomarkers

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