



K49 The Quantitation of Mitragynine in Human Whole Blood by Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) and Its Application to the Analysis of Toxicological Samples

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After attending this presentation, attendees will be able to describe the pharmacology and toxicology of the psychoactive drug mitragynine and will be able to implement a quantitative Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for testing mitragynine in biological samples.

This presentation will impact the forensic science community by providing insight into the analysis of the naturally occurring, emerging psychoactive substance mitragynine, the challenges associated with its analysis and interpretation, and the quantitative results from authentic casework with associated case histories.

Mitragynine is an indole alkaloid and the major constituent found in *Mitragyna Speciosa*, a tropical plant of the Rubiaceae family known as “kratom” in Thailand and “biak-biak” or “ketum” in Malaysia. The leaves of the plant contain a variety of alkaloids and are used recreationally for their stimulatory and narcotic analgesic effects during manual labor. Medicinally, kratom is used as an opioid substitute for drug withdrawal or to treat pain and diarrhea. Its use has recently been implicated in deaths following its use; however, there are few reports of quantitative results from these cases. The Center for Disease Control (CDC) has reported that the exposure-related calls about kratom have increased tenfold from 2010 to 2015 and the Drug Enforcement Administration (DEA) includes it on its Drugs of Concern list. Kratom use appears to be increasing in the United States and is considered an emerging public health threat; to be able to better differentiate concentrations associated with adverse effects compared to fatalities, a quantitative method for the toxicological determination of mitragynine was needed. This presentation describes the development and validation of a quantitative analytical method for mitragynine and its application to testing blood samples from forensic investigations.

A liquid-liquid extraction method was developed to recover mitragynine from human whole blood. Blood, 0.5mL, was extracted using borax buffer (0.1M, pH 4.0) and n-butyl chloride/ethyl acetate (70:30). Extracts were evaporated to dryness and reconstituted in mobile phase for analysis by LC/MS/MS. Analysis was performed using a UPLC-Tandem Quadrupole Detection (TQD) in positive electrospray ionization mode utilizing a gradient of ammonium formate (pH 4.0) and acetonitrile (10:90) isocratically on a silica column. D₃-Mitragynine was used as internal standard. Validation of the method followed the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines and included assessment of precision, accuracy, limits of detection (LOD) and Quantitation (LOQ), recovery, ion suppression, interference, and stability. The method has an LOD of 0.16ng/mL, LOQ of 5.0ng/mL, and Upper Limit of Quantitation (ULOQ) of 500ng/mL. Precision of the controls revealed a maximum % CV of 4.9% and accuracy was limited to a 5.4% difference.

Samples from forensic investigation cases were analyzed using the validated method. The method successfully resolved mitragynine from three other inactive alkaloids (speciogyne, mitracillatine, and speciocillantine) present in the kratom plant and detected in authentic toxicological samples.



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The method successfully passed validation, and 19 cases submitted for forensic toxicology analysis that had qualitatively screened positive by Liquid Chromatography/Time Of Flight/Mass Spectrometry (LC/TOF/MS) using an independently validated method were analyzed using the procedure as described. Mitragynine was not confirmed in four cases, which could be attributed to stability. Of the 15 remaining cases, mean and median mitragynine concentrations were 59ng/mL and 25ng/mL, respectively, with a range of 10ng/mL to 220ng/mL; these values are consistent with concentrations of mitragynine reported in fatalities, which ranged from 20ng/mL to 600 ng/mL.

Kratom, Mitragynine, UPLC-MS/MS