K41 FAST Analysis of 6-Monoacetyl Morphine (6-MAM) and Acetylcodeine (AC) in Urine of Opiate-Positive Drugs and Driving Cases

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After attending this presentation, attendees will understand a novel yet efficient method for extracting 6-MAM and AC from opiate-positive urine employing commercially available Solid Phase Extraction (SPE) cartridges and analyzing them by Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS). Metabolism of heroin (diacetylmorphine) is known to proceed via the well-recognized de-acetylation route to 6-MAM, from which morphine and the respective glucuronides (3 and 6 morphine glucuronides) are formed. Heroin use rather than opiate use/misuse may be differentiated by the confirmation of 6-MAM and/or AC in such matrices as urine. With this minimal sample preparation procedure, both 6-MAM and AC can be confirmed in opiate-positive urines by LC/MS/MS for confirmation of heroin use as a novel and efficient alternative to GC/MS.

This presentation will impact the forensic science community by offering analysts operating in forensic facilities information regarding the extraction and analysis of 6-MAM and AC in urine samples obtained in drugs and driving cases using LC/MS/MS. These drugs are used as confirmatory biomarkers for heroin being used by subjects within a short time frame (i.e., within an hour of administration). This information will allow analysts to differentiate opiate use (morphine, codeine) from heroin administration and offer submitting agencies more appropriate interpretation when only urine is the matrix of analysis.

Method: 0.5mL samples of urine (calibrators, controls, and test samples each containing deuterated internal standards) were diluted with 0.5 mL of diluent solution (50% aqueous methanol), vortex mixed, and centrifuged. The supernatant liquid was applied to a mixed mode ion exchange column (3mL, 200mg) and positive pressure was applied (80psi, flowrate 1mL/minute). The eluates were collected in autosampler vials (2mL) for LC/MS/MS. LC was performed in gradient mode employing a 50mm x 2.1mm (3µm) aromatic phase LC column using mobile phase consisting of acetonitrile and 0.1% aqueous formic acid at a flowrate of 0.5mL/minute.

MS/MS was performed in positive multiple reaction (Multiple Reaction Monitoring (MRM)) mode. The following transitions were monitored (quantification transition ions underlined): 6 MAM (328.1 to 165.1 and 211.1), 6-MAM-d_6 (334.1 to 165.1 and 216.1), AC (342.1 to 225.1 and 165.1), AC-d_3 (345.1 to 228.1 and 165.1), respectively In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis of 6-MAM and AC from 20 completed drugs and driving cases.

Results: The limits of detection/quantification for this method were determined to be 0.5ng/mL and 1ng/mL, for 6-MAM and AC, respectively. The method was found to be linear from 1ng/mL to 1,000ng/mL (r^2>0.999). The analyte recoveries were found to be greater than 95% for all of the noted compounds. Inter-day and intra-day variation of the method were found to <8% and <10 %, respectively. Matrix effects were determined to be <6%. Details regarding the concentrations of 6-MAM and AC found in 20 genuine urine cases are presented.

Conclusion: This method demonstrates the efficient use of non-conventional SPE coupled with the use of LC/MS/MS for the analysis of 6-MAM and AC in cases of driving under the influence of drugs. The ability to analyze and confirm these compounds rapidly (i.e., extraction <1 minute per sample and analysis <5 minutes) in drugs and driving cases will clearly assist toxicologists in differentiating between opiates cases involving regular opiates (e.g., morphine, codeine) and those that involve heroin, thus offering the appropriate interpretation to the respective submitting agencies.

6-MAM, Acetlcodeine, LC/MS/MS