



K6 The Results of Analysis of 6-Monoacetyl Morphine (6-MAM) in Operating Under the Influence (OUI) Drug and Driving Cases and Drug-Facilitated Crimes (DFC) in the Commonwealth of Massachusetts During a Five-Year Period

*Jeffery Hackett, PhD**, 89 Finch Lane, Dovecot, Liverpool L14 9PY, UNITED KINGDOM; and *Albert A. Elian, MS**, Massachusetts State Police Crime Laboratory, 59 Horsepond Road, Sudbury, MA 01776

After attending this presentation, attendees will better understand the extraction and analysis method for 6-MAM from opiate-positive urine cases involving both drug/driving and drug-facilitated cases employing commercially available Solid Phase Extraction (SPE) cartridges, then analyzing the samples by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

This presentation will impact the forensic science community by offering analysts working in forensic facilities information regarding the extraction and analysis of 6-MAM in urine samples obtained in OUI cases and comparing the results with those obtained from DFC cases using SPE and LC/MS/MS. This compound is used as a confirmatory biomarker for recent heroin (diacetyl morphine) use (i.e., within one hour of administration). It is known that heroin is quickly metabolized in the human body to morphine and its glucuronides via the production of 6-MAM. The forensic toxicology community recognizes that 6-MAM may not be detected in whole blood samples, while it is able to be confirmed in urine samples in the same case. The information presented will allow analysts to differentiate opiate use (morphine, codeine) from heroin administration in populations of OUI and DFC cases.

Method: Prior to analysis, samples of patient urine (>10mL) were collected at local hospitals by medical staff using forensically sealable containers. The samples were transferred under chain of custody via police agencies to the laboratory where they were stored in unpreserved conditions at 4°C. For analysis, 1.0 mL samples of urine (calibrators, controls, and test samples each containing deuterated internal standard (6-MAM-d₃)) were diluted with pH 6 buffer. The samples were each applied to conditioned mixed mode SPE columns. Each SPE column was washed with DI H₂O, aqueous acetic acid, and methanol, then dried. Each SPE column was eluted with of a solution consisting of methylene chloride-isopropanol-ammonium hydroxide (78-20-2) and the eluates were dried under a gentle stream of nitrogen. The dried residues were dissolved in 100µL of mobile phase for analysis by LC-MS/MS. Tandem mass spectrometry was performed in positive multiple reaction monitoring mode (MRM). Liquid chromatography was performed using a polyaromatic column in gradient mode with a mobile phase consisting of acetonitrile and 0.1% aqueous formic acid at a flowrate of 0.55mL/minute. The following MRM transitions were monitored (quantification transition ions underlined): 6-MAM (328.1 to 165.1 and 211.1), 6-MAM-d₃ (331.2 to 165.0 and 211.3), respectively. In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis of 6-MAM in both OUI and DFC cases.

Results: The limits of detection/quantification for this method were determined to be 0.5ng/mL and 1.0ng/mL for 6-MAM. The method was found to be linear from 1.0ng/mL to 1,000ng/mL ($r^2 > 0.999$) for 6-MAM. The analyte recoveries were found to be >95% for 6-MAM and 6-MAM-d₃. Interday/Intraday variation of the method was found to be <8% and <10%, respectively. Matrix effects were determined to be <6%. Details regarding the concentrations of 6-MAM found in five years' worth of genuine OUI cases ranged from: 178 Males: mean age=42 years (45ng/mL-365ng/mL: median=275ng/mL); 82 Females: mean age=29 years (23ng/mL-137ng/mL: median=82ng/mL). With respect to DFC cases, 31 Males: mean age=35 years (11ng/mL-93ng/mL: median=45ng/mL); 130 Females: mean age-24 years (5ng/mL-67ng/mL: median=18ng/mL).

Copyright 2017 by the AAFS. Unless stated otherwise, noncommercial *photocopying* of editorial published in this periodical is permitted by AAFS. Permission to reprint, publish, or otherwise reproduce such material in any form other than photocopying must be obtained by AAFS.



Toxicology - 2017

Conclusion: The data obtained in this study compares the concentrations of the biomarker of heroin use (6-MAM) found in the urine of opiate positive cases obtained from two populations of samples often presented to forensic toxicology laboratories. The information obtained from the analysis of these antemortem samples (in terms of 6-MAM concentrations) can offer analysts involved with OUI and DFC cases valuable information regarding the previous administration of heroin (diacetylmorphine) by the subjects vs regular opiates (morphine/codeine). It is accepted by the forensic toxicology community that the presence of 6-MAM is an indicator of heroin being used by subjects within a short period of time of administration. This information enables the toxicology analysts to offer submitting agencies more appropriate interpretation regarding the ingestion of heroin.

Heroin, LC/MS/MS, SPE