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Objective: As of October 2010, new guidelines established by the Department of Health and Human Services (HHS) concerning the analysis of 6-acetylmorphine (6-AM) became effective. A unique biomarker indicative of heroin use, 6-AM is typically present at approximately 1-3% of the concentration of total morphine after administration of heroin. In the past, 6-AM was confirmed when an initial screening for morphine yielded a result >2000 ng/mL. However, due to concerns of false negative results for heroin use and to improve laboratory processing, the new guidelines allow for initial testing for 6-AM specifically. At the request of the Office of Drug & Alcohol Policy & Compliance, Department of Transportation and with the concurrence of the Substance Abuse and Mental Health Administration, Department of Health and Human Services from November 2010 to January 2011 the National Laboratory Certification Program (NLCP) was presented with 44 urine samples from federally regulated workplace drug testing (WPDT) labs that tested positive for 6-AM and screened negative for morphine/codeine. Upon confirmatory testing by the labs, many of the samples contained morphine below the detection levels of the labs. These samples were released by the Department of Transportation and sent to RTI International for further testing by the NLCP. The goal of this research was to investigate these atypical results and to determine if other drugs or metabolites present in the samples could explain the lack of morphine present, or if the source of the 6-AM could have been something other than heroin.

Methods: The samples were analyzed by three separate LC/MS/MS techniques 1) a dilute and shoot method for 6-AM, codeine, morphine, morphine-3- β -D-glucuronide, morphine-6- β -D-glucuronide, and codeine-6- β -D-glucuronide 2) an acid hydrolysis with solid phase extraction method for total morphine and codeine and 3) a solid phase extraction without hydrolysis for 6-AM. For all methods, deuterated internal standards for the appropriate analytes were added to the samples prior to analysis. Samples analyzed for total morphine and codeine were subjected to acid hydrolysis at 120 °C prior to solid phase extraction. Extractions were performed on each sample (volume permitting) by two different analysts. All samples received at RTI were analyzed on an Agilent quadrupole Technologies (Santa Clara, CA) 1200 Series liquid chromatography system coupled to a 6410 triple mass spectrometer (QQQ), operated in positive ESI mode. The samples from both the dilute and shoot and extraction methods were also screened on an Agilent 1200 Series liquid chromatography system coupled to an Agilent 6230 time-of-flight (TOF) mass spectrometer. A set of 18 other opioid and opioid-like drug standards were analyzed by the TOF system, and this data was used to screen the urine samples for the presence of these other drugs.

Results: The presence of 6-AM was re-confirmed in all samples by both the dilute and shoot and extraction methods. Of the 44 samples received at RTI, 9 were blind QC samples expected to contain only 6-AM. Of the 35 remaining donor specimens analyzed with the "dilute and shoot" method, 30 had detectable morphine or codeine (free or conjugated) present at concentrations greater than 5 ng/mL. After hydrolysis and extraction, detectable amounts of free morphine were found in all samples as would be expected from converting the 6-AM known to be present in the samples. TOF screening results for some specimens indicated the potential presence of other opioid compounds, but appeared to be impacted by matrix effects.

Conclusions: Since all samples had 6-AM reconfirmed by an analytical method utilizing mild conditions (dilute and shoot), the observed 6-AM in the samples is not the result of an analytical artifact. The TOF screening results were more equivocal for the presence of other compounds and were not as useful. Differences in variability in 6-AM results between the dilute and shoot and the extracted samples, suggest that matrix effects may limit the utility of dilute and shoot methods for opiates and 6-AM analysis.

Keywords: 6-acetylmorphine, workplace drug testing, NLCP