



K5 Validation of Opiate Detection and Quantification in Human Urine Using Liquid Chromatography and Tandem Mass Spectrometry (LC/MS/MS)

Chelsy L. Wingate, BS, 1517 Hawk Tree Drive, College Station, TX 77845*

After attending this presentation, attendees will have assessed the validity of a new method used in the detection and quantification of opiates in urine specimens using LC/MS/MS.

This presentation will impact the forensic community by demonstrating the development of a highly sensitive method for detection and quantification of opiates that provides rapid results, which can be utilized in both clinical and forensic toxicological settings.

The abuse of prescription pain medication has increased dramatically over recent years. Opiates, which have a high potential for addiction, are among several classes of drugs commonly used in the treatment of chronic pain. With the growing amount of opiates being used to treat pain, it is important for physicians to have the ability to monitor patient prescription use to determine if abuse has occurred. A new highly sensitive method has been developed that detects the presence of opiates in human urine specimens using liquid chromatography and tandem mass spectrometry (LC/MS/MS). This method can analyze a large quantity of samples in a short period of time due to simple sample preparation and online extraction. Alternative methods such as Gas Chromatography/Mass Spectrometry (GC/MS) require longer sample preparation time given that the sample must be extracted from the biological matrix before analysis can occur. The liquid chromatography instruments used to perform this study are multiplex systems having two to four injection ports (Thermo Scientific Aria TLX2 and TLX4) coupled with a triple quadrupole mass spectrometer (TSQ Quantum Access). This multiplex system allows for analysis of a much larger number of samples than standard LC/MS/MS systems and the combination of the LC system with tandem mass spectrometry eliminates the need for derivatization, also decreasing analysis time.

In order to report toxicological results, it is crucial that the method utilized can provide reliable, reproducible results. A validation study was performed to assess the ability of this method to detect and quantify opiates accurately in urine specimens. The opiates analyzed include morphine, oxycodone, hydromorphone, codeine, oxycodone, and hydrocodone. These six analytes are the most common opiates used in prescription pain medication. The validation parameters evaluated in this study consist of accuracy, inter and intra-assay precision, linearity, carryover, lower and upper limit of quantification, limit of detection, and specificity.

The linearity or calibration model contained ten calibrators ranging from 50ng/ml to 50,000 ng/ml and all analytes produced an R^2 value above 0.99. The precision and accuracy was performed by analyzing five replicates at three concentrations. The precision study was performed over a three day period on two different instruments. The % CV was calculated for each day and was not to exceed 10%. All of the analytes passed this criterion except for morphine on days one and two with the lowest concentration having a CV of 13.7% and 11.94% as well as hydrocodone on day one with a CV of 11.08% for the highest concentration. Accuracy calculated based on the value determined by analysis and the true value of each analyte. All of the analytes were within 10% of the target except for oxycodone on day one for all three concentrations and oxycodone on day two for the middle concentration with a percent accuracy of 112%. Oxycodone is the least stable of the six opiates.

The lower limit of quantification for all six analytes was determined to be 100 ng/ml where as the limit of detection was determined to be 50 ng/ml. The upper limit of quantitation was found to be 50,000 ng/ml for all of the analytes and minute carryover was observed from extremely high concentrations of the analytes. Several different drugs such as acetaminophen, 6-acetylmorphine, methadone, buprenorphine, norbuprenorphine, morphine-3-glucuronide, oxazepam, lorazepam, alprazolam, α -hydroxyalprazolam, diazepam, and nordiazepam were analyzed for interference purposes. It was determined that 6-acetylmorphine gave positive results for morphine. These results are most likely due to the acid hydrolysis step required for deconjugation. To avoid conversion from 6-acetylmorphine to morphine, it may be advantageous to use an enzyme such as beta glucuronidase for hydrolysis rather than an acid. The results from this study indicate that this LC/MS/MS method can provide reliable, reproducible results for the detection and quantification of opiates in a short period of time.

Liquid Chromatography Coupled With Tandem Mass SPE, Method Validation, Opiates