K32 Quantification of Buprenorphine and Norbuprenorphine in Postmortem Blood and Urine by Ultra High-Performance Liquid Chromatography/Tandem Mass Spectrometry (UHPLC/MS/MS)

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After attending this presentation, attendees will gain insight into a highly sensitive UHPLC/MS/MS approach for the quantifications of buprenorphine and norbuprenorphine in blood and urine specimens that have been prepared by a liquid-liquid extraction process.

This presentation will impact the forensic science community by describing how the development and validation of the UHPLC/MS/MS method will improve forensic laboratories' abilities in the quantification of buprenorphine and norbuprenorphine in postmortem specimens.

Buprenorphine is prescribed for patients in heroin treatment programs in Taiwan. It is also used for treating moderate to severe chronic pain. In humans, buprenorphine is metabolized to norbuprenorphine by N-dealkylation. The purpose of this study was to develop an effective UHPLC/MS/MS-based methodology that is simple, accurate, and sensitive for the quantification of buprenorphine and norbuprenorphine in blood and urine at low concentration levels.

Blood or urine (1mL) were mixed with sodium carbonate/bicarbonate buffer (pH=9.5) and extracted with ethyl acetate. Extracts were evaporated and reconstituted in the mobile phase (initial gradient composition) for injection onto the UHPLC/MS/MS system. Deuterated analogues of the analytes were used as internal standards. Chromatographic separation was achieved using an Agilent® ZORBAX® SB-Aq (100mm ´ 2.1mm i.d., 1.8-µm particle) analytical column at 50oC. The mobile phase included 0.1% formic acid (v/v) in water (A) and methanol (B), with a flow rate of 0.32mL/min. The initial gradient composition (A/B 90:10, v/v) was held for 1.5min; decreased to 0% A in 10min and held for 2min, then increased to 90% A in 1min and held for 2min. Parameters for mass spectrometric analysis included: (1) Agilent®Jet Stream technology electrospray ionization in positive-ion Multiple Reaction Monitoring (MRM) mode; (2) optimized collision energy levels for selected precursor ions; and, (3) monitoring two or three transitions for analytes and internal standards.

Method validation was performed using drug-free blood and urine that were fortified with 1ng/mL-20ng/mL of the analytes. The following analytical parameters were obtained: (1) average extraction recovery, derived from four different sources of blood and urine, was higher than 60%; (2) matrix effect (ion enhancement) was observed, except for urine samples at the 10ng/mL and 20ng/mL concentration levels, but was adequately compensated for by respective deuterated internal standards; (3) intra-/inter-day precision (%CV) and accuracy ranges for blood were 0.45%-8.6% / 1.7%-10% and 95%-108% / 97%-105%, while the corresponding ranges for urine were 0.49%-4.1% / 2.0%-7.9% and 96%-107% / 94%-113%; and, (4) calibration linearity (r^2) for both analytes were >0.997; the limits of detection and quantification for buprenorphine and norbuprenorphine were 0.01ng/mL and 0.025ng/mL (urine) and 0.075ng/mL and 0.075ng/mL (blood), respectively. When applied to case samples, postmortem urine specimens were first hydrolyzed by an enzymatic method prior to the extraction step. In conclusion, this relatively simple protocol was found to be effective and reliable for routine identification and quantification of buprenorphine and norbuprenorphine in blood and urine. This method was successfully applied to the analysis of postmortem and antemortem specimens from forensic cases.

Buprenorphine, Postmortem, UHPLC/MS/MS

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