K3 Validation of Liver Oxymorphone Analysis Using LC/MS/MS: Comparison With Associated Blood Concentrations in Fatal Intoxications

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The goal of this presentation is to present a validated method for the detection and quantification of oxymorphone in liver tissue.

This presentation will impact the forensic science community by demonstrating an assay which provides reproducible quantification of oxymorphone in liver tissue. The assay has application in forensic and postmortem toxicology laboratories.

Oxymorphone, a semi-synthetic opioid analgesic derived from thebaine, is a μ-opioid receptor agonist and indicated for the relief of moderate to severe pain. It is also an active metabolite of oxycodone and has about six to eight times the analgesic potency of morphine. With the introduction of oral formulations in 2006, oxymorphone has become widely abused. The West Virginia Office of the Chief Medical Examiner has seen a dramatic increase in incidence of accidental deaths related to oxymorphone. Most quantification methods focus on the analysis of oxymorphone in blood or urine. The objective of this study was to develop and validate a reliable method for the quantification in a common supplemental specimen, liver tissue.

Oxymorphone standards ranging from 0.5 – 500 μg /kg and four control samples ranging from 6 – 300 μg /kg were prepared in drug and ethanol negative liver homogenate. Internal standard, d3-oxymorphone, was added prior to extraction. Solid phase extraction utilizing Trace-B columns was employed to process the calibrators and controls. Dried eluents were reconstituted in 100 μL of a mixture of water/acetonitrile/formic acid (99:1:0.1) and transferred to high recovery autosampler vials for LC-MS/MS analysis. All chromatography was performed using a ultra-performance liquid chromatography (UPLC) system with separation achieved on an UPLC HSS T3 2.1 x 100 mm (1.8 μm) column. For each analysis, column temperature was maintained at 40°C. All chromatographic runs were performed using linear gradients where mobile phase A was water with 0.1% formic acid and mobile phase B consisted of acetonitrile with 0.1% formic acid. Initial conditions of mobile phase A/B (99:1) were maintained for 0.5 minutes. Mobile phase B was then increased to 30% over 1.5 minutes. It was then increased to 100% over 1.0 minute and maintained for 1.0 minute. The system was then returned to initial starting conditions and held for 0.9 minutes until the subsequent injection. Total run time for each injection was 5.0 minutes. Tandem MS analysis was carried out using a TQ Detector with ionization in electrospray positive mode. Oxymorphone and d3-oxymorphone were analyzed using multiple reaction monitoring (MRM) with argon as the collision gas. The source temperature was set at 150°C and desolvation temperature was 400°C. Desolvation gas was maintained at 800 L/h and cone gas was set to 11 L/h. One quantification and two target transitions were monitored for both oxymorphone (302->284, 302->227, 302->242) and the deuterated internal standard (305->230, 305->245, 305->287).

As part of the validation, studies to determine potential interference, ion suppression/enhancement and carryover were conducted. The calibration model, limit of detection, lower limit of quantitation, linear range, precision and accuracy were established from calibrators and controls prepared and analyzed on five different days with four replicates each day. The linear range was shown to be 5 to 500 μg/kg for oxymorphone in liver homogenate. The limit of detection (LOD) and lower limit of quantitation (LLOQ) were determined to be 5 μg/kg.

Liver specimens from thirty-three cases were analyzed using this validated method. For each, 3.0 g of water was added to 1.0 g of liver tissue. Samples were homogenized, extracted and analyzed. The oxymorphone concentrations ranged from 39 to 1740 μg/kg for liver and 5 to 546 μg/L for blood. A comparison of blood and liver oxymorphone data in fatal intoxications will also be presented.

Oxymorphone, Validation, Liver

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