K77 Optimization of Extraction Parameters Using IMCSzyme™ ß-Glucuronidase for Opiate Analysis of Various Toxicological Matrices

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After attending this presentation, attendees will be able to evaluate the optimal parameters of IMCSzyme™ ß-glucuronidase and its use for opiate analysis in whole blood. Attendees will also be able to evaluate the use of those parameters in the analysis of opiates in various toxicological matrices (Antemortem (AM) blood and Postmortem (PM) blood, urine, and liver.)

This presentation will impact the forensic science community by offering analysts information on an enzyme that could shorten the analysis required for total opiate analysis.

Samples of whole porcine blood were spiked with various concentrations, representing the lower and upper ends of the calibration standards used, with morphine-3β-glucuronide, morphine-6β-glucuronide, codeine-6β-glucuronide, hydromorphone-3β-glucuronide, and oxymorphone-3β-glucuronide. Samples (1mL) were hydrolyzed at 55°C using a 1:1 mixture of IMCSzyme™ β-glucuronidase:pH 6.8 phosphate buffer. The enzyme volume was optimized by examining the recovery using 100µL or 200µL IMCSzyme™ β-glucuronidase (52,000 U stock). The hydrolysis time was also optimized by testing 30-minute or 1-hour hydrolysis time with the different enzyme amounts. After hydrolysis was complete, deuterated internal standard (100µL) was added to each sample and standard. Samples were then sonicated, centrifuged, and the supernatant was derivitized using 100µL of 5% methoxylamine and cleaned up via solid phase extraction. Samples were then dried, derivitized again using 100µL of a 1:1 mixture of propionic anhydride and pyridine, then analyzed via gas chromatography/mass spectrometer with selective ion monitoring.

Once optimal parameters were established, those parameters were used to analyze the IMCSzyme™ β-glucuronidase efficiency in various toxicological matrices commonly tested for opiates. All matrices were tested to obtain results for the free opiate content and the total opiate content using IMCSzyme™ β-glucuronidase and the currently used *H. pomatia* β-glucuronidase.

It was determined that 100µL of IMCSzyme™ β-glucuronidase with 30-minute hydrolysis time produced sufficient hydrolysis in the spiked porcine samples. The enzyme produced comparable or better hydrolysis efficiency with all five glucuronide forms studied than the currently used *H. pomatia* β-glucuronidase in the initial optimization experiments done on porcine blood. Based on the matrix studies, the IMCSzyme™ β-glucuronidase returned comparable or better hydrolysis efficiency in AM blood, PM blood, and urine than the *H. pomatia* β-glucuronidase; however, IMCSzyme™ β-glucuronidase appeared to underperform in liver samples, with codeine-6β-glucuronide proving to be the most difficult glucuronide to hydrolyze in all matrices.

Based on manufacturer recommendations, a 2:3 ratio of IMCSzyme™ β-glucuronidase: pH 7.4 phosphate buffer, with 30-minute hydrolysis time at 55°C was used for hydrolysis in urine to increase overall extraction efficiency, especially for codeine-6β-glucuronide.

Future work will be conducted to attempt to increase hydrolysis efficiency in liver samples. Due to the consistency of the sample matrix, longer hydrolysis times may be tested to allow the enzyme more time to effectively hydrolyze the opiates. Other tests will consist of sonicating the samples prior to hydrolysis or agitating the samples during the hydrolysis step.

Toxicology, Opiate, Glucuronide