A summary of “Buprenorphine Hydrolysis Using a Novel Recombinant \( \beta \)-glucuronidase for Urine Drug Testing”

Overview:
Ameritox, recognizing that enzyme hydrolysis can be costly and time-consuming, tested IMCSzyme® as an alternative \( \beta \)-glucuronidase enzyme to be used for rapid hydrolysis in buprenorphine urinalysis. Ameritox discovered the superior potential of IMCSzyme’s rapid hydrolysis, which will decrease incubation time and as a whole, shorten the lab’s processing times.

Material and Methods:
All drug standards were purchased from Cerilliant Corporation. \( \beta \)-Glucuronidase enzymes were from Integrated Micro-Chromatography Systems, INC (IMCSzyme). Randomly selected authentic buprenorphine urine specimens, that were previously confirmed positive for buprenorphine glucuronide and/or norbuprenorphine glucuronide using a method monitoring the intact glucuronides, were analyzed. Specimens were hydrolyzed with IMCSzyme for 15, 30, 45, and 60 minutes at 55 °C and 65 °C. Hydrolyzed samples were centrifuged and then injected onto the instrument without any further sample preparation. Analysis was performed on Waters Acquity TQD UPLC/MS/MS.

Results:
Mean hydrolysis percentage of specimens by IMCSzyme is shown in Figure 1. Recovery of norbuprenorphine from the glucuronide control improved with heat activation and longer incubation times. A complete hydrolysis was achieved at 65 °C in 30 minutes, as opposed to at 55 °C in 45 minutes. The maximum buprenorphine hydrolysis was obtained without heating and incubation time. It is considerably faster than the optimized incubation time of 60 minutes for glusulase and 4 hours for Helix pomatia, both at 60 °C [1-2]. Total buprenorphine and norbuprenorphine compared well between the enzyme treatment and total measurements from monitoring the intact glucuronides (Table 2).

Conclusions:
The superior potential of IMCSzyme was demonstrated with complete buprenorphine and norbuprenorphine hydrolysis faster than has been previously reported. Percent hydrolysis exceeded 93% for all target analytes in patient samples. The use of IMCSzyme will decrease processing time due to the shorter hydrolysis incubation time.