

## 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<sup>®</sup> 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.

**Table 1. Hydrolysis Parameters**

| Urine       | Enzyme              | Buffer         | Water       |
|-------------|---------------------|----------------|-------------|
| 100 $\mu$ L | 20 $\mu$ L IMCSzyme | 38 $\mu$ L RHB | 342 $\mu$ L |

### 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).

**Table 2. Analyte Recovery after Hydrolysis of Authentic Urine Specimens with Recombinant  $\beta$ -glucuronidase at 30 mins. at 65°C.**

| Analyte          | Intact Glucuronide Target Range (ng/mL) | IMCSzyme <sup>®</sup> Range (ng/mL) | % Hydrolysis* | $\pm$ Standard Deviation | Correlation (R <sup>2</sup> ) | p value at 95% Confidence Interval |
|------------------|-----------------------------------------|-------------------------------------|---------------|--------------------------|-------------------------------|------------------------------------|
| Buprenorphine    | 19-862                                  | 16-1011                             | 103.8         | 18.5                     | .9936                         | 0.188                              |
| Norbuprenorphine | 27-2498                                 | 32-2377                             | 92.6          | 17.1                     | .9873                         | 0.018                              |

\* % hydrolysis relative to target concentrations from monitoring the intact glucuronides

### 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.

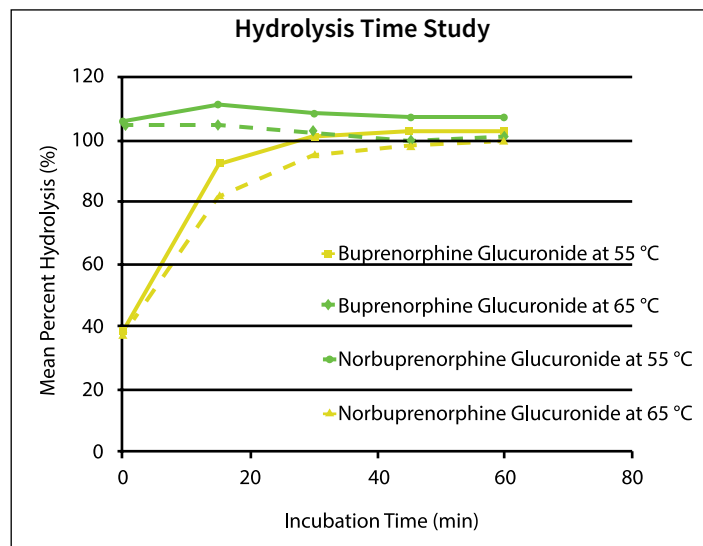


Figure 1. Effect of Incubation Time and Temperature on the hydrolysis of Glucuronides of Buprenorphine and Norbuprenorphine with Recombinant  $\beta$ -glucuronidase