

## A summary of “Accurate Quantitation of Heroin Metabolite, 6-Monoacetylmorphine in Urine using IMCSzyme® for Hydrolysis”

### Overview

Hydrolysis of drug metabolite conjugates is common practice to analyze drugs of abuse in human matrices on LC-MS/MS.  $\beta$ -glucuronidase enzyme is generally used to hydrolyze glucuronide conjugates. However, in the case of heroin use, where 6-monoacetylmorphine (6-MAM) may be present in urine, the conversion of 6-MAM to morphine may occur during hydrolysis. This study was performed to investigate the 6-MAM conversion by four commercially available  $\beta$ -glucuronidase enzymes.

### Materials and Methods

All drug standards were purchased from Cerilliant Corporation.  $\beta$ -Glucuronidase enzymes were from Integrated Micro-Chromatography Systems, LLC (IMCSzyme®), Campbell Science (*Helix pomatia* and *Patella vulgata*). 200  $\mu$ L of urine was hydrolyzed with 100  $\mu$ L of enzyme solution (containing hydrolysis buffer and internal standard) at 55 °C with shaking. The hydrolysis times were 1 and 2 hours for each enzyme. The hydrolyzed sample was extracted with RP-S tip, provided by DPX Labs, LLC, and then analyzed by LC-MS/MS.

### Results

In the urine sample hydrolyzed with IMCSzyme, there was less than 10 ng/mL, or less than 1% increase in morphine concentration and no significant difference in 6-MAM concentration over the 2 hour time incubation period. Comparing to other enzymes, the levels of morphine detected increased over the hydrolysis time, corresponding with the decrease in 6-MAM. The enzyme that converts 6-MAM into morphine the highest to the lowest is in the order: *Helix pomatia*, *Patella vulgata*, *Haliothis rufescens*, and IMCSzyme (Figure 1)

### Conclusions

Due to the possible conversion of 6-MAM, most laboratories have to treat 6-MAM positive samples differently than other opiate positive samples. The results showed that while the three other commercially available enzymes significantly converts 6-MAM to morphine, IMCSzyme recombinant enzyme did not. The decrease in conversion of 6-MAM to morphine would allow for more accurate analytical runs of heroin positive samples even in the presence of a glucuronidase.

### References

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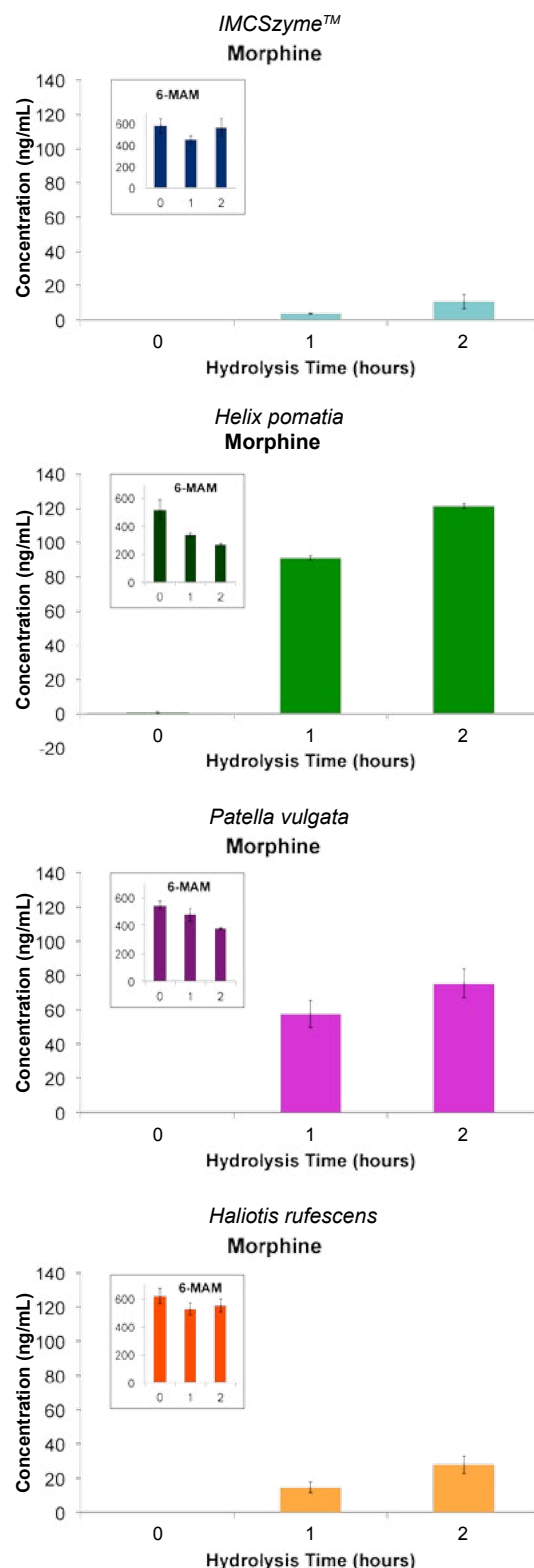


Figure 1. Concentration, in ng/mL, of 6-MAM and morphine after 0, 1, and 2 hours of incubation with the respective enzyme in previously drug free urine spiked with 6-MAM.