POSTER SUMMARY

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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. β-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 β-glucuronidase enzymes.

Materials and Methods

All drug standards were purchased from Cerilliant Corporation. β-Glucuronidase enzymes were from Integrated Micro-Chromatography Systems, Inc (IMCSzyme®), Campbell Science (*Haliotis rufescens*), and Sigma-Aldrich (*Helix pomatia* and *Patella vulgata*). 200 µL of urine was hydrolyzed with 100 µL 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*, *Haliotis 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 coversion of 6-MAM to morphine would allow for more accurate analytical runs of heroin positive samples even in even in the presence of a glucuronidase.

References

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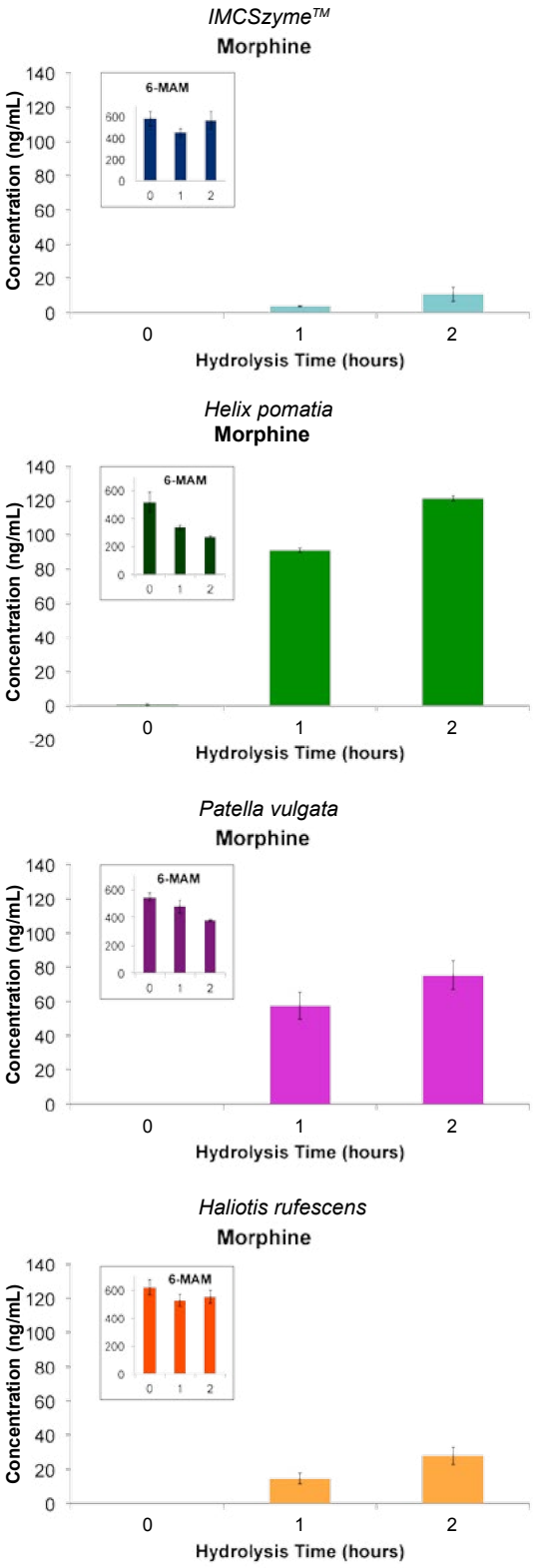

Figure 1. Concentration, in ng/mL, of 6-MAM and morphine after 0, 1, and 2 hours of incubation with the respective enzyme in previously drug free urine spiked with 6-MAM.

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This information was summarized by IMCS from the poster "Accurate Quantitation of Heroin Metabolite, 6-Monoacetylmorphine in Urine using IMCSzyme for Hydrolysis" presented by Kaylee McDonald - University of South Carolina at SOFT 2014