POSTER SUMMARY

integrated micro-chromatography systems

A summary of "Opiate Hydrolysis by a Novel Recombinant β- Glucuronidase for Urine Analysis"

Overview:

A pain medication monitoring laboratory presented the effectiveness of IMCSzyme® in hydrolyzing glucuronidated opiates in comparison to acid hydrolysis method at SOFT in 2014. The research showed that unlike conventional hydrolyses, IMCSzyme® did not convert 6-acetylmorphine (6-AM) to morphine and required less time than the other commercially available products from other sources (snail, limpet, bovine or abalone).

Material and Methods:

All drug standards were purchased from Cerilliant Corporation. β-Glucuronidase enzyme (IMCSzyme®) was purchased from Integrated Micro-Chromatography Systems, Inc. Aliquots of blank urine were spiked with 2,5000 ng/mL of glucuronides of oxymorphone, hydromorphone, codeine and both morphine-3 and -6. Randomly selected authentic patient samples that were confirmed positive were tested. For hydrolysis, 125 uL of urine samples were incubated with 3,000 U for 15, 30, 45, and 60 minutes at 55 °C or 65 °C. Following hydrolysis, samples were centrifuged and injected onto LC/MS-MS instrument without any further sample preparation. Analysis was performed on TLX-4 multiplexed HPLC with Agilent 1200 Series Binary Pumps coupled to a Thermo Scientific TSQ Quantum Triple-stage Quadrupole Mass Spectrometer. Analytes were separated chromatographically in a 4.5 mins gradient on a Phenomenex® Kinetex C18 50 x 3 mm analytical column. Hydrolysis efficiency was calculated by dividing the resulting analyte concentration by the target concentration of the test compound and multiplying by 100%.

Figure 1. Hydrolysis Time Study

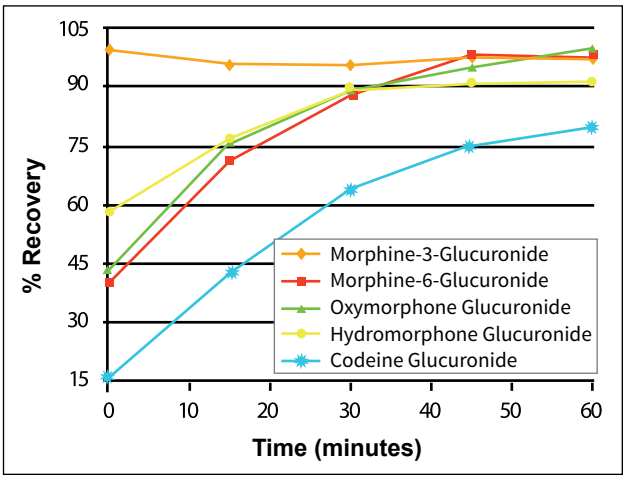

Figure 1: Effect of Incubation Time on the Hydrolysis of Glucuronides of Morphine-3, Morphine-6, Oxymorphone, Hydromorphone and Codeine with Recombinant β-Glucuronidase at 65 °C.

Results

Morphine-3-glucuronide was more responsive to the enzyme than any other tested glucuronide. This differential activity is consistent with observations with other β-glucuronidase enzymes [1]. IMCSzyme® was able to completely hydrolyze morphine-3-glucuronide without incubation (Figure 1). This is substantially faster than the optimized incubation time of 2, 3, or 16 hours for *Patella vulgate* (limpet), *Haliotis rufescens* (abalone), and *Helix pomatia* (snail), respectively [1-3]. Heat activation of IMCSzyme® and longer incubation time improved the recovery of morphine-6-glucuronide, codeine-6-glucuronide, oxymorphone-3-glucuronide and hydromorphone glucuronide controls (Figure 1). Overall, percent hydrolysis of IMCSzyme® compared to acid hydrolysis exceeded 77% for all target analytes in patient samples (Table 1).

Table 1.

Analyte	Acid Target Range (ng/ml)	IMCSzyme Range (ng/mL)	% Hydrolysis	+ Standard Deviation	Correlation (R2)	P value at 95% Confidence Interval
Morphine	180-35474	165-40726	100.3	12.5	.9846	.724
Codeine	134-48963	125-41580	81.7	24.4	.8731	.089
Oxymorphone	180-25840	154-13648	78.4	10.4	.9854	.030
Hydromorphone	134-24894	106-16838	77.3	11.9	.9961	.053

Analyte Recovery after Hydrolysis of Authentic Urine Specimens with Recombinant β-Glucuronidase at 60 mins at 65°C.
** % hydrolysis relative to target concentrations from acid hydrolysis*

Conclusions

Based on the tested parameters, IMCSzyme® performed less effective than acid hydrolysis of opiate glucuronides. However, the purified enzyme did not convert the heroin metabolite (6-AM) to morphine and did not require a high temperature (>90 °C) with highly caustic reagent (8 M HCl) in order to achieve its hydrolysis. While the control samples using drug-free urine suggested near complete hydrolysis (>90%) of morphine, oxymorphone and hydromorphone, the recoveries from patient samples were lower for oxymorphone and hydromorphone. The presenters suggest that the variability within the patient samples may affect the performance of the enzyme thereby decreasing recoveries of analytes when using patient samples.

References

1. P. Wang, J. A. Stone, K. H. Chen, S. F. Gross, C. A. Haller and A.H. B. Wu. Incomplete recovery of prescription opioids in urine using enzymatic hydrolysis of glucuronide metabolites. J. Anal. Toxicol. 30: 570-575 (2006).
2. B. Malik-Wolf, S. Vorce, J. Holler and T. Bosy. Evaluation of abalone β-glucuronidase substitution in current urine hydrolysis procedures. J. Anal. Toxicol. 1-6 doi:10.1093/jat/bku003 (2014).
3. R. W. Romberg and L. Lee. Comparison of the hydrolysis rates of morphine-3-glucuronide and morphine-6-glucuronide with acid and β-glucuronidase. J. Anal. Toxicol . 19: 157-162. (1995).

This information was summarized by IMCS from the poster "Opiate Hydrolyis Using a Novel Recombinant β-glucuronidase for Urine Analysis" presented by Ayodele A. Morris - Ameritox, Ltd. at SOFT 2014