POSTER SUMMARY

imcs
integrated micro-chromatography systems

A summary of "Enzyme Hydrolysis using a Novel Recombinant β-Glucuronidase for Pain Management Urine Drug Testing"

Overview

Precision Toxicology presented the use of recombinant β-glucuronidase (IMCSzyme®) in pain management urine drug testing at MSACL in March 2015. The researchers compared their initial hydrolysis parameters which used abalone extracts to a diluted and shoot process using IMCSzyme®. The comparison between two different enzyme sources indicated IMCSzyme® had comparable or better recoveries of the glucuronide metabolites in patient samples despite the decrease in enzyme volume and incubation time. The study demonstrated that IMCSzyme® is a viable and cost-effective alternative to their previous hydrolysis procedures using abalone β-glucuronidase.

Materials and Methods

For this study, patient urine specimens previously confirmed positive by LC-MS/MS for the different drugs were randomly selected and used as test samples. The study qualitatively compared the current hydrolysis process to a dilute and shoot process (Table 1). The differences between the different sample processing methods (ie. pH, ionic strength) were not methodically controlled, which will skew these comparisons.

Table 1. Sample Preparation hydrolysis condition comparisons

	IMCSzyme®	Abalone β-glucuronidase
Sample Prep.	Dilute and Shoot	Solid Phase Extraction
Enzyme Units	> 50,000 units/mL	25,000 units/mL
Enzyme Amount	30 µL	200 µL
Sample Amount	140 µL	200 µL
Buffer Amount	30 µL	500 µL
Internal Standard	50 µL	100 µL
Temperature	60 °C	60 °C
Incubation Time	30 min.	1 hr.

Results

IMCSzyme® hydrolyzed the glucuronide drug metabolites in patient samples in shorter incubation time of 30 min. The decreased enzyme use from 200 µL to 30 µL was primarily dictated by the overall total sample volume decreasing from 1 mL to 250 µL. Codeine, morphine, oxymorphone, fentanyl, tramadol, cyclobenzaprine, amitriptyline, naloxone, and tapentadol, showed higher quantities following hydrolysis with IMCSzyme®, indicating more complete conversion than was previously obtained with abalone β –Glucuronidase (Figure 1). Hydrocodone, butalbital, phenobarbital and gabapentin are not glucuronidated. The increase in concentration for these analytes can be attributed to differences in the sample processing with solid phase extraction versus dilute and shoot process.

Hydrolysis Recoveries

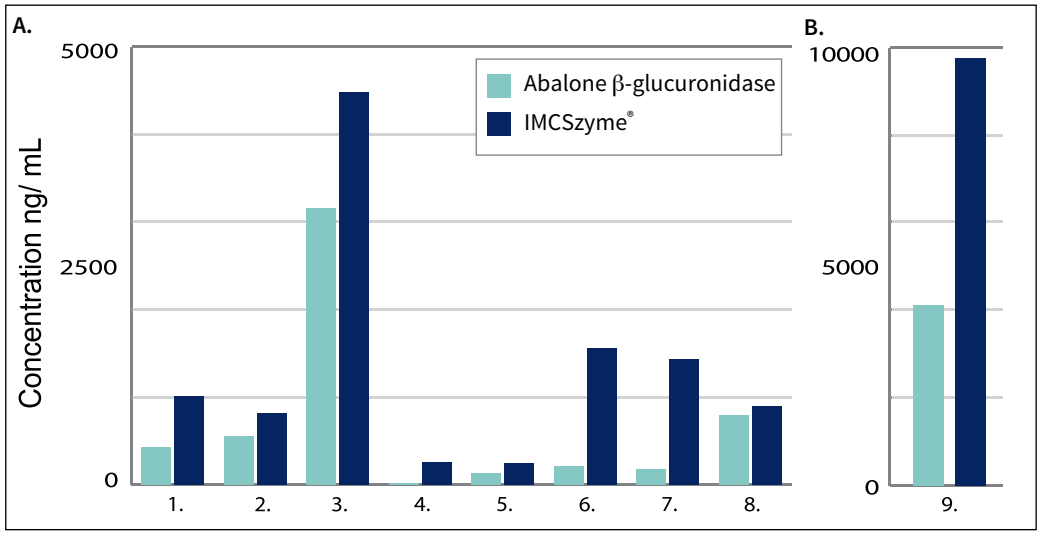

Figure 1 A.
1.Codeine
2. Morphine
3. Oxymorphone
4. Fentanyl
5. Tramadol
6. Cyclobenzaprine
7. Amitriptyline
8. Naloxone
Figure 1 B.
9. Tapentadol

Figure 1. Drug targets with higher recoveries when using IMCSzyme® versus the abalone extract from patient urine samples. Only a select few targets are shown for briefness and full poster can be requested from Precision Toxicology. Not all analytes show higher recoveries.

Conclusions

IMCSzyme® is a novel recombinant β-glucuronidase designed for rapid hydrolysis of (R)-D-glucuronic acid from drug metabolites in various biological fluids. Enhanced catalytic activity is maintained by the stringent purification of a genetically modified β-glucuronidase. The researchers concluded that IMCSzyme® is ideal for high-throughput requiring no additional steps prior to hydrolysis.

This information was summarized by IMCS from the poster "Enzyme Hydrolysis using a Novel Recombinant β-Glucuronidase for Pain Management Urine Drug Testing" presented by Agnes Cua - Precision Toxicology at MSACL 2015.