JOURNAL SUMMARY

imcs
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A summary of "Rapid Enzyme Hydrolysis Using a Novel Recombinant β-Glucuronidase in Benzodiazepine Urine Analysis"

Overview:

A pain medication-monitoring lab presented their evaluation of IMCSzyme® at AACC in 2014 to determine the efficiency and effectiveness of the recombinant β-glucuronidase to use in their drug analyses. The original method utilized β-glucuronidase from abalone extracts which has been reported to be more effective than the enzyme from other sources. In the poster, the researchers presented both the speed of IMCSzyme for hydrolysis of benzodiazepines and percent of hydrolysis relative to recoveries from abalone treated samples.

Material and Methods:

Drug free urine fortified with glucuronides of oxazepam, lorazepam and temazepam at 2500 ng/mL. Hydrolysis efficiency was assessed in triplicate with individual glucuronide controls at the recommended optimum temperature of 55˚C at incubation times of 5, 15, 30 and 60 minutes and at room temperature at incubation times of 0, 5, 10 and 15 minutes. Randomly selected authenticated patient urine samples that were previously confirmed positive for benzodiazepines had been used to find hydrolysis efficiency of IMCSzyme relative to the original abalone enzyme. Analysis was completed on a TLX-4 Multiplexed HPLC with Agilent 1200 Series Binary Pumps coupled to a Thermo Scientific TSQ Quantum Ultra Triple-Stage Quadrupole Mass Spectrometer using a previously validated multiplexed method. Analytes were separated chromatographically in a six minute gradient following online sample purification using Cohesive Technologies Cyclone-P 0.5 x 50 mm Turboflow column.

Results:

Table 1

Analyte	Abalone Target Range (ng/mL)	IMCSzyme Target Range (ng/mL)	Mean % Hydrolysis* ± standard deviation
Oxazepam	45-2788	35-2708	90.5 ± 10.1
Lorazepam	79-3838	37-5808	87.6 ± 29.3
Temazepam	33-4105	32-4361	99.1 ± 7.8
Alphahydroxyalprazolam	34-3016	54-5039	156.3 ± 80.2
Alprazolam	38-1131	31-1437	110.7 ± 20.3
Nordiazepam	24-1219	32-1512	150.8 ± 89.7
7-Amino-clonazepam	489-1882	25-1740	86.0 ± 15.9

Analyte recovery after hydrolysis of authentic urine specimens with recombinant β-glucuronidase at 0 mins. at RT.

** % Hydrolysis relative to target concentrations from abalone hydrolysis*

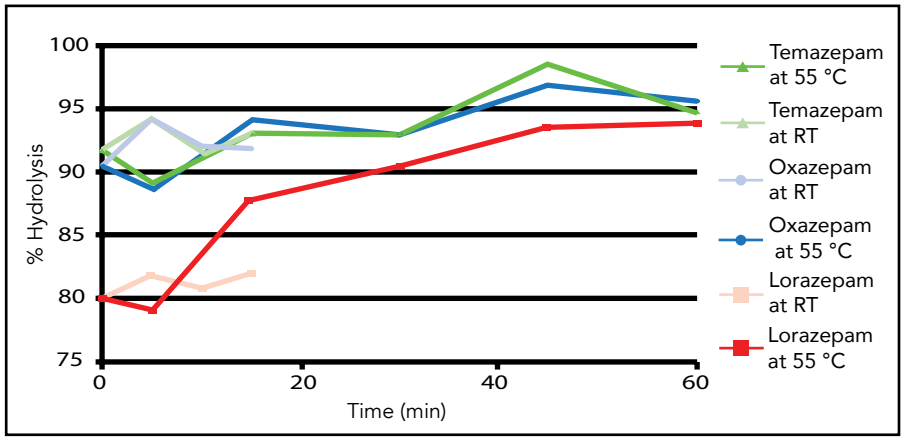

Figure 1: Effect of Incubation Time and Temperature on the Hydrolysis of Glucuronides of Oxazepam, Lorazepam, and Temazepam with Recombinant β-Glucuronidase

Conclusions:

The study showed that hydrolysis with IMCSzyme was both accurate and efficient in comparison to β-glucuronidase solutions from traditional sources like bovine liver, *Helix pomatia*, *Escherichia coli* and *Patella vulgata*. The results from the study also indicated how quickly benzodiazepine hydrolysis could be completed at room temperature before samples are injected onto the LC/MS-MS using the recombinant enzyme versus the abalone enzyme, whereas prior method required a 45 minute incubation time at 55 ˚C. IMCSzyme has decreased the laboratory processing time by eliminating the need for incubation and not requiring heat activation.

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This information was summarized by IMCS from "Rapid Enzyme Hydrolyis Using a novel recombinant β-glucuronidase in Benzodiazepine Urine Analysis" by Ayodele A. Morris, Scot A. Cester, Erin C. Strickland and Gregory L. McIntire in the Journal of Analytical Toxicology 2014; 38:6 10 - 614