Effect of Enzyme Source, Form and Hydrolysis Conditions on the Conversion of Glucuronide Drug Metabolites in Urine to Parent Drugs by β-Glucuronidase

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β-Glucuronidase (β-D-glucuronide glucuronosohydrolase) enzymes play an important role in the analysis of biological fluids for the presence of drug metabolites for drug screening and drug metabolism studies. β-Glucuronidase hydrolyzes glucuronide metabolites back to the native parent drug. Hydrolysis using β-glucuronidase is often necessary when extensive drug metabolism complicates drug detection in biological samples. For liquid chromatographic analytical methods, the highly-polar glucuronide metabolites that form cannot be easily retained in reversed phase chromatographic separation thus hindering the quantitative ability of the analytical method. Hydrolysis of glucuronide metabolites is necessary for gas chromatographic methods; this also commonly includes the addition of secondary derivitisation for effective analysis and detection.

Although there are numerous β-glucuronidase enzymes available, each enzyme has optimum conditions to which hydrolysis of glucuronide metabolites can be effectively conducted. Variables such as β-glucuronidase enzyme concentration, digestion pH, incubation time and temperature all play an important role for effective hydrolysis of glucuronide metabolites.

This study addresses some of the critical variables that come into play when choosing an appropriate β-glucuronidase enzyme and incubation conditions to perform effective hydrolysis of glucuronide metabolites to the parent drug form. In this study, a set of compounds that are commonly hydrolyzed for analysis were used to evaluate the conditions for effective hydrolysis over a range of sources and forms of β-glucuronidase. Various strains of β-glucuronidase enzymes were evaluated to determine proper hydrolysis conditions and the optimum combination for corresponding drug classes. Five drugs classes covering opioids, benzodiazepines, steroids, cannabinoids, and pain management drugs and eleven β-glucuronidases that differ in source, type, or formulation were included in the study.

To optimize hydrolysis using β-glucuronidase, factors such as incubation time, temperature, hydrolysis pH, and enzyme source and concentration must be evaluated for each glucuronide metabolite to be analyzed. In the study reported here, the hydrolysis pH was determined to be the most important factor for effective hydrolysis of glucuronide metabolites in urine. In some cases, a specific β-glucuronidase enzyme was effective for hydrolysis across a class of glucuronide metabolites. However, in most cases optimal hydrolysis conditions were compound dependent.

References

1. McCarter, J.D.; Withers, S. G. Mechanisms of enzymatic glycoside hydrolysis. *Curr. Opin. Struct. Biol.,* **1994,** *4(6)*, 885-92.