Enzyme Activities (Fishman units) Correlate Poorly with Hydrolysis Efficiencies


ABSTRACT

• Activity of five different β-glucuronidases was tested via a phenolphthalein-glucuronidase chromogenic assay.
• Hydrolysis efficiency of ten drugs was monitored for all five β-glucuronidases at their respective pH optimum.
• Activity level measured with phenolphthalein-glucuronide did not correlate well with drug hydrolysis efficiency.

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METHODS

Activity of each glucuronidase was monitored over three nonconsecutive days to determine their respective enzymatic activity. The activity protocol performed was specific to each vendor using their respective buffers, pH, and temperatures. The three activities for each enzyme were averaged and converted to vendor-specific activity values. The effects of ethanol concentration on activity were evaluated, and the pH profile of each enzyme was determined by testing each enzyme’s activity at 37°C over a range of buffer pH values from 4.5 to 8.5.

For the hydrolysis efficiency test, drug-free synthetic urine was spiked with 1000 ng of amitriptyline, codeine, diazepam, dihydrocodeine, morphine, oxymorphone, and temazepam glucuronides. IMCSzyme showed a significantly higher rate of hydrolysis, with little to no hydrolysis of amitriptyline, codeine, diazepam, dihydrocodeine, morphine, oxymorphone and temazepam glucuronides. IMCSzyme glucuronidase and one commercially available β-glucuronidases construct were compared in this study.

RESULTS

Table 1. Activity of five different β-glucuronidases analyzed in this study.

Enzyme | Activity (units) | Activity (units) | Activity (units) | Activity (units) | Activity (units) |
--- | --- | --- | --- | --- | --- |
Pure Abalone 1 | 45,416 | 5421 | >50,000 | 37 | 4.5 |
Pure Abalone 2 | 113,731 | 5367 | ≥100,000 | 37 | 3.8 |
Pure Limpets | 88,484 | 534 | 100,000–200,000 | 37 | 3.8 |
IMCSzyme | 82,702 | 10,557 | >50,000 | 25 | 6.8 |
#8282 | 398,290 | 24,643 | N/A | 25 | 5.2 |

Table 2. Activities of five different β-glucuronidases in Fishman units.

Enzyme Activity (Fishman units) | Activity (units) | Activity (units) | Activity (units) | Activity (units) | Activity (units) |
--- | --- | --- | --- | --- | --- |
Pure Abalone 1 | 0.58 | 100,000–200,000 | 37 | 3.8 |
Pure Abalone 2 | 1.05 | Not commercially available | | |
Pure Limpets | 0.77 | >50,000 | 37 | 4.5 |
IMCSzyme | 0.24 | ≥100,000 | 37 | 3.8 |
#8282 | 1.24 | N/A | |

Figure 1. 1. 2. 3. 4. 5. 6. Figure 2. 1. 2. 3. 4. 5. 6. 0.58 100,000 – 200,000 0.24 ≥ 100,000 0.77 > 50,000 1.05 Not commercially available 1.24 N/A

CONCLUSIONS

• Activity measured with the chromogenic substrate phenolphthalein-glucuronide is not a good indicator of drug hydrolysis due to substrate specificity.
• Laboratories should ensure when choosing a β-glucuronidase that high activity in Fishman units will not necessarily correlate with drug hydrolysis efficiency.
• Laboratories should consider β-glucuronidases in-house to ensure effectiveness of drugs being monitored and compatibility with patient samples.

REFERENCES


Figure 3. Graph demonstrating the linear decrease in phenolphthalein absorbance with increasing percentage of organic solvents.

Figure 4. Graph showing standard curves for phenolphthalein-glucuronide.

Figure 5. Graph demonstrating the optimized pH values for each enzyme. pH is a critical factor when choosing a β-glucuronidase for a given glucuronide since patient urine samples are found to be within a wide range of pH values. Typically, fish enzyme has an optimum pH range of 4.5 to 6.5. However, the glucuronidase assay for drug hydrolysis can be performed with a pH range of 4 to 9.5 or higher.

Figure 6. Graph demonstrating the optimized pH values for each enzyme. pH is a critical factor when choosing a β-glucuronidase for a given glucuronide since patient urine samples are found to be within a wide range of pH values.