Hydrolysis Efficiency Evaluation of Novel Recombinant Limpet and *E. coli* β-Glucuronidase Enzymes

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Overview

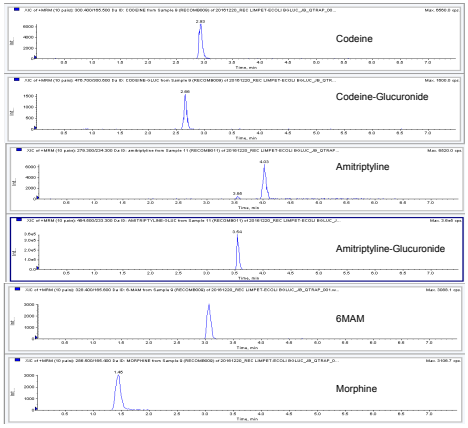
A trend in recent years is that many prescription drugs are being increasingly diverted and abused by all age groups. Urine drug testing has thus become an important tool to monitor compliance and detect the presence of illicit drugs as well as pain management monitoring.

The primary metabolites of many such drugs are glucuronides, which can be challenging to analyze due to their highly polar nature and poor ionization efficiencies. β-glucuronidase is often utilized for enzymatic hydrolysis of glucuronide metabolites to their parent drug, simplifying the analytical workflow. β-glucuronidase enzymes are derived from many native and recombinant sources which have been demonstrated to exhibit variable substrate-dependent hydrolysis efficiencies.

Here we evaluate hydrolysis efficiencies of two novel recombinant β-glucuronidase enzymes as well as a native enzyme against a traditionally difficult opioid substrate, Codeine-6-β-D-glucuronide, and a unique quaternary amine substrate, Amitriptyline-N-β-D-glucuronide. Additionally we evaluated esterase activity for the three enzymes by measuring conversion of 6-Monoacetylmorphine (6MAM) to Morphine.

LC-MS/MS Analysis

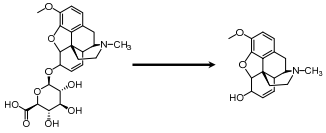
- MS: AB Sciex 4000 Qtrap
- Column: Supelco Ascentis Express C18, 50 x 3.0 mm, 5 um, PN50523-U
- MP A: 4 mM Ammonium Formate in Water
- MP B: 4 mM Ammonium Formate in 90% ACN



Enzyme Digestion

- Target glucuronides and 6MAM were spiked into synthetic urine matrix.
- Heavy parent (non-glucuronide) drug was spiked as internal standard.
- Enzyme stocks prepared at 65,000 Units/mL at pH 5.2 and 6.8 for Limpet and *E. coli*, respectively.
- Mixed equal volumes of sample and enzyme stock, incubated at 60 °C.
- At each time-point, pulled sample, precipitate protein with TCA, and clarify by centrifugation.
- Analyzed by LC-MS/MS

Codeine-6-β-D-glucuronide



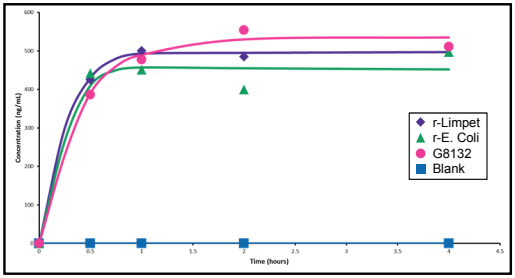
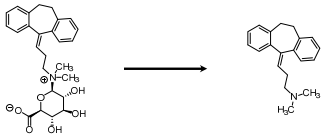


Figure 1. Codeine-Glucuronide to Codeine conversion with rLimpet, rEcoli, and native Limpet enzymes.

Amitriptyline-N-β-D-glucuronide



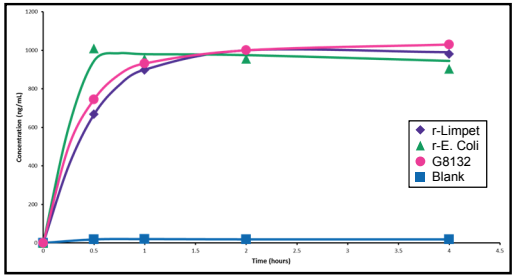
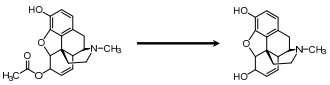


Figure 2. Amitriptyline-Glucuronide to Amitriptyline conversion with rLimpet, rEcoli, and native Limpet enzymes.

6-Monoacetylmorphine (6MAM)



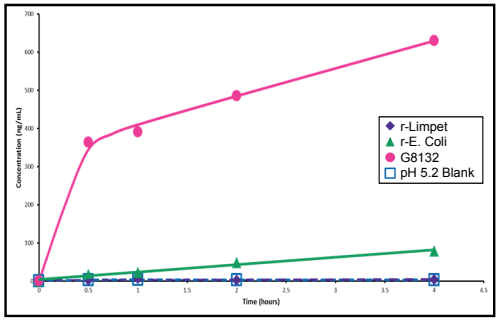


Figure 3. 6MAM to Morphine conversion with rLimpet, rEcoli, and native Limpet enzymes.

Results Summary

- Recombinant Limpet enzyme (SRE0093): Codeine-Glucuronide and Amitriptyline-Glucuronide conversion was >90% after 1 hour and >95% after 2 hours. 6MAM conversion to Morphine was <1% after 2 hours.
- Recombinant *E. coli* enzyme: Codeine-Glucuronide and Amitriptyline-Glucuronide conversion was >90% after 1 hour and >95% after 2 hours. 6MAM conversion to Morphine was <7% after 2 hours.
- Native Limpet enzyme (G8132): Codeine-Glucuronide and Amitriptyline-Glucuronide conversion was >90% after 1 hour and >95% after 2 hours. 6MAM conversion to Morphine was significant at approximately 70% after 2 hours.

Conclusions

The total protein loads of the recombinant enzyme reactions (~ 0.4 mg/mL) investigated here are substantially lower compared to native enzyme reactions (~ 30 mg/mL). No significant loss in conversion rates of difficult glucuronide substrates was observed with the recombinant enzymes. These new reagents will positively impact hydrolysis workflows and provide great improvement compared to native enzyme preparations regarding 6MAM to Morphine conversion from esterase activity.

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