Rapid Enzyme Hydrolysis Using a Novel Recombinant β-glucuronidase in β-agonists Meat Analysis

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Introduction

β_2-Adrenergic agonists (β-agonists) have been legally used in the U.S. since the mid 1980s to increase lean muscle mass in meat animals (1-2). In many countries, including European Union countries (3) and China (4), these drugs have been banned due to their adverse effects on humans, such as food poisoning (5), cardiovascular and central nervous diseases (6). Residue analysis on these target compounds is carried out by several control laboratories in order to protect the consumer, guarantee fair trade and enforce the ban (7).

It is common practice to include a hydrolysis reaction to cleave the glucuronide linkage when extracting conjugated analytes from biological samples to measure the total drug concentration. Various sources of β-glucuronidase exist such as *Helix pomatia (H. pomatia), Escherichia coli (E. coli)*, bovine liver, *Patella vulgatta* and abalone, with each exhibiting different hydrolysis efficiencies and rates. The purpose of this study was to compare two different enzyme sources, recombinant β-glucuronidase (IMCSzyme) and *Helix pomatia* β-glucuronidase (Helix B-Gus) and optimized hydrolysis time to achieve the most efficient hydrolysis of beta-agonists in meat matrix.

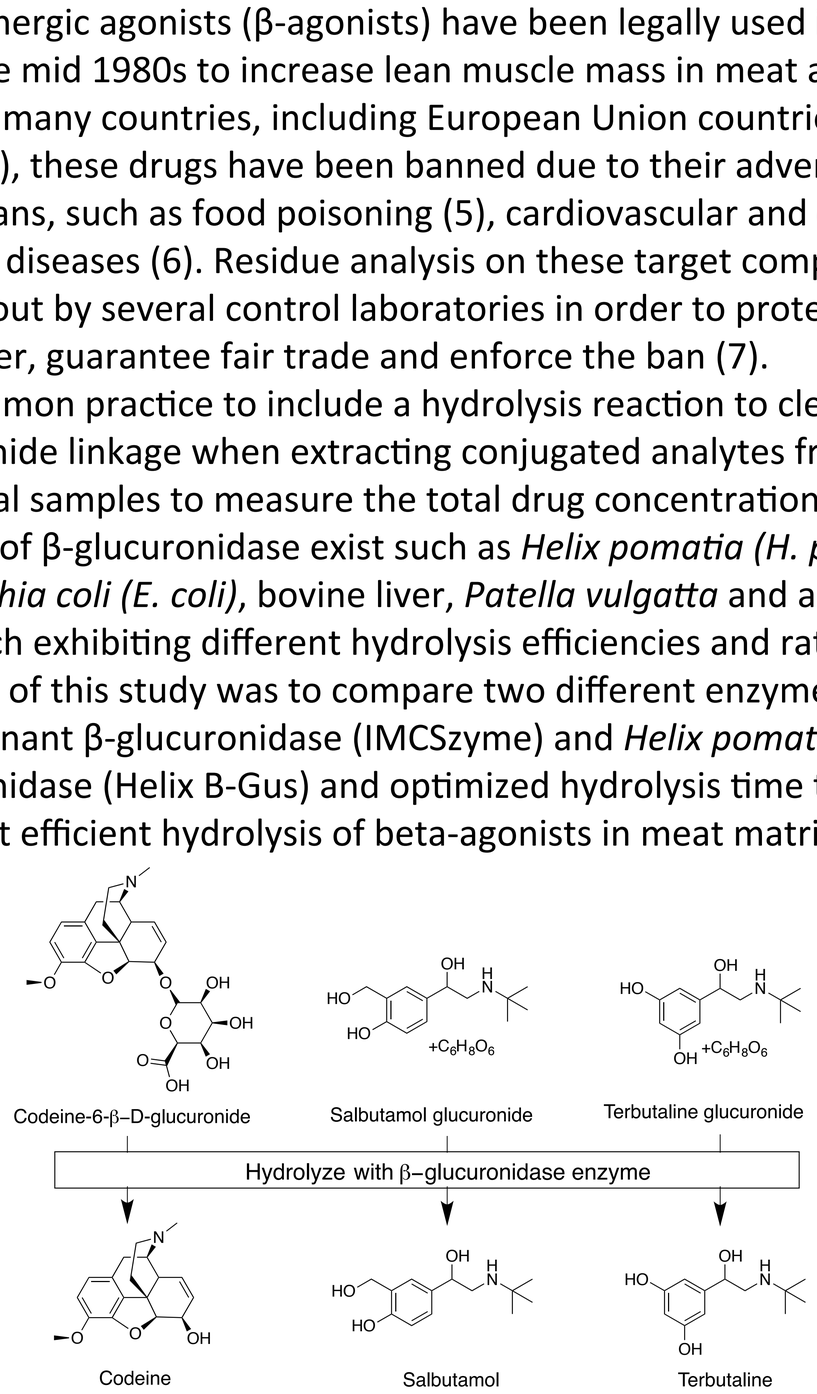

Figure 1. *Hydrolysis scheme of glucuronide substrates used in this study*

Instrumentation and Methods

To compare the activity of IMCSzyme and Helix B-Gus, β-agonists–free bovine liver standard was spiked with 10 ng/mL of codeine-6-β-D-glucuronide (C6G). 250 mg of spiked sample was hydrolyzed with 50 µL of enzyme in 250 µL of enzyme appropriated buffer. Incubation temperature was fixed at 37° C for 6 or 24 hours. The hydrolyzed samples were extracted and cleaned with DPX CX tips. Then the cleaned samples were analyzed by LC-MS/MS.

To optimize for rapid yet efficient hydrolysis of β-agonists in organ meat, 250 mg of reconstituted β-agonists positive lyophilized bovine liver standard was hydrolyzed with 50 µL of IMCSzyme in 250 µL 20 mM potassium phosphate buffer pH 7.4 at 37° C for 1, 2, 6 or 24 hours. The hydrolyzed samples were extracted and cleaned with DPX CX tips. Then the cleaned samples were analyzed by LC-MS/MS.

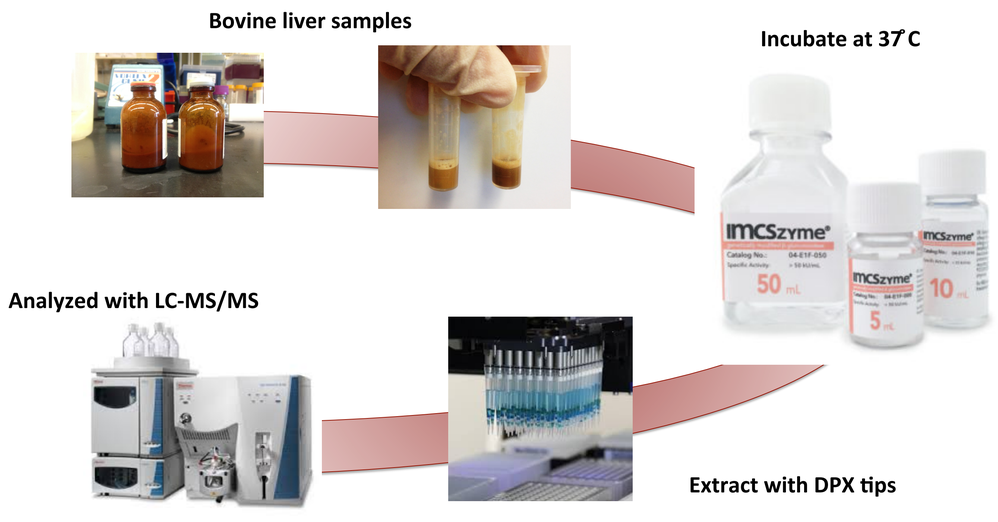

Figure 2. *Schematic representation of hydrolysis and sample preparation procedures*

Results and Discussion

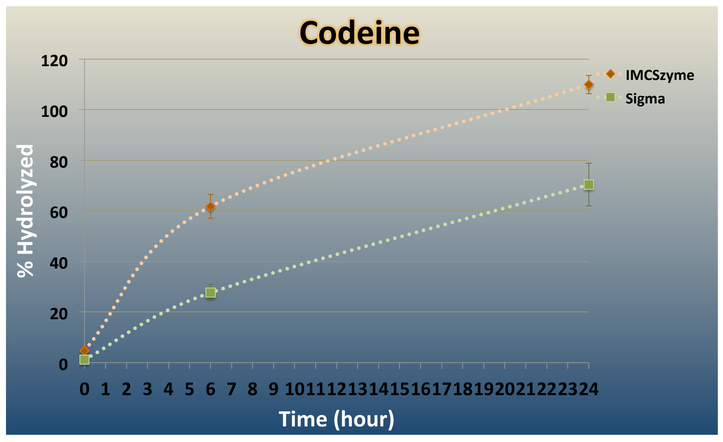

Figure 4. *Average percent hydrolysis of Codeine after 6 and 24 hours incubation with IMCSzyme or Helix B-Gus*

When the spiked bovine liver samples were incubated for 6 hours, samples hydrolyzed with IMCSzyme and Helix B-Gus recovered 62% and 28% of codeine, respectively. For samples with an incubation time of 24 hours, 100% hydrolysis was achieved with IMCSzyme, while 70% recovery of codeine was obtained by Helix B-Gus (Figure 3). The results proved the hydrolysis capability of IMCSzyme in highly complex matrix and one of the most challenge glucuronide substrates, C6G. Therefore, we selected IMCSzyme for rapid and efficient hydrolysis of β-agonists in organ meat matrix.

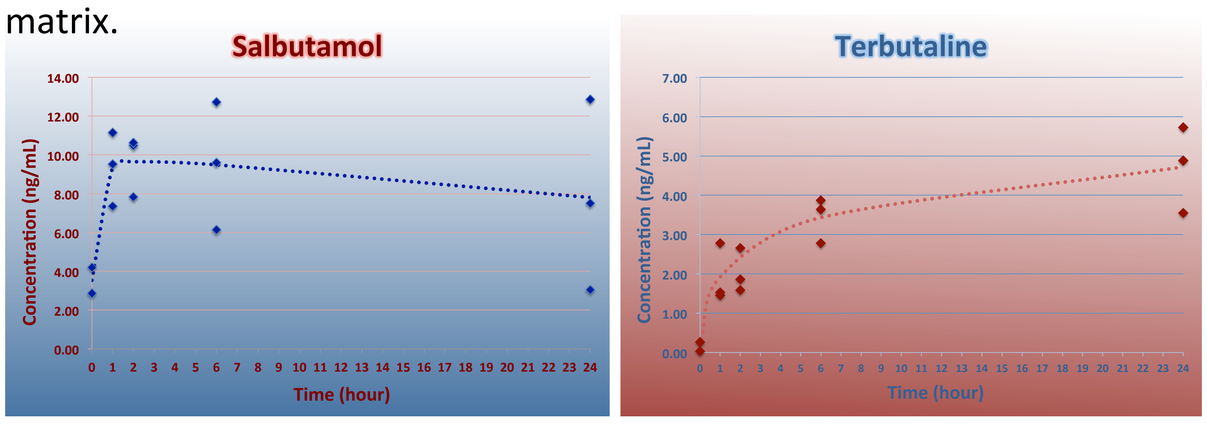

Figure 4. *Average concentration recovered after 1, 2, 6, and 24 hours of hydrolysis of salbutamol and terbutaline by IMCSzyme*

When the β-agonists positive samples were incubated for 1 hour, salbutamol concentration reached plateau level, which implies complete hydrolysis of salbutamol-glucuronide in the samples. In addition, more than 50% of terbutaline-glucuronide was hydrolyzed within the first hour. For samples with an incubation time of 2 hours, greater than 70% hydrolysis of terbutaline was achieved. At 6 hours incubation, 100% hydrolysis for both β-agonists were completed (Figure 4).

Conclusion

IMCSzyme can achieve efficient hydrolysis of challenging glucuronide substrate, Codeine, in complex matrices such as organ meat within 24 hours. The comparison between two different enzyme sources, recombinant β-glucuronidase (IMCSzyme) and *H. pomatia* β-glucuronidase (Sigma B-Gus), indicated IMCSzyme had better recoveries of the glucuronide metabolites in organ meat samples. Complete hydrolysis of β-agonists in organ meat samples can be accomplished by IMCSzyme within 6 hours or earlier. The study demonstrated that IMCSzyme is a viable and cost-effective alternative to the previous hydrolysis procedure using *Helix pomatia* B-Gus.

References

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