TECHNICAL NOTE

integrated micro-chromatography systems

A summary of "Hydrolyze Your Way to Compliance – A Call for Pain Management Certified Reference Materials"

Overview

β-glucuronidase enzymes can have varying degrees of hydrolysis efficiency based on which analytes are being tested. Three β-glucuronidase enzymes were compared and optimized to achieve the most efficient hydrolysis of various glucuronidated opioids. Enzyme concentration, incubation time, and incubation temperature were varied in the hydrolysis studies of enzymes from *Helix pomatia* (Sigma), *Haliotis rufescens* (Kura Biotec®), and recombinant IMCSzyme® (IMCS). The effects of these variances were noted as well as the importance of including multiple glucuronidated analytes in the validation of each enzyme's hydrolysis method.

Materials and Methods

Prior to testing 15 patient samples, hydrolysis conditions were optimized separately for each enzyme. The conditions tested were enzyme concentration (ranging from 5,000 to 25,000 U/mL), incubation time (30, 60, and 90 minutes), and incubation temperature (50 °C, 55 °C, and 68 °C). After optimal conditions were established, the patient samples were tested in triplicate in batches over three days. A five point calibration curve and two QC samples were included with each batch of patient samples. Samples were analyzed using a Waters TQ-S mass spectrometer operated in positive ion electrospray mode. Mobile phase A consisted of 5 mM ammonium formate, pH 3.0, and mobile phase B was 0.1% formic acid in acetonitrile. The column used was Waters XSelect HSS C18 2.5 µm, 2.1x150 mm XP with a Phenomenex C18 SecurityGuard ULTRA Cartridge.

Results

The hydrolysis optimization study showed that hydrolyzed drug recovery was influenced by incubation time for *Helix pomatia* and *Haliotis rufescens* β-glucuronidases, but it was influenced by incubation temperature for IMCSzyme®. The optimized hydrolysis time for *Helix pomatia* and *Haliotis rufescens* was 90 minutes, while optimal hydrolysis time for IMCSzyme® was only 30 minutes. The optimal temperatures and enzyme concentrations were 50°C at 5,000 U/mL for *Helix pomatia*, 68°C at 25,000 U/mL for *Haliotis rufescens*, and 55°C at 14,667 U/mL for IMCSzyme®. A comparison of codeine-6-β-D-glucuronide recovery using the optimized conditions for each enzyme showed that *Helix pomatia* had only reached 50 % recovery while *Haliotis rufescens* and IMCSzyme® reached 100 % recovery. From 15 patient samples tested, the analytes which showed the greatest differences in hydrolysis efficiency were codeine, norcodeine, morphine, hydromorphone, and oxymorphone. Increased recovery of parent drugs was seen with *Haliotis rufescens* and IMCSzyme. Percent CV for all analytes was no more than 11% for each patient sample batch and calibration curves had R^2 greater than or equal to 0.995. QC samples were all within 20% of assigned value except for morphine. A 40% bias was seen for morphine with *Helix pomatia* while less than 2% bias was observed between *Haliotis rufescens* and IMCSzyme®. Less than 10% bias was observed between *Haliotis rufescens* and IMCSzyme® for hydromorphone and oxymorphone. *Haliotis rufescens* and IMCSzyme® correlated very well as seen in **Figure 1**.

In regards to heroin exposure, morphine-to-codeine ratios were monitored for two patients and the results from the three enzymes were compared.

Table 1. Morphine to Codeine Ratios

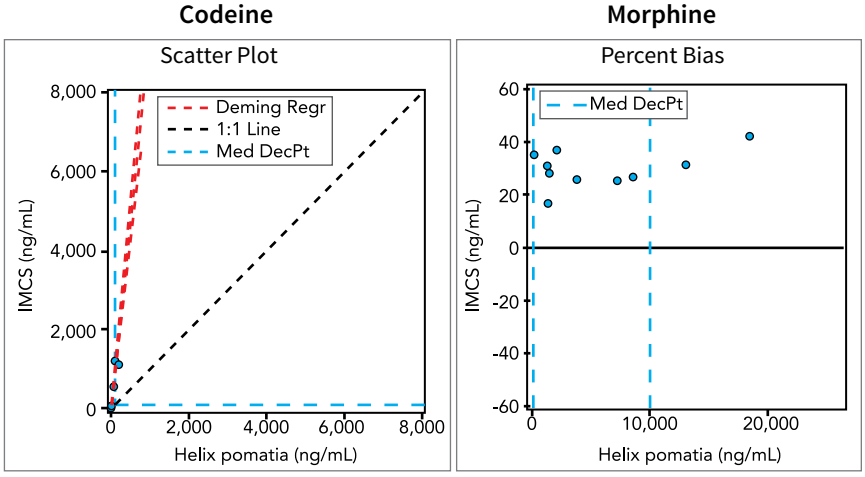
	Patient 1	Patient 2
Helix pomatia	91.4	1.7
Haliotis rufescens	24.2	0.24
IMCSzyme®	24.0	0.24
6-MAM Positive?	Yes	No

As seen in Table 1, the ratios obtained with *Helix pomatia* for patients 1 and 2 were 4- and 7-fold greater than those obtained with *Haliotis rufescens* and IMCSzyme. The morphine-to-codeine ratio is important in distinguishing heroin exposure from codeine exposure since 6-acetylmorphine has a short half-life.

Conclusions

Without hydrolysis optimization, drug testing may result in an incomplete hydrolysis leading to false negative interpretations. It is important for laboratories to test various conditions with their enzyme of choice and include multiple if not all analytes of interest in their validation study. By following these two recommendations, clinical laboratories will achieve more reliable results, which will subsequently lead to improved patient care.

Figure 1.



Codeine slope=9.5, slopes for all other analytes ranged from 0.9-1.4
Morphine - 40% Bias, <2% bias between Haliotis rufescens and IMCSzyme

This information was summarized by IMCS from the technical poster "Hydrolyze Your Way to Compliance– A Call for Pain Management Certified reference Material" presented by Heather Hochrein – UC San Diego Health at MSACL 2016.