RAPID AND EFFICIENT ENZYMATIC HYDROLYSIS OF CODEINE AND MORPHINE GLUCURONIDES IN URINE
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Introduction
LCMS procedures for the confirmation of drugs of abuse in urine are now used in most laboratories, because multiple drugs can be assayed at the same time and sample processing is quick and easy, resulting in faster turn around times. For opiate analysis, hydrolysis of the glucuronide bonds is required and enzymatic hydrolysis is the preferred option. However, up to date the yields of codeine from the enzymes used have been relatively low even with long incubation times, as the codeine 6 glucuronide bond is difficult to cleave. This study examines the Kura BG 100 enzyme and Patella Vulgata to breakdown the glucuronide bonds of codeine and morphine under various incubation conditions.

Methods
Enzymatic hydrolysis was performed on urine spiked with codeine and morphine glucuronides over the range 500-10000ug/l and codeine and morphine concentrations determined by LCMS. These results and results from authentic urines were compared to acid hydrolysis and assayed by GCMS.

Results
The Kura enzyme BG 100 (Haliotis Rufescens) obtained from abalone gave recoveries of codeine from urine spiked with codeine 6 glucuronide of greater than 90%. Morphine recoveries for both the 3 and 6 glucuronides were similar. Incubation was carried out at 68 degrees C for one hour using an enzyme concentration of 20000 units per ml of urine. Results for non-spiked urine specimens in the Austox Quality Assurance Program and authentic patient urine specimens from subjects on codeine medication have shown an acid hydrolysis to Kura enzyme ratio of 0.95 for codeine and 1.27 for morphine, indicating significant agreement.

Conclusions
Since high yields of codeine and morphine can be obtained with a relatively short incubation time, the Kura enzyme is ideal for the hydrolysis of opiates, and can be used when assaying for multiple drugs in the same run using LCMS.