POSTER SUMMARY

integrated micro-chromatography systems

A summary of "Evaluation of New β-glucuronidase for Urine Drug Testing"

OVERVIEW

This study compared the hydrolysis efficiencies of IMCSzyme® RT and an All-in-One β-glucuronidase (Current Enzyme, CE). Both enzyme products are recombinant room temperature β-glucuronidases widely used in urine drug testing. The first set of experiments looked at the optimal β-glucuronidase amounts and incubation times for enzyme hydrolysis. Next, benzodiazepines and opioids were evaluated for deconjugation efficiency using the different β-glucuronidases.

MATERIALS AND METHODS

Urine samples were pooled from patients previously confirmed positive for benzodiazepines and opioids. The samples were mixed with internal standard (IS) and a range of enzyme volumes (0 – 100 µL). After incubation at room temperature (0 to 30 minutes), the urine was diluted using a methanol water diluent. Samples were run using the Altis LC MS/MS (Thermo Fisher Scientific). Data were collected and entered into an Excel spreadsheet for analysis.

RESULTS

Benzodiazepine analyte concentrations (ng/mL) between IMCSzyme RT and CE after 15-minute room temperature hydrolysis were compared (**Figure 1A, 1B**). Benzodiazepine recoveries were comparable between the two enzyme products.

Individual relative opioid recoveries were determined after 15-minute room temperature hydrolysis with varying amounts of IMCSzyme RT and CE (0 – 100 µL). Relative recovery was normalized to maximum recovery. All opioid recovery levels plateaued using ≥20 µL IMCSzyme. CE offered similar hydrolysis performance to IMCSzyme RT for six opioids. However, the other six opioids required the addition of more enzyme to achieve similar efficiency: codeine recovery for CE did not plateau even after 100 µL whereas IMCSzyme RT reached its plateau at 20 µL; hydromorphone recovery plateaued at >50 µL; morphine, oxymorphone, and o-desmethyltramadol recoveries were lower for CE and did not plateau until 65 µL of enzyme was added.

A deeper look into opioid recovery amounts (ng/mL) for codeine, norbuprenorphine, and o-desmethyltramadol demonstrated consistent recoveries from IMCSzyme RT (from 20 µL to 100 µL, **Figure 1C, 1D**). CE showed a trend towards improved recovery proportional to the increase in the enzyme volume.

Figure 1

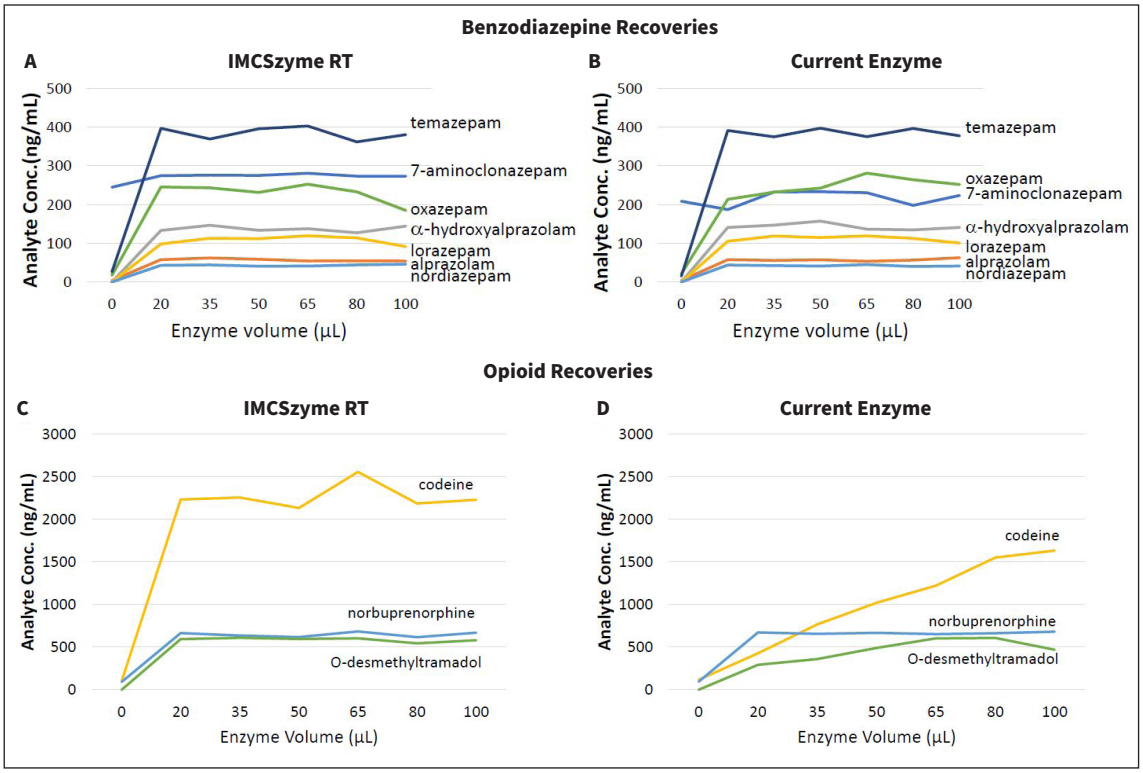

Figure 1: *Comparison of benzodiazepine analyte concentrations (ng/mL) between IMCSzyme RT and CE after 15-minute room temperature hydrolysis (**A,B**). A fixed urine sample volume (100 µL) was hydrolyzed with enzyme volumes ranging from 0 µL (no enzyme control) to 100 µL. Comparison of opioid recoveries (ng/mL) for codeine, norbuprenorphine, and o-desmethyltramadol between IMCSzyme RT and CE (**C, D**). A fixed urine sample volume (100 µL) was incubated with various volumes of enzymes from 0 µL (no enzyme control) to 100 µL.*

POSTER SUMMARY

RESULTS CONTINUED

Finally, time and enzyme-dependent enzyme differences were observed (**Figure 2**). Recoveries were determined from pooled urine samples hydrolyzed at 5 or 30 minutes with IMCSzyme RT or CE. There is a dramatic difference in codeine recovery shown between IMCSzyme RT and CE for both the 5-minute and 30-minute incubations. Lower concentration targets (hydromorphone, morphine, o-desmethyltramadol, and oxymorphone) show recovery differences for both enzymes and incubation times. Higher recoveries for CE were observed for the initial 5 to 15 minutes of incubation (buprenorphine, norbuprenorphine, EDDP, hydrocodone, and oxycodone); however, recoveries decreased after 30 minutes. EDDP, hydrocodone, and oxycodone are non-glucuronidated targets and warrant further investigation. Recovery inconsistencies may be due to LC and ion-peak detection.

Figure 2

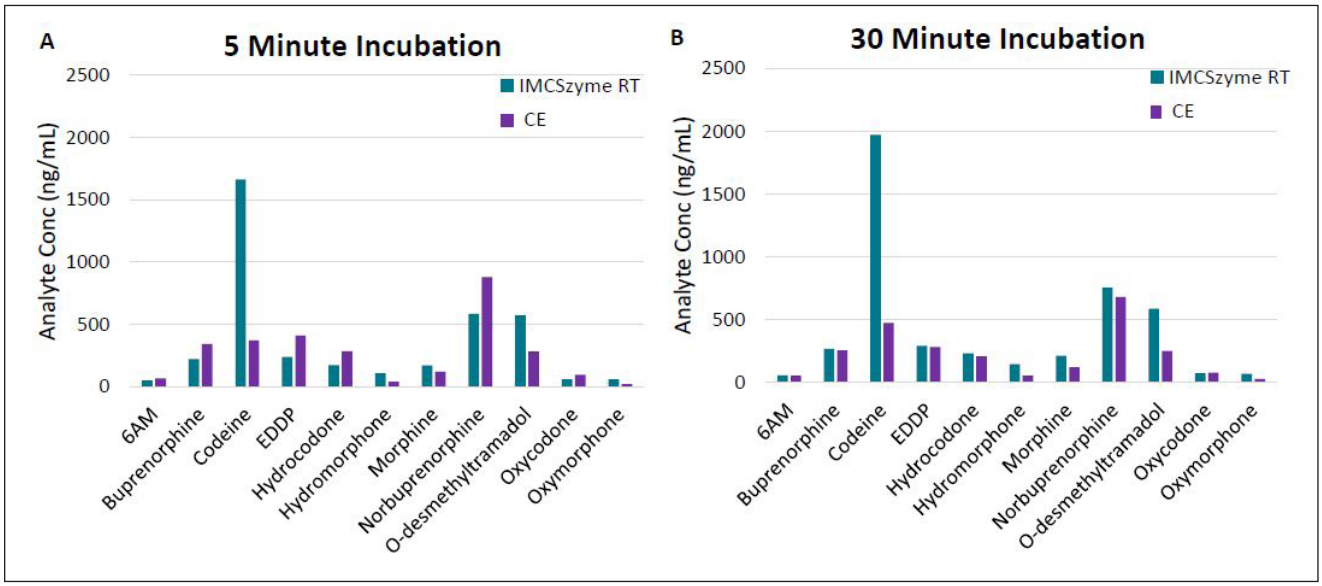

Figure 2: *Recoveries from pooled urine samples hydrolyzed at 5 or 30 minutes with IMCSzyme RT or CE.*

CONCLUSIONS

Similar performance was seen for hydrolyzing benzodiazepines using either enzyme at 20µL. For opioids, IMCSzyme RT showed better recoveries for codeine using less enzyme compared to CE. Reduced recoveries may be attributable to urine inhibitors, but further investigation is warranted. Recovery discrepancies between the two enzymes require further studies for drug levels near the limit of quantitation. For instance, if a codeine cutoff is at 50 ng/mL, a low codeine level would show positive with IMCSzyme RT and negative with CE.

A fixed enzyme amount of 100 µL for RT and CE and varying incubation times did not improve recoveries for CE. A possible explanation for this may be attributed to a recent JAT publication (Lee et al., 2021) revealing that urine inhibits or reduces the efficiency of enzymes to varying degrees, resulting in lower recoveries of certain analytes. Future studies identifying the specific inhibitors are underway.

IMCSzyme RT has separated enzyme and buffer solutions, whereas CE is a combined “all-in-one” enzyme buffer format. Since the relative enzyme:buffer ratio of CE is unknown to users, future direct comparison studies will follow each manufacturer’s recommended conditions. In addition, a larger sample size of various patient urine would allow better evaluation of these enzymes.

This information was summarized by IMCS from the poster “Evaluation of New β glucuronidase for Urine Drug Testing” presented by Rasha Abddelgader, MD - Baylor Scott & White Medical Center at MSACL 2022