JOURNAL SUMMARY

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integrated micro-chromatography systems

Evaluation of Enzymatic Hydrolysis Efficiencies for Amitriptyline and Cyclobenzaprine in Urine

A summary of the article: "Variations in enzymatic hydrolysis efficiencies for amitriptyline and cyclobenzaprine in urine" Kaylee R. Mastrianni, L. Andrew Lee, William E. Brewer, Nagarju Dongari, Michael Barna and Stephen L. Morgan (2016) Journal of Analytical Toxicology doi: 10.1093/jat/bkw062.

Overview:

Amitriptyline, a widely used tricyclic antidepressant, and cyclobenzaprine, a tricyclic skeletal muscle relaxant, are metabolized to form quaternary ammonium linked glucuronides. In order to accurately quantify these metabolites by LC-MS/MS, the glucuronide conjugates are hydrolyzed. The use of IMCSzyme® for hydrolysis of amitriptyline and cyclobenzaprine glucuronides against three other commercially available enzymes was investigated.

Material and Methods:

Drug standards were purchased from Cerilliant Corporation. β-Glucuronidase enzymes were from Integrated Micro-Chromatography Systems, Inc (IMCSzyme®), Campbell Science (Abalone), and Sigma-Aldrich (*Helix pomatia* and *Patella vulgata*). 200 µL of urine was hydrolyzed with 100 µL of hydrolysis solution (containing buffer, enzyme and internal standard) at 60 °C with shaking. The hydrolysis times were 30 minutes for IMCSzyme® and 60 minutes for three other enzymes. The hydrolyzed samples were extracted with RP-S tip, provided by DPX Labs, LLC, and then analyzed by LC-MS/MS.

Results

Amitriptyline and cyclobenzaprine glucuronides were completely hydrolyzed by IMCSzyme®, while they were only partially hydrolyzed by three other enzymes, which also required longer hydrolysis time (Figure 1). Chromatograms also showed peaks of unhydrolyzed glucuronides in these three enzyme samples (Figure 2 b,e). In comparison, no glucuronide peak was observed in the IMCSzyme® treated sample (Figure 2 c,f).

Conclusions

IMCSzyme® hydrolyzes amitriptyline and cyclobenzaprine glucuronides more efficiently than the other commercially available enzymes with a shorter incubation time. The results suggest that the other enzymes are ineffective due to several possible factors, impurities in the enzyme extract, differences in substrate binding affinities, and superior catalytic efficiency of IMCSzyme® genetically modified enzyme.

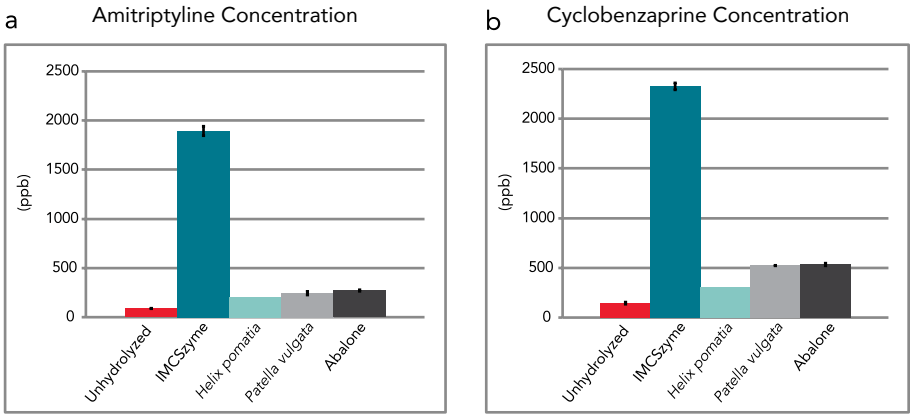

Figure 1. The concentration of the two different tricylics (a) amitriptyline and (b) cyclobenzaprine determined by LC-MS/MS. Red bar represents concentrations of the two analytes in urine prior to hydrolysis. Concentrations of two tricyclics after hydrolysis for IMCSzyme®, H. pomatia, P. Vulgata and Abalone

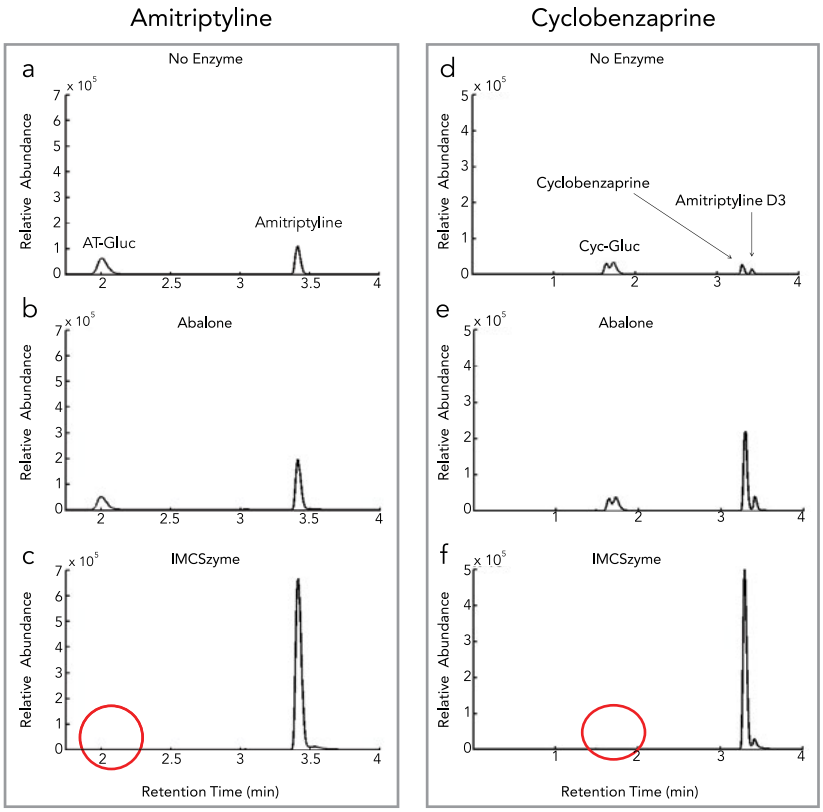

Figure 2. The comparison of chromatograms from amitriptyline (a-c) and cyclobenzaprine (d-f) glucuronides hydrolysis, using no enzyme (a, d), Abalone enzyme (b, e), and IMCSzyme® (c, f).

This information was summarized by IMCS

Reference: Kaylee R. Mastrianni, L. Andrew Lee, William E. Brewer, Nagarju Dongari, Michael Barna and Stephen L. Morgan (2016)Variations in enzymatic hydrolysis efficiencies for amitriptyline and cyclobenzaprine in urine. Journal of Analytical Toxicology doi: 10.1093/jat/bkw062.

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