JOURNAL SUMMARY

integrated micro-chromatography systems

A summary of "Advancing the analysis of terbutaline in urine samples using novel enzyme hydrolysis"

OVERVIEW

Terbutaline is a typical short-acting beta-agonist primarily used to treat pulmonary diseases and premature labor. Off-label and illicit uses of terbutaline include administering the drug to livestock as a growth promoter and as a performance-enhancing substance used by athletes. Two fast-acting enzymes were tested and compared to β-glucuronidase from crude enzyme on efficiency in hydrolyzing terbutaline metabolites in human urine samples. Mutant β-glucuronidase from *Escherichia coli* (IMCSzyme®) and recombinant aryl-sulfatase IMCS-PSF (now known as Sulfazyme™ PaS) were compared to *Helix pomatia* β-glucuronidase (Sigma). This study demonstrated that among all enzymes tested, IMCS-PSF (Sulfazyme™ PaS) hydrolyzed terbutaline conjugates in human urine samples with the highest efficiency, leading to accurate quantitation and detection of terbutaline metabolites.

METHODOLOGY

Hydrolysis efficiencies of three enzymes were compared: IMCSzyme®, IMCS-PSF (Sulfazyme™ PaS), or crude β-glucuronidase from *Helix pomatia* (Sigma). Urine samples from healthy volunteers were collected before and after oral administration of terbutaline tablets. Samples were subjected to four enzyme and sample mixture conditions (***Table 1***). The following were determined: (1) metabolite profiling with enzyme hydrolysis, (2) comparison of enzyme hydrolysis efficiencies between the three enzymes, and (3) the urine concentration profile of terbutaline and each metabolite.

Table 1. *The four enzyme and sample mixture conditions.*

Test condition	Urine	Water	Buffer	Enzyme
Blank urine sample	100 µL	75 µL	25 µL (tris buffer)	None
IMCS Sulfazyme™ PaS (IMCS-PSF)	100 µL	50 µL	25 µL (tris buffer)	25 µL
IMCS β-glucuronidase (IMCSzyme®)	100 µL	50 µL	25 µL (tris buffer)	25 µL
Sigma H. pomatia β-glucuronidase (crude enzyme)	100 µL	50 µL	25 µL (NaOAc buffer)	25 µL

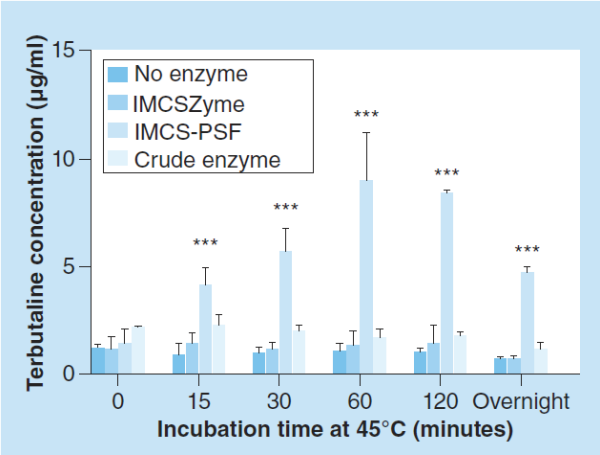

Figure 1. Comparison of the temporal hydrolysis profile of terbutaline metabolites upon hydrolysis with no enzyme, IMCSZyme, IMCS-PSF (Sulfazyme PaS) and crude enzyme. *The data are expressed as mean ± SD (n = 3). ***p ≤ 0.001 is based on ANOVA. ANOVA: Analysis of variance; SD: Standard deviation.*

RESULTS

This study demonstrated that IMCS-PSF (Sulfazyme™ PaS) enzyme shows higher hydrolysis efficiency in comparison with other enzyme reagents typically used for the analysis of terbutaline (***Figure 1***). The IMCS-PSF (Sulfazyme™ PaS) enzyme alone is sufficient to hydrolyze terbutaline conjugates in urine for the analysis of terbutaline. Terbutaline sulfoconjugates are the major metabolite of terbutaline, while glucuronide conjugates appear as minor metabolic products. Prolonged incubation of hydrolysis enzyme and urine sample should be avoided to prevent terbutaline degradation.

CONCLUSION

This study demonstrated the high efficiency of the IMCS-PSF enzyme (Sulfazyme™ PaS) in hydrolyzing terbutaline conjugates in comparison with other enzyme reagents typically used for the analysis of terbutaline. Additionally, this study also found that sulfoconjugates are the main terbutaline metabolites in urine. This novel approach of analyzing terbutaline metabolites in urine may potentially aid regulatory agencies in enforcing accurate antidoping screening. Additionally, this method could be applied to the regulation of terbutaline and similar beta-agonists that are misused in livestock.

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This information was summarized by IMCS from from "Advancing the analysis of terbutaline in urine samples using novel enzyme hydrolysis" by Florence R. Wang, Jing Fei, Xiao-Lan Yu, Xi-Cheng Zhao, Qian Wang, & Kalmorat Metavarayuth in *Bioanalysis* (2018). DOI: 10.4155/bio-2018-0145