TECHNICAL NOTE

imcs integrated micro-chromatography systems

MicroSep Biological Science

A summary of "Comparison of Novelβ-Glucuronidases for Process Improvement in Urine ToxicologyWorkflows"

Overview:

Quantification and detection of metabolized drugs is made simpler with the use of a β-glucuronidase to hydrolyze glucuronides before detection of the drug compounds via LC-MS/MS. New recombinant glucuronidases have been introduced which are much cleaner and more efficient, avoiding many issues that are present with crude enzyme solutions. This study compares the substrate specificity and conversion efficiency of several different purified β-glucuronidases, as well as assessing side reactions from acetylase and sulfatase activity.

Material andMethods:

Nine different glucuronide standards were used as test substrates, and 6-acetylmorphine and 6-acetylcodeine were added to assess acetylase activity, while tapentadol-O-sulfate was used to assess sulfatase activity. All substrates were prepared at 1,000 ng/mL in S-urine. Samples were prepared in triplicate and hydrolyzed with optimum buffer and pH conditions at 55°C for 30 minutes. All products where assayed on a LCMS-8050 Triple Quad with a single injection dilute and shoot method, ESI mode, pos/neg mode switching, Restek Raptor Biphenyl2.7 µm 50 mm X 2.1 mm with water and methanol with 1% acetic acid as mobile phases. The enzymes tested in this study were IMCSzyme® (IMCS), BGTurbo™ (Kura Biotec), SRE0093 (Sigma-Aldrich), Abalonase™ and Abalonase™+ (UCT).

Results

Figure 1. Glucuronidase Substrate Specificity

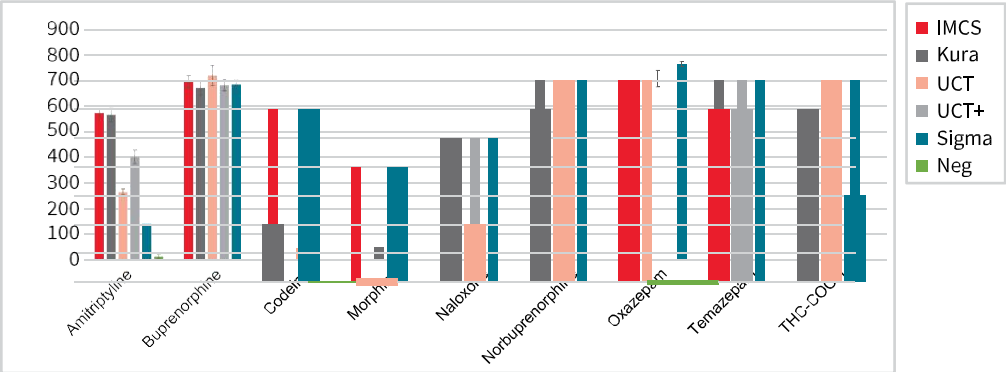

Table1

Substrate	IMCS	Kura	UCT	UCT+	Sigma	Neg	100% Yield (ng/mL)
Amitriptyline N-β-D-gluc	94%	93%	43%	66%	23%	2%	612
Buprenorphine-3β-D-gluc	114%	110%	118%	112%	112%	0%	727
Codeine-6β-D-gluc	94%	29%	7%	93%	91%	1%	630
Morphine-6β-D-gluc	60%	8%	4%	57%	54%	0%	618
Norbuprenorphine-gluc	76%	74%	34%	81%	73%	0%	701
Naloxone-3β-D-gluc	100%	102%	105%	103%	107%	0%	650
Oxazepam-gluc	108%	115%	116%	117%	125%	3%	619
THC-COOH-gluc	99%	104%	99%	104%	113%	51%	662
Temazepam-gluc	97%	100%	104%	104%	108%	0%	632
Average	94%	82%	70%	93%	90%	6%	

Conclusions

The substrate specificity varied greatly among the tested enzymes (**Figure 1**). Hydrolysis efficiency of amitriptyline, codeine, and morphine in particular was wide-ranging, with IMCSzyme hydrolyzing the highest percentage of these substrates (**Table 1**). No significant acetylase or sulfatase activity was observed for any of the enzymes.

This information was summarized by IMCS

Reference: Phillip R. Gibbs, Hannah M. Milano, and Matthew D. Kibbons (2017)Comparison of Novel β-Glucuronidases for Process Improvement in Urine Toxicology Workflows. American Society of Mass Spectrometry conference -Technical poster presentation

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