CONSERVING BIORELEVANT ENZYMATIC ACTIVITY DURING *IN VITRO* ANALYSIS OF BIOTRANSFORMABLE DRUGS

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INTRODUCTION

All classes of biotransformable drugs (prodrugs) are typically analyzed in dissolution apparatuses[1] that do not include the machinery required to catalyze the biotransformation. With 94% of prodrugs relying on the patient's enzymatic activity,[2] we looked to include these constituents within our Subcutaneous Injection Site Simulator (SCISSOR™) platform,[3,4] designed to probe the pharmacokinetic properties of injectable drugs. Herein, we describe the successful integration of enzymes within the platform and the ways in which this methodology can be used to analyze the release of prodrugs.

METHOD(S)

Esterase from bovine pancreas was obtained from Sigma, USA and homogenized within an artificial extracellular matrix (aECM) obtained from Pion Inc.

The enzymatic activity was calculated by monitoring the conversion of methylparaben (MP) to 4-hydroxybenzoic acid (4H) via Acquity Arc HPLC (Waters®) after injection of an MP solution into multiple esterase-containing media.

After confirmation of the enzymatic activity and stability, representative SCISSOR assays were carried out to demonstrate the applicability of this methodology within the pre-clinical drug development pipeline.

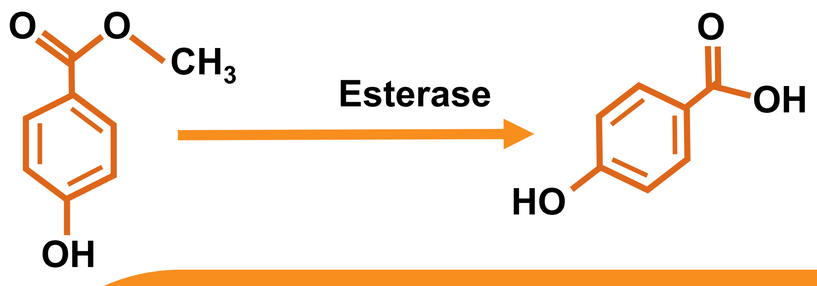

Table 1. *Each samples designated medium and conditions in which the esterase was incubated before HPLC analysis to monitor the conversion of methylparaben to 4-hydrxybenzoic acid.*

Sample	Esterase Medium	Notes
Control	**Buffer**	Immediate transfer to HPLC
Control + azide	**Buffer + Sodium azide**	aECM with Sodium azide preservative. Immediate transfer to HPLC
aECM w/o azide	**aECM without Sodium azide**	aECM without Sodium azide preservative. Immediate transfer to HPLC
24-aECM Storage	**aECM**	24 hours at storage conditions (8-12˚C, fridge) before transferring to HPLC
2-aECM	**aECM**	2 hours in SCISSOR assay (bicarbonate buffer + sodium azide) at pH 7.4, 34˚C
20-aECM	**aECM**	20 hours in SCISSOR assay (bicarbonate buffer + sodium azide) at pH 7.4, 34˚C

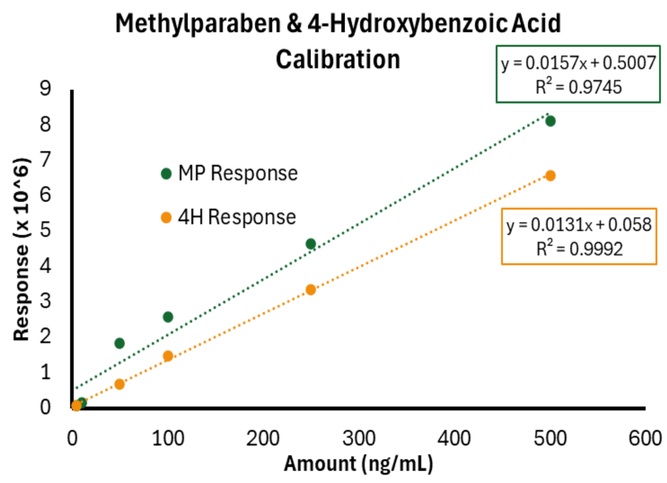

Figure 1*. Calibration curve of methylparaben (MP, green)) and 4-Hydroxybenzoic Acid (4H, orange) with their linear regression fits.*

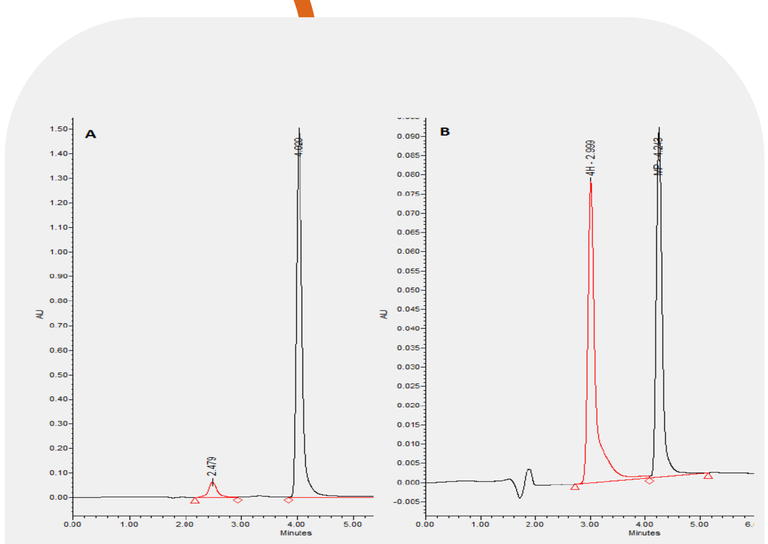

Figure 2. *A) Representative chromatogram of the Control sample in HPLC. B) Representative final chromatogram from Control sample in HPLC.*

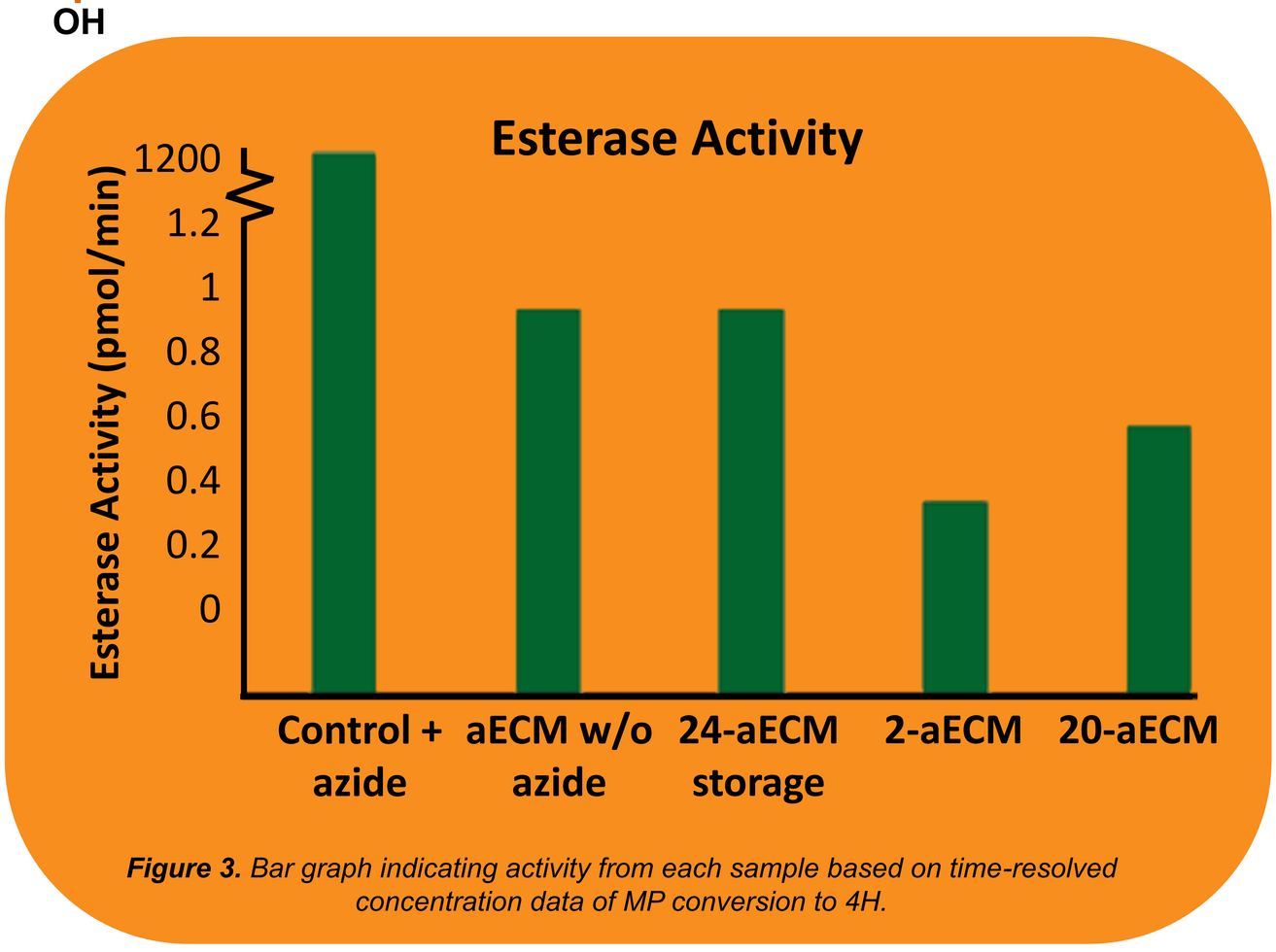

Figure 3. *Bar graph indicating activity from each sample based on time-resolved concentration data of MP conversion to 4H.*

RESULT(S)

Monitoring the esterase-mediated conversion of MP into 4H in biorelevant bicarbonate buffer, a glycosaminoglycan-based ECM, and within a SCISSOR assay all resulted in enzymatic activities of 0.5-1 pmol/min for a minimum of 24 hours. Without the steric hinderance caused by the hydrogel-like environment the esterase achieved activity 3 orders of magnitude greater, highlighting the differences between the SCISSOR assay and traditional solubilization techniques.

The SCISSOR's glycosaminoglycan-based ECM allowed the stable integration of active enzymes, as well as maintained the mechanical and optical properties that allow for reproducible bolus formation and seamless post-injection analysis.

Control samples in buffer showed activity three orders of magnitude higher than artificial ECM samples with MP, highlighting the impact of the hydrogel-like environment on esterase function.

Enzymatic activities of 0.5–1 pmol/min were sustained for at least 24 hours in all tested environments.

CONCLUSION(S)

This study demonstrates that esterase (acting on over 54% of FDA-approved prodrugs)[2] can maintain its activity throughout a SCISSOR assay, highlighting the ability to use enzymes in a dynamic *in vitro* setting to better establish the *in vitro-in vivo* relationship for prodrugs.[3,4] Current and future efforts are looking to model the release of commercial prodrug formulations.

Unlike traditional methods of *in vitro* analysis, which solely focus on solubility or dissolution in a non-biomimetic buffer, these findings provide initial validation of the platform for *in situ* analysis of biotransformable drugs (e.g. prodrugs) in an *in vitro* environment.

FUNDING / GRANTS / ENCORE / REFERENCE OR OTHER USE

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