Approaches to Account for Colon Absorption in Physiologically Based Biopharmaceutics Modeling of Extended Release Drug Products

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ABSTRACT

The rate and extent of colon absorption are important determinants of the *in vivo* performance of extended release (ER) drug products. The ability to appropriately predict this at different stages of development using mechanistic physiologically based biopharmaceutics modeling (PBBM) is highly desirable. This investigation aimed to evaluate the prediction performance of three different approaches to account for colon absorption in predictions of the *in vivo*

performance of ER drug product variants with different *in vitro* release profiles. This was done by mechanistic predictions of the absorption and plasma exposure of the ER drug products using GastroPlus® and GI-Sim for five drugs with different degrees of colon absorption limitations in humans. Colon absorption was accounted for in the predictions using three different approaches: (1) by an *a priori* approach using the default colon models, (2) by fitting the colon absorption scaling factors to the observed plasma concentration-time profiles after direct administration to the colon in humans or (3) from the ER drug product variant with the slowest *in vitro* release profile. The prediction performance was evaluated based on the percentage prediction error and the average absolute prediction error (AAPE). Two levels of acceptance criteria corresponding to highly accurate (AAPE ≤ 20%) and accurate (AAPE 20-50%) predictions were defined prior to the evaluation. For the *a priori* approach, the relative bioavailability (F_{rel}), $AUC_{0-t,}$ and C_{max} of the ER drug product variants for the low to medium colon absorption limitation risk drugs was accurately predicted with an AAPE range of 11-53% and 8-59% for GastroPlus® and GI-Sim, respectively. However, the prediction performance was poor for the high colon absorption limitation risk drugs. Moreover, accounting for the human regional colon absorption data in the models did not improve the prediction performance. In contrast, using the colon absorption scaling factors derived from the slowest ER variant significantly improved the prediction performance regardless of colon absorption limitation, with a majority of the predictions meeting the high accuracy criteria. For the slowest ER approach, the AAPE range was 5-24% and 5-32% for GastroPlus® and GI-Sim, respectively, excluding the low permeability drug. In conclusion, the *a priori* PBBM can be used during candidate selection and early product design to predict the *in vivo* performance of ER drug products for low to medium colon absorption limitation risk drugs with sufficient accuracy. The results also indicate a limited value in performing human regional absorption studies where the drug is administered to the colon as a bolus to support PBBM development

for ER drug products. Instead, by performing an early streamlined relative bioavailability study with the slowest relevant ER *in vitro* release profile, a highly accurate PBBM suitable for ER predictions for commercial and regulatory applications can be developed, except for permeability-limited drugs.

INTRODUCTION

Extended-release (ER) drug products may offer several benefits compared to immediate-release (IR) counterparts, such as minimizing side effects associated with peak plasma concentrations, delivering drugs to specific locations in the gastrointestinal tract, reducing dosing frequency, and ultimately improving patient compliance[1-5]. This is achieved by designing the ER drug product to release the drug content over an extended period, often exceeding the small intestinal transit time. Consequently, the performance of an ER drug product also relies on sufficient drug release and absorption in the colon. Therefore, accurate assessment and predictions of colon absorption are crucial for the successful development of ER drug products[1, 6, 7]. However, the colon presents challenges for drug absorption due to its small surface area, tight junctions in the epithelial cell layer affecting permeability and membrane transport, low water content, very low bile salts, and irregular motility pattern, which can restrict drug dissolution[7-13]. Furthermore, drugs may undergo bacteria-mediated luminal degradation as well as bind to the solid/semisolid intracolonic contents, potentially hindering drug absorption[14-16]. Therefore, the current *in vitro* and *in vivo* methods focus on assessing the rate and extent of colon absorption in humans[17-23]. Recently, the usefulness of physiologically based biopharmaceutics modelling (PBBM) to predict human colon absorption has been demonstrated[24]. However, it has not been evaluated how useful this is in predicting the *in vivo* performance of ER drug products. PBBM, or physiologically based pharmacokinetics modelling (PBPK) for biopharmaceutics applications, is increasingly being

used to guide oral drug product development[25-39], and FDA has issued a draft guidance to support the use of PBBM for regulatory submissions[33]. PBBM can be used throughout the drug development process to optimize the *in vivo* performance of the drug product, enabling dose optimization, risk assessment, and supporting regulatory decision-making. Several software packages, such as GastroPlus®, Simcyp®, PK-Sim®, and GI-Sim, are used to predict the *in vivo* performance of a drug product. The majority of the PBBM applications have focused on predicting the absorption after administration of IR drug products, where absorption predominantly occurs in the proximal small intestine. Less emphasis has been put on predicting the absorption of modified/extended-release (MR/ER) drug products, which pose additional challenges due to the need for modeling regional intestinal differences in absorption, and it has been stated that the prediction of colon absorption using PBBM is a current modeling and simulation capability gap[26, 40, 41]. To date, only a few investigations have been made on using PBBM to predict the *in vivo* performance of ER drug products. Most of the applications have been based on top-down approaches, where an *in vivo* understanding of the drug product was known, and few investigations accounted for colon absorption by adjusting the colon physiology parameters[8, 22, 25, 42, 43]. In addition, there has been no systematic investigation of the prediction performance for different approaches to account for colon absorption in PBBM of ER drug products for drugs with different risk levels of colon absorption limitations. Therefore, this study aimed to evaluate the prediction capability of GastroPlus® and GI-Sim for ER drug products where colon absorption was accounted for using three different approaches: (1) by an *a priori* approach using the default colon models or (2) by fitting the colon absorption scaling factors to the observed plasma concentration-time profiles from direct human colon administration or (3) from ER drug product variants with the slowest *in vitro* release profile. The prediction results were evaluated in relation to the assigned colon absorption limitation risk.

MATERIALS AND METHODS

Model drug selection & dataset establishment

The model drugs in this study were selected based on the availability of colon absorption data from human regional absorption studies and *in vitro* release profiles for several ER drug product variants with corresponding human *in vivo* pharmacokinetic data together with a mechanistic understanding of the factors affecting their regional and colon absorption (**Table 1**).

Table 1. Drug-specific model input parameters used for the PBBM of the model drugs

Parameter	Unit	Metoprolol[44]	Oxprenolol[44]	AZ2[44]	AZ1[44]	Ximelagatran[44]
Molecular weight	g/mol	267.36	265.34	250±5	500±5	429.5
LogD (pH)		1.74 (logP)	0.14 (7.4)	0.8 (6.7)	4.3 (7.4)	-1.3 (7.4)
pKa		9.18 (base)	9.5 (base)	10.7 & 7.18 (acid)	3.05 (base)	2 (acid), 7 & 11.5 (base)
Solubility (pH)	mg/ml	43 (6.5)	15 (6)	0.06 (1.8)	0.007 (7.4)	215 (7)
Diffusion coefficient	$10^{-5}\times cm^2/s$	0.8169	0.8203	0.8427	0.5704	0.6336
Drug particle density	g/ml	1.2	1.2	1.2	1.24	1.2
Particle radius	µm	25	25	25	5	25
Human jejunal P_{eff}	$10^{-4}\times cm/s$	1.34	5.1	2.03	8.8	0.10
FPE	%	40.126	29.15	0	64	64
B:P		1.07	0.9	1	0.6	0.6
F_{up}	%	90	12	26	0.1	86
CL_H	l/h/kg	0.552024	0.33731	0.16194	0.1569	0.02944
CL_R	l/h/kg	0.061336	0	0	0.00008342	0.08256
V_c	l/kg	1.5304	0.22416	0.03808	0.174	0.134
k_{12}	1/h	1.3633	9.0486	16.018	3.267	0.31624
k_{21}	1/h	1.4717	5.9581	9.5998	0.84	0.11501
k_{13}	1/h	20.639	0.31433	5.7453	0.373	0
k_{31}	1/h	27.641	0.03505	1.1587	0.0142	0

FPE- First pass extraction, B:P- blood to plasma ratio, F_{up}- fraction unbound in plasma, CL_H- hepatic clearance, CL_R- renal clearance.

The selected drugs were categorized into low, medium, and high colon absorption limitation risk based on the *in vitro* permeability and dose to solubility ratio. A drug is considered to be highly permeable when the *in vitro* permeability is higher than that of a high permeability marker, such as metoprolol or minoxidil, or when the permeability corresponds to a fraction

absorbed in the colon ($Fabs_{colon}$) of 85% or higher according to previously established *in vitro* apparent permeability (P_{app})-$Fabs_{colon}$ correlation[7, 44]. The estimated dose to solubility ratio is based on the dose of non-solution formulations administered directly to the colon in the human regional absorption studies, in relation to the buffer solubility in the pH 6.3-6.8 range determined according to internal AstraZeneca best practice (**Table 2**)[7].

Table 2. Classification of the model drugs based on colon absorption limitation risk

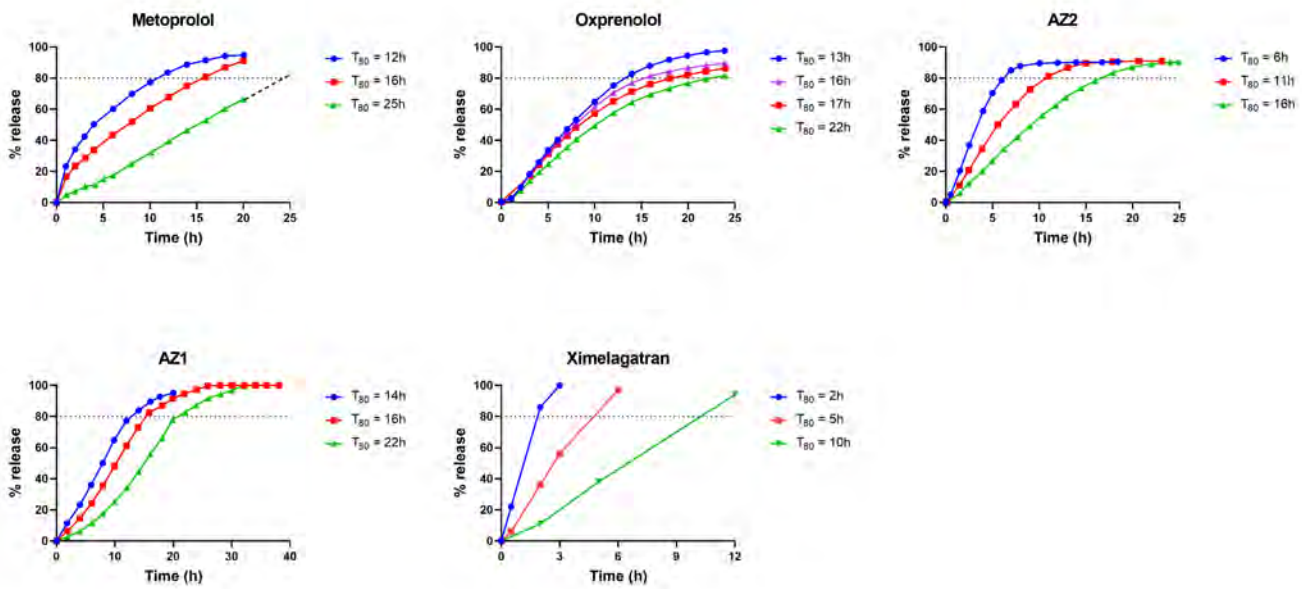
Drug	**Permeability class**	**Dose: solubility ratio**	**Colon absorption risk (Limiting factor)**
Metoprolol	High	Low	Low (-)
Oxprenolol	High	Low	Low (-)
AZ2	High	Medium	Medium (Solubility/Dissolution)
AZ1	High	High	High (Solubility/Dissolution)
Ximelagatran	Low	Medium	High (Permeability)

All clinical data were gathered from previously published work or studies performed in-house at AstraZeneca. The human effective permeability (P_{eff}) for the model drugs used in the predictions was either the measured clinical value or estimated using measured *in vitro* intrinsic Caco-2 apparent permeability (P_{app}) values combined with a previously established Caco-2 P_{app} – human P_{eff} correlation[44-46]. *In vitro* buffer solubility data combined with the pKa values were used to establish a pH-solubility profile used in the predictions according to the Henderson-Hasselbalch equation (**Table 1)**. The mean particle radius of 25 µm was used for drugs where particle size data were not available. In GastroPlus®, the default solubility (Sol) factor of 50 was used for all drugs[44]. It was assumed that no precipitation occurred *in vivo*, and the mean precipitation time was adjusted accordingly[44]. All the pharmacokinetic/disposition parameters used in the model development were estimated by fitting the intravenous and/or oral solution data to either two or three compartment pharmacokinetic models. The same pharmacokinetic

parameters were used in each software during the predictions. For the prodrug ximelagatran, the disposition model was developed based on the appearance of the active drug in plasma after absorption[47]. The hepatic extraction ratio (E_H) was either obtained directly from literature or estimated by **Equation (1)**[48].

$$E_H = \frac{CL_H}{Q_H \times B{:}P} \quad \textbf{(1)}$$

where Q_H (1.5 L/min), CL_H, and B:P are the hepatic blood flow, hepatic clearance, and blood to plasma ratio, respectively. CL_H was calculated by $CL = CL_H + CL_R$, where CL_R is the renal clearance. The physicochemical, biopharmaceutics, and pharmacokinetic input parameters for all drugs are summarized in **Table 1**. The *in vitro* dissolution profiles used in the modeling of ER drug product variants were generated using discriminatory USP I or II methods, which all were the established methods to support the development of each drug product[44]. In addition, the *in vitro* dissolution method for oxprenolol was used to establish a relationship with *in vivo* drug release[49]. The ER drug product variants for the model drugs were categorized as fast, medium, and slow based on the time to release 80% of the drug content *in vitro* (T_{80}) (**Figure 1**). Each *in vitro* release profile was fitted to a Weibull function prior to the ER predictions.



147

148 **Figure 1.** Mean *in vitro* release profiles for the extended-release drug products of the model

149 drugs with fast (blue), medium (red & purple), and slow (green) release rates, which were used

150 in the predictions after fitting to a Weibull function.

151

152 **Investigated absorption models**

153 *GastroPlus*® 9.8.1

154 GastroPlus® is based on the advanced compartmental absorption and transit (ACAT) model

155 consisting of nine compartments to mimic the human GI tract. The detailed description of the

156 model is described elsewhere[50, 51].

157 In GastroPlus®, the absorption of a drug from a compartment is based on **Equation (2)**:

158

159 $$\frac{dM_{i(absorbed)}}{dt} = Ka'_i \times V_i \times (C_{lumen_i} - C_{ent,u_i}) \qquad \textbf{(2)}$$

160

161 where $dM_{i(absorbed)}/dt$ is the rate of absorption, Ka'_i is the absorption rate coefficient, $C_{lumen\ i}$ is

162 the concentration in the lumen, $C_{ent,u\ i}$ is the unbound concentration of drug in the enterocyte

163 subcompartment, and *i* indicates a particular compartment[51]. In addition, GastroPlus® includes

an Absorption Scale Factor (ASF) in its model. The ASF multiplier scales the effective permeability to adjust for variations in surface-to-volume ratio, pH effects, and other absorption rate-determining effects that may vary from one gastrointestinal compartment to another according to **Equation (3)**:

$$Ka_i' = ASF_i \times P_i \quad \textbf{(3)}$$

where ASF_i and P_i are the absorption scale factor and effective human permeability of the i^{th} compartment, respectively[51]. The default Opt LogD V6.1 model was selected and used in this study.

GI-Sim 5.6

GI-Sim is a mechanistic PBBM software developed within AstraZeneca. The GI-Sim absorption model is divided into nine physiological compartments coupled in series: stomach (1), the small intestine (2-7), and the colon (8-9). The detailed description of each compartment is described elsewhere[52]. The current GI-Sim model considers the unidirectional transfer of uncharged drugs through the intestinal wall. The absorptive flux (dM/dt) is calculated by **Equation (4)**:

$$\frac{dM_{(absorbed)}}{dt} = A \times P_{mem} \times (C_{lumen} - C_{ent,u}) \quad \textbf{(4)}$$

Where A is the surface area available for absorption, P_{mem} is the membrane permeability of uncharged molecule, C_{lumen} is the total drug concentration in the lumen, and $C_{ent,u}$ is the unbound

drug concentration in the enterocyte sub-compartment. The current GI-Sim model has an option enabling drug administration to any of the nine compartments for regional drug absorption investigations as described earlier[24].

Modelling strategy

The overall modeling strategy was to evaluate the prediction performance of three different approaches to account for colon absorption in the prediction of the vivo performance of ER drug product variants of the model drugs. Firstly, an *a priori* approach using the current default colon models was evaluated to reflect an early ER product development scenario when no *in vivo* understanding exists, e.g., during candidate selection or product design. Secondly, the ASFs or area in the colon model in GastroPlus® and GI-Sim, respectively, were optimized to fit the mean plasma concentration-time profile observed after direct administration of the drug to the colon to reflect a situation where an early human regional absorption study was performed to generate a mechanistic understanding of the extent of colon absorption. Finally, the ASFs or area in the colon model in GastroPlus® and GI-Sim, respectively, were optimized to fit the observed mean plasma concentration-time profile of the slowest ER variant for each model drug to mimic a situation where *in vivo* data of prior ER prototypes are available. The slowest release profile was used since it represents the scenario when the highest fraction is released in the colon, and the colon absorption limitation is the highest. Mechanistic models were developed for the prediction of the relative bioavailability (F_{rel}), the area under the curve (AUC_{0-t}), and maximum plasma drug concentration (C_{max}) for the ER drug product variants in both GastroPlus® (ver. 9.8.1003, Simulations Plus Inc., Lancaster, CA) and GI-Sim (ver. 5.6, AstraZeneca). Initially, an intravenous model was developed using PKPlus® compartmental modeling in GastroPlus®. All simulations were performed using compartmental modeling

without the addition of any specific enzymes or transporters. Furthermore, it was assumed that no luminal degradation occurred in the GI tract. The compartment model with the best-fitted weighting function, based on lowest value of the Akaike Information Criterion (AIC) and Schwarz Criterion (SC), was used to calculate the disposition parameters. The human fasted physiology model with a body weight of 70 kg was used throughout the GastroPlus® simulations. Oral immediate-release models for solution and tablet drug products were developed based on the intravenous disposition parameters (except for AZ2, where disposition parameters were calculated based on oral solution data) and the other input parameters (**Table 1**). Lastly, an ER drug product model was developed where three different approaches to account for colon absorption in the predictions were evaluated (**Figure 2**).

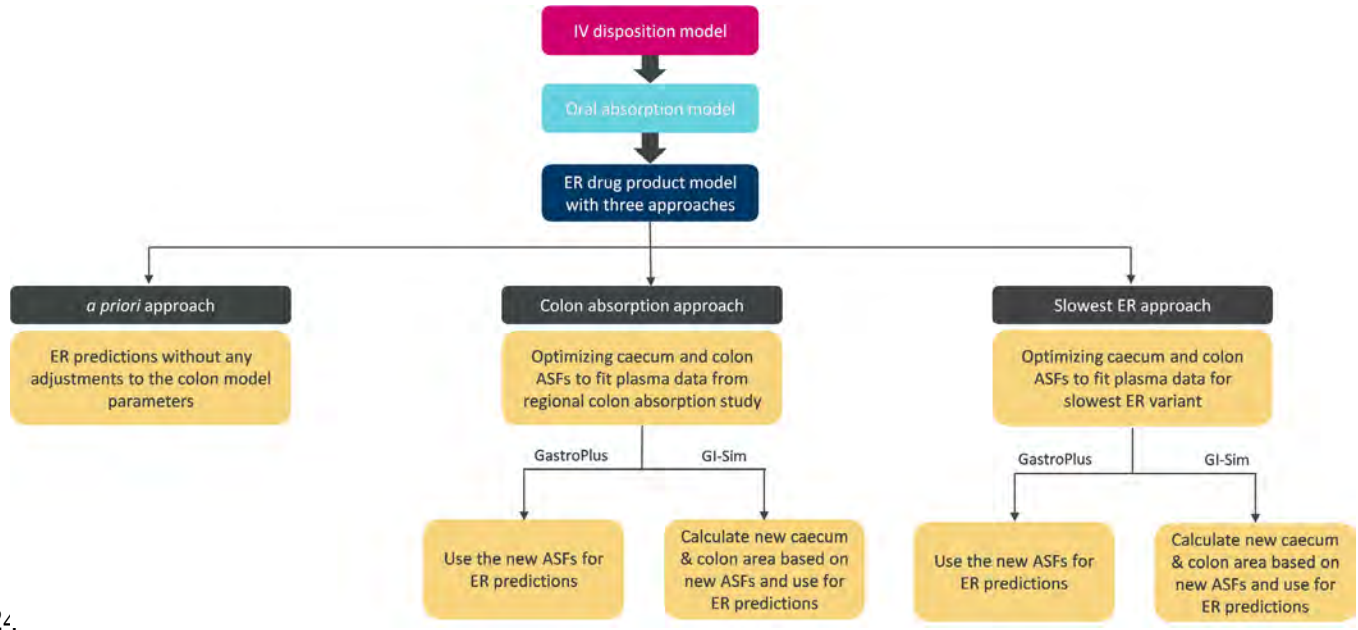


Figure 2. PBBM strategy for the ER drug products, including the three approaches to account for colon absorption. ASF: Absorption scale factor

a priori prediction approach

The oral absorption models for either solutions or IR tablets, in combination with *in vitro* release data, were used for model development for each ER drug product variant. No fittings were performed to the observed data in either software package.

Colon absorption approach

In the colon absorption approach, the *a priori* developed ER model for each model drug was modified to account for the observed plasma data after direct colon administration of the model drugs generated in the human regional absorption studies. This was done by initially developing a regional colon absorption model for the model drugs as previously described[24]. To mimic colon administration in GastroPlus®, the transit times of all compartments before the caecum were set to 0.0001 hour, and the percentage of fluid in the stomach and small intestinal compartments changed to 0.001%. In GI-Sim, this was achieved by administering the model drugs directly to the caecum compartment (8). The developed regional colon absorption model for each drug was further optimized with caecum and colon ASFs in GastroPlus® to capture the rate and extent of drug absorption through the colon. This was done by using the 'Optimization module' of GastroPlus®. These adjustments were analogues to adjustments of ASF values in previous publications[22, 42]. The optimized ASF values were then used to predict the *in vivo* plasma exposure of the ER drug product variants for the model drugs. To replicate the GastroPlus® optimization of ASFs in GI-Sim, the area of the caecum and colon compartment were modified based on **Equation (5):**

$$A_i = ASF_i \times V_i \quad \textbf{(5)}$$

where A_i and V_i are the area and volume of the i^{th} compartment, respectively; the default GastroPlus® and GI-Sim volumes used for the caecum (V_8) and ascending colon (V_9)

compartments were 47.49 ml and 50.33 ml, respectively. The new area for the eighth and ninth compartments was then used in GI-Sim simulations for each drug (**Equation 3**). The optimized ASFs and calculated new caecum and ascending colon areas for each drug are shown in **Table 3**.

Table 3. Absorption scale factors and corresponding surface areas for the different approaches to account for colon absorption in the predictions.

Drug	Compartment	Approach					
		a priori		Colon absorption		Slowest ER	
		ASF (G^+) (1/cm)	Area (GS) (cm^2)	ASF (G^+) (1/cm)	Area (GS) (cm^2)	ASF (G^+) (1/cm)	Area (GS) (cm^2)
Metoprolol	Caecum	0.05	28.02	2.23	105.95	0.99	46.81
	Colon	0.14	41.77	0.00	0.04	0.37	18.41
Oxprenolol	Caecum	0.07	28.02	0.28	13.39	0.01	0.38
	Colon	0.17	41.77	0.00	0.00	0.21	10.72
AZ2	Caecum	0.31	28.02	1.12	53.24	9893	469832
	Colon	0.58	41.77	1.88	94.77	0.18	9.16
AZ1	Caecum	13.00	28.02	0.07	3.37	9507	451511
	Colon	25.33	41.77	0.02	1.21	0.01	0.60
Ximelagatran	Caecum	0.02	28.02	1.57	74.70	0.21	10.02
	Colon	0.05	41.77	0.00	0.05	0.00	0.02

Slowest ER approach

For the slowest ER approach, the same methodology as for the colon absorption approach was used, but the physiology in the caecum and colon compartments used in the predictions were generated by fitting the ASFs or surface areas to the plasma concentration-time profile of the slowest ER variant for each model drug.

Model prediction performance assessment

The predictive performance of the three different approaches was evaluated based on the prediction of the maximum plasma drug concentration (C_{max}), the area under the curve (AUC_{0-t}), and relative bioavailability (F_{rel}) ($F_{rel} = AUC_{ER}/AUC_{IRreference}$) for the different ER drug product variants of the model drugs. The prediction performance was calculated individually

using percent prediction error (%PE) for each ER drug product variant and as the average absolute prediction error (%AAPE) for each model drug and colon modeling approach according to **Equation 6**:

$$\%\text{AAPE} = \frac{1}{N}\left(\sum_{i}^{n}\left|\frac{\text{predicted}_i - \text{observed}_i}{\text{observed}_i}\right| \times 100\right) \quad \textbf{(6)}$$

Also, a visual comparison between observed and predicted plasma concentration-time profiles was performed to assess the predictive performance qualitatively for all drugs.

Two levels of acceptance criteria were selected for the current ER drug product prediction performance assessment: highly accurate and accurate. An AAPE less than or equal to 20% was set for highly accurate predictions, while an AAPE of 20-50% was set for accurate predictions. Predictions with AAPE > 50% were considered to be poor. The acceptance criteria were justified as the PBBM development was based on independent studies and based on previous investigations considering different stages of drug product development[24, 38, 51].

RESULTS

The observed and predicted C_{max}, $AUC_{0\text{-}t}$, and F_{rel} of the investigated ER drug product variants for the three different approaches to account for colon absorption are presented in **Table 4**, and the observed and predicted plasma concentration-time profiles are shown in **Figures 3-7**. The overall prediction accuracy of the different approaches is summarized in **Table 5** and presented in **Figures 8-10**.

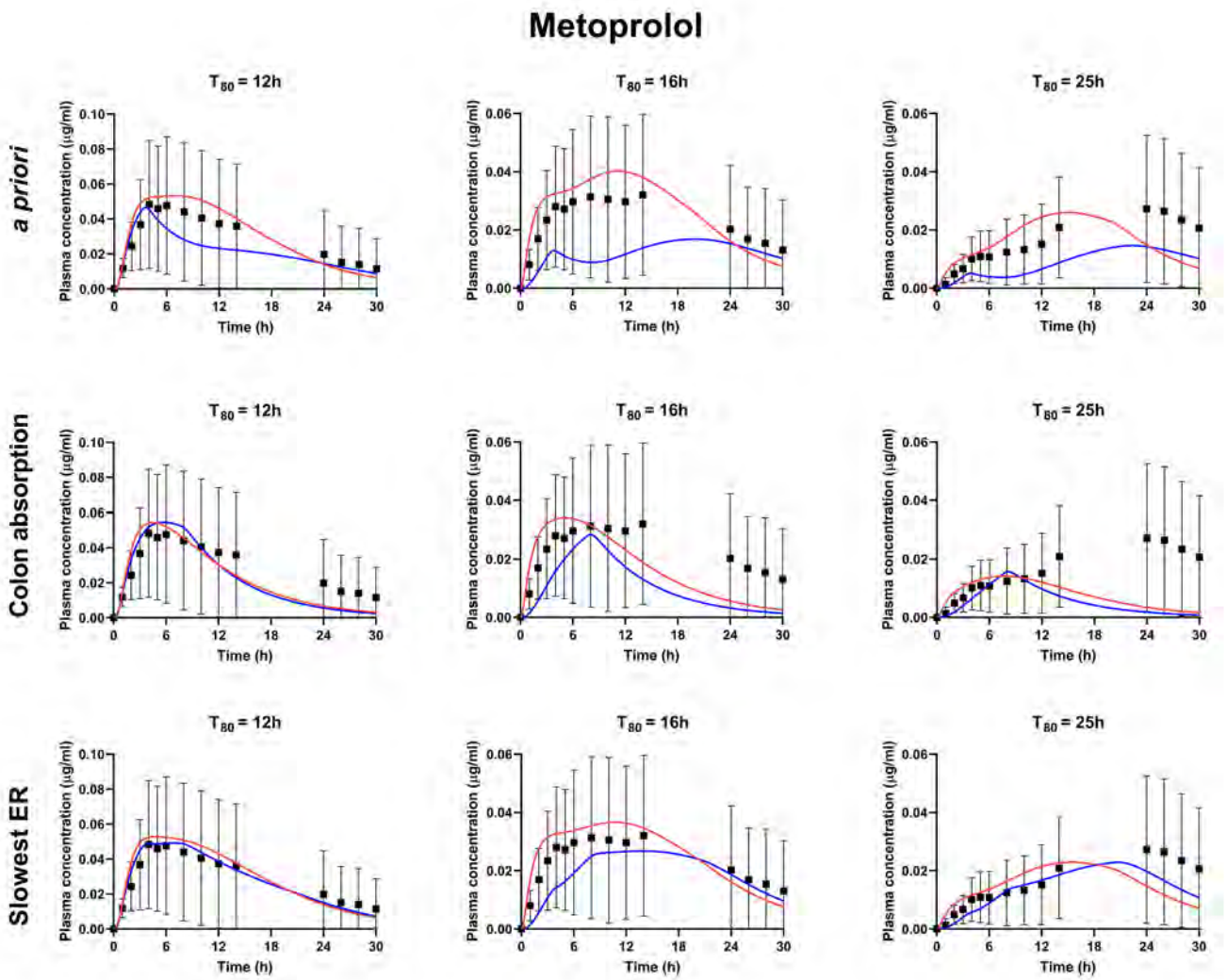
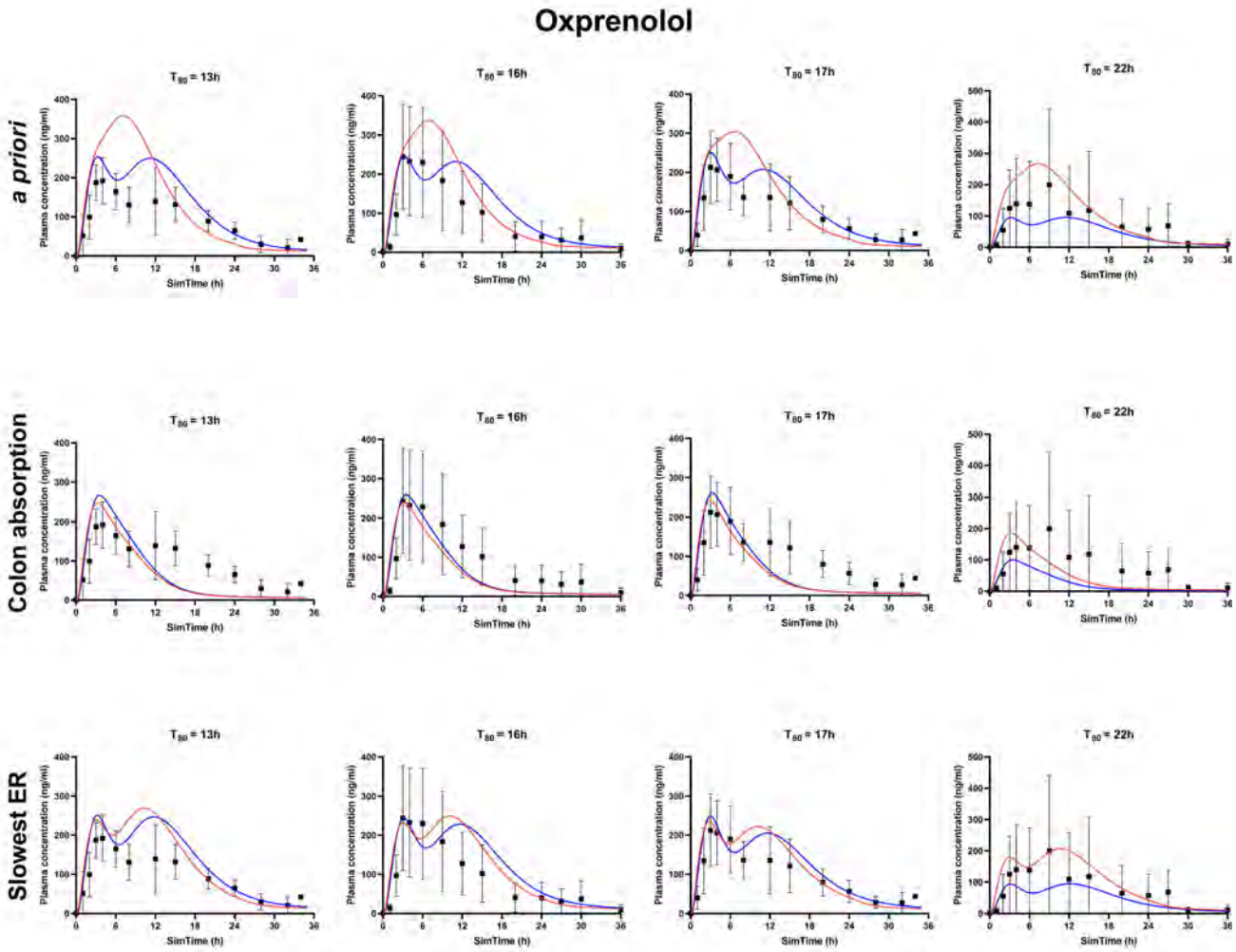


Figure 3. Mean (±CV) observed (symbols) and predicted (solid lines) plasma concentration-time profiles of the three Metoprolol ER drug product variants with different *in vitro* release rates (T_{80} of 12, 16, and 25 hours) in GastroPlus® (blue) and GI-Sim (red). The *a priori*, colon absorption, and slowest ER approaches are displayed on the top, middle, and bottom rows, respectively.



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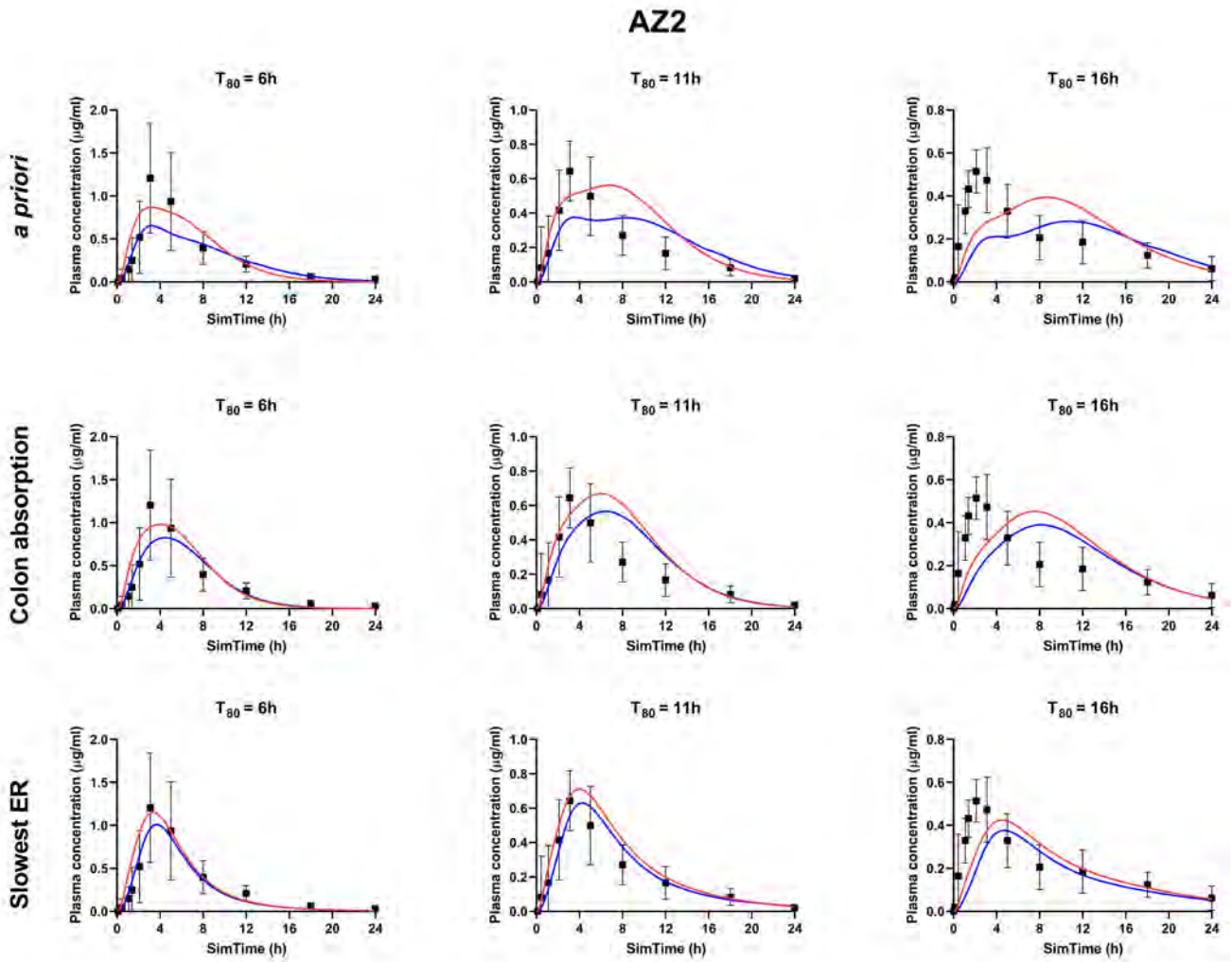
310 **Figure 4.** Mean (±CV) observed (symbols) and predicted (solid lines) plasma concentration-

311 time profiles of the four Oxprenolol ER drug product variants with different *in vitro* release

312 rates (T_{80} in 13, 16, 17, and 22 hours) in GastroPlus® (blue) and GI-Sim (red). The *a priori*,

313 colon absorption, and slowest ER approach are displayed on the top, middle, and bottom rows,

314 respectively.



315

316 **Figure 5.** Mean (±CV) observed (symbols) and predicted (solid lines) plasma concentration-

317 time profiles of the three AZ2 ER drug product variants with different *in vitro* release rates (T_{80}

318 in 6, 11, and 16 hours) in GastroPlus® (blue) and GI-Sim (red). The *a priori*, colon absorption,

319 and slowest ER approach are displayed on the top, middle, and bottom rows, respectively.

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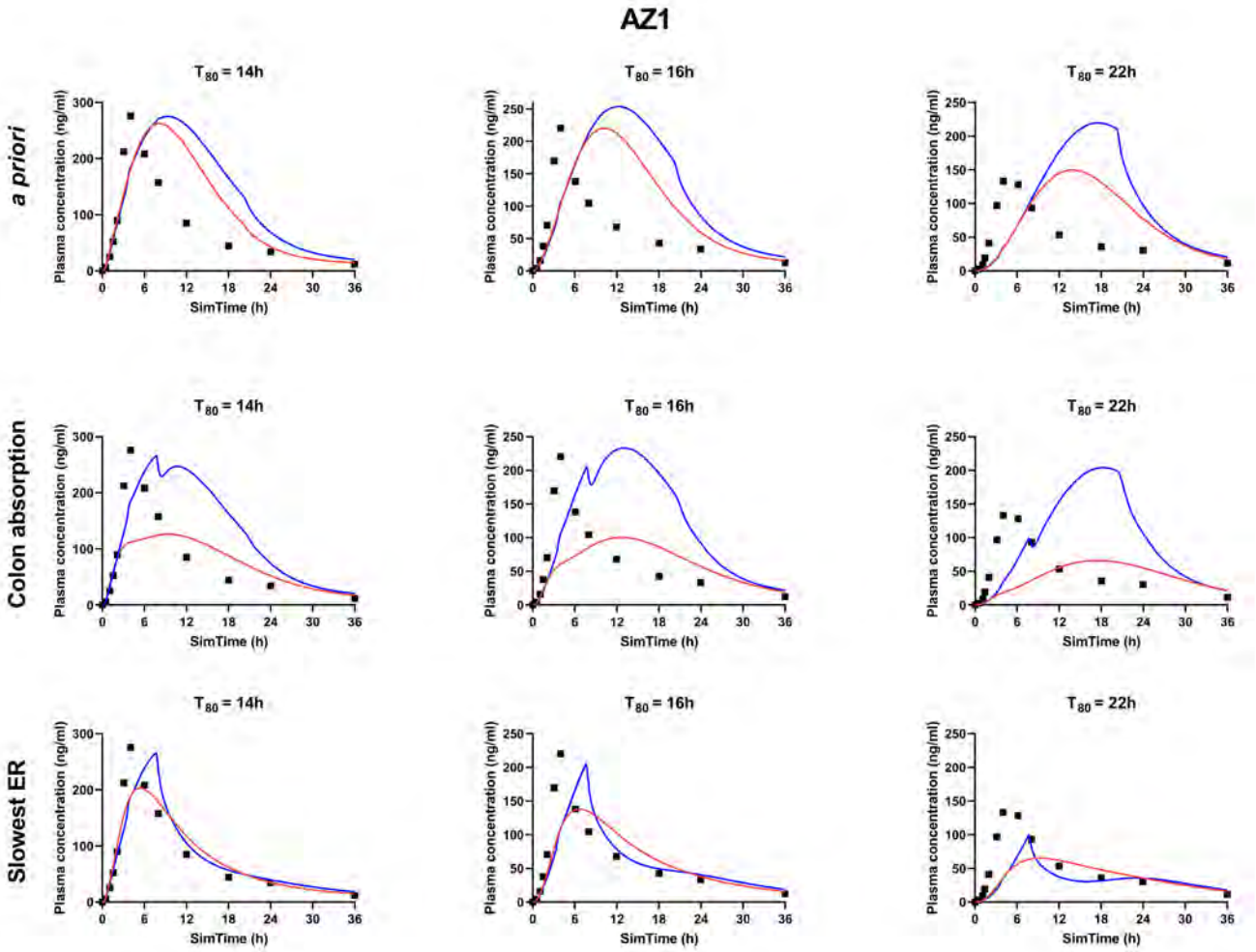

Figure 6. Mean observed (symbols) and predicted (solid lines) plasma concentration-time profiles of the three AZ1 ER drug product variants with different *in vitro* release rates (T_{80} in 14, 16, and 22 hours) in GastroPlus® (blue) and GI-Sim (red). The *a priori*, colon absorption, and slowest ER approach are displayed on the top, middle, and bottom rows, respectively.

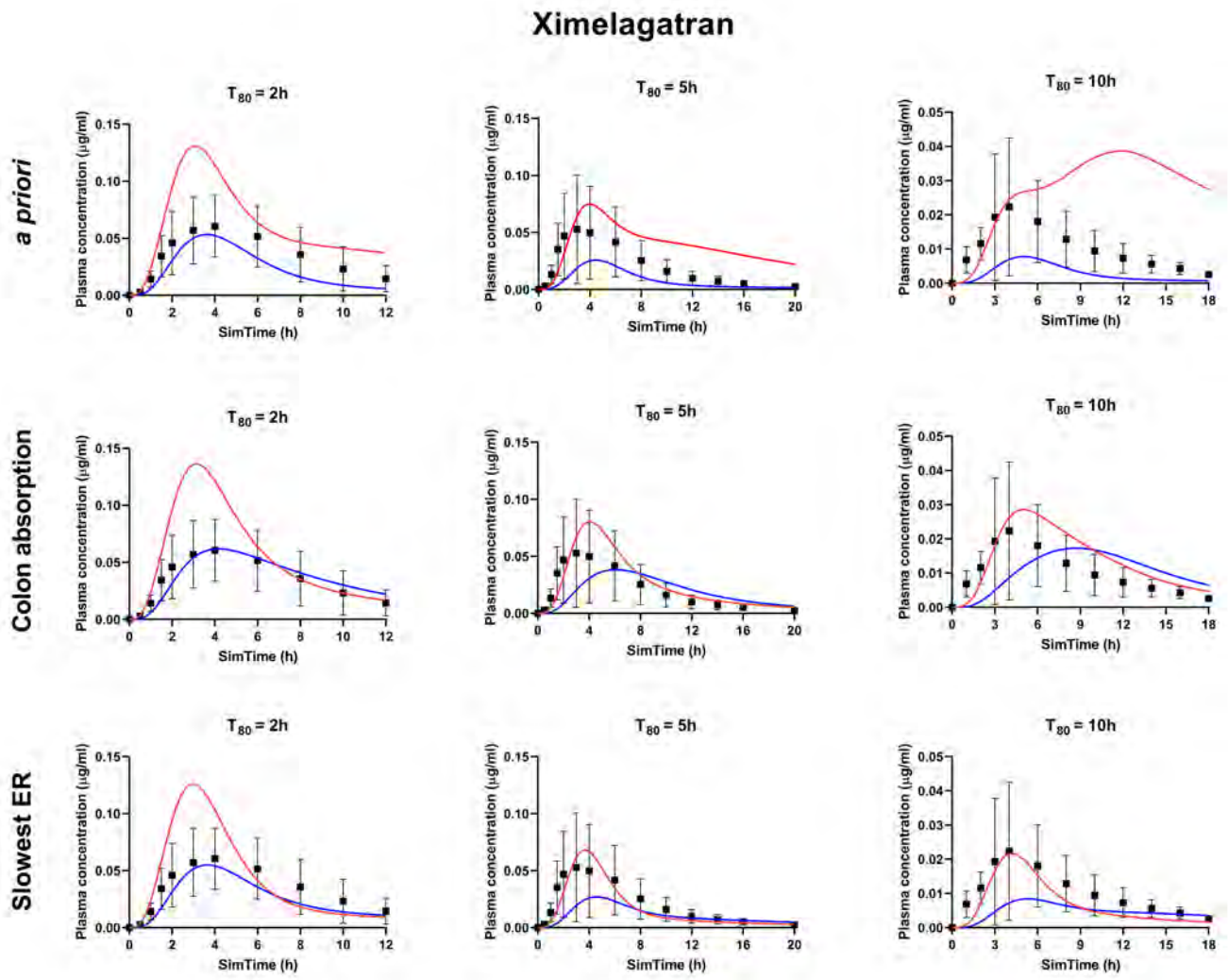

Figure 7. Mean (±CV) observed (symbols) and predicted (solid lines) plasma concentration-time profiles of the three Ximelagatran ER drug product variants with different *in vitro* release rates (T_{80} in 2, 5, and 10 hours) in GastroPlus® (blue) and GI-Sim (red). The *a priori*, colon absorption, and slowest ER approach are displayed on the top, middle, and bottom rows, respectively.

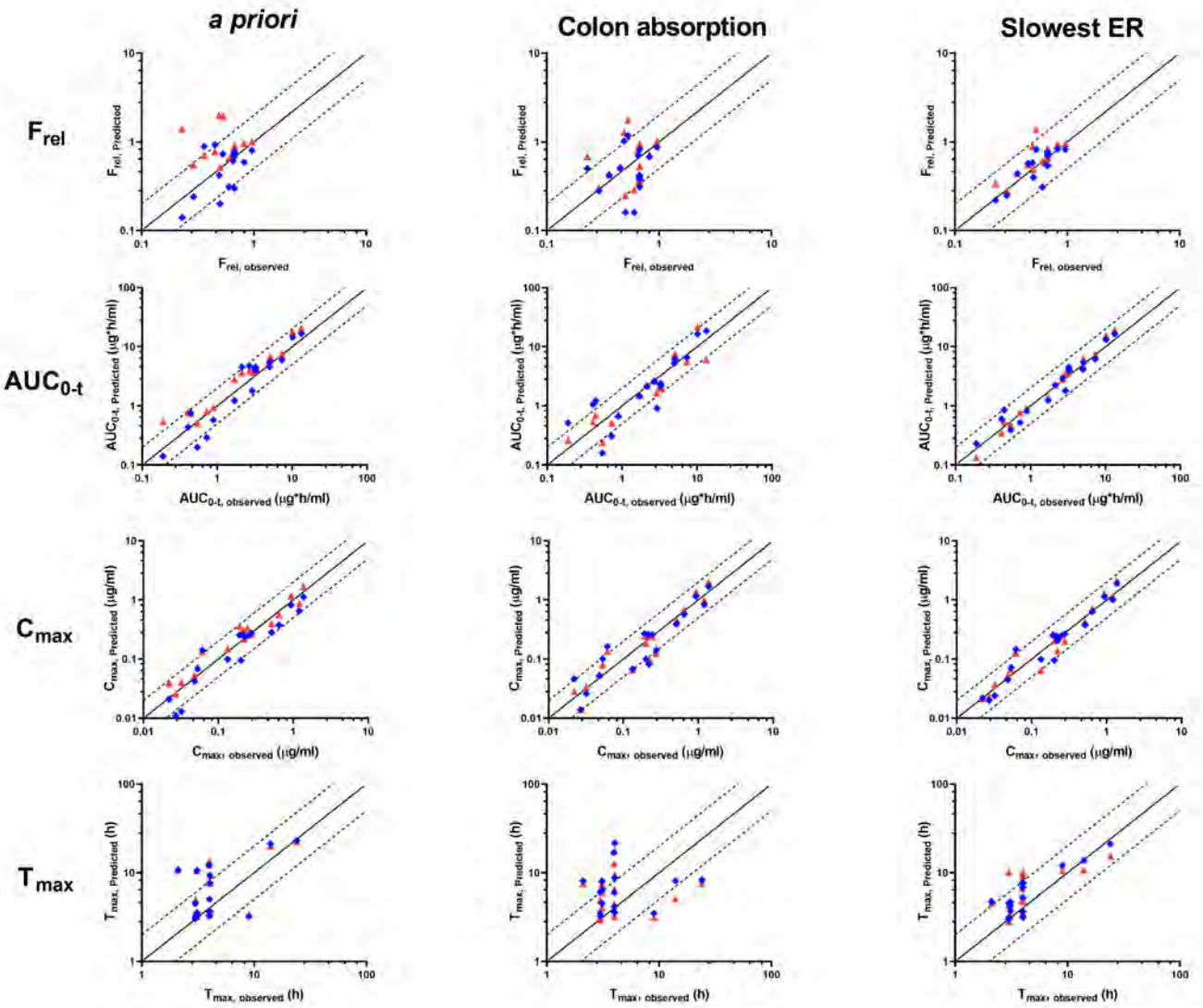

Figure 8. Overall prediction performance of F_{rel}, AUC_{0-t}, C_{max}, and T_{max} for the three different approaches to account for colon absorption in the predictions of the *in vivo* performance of the ER drug product variants for the model drugs in GastroPlus® (blue) and GI-Sim (red). Solid and dotted lines represent the line of unity and a 2-fold difference, respectively.

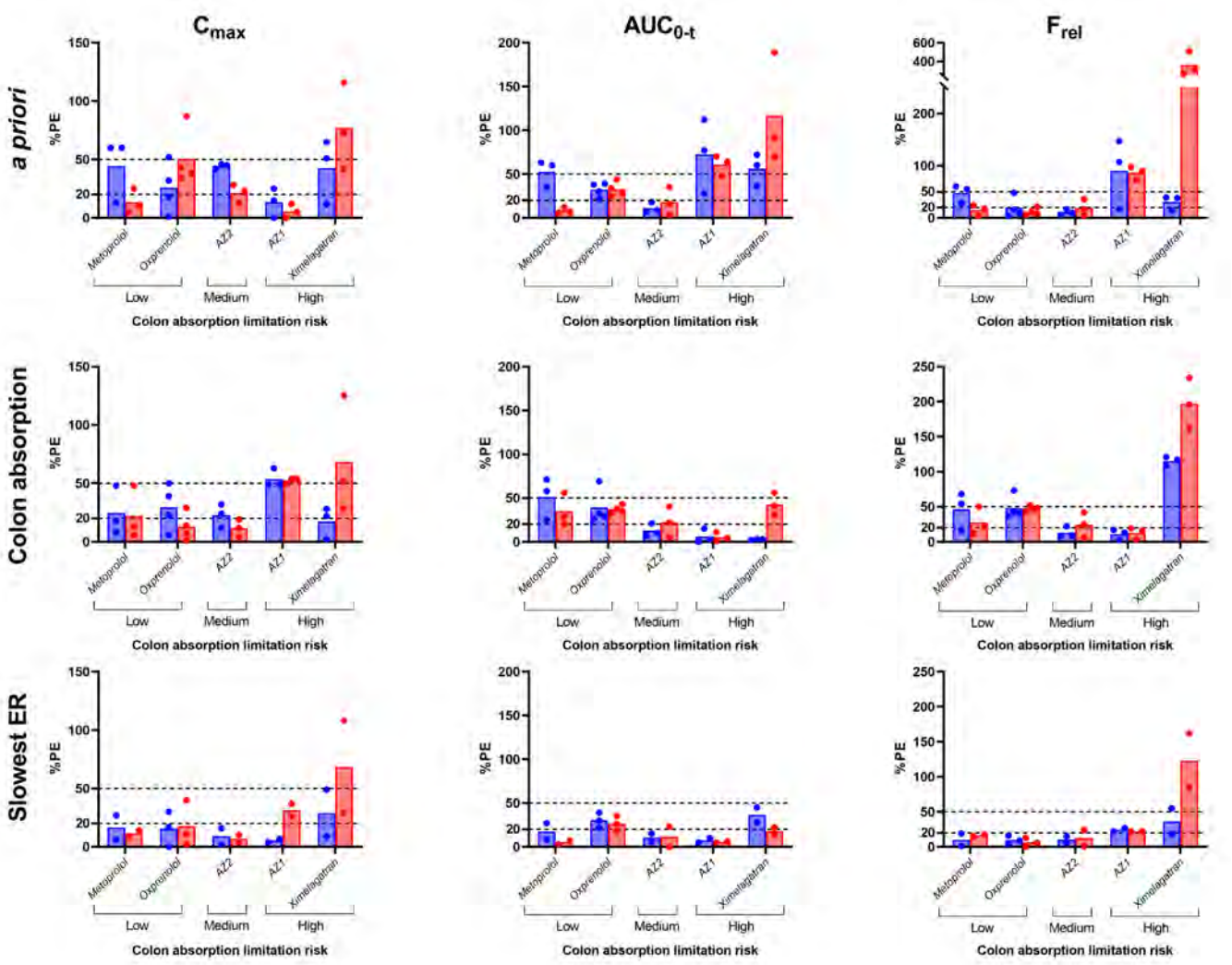

Figure 9. Prediction error of C_{max}, AUC_{0-t}, and F_{rel} for the three approaches to account for colon absorption in the predictions of the *in vivo* ER drug product performance in relation to the assigned colon absorption limitation risk for the model drugs in GastroPlus® (blue) and GI-Sim (red). The dotted lines represent the predefined criteria for highly accurate (AAPE≤20%) and accurate (AAPE 20%-50%) predictions.

Table 4. Observed and predicted human absorption and plasma pharmacokinetic parameters for the ER drug product variants of the model drugs for the different approaches to account for colon absorption in GastroPlus® & GI-Sim

Drug	Dose	Formulation	Observed values			Predicted values																	
						a priori						Colon absorption						Slowest ER					
			C_{max} (µg/ml)	AUC_{0-t} (µg.h/ml)	F_{rel} (%)	C_{max} (µg/ml)		AUC_{0-t} (µg.h/ml)		F_{rel} (%)		C_{max} (µg/ml)		AUC_{0-t} (µg.h/ml)		F_{rel} (%)		C_{max} (µg/ml)		AUC_{0-t} (µg.h/ml)		F_{rel} (%)	
						G^+	GS	G^+	GS	G^+	GS	G^+	GS	G^+	GS	G^+	GS	G^+	GS	G^+	GS	G^+	GS
Metoprolol[53]	95	Solution	0.1228	1.086	100	0.109	0.1276	0.9783	1.127	100	100												
	95	ER T_{80}:12h	0.0483	0.8867	82	0.042	0.0533	0.5734	0.9463	59	97	0.0521	0.0545	0.6659	0.7084	68	72	0.0452	0.0528	0.8118	0.9161	83	94
		ER T_{80}:16h	0.0321	0.7233	67	0.013	0.0402	0.2914	0.8122	30	83	0.0262	0.0341	0.3055	0.5151	31	53	0.0235	0.0367	0.5245	0.7739	54	79
		ER T_{80}:25h	0.0273	0.5418	50	0.011	0.0260	0.1992	0.5112	20	52	0.0142	0.0142	0.1591	0.2400	16	25	0.0195	0.0229	0.3878	0.4752	40	49
Oxprenolol[49]	105.5	IR tablet	0.6581	2.4135	100	0.9902	1.1810	2.8569	2.8667	100	100												
	212.7	ER T_{80}:13h	0.1922	3.2492	67	0.2532	0.3591	4.5050	4.6700	78	81	0.2678	0.2486	2.3949	2.1960	42	38	0.2508	0.2690	4.5064	4.3820	78	76
		ER T_{80}:16h	0.2126	3.2712	67	0.2506	0.3041	3.9963	4.1033	70	71	0.2612	0.2421	2.2085	2.0140	38	35	0.2490	0.2355	3.9980	3.8600	70	67
		ER T_{80}:17h	0.2439	3.2312	67	0.2461	0.3371	4.2027	4.3333	73	75	0.2596	0.2409	2.2868	2.0920	40	36	0.2439	0.2489	4.2023	4.0720	73	71
		ER T_{80}:22h	0.1998	2.9219	60	0.0954	0.2673	1.8014	3.7517	31	65	0.1004	0.1863	0.9113	1.6760	16	29	0.0949	0.2063	1.8061	3.5180	31	61
AZ2[44]	75	Solution	1.7887	5.6875	100	1.7792	1.8160	5.6487	6.5920	100	100												
	100	ER T_{80}:6h	1.206	7.3068	96	0.6537	0.8682	5.9933	7.514	80	100	0.8241	0.9812	6.5707	7.695	87	102	1.0106	1.153	6.1925	7.306	82	97
		ER T_{80}:11h	0.6446	5.0902	67	0.3752	0.5618	5.5709	6.872	74	91	0.5667	0.6683	6.1722	7.137	82	95	0.6303	0.7115	5.3828	6.275	71	83
		ER T_{80}:16h	0.5142	4.9578	65	0.2818	0.3941	4.6076	5.747	61	76	0.3902	0.4532	5.2866	6.069	70	81	0.3768	0.4245	4.1525	4.883	55	65
AZ1[44]	100	IR tablet	0.3192	2.981	100	0.5813	0.6170	2.5538	2.5650	100	100												
	200	ER T_{80}:14h	0.2757	2.6802	45	0.2751	0.2628	4.7548	3.9780	93	78	0.1410	0.1261	2.5981	2.6650	51	52	0.2658	0.2032	2.9353	2.8150	57	55
		ER T_{80}:16h	0.2204	2.1389	36	0.2539	0.2200	4.5381	3.6330	89	71	0.0824	0.1003	2.1356	2.2270	42	43	0.2053	0.1381	2.2413	2.2590	44	44
		ER T_{80}:22h	0.1331	1.7062	29	0.0992	0.1496	1.2269	2.8060	24	55	0.0684	0.0662	1.4518	1.5220	28	30	0.0992	0.0652	1.2540	1.4100	25	28
Ximelagatran[44]	50	Solution	0.1611	0.8304	100	0.1923	0.1459	1.0360	0.8565	100	100												
	50	ER T_{80}:2h	0.0606	0.4434	53	0.0533	0.1309	0.2846	0.7537	73	193	0.0618	0.1363	0.4567	0.6911	117	177	0.0550	0.1261	0.3206	0.5414	82	139
		ER T_{80}:5h	0.0528	0.4091	49	0.0257	0.0749	0.1635	0.7857	42	202	0.0381	0.0803	0.3958	0.5373	102	128	0.0269	0.0679	0.2268	0.3517	58	90
		ER T_{80}:10h	0.0223	0.1888	23	0.0078	0.0387	0.0527	0.5464	14	140	0.0173	0.0286	0.1935	0.2659	50	68	0.0084	0.0216	0.0866	0.1337	22	34

Table 5. Prediction error (PE, %) for the primary pharmacokinetic parameters for the three different approaches to account for colon absorption in the ER *in vivo* performance predictions in GastroPlus® and GI-Sim

Drug	T_{80} (h)	*a priori*							Colon absorption							Slowest ER						
		GastroPlus®				GI-Sim			GastroPlus®				GI-Sim			GastroPlus®				GI-Sim		
		C_{max}	AUC_{0-t}	F_{rel}		C_{max}	AUC_{0-t}	F_{rel}	C_{max}	AUC_{0-t}	F_{rel}		C_{max}	AUC_{0-t}	F_{rel}	C_{max}	AUC_{0-t}	F_{rel}		C_{max}	AUC_{0-t}	F_{rel}
Metoprolol	12	-13	-35	-28		10	7	18	8	-25	-17		13	-20	-12	-6	-8	1		9	3	15
	16	-60	-60	-55		25	12	24	-18	-58	-54		6	-29	-21	-27	-27	-19		14	7	18
	25	-60	-63	-60		-5	-6	4	-48	-71	-68		-48	-56	-50	-	-	-		-	-	-
	AAPE	44	53	48		13	8	15	25	51	46		22	35	28	16	18	10		12	5	16
Oxprenolol	13	32	39	16		87	44	21	39	-26	-37		29	-32	-43	30	39	16		40	35	13
	16	18	22	4		43	25	6	23	-32	-43		14	-38	-48	17	22	4		11	18	0
	17	1	30	9		38	34	12	6	-29	-40		-1	-35	-46	0	30	9		2	26	6
	22	-52	-38	-48		34	28	8	-50	-69	-73		-7	-43	-52	-	-	-		-	-	-
	AAPE	26	32	20		50	33	12	30	39	49		13	37	47	16	30	10		18	26	6
AZ2	6	-46	-18	-17		-28	3	4	-32	-10	-9		-19	5	6	-16	-15	-15		-4	0	1
	11	-42	9	10		-13	35	36	-12	21	22		4	40	42	-2	6	6		10	23	24
	16	-45	-7	-6		-23	16	17	-24	7	8		-12	22	25	-	-	-		-	-	-
	AAPE	44	11	11		21	18	19	23	13	13		11	23	24	9	10	10		7	12	12
AZ1	14	0	77	107		-5	48	73	-49	-3	13		-54	-1	16	-4	10	27		-26	5	22
	16	15	112	147		0	70	97	-63	0	17		-54	4	19	-7	5	22		-37	6	22
	22	-25	-28	-17		12	64	90	-49	-15	-3		-50	-11	3	-	-	-		-	-	-
	AAPE	14	73	90		6	61	87	53	6	11		53	5	13	5	7	24		32	5	22
Ximelagatran	2	-12	-36	38		116	70	264	2	3	121		125	56	234	-9	-28	55		108	22	162
	5	-51	-60	-14		42	92	312	-28	-3	108		52	31	161	-49	-45	18		29	-14	84
	10	-65	-72	-39		73	189	509	-22	2	117		28	41	196	-	-	-		-	-	-
	AAPE	43	56	30		77	117	362	17	3	115		68	43	197	29	36	37		68	18	123

359 ***a priori* prediction of the *in vivo* performance of ER drug product variants**

360 With the *a priori* modelling approach, the current absorption models in GastroPlus® and GI-
361 Sim were assessed for predicting the C_{max}, AUC_{0-t}, and F_{rel} of ER drug product variants of the
362 model drugs with different *in vitro* release rates without changing any colon physiology
363 parameters. The predefined criteria for accurate predictions (AAPE 20-50%) were met for the
364 low-medium colon absorption risk drugs for all primary prediction parameters (**Figures 8-9,**
365 **Table 5**), except for the metoprolol GastroPlus® predictions, which were borderline to accurate.
366 In predicting C_{max}, AUC_{0-t}, and F_{rel} for the low-medium colon absorption risk drugs, the AAPE
367 range was 11-53% and 8-59% for GastroPlus® and GI-Sim, respectively (**Table 5**).
368 Qualitatively, the predicted plasma concentration-time profiles were generally within the
369 interindividual variability for metoprolol and oxprenolol while less well predicted for the low
370 solubility model drug AZ2, especially for the slower ER variants (**Figures 3-5**). In contrast,
371 the criteria for accurate predictions were not met for the high colon absorption risk drugs AZ1
372 and Ximelagatran (**Figures 6-7 and 9, Table 5**), resulting in poor qualitative predictions of the
373 mean plasma concentration-time profiles. For the high colon absorption limitation risk drugs,
374 the AAPE range was 14-90% and 6-362% for GastroPlus® and GI-Sim, respectively. By
375 combining all prediction parameters for the *a priori* approach, 36% of the predictions were
376 categorized as highly accurate, 33% as accurate, and 31% as poor predictions in GastroPlus®,
377 while the corresponding values for GI-Sim were 38%, 33%, and 29% (**Figure 10**).
378

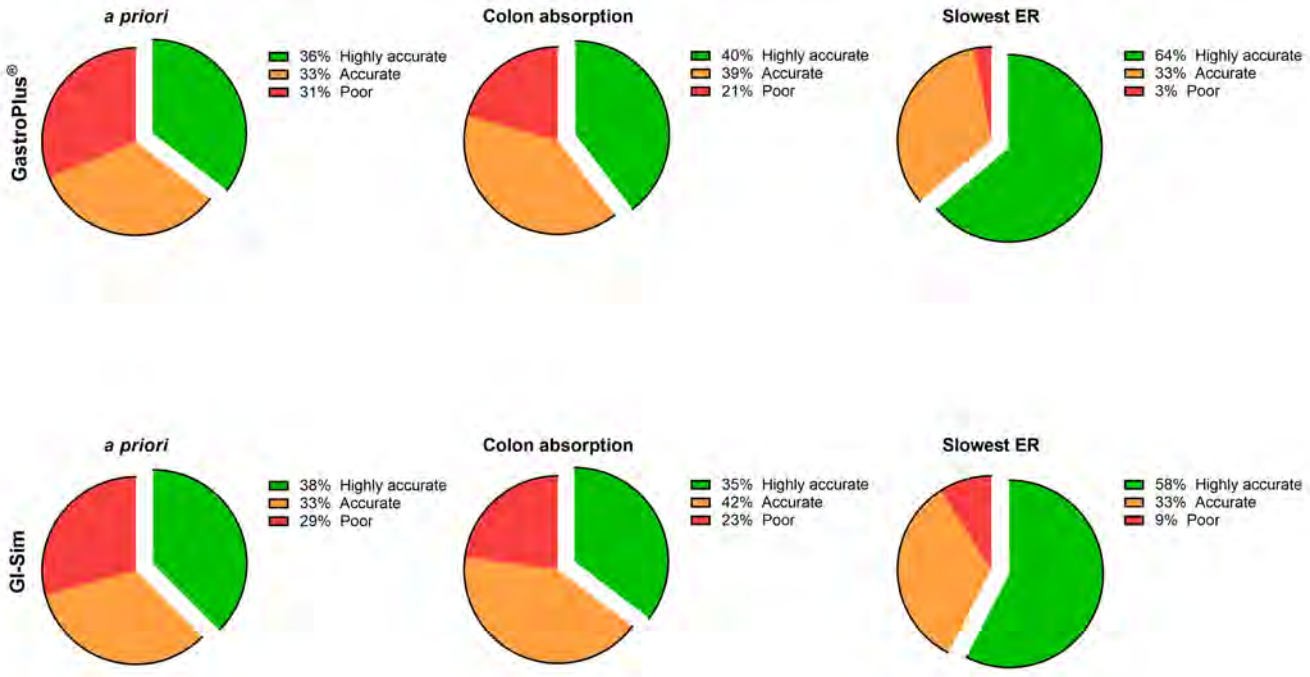

Figure 10. Percentage-distribution of the degree of accuracy of predicted pharmacokinetic parameters (C_{max}, $AUC_{0\text{-}t,}$ and F_{rel}) combined into highly accurate, accurate, and poor for the *a priori*, colon absorption, and slowest ER approaches to account for colon absorption in the prediction of the ER drug product variants using GastroPlus® and GI-Sim.

Predictive performance of ER drug product variants with the colon absorption approach

The colon absorption approach aimed to evaluate the prediction performance of the ER drug product variants when the observed plasma exposure from a human regional colon absorption study was accounted for in the PBBM development. Using this approach, the prediction performance remained unchanged compared to the *a priori* approach, and the predefined criteria for accurate predictions were met for the low-medium colon absorption limitation risk drugs (**Figure 8-9, Table 5**). There was no obvious/significant change in the prediction performance for the high colon absorption limitation risk drugs either (**Figure 8-9, Table 5**). Qualitatively, the predicted plasma concentration-time profiles for metoprolol and oxprenolol were within the observed interindividual variability but with a clear tendency toward

underprediction at the later time points, while the prediction of the plasma concentration-time profiles for the solubility/dissolution limited drug AZ2 was slightly improved compared to the *a priori* approach (**Figures 3-5**). For the high colon absorption limitation risk drugs, the predictions of the plasma concentration-time profiles were not improved for the solubility/dissolution limited AZ1, while an improvement was seen for the permeability-limited drug ximelagatran (**Figures 6-7**). In predicting C_{max}, AUC_{0-t}, and F_{rel} for low-medium colon absorption risk drugs, the AAPE range was 13-51% and 11-47% for GastroPlus® and GI-Sim, respectively (**Table 5**). On the other hand, for the high colon absorption limitation risk drugs, the AAPE range was 3-115% and 5-197% for GastroPlus® and GI-Sim, respectively. By combining all prediction parameters for the colon absorption approach, 40% of the predictions were categorized as highly accurate, 39% as accurate, and 21% as poor predictions in GastroPlus®, while the corresponding values for GI-Sim were 35%, 42%, and 23% (**Figure 10**).

Predictive performance of the ER drug product variants with the slowest ER approach

For the slowest ER approach, the observed plasma exposure for the slowest ER drug product variant was utilized to account for colon absorption in the ER predictions of the model drugs. With this approach, the prediction performance was significantly improved for all model drugs compared to the *a priori* approach, and the vast majority of the predictions of C_{max}, $AUC_{0-t,}$ and F_{rel} met the predefined criteria for high accuracy (PE≤20%) in both GastroPlus® and GI-Sim (**Figure 3-9**, **Tables 4-5**). In predicting C_{max}, AUC_{0-t}, and F_{rel} for all model drugs, except for the permeability-limited drug ximelagatran, the AAPE range was 9-30% and 5-26% for GastroPlus® and GI-Sim, respectively (**Table 5**). Qualitatively, the mean plasma concentration-time profiles were well predicted for all ER drug product variants of all drugs except for Ximelagatran. By combining all prediction parameters for the slowest ER variant

approach, 64% of the predictions were categorized as highly accurate, 33% as accurate, and 3% as poor predictions in GastroPlus®, while the corresponding values for GI-Sim were 58%, 33%, and 9% (**Figure 10**).

DISCUSSION

The main objective of this work was to evaluate how well PBBM could predict the *in vivo* performance of ER drug product variants with different *in vitro* release profiles by using three different approaches to account for colon absorption in the model development. The rationale for this approach was that it is well-established that sufficient colon absorption is a key determinant for successful ER development[1, 6, 7], and the necessity to apply different PBBM approaches at different stages in drug product development. In predictions of ER drug product performance during candidate profiling, product design, and early product development, represented by the *a priori* approach in this evaluation, the prediction accuracy relies on the general prediction performance of the model using default model settings and *in vivo* predictive input parameters since clinical *in vivo* data is not available. As the early ER PBBM applications mainly include predictions of potential colon absorption limitations, providing initial proof-of-concept ER predictions to support the high-level formulation strategy as well as defining the initial *in vitro* ER release target profile, it is appropriate to assign lower predictive performance requirements of the model at this stage. Since mechanistic human regional absorption studies, where the study drug is administered directly to the colon, can be performed to enable early clinical colon absorption and ER development suitability assessments, the “colon absorption approach” evaluated the predictive performance when the colon absorption data generated in such studies was accounted for in the model development. Finally, the evaluation of the prediction performance for the “slowest ER approach” reflected a scenario at a later stage in

the drug product development process when *in vivo* data on ER prototypes is available and can be accounted for in the colon model of ER predictions. For the latter two approaches, it is appropriate to assign higher prediction performance requirements of the model given the availability of *in vivo* data to inform model development, especially for commercial drug products and regulatory PBBM applications. Availability of PBBMs with stage-appropriate accuracy in the prediction of the *in vivo* performance of ER drug products would provide opportunities to reduce product development time, and cost since the selection of candidate drugs, the decision to initiate development or not, as well as a definition of target release profiles and *in vitro* release methods would be greatly facilitated. Accurate PBBMs would also reduce the need for clinical relative bioavailability studies. The results demonstrated that, in relation to the predefined prediction performance criteria, the *a priori* approach was sufficiently accurate to be used to predict the *in vivo* performance of ER drug products during candidate selection and early product design and development for low and medium colon absorption limitation risk drugs. In addition, the "slowest ER approach" significantly improved the prediction performance and provided the opportunity to use this methodology for ER PBBM development for commercial drug products and regulatory applications, except for low permeability drugs. In contrast, the "colon absorption" approach did not improve the prediction performance compared to the *a priori* approach.

The results also showed that the prediction performance for the *a priori* approach did not meet the predefined criteria for highly accurate prediction. This is in agreement with a recently published evaluation of the ability of PBBM to predict regional and colon absorption in humans, where accurate predictions were achieved for high permeability drugs while the prediction performance was poor for low permeability drugs[24]. The relatively poor predictions of the plasma concentration-time profiles for the ER drug product variants of the low

permeability compound ximelagatran and the borderline high permeability compound metoprolol in both GastroPlus® and GI-Sim and in GastroPlus®, respectively, are also in agreement with the previous evaluation, where it was shown that GastroPlus® underpredicted while GI-Sim overpredicted the colon absorption of low permeability drugs[24]. The fact that all ER drug product variants of all model drugs displayed various degrees of regional dependent absorption as indicated by the lower F_{rel} observed compared to the IR reference formulation. This demonstrates that PBBM of ER drug products will always be more complex than the IR counterpart for the same compound. Based on this, it was not surprising that highly accurate predictions were not achieved with the *a priori* approach. Furthermore, the results highlighted that more efforts are needed to develop more mechanistically and physiologically based colon models to achieve highly accurate *a priori* predictions of the *in vivo* performance of ER drug products.

Human regional absorption studies where the study drug is administered directly to the colon have been used to assess the extent of colon absorption and guide decisions to initiate ER drug product development[7]. Recently, the colon absorption data generated in these studies have been used to establish *in vivo* predictive *in vitro* models to enable colon absorption limitation assessments already during candidate selection[7, 23]. Interestingly, accounting for the observed plasma exposure after direct colon administration in the colon models did not improve the prediction performance. It seems that the colon ASFs generated for the low colon absorption limitation risk drugs metoprolol and oxprenolol predicted the initial part of the ER plasma concentration-time profiles well, while the later part was underpredicted. This can be explained by the fact that these compounds are rapidly and completely absorbed within a limited region after a bolus dose to the colon, whereas an ER drug product is released and absorbed through a broader region of the colon. In contrast, the colon ASFs derived for the dissolution/solubility

limited compounds AZ2 and AZ1 predicted the relative bioavailability of the ER drug product variants well, but the predicted absorption rate was slower than the observed. The colon absorption approach surprisingly predicted the ER drug product variants with 5 and 10h release profiles of the low permeability drug ximelagatran. The likely explanation for this is that the permeability rather than the release from the drug product variants determines the absorption rate, which is well captured by the colon ASFs generated based on data after a bolus dose to the colon. All in all, there seems to be a limited value in performing human regional absorption studies where the drug is administered to the colon as a bolus to support PBBM development for ER drug products. On the other hand, it should be stressed that the application of telemetric capsule techniques where the drug content is released at a defined rate throughout the gastrointestinal tract would likely be beneficial in PBBM development and predictions of ER drug products.

For the “Slowest ER approach”, the extracted ASFs from the plasma concentration-time profiles of the slowest ER drug product variants seemed to capture all the key factors related to permeability, dissolution, and release throughout the colon, which resulted in significantly improved predictions compared to the *a priori* approach for all model drugs regardless of colon absorption limitation risk. Even though this approach did not meet the high accuracy criteria in all cases, it should be considered as a very promising methodology to achieve highly predictive compound specific PBBMs qualifying for commercial and regulatory applications until highly accurate *a priori* models are available. This approach also presents opportunities to reduce product development time and costs, which includes opportunities to reduce the number or streamline the design of relative bioavailability studies. Since this evaluation aimed to directly compare the three different approaches to account for colon absorption in the predictions of the *in vivo* performance of the ER drug product variants, the number of suitable model drugs were

limited. Therefore, an additional evaluation with a larger dataset could be considered to gain further confidence in the predictive performance using the slowest ER approach. A limited dataset of drugs with distinct biopharmaceutics properties and different degrees of colon absorption limitation risks was included in this evaluation, which needed to be taken into consideration in the justification of the selected prediction performance parameter and acceptance criteria. Therefore, it was decided to treat the selected drugs as separate case studies rather than a complete dataset, which also warranted the selection of %AAPE as the prediction performance parameter in line with traditional IVIVC development[54]. Furthermore, the assigned acceptance criteria for accurate predictions were considered appropriate for a model at an early product development stage in line with the prediction performance criteria applied for other pharmacokinetic parameters at this stage of development[24, 55]. The assigned criteria of AAPE less than or equal to 20% for highly accurate predictions, which reflected a prediction performance sufficient to predict the *in vivo* performance of an ER drug product from a commercial drug product applications perspective, differs from the 10% criteria applied in traditional IVIVC development[54]. However, in this evaluation, the developed mechanistic models utilized data from several different clinical studies compared to traditional IVIVC, where the clinical data is generated in a single cross-over study. Moreover, in this evaluation the ER drug product variants used in the predictions spanned across a significantly wider *in vitro* release and pharmacokinetic parameter range than the 10% difference recommended for traditional IVIVC development, which is more challenging from a regional absorption prediction perspective. Based on this, it was considered appropriate to assign AAPE $\leq$ 20% as the criteria for highly accurate predictions in this evaluation.

Finally, the importance of the selection and development of an appropriately biorelevant and discriminatory *in vitro* release/dissolution method for successful PBBM development for ER

drug product predictions should be emphasized since a wrongly selected dissolution method could lead to failed PBBM[56]. The selection should be made in close collaboration between *in vitro* dissolution and biopharmaceutics scientists, and a framework for key considerations in the selection of an *in vitro* dissolution method has been described previously[56]. A successful PBBM development will establish a link between the *in vitro* release rate, ideally the quality control (QC) dissolution method, and the clinical *in vivo* performance for the ER drug product, which potentially will allow instant acceptance or rejection of manufactured batches, enable clinically relevant release specifications, and waive *in vivo* studies during product development and for post-approval changes. In the current study, we assumed that discriminatory and potentially biorelevant dissolution method was used to generate the data and same was used as an input in the PBBM. Moreover, there is a need for a more mechanistic way to incorporate the dissolution data of ER drug products like product particle size distribution (P-PSD) as available for IR drug products[57, 58]. Additionally, the volume of fluid available in colon compartments, hydrodynamics, luminal degradation, binding of drugs to fecal or luminal contents, and mucus diffusion are some of the critical parameters for ER drug product performance where clarity is still needed. A comprehensive systematic evaluation of each parameter independent of the other could help to understand these grey areas.

CONCLUSION

The physiologically based biopharmaceutics modeling of ER drug products with *a priori* modeling demonstrated sufficient accuracy for low-medium colon absorption limitation risk drugs enabling use during candidate selection and early product development. The results also indicate a limited value in performing human regional absorption studies where the drug is administered to the colon as a bolus to support PBBM development for ER drug products. Instead, performing an early streamlined relative bioavailability study with the slowest relevant

ER *in vitro* release profile may develop a highly accurate PBBM suitable for ER predictions for commercial applications, except for permeability-limited drugs.

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H.J. and C.T. are both employees of AstraZeneca. GI-Sim has been developed by AstraZeneca for internal and academic use. AstraZeneca has ongoing license agreements for GastroPlus®.

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GRAPHICAL ABSTRACT