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On absorption modeling and food effect prediction of rivaroxaban, a BCS II drug orally administered as an immediate-release tablet

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Abstract: The present work evaluates the food effect on the absorption of rivaroxaban (Riva), a BCS II drug, from the orally administered commercial immediate-release tablet (Xarelto IR) using physiologically based pharmacokinetic (PBPK) and conventional in vitro- in vivo correlation (IVIVC) models. The bioavailability of Riva upon oral administration of Xarelto IR tablet is reported to exhibit a positive food effect. The PBPK model for Riva was developed and verified using the previously reported in vivo data for oral solution (5 and 10 mg) and Xarelto IR tablet (5 and 10 mg dose strength). Once the PBPK model was established, the in vivo performance of the tablet formulation with the higher dose strength (Xarelto IR tablet 20 mg in fasted and fed state) was predicted using the experimentally obtained data of in vitro permeability, biorelevant solubility and in vitro dynamic dissolution data using United States Pharmacopeia (USP) IV flow-through cell apparatus. In addition, the mathematical IVIVC model was developed using the in vitro dissolution and in vivo profile of 20 mg strength Xarelto IR tablet in fasted condition. Using the developed IVIVC model, the pharmacokinetic (PK) profile of the Xarelto IR tablet in fed condition was predicted and compared with the PK parameters obtained via the PBPK model. A virtual in vivo PK study was designed using a single-dose, 3-treatment cross-over trial in 50 subjects to predict the PK profile of the Xarelto® IR tablet in the fed state. Overall, the results obtained from the IVIVC model were found to be comparable with that from the PBPK model. The outcome from both the model pointed to the positive food effect on the in vivo profile of the Riva. The developed models thus can be effectively extended to establish bioequivalence for the marketed and novel complex formulations of Riva such as amorphous solid dispersions.

Keywords: *In vitro–In vivo* Correlation; Physiologically Based Pharmacokinetic Model; BCS Class II; Rivaroxaban; Xarelto; Food Effect; Population Kinetics

1. Introduction

Developing and deploying approaches that enable predicting the *in vivo* efficacy and safety profile of pharmaceutical drug products enormously expedite the product and process development effort as well as reduce the expensive clinical studies. For solid oral dosages, a thorough biopharmaceutical characterization at the *in vitro* level, such as solubility and dissolution testing using biorelevant media, study of food-formulation in-

teraction, *in vitro* membrane permeability and drug transport studies etc., provides the input data for *in vivo* absorption prediction. In recent years, prominent progress has been made in the *in vitro* biopharmaceutics profiling as well as in silico modeling for solid drug products[1]. A set of biorelevant and clinically relevant in silico models are expected to account for the critical formulation and physiological factors to facilitate the correlation between *in vitro* drug dissolution and *in vivo* pharmacokinetic profiles.

A widely accepted approach to assess the correlation between in vitro dissolution and in vivo bioavailability of an immediate release (IR) drug product is based on the Biopharmaceutics Classification System (BCS). The BCS categorizes drug substances into one of four classes based on their solubility and permeability. In general, BCS Class I (highly soluble and highly permeable) drugs are well absorbed. The rate limiting step to absorption is dissolution or gastric emptying. In vitro- in vivo correlation (IVIVC) is expected if dissolution rate is slower than gastric emptying rate. In case of IR drug products containing BCS Class II drug substance (dissolution as a rate-limiting step for absorption), conventional IVIVC can be used to establish the (cor)relation between the in vitro drug release and in vivo plasma concentration. Dissolution media and methods that reflect the in vivo controlling process are particularly important in this case if good in vitro-in vivo correlations are to be obtained. For BCS Class III drugs (permeability is the rate-limiting step for absorption) limited or no IVIV correlation is expected with the dissolution rate. Drug products containing BCS Class IV drug substance (low soluble and low permeable) has to be evaluated case by case, however, these drugs exhibit poor and variable bioavailability [2,3].

IVIVC is a predictive mathematical model describing the relationship between an in vitro property and a relevant in vivo response. In case of immediate release dosage forms, the main objective of an IVIVC is to reduce the number of BE studies via supporting the optimization of the drug formulation. A Level A IVIVC is usually established by a two-stage procedure: in vivo absorption is estimated using an appropriate deconvolution technique (e.g., Wagner-Nelson, Loo-Riegelman, numerical deconvolution) followed by comparison of drug absorbed to the fraction of drug dissolved. Even though the deconvolution method is often applied for the regulatory submission, the method is limited to linear pharmacokinetics (PK) regimen [4].

In general, IVIVC is developed using two approaches, i.e. convolution and deconvolution [5]. Convolution is a one-step method to predict the *in vivo* plasma concentrations using the *in vitro* release profile. However, the convolution method is limited to the compounds exhibiting linear or point to point correlation with no dose and time-dependent disposition. Whereas, in the case of the two-step deconvolution method, initially the *in vivo* plasma drug concentration-time course is estimated using mathematical methods such as Loo-Riegelmann, Wagner-Nelson, or numerical methods. Thereafter, the *in vivo* absorption time profile is related to the *in vitro* drug release rate [6–9].

Alternatively, the mechanistic deconvolution using the physiologically based pharmacokinetic (PBPK) modeling popularly known as Physiologically based IVIVC (PB-IVIVC) is nowadays extensively utilized for biopharmaceutics modeling [10,11]. Besides its applicability to the nonlinear PK, the PBPK model also considers the different factors governing the drug release and absorption such as particle size of the API, food effect, pH-dependent solubility profile, precipitation, gastric emptying time, drug degradation, drug solubilization in presence of excess bile acids and permeation across the intestinal membranes [12–15].

In the present work, we established both PB-IVIVC and conventional (numerical) IVIVC models for the immediate release oral tablet (Xarelto) formulations containing Rivaroxaban (Riva), BCS II anti-coagulant drug. Riva is reported to exhibit dose-dependent food effects. More precisely, while the lower dose (10 mg) can be taken with or without food, the highest dose strength tablet (20 mg) should be taken with food to attain the positive food effect for oral absorption and systemic availability [11]. The clinical trials are the regulatory requirement for the generic drug products containing

BCS II drug, and especially exhibiting food effect, to show the bioequivalence with the innovator product. As the critical formulation and drug product information of Riva is still covered by patent protection and no biowaiver exists, the design of a generic formulation containing this drug can be a challenge, especially considering the food effect displayed by the highest dose strength [16,17]. Here, we first developed in silico PBPK model using the fasted conditions and low dose formulations. On the basis of developed models, the PK profile of Xarelto formulations of the highest dose strength was predicted in fed conditions. The *in vitro* permeability of Riva as the pure API alone, and in the reference formulations (Xarelto® IR tablet) was determined using Caco-2 cell lines. Using combined *in vitro* experimental data on solubility, dissolution, and permeation with literature data as the input in the developed PBPK model, the extent of food effect *in vivo* on the oral absorption of the drug was predicted by mimicking fed state condition using biorelevant media. In addition, the results of the IVIVC model were compared with the PBPK model. Lastly, a virtual bioequivalence trial was performed to assess the performance of formulation in the fed state taking into consideration the population variability.

2. Materials and Methods

2.1 Chemical and Reagents

The reference formulations (Xarelto™ of 20 mg strength IR tablet) were acquired from the market. Caco-2 cell line was purchased from American Type Culture Collection (ATCC) (Rockville, MD). Minimal Essential Medium (MEM), fetal bovine serum (FBS), L-glutamine, trypsin (0.25%)-EDTA (1 mM), and penicillin-streptomycin mixture were purchased from Sigma. Simulated fluid powders were purchased from Biorelevant.com Ltd, the UK for the cell culture studies. Whereas for the solubility and dissolution studies, the simulated media were prepared using sodium taurocholate, lecithin and pepsin purchased from Sigma. The milk used for the solubility studies were purchased from the local market with the natural fat content of 3.5% fat. All other chemicals were of analytical reagent grade.

2.2 Software

Simulations were performed using the advanced compartmental absorption and transit model (ACAT) model implemented in the GastroPlus™ (version 9.0., Simulation Plus, Inc., Lancaster, CA, USA). The ACAT model serves as a bridge between the formulation performance and PK parameters of the drug products and hence, provides a valuable tool to guide formulation development to achieve the desired quality target product profile. In addition, the simulations from the ACAT models were verified using the conventional IVIVC model developed using the IVIVC Toolkit of Phoenix Win-Nonlin, Certara, New Jersey.

2.3 Chromatographic quantitative analysis

Ultra-High-Performance Liquid Chromatography (UHPLC) was used to quantify Riva in the samples obtained during *in vitro* dissolution, solubility, and permeability. A Waters Acquity H-Class instrument equipped with a PDA detector (operating at a wavelength value of 248 nm) was used. The column used was Acquity UPLC BEH C18, 1.7 μm, 2.1 mm x 50 mm (Waters) and the mobile phase was 3:7 (V/V) mixture of acetonitrile and 0.1M Ammonium-acetate buffer. The analysis was performed applying isocratic elution at the flow rate of 0.5 mL min^{-1}. The injection volume was 2 μl and the total run time was 5 min. The dissolved amount of Riva was determined based on the area under the appropriate peak and using external standard calibration.

2.4 Biopharmaceutical properties of Riva

2.4.1 Biorelevant Solubility Determination

Equilibrium solubility of Riva was determined in water, fasted state simulated gastric fluid (FaSSGF), fed state simulated gastric fluid (FeSSGF), fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF). All the simulated fluids (version 01) were prepared freshly on the day of the experiments conducted. In the case of FaSSIF media, 3 mM and 0.75 mM and for FeSSIF, 15 mM and 3.75 mM of the so-

dium taurocholate and lecithin were used, respectively. The FaSSGF media contains 0.08 mM and 0.02 mM sodium taurocholate and lecithin, whereas the FeSSGF media was prepared using an equivalent amount of milk and monobasic sodium phosphate buffer of pH 5. The concentration of different components of the media in detail is mentioned in Table A1 of the Appendix.

Further for the solubility determination, an excess amount of the pure drug was added to 5 mL of different simulated fluids. Thereafter, the sample was incubated at RT for 24 h with gentle shaking at 100 rpm using a rotatory shaker. The samples were then filtered using 0.22 μm syringe filter and the filtrate was analyzed using UHPLC.[18,19] The solubility results of Riva obtained in different simulated media were incorporated in GastroPlus™.

2.4.2 In Vitro Dynamic Biorelevant Dissolution

Dynamic dissolution of Xarelto™ (20 mg) tablets were carried out using USP Apparatus 4 fitted with 12 mm tablet cell using the media mimicking both the fasted and fed gastrointestinal state. In *in vitro* fasted state, the tablet was exposed to FaSSGF (pH 1.6) for 0.5 h, followed by exposure of the same tablet to FaSSIF (pH 6.5) for 5.5 h. The dissolution study was evaluated with 12 replicates. During the dissolution test, samples were withdrawn at predetermined time points and then filtered with 0.7 μm GF/F disc filter (Whatman, Maidstone, UK). The *in vitro* fed state experiments were conducted in a similar manner as that of the fasted state, using FeSSGF and FeSSIF as dissolution media. The dissolution media (FaSSGF, FeSSGF, FaSSIF, FeSSIF) were prepared based on the composition available at biorelevant.com, however, enzymes were excluded and SIF powder was replaced by surfactants such as tween80 and sodium lauryl sulfate.

2.4.3 In Vitro Caco-2 Permeability Determination

Caco-2 cells (ATCC) were cultured in Minimal Essential Medium (MEM), 20% fetal bovine serum, 2 mM L-glutamine, and 1% penicillin-streptomycin at 37°C in humid air atmosphere containing 5% CO_2 in 75 cm^2 cell culture flasks. Freshly prepared FaSSIF was used for the study.

For the transport studies, 0.5×10^6 cells were seeded per 12-well transwell insert (translucent, 0.4 μm pore size, Greiner Bio-one®). Cells were cultured with 500 μl medium in the upper compartment and 1500 μl in the lower compartment. The medium was changed every 2 or 3 days and transepithelial electrical resistance was measured via EVOM STX-2-electrode (World Precision Instruments). The cell monolayers were used for the experiments, once the resistance reached a TEER value of >300 Ω^*cm^2 (18-21 days) [20,21].

Thereafter, the medium was removed and the cells were coated with 90 μl gastric porcine mucin (40 mg/ml in MEM + 10%FBS for 30 min. A concentration of 10 μM of Riva and Xarelto™ (20 mg) tablets (equivalent to 10 μM of Riva) was used. For the preparation of Xarelto™ (20 mg) samples, the tablets were ground using a mortar and pestle and the amount of the formulation containing the desired amount of Riva was dispersed in FaSSIF and stirred for 30 min at RT. The respective suspensions (510 μl) were applied to the upper compartment of the transwell and 1500 μl Krebs Ringer buffer added in the lower compartment. Ten μl were immediately withdrawn from the apical compartment to determine the total amount applied. Plates with transwell were incubated upon agitation for a total of 120 min. 100 μl samples were taken from the lower compartment at predetermined time points of 0, 30, 60, 90, and 120 min and replaced by pre-warmed Krebs-Ringer buffer. In addition, at the end of the experiment, 10 μl of the upper compartment were collected for the calculation of the recovery rate. TEER values were measured before and after the transport study to identify potential damage to the cell layer. The permeability of sodium fluorescein (10 μg/ml) in Krebs-Ringer buffer was determined to verify the barrier properties of the Caco-2 monolayer. All samples were stored at -20° C till further analysis. On the day of analysis, the samples were thawed at room temperature, and the content was measured using UHPLC.

For the determination of the apparent permeability coefficient (P_{app}), the following equation, where dQ/dt is the flux across the cell monolayer (ng/sec), A the surface of the monolayer (cm²), and C the initial concentration in the donor compartment (ng/ml), was used:

$$P_{app} = \frac{dQ}{dt * A * c}$$

equation 1

2.4.4 Determination of systemic disposition parameters of Riva

The *in vivo* PK profile of Riva after intravenous administration was not found in the literature. Therefore, plasma concentration-time profile after 10 mg oral solution administration under the fasted state was used into the PKPlus™ to obtain the systemic clearance, volume of distribution, half-life and distribution constants between the central and peripheral compartments [22,23]. Models were fitted empirically in the PKPlus™ employing 1-, 2- and 3- compartment separately. The Hooke & Jeeves pattern search method was used during the fitting and the weighing was equal to 1/Yhat². Akaike information criterion (AIC) and Schwarz criterion (SC) were used to select the best-fitted compartment model. The obtained PK parameters were then fixed and employed in the simulation of solid oral dosage forms.

2.5 Physiologically based Gastrointestinal Absorption Modeling

2.5.1 Model Compound Parameters

Physiochemical properties of Riva such as molecular weight, and lipophilicity were compiled from the literature (Table 2) [24]. Human duodenum effective permeability (P_{eff}) was estimated from *in vitro* CaCo-2 permeability data using the in-built relation present in the GastroPlus™. The experimentally obtained values of solubility, particle size and dissolution parameters were used.

2.5.2 Development of In-silico Physiology based Gastrointestinal Absorption Model

The ACAT model implemented in GastroPlus™ was used for all the simulations in the current study. Input parameters for the ACAT model can be categorized into three classes, i.e., formulation properties (such as particle size distribution, density, and release profiles of drug products), physicochemical properties of drug substances (such as diffusion coefficient, lipophilicity, pKa, solubility, and permeability) and pharmacokinetic parameters (such as clearance, the volume of distribution and the disposition model). All other parameters were set at default values in GastroPlus™. The simulations were performed using the default 'Human Physiological-Fasted' and 'Opt LogD Model SA/V6.1', in order to simulate the plasma concentration profiles of Riva following oral administration of tablet dose in fed condition.

2.5.2.1 Model Verification

Initially, the predictive power, robustness, and the effect of Absorption Scale Factors (ASF) optimization on the absorptive phase of Riva were evaluated. For that, the predicted pharmacokinetic parameters were compared with the *in vivo* data from literature. The pharmacokinetic model was developed using oral solution doses (5 and 10 mg) as well as for oral IR tablet doses (5 and 10 mg) of Riva under the fasted state and compared with the published data [22,23]. The percent prediction error value was calculated to evaluate the accuracy of the model. The validated ACAT model for Riva was then applied to simulate the plasma concentration-time profile of Xarelto tablet (20 mg) in the fed state.

2.5.2.2 Parameter Sensitivity Analysis (PSA)

PSA was performed for the uncertain and key parameters in formulations such as mean particle radius, dose volume, particle density, effective permeability, precipitation time, and diffusion coefficient for the dosage forms investigated under the fasted and fed state.

2.6 IVIVC Studies

In addition to the PBPK model, the conventional IVIVC was used to predict the PK profile after oral administration of formulation in the fed state using IVIVC Toolkit of

WinNonlin software. The IVIVC model was developed and validated using the *in vitro* dissolution and *in vivo* profile of 20 mg strength of Xarelto IR tablet in fasted condition. Thereafter, using the established IVIVC, the PK profile of the Xarelto IR tablet in fed condition was predicted and compared with the simulation results obtained from the PBPK model.

IVIVC was based on a two-step deconvolution method, initially, the Weibull function was fitted to the *in vitro* data of the Xarelto IR tablet (20 mg tablet) in fasted condition. Moreover, the time course of *in vivo* absorption was derived using deconvolution. Thereafter, in the second step, a correlation was built between the *in vitro* drug release and *in vivo* drug absorption. The *in vivo* data of the 10 mg oral solution published by Kubitza was used as a reference for calculating the unit impulse response (UIR) function [22,23].

The *in vivo* data of the PK profiles of the Xarelto IR tablet (20 mg tablet) was then deconvolved and compared to the *in vitro* dissolution profiles using the Levy plot. The IVIVC model was established based on *in vitro* dissolution profile of the Xarelto IR tablet, and *in vivo* results were obtained from the literature. As per the condition, of regression slope line most closely aligned with a value of 1.0. Whereas, the elimination phase of Riva was calculated using the PK profile of 10 mg oral solution.

Thereafter, as the final step, validation of the developed IVIVC model is required, in order to establish quantitative resilient of predictive capacity of the model. The validation of the model is performed by using the results of the *in vivo* fate of the formulation used to establish the model, known as internal validation and/or by the application of a different formulation, known as external validation. In the present model development, the internal validation of the IVIVC model was performed by comparing the predicted and observed PK profile of the reference product Xarelto 20 mg tablet in fasted condition. Thereafter, the predictability of the model was evaluated using the percentage prediction error (%PE) from the following equation:

$$\%PE = 100 \times \left(\frac{\text{Predicted Value} - \text{Observed Value}}{\text{Observed Value}} \right)$$

equation 2

For the development of a robust model, the % PE for each PK parameter should be less than 15, whereas the average %PE should be not more than 10%. If the % PE conditions are not met for the internal validation, further validation using the external formulation is required [5,25].

2.7 Food Effect (FE) Studies of Riva in Simulated Healthy Population

Simulations were carried out for 20 mg dose strength using the fed state physiology of GastroPlus™ to evaluate the quantitative prediction of FE based on the measurements of *in vitro* biorelevant solubility and dissolution. The percent prediction error for the predicted PK parameters was calculated in comparison with the predicted values Xarelto IR tablet (20 mg tablet).

A single dose (20 mg), three-period virtual trial in 50 subjects was carried out. GastroPlus™ randomly generates subjects by varying physiological factors such as gastrointestinal transit times, pH, fluid volumes, PK parameters as well as compound parameters. Three populations namely A, B, and C with 50 subjects each (in order to gain a thorough sampling across all the variables) were given the same treatment (20 mg strength of Xarelto IR tablet).

3. Results and discussion

3.1 Biopharmaceutical properties of Riva

3.1.1 Equilibrium solubility in simulated media

Table 1 depicts the solubility of the Riva in different biorelevant media. The solubility of the Riva was found to be comparable among different media, i.e., water, FaSSGF (pH 1.2), and FaSSIF (pH 6.5). Whereas in the case of FeSSIF and FeSSGF (pH 5.0), the solubility of the Riva was found to be markedly higher as compared to other simulated

biorelevant media. The FeSSIF contains 15 mM sodium taurocholate and 3.75 mM lecithin as compared to 3 mM sodium taurocholate and 0.75 mM lecithin in the case of FaSSIF. Thus, approx. 2-fold increase in the solubility in the Fed conditions could be attributed to the increase in the lipidic component of the media with a higher fraction solubilized in taurocholate and lecithin micelles. The increased solubility of Riva in Fed state is in accordance with the literature, demonstrating higher bioavailability of equal of more than 80% when taken with food (for 20 mg dose of Riva) [24,26].

Table 1: Solubility of Riva in biorelevant media

Solubility in	pH	Values (µg /mL)
Unbuffered water	7.0	10.0
FaSSGF	1.2	11.0
FaSSIF	6.5	9.9
FeSSGF	5.0	24.0
FeSSIF	5.0	16.8

3.1.2 In vitro release profile of Riva in fasted and fed conditions

Figure 1 demonstrates the in vitro release profiles of the Xarelto IR tablet (20 mg) in fasted and fed conditions using the dynamic dissolution method. After 30 mins, in vitro release of Xarelto IR tablet in the fed state and fasted simulated gastric fluids were found to be 27.7 and 11.0%.

Thereafter, the Xarelto IR tablets were shifted to the fed state, and fasted simulated intestinal fluids and the *in vitro* release and the time to 80% drug release was found to be 360 and 210 mins in case of fasted and fed state conditions, respectively. Furthermore, the $f1$ (the difference factor) and $f2$ (the similarity factor) was also calculated and was found to be 28 and 38, respectively. For bioequivalent *in vitro* release profile, the values of $f1$ should be between 0 and 15, whereas the value of $f2$ should be between 50 and 100 [27,28]. Thus, the release profile in the case of the fed condition was found to be significantly higher as compared to the fasted condition. The results demonstrated that the lower solubility in the absence of food components could be the rate-limiting factor for the dose-proportional absorption of Riva from Xarelto IR tablets, independent of the formulation. The significantly higher dissolution in presence of a food-induced increase in bile salt concentration was found to be in accordance with the solubility study and is the key parameter for the establishment of the PBPK model.
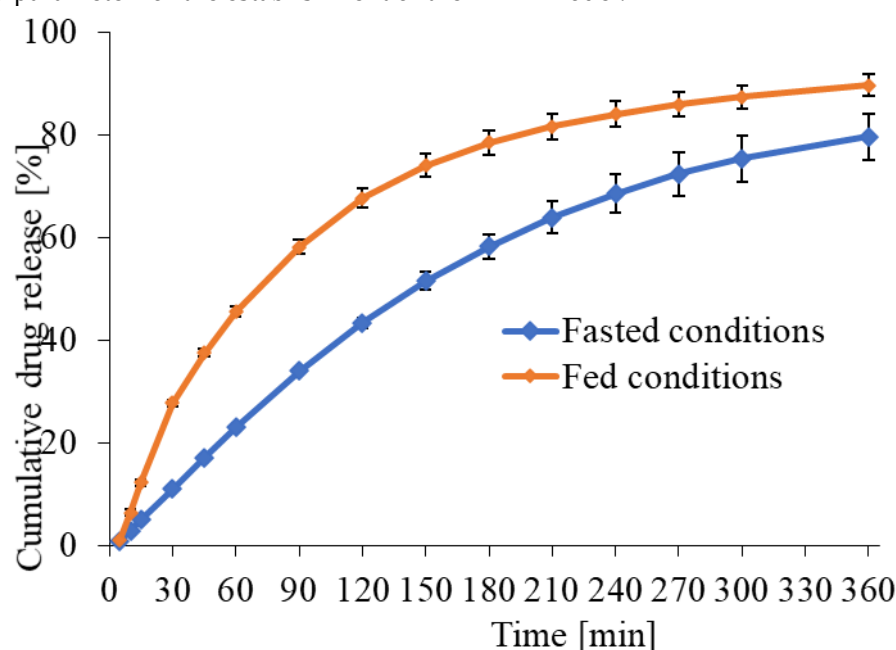

Figure 1: In-vitro release profile of Riva from Xarelto IR tablet (20 mg strength)
3.1.3 In Vitro Caco-2 Permeability

Permeation of the marker substance fluorescein did not cause any alterations of transepithelial electrical resistance (TEER) values compared to controls (no permeation performed). The API powder and, to a greater extent, the formulations caused a decrease in TEER values (Figure 2 (A)). Despite the decrease in TEER values induced by the formulations, there was no increased transport of Riva across the monolayers. P_{app} values (2.69 ± 0.72 x10^{-6} cm/s) for Xarelto was not increased in the formulations compared to standard Riva (3.11 ± 0.24 x10^{-6} cm/s, (Figure 2 (B)). The P_{app} of fluorescein was 0.72 ± 0.13 x10^{-6} cm/s, indicating a good barrier function of the Caco-2 monolayer and absence of damage by FaSSIF.
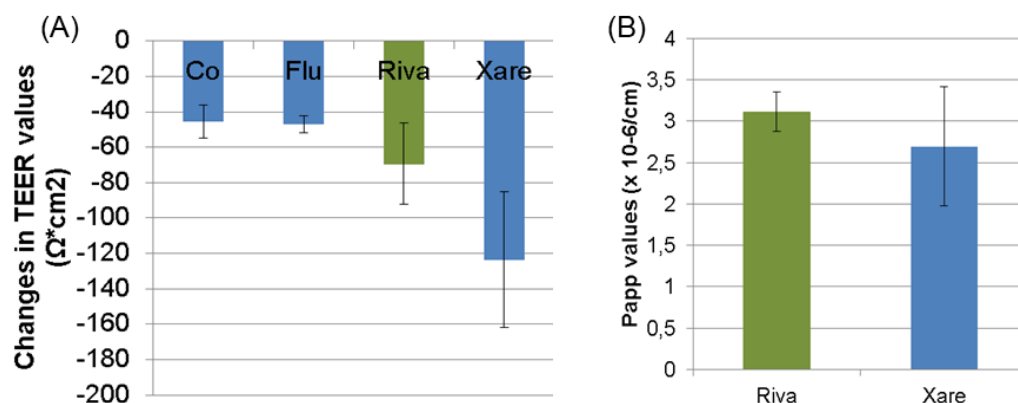
Figure 2: (A) Differences in TEER values between start value and measurement at the end of the permeation studies. Abbreviations: Control (Co), fluorescein (Flu), rivaroxaban (Riva), Xarelto (Xare). (B) Papp values of rivaroxaban (Riva) and Xarelto (Xare)

P_{app} and transport rates were identical for formulated products and standard Riva. The decrease in TEER values was slightly higher in the formulations than in the unformulated Riva but was not reflected in changes of the P_{app} values. Excipients in the formulations more likely decrease the TEER values. However, taking into consideration that there is no difference in the P_{app} of pure Riva in comparison with Xarelto (presence of excipients) in spite of change in TEER values of CaCo-2 cells in Xarelto, it can be suggested that passage of Riva is mainly transcellular through the CaCo-2 cells. P_{app} values determined in the study were lower than the values published by Gnoth et al. (8.0 ± 0.6 x10^{-6} cm/s) [29]. The most likely reason for this difference is the lack of mucus production of Caco-2 cells, which can affect permeation. To reproduce the physiological situation, a mucus layer has been added to the Caco-2 monolayer in this study. The effect of mucus on the permeation of active pharmaceutical ingredients (APIs) has been reported controversially [30,31]. The comparison between Caco-2 cells and mucus-producing HT29-MTX did not show prominent differences for many lipophilic and hydrophilic compounds suggesting that mucus does not represent a strong barrier for the permeation [32]. The exclusive assessment of the role of mucus, however, was not possible because HT29-MTX cells lack P-glycoprotein expression and the lack of reverse transport will increase the measured P_{app} values. Although the same cell types were used, another study reported that the permeability of drugs with a partition coefficient (log P) > 1 was decreased in the mucus-producing cell lines [32]. The passage of Riva might be hindered by mucus because its logP value of 1.36 [32]. It can be concluded that Xarelto formulations reacted very similar and did not display a permeation-enhancing effect on the permeability of Riva.

Thus, the various physicochemical parameters such as solubility study, Caco-2 permeability, and published literature required as an input for the PBPK model are mentioned in Table 2 [24,33–37].

Table 2: Input parameters of Riva for building the PBPK model in GastroPlus™

Physiochemical Parameter	Values
Molecular Weight (g/mol)	435.89
logP	1.36

pKa	strongest acidic: 13.6
	strongest basic: -1.6
Solubility vs pH	water solubility (pH=7) = 10 µg /mL
	pH 1.2, FaSSGF = 11 µg /mL
	pH 6.5, FaSSIF = 9.9 µg/ml
	pH 5.0, FeSSIF =16.8 µg/ml
Particle Size (Radius)	7.5 µm (Xarelto tablet, 20 mg)
	d_{90}= 9.4 µm; d_{50}=3.8 µm; d_{10}=0.7 µm
Caco-2 Permeability	$2.69 \pm 0.72 \times 10^{-6}$ cm/s (Xarelto)
Dissolution Profiles (USP 4)	Xarelto IR tablet (20 mg)

3.1.4 Systemic disposition parameters of Riva

The two-compartment model was found to be the best fit model according to the AIC and SC criteria to describe the Riva pharmacokinetics following the administration of oral solution dose (10 mg). Values of clearance (L/h), the volume of distribution (Vc) and, $T_{1/2}$ were in accordance with the literature values. Other parameters such as the peripheral volume of distribution, distribution constant from the central to peripheral compartment, and distribution coefficient from the peripheral to central compartment determined from the two-compartment model fitting were used for further simulations. Table 3 depicts the mean baseline values used for the simulation of Riva plasma concentration time profile following the administration of oral solution and IR formulation doses. The value of clearance and elimination half-life obtained through fitting is found to be in accordance with the value reported in the Xarelto product information after intravenous administration of Riva at a dose of 1 mg [38,39].

Table 3: PK parameters obtained from two compartment model fitting of oral solution (10 mg) of Riva

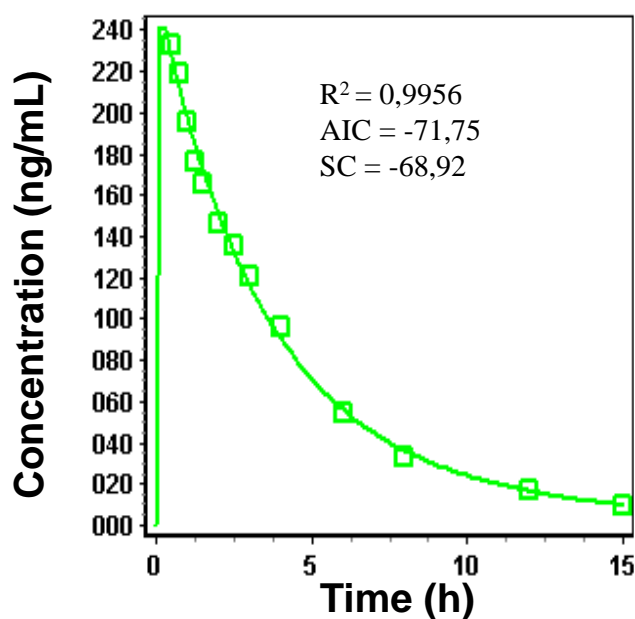| Parameter	Values
Clearance (L/h)	9.43
V_c (L/kg)	0.47
$T_{1/2}$ (h)	4.62
K_{12} (1/h)	0.04
K_{21} (1/h)	0.21
V_2 (L/kg)	0.09

$R^2 = 0,9956$
AIC = -71,75
SC = -68,92

Figure 3: Pharmacokinetic data fitting 2- compartment of oral solution dose (10 mg) of Riva

3.2 Physiology based Gastrointestinal Absorption Model of Riva formulation

3.2.1 Prediction of PK profiles and optimization of ACAT model

Taking into consideration the input parameter as mentioned in Table 2 and the pharmacokinetic parameters mentioned in Table 3, the PK profile of oral solution dose (10 mg) of Riva was simulated using the ACAT model in GastroPlus™ with default fasted human physiology. Simulation of 10 mg oral dose with the default Gastroplus™ Human Physiology Fasted largely underestimated the absorption phase of Riva resulting in the poor fitting of the extracted plasma concentration-time profile obtained for 10 mg oral dose (Figure 4A). This observation suggested that the default fasted human physiology in GastroPlus™ was not able to capture the absorption phase of Riva. Thereafter, the influence of effective permeability (P_{eff}) on the C_{max} and T_{max} predictions under fasted conditions for oral solution dose (10 mg) of Riva were evaluated. The results showed that even though the P_{eff} was increased by 10 folds to 3.1, T_{max} was overpredicted by 5 folds and C_{max} was underpredicted by 1.78 folds than the average observed values (Table 5) [23].

Furthermore, it is also important to consider the solubility of Riva in gastric medium and concentration of Riva attained with 5 mg and 10 mg of oral solutions. Table 1 reports fasted state gastric solubility of 11 μg/mL (i.e., 0.011 mg/mL) for Riva. Considering 250 mL of dosing volume with instantaneous saturation translates to a solubilization capacity of 2.75 mg in the medium. At a dose of 10 mg, this can generate ~3.6-fold supersaturation, which can lead to faster absorption as reflected by high C_{max} and lower T_{max} in the observed profile (assuming absence of precipitation from the supersaturated state). As simulations were conducted with solubility values of ~11 μg/mL, it could have led to underpredictions.

As a result, the ASF values were optimized using the optimization module in the GastroPlus™ which could capture the absorption phase of the plasma concentration-time profile of oral solution (10 mg) (Figure 4B). ASFs in GastroPlus™ is a multiplier used to scale the effective permeability to account for variations in surface-to-volume ratio, pH effects, influx, or efflux transporter differences, and other absorption-rate-determining effects.

The ASFs were optimized using the PK data set of single-dose oral solution (10 mg) in the fasted state changing the C1 and C2 coefficients of Opt logD Model SA/V 6.1, which determines absorption from the small intestinal compartments. However, the C3 and C4 coefficients which determine absorption from the colon were kept at their default values. The optimized ASF were nearly 14 folds higher than the default values leading to the faster absorption of Riva in the small intestine. In addition, the default value for compartment volume occupation by water in the colon was reduced from 10% to 2% to better account for measured free water content in the colon [40,41]. All other parameters were set at default values in GastroPlus™, the default and optimized ASF values are mentioned in Table 4.

Table 5 shows the pharmacokinetic parameters obtained from the simulated PK profile of solution oral dose (10 mg) of Riva before and after optimization of ASF and compared with the literature data.

In order to verify that the optimized ASF values for the small intestine can reasonably capture the absorption phase of Riva, simulations of mean plasma concentration-time profile of Riva following administration of oral solution dose (5 mg and 10 mg) and IR tablet formulation (5 mg and 10 mg) under fasted condition were also carried out. Simulated mean plasma concentration-time profiles of Riva from solution and IR tablet formulation and corresponding pharmacokinetic parameters (C_{max}. T_{max} and $AUC_{0-\infty}$) calculated are demonstrated in Table 5 and Table 6. All the predictions of pharmacokinetic parameters for different formulation and doses of Riva were within the two folds of the reported values. This fosters our confidence in the predictive ability of the developed ACAT model for Riva.

Parameter sensitivity studies were performed investigating the impact of key factors on the bioavailability of Riva. Mean particle size of the API in the IR tablet formulation was found to be the most important factor influencing the bioavailability of Riva (Data not shown) irrespective of fasted and fed state. Other factors such as dose volume, particle density, precipitation time, diffusion coefficient, and P_{eff} seem to have a relatively minor influence on the bioavailability of Riva.
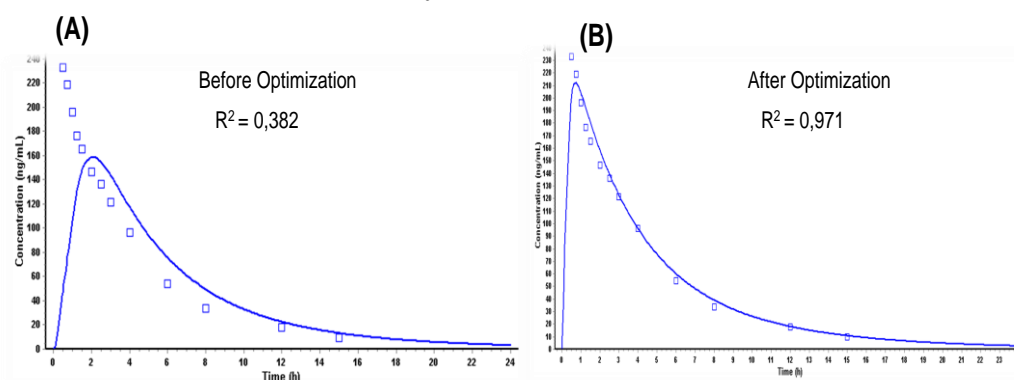

Figure 4: Riva plasma concentration time profile from solution oral dose (10 mg) before and after optimization

Table 4: ASF values before and after optimization of ACAT model

Compartment	Default (GastroPlus) ASF	Optimized ASF
Stomach	0	0
Duodenum	2.673	36.44
Jejunum 1	2.658	36.25
Jejunum 2	2.629	35.85
Ileum 1	2.592	35.35
Ileum 2	2.568	35.02
Ileum 3	2.505	34.16
Caecum	0.535	0.535
Asc Colon	1.038	1.038

Table 5: PK parameters obtained from simulated PK profiles

Parameter	Actual (Reported)[a]	Predicted (before optimization)	Predicted (after optimization)
PK parameters obtained from simulated PK profile of solution oral dose (10 mg) of Riva before and after optimization			
C_{max} (µg/L)	266/25.1 (187-412)	159.27	212.23
T_{max} (h)	0.50 (0.25-1.00)	1.92	0.72
AUC (µg.h/L)	997/25.1 (613-1383)	1056	1058
F%	>90%	99.54	99.79
Pharmacokinetic parameters for 5 mg oral (solution) dose of Riva in fasted conditions			
C_{max} (µg/L)	119/18.5 (97.2-158)	80.13	107.06
T_{max} (h)	0.63 (0.5-0.75)	1.92	0.66
AUC (µg.h/L)	461/17.2 (348-587)	528.24	529.37
F%	>90%	99.58	99.80

a – taken from literature

Once the ACAT model was optimized with modified ASF values, pharmacokinetic parameter predictions were carried out for Riva 5 mg oral solution dose (Table 5) and Xarelto IR tablet for 5 mg and 10 mg (Table **6**) dose in the fasted condition. The *in vitro* release profile and the physicochemical parameters of the API was found to be bio-predictive and was capable to describe the plasma concentration profiles and the

predicted values of C_{max}, AUC, and T_{max} were found to be in agreement with that of the literature, which increased the confidence in the developed ACAT model.

Table 6: Pharmacokinetic parameters for IR Tablet in fasted conditions

Parameter	Actual (Reported) [a]	Predicted (Optimized ASF)
Pharmacokinetic parameters for 5 mg oral (IR Tablet) dose of Riva in fasted conditions		
C_{max} (µg/L)	72/19.7 (55-96)	76.13
T_{max} (h)	1.88 (0.5-4.00)	2.1
AUC (µg.h/L)	466/23.0 (348-677)	524.42
F%	80-100%	98.86
Pharmacokinetic parameters for 10 mg oral (IR Tablet) dose of Riva in fasted conditions		
Parameter	Actual (Reported) [a]	Predicted (Optimized ASF)
C_{max} (µg/L)	141/15.5 (112-184)	149
T_{max} (h)	2.00 (0.5-2.50)	2.20
AUC (µg.h/L)	1020/14.9 (797-1217)	1037
F%	80-100%	97.75

In silico simulation of Riva-Xarelto (20 mg dose strength) in fasted and fed state

The developed ACAT model was used to simulate the plasma concentration-time profile of Xarelto (20 mg dose strength) in the fasted and fed state with the dissolution profiles using the USP 4 flow-through apparatus and biorelevant dissolution medium. It was observed that the inclusion of dissolution profiles of Xarelto IR Tablet (20 mg) during modeling led to an improvement in the simulations with predicted values close to the observed values, as reported in the literature. Table 7 and Figure 5 represent the simulated plasma concentration-time profile and key pharmacokinetic parameters predicted from the simulation of Xarelto (20 mg) IR tablet in the fasted and the fed state.

Table 7: Key pharmacokinetic parameters predicted from the simulation of Xarelto (20 mg) IR tablet in the fasted and fed state

| Parameter | Fasted State | | Fed State | |
	Actual (Reported)a	Predicted (Optimized ASF)	Actual (Reported)b	Predicted (Optimized ASF)
Cmax (µg/L)	173/35.6 (111-294)	171.15	294.4/15 (225.4-360.6)	236.64
Tmax (h)	1.50 (0.5-4.00)	3	3.00 (0.5-6.00)	2.9
AUC (µg.h/L)	1612/36.1 (859-2193)	1433.8	2294/19 (1464-3227)	1857.3
F%	66%	67.57	80-100%	87.54

a – taken from Reference 1 b- taken from Reference 2; data is represented as geometric means/percent geometric coefficient of variation and range in case of reported data

The results clearly depicted the presence of food effects when Xarelto (20 mg) is administered in the fasted and fed state. However, the food effect was a bit underestimated as compared to that reported in the literature.
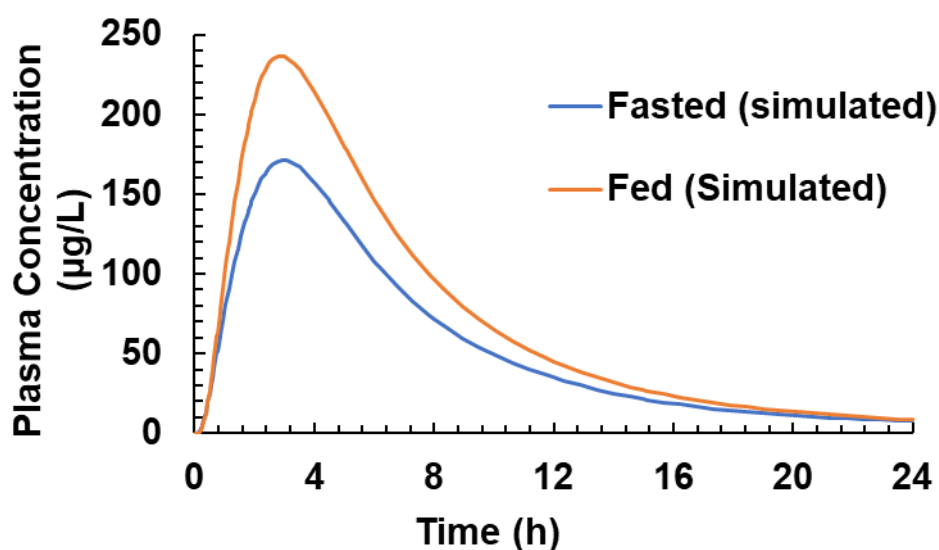
Figure 5: Simulated plasma concentration time profile of Xarelto (20 mg) in Fasted and Fed State using GastroPlus™ ACAT model
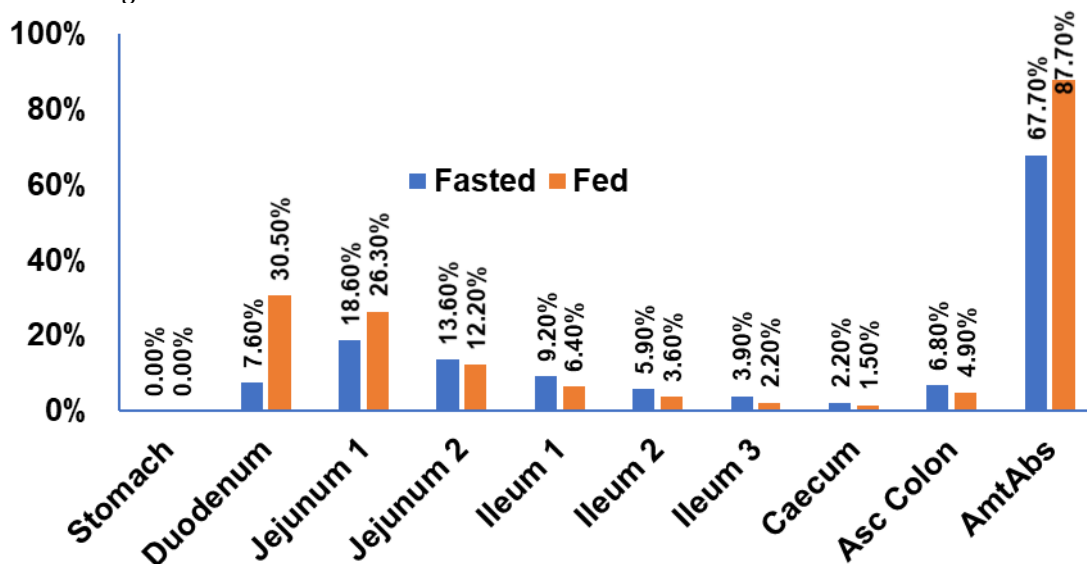

Figure 6: Regional amount absorbed from Xarelto in Fasted and Fed State in the gastrointestinal tract

As evident from Figure 6, the increase in bioavailability of Xarelto during the fed state simulation was found, which could be due to the enhanced dissolution of Riva in the fed state. The increase in solubility in the fed state resulted in a greater fraction of Riva to be absorbed from the duodenum and Jejunum 1, as compared to the fasted state. The simulated results are in accordance with the Xarelto product literature outlining site-specific absorption of Riva [38,39].

3.3 IVIVC Studies

3.3.1 Modelling

3.3.1.1 In vitro and in vivo raw data

Due to the availability of the *in vivo* pharmacokinetic profile in fasted conditions only for Xarelto 20 mg tablet, the *in vitro* release profile of fasted state was used for the establishment of the IVIVC model. As evident from the dynamic *in vitro* dissolution of the Xarelto (Figure 1), after two hours of incubation in a simulated gastric medium, approx. 43% of the drug was released. Thereafter, the simulated gastric medium was replaced by a simulated intestinal medium. On incubating the tablets for 4 hours, the amount of API release was found to be approx. 80%.

Whereas, the *in vivo* data of the Xarelto 20 mg tablet and 10 mg oral solution in fasted condition was obtained by literature published by Kubitza and co-workers [22,23]. Apparently, the T_{max} in the case of solution and tablet was found to be 0.5 and 3h, respectively. In-addition, the C_{max} was reported to be markedly higher in the case of the solution as compared to the tablet. The observation suggests higher absorption of the Riva in presence of solutions, which is reported to be due to the faster and higher amount of Riva available to be absorbed. Thus, suggesting the absorption of Riva to be not limited by permeability.

3.3.1.2 Dissolution curve fitting

The fasted state *in vitro* release profile of the Xarelto 20 mg tablet was fitted with the Weibull function. As evident from Figure 7, using the Weibull function, the predicted *in vitro* release or more precisely, the fraction of API dissolved was found to be overlapping with the observed *in vitro* release data as a function of time, suggesting the Weibull function was suitable to fit the dissolution data.

$$\text{Weibull function, } y(t) = F_{inf}\left[1 - e^{-(t/MDT)^b}\right]$$

equation 3
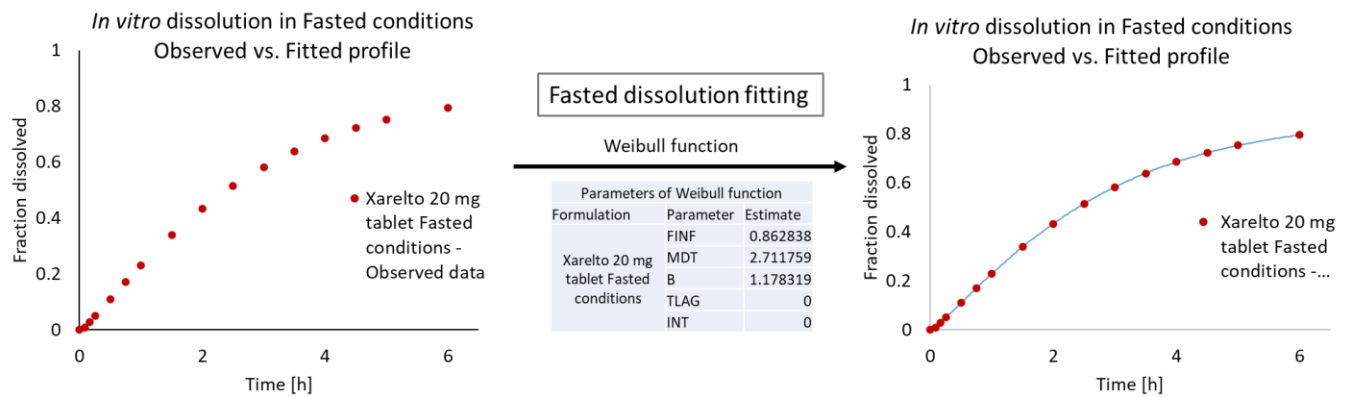
Figure 7: Observed *in vitro* profile vs fitted curves of the Xarelto 20 mg tablet

3.3.2 Calculation of Unit impulse response (UIR) function

To obtain the *in vivo* data, the pharmacokinetic parameters of 10 mg oral solution was used. Thus, the UIR function was used to calculate the oral pharmacokinetic data. As evident from Figure 8, using the UIR function, the observed pharmacokinetic profile was found to be overlapping with the predicted pharmacokinetic profile and a linear relation was established suggesting the best fit model. The UIR function obtained were then used to deconvolute the *in vivo* pharmacokinetic profile of Xarelto 20 mg tablet in fasted condition, for the assessment of *in vivo* absorption profile (Figure 9).

$$\text{UIR function, } Cp(t) = A_1 e^{\alpha 1 t} + A_2 e^{\alpha 2 t} + A_3 e^{\alpha 3 t}$$
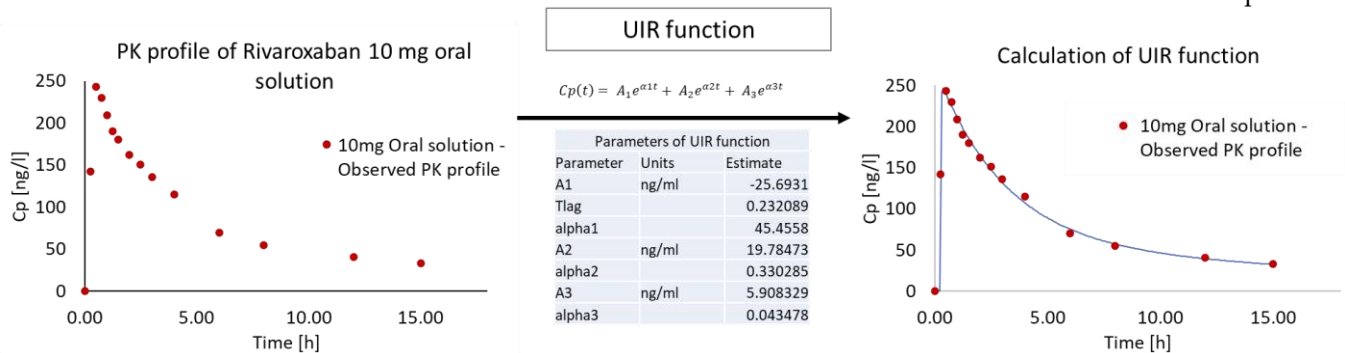
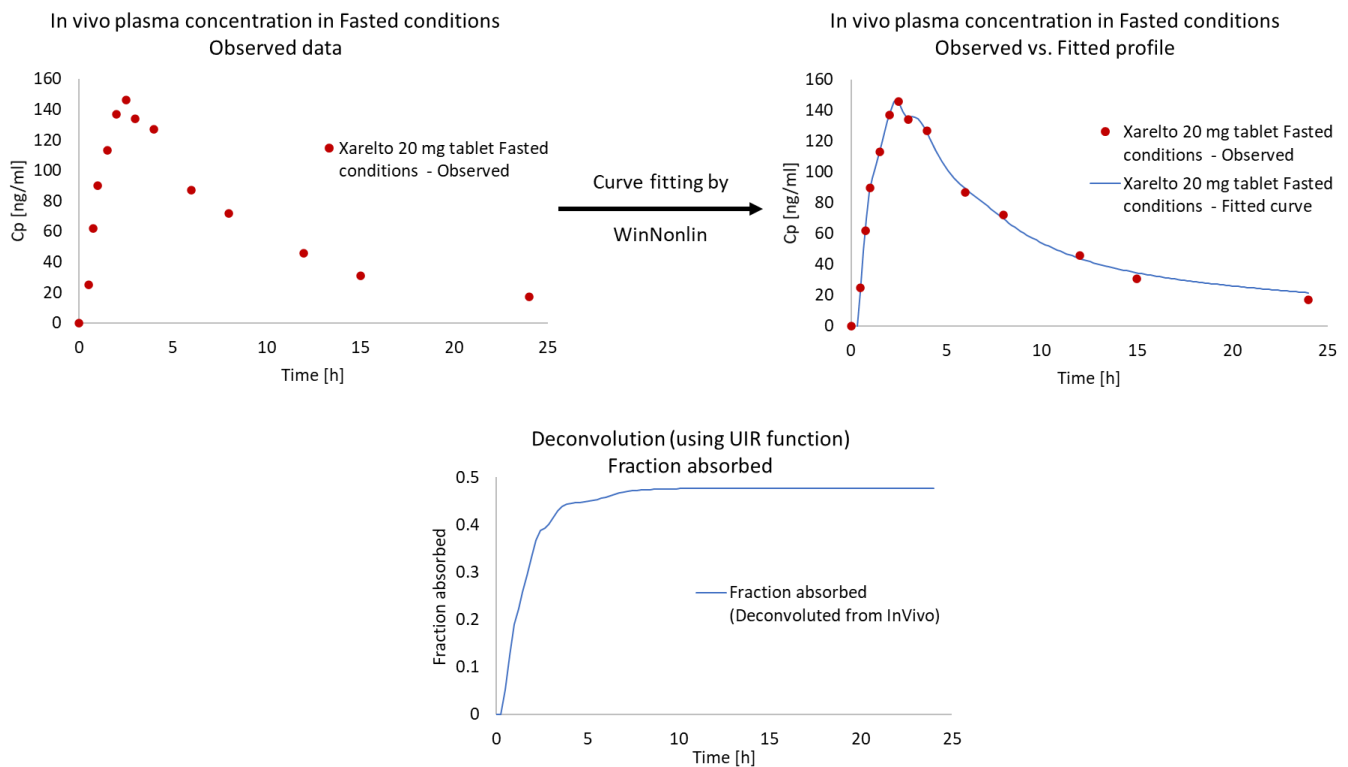equation 4

Figure 8: Properties of UIR function

Figure 9: Calculated absorption after deconvolution of Xarelto 20 mg tablet

3.3.3 Correlation

The dissolved API fraction (obtained from the fitting of *in vitro* release profile) was then related with the absorbed *in vivo* fractions (obtained from the deconvolution of plasma concentration) using Levy's plot. Levy's plot is a graphical presentation of time required for the *in vivo* absorption of API against the time required for the same amount of API to get dissolved or released from the formulation. As evident from Figure 10, the *in vivo* fraction absorbed was found to be linearly correlated with the fraction of API dissolved *in vitro*. The observed relation was found to be aligned with the linear regression analysis. Thus, the slope and regression values of Levy's plot suggest the development of a robust mathematical IVIVC model.

Correlation equation, $F_{abs}= AbsScale*Diss(T_{scale}*T_{vivo}-T_{shift})$ equation 5
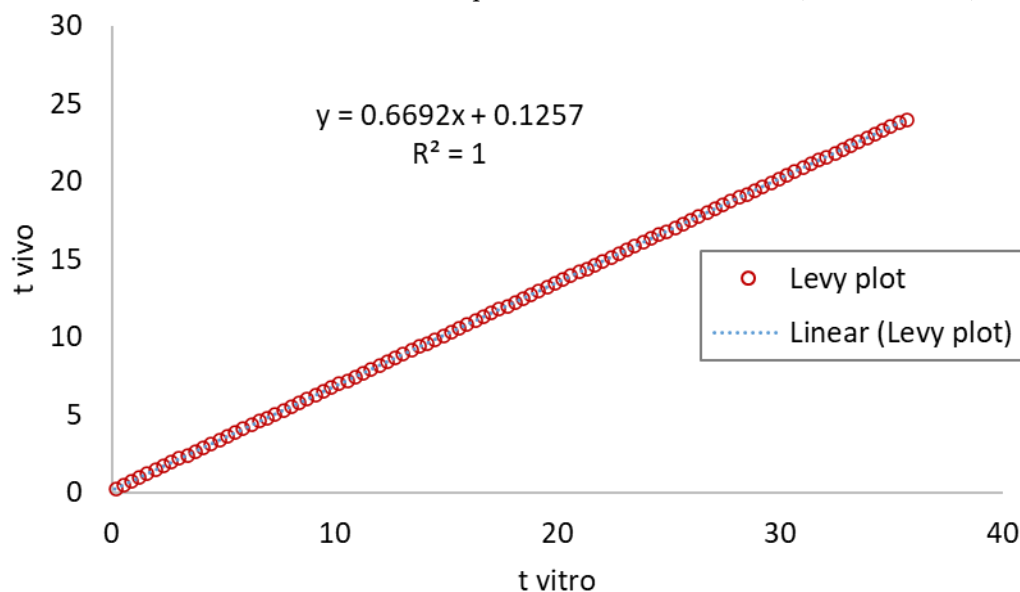
Figure 10: Levy plot (fraction absorbed *in vivo* vs fraction dissolved *in vitro*) for the Xarelto 20 mg tablet in fasted condition

3.3.4 Internal validation and Prediction

An IVIVC model was established based on the USP IV fasted state dissolution data and using published *in vivo* data as an internal validation of the reference product Xarelto 20 mg tablet (Figure 11). The result of the internal validation was promising (<2% error). However, the external validation was not performed due to very limited *in vivo* data.

The IVIVC model, developed based on the fasted *in vitro* and *in vivo* profile of Xarelto 20 mg tablet, was then used for the prediction of *in vivo* performance of the Xarelto 20 mg tablet in the fed state. As evident from Figure 12, the C_{max} and AUC were found to be distinctly higher, whereas no significant difference in the T_{max} was predicted in the case of the fed state as compared to the fasted condition. The increase in absorption is found to be in accordance with the increase in solubility of Riva in presence of simulated fed media. Thus, the developed IVIVC was found to biased and was able to adapt the effect of fed media, predicting the *in vivo* profile in the fed state. The developed IVIVC model (developed for reference product) can also be used as an effective tool to predict the *in vivo* fate of formulations with different strength and release profiles, considering an average percentage error of less than 10% and tolerance limit in the range of 0.8-1.25.

In the present report, both PBPK absorption and IVIVC model were developed to predict the food effect on the *in vivo* fate of the Riva released from Xarelto 20 mg tablet. Both models predicted a higher amount of Riva absorption in case of fed conditions, which could be due to higher solubility and release profile in simulated fed media [22]. The findings are in accordance with the literature, which reported an increase in Riva AUC and the mean C_{max} by 39% and 76%, respectively, when orally administered 20 mg tablet with food [42]. Interestingly, both 10 and 20 mg strength of the marketed formulation comprise of sodium lauryl sulphate (SLS), in order to increase the solubility of the API in the *in vivo* conditions. However, as evident from the lower bioavailability, the solubilization efficiency of SLS in increasing the solubility of Riva in 20 mg tablet was found to be less as compared to the Riva in 10 mg tablet [42]. This decrease in solubilization efficiency in the fasted state could be due to a higher amount of API above the saturation solubility in the case of 20 mg Riva, as the volume of the *in vivo* fluid remains the same, potentially resulting in local precipitation and thus reduced bioavailability. Now in the case of the fed state, the excess of API higher than the saturation solubility could dissolve in lipidic components of the food and thus resulted in higher AUC and mean C_{max}. On further increasing the dose to supra-therapeutic levels of 50 mg of Riva, no further increase in the AUC and mean C_{max} values was observed even in the presence of fed conditions [37,43]. The ceiling effect observed in the case of 50 mg of Riva dosing, could be due to attainment of maximum solubility in fed conditions, assuming the volume of food nearly alike.

The C_{max} and AUC values predicted by the PBPK and IVIVC model were found to be comparable. However, the C_{max} and AUC values in the case of the PBPK model were slightly higher as compared to the IVIVC model. The increase in C_{max} and AUC values in the case of PBPK model, could be due to the incorporation of different physicochemical and formulation properties, for the development of the model, whereas the IVIVC model lacks integration of such parameters. In the present case, as evident from the parameter sensitivity studies, the particle size of the API was found to be a critical factor affecting the release profile and thus the bioavailability of the Riva. The impact of particle size on the bioavailability was found to be in accordance with the product filling, as the reference formulation was developed using the micronized Riva, in order to improve oral bioavailability via increasing solubility [43]. Thus, the development/optimization of the PBPK model using the particle size distribution of API could be responsible for the slight difference in the predicted C_{max} and AUC values, compared to the IVIVC model. Thus, the developed model can further be used to develop and optimize the formulation parame-

ters, mainly in the early-stage development phase, reducing preclinical and clinical time and cost.

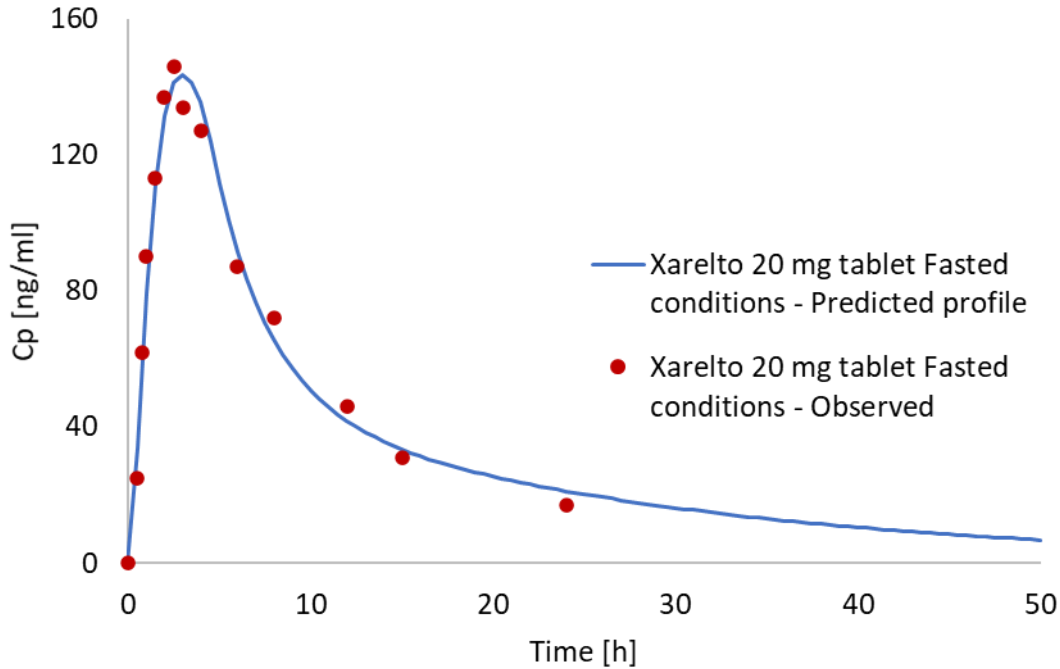## Simulated vs Observed PK profile in Fasted conditions

Figure 11: Internal validation (Xarelto 20 mg tablet Fasted condition) of the IVIVC model
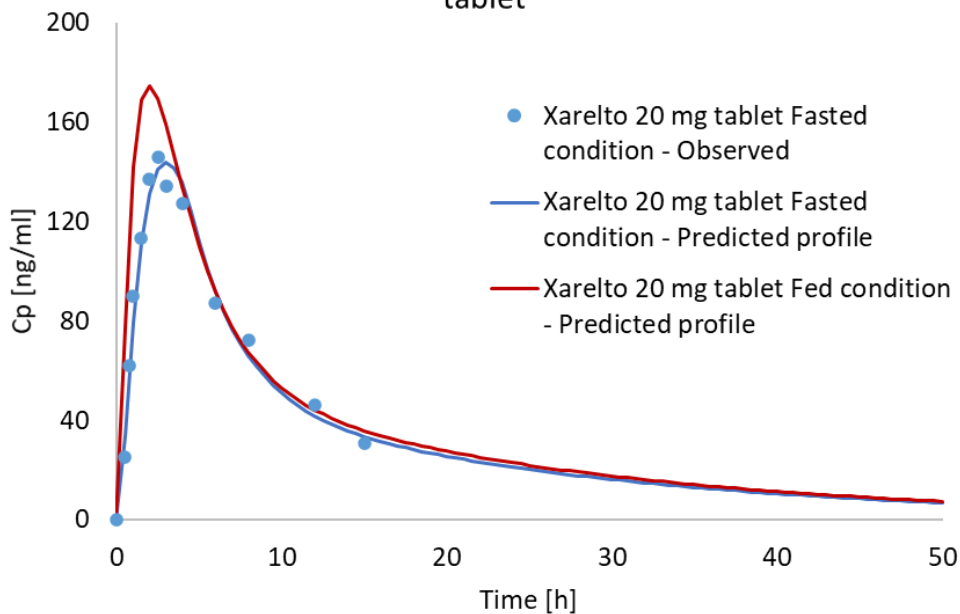
Observed vs Predicted PK profiles of Xarelto 20mg tablet

**Figure 12: Predicted mean *in vivo* profiles of Xarelto 20 mg tablet**

Table 8: Observed and predicted values of the internal validation (Xarelto 20 mg tablet Fasted condition) and predicted values of Xarelto 20 mg tablet Fed condition

Formulation	Parameter	Predicted	Observed	%PE	Ratio
Xarelto 20 mg tablet Fasted condition	AUClast	1381.946	1361.125	1.52966	1.015297
Xarelto 20 mg tablet Fasted condition	Cmax	143.567	146.000	-1.66657	0.983334

Xarelto 20 mg tablet Fed condition	AUClast	**1543.120**
Xarelto 20 mg tablet Fed condition	Cmax	**174.566**

3.4 Food Effect (FE) Studies of Riva in Simulated Healthy Subjects

A virtual trial is a stochastic simulation that randomly samples parameters from predefined distributions. In order to take into account, the effect of population variability on the plasma concentration profile of Riva following administration of Xarelto, a single dose (20 mg) 3-period virtual study design carried out in the fed state. Three virtual populations of 30-year American Male/Female A, B and C, each of 50 subjects, were created and subjected to Xarelto IR tablet (20 mg tablet). GastroPlus™ randomly generates subjects by varying the physiological factors such as gastrointestinal transit times, pHs, fluid volumes, pharmacokinetics parameters as well as compound parameters [44]. Table 9 provide a summary of virtual BE studies for population A, B, and C.
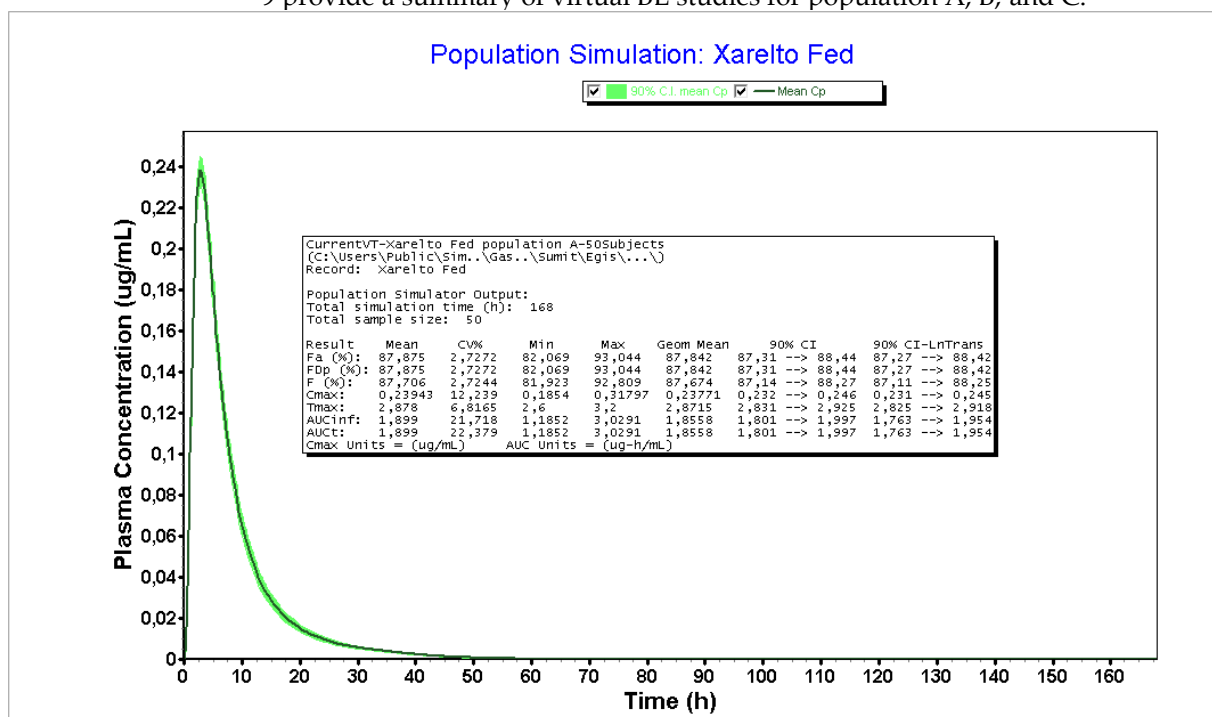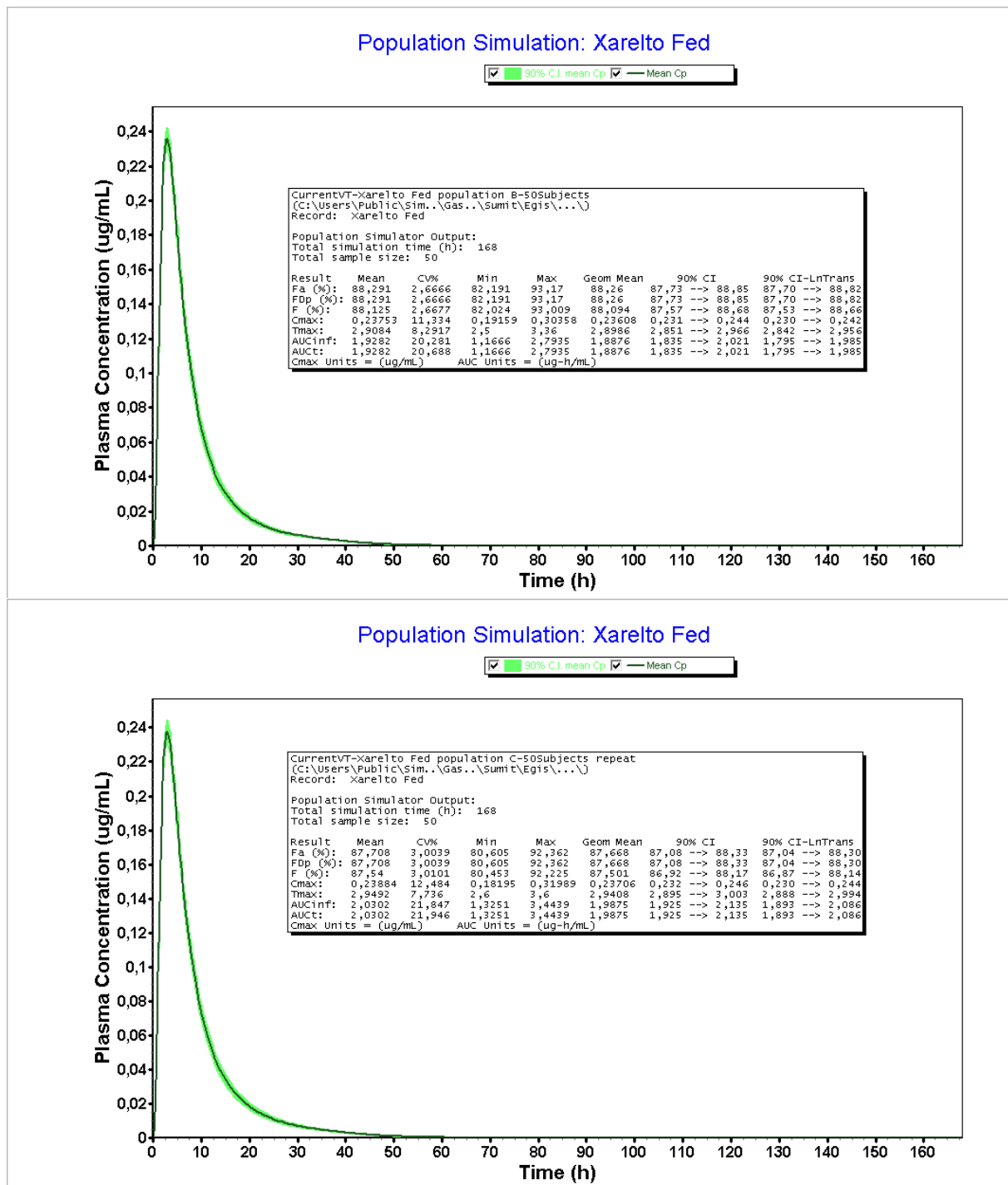
Figure 13: Virtual *in vivo* pharmacokinetic profile of Xarelto IR tablet (20 mg Strength) for Population A, B and C in fed conditions

Table 9: Summary *in vivo* pharmacokinetic profile of Xarelto IR tablet (20 mg Strength) for Population A, B and C in fed conditions

	Population A	Population B	Population C
C_{max} (ng/mL)	238	236	237
$AUC_{0-\infty}$ (ng.h/mL)	1856	1888	1988

The predicted population *in vivo* pharmacokinetic profile of Xarelto IR tablet (20 mg strength) in fed condition was found to be bioequivalent in population A, B, and C in the conditions, i.e., the average value of population geometric means were found to be within the range of 80-125% compared to the mean predicted *in vivo* profile mentioned in

Table 7, Figure 5 (C_{max} and $AUC_{0-\infty}$ value of 236.64 and 1857.3, respectively). Whereas, as compared to fasted conditions (C_{max} and $AUC_{0-\infty}$ value of 171.15 and 1433.8, respectively), the predicted *in vivo* population pharmacokinetics of Xarelto IR tablet (20 mg strength) in fed conditions, the bioequivalence criteria of 80 – 125% was found to be not satisfied.

During the virtual simulations, the physiological and pharmacokinetic parameters of the same subjects were identical for reference formulations. However, in reality, physiological and pharmacokinetic parameters could fluctuate within the same subjects if they were given different formulations on different occasions. Thus, by incorporating this intra-subject variability, it is possible that *in vivo* profile of Xarelto IR tablet in fasted condition might be bioequivalent to the population kinetics of Xarelto IR tablet (Fed) in population A since its 80% confidence interval for the AUC value (77%) is close to the edge of the BE limits.

4. Conclusions

In the present manuscript, the mechanistic physiology-based model for the Xarelto IR tablet was developed considering different physicochemical and pharmacokinetic parameters. In addition, the conventional IVIVC model was also developed in order to verify the *in vivo* profile obtained via the PBPK model. The validation results demonstrated the development of successful models and the predicted *in vivo* profiles from both models were found to be comparable. The results demonstrated a significant food effect increasing the C_{max} of the Riva and which could be due to higher solubility in Fed conditions. The developed model strategy can be effectively adopted to increase the confidence of the model. Furthermore, the PBPK model can also lead to the establishment of the biased dissolution methods crucial for the generic company to establish bioequivalence mainly focusing on complex formulations such as amorphous solid dispersions.

Appendix:

Table A1: Concentration of different components of the simulated media used in the solubility and dissolution studies

	FaSSGF	FaSSIF	FeSSGF	FeSSIF
pH	1.6	6.5	5.0	5.0
Solubility [µg/ml]	11.0	9.9	24.0	16.8
Sodium taurocholate (mM)	0.08	3.0	-	15
Lecithin (mM)	0.02	0.75	-	3.75
Sodium chloride (mM)	34.2	105.9	237.02	203.2
Sodium hydroxide (mM)	-	8.7	-	101
Hydrochloric acid (mM)	25.1	-	-	-
Monobasic sodium phospate (mM)	-	28.4	-	-
Acetic acid (mM)	-	-	17.12	144.1
Pepsin (mg/ml)	0.1	-	-	-
Sodium acetate anhydrous (mM)	-	-	29.75	-
Milk/Buffer	-	-	1:1	-

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