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Towards Virtual Bioequivalence Studies for Oral Dosage Forms Containing Poorly Water-Soluble Drugs: A Physiologically Based Biopharmaceutics Modeling (PBBM) Approach

Atsushi Kambayashi[a,b,*], Jennifer B. Dressman[c]

[a] Pharmaceutical Research and Technology Labs, Astellas Pharma Inc., 180 Ozumi, Yaizu, Shizuoka 425-0072, Japan
[b] School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
[c] Fraunhofer Institute for Translational Medicine and Pharmacology, Theodor Stern Kai 7, 60596 Frankfurt am Main, Germany

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A B S T R A C T

The objective of the present study was to develop a physiologically based biopharmaceutics (PBBM) approach to predict the bioequivalence of dosage forms containing poorly soluble drugs. Aripiprazole and enzalutamide were used as model drugs. Variations in the gastrointestinal (GI) physiological parameters of fasted humans were taken into consideration in *in vitro* biorelevant dissolution testing and in an *in silico* PBBM simulations. To estimate bioequivalence between dosage forms, the inter-individual variabilities in their performance in virtual human subjects were predicted from the *in vitro* studies and variability in e.g. gastric emptying and fluid volume in the stomach was also taken into account. Formulations with different *in vitro* dissolution performance, a solution and a tablet formulation, were used in order to evaluate the accuracy of bioequivalence prediction using the PBBM approach. The bioequivalence parameters, i.e. geometric mean ratio and 90% confidence interval, for both drugs were predicted well in the virtual studies. In order to achieve even more precise predictions, it will be important to continue characterizing GI physiological parameters, along with their variabilities, on both an inter-subject and inter-occasion basis.

Introduction

Drug formulations are often changed during clinical development and even after pivotal clinical studies and product registration. After formulation and/or manufacturing changes, it is important to establish bioequivalence between the two dosage forms (i.e. original vs. new) in order to assure the drug's safety and effectiveness. A bioequivalence study in humans between the original and new formulation can be replaced with a similarity assessment in *in vitro* dissolution testing for minor formulation changes and for drug formulations containing biopharmaceutics classification system (BCS) class I and III drugs.[1,2] However, for major formulation and manufacturing changes, including generic drug product development, a bioequivalence study in humans is mandatory throughout most of the world.

Risk factors that can affect the success of bioequivalence studies in humans have been discussed in the literature to date. BCS class II drugs (poorly soluble but highly permeable) were reported to have a risk of failure approximately four times higher than class I and III (both highly soluble) in an evaluation of 500 bioequivalence studies.[3] Another investigation using data sets of human bioequivalence studies of 113 drug products revealed that drugs with solubility-limited absorption would present one of the biggest risks of variable absorption in the gastrointestinal (GI) tract.[4] These reports suggest that low solubility of the active pharmaceutical ingredient (API) would be a major risk factor in human bioequivalence studies. This might be because solubility and dissolution rate of poorly soluble drugs in the GI tract can be highly affected by inter-individual and intra-individual variations in GI physiology such as pH, bile levels, fluid volume, transit time, etc. Therefore, an approach that can take into consideration of these variations to precisely predict bioequivalence for poorly soluble drugs would be very useful in drug product development.

Over the last few years, model informed drug development has increasingly been implemented in various pharmaceutical research areas ranging from early phase drug discovery to the submission of new drug applications to regulatory authorities. Physiologically based pharmacokinetic (PBPK) models have been widely used when predicting drug-drug interactions (DDIs).[5] Among these prediction models, physiologically based biopharmaceutics models (PBBM) have

* Corresponding author at: Pharmaceutical Research and Technology Labs, Astellas Pharma Inc., 180 Ozumi, Yaizu, Shizuoka 425-0072, Japan.
E-mail address: atsushi.kambayashi@astellas.com (A. Kambayashi).

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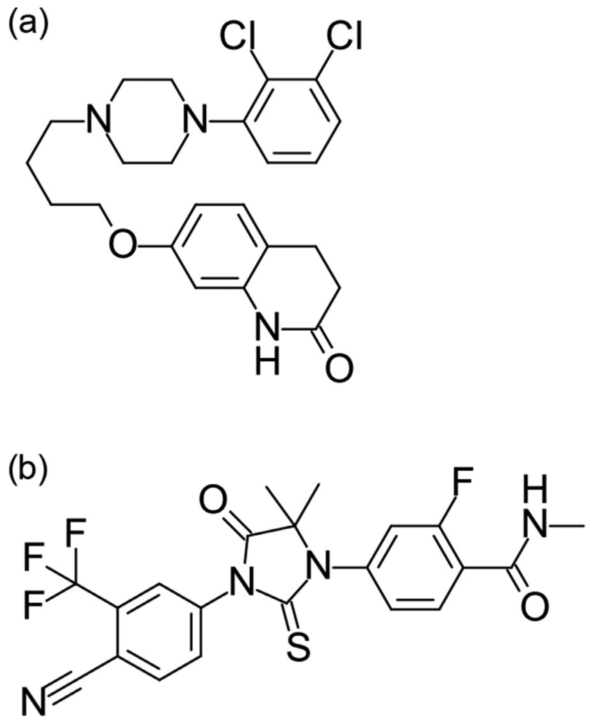

Fig. 1. Chemical structure of (a) aripiprazole and (b) enzalutamide.

focused on drug absorption in the GI tract, incorporating factors such as drug release and dissolution from the administered dosage form, the gastrointestinal transit of the drug and formulation, and the membrane permeation of the drug in the small intestine appropriately. To date, PBBM simulation approaches have been used in predictions of gastric pH-dependent DDIs,[6−9] effects of drug particle diameter on PK profile,[10] virtual bioequivalence and clinically relevant dissolution specifications,[11−15] and so on. Among these applications of PBBM simulations, it has been recognized by regulatory authorities and pharmaceutical companies that virtual bioequivalence would constitute the greatest challenge.[16]

The objective of the present study was to develop a PPBM approach to predict the bioequivalence of dosage forms containing poorly soluble drugs. Aripiprazole, a weak base drug, and enzalutamide, a neutral drug but formulated in amorphous solid dispersion with acidic polymer HMPC-AS (Fig. 1), were used as model drugs for these studies. Variations in the GI physiological parameters of fasted humans were taken into consideration in *in vitro* biorelevant dissolution testing and in an *in silico* PBBM simulations. Not only the average performance of dosage forms but also inter-individual variabilities in their performance in virtual human subjects were predicted to estimate bioequivalence between dosage forms. Formulations with apparently different *in vitro* dissolution performance, a solution and a tablet formulation, were used in order to validate the accuracy of bioequivalence prediction using the PBBM approach.

Materials and methods

Materials

Commercially available tablets of aripiprazole (Abilify® tablets 3 mg, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) and enzalutamide (Xtandi® tablets 80 mg, Astellas Pharma Inc., Tokyo, Japan) were used in this study. Acetonitrile, hydrochloric acid solution (1 mol/L), maleic acid, perchloric acid, sodium chloride, sodium hydroxide pellets, sodium hydroxide solution (1 mol/L) were all of analytical grade and purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). FaSSIF/FeSSIF/FaSSGF powder and FaSSIF-V2 powder were purchased from Biorelevant.com Ltd (London, United Kingdom). Pepsin was purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA).

In vitro dissolution testing

In the current study, biorelevant media that have been designed for humans were used for *in vitro* dissolution testing: 300 mL of fasted state simulated gastric fluid (FaSSGF) and 500 mL of fasted state simulated intestinal fluid version 2 (FaSSIF-V2). For testing of 160 mg of enzalutamide (2 tablets, Xtandi®), the USP apparatus II (paddle) at 50 rpm was used. The dissolution apparatus for 3 mg of aripiprazole (1 tablet, Abilify®) was USP apparatus I (basket) at 100 rpm, since significant coning was observed when these tablets were tested in USP apparatus II at 50 rpm. The basket method at 100 rpm is generally interchangeable with the paddle method at 50 rpm.[17] In both cases experiments were conducted at 37 ± 0.5°C. At 5, 10, 15, 20, 30, and 60 min, 5 mL dissolution samples for aripiprazole, or 7.5 mL for enzalutamide, were withdrawn using a stainless steel cannula and a plastic syringe. Immediately after the sampling, the aripiprazole samples were filtered through PVDF 0.45 μm (Whatman GD/X 13 mm) after discarding the first 3.5 mL, while enzalutamide samples were filtered through PES 0.45 μm (Whatman GD/X 25 mm) after discarding the first 5 mL. After filtration, the filtrates were mixed 1:1 with acetonitrile to avoid further precipitation before HPLC analysis.

In order to evaluate potential variability in dissolution performance of the tablets *in vivo*, not only the standard composition of the biorelevant media but also variants of the biorelevant media (pH, buffer capacity, and bile concentration) were employed in the present study (Table 1). Dissolution testing was conducted in triplicate in each study condition.

Solubility measurements of aripiprazole were performed by adding excess amount of the drug formulations in FaSSGFs and FaSSIF-V2 (pH 5) and shaking for 6 hours at 37 ± 0.5°C. The solubility samples were pre-treated according to the same procedure used for the dissolution samples of the drug. The solubility in each biorelevant medium was measured in triplicate.

The dissolved concentration of aripiprazole and enzalutamide in the biorelevant media in both dissolution testing and solubility measurements were quantitatively analyzed using a HPLC system (Alliance Separations Module 2695 with detector of type 2487, Waters Corporation, Milford, MA, USA). The analytical column was TSKgel ODS 100Z 5 μm (4.6 mm × 15 cm, Tosoh Corporation, Tokyo, Japan), which was maintained at 40°C or 30°C for aripiprazole and enzalutamide, respectively. The mobile phases were a mixture of acetonitrile and 100 mM sodium perchlorate solution 1:1 v/v for aripiprazole, and 60% acetonitrile for enzalutamide. The flow rate of the mobile phase was set at 1.0 mL/min. The injection volume was 10 μL and the detection wavelengths were 254 nm and 260 nm for aripiprazole and enzalutamide, respectively. The lower limit of quantifications was < 0.2 μg/mL for ariprípazole and < 3 μg/mL for enzalutamide.

In silico modeling and simulation for estimating bioequivalence

An *in silico* prediction model was developed and the pharmacokinetic profiles of aripiprazole and enzalutamide after oral administration were simulated using Stella Professional version 1 (isee systems, Lebanon, NH, USA) software. The basic model structure and theory of the simulation have been reported previously.[9,11]

Table 1
Composition and characteristics of biorelevant dissolution media and variations thereof.

	FaSSGF			FaSSIF-V2						
	Standard	pH 1.2	pH 2.3	Standard	Buffer capacity 3	Buffer capacity 6	pH 5.0	pH 7.0	Bile 2	Bile 4
Hydrochloric acid (mM)	25.1	63.1	5.0	—	—	—	—	—	—	—
Sodium chloride (mM)	34.2	34.2	34.2	68.62	20.59	41.17	68.62	68.62	68.62	68.62
Pepsin (mg/mL)	0.1	0.1	0.1	—	—	—	—	—	—	—
Maleic acid (mM)	—	—	—	19.12	5.74	11.47	19.12	19.12	19.12	19.12
Sodium hydroxide (mM)	—	—	—	34.80	10.44	20.88	34.80	34.80	34.80	34.80
Sodium taurocholate (mM)	0.08	0.08	0.08	3	3	3	3	3	2	4
Lecithin (mM)	0.02	0.02	0.02	0.2	0.2	0.2	0.2	0.2	0.13	0.27
pH	1.6	1.2	2.3	6.5	6.5	6.5	5.0	7.0	6.5	6.5
Buffer capacity (mM/ΔpH)	—	—	—	10	3	6	10	10	10	10

In the present study, dissolution rate of the drugs in the biorelevant media and in the GI tract of fasted humans were assumed to follow the modified Noyes-Whitney equation:

$$\frac{dW_t}{dt} = z \cdot W^{2/3} \cdot \left(C_S - \frac{W_t}{V_t}\right) \tag{1}$$

where W_t is the amount of the dissolved drug at time t, z is the dissolution rate parameter, W is the amount of drug still undissolved at time t, C_s is the saturate solubility of the drugs in each biorelevant medium, and V_t is the fluid volume of the *in vitro* dissolution testing or the GI tract. The z values of aripiprazole and enzalutamide in each biorelevant dissolution medium were estimated from the results of *in vitro* dissolution testing in 300 mL FaSSGF and 500 mL FaSSIF-V2. In the GI tract of fasted humans, the initial fluid volume of 50 mL and 100 mL in the stomach and small intestine,[18] respectively, were assumed. Ingested water volume in the simulations were 150 mL[19] and 240 mL for aripiprazole and enzalutamide, respectively.

The gastric emptying rate of the drugs (both dissolved and undissolved) and stomach fluid were assumed to follow the first order equation:

$$\frac{dG_t}{dt} = GER \cdot X \tag{2}$$

where G_t is the amount of drug or fluid volume that are already emptied from the stomach at time t, GER is the first order gastric emptying rate constant, and X is the amount of drug or fluid volume still remaining in the stomach at time t. A GER value of 2.8 h^{-1}[20] and a small intestinal transit time of 4 h[21] were assumed in the current study.

In the present simulation, it was assumed that dissolved drug in the small intestine, but not in the stomach and colon, can be permeated through the intestinal epithelium to reach the blood circulation using the following equation:

$$\frac{dA_t}{dt} = P_{eff} \cdot SA \cdot \frac{W_t}{V_t} \tag{3}$$

where A_t is the drug amount already absorbed in the small intestine at time t, P_{eff} is the permeability coefficient of each drug, and SA is the effective surface area in the permeation. The P_{eff} value of aripiprazole in the small intestine of humans was estimated using its permeability data through an artificial membrane[22] and an equation in the literature (PAMPA *vs.* human intestinal P_{eff}).[23] By contrast, diffusion rate constant through the unstirred water layer, which was calculated using an equation in the literature,[24] was used for enzalutamide, due to its permeability value being much higher than the "highly permeable" model drug propranolol in Caco-2 cell monolayers.[25] The effective surface area in the small intestine was assumed to be 800 cm^2[26] in the present study.

After intestinal absorption, both drugs were assumed to follow two-compartmental distribution and elimination properties. The post-absorptive pharmacokinetic parameters of each drug was estimated using Phoenix WinNonlin version 8.0 (Certara, L.P., Princeton, NJ, USA) from published plasma concentration profiles: for aripiprazole after administration of an oral solution[19] and for enzalutamide after administration of a liquid-filled capsule.[27] Table 2 summarizes the post-absorptive pharmacokinetic parameters of aripiprazole and enzalutamide used in the simulations in the present study. As it has been reported that >87% of aripiprazole is absorbed in humans and almost all enzalutamide can be absorbed in humans, the distribution volume in the central compartment divided by oral bioavailability (V_1/F) was assumed to be the volume divided by the fraction surviving the first pass metabolism in the gut and liver (Fg and Fh) for both drugs.

Simulations of the plasma concentration profiles of aripiprazole and enzalutamide were performed using Stella Professional software with the above-mentioned theory and parameters. The plasma concentrations were calculated up to 24 h after oral administration with a time interval of 0.05 h.

In addition to applying the standard values for the physiological variables, inter-individual variability in the GI physiology of fasted humans were taken into consideration to perform a sensitivity analysis of the simulations. Table 3 summarizes the lowest and the highest possible values of each GI physiological parameter used in the present study. In the parameter sensitivity analysis of the simulations, the plasma concentration profile and pharmacokinetic parameters for each study condition was calculated and compared with those of the standard values in order to understand the impact of each parameter on the oral absorption behavior of the two APIs.

In order to perform virtual bioequivalence studies for aripiprazole and enzalutamide, plasma concentration profiles for the virtual subject (each with different GI physiology) were simulated prospectively. According to the lowest and the highest values in the sensitivity analysis (Table 2), Fig. 2 describes the cumulative % probability curves for the GI physiological parameters. Random numbers between 0 and 100 were generated using Microsoft Excel Office 365 (Microsoft Corporation, Redmond, WA, USA), and then each GI physiological parameter for each virtual subject was calculated using separated linear regression (Fig. 2) and the

Table 2
Post-absorptive pharmacokinetic parameters used in PK simulations for aripiprazole and enzalutamide.

	Aripiprazole	Enzalutamide
V_1/(Fg Fh) (mL)	184375	24489
K10 (h^{-1})	0.0193	0.0224
K12 (h^{-1})	0.0444	0.2975
K21 (h^{-1})	0.0984	0.1541

Table 3
Physiological parameters used in the sensitivity analysis of PK simulations.

	Standard value	The lowest value in the sensitivity analysis	The highest value in the sensitivity analysis	References
Stomach				
Gastric emptying rate constant (h^{-1})	2.8	1.9	14	28,29
Fluid volume (mL)	50	27	63	18
pH	1.6	1.2	2.3	30
Small intestine				
Transit time (h)	4.0	3.2	4.4	21
Fluid volume (mL)	100	74	112	18
pH	6.5	5.0	7.0	30
Buffer capacity (mM/ΔpH)	6	3	10	30
Bile concentration (mM)	3	2	4	30

random numbers. Each virtual subject had 8 generated GI physiological parameters, each of which were assumed to be independent to the others, recognizing that there may be some covariate effects among GI parameters. As the focus of the current virtual bioequivalence simulations was the detection of differences in formulation performance in the GI tract and their effect on the plasma profile, the same values of permeability coefficient and post-absorptive distribution − elimination parameters were used in all the virtual subjects. Thirty virtual subjects were enrolled in this study, which was almost the same number participating in the bioequivalence study of enzalutamide.[31]

In the current study, plasma concentration profiles of two dosage forms: (i) solution formulations with no precipitation in the GI tract and (ii) tablet formulations of aripiprazole and enzalutamide were simulated. It was assumed that no intra-individual variability in the GI physiology occurs between administration of the two dosage forms in the virtual subjects, therefore, the same combination of the GI parameters was used for each subject when simulating the performance of two dosage forms (i.e. solution and tablet). Pharmacokinetic parameters, Tmax, Cmax, and AUC_{inf}, were calculated with each plasma profile using Phoenix WinNonlin. The geometric mean ratio (GMR) and 90% confidential intervals (CI) of Cmax and AUC_{inf} ratios (tablet to solution) were then calculated using GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA). The results of GMRs and 90% CIs of the virtual studies for aripiprazole and enzalutamide were compared with the observed data. The virtual bioequivalence study (N = 30) was performed in triplicate for aripiprazole and enzalutamide.

Results and discussion

Virtual bioequivalence for aripiprazole

Fig. 3 shows the dissolution profiles of aripiprazole from Abilify® 3 mg tablets in various biorelevant dissolution media. In addition to the standard compositions of the dissolution media, FaSSGF with pH values of 1.2 − 2.3 and FaSSIF-V2 with different variations in buffer capacity, pH, and bile concentration were used in the present study. Aripiprazole dissolved very rapidly, with >85% dissolution at 10 minutes in all the FaSSGF variants (pH 1.2 − 2.3) tested (Fig. 3a). Although a slight difference in the solubility of the drug between pH 1.2 and 2.3 was seen, possibly due to a common ion effect of chloride, all the FaSSGF variants had sufficient solubility to dissolve the entire 3 mg of aripiprazole. By contrast, dissolution behavior of the drug was highly affected by the composition of FaSSIF-V2 (Fig. 3b). Almost all the drug dissolved in FaSSIF-V2 at a pH of 5.0, while less than 50% of the drug dissolved at higher pH values (i.e. pH 6.5 and 7.0). The dependency of dissolution on pH is likely related to the dissociation behavior of aripiprazole, which is a weak base compound with a pKa of 7.7.[32] The extent and rate of dissolution of aripiprazole were not significantly affected by the buffer capacity or the bile component concentration of FaSSIF-V2.

The Noyes-Whitney dissolution rate parameter z for aripiprazole in each composition of biorelevant media in the study was estimated for the following simulations of plasma concentration profiles (Table 4). In the parameter estimation, the dissolution profiles of the drug in Fig. 3 and the solubility values in each medium were used. As only a limited amount (< 50%) of the drug dissolved in FaSSIF-V2, except at pH 5.0, an infinity point was invoked to estimate the solubility in each respective medium. After subjecting the formulation to the dissolution test for one hour, the revolution rate was raised to 250 rpm for a further hour and the end concentration was used in simulations to represent the solubility, rather than the concentration achieved in separate solubility measurements.

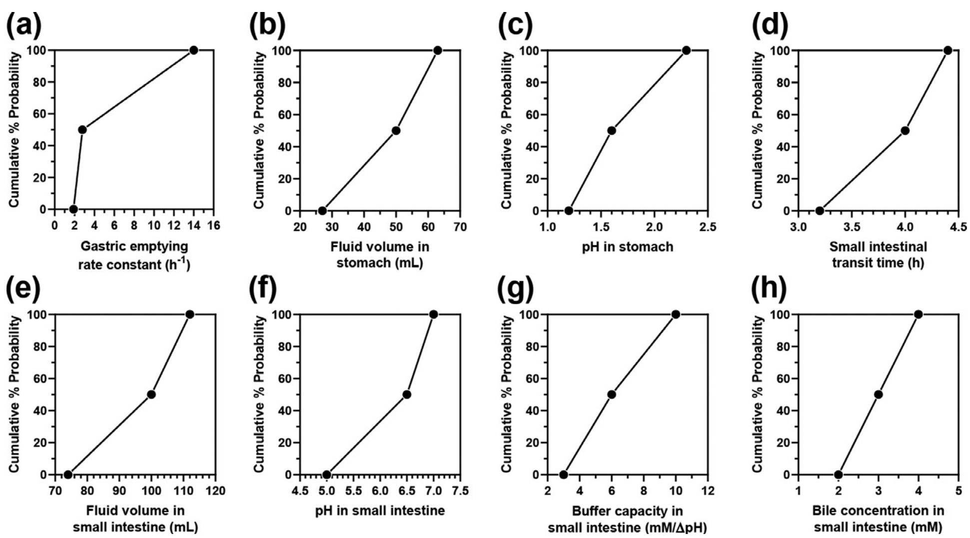

Fig. 2. Cumulative percent probability curves for the physiological parameters in the gastrointestinal tract in the PK simulations.

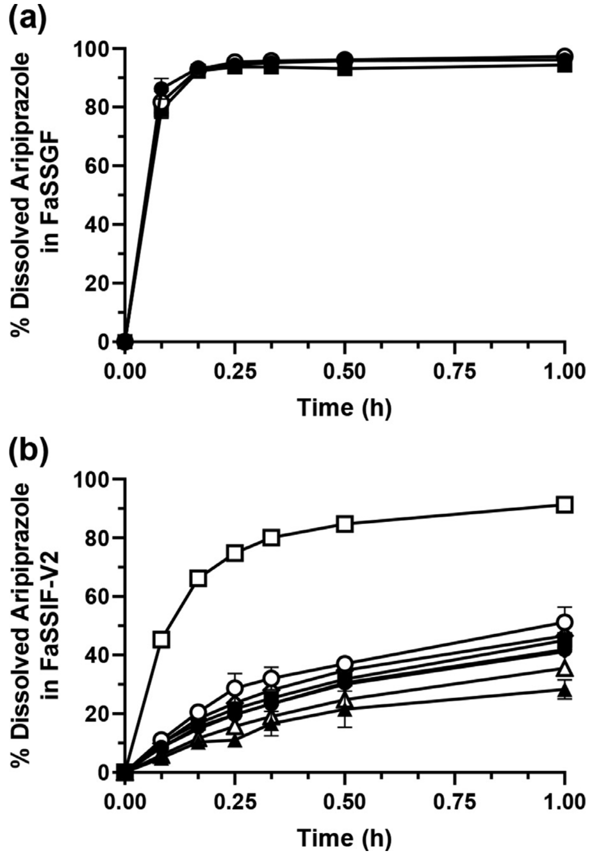

Fig. 3. Dissolution profiles of aripiprazole from Abilify® tablets in (a) FaSSGF with (●) the standard composition, (○) pH 1.2, and (□) pH 2.3, and in (b) FaSSIF-V2 with (●) the standard composition, (○) buffer capacity of 3 mM/ΔpH, (□) 6 mM/ΔpH, (□) pH 5, (▲) pH 7, (△) bile salt concentration of 2 mM, (◆) 4 mM, and (◇) buffer capacity of 6 mM/ΔpH with bile salt concentration of 4 mM.

In pharmacokinetic simulations for virtual bioequivalence assessment in the present study, it was assumed that, in terms of dissolution in the stomach, the pH value is of primary importance, but that in the small intestine, dissolution can be affected by several parameters such as buffer capacity, pH, and bile concentration, all of which vary among virtual subjects. Therefore, it is necessary to establish relationships between the essential parameters for drug dissolution (solubility and *z* value) and the three parameters of the intestinal fluid for each virtual subject to predict the bioperformance of dosage forms. In the present study, a multiple linear regression was performed for solubility and *z* value with variables of buffer capacity, pH, and bile concentration in the small intestinal fluid using GraphPad Prism. The following equations are the results of the analyses for solubility and *z* value of aripiprazole in the small intestinal fluid:

$$Solubility = 0.1479 - 0.02251 \times pH + 0.0004678 \times BC - 0.0001713 \times C_{bile} \tag{4}$$

$$z\ value = -1.133 + 0.3699 \times pH - 0.05582 \times BC + 0.09396 \times C_{bile} \tag{5}$$

where *BC* is the buffer capacity (mM/ΔpH) and C_{bile} is the bile concentration (mM) in the small intestine. The R-squared values of the analyses were 0.9327 and 0.9420 for the solubility and *z* value,

Table 4
Solubility and *z* value of aripiprazole in FaSSGF and FaSSIF-V2.

	Characteristics of biorelevant media	Solubility (mg/mL)	Z value (mL mg$^{-2/3}$ h^{-1})
FaSSGF	Standard	0.9390	0.093
	pH 1.2	0.3695	0.213
	pH 2.3	1.0513	0.069
FaSSIF-V2	Standard	0.0029*	1.08
	Buffer capacity 3 mM/ΔpH	0.0034*	1.38
	Buffer capacity 6 mM/ΔpH	0.0030*	1.22
	pH 5.0	0.0416	0.39
	pH 7.0	0.0019*	1.03
	Bile salts 2 mM	0.0025*	0.96
	Bile salts 4 mM	0.0028*	1.14
	Buffer capacity 6 mM/ΔpH with bile salts 4 mM	0.0031*	1.32

* Estimated from the concentration at the infinity sampling point in the dissolution test.

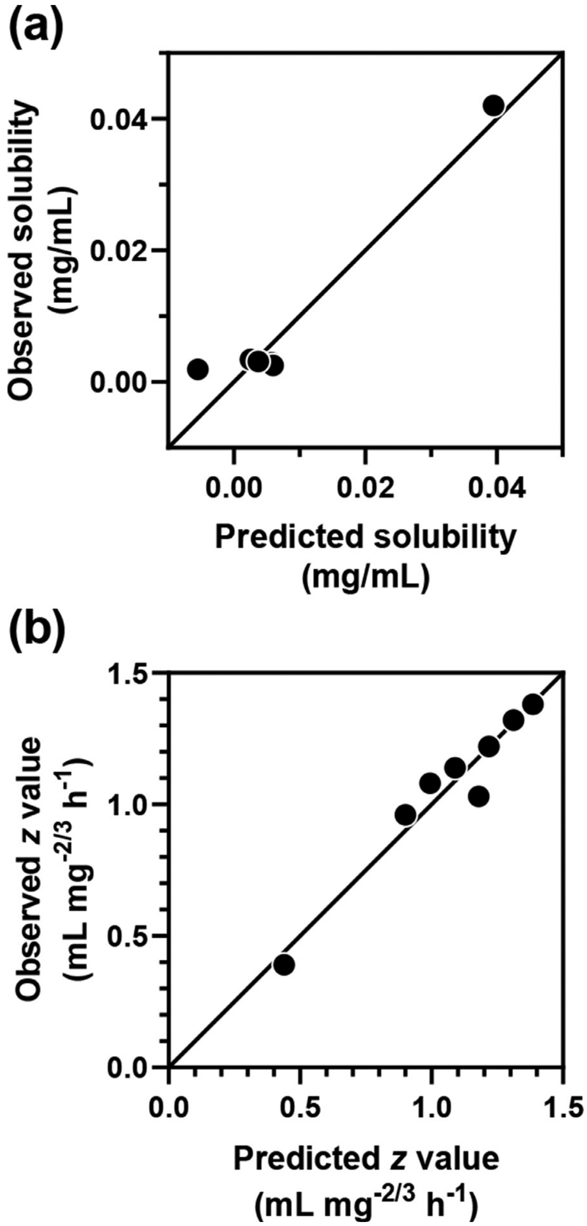

Fig. 4. Relationship between observed data and predicted data using multiple linear regression analysis for aripiprazole tablet in the simulated intestinal fluid: (a) solubility and (b) dissolution rate parameter *z* value. The solid lines represent a straight line with slope of 1.

respectively. Fig. 4 shows comparisons of the predicted data using Eq. 4 and 5 with the observed solubility and z value. The results suggest that these equations are appropriate to estimate the solubility and z value in each virtual subject since almost all the plots in the figures were close to the line of unity.

Before performing a virtual bioequivalence study, parameter sensitivity analysis in the pharmacokinetic simulations was conducted in order to understand which parameters in the GI tract are critical to oral absorption of aripiprazole. Fig. 5 represents the results of the parameter sensitivity analysis of Cmax and AUC_{inf} for the aripiprazole oral solution and tablet formulations, in which various GI parameters such as gastric emptying rate, stomach fluid volume, stomach pH, small intestinal transit time, intestinal fluid volume, pH, buffer capacity, and bile concentration were employed in the PBPK simulations.

Almost the same values of Cmax and AUC_{inf} were observed with the oral solution of aripiprazole under all conditions of the parameter sensitivity analysis, noting that aripiprazole was assumed not to precipitate from the oral solution in these simulations. By contrast, changes in the GI physiological parameters affected the oral absorption of aripiprazole from the tablet formulation. In particular, the Cmax and AUC_{inf} of the tablet decreased at more rapid gastric emptying rates. As aripiprazole has pH-dependent aqueous solubility, with high solubility in acidic conditions but poor solubility towards neutral pH, a short residence time in the stomach may limit the overall drug dissolution in the GI tract. Further, the tablet showed higher Cmax value in FaSSIF-V2 with pH 5.0 than for the higher pH conditions. This is consistent with the higher dissolution of aripiprazole from the tablet in pH 5.0 than in versions of FaSSIF-V2 with a pH of 6.5 or 7.

A virtual bioequivalence study was then performed three times to compare the *in vivo* performance of solution and tablet formulations of aripiprazole in 30 virtual subjects who were assigned various GI physiological parameters, as described above in Fig. 2. Fig. 6 shows the predicted plasma concentration profiles of aripiprazole after administration of the solution (Fig. 6a, c, and e) and the tablet (Fig. 6b, d, and e) in comparison with the observed plasma profiles.

The mean predicted plasma profiles in the three virtual studies were in excellent agreement with the observed profile of the solution. However, the inter-individual variabilities in the predicted plasma profiles for the solution were much smaller than that of the observed profile. The likely reason for this is that inter-individual variability in the post-absorptive PK parameters was not taken into consideration in the present PK simulations.

The predicted plasma concentration profiles for the tablet showed larger inter-individual variabilities than for the oral solution. As observed in the parameter sensitivity analysis for the tablet, the gastric emptying rate and the small intestinal pH are expected to have a big impact on the *in vivo* dissolution performance of the aripiprazole

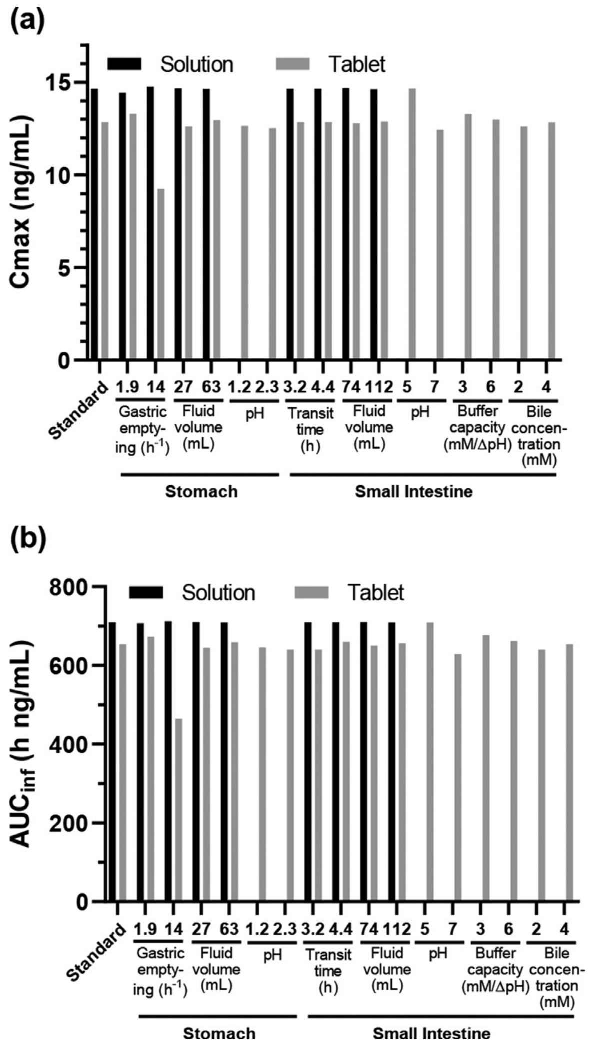

Fig. 5. Sensitivity analysis for the gastrointestinal physiological parameters in simulating (a) Cmax and (b) AUC of aripiprazole solution and tablet.

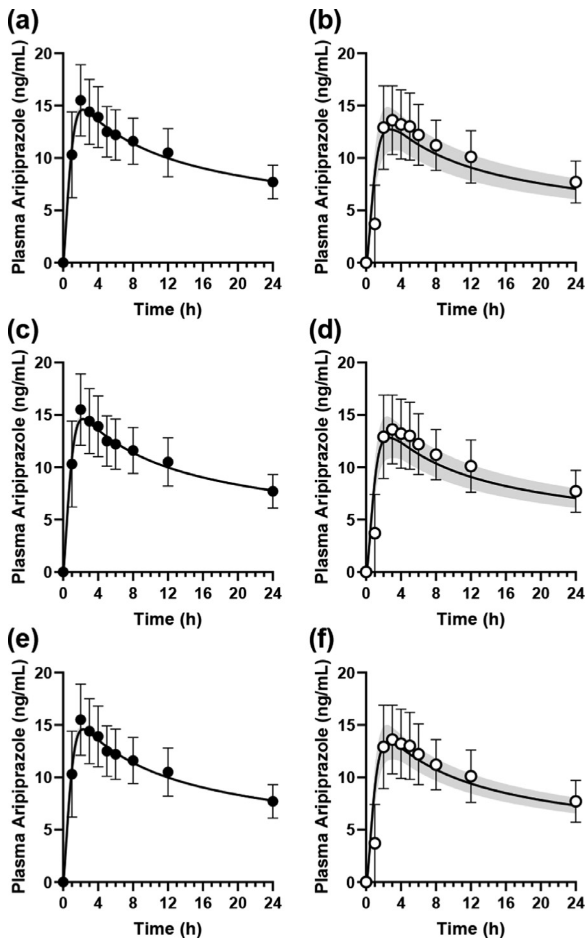

Fig. 6. Predicted and observed PK profiles of aripiprazole after oral administration of (a) solution and (b) tablet formulations in the first run of virtual BE study, (c) solution and (d) tablet in the second run, and (e) solution and (f) tablet in the third run. Mean ± SD (error bars and area shaded in gray). The observed profiles were taken from the literature.[19]

Table 5
Predicted and observed PK parameters of aripiprazole after oral administration of solution and tablet.

	Solution				Tablet			
	Observed	Predicted (Run 1)	Predicted (Run 2)	Predicted (Run 3)	Observed	Predicted (Run 1)	Predicted (Run 2)	Predicted (Run 3)
Tmax (h)	2.00 [2.00 − 6.00]	2.30 [1.85 − 2.55]	2.25 [2.00 − 2.65]	2.10 [1.90 − 2.55]	3.00 [2.00 − 8.00]	2.73 [2.00 − 4.25]	2.73 [2.15 − 4.05]	2.68 [2.00 − 4.15]
Cmax (ng/mL)	15.8 ± 3.3	14.7 ± 0.1	14.6 ± 0.1	14.6 ± 0.1	15.3 ± 2.5	12.9 ± 1.9	12.9 ± 1.9	13.4 ± 1.6
AUC_{inf} (h ng/mL)	762.1 ± 188.2	710.1 ± 1.7	710.0 ± 1.5	709.8 ± 1.7	743.1 ± 196.6	639.5 ± 88.0	640.1 ± 81.8	662.8 ± 65.3
Cmax GMR with 90% CI (tablet to solution)	—	—	—	—	0.98 (0.92 − 1.03)	0.87 (0.82 − 0.92)	0.87 (0.83 − 0.92)	0.91 (0.87 − 0.95)
AUC_{inf} GMR with 90% CI (tablet to solution)	—	—	—	—	0.97 (0.93 − 1.01)	0.89 (0.85 − 0.94)	0.89 (0.85 − 0.93)	0.93 (0.90 − 0.96)

The observed data were taken from the literature.[19]

tablet. Therefore, these GI physiological parameters also affected the plasma profiles of the drug in the virtual 30 subjects, resulting in variable plasma profiles. Interestingly, the simulated variability appeared to be similar to (although slightly lower than) the observed variability, suggesting that events in the GI tract are a major source of variability in the plasma levels for this formulation. It should be also noted that the variability in the tablet PK cannot be predicted completely using the current prediction model since the variability in the post-absorptive PK of drug was not considered.

Table 5 summarizes the predicted and observed PK parameters of aripiprazole following oral administration of the solution and tablet. The predicted 90% CI for GMRs (tablet to solution) of Cmax and AUC_{inf} of aripiprazole fell within 0.80 − 1.25, indicating that the formulations were bioequivalent, not only in the actual study in humans but also in the three virtual clinical studies.

It is noted that the GMRs for Cmax and AUC_{inf} were slightly underestimated (ca. -10%) in the virtual studies, although the results in the third run were slightly higher than in the other two runs. This might be due to some outliers of the predicted Cmax and AUC_{inf} in the virtual studies. For example, in the first study, three virtual subjects had very rapid gastric emptying and high intestinal pH, which resulted in much lower Cmax (ratio of 0.52 − 0.66) and AUC_{inf} (ratio of 0.55 − 0.70) values compared with the other subjects. Although the ranges of GI physiological parameters employed in the present virtual bioequivalent study were derived from the literature, it would be unusual to have three outliers with extremely low bioavailability in an actual subject group of 24 volunteers in a bioequivalent study of aripiprazole tablets, and in fact, the observed minimum values of Cmax and AUC of aripiprazole for the solution and tablet dosage forms were reported to be very similar in the clinical study that was published in the open literature.[19]

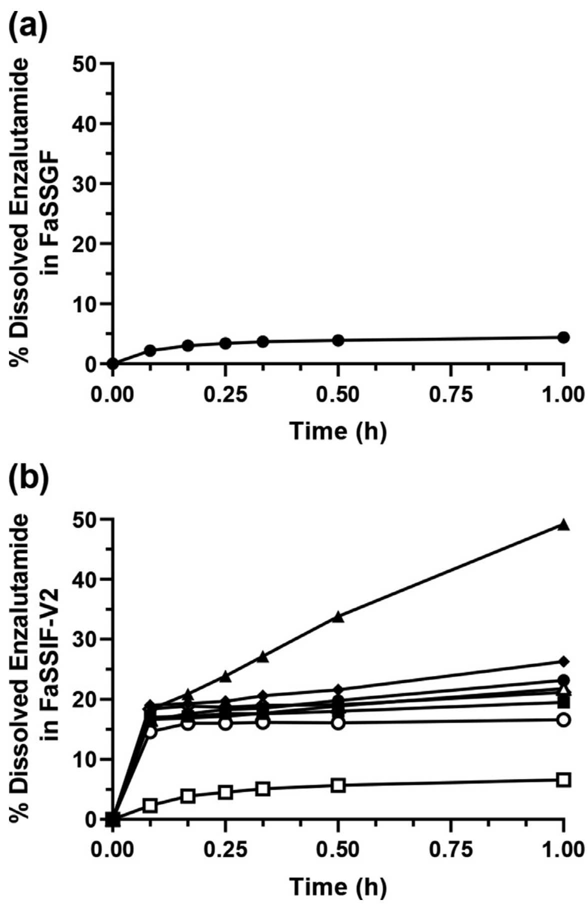

Fig. 7. Dissolution profiles of enzalutamide from Xtandi® tablets in (a) FaSSGF with (•) the standard composition and in (b) FaSSIF-V2, with (•) the standard composition, (◯) buffer capacity of 3 mM/ΔpH, (□) 6 mM/ΔpH, (□) pH5, (▲) pH7, (△) bile salt concentration of 2 mM, (◆) 4 mM, and (◇) buffer capacity of 6 mM/ΔpH with bile salt concentration of 4 mM.

Virtual bioequivalence for enzalutamide

Various compositions of biorelevant media for the stomach and small intestine of humans were also used in the *in vitro* dissolution testing of the enzalutamide formulation, which is an amorphous solid dispersion (ASD) formulation comprising hypromellose acetate succinate (HPMC-AS) as the carrier polymer (Fig. 7). In FaSSGF (Fig. 7a) and FaSSIF-V2 with pH 5 (Fig. 7b), a very limited amount of the drug was dissolved in the dissolution media, consistent with the solubility data in these two media (Table 6). The low solubility of the ASD formulation at pH 5 or below, ca. 0.02 mg/mL, is consistent with the solubility properties of HPMC-AS. Due to the poor dissolution of the ASD in FaSSIF-V2 (pH 5) and in the standard composition of FaSSGF, dissolution in the pH variants of FaSSGF (pH 1.2 and 2.3) was not performed.

Table 6
Solubility and z value of enzalutamide in FaSSGF and FaSSIF-V2.

		Solubility (mg/mL)	Z value (mL mg$^{-2/3}$ h^{-1})
FaSSGF	Standard	0.021*	0.28
FaSSIF-V2	Standard	0.074*	0.31
	Buffer capacity 3 mM/ΔpH	0.053*	0.97
	Buffer capacity 6 mM/ΔpH	0.062*	0.96
	pH 5.0	0.021*	0.18
	pH 7.0	0.157*	0.12
	Bile salts2 mM	0.070*	0.32
	Bile salts 4 mM	0.084*	0.29
	Buffer capacity 6 mM/ΔpH with bile salts 4 mM	0.068*	0.93

* Estimated from the concentration at the infinity sampling point in the dissolution test.

In FaSSIF-V2, the pH value had a big impact on the dissolution performance of enzalutamide. The higher the pH value in FaSSIF-V2, the greater the dissolution from the formulation. This result can also likely be attributed to the dissolution characteristics of HPMC-AS, with its nominal dissolution pH of 5.5 – 6.8. The effect of the buffer capacity of FaSSIF-V2 on the dissolution profile of enzalutamide was also evaluated. The solubility of the drug tended to decrease under the low buffer capacity condition. This might be because the dissolved HPMC-AS can acidify the dissolution fluid in the microclimate around the undissolved drug particles and prevent from further dissolution from the particles under lower buffer capacity conditions. By contrast in a FaSSIF-V2 version with a higher buffer capacity, the bulk pH value of 6.5 can be maintained in the dissolution microclimate around the drug particles even during dissolution of the acidic HPMC-AS. The effect of the bile component concentration in FaSSIF-V2 on the dissolution performance of enzalutamide was also evaluated. A higher concentration of the bile salts tended to increase the solubility of the drug, as would be predicted from its log P value of 3.0.[31,33]

Table 6 summarizes the solubility and the dissolution rate parameter z values estimated from each dissolution profile (Fig. 7). These values were used in the following PBPK simulations for enzalutamide.

Multiple linear regressions were also performed in order to analyze the relationships between dissolution performance (solubility and z value) of enzalutamide in FaSSIF-V2 and the characteristic parameters (i.e. buffer capacity, pH, and bile concentration) of the dissolution medium using GraphPad Prism. The following equations were obtained with R-squared values for solubility and z value of 0.7717 and 0.8768, respectively, under the equal weight regression:

$$Solubility = -0.3460 + 0.05542 \times pH + 0.006205 \times BC + 0.005090 \times C_{bile} \tag{6}$$

$$z\ value = 1.218 + 0.02611 \times pH - 0.1241 \times BC + 0.04281 \times C_{bile} \tag{7}$$

Fig. 8 shows a comparison of the predicted data using Eq. 6 and 7 with the observed solubility and z value. Although some deviations from the straight line with the slope of 1 were seen, almost all the plots fell within the range of ± 20%. Therefore, using these equations, it should be possible to estimate solubility and z value in the small intestine for each GI physiological parameter in the virtual subjects.

Plasma concentration profiles of enzalutamide after oral administration of liquid filled capsule and tablet were predicted under the standard GI physiological condition. Furthermore, the sensitivity of the PK parameters Cmax and AUC_{inf} to physiological parameters such as gastric emptying rate, fluid volume in the stomach, small intestinal transit time, fluid volume in the small intestine, pH, buffer capacity, and bile concentration in the intestinal fluid was determined.

In the PBPK simulations, the liquid filled capsule formulation of enzalutamide was regarded as a simple solution. The sensitivity analyses indicated that AUC_{inf} of the liquid filled capsule is not affected by the GI physiological parameters, with the exception that the gastric emptying rate can affect Cmax of the formulation. It was assumed that enzalutamide does not precipitate after releasing from the capsule in the GI tract of humans. This assumption is supported by the fact that the drug is absorbed almost completely in humans.[25]

In many conditions of the sensitivity analysis, with the exception of pH 5 in the small intestine, the enzalutamide tablet showed ca. 70% Cmax and comparable AUC_{inf} to the liquid capsule of the drug. At

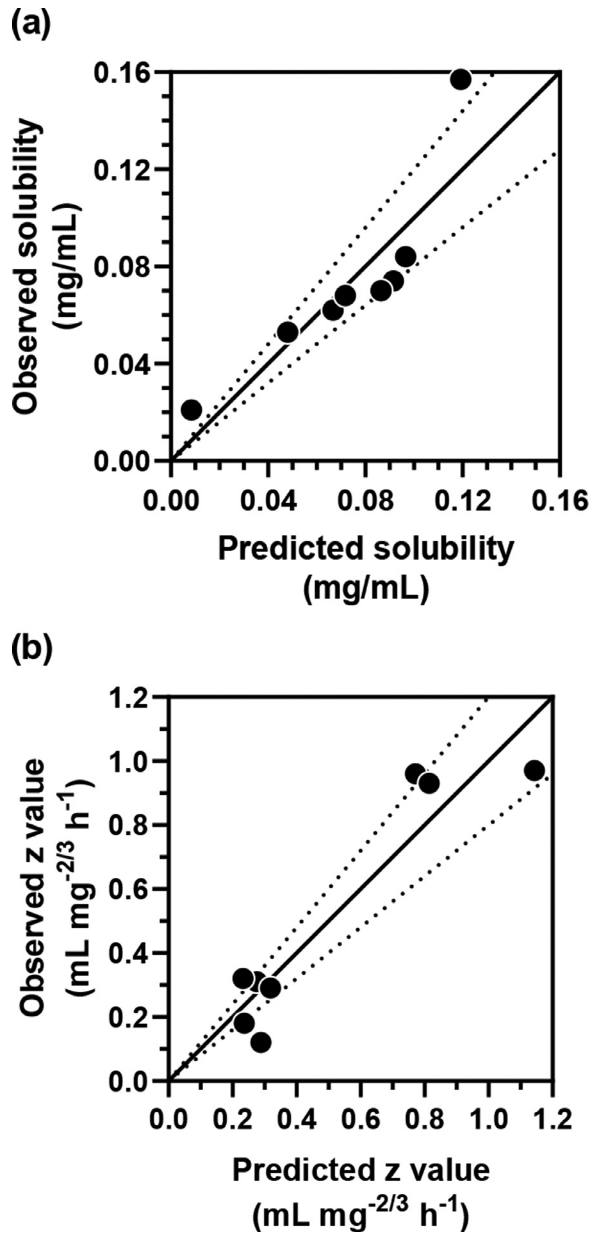

Fig. 8. Relationship between observed data and predicted data using multiple linear regression analysis for enzalutamide tablet in the simulated intestinal fluid: (a) solubility and (b) dissolution rate parameter z value. The solid and dot lines represent slope of 1 and ± 20% difference, respectively.

pH 5 in the small intestine, the predicted Cmax and AUC_{inf} of the tablet were much lower than under other GI conditions. This observation can be linked to the decreased solubility and rate of dissolution from the ASD formulation in pH 5 media.

Virtual bioequivalence studies for enzalutamide were performed using 30 virtual subjects, whose GI physiological parameters were varied using the same values as in the virtual studies of aripiprazole. Fig. 10a, c, and e show the predicted and observed plasma concentration profiles of enzalutamide after oral administration of the liquid filled capsule. The predicted mean PK profiles of the drug were close to the observed profile. Inter-individual variabilities of the plasma profile in the predicted data were much lower than the observed variability. Similar to aripiprazole, in the virtual BE studies, the post-absorptive variability was not accounted for in the simulations. Here too, the discrepancy in variability between the simulated and

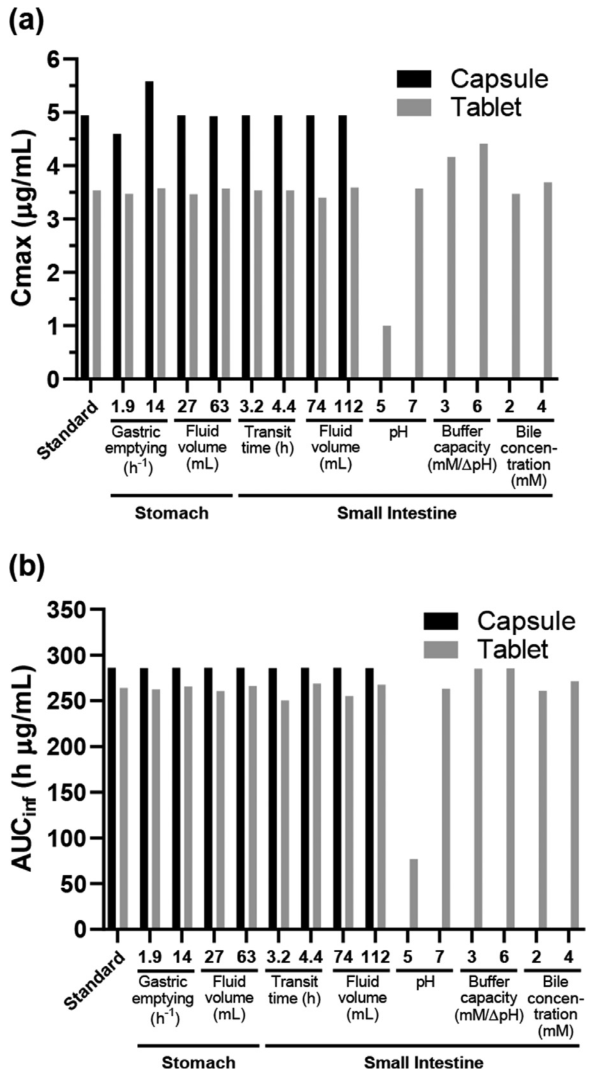

Fig. 9. Sensitivity analysis for the gastrointestinal physiological parameters in simulating (a) Cmax and (b) AUC_{inf} of enzalutamide liquid filled capsule and tablet.

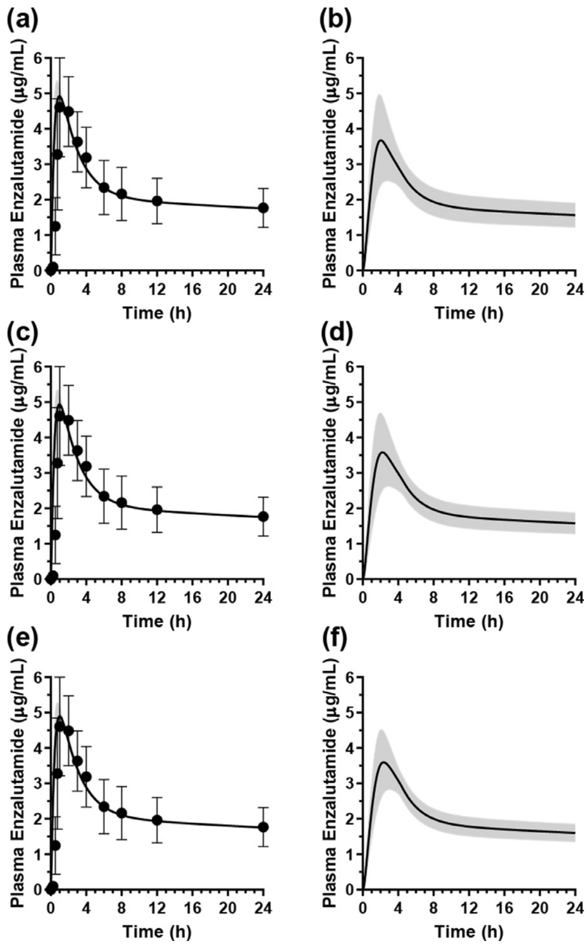

Fig. 10. Predicted and observed PK profiles of enzalutamide after oral administration of (a) liquid filled capsule and (b) tablet formulations in the first run of virtual BE study, (c) liquid filled capsule and (d) tablet in the second run, and (e) liquid filled capsule and (f) tablet in the third run. Mean ± SD (error bars and area shaded in gray). The observed profile was taken from the literature [27].

observed profiles suggests that post-absorptive variability is substantial for enzalutamide.

Fig. 10b, d, and f show the predicted plasma profiles with the standard deviations of enzalutamide after oral dosing of the tablet formulation in the three virtual studies. Since a plasma profile of the enzalutamide tablet was not available in the open literature, only the predicted profiles are shown in the figure. Unlike in the case of the simulations for the liquid filled capsule, large inter-individual variabilities in the plasma profile were generated. As in the parameter sensitivity analysis of enzalutamide (Fig. 9), some GI physiological parameters (intestinal pH and buffer capacity) would be expected to highly affect the *in vivo* performance of the enzalutamide tablet and virtual subjects with variations in these parameters would be primarily responsible for the large variability in the simulated plasma profiles of the tablet.

Table 7
Predicted and observed PK parameters of enzalutamide after oral administration of liquid filled capsule and tablet.

	Liquid filled capsule				Tablet			
	Observed	Predicted (Run 1)	Predicted (Run 2)	Predicted (Run 3)	Observed	Predicted (Run 1)	Predicted (Run 2)	Predicted (Run 3)
Tmax (h)	1.00 [0.50 – 3.02]	1.10 [0.55 – 1.30]	1.08 [0.60 – 1.35]	1.10 [0.55 – 1.30]	2.00 [0.50 – 6.02]	2.13 [1.60 – 4.55]	2.55 [1.55 – 4.65]	2.50 [1.75 – 4.25]
Cmax (μg/mL)	4.8 ± 0.9	5.1 ± 0.4	5.1 ± 0.3	5.0 ± 0.3	3.5 ± 0.8	3.9 ± 1.1	3.8 ± 1.0	3.7 ± 0.8
AUC_{inf} (h μg/mL)	234 ± 61	286 ± 0	286 ± 0	286 ± 0	246 ± 80	253 ± 56	255 ± 48	258 ± 40
Cmax GMR with 90% CI (tablet to liquid filled capsule)	—	—	—	—	0.72 (0.67 – 0.77)	0.73 (0.65 – 0.81)	0.72 (0.65 – 0.79)	0.73 (0.67 – 0.79)
AUC_{inf} GMR with 90% CI (tablet to liquid filled capsule)	—	—	—	—	1.01 (0.96 – 1.06)	0.86 (0.79 – 0.93)	0.87 (0.81 – 0.94)	0.89 (0.84 – 0.94)

The observed data were taken from the literature.[31]

Table 7 summarizes the PK parameters of enzalutamide of the liquid filled capsule and tablet. In the clinical study, the two products were bioequivalent with respect to AUC_{inf} but failed with respect to Cmax. These results were mirrored in the simulations, with the GMR and CI for Cmax in the three virtual studies being almost identical to the results in the clinical studies.

The simulations underestimated the GMR (tablet:capsule) for AUC_{inf} in the three virtual studies, such that the CI narrowly missed the lower bound for bioequivalence in the first run. The underestimate can be linked to a narrower absorption window in the GI tract for enzalutamide in the PK simulations than in the clinical study subjects. In the PK simulations, it was assumed that the drug can be permeated through the intestinal epithelium only in the small intestine. However, *in vivo*, a certain fraction of the drug might be absorbed from the colon. This would contribute to increasing AUC_{inf} values from the tablet formulations in humans, since the drug dissolution from the tablets starts in the small intestine. In fact, although the AUC_{inf} ratio (tablet:capsule) in the simulations with the small intestinal transit time (SITT) of 4.0 h, which is the average SITT used in simulations, was 0.92, an additional simulation assuming permeability in the proximal colon as well as in the small intestine (total permeation time 7 hours), resulted in an AUC_{inf} ratio of 1.00.

The predicted absolute values of AUC_{inf} of the liquid filled capsule in the virtual studies were overestimated (ca. + 20%) compared to the observed value. This was because the post-absorptive PK parameters used in the simulation was estimated from a different human study than the human bioequivalence study comparing the liquid filled capsule and tablet (the profiles not shown in literature). But since the present study focused on bioequivalence of the two dosage forms of enzalutamide, the discussion around the accuracy of simulating GMR and 90% CI for the PK parameters is more important than the absolute comparison of the predicted with observed AUC_{inf} values.

Intra- and inter-subject variability

Although the (non)-bioequivalence of both drugs between formulations was predicted well using the present modeling and simulation approach, the Cmax or AUC_{inf} values for the solid formulations were underestimated slightly.

The inter-individual variabilities in GI physiology in the present study were based on values from the literature (Table 3). In addition to those human data, other observations of the variations in the GI physiology, such as gastric pH,[34] buffer capacity in the jejunum[35] etc., have been reported. With further investigation of inter-subject variability in these parameters, more precise virtual bioequivalence studies could be run. A long-term goal would be to characterize the intra-occasion variability of these same parameters, enabling such effects to also be included in the PBBM model. At the same time, the effects of ethnic background, age, and disease on the GI parameters should be more vigorously investigated to make a virtual bioequivalence studies possible in specific target populations.

In the present study, the predicted variability in plasma profile, Cmax, and AUC for the solution or liquid capsule of both drugs were underestimated due to lack of consideration of variation in postabsorptive PK parameters of the drugs. Since the main purpose of the current simulations was to predict the bioequivalence of dosage forms (i.e. GMR with 90% CI for Cmax and AUC) in virtual subjects, adding variability in the postabsorptive PK would be of primary benefit in calculating the study size.

As almost complete absorption could be assumed for both model drugs in the present study, precipitation in the GI tract was not taken into consideration in the current PBBM approach. We note that, in general, precipitation kinetics for poorly soluble weak base drugs[9] and ASDs[36] should be incorporated in the prediction model whenever performing a virtual BE study for drugs that are likely precipitate in the GI tract.

Conclusion

In the present study, virtual bioequivalence assessments for two poorly soluble drugs, aripiprazole (oral solution vs. tablet) and enzalutamide (liquid capsule vs. ASD tablet), were performed. Variabilities in the GI physiological parameters were taken into consideration in the *in vitro* biorelevant dissolution testing and in the *in silico* modeling and simulations. Virtual subjects with various GI physiological parameters were used in the virtual bioequivalence studies. The *in vivo* performance of solution and tablet formulations of both model drugs were adequately predicted. The bioequivalence parameters, i.e. GMR and 90% CI, for the both drugs were predicted well in the virtual studies. In order to perform more precise predictions, it will be important to continue characterizing GI physiological parameters, along with their variabilities, on both an inter-subject and inter-occasion basis.

Declaration of Competing Interest

Atsushi Kambayashi is an employee of Astellas Pharma Inc. and this study was supported by Astellas Pharma Inc.

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