European Journal of Pharmaceutical Sciences 198 (2024) 106782

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Bioequivalence prediction with small-scale biphasic dissolution and simultaneous dissolution-permeation apparatus—An aripiprazole case study

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ARTICLE INFO

Keywords:
Dissolution-permeation
Biphasic dissolution
Absorption
in vivo predictive
Aripiprazole

ABSTRACT

Both biphasic dissolution and simultaneous dissolution-permeation (D-P) systems have great potential to improve the in *vitro-in vivo* correlation compared to simple dissolution assays, but the assay conditions, and the evaluation methods still need to be refined in order to effectively use these apparatuses in drug development.

Therefore, this comprehensive study aimed to compare the predictive accuracy of small-volume (16–20 mL) D-P system and small-volume (40–80 mL) biphasic dissolution apparatus in bioequivalence prediction of five aripiprazole (ARP) containing marketed drug products.

Assay conditions, specifically dose dependence were studied to overcome the limitations of both small-scale systems. In case of biphasic dissolution the *in vivo* maximum plasma concentration (C_{max}) prediction greatly improved with the dose reduction of ARP, while in case of the D-P setup the use of whole tablet gave just as accurate prediction as the scaled dose. With the dose reduction strategy both equipment was able to reach 100 % accuracy in bioequivalence prediction for C_{max} ratio. In case of the *in vivo* area under the curve (AUC) prediction the predictive accuracy for the AUC ratio was not dependent on the dose, and both apparatus had a 100 % accuracy predicting bioequivalence based on AUC results.

This paper presents for the first time that not only selected parameters of flux assays (like permeability, initial flux, AUC value) were used as an input parameter of a mechanistic model (gastrointestinal unified theory) to predict absorption rate but the whole *in vitro* flux profile was used. All fraction absorbed values estimated by Predictor Software fell within the ±15 % acceptance range during the comparison with the *in vivo* data.

1. Introduction

In recent years, several devices including the biphasic dissolution setup have been developed to enable better *in vivo* predictions compared to simple dissolution tests (Berben and Borbás, 2022; Mudie et al., 2012; Pestieau and Evrard, 2017; Pillay and Fassihi, 1999). The dissolution of the drug is happening in the aqueous phase and the kinetic process of drug partitioning into an organic phase is monitored. As an organic layer, the use of octanol is traditional, but nowadays, nonanol and decanol are usually applied for practical reasons (Jankovic et al., 2019; O'Dwyer et al., 2020a). Over the years, many different biphasic configurations were developed to investigate specific formulations and the apparatus design was optimized for better *in vitro-in vivo* correlation (IVIVC) (Abeele et al., 2020; Denninger et al., 2020; Heigoldt et al., 2010; Mann et al., 2017; Tsume et al., 2020, 2018). One such commercially available device, InForm (Pion Inc., Billerica MA, USA) operating on a small scale, enabling efficient experimentation with reduced material consumption and medium area-to-volume ratio (17.35 cm^2/40 cm^3 = 0.434 cm^{-1}) Additionally, their high level of automation accurately replicates gastric to intestinal transfer, closely mirroring physiological drug absorption processes. However, these systems may encounter limitations such as direct transfer of floating particles and

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https://doi.org/10.1016/j.ejps.2024.106782
Received 26 January 2024; Received in revised form 8 April 2024; Accepted 29 April 2024
Available online 1 May 2024

turbidity in the aqueous phase, impacting data reliability (Reppas et al., 2023).

Another frequently used method for improving IVIVC is *in vitro* dissolution-permeation (D-P) system. These experiments have become widely used, as numerous studies have demonstrated their ability to predict the bioavailability of a drug more accurately compared to traditional dissolution tests. Moreover, this approach offers improved insights into the effects of drug excipients and the impact of supersaturation and precipitation (Carlert et al., 2010; Gu et al., 2005; O'Dwyer et al., 2019; Tőzsér et al., 2023). Notably, the application of *in vitro* D-P experiments has facilitated the development of generic formulations by enabling the investigation of excipient effects on absorption throughout the entire development process (Kádár et al., 2022).

The instruments for simultaneous D-P measurements are available in different designs and volume ranges. The vertical small-volume apparatus is represented by the Vertical Diffusion Cell (Franz diffusion cell) that is frequently used to investigate semi-solids, creams, and gel formulations (Ng et al., 2010; Salamanca et al., 2018). Small volume apparatus can be side-by-side as well, and they can be applied to investigate pre-formulations and mini tablets. One limitation of such side-by-side small-volume D-P systems is their small area-to-volume ratio (1.54 cm^2/20 cm^3 = 0.077 cm^{-1}) compared to flow-through type D-P systems (A/V ratio = 1.3 cm^{-1}) (Holzem et al., 2022; Nunes et al., 2023; Raines et al., 2023; Sironi et al., 2017). This presents a challenge in D-P testing, as the limited permeation area can become the rate-limiting step, making it difficult to accurately predict how the dissolution kinetics of the formulation affects absorption *in vivo*. However, the membrane properties if the *in vitro* play an equally important role and can diminish the advantage of the larger A/V ratio as demonstrated in a study by Holzem (Holzem et al. (2022). Enlarged versions of this instrument have been developed, which incorporate an absorption compartment into a USP II dissolution apparatus, which have been successfully applied to predict the bioavailability of various formulations (Borbás et al., 2019, 2018, 2016; Eliasen et al., 2020; Kádár et al., 2022; Tsinman et al., 2018).

In recent times, the focus from developing new, more biorelevant *in vitro* apparatuses to improve IVIVC shifted to method development and validation for the existing apparatuses and exploring their limits and usefulness in drug development (Abrahamsson et al., 2020; O'Dwyer et al., 2019; Vinarov et al., 2021).

Integrating the data generated with these apparatuses *in silico* predictions is also a new challenge. According to a recent study the result of biphasic dissolution assays incorporated in a physiological based pharmacokinetic (PBPK) model can improve the *in silico* prediction of plasma concentration-time profile. Furthermore, they found that the result of biphasic dissolution data was better suited to estimate *in vivo* precipitation kinetics than side-by-side D-P data presumably because of the too small membrane surface to dissolution volume ratio in case of the small-scale D-P apparatus (O'Dwyer et al., 2020a). However, in a later study they found that in case of disintegrating tablets the results of biphasic dissolution tests can be very misleading, because tablet particles appeared to float on the surface of the aqueous media partitioning into the decanol layer before even dissolving in the aqueous layer (Tsakiridou et al., 2022). These studies utilize a PBPK model to predict *in vivo* behavior, enabling the incorporation of discrete values from *in vitro* measurements.

However, the direct incorporation of *in vitro* flux profiles generated by D-P systems instead of discrete permeability values calculated from *in vitro* data might be useful for the prediction of *in vivo* absorption rates. The Predictor™ software applies mechanistic principles outlined in the GUT framework (Sugano, 2011, 2009) to rescale *in vitro* flux measurements to represent *in vivo* absorption, integrates to determine the absorbed mass of the drug, and provides estimates of the absorbed fraction of a dose. The software is able to compensate for poor permeability clearance in low surface area to volume ratio D-P systems, which is one of the main advantages of this software. However, as the basis for the predictions is the D-P assay acceptor concentration vs. time profile, the method is subject to the same limitations as the D-P apparatus and the membrane it employs e.g. only passive transport can be estimated.

In conclusion, both biphasic dissolution and D-P systems have great potential to improve the IVIVC compared to simple dissolution tests, but the assay conditions, and the evaluation methods still need to be refined in order to effectively use these apparatuses in drug development.

This comprehensive study aimed to compare the predictive accuracy of a small-volume (16–20 mL) D-P system and a small-volume (40–80 mL) biphasic dissolution apparatus in bioequivalence prediction of five aripiprazole (ARP) containing marketed drug products. Assay conditions, specifically dose dependence were studied to overcome the limitations of both small-scale systems. Furthermore, the direct integration of flux profile to an *in silico* model aimed to predict *in vivo* absorption rates and ascertain the importance of appropriate donor compartment dosing. Only D-P data is used for this purpose in the course of this study, as the *in silico* software used (Predictor™) does not support the use of biphasic dissolution data.

2. Materials and methods

2.1. Materials

Aripiprazole (ARP, 448.39 g/mol, structure shown in Fig. 1) is a BCS Class II third-generation atypical antipsychotic, which is a dopamine D_2 receptor and $5HT_{1A}$ receptor partial agonist, therefore, using it for the treatment of schizophrenia and bipolar disorder (Jordan et al., 2002; Stelmach et al., 2022). ARP is a lipophilic weak base (pK_a = 7.46) with low aqueous solubility and pH-dependent permeability (Butreddy et al., 2021; Zhou et al., 2021). ARP and buffer components (NaH_2PO_4, NaOH, NaCl, KCl, HCl were purchased from Sigma-Aldrich Co. Llc. (St. Louis, MO, USA). The gastrointestinal tract (GIT) and acceptor sink buffer (ASB) were obtained from Pion Inc. (Billerica, MA, USA). Simulated Intestinal Fluid (SIF) powder was purchased from Biorelevant.com (London, UK). ARP immediate-release tablets and oral solution were sourced from a local pharmacy in Hungary, the composition of the marketed formulations (the brand- Abilify, and five generic formulations namely Restigulin, Sandoz, Piprason, Explemed and Abilify oral solution) is shown in Table 1.

2.2. Biphasic dissolution assay

The biphasic experiments were carried out using the inForm (Pion Inc.) instrument at 37 °C, with the experimental setup shown in Fig. 2. The formulations were introduced at $t = 0$ via basket using an automated picking fork to 32 mL of pH 1.6 HCl. The instrument stirring speed was set to 100 rpm for the gastric sector, which was held for 30 min. To simulate the transition into an intestinal environment, the instrument added concentrated FaSSIF (8 mL) and a secondary lipid layer consisting of 39.8 mL 1-decanol; stirring was halted during the addition of these reagents so as to limit the degree of partitioning to the 1-decanol layer

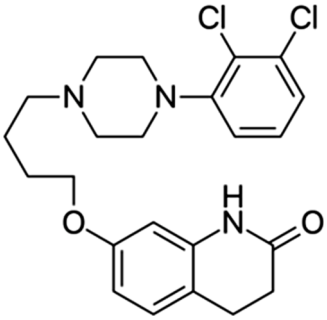

Fig. 1. Structure of aripiprazole.

Table 1
Composition of Aripiprazole containing marketed formulations.

Name	Abilify 15 mg tablets	Restigulin 15 mg tablets	Sandoz 15 mg tablets	Piprason 15 mg tablets	Explemed 15 mg tablets	Abilify oral solution (1 mg/mL)
	lactose monohydrate (54.15 mg)	**lactose monohydrate (94.62 mg)**	**lactose monohydrate (97.75 mg)**	**lactose monohydrate (160.48 mg)**		Disodium edetate
	maize starch	maize starch	maize starch	maize starch	pregelatinised	Fructose
						Glycerin
						Lactic acid
	microcrystalline cellulose	microcrystalline cellulose	microcrystalline cellulose	microcrystalline cellulose	maize starch	Methyl parahydroxybenzoate
	hydroxypropyl cellulose	hydroxypropyl cellulose	hydroxypropyl cellulose	hydroxypropyl cellulose	microcrystalline cellulose	Propylene glycol
	magnesium stearate	magnesium stearate	magnesium stearate	magnesium stearate	colloidal anhydrous silica	Propylparahydroxy-benzoate
			red iron oxide (E172)	red iron oxide (E172)	magnesium stearate	Sodium hydroxide
						Sucrose
						Purified water
						Orange flavour

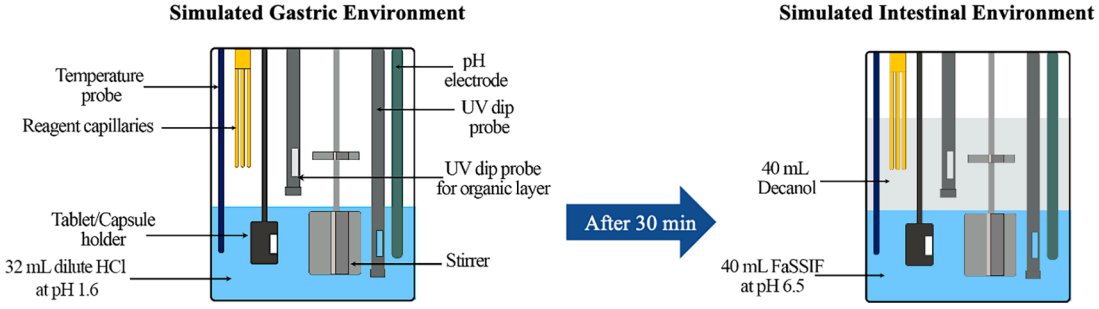

Fig. 2. Schematic of the biphasic dissolution setup.

prior to data collection resuming. Any deviation in pH of the media from the pH 6.50 target was automatically corrected by addition of 0.5 M HCl or 0.5 M NaOH titrant as required. Stirring was set to 100 rpm for the intestinal sector and partitioning of the API to 1-decanol was monitored for 60 min. The drug concentrations in the decanol layer were quantified using a secondary in situ fiber-optic immersion probe. Concentration values in the aqueous and lipid layers were obtained using pre-determined multiwavelength molar extinction coefficients. Molar extinction coefficients (MECs) were analysed in the same media as the experimental setup and acquired as an average over series of different concentrations by serial addition of API stock solution. The acquired MECs were iteratively fitted to the assay spectra to minimise the residual absorbance between them using the inForm Refine program (V1.60). The factor required to transform the MEC to achieve the resultant fit was used to calculate concentration for each spectrum collected, in order to construct the concentration vs. time profiles. Concentration calculations were performed normalised to a 10 mm path length. Values of flux were calculated from the rate of sample appearance in the 1-decanol layer, acquired by a least squares linear regression ($R^2 > 0.99$) to the initial section ($T = <70$ min) of the obtained profiles. To facilitate flux calculations per unit area from the biphasic partitioning data, the area of the interface between the aqueous and decanol layers was taken as the area of a circle with the diameter of the assay vessel (17.35 cm^2).

2.3. Small volume dissolution-permeation measurements with MicroFLUX apparatus

The formulations were tested using MicroFLUX™ apparatus (Pion Inc., Billerica MA, USA), which consists of a donor and an acceptor chamber separated by an artificial membrane (PVDF, polyvinyliden-fluoride, 0.45 μm, 1.54 cm^2) impregnated with 25 μL GIT lipid solution to form a lipophilic barrier between the donor and acceptor chambers. In cases of oral drug delivery, the donor chamber represents the stomach's condition, while the 20 mL pH 7.4 ASB buffer of acceptor chamber represents the blood circulation. The attempt was started in 16 mL pH 1.6 simulated gastric fluid (SGF) buffer solution then after 30 min, media in the dissolution vessel was converted to fasted state simulated intestinal fluid (FaSSIF) (pH 6.5) by adding 4 mL of SIF concentrate. Both chambers were stirred at 250 rpm at 37 °C. In both chambers, the API concentration was followed by immersed UV-probes connected to the Rainbow instrument (Pion Inc., Billerica MA, USA).

The flux (J) across the membrane was calculated using the Eq. (1):

$$J\ (t) = \frac{dm}{A \cdot dt} \tag{1}$$

where the flux of a drug through the membrane is defined as the amount (m) of drug crossing a unit area (A) perpendicular to its flow per unit time (t).

2.4. In silico prediction

In silico prediction was carried out using the GUT (Gastrointestinal Unified Theoretical) framework by Kiyohiko Sugano, which is available as part of the Predictor software (Pion Inc., Billerica MA, USA).

Apparent permeability was calculated based on the initial flux of the acceptor profile and the highest observed donor concentration and used to estimate the *in vivo* accessible intestinal surface. For the ARP formulations the observed permeability classified them as highly permeable and therefore only the plicate expansion factor has been considered, as the *in vivo* absorption could be presumed to be unstirred water layer limited. After correcting the data for an assumed *in vivo* unstirred water layer thickness of 300 μm, the fluxes through the available surface were summed for an average intestinal transit time of 210 min, providing the total mass absorbed (Eq. (2)). Dividing this result by the *in vivo* dosage provided the fraction absorbed ratio.

$$Mass_{ABS} = J_{\text{in vivo}} \cdot SA_{GI} \cdot T_{transit} \quad (2)$$

where $J_{\text{in vivo}}$ is the *in vivo* scaled flux, SA_{GII} is the intestinal surface area, and $T_{transit}$ is the intestinal transit time. F_a% values were calculated relative to the mass absorbed evaluated at the end of the intestinal transit time and the dose administered *in vivo*.

The calculation was carried out for the small-volume flux measurements only (full and scale dose), because some of the necessary parameters (e.g. unstirred water layer thickness) are not available in the case of biphasic dissolution system, these measurements therefore could not be involved in the prediction.

In the case of ARP, the absorption of the drug is not dissolution rate limited, therefore the F_a can be predicted solely from the flux-time curve, without the need for dissolution data.

The input parameters required for modeling the Predictor software in the case of ARP formulations are shown in Table 2.

3. Results

3.1. Testing of formulations with biphasic dissolution apparatus

The biphasic dissolution assay was performed on the inForm instrument using a media conversion protocol as described in 2.2 Section. Biphasic dissolution measurements were performed with full (15 mg of API/32 mL) and scaled doses (2.4 mg of API/32 mL) of 5 ARP containing tablets. Additionally, an oral solution was incorporated into the measurements, but due to volume limitations in the full-dose experiments, testing the complete dose of the oral solution (15 mL) was not feasible. Consequently, the measurements were only carried out with scaled dose.

3.1.1. Testing of full dose formulation

The first step in the biphasic dissolution studies was to investigate the full dose of ARP formulations. The dissolution and appearance profiles are shown in Fig. 3. For all of the formulations, the donor media became very turbid right after the pH change, disabling the *in situ* UV detection. For this reason, only the first 30 min of dissolution of the formulations are depicted in Fig. 3. There is no significant difference between the dissolution of the investigated formulations. The left graph shows that all formulations dissolved completely in the first 30 min. On the absorption profiles, the tendency towards saturation can be observed as a consequence of precipitation on the donor side.

For this type of measurement, the flux evaluation range was chosen as 45–60 min, and the AUC was calculated for the entire measurement range (0–95 min). The calculated results were contained in Table 3. Except for Piprason all formulations had a significant difference in flux and AUC value compared to the brand, Abilify tablet (p-value less than 0.05).

Table 2
Values of physicochemical and *in vitro* assay parameters used in the Predictor modeling for ARP.

Parameter (units)	Values used/comments
In vitro flux assay parameters	
Acceptor media	ASB
Acceptor vessel volume (mL)	20.0
API weight (mg)	15.0 or 1.2
Molecular weight (g/mol)	448.39
Density (g/cm^3)	1.2
Temperature (°C)	37.0
pH	7.4
Membrane type	GIT-0 lipid
Membrane surface area (cm^2)	1.54
Biorelevant media	FaSSIF
Stirring speed (rpm)	250
Absorption parameters	
Species model	Human
Dose (mg)	15
Effective permeability (cm/sec)	calculated based on fitting flux and dissolution curves in Predictor to data of the same assay
H_{UWL} (μm)	~100
Flux type	MicroFLUX
Bile micelle term	1
S dissolution media (μg/mL)	the maximum available donor concentration, calculated based on dissolution curves in Predictor
Time markers	
T_0 (min)	30
Linear 1 (min)	50
Polynomial (min)	70
Linear 2 (min)	150

3.1.2. Testing of scaled dose formulation

Due to the great difference between the flux results of full dose measurements, tests of scaled-down dose of ARP formulation were performed. To achieve this, the concentration in the aqueous phase was calculated based on 250 mL being the biorelevant volume in the stomach (McConnell et al., 2008). The dissolution and appearance profiles of the scaled dose of ARP formulations are shown in Fig. 4. Similarly, to the full dose experiments, only the first 30 min of the dissolution was detectable because of the turbidity caused by the pH change. As depicted in the left graph, all tablet formulations completely dissolved within the initial 30 min. The lower donor concentration observed with Explemed may be attributed to tablet pulverization, occurrences resulting from inadequate homogeneity and measurement inaccuracies.

Table 4 contains the calculated initial flux and AUC result from the scaled dose biphasic dissolution measurements. It can be concluded that there is no significant difference between the flux values of investigated generic tablets and original product, while the AUC value increased significantly in the case of Explemed tablet (p-value less than 0.05).

The Abilify oral solution showed incompatibility with the decanol layer, therefore the UV signal could not be evaluated properly. For that reason, the data are not shown on Fig. 4.

3.2. Testing of formulations with small volume dissolution-permeation apparatus

The small volume flux assays were performed on MicroFLUX setup using a media conversion protocol as described in Section 2.3. Small volume flux measurements were performed with full (15 mg of API/ 16 mL SGF) and scaled doses (1.2 mg of API/ 16 mL SGF) of ARP formulations. In full-dose MicroFLUX measurements, the maximum achievable concentration is twice as high (937.5 μg/mL) compared to inForm (468.75 μg/mL), because the MicroFLUX apparatus has half the working volume than the InForm. In case of the scaled down dose measurements the maximum concentration was 75 μg/mL in SGF and 60 μg/mL in the intestinal condition. Additionally, an oral solution was incorporated into the measurements. Due to volume limitations (16–20 mL) in the full-dose experiments, testing the complete dose of the oral solution (15 mL) was not feasible. Consequently, the results of this formulation are only presented for the scaled-dose measurements.

3.2.1. Testing of full dose formulation

The dissolution and appearance profiles with the full dose of ARP formulations are shown in Fig. 5. As shown on the left graph, most formulations dissolved completely in simulated gastric fluid (SGF), but precipitation was observed in the first 30 min. The kinetics of the precipitation were different for each formulation. After 30 min, the media in the donor cell was converted to FaSSIF, therefore the change in the ionization state of the ARP resulted in a further, fast precipitation. The right-side graph demonstrates that the permeation only started after the media conversion. The precipitation on the donor side caused the progressively decreasing flux, as it is visible on the appearance profiles. The most noticeable difference compared to the brand tablet was observable in the case of Restigulin and Explemed.

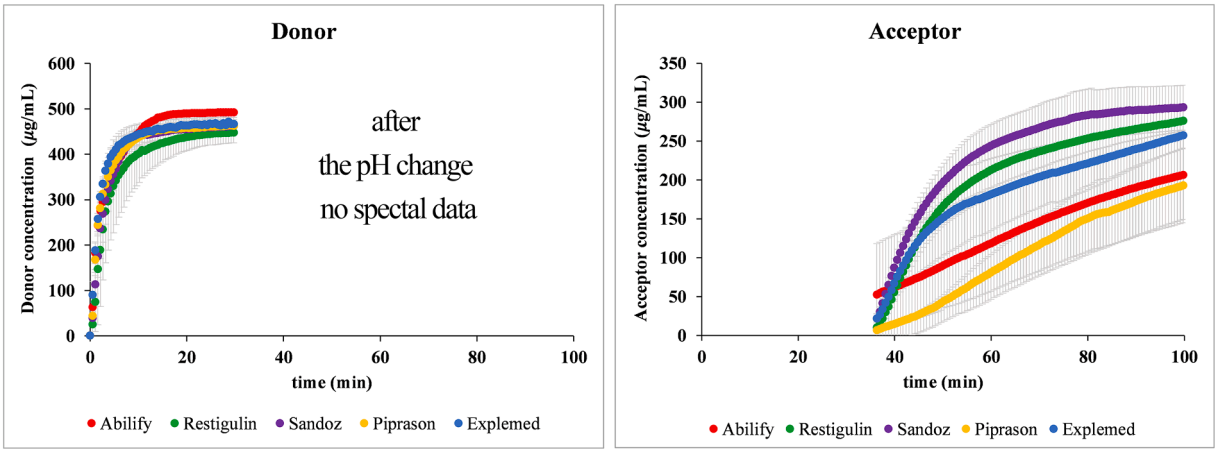

Fig. 3. Dissolution in SGF (left) and appearance profile in decanol (right) of ARP measured in the biphasic assay at full dose (maximum donor concentration = 468.75 µg/mL).

Table 3
Initial flux data and AUC (0–95 min) values of full dose ARP formulations by biphasic dissolution assay.

Formulation	Initial flux ± SD (µg/min cm^2)	AUC_{0-95} ± SD
Abilify	6.68 ± 1.16	19,535 ± 2087
Restigulin	30.87 ± 3.40	29,390 ± 2060
Sandoz	35.37 ± 4.55	31,877 ± 3081
Piprason	8.49 ± 2.37	19,323 ± 4291
Explemed	27.28 ± 8.75	27,660 ± 2289

Due to the impact of precipitation on the donor side and its consequential influence on absorption dynamics, the initial flux was computed at the onset of absorption (50–70 min). To comprehensively analyze the absorption profile across the entire time span the area under the curve (AUC) was calculated. The summarized outcomes are provided in Table 5. Notably, based on the results of initial flux and AUC, a significant divergence (p-value less than 0.05) was observed between the Explemed formulation and the Abilify tablet. Conversely, the remaining formulations - Restigulin, Sandoz, and Piprason - demonstrated similar initial flux and AUC values.

3.2.2. *Testing of scaled dose formulation*

The dissolution and appearance profiles of the scaled dose ARP formulations, including the oral solution, are depicted in Fig. 6. Unlike the dissolution behavior observed in the full dose experiments, for scaled dose measurements, the API exhibits minimal or no precipitation in the stomach condition due to lower supersaturation. However, following the pH change, the ionization state of the ARP changes, leading to a higher degree of precipitation. Notably, no observable differences were found in the appearance profiles, the lowest concentration on the acceptor side was observed in the case of the Sandoz and Explemed formulations.

Similar to the full dose experiments, the assessment of initial flux and AUC values was performed for these measurements. The outcomes of these calculations are outlined in Table 6. Based on the initial flux and AUC results, the generic tablets had no significant difference compared to the brand tablet. (*p*-value more than 0.05) The AUC value of the Abilify oral solution did not differ significantly, while it exhibited a significant increase in initial flux compared to the Abilify tablet.

Table 4
Initial flux data and AUC (0–95 min) values of scaled dose ARP formulations for biphasic dissolution assay.

Formulation	Initial flux ± SD (µg/min·cm^2)	AUC_{0-95} ± SD
Abilify	0.923 ± 0.15	787 ± 225
Restigulin	0.879 ± 0.08	706 ± 112
Sandoz	0.921 ± 0.17	742 ± 168
Piprason	0.721 ± 0.12	610 ± 140
Explemed	1.106 ± 0.10	937 ± 127

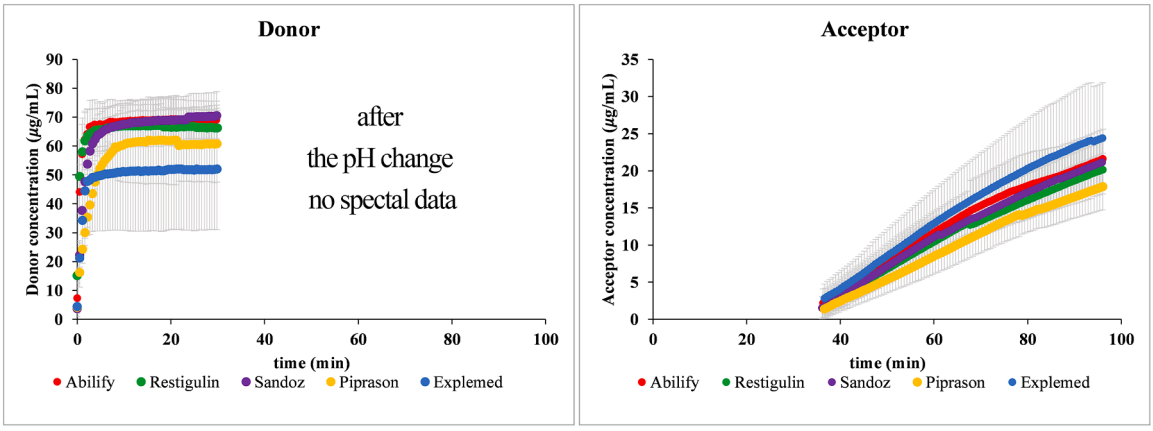

Fig. 4. Dissolution in SGF (left) and appearance profile in decanol (right) of ARP measured in the biphasic assay at scaled dose (maximum donor concentration = 75 µg/mL).

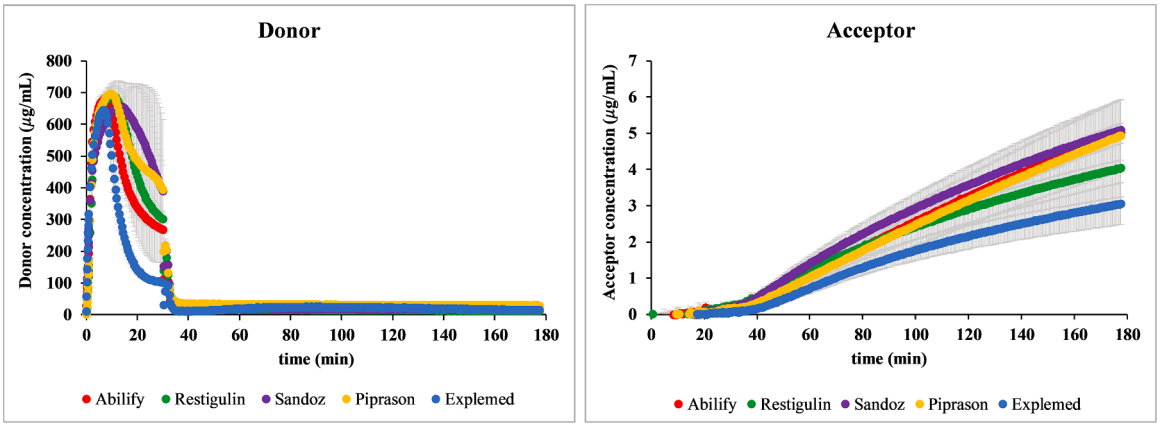

Fig. 5. Dissolution (left) and appearance profile (right) of different ARP formulations measured by small volume dissolution-permeation apparatus at full dose (maximum donor concentration = 937.5 μg/mL).

Table 5
Calculated initial flux and AUC (0–180 min) values of small volume dissolution-permeation assay of ARP full dose formulations.

Formulation	Initial flux ± SD (μg/min·cm^2)	AUC_{0-180} ± SD
Abilify	0.485 ± 0.05	509 ± 32
Restigulin	0.457 ± 0.05	544 ± 72
Sandoz	0.581 ± 0.06	453 ± 139
Piprason	0.481 ± 0.09	445 ± 104
Explemed	0.393 ± 0.01	3 ± 44

3.3. Determining absolute fraction absorbed from in vitro flux by predictor software

Predictor software was used to predict the *in vivo* fraction absorbed rate-time profile. For the prediction, concentration vs. time profiles in the acceptor chamber of the *in vitro* flux assay (flux profile) were imported as the main parameter. The calculation was carried out for the small-volume flux measurements only (full and scale dose), because some of the necessary parameters (e.g. unstirred water layer thickness) are not available in the case of biphasic dissolution system, these measurements therefore could not be involved in the prediction.

3.3.1. Prediction based on full dose dissolution-permeation measurements

The predicted F_a-time profiles of full dose flux measurements are shown on Fig. 7. It can be seen that two generic formulations—Restigulin and Piprason—have a similar F_a-time profiles to the original product, Abilify. The other two formulation show different characteristics—Explemed deviate in negative direction, while the Sandoz has positive deviation compared to the reference product.

3.3.2. Prediction based on scaled-dose dissolution-permeation measurements

The Fig 8. shows the predicted F_a-time profiles in the case of scaled dose flux measurements. There are no significant differences observed in the final fraction absorbed value between the generic formulations and the reference product. However, the lag time of the original tablet is notably longer.

4. Discussion

The ratio of maximum plasma concentration (C_{max}) for the test

Table 6
Calculated initial flux and AUC (0–180 min) values of small volume dissolution-permeation assay of ARP scaled-dose formulations.

Formulation	Initial flux ± SD (μg/min·cm^2)	AUC_{0-180} ± SD
Abilify	0.417 ± 0.04	350 ± 51
Restigulin	0.392 ± 0.13	301 ± 87
Sandoz	0.391 ± 0.06	269 ± 44
Piprason	0.370 ± 0.10	329 ± 142
Explemed	0.380 ± 0.05	265 ± 45
Abilify oral solution	0.508 ± 0.06	334 ± 50

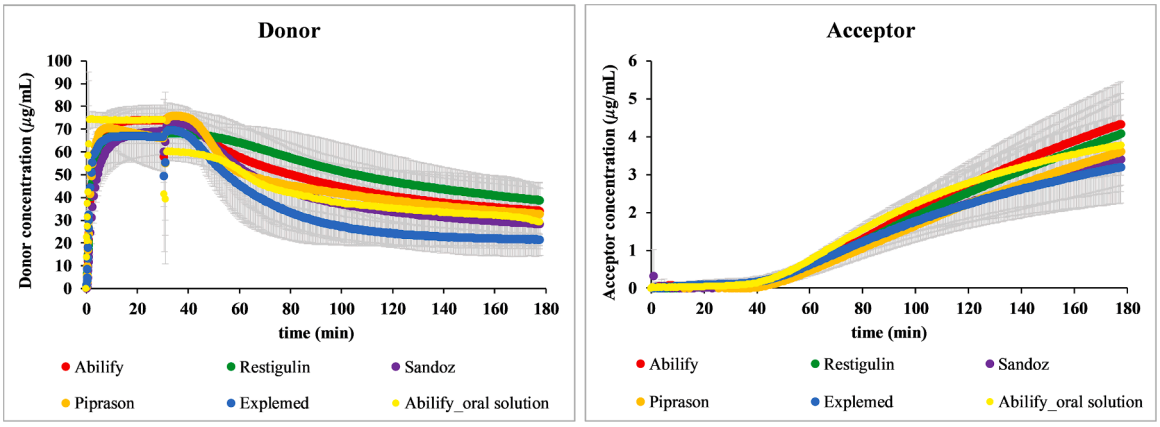

Fig. 6. Dissolution (left) and appearance profile (right) of different ARP formulations measured by small volume dissolution-permeation apparatus at scaled dose (maximum donor concentration = 75 μg/mL).

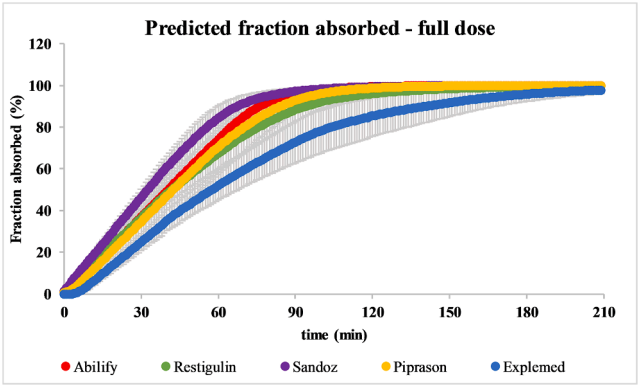

Fig. 7. Predicted human fraction absorbed value based on full dose dissolution-permeation measurements.

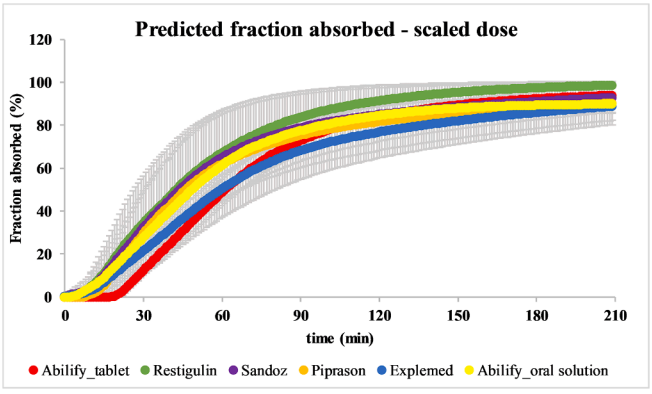

Fig. 8. Predicted human fraction absorbed value based on scaled dose dissolution-permeation measurements.

products was predicted from *in vitro* flux data and *in vitro* area under the acceptor concentration curve (AUC) was utilized to estimate *in vivo* AUC ratios by dividing the results of the generic formulation by those of the brand formulation (Abilify tablet) to determine an *in vitro* flux or AUC ratio. Similarly, to bioequivalence test evaluation, if the resulting ratio of ln transformed value ±90 % confidence interval fell within 80–125 % of the reference, the formulation was classified as likely to pass the bioequivalence study. All predictions were compared to *in vivo* results published by the European Medicines Agency (EMA) (Abilify Public assessment report, n.d.; Aripiprazole Sandoz Public assessment report, n.d.; Explemed Public assessment report, n.d.; Piprason Public assessment report, n.d.; Restigulin Public assessment report, n.d.).

4.1. Prediction of bioequivalence based on biphasic dissolution assays

In the results section Table 3 showed that in case of full dose experiments the flux of the formulations were in many cases an order of magnitude higher than the reference. Consequently, the flux ratios calculated from this data fell out of the acceptance range for Restigulin, Sandoz and Explemed, only the Piprason data was in good agreement with the *in vivo* C_{max} ratios, meaning only 25 % predictive accuracy (Fig. 9). In case of the scaled dose experiments in all four cases the flux ratio fell within the acceptance range and with that showed 100 % predictive accuracy. This difference in predictive accuracy of the different doses may be explained with solid particles floating on the surface of the aqueous layer and partitioning to decanol before even dissolving in the dissolution media. In case of full dose measurements, this effect is more pronounced, while with scaling down the dose, the amount of solid particles are reduced, therefore this effect may be negligible.

While full dose experiments showed great difference in flux values, the AUC values did not differ to the same extent. Although the absolute values differed significantly (Table 3), the ln transformed ratios fell within the acceptance range in all four cases, meaning 100 % predictive accuracy. In case of the scaled dose experiments (Table 4) in all four cases the AUC ratio fell within the acceptance range and with that showed 100 % predictive accuracy (Fig. 10). Based on these results it seems that the disturbance caused by solid particles have a greater effect on initial flux and therefore the prediction of C_{max} ratio, while it does not affect the predictive accuracy of AUC ratio estimation to the same extent.

In previous studies, the biphasic dissolution system was employed to investigate various modified release formulations of tacrolimus. However, based on the total exposure of the formulations, the system failed to predict differences between the different release formulations. The authors attributed this discrepancy to the observation that particles from the formulation floated on the surface of the aqueous media, resulting in direct mixing with the decanol layer and the transfer of the entire dose into that layer. Similarly, this conclusion can be drawn from the results of full dose measurements of ARP. Nonetheless, it was established that this phenomenon can be minimalized by reducing the dose, hereby resulting in good predictive accuracy (Tsakiridou et al., 2022).

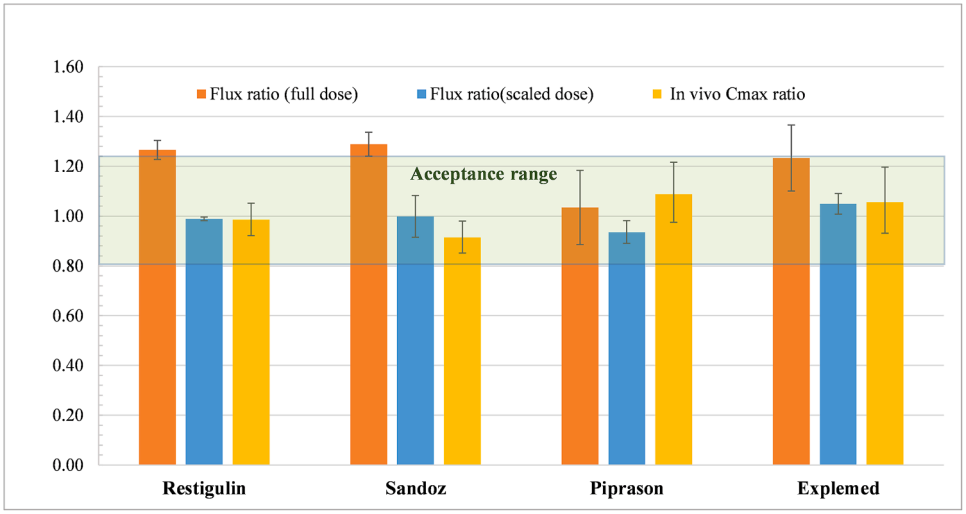

Fig. 9. Comparison of *in vitro* flux ratios (+/−90 % confidence interval) of biphasic dissolution test and *in vivo* C_{max} ratios (+/−90 % confidence interval).

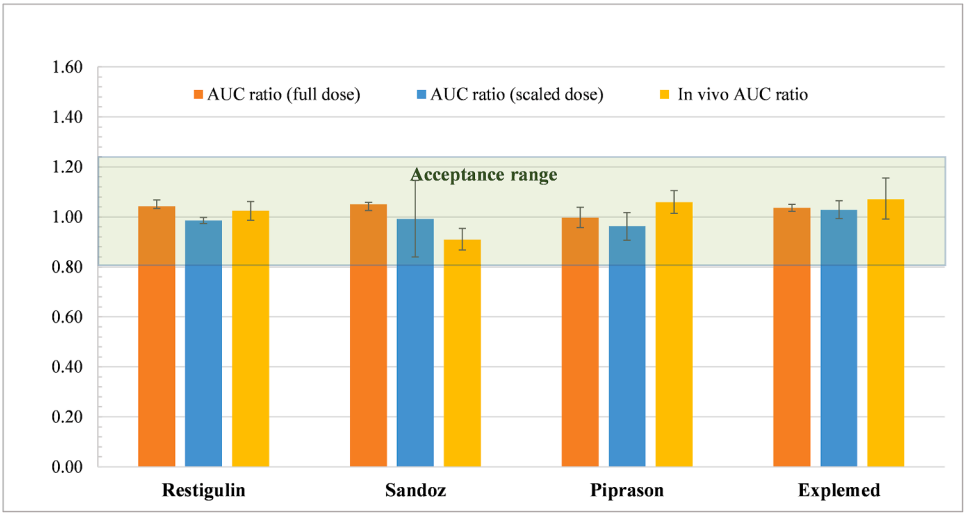

Fig. 10. Comparison of *in vitro* AUC ratios (+/−90 % confidence interval) of biphasic dissolution test and *in vivo* AUC ratios (+/−90 % confidence interval).

4.2. Prediction of bioequivalence based on small volume dissolution-permeation assays

In the results section Tables 5 and 6 showed the results of the full and scaled dose experiments carried out with the D-P system. The use of UV-probes and using second derivative spectra for UV analysis (Kádár et al., 2023; Nir and Lu, 2018; Tsinman et al., 2013) enabled real-time monitoring, the examination of dissolution and precipitation following pH changes and their kinetics, as well as the determination of the API in the presence of various excipients (Csicsák et al., 2021; O'Dwyer et al., 2019). In case of the full dose data, only Explemed showed significant deviance from the brand tablet, while with dose reduction all formulation were found similar (with *t*-test) to the brand tablet. The flux ratios calculated from the scaled dose and also from the full dose data fell in the acceptance range of bioequivalence calculations, meaning 100 % predictive accuracy independent from the applied dose (Fig. 11).

After the C_{max} ratios, AUC ratios were compared. Very similarly to the C_{max} comparison, only Explemed showed significant deviance from the brand tablet in case of full dose measurements, while with dose reduction all formulation were found similar (with *t*-test) to the brand tablet (Table 5 and 6). The flux ratios calculated from the scaled dose and from the full dose data fell in the acceptance range, it can be observed that Restigulin is at the bottom of the range regardless of the dose. With that 100 % predictive accuracy was reached independent from the applied dose (Fig. 12).

With the D-P setup it was possible to analyze the oral solution of ARP with scaled dose. *In vitro* data showed significantly higher flux (121.8 %), on the other hand the AUC results were found similar (Table 6). These results are in good agreement with the information in the public assessment report: the *in vivo* C_{max} value of the solution was found to be somewhat higher (122 % of the geometric means) than in case of the tablet form (Fig. 11), but no difference could be seen in the AUC results (Fig. 12).

In previous studies, the device was tested for other different APIs (ritonavir, itraconazole and tacrolimus), where the D-P system proved to be successful in the rank-order of the *in vivo* results and was able to distinguish between different pharmaceutical formulations (O'Dwyer et al., 2022, 2020b; Tsakiridou et al., 2022; Tsinman et al., 2018). In another previous study, the D-P system was utilized to investigate amorphous solid dispersions (ASDs) containing aripiprazole, which included citric acid as a micro-environmental pH-lowering agent. The significance of this was that, as a result of lowering the pH, ARP was mainly present in an ionized state, thereby increasing its solubility. While complete dissolution of ASD was achieved, some precipitation was observed, albeit at a slower and less drastic rate compared to traditional dissolution tests, as observed during simultaneous dissolution-absorption tests (Borbás et al., 2015).

Additionally, the small-volume D-P system was found to be

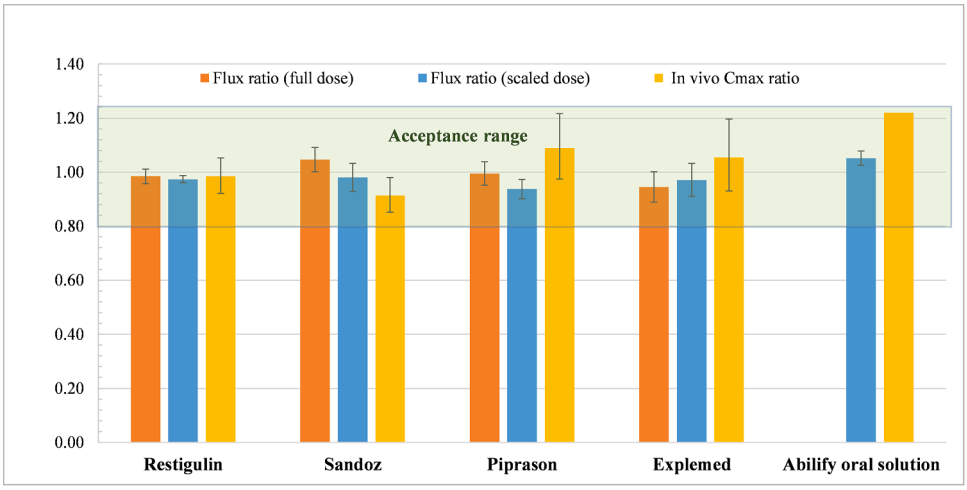

Fig. 11. Comparison of *in vitro* flux ratios (+/−90 % confidence interval) of dissolution-permeation test and *in vivo* C_{max} ratios (+/−90 % confidence interval).

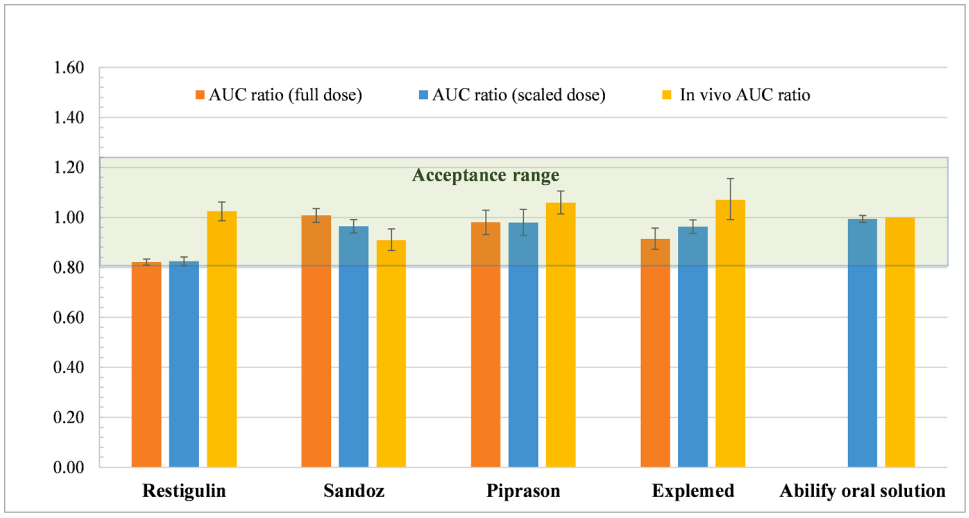

Fig. 12. Comparison of *in vitro* AUC ratios (+/−90 % confidence interval) of dissolution-permeation test and *in vivo* AUC ratios (+/−90 % confidence interval).

applicable for studying the effect of pH modifiers on formulations containing pimobendan (Tőzsér et al., 2023). Overall, it can be concluded that the D-P system enabled the examination of different drug formulations and is capable of predicting key parameters in bioequivalence studies.

4.3. Determining absolute fraction absorbed from in vitro flux by predictor software

Absolute fraction absorbed values were calculated from the measured *in vitro* flux appearance profiles using Predictor™ software. Predictions were performed from results of both full and scaled dose flux experiments. The calculated F_a% was compared to the *in vivo* F_a% - the published *in vivo* data indicates the original solid dosage form provided a 87 % fraction absorbed, while the F_a% of generic formulations was calculated from AUC ratio from public assessment reports. Comparing the predicted and measured *in vivo* F_a%, the deviation of ±15 % was considered to be an accurate prediction.

4.3.1. Prediction based on full dose dissolution-permeation measurements

Table 7 shows the calculated and observed *in vivo* F_a%. It can be seen that the software overpredicted the absolute fraction absorbed in all cases, Sandoz also was over ±15 % acceptance range.

4.3.2. Prediction based on scaled dose dissolution-permeation measurements

The results of the F_a% calculation at the end of the intestinal transit are presented against human *in vivo* data in Table 8. As it was already visible from the results of the flux measurements, dose reduction significantly improved the prediction of bioavailability. This tendency also appears in the software prediction, as a reduced degree of overprediction can be observed, all of the investigated formulations are in the ±15 % acceptance range.

Table 7
Calculated absolute fraction absorbed values presented based on full dose measurements relative to *in vivo* fraction absorbed values for fasted-state conditions in humans.

Formulation	*in vivo* F_a%	Acceptance range	Calculated F_a%	Acceptance
Abilify	87	72–102	100.0	✓
Restigulin	89	74–104	99.7	✓
Sandoz	79	64–94	100.0	–
Piprason	92	77–107	100.0	✓
Explemed	93	78–108	97.9	✓

Table 8
Calculated absolute fraction absorbed values presented based on scaled dose measurements relative to *in vivo* fraction absorbed values for fasted-state conditions in humans.

Formulation	*in vivo* F_a%	Acceptance range	Calculated F_a%	Acceptance
Abilify	87	72–102	93.9	✓
Restigulin	89	74–104	98.6	✓
Sandoz	79	64–94	91.1	✓
Piprason	92	77–107	99.6	✓
Explemed	93	78–108	87.7	✓
Abilify oral solution	87*	72–102	89.8	✓

* the AUC value was not available, the public assessment report indicated that the AUC value of the oral solution was statistically considered equivalent to that of the Abilify tablet.

5. Conclusion

This study was the first to compare the small-volume D-P system with small-volume biphasic dissolution experiments in their predictive accuracy for bioequivalence prediction through the example of five marketed formulations of ARP.

Assay conditions, specifically dose dependence were studied to overcome the limitations of both small-scale systems. In case of biphasic dissolution the *in vivo* C_{max} prediction greatly improved with the dose reduction of ARP, while in case of the D-P setup the use of whole tablet gave just as accurate prediction as the scaled dose. With the dose reduction strategy both equipment was able to reach 100 % accuracy in bioequivalence prediction for C_{max} ratio. In case of the *in vivo* AUC prediction the predictive accuracy for the AUC ratio was not dependent on the dose, and both apparatus had a 100 % accuracy predicting bioequivalence based on AUC results.

The D-P setup was able to handle the oral solution of ARP as well and gave a similar result to the *in vivo* data: the C_{max} value was found to be higher (122 % of the geometric means of *in vivo* C_{max}) than in case of the tablet form, but no difference could be seen in the AUC results. It can be concluded that, in addition to the previously used pre-formulations and mini-tablets, the D-P system is suitable for predicting the bioequivalence test result of the full dose tablet with sufficient accuracy in terms of both C_{max} and AUC. As a result, the device can be well used in the development of generic pharmaceutical products, either in the early stages of development or in the comparison of the developed product with the reference product.

This paper presents that not only selected parameters of flux assays (like permeability, initial flux, AUC value) can be used as an input parameter of a mechanistic model (GUT framework) to predict absorption rate, but the whole *in vitro* flux profile as well. Predictor™ software exhibited a tendency to overestimate the F_a% for full dose formulations. Using the scaled dose appearance profiles improved the correlation between *in silico* and *in vivo* F_a% values significantly, although a minimal overprediction persisted in this case as well, but all estimated F_a% values for the investigated formulations fell within the ±15 % acceptance range. The *in silico* prediction of the absorption rate and the F_a% during product development may promote the success of the bioequivalence study, as well as the deeper understanding of the *in vivo* behavior.

CRediT authorship contribution statement

Szabina Kádár: Writing – original draft, Software, Methodology, Investigation, Formal analysis. **Andrew Kennedy:** Methodology, Investigation. **Samuel Lee:** Software, Methodology, Investigation, Formal analysis. **Rebeca Ruiz:** Methodology, Investigation. **Attila Farkas:** Supervision, Software, Formal analysis. **Petra Tőzsér:** Investigation. **Dóra Csicsák:** Writing – original draft, Methodology, Investigation. **Gergő Tóth:** Writing – review & editing, Visualization, Investigation. **Bálint Sinkó:** Writing – original draft, Supervision, Software, Conceptualization. **Enikő Borbás:** Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

No data was used for the research described in the article.

Acknowledgment

This work was financially supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (EB). This work was funded by the National Research, Development and Innovation Office, Hungary (grant: NKFIH FK 146930). This project was supported by the ÚNKP-23-3-II-BME-88 New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund (SK). Project no. RRF-2.3.1-21-2022-00015 has been implemented with the support provided by the European Union.

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