molecular pharmaceutics

Article

Cite This: Mol. Pharmaceutics 2018, 15, 2307−2315

In Vitro Dissolution as a Tool for Formulation Selection: Telmisartan Two-Step IVIVC

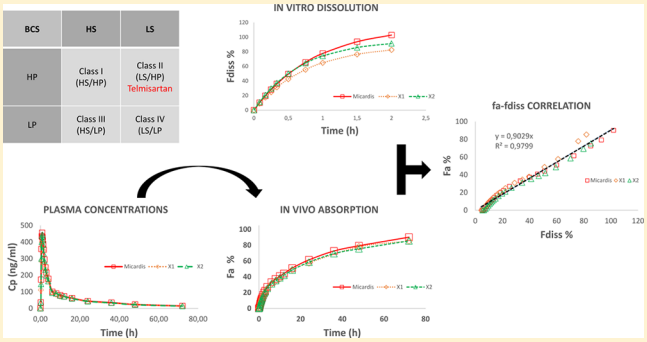
Alejandro Ruiz Picazo,[†] Mª Teresa Martinez-Martinez,[†] Sarin Colón-Useche,[†,∥] Ramon Iriarte,[†] Bárbara Sánchez-Dengra,[†] Marta González-Álvarez,[†] Alfredo García-Arieta,[‡] Isabel González-Álvarez,*[,†] and Marival Bermejo[†]

[†]Engineering: Pharmacokinetics and Pharmaceutical Technology Area, Miguel Hernandez University, Alicante 03550, Spain

[∥]Analysis and Control Department, University of Los Andes, Mérida 5101,Venezuela

[‡]Service on Pharmacokinetics and Generic Medicines, Division of Pharmacology and Clinical Evaluation, Department of Human Use Medicines, Spanish Agency for Medicines and Health Care Products, Madrid, Spain

ABSTRACT: The purpose of this investigation was to develop an exploratory two-step level A IVIVC for three telmisartan oral immediate release formulations, the reference product Micardis, and two generic formulations (X1 and X2). Correlation was validated with a third test formulation, Y1. Experimental solubility and permeability data were obtained to confirm that telmisartan is a class II compound under the Biopharmaceutic Classification System. Bioequivalence (BE) studies plasma profiles were combined using a previously published reference scaling procedure. X2 demonstrated in vivo BE, while X1 and Y1 failed to show BE due to the lower boundary of the 90% confidence interval for C_{max} being outside the acceptance limits. Average plasma profiles were deconvoluted by the Loo-Riegelman method to obtain the oral fractions absorbed (f_a). Fractions dissolved (f_{diss}) were obtained in several conditions in USP II and USP IV apparatus, and later, the results were compared in order to find the most biopredictive model, calculating the f_2 similarity factor. The apparatus and conditions showing the same rank order than in vivo data were selected for further refinement of conditions. A Levy plot was constructed to estimate the time scaling factor and to make both processes, dissolution and absorption, superimposable. The in vitro dissolution experiment that reflected more accurately the in vivo behavior of the different formulations of telmisartan employed the USP IV dissolution apparatus and a dissolution environment with a flow rate of 8 mL/min and a three-step pH change, from 1.2 to 4.5 and 6.8, with a 0.05% of Tween 80. Thus, these conditions gave rise to a biopredictive dissolution test. This new model is able to predict the formulation differences in dissolution that were previously observed in vivo, which could be used as a risk-analysis tool for formulation selection in future bioequivalence trials.



KEYWORDS: *telmisartan, IVIVC, bioequivalence, BCS, in vivo predictive dissolution*

INTRODUCTION

In vivo predictive dissolution methods (iPD) are useful tools for product development, since they have the ability to predict with accuracy and precision the bioequivalence of new formulations of the innovator or generic products after patent expiration.

In vitro−in vivo correlations (IVIVC) are developed to confirm that in vitro dissolution methods are predictive within the design space under investigation. IVIVC corresponds with a mathematical model that allows, after the validation of its predictability by comparison with human in vivo results, to pass from an in vitro characteristic of a drug product to its in vivo biological behavior, such as from the in vitro dissolution profiles to in vivo absorption profiles.

Class II drugs are characterized by low solubility and high permeability, and in this case, dissolution is the limiting process in the absorption rate. For low solubility drugs, bioequivalence should in principle be demonstrated by means of human pharmacokinetics studies unless a class A IVIVC is developed and validated. Level A correlation is a point to point relationship between the in vitro dissolution and the in vivo absorption rate of a drug from the dosage form.[1]

The main objective of this investigation was to develop an exploratory two-step level A IVIVC for three telmisartan oral immediate release formulations, the reference product Micardis, and two generic formulations (X1 and X2). X2 demonstrated in vivo bioequivalence (BE), while X1 failed to show bioequiva-

Received: February 11, 2018
Revised: April 15, 2018
Accepted: May 10, 2018
Published: May 10, 2018

DOI: 10.1021/acs.molpharmaceut.8b00153
Mol. Pharmaceutics 2018, 15, 2307−2315

lence due to the lower boundary of the 90% confidence interval for C_{max} being outside the acceptance limits.

Telmisartan belongs to class II of the Biopharmaceutic Classification System. It is a low solubility, high permeability compound (log*P* of 7.23[2]) with pH-dependent solubility.[3] Telmisartan is an ionizable substance with pK_a values of 3.5, 4.1, and 6.0. Its solubility is extremely low between pH 3 and 9 (intrinsic solubility = 0.00469 μg/mL).[4]

In the present work, telmisartan BCS classification was experimentally confirmed through solubility−pH profile characterization and permeability estimation in vitro. The work included the in vivo BE assay provided by a pharmaceutical company, the development of the in vitro dissolution method, the analysis of the potential reasons of the BE failure on the basis of drug and formulation characteristics, and the development of the in vitro−in vivo correlation.

EXPERIMENTAL SECTION

1. Drug and Formulations. Telmisartan (MW = 514.64 g/mol; log*P*= 7.23[2]) was given by a pharmaceutical company. Test formulations and the reference product were kindly provided by two pharmaceutical companies. All of them consist of immediate release telmisartan formulations with conventional excipients in customary amounts. Metoprolol and HPLC liquids were purchased from Sigma (Barcelona, Spain).

2. In Vivo Studies. Study 1 was a single-blind, controlled, balanced, randomized, two-period crossover BE study using 71 healthy subjects. Study 2 was an open label, balanced, randomized, two-treatment, three-period, three sequence, single-dose, reference-replicated, crossover, BE study in 55 healthy subjects. In each study, the volunteers received two formulations, one immediate release (IR) dose of the test formulation (X1 or X2, 80 mg) and one dose of the reference formulation (Micardis, 80 mg) in a sequence determined by randomization. A washout period of 14 and 10 days, respectively, was set between periods in each study. Blood samples were taken up to 72 h. Telmisartan concentration in blood samples was determined by a validated HPLC method in both studies. C_{max} and AUC were calculated from the average or individual plasma concentration time profiles. AUC were estimated individually by noncompartmental methods from the in vivo observations. On the other hand, public data from the 90% confidence interval as the outcome of a third BE study was available as well as the employed formulation Y1, which was also kindly provided by the manufacturing pharmaceutical company.

The results obtained in all of the crossover BE studies are reported in Table 1. The nonbioequivalent formulations X1 and Y1 (NBE) were not bioequivalent formulation in C_{max}, as their 90% confidence interval was not inside the acceptance limits (0.8−1.25). In addition, the 90% confidence intervals of the X1 and Y1 C_{max} did not include the 100% value, showing that there was a statistical significant difference with the reference products at the significance level employed.

Table 1. In Vivo Bioequivalence Results of the Test Formulations[a]

point estimate and 90% CI (%)	pharmacokinetic parameter	
	C_{max}	$AUC_{0\text{-tlast}}$
X1 (NBE)	86.31 (71.11−95.37)	93.99 (89.27−98.97)
X2 (BE)	**93.80 (82.77−106.33)**	**93.50 (89.30−97.94)**
Y1 (NBE)	81.34 (72.31−91.51)	97.04 (92.43−101.97)

[a]CI: confidence interval; BE: bioequivalent formulation; NBE: nonbioequivalent formulation.

3. Experimental Techniques. *Solubility Assays: Saturation Shake-Flask Procedure.* The solubility of telmisartan was estimated by adding an excess of solid in a standard buffer solution at 37 °C (pH 1.2, 4.5, and 6.8) and taking samples until saturated conditions were reached, when concentration remained unchanged. Flasks were shaken for 4, 8, 24, and 48 h. The determination of sample concentration was done by fluorescence detection using a validated HPLC method.

Permeability Assays: Cell Culture and Transport Studies. Dubelcco's Modified Eagle's Media supplemented with L-glutamine, fetal bovine serum (10%), and penicillin/streptomycin (5%) was the media used to grow Cace-2 cells. They were maintained in an incubator at 37 °C, 90% relative humidity, and 5% CO_2 during the growth of cells in flasks, and they were seeded on polycarbonate membranes at 250 000 cells/cm^2 and maintained during 20 days until the experiment to ensure transporters were expressed. Afterward, the trans-epithelial electrical resistance (TEER) was measured to evaluate the integrity of each cell monolayer. Standard operating procedures (SOPs) were described and validated previously in our laboratory.[5−9] To fill the receiver chamber and to prepare the drug solution (API or formulation) that was placed in the donor chamber, Hank's balanced salt solution (HBSS) supplemented with HEPES was used. The transport studies in the presence of formulation excipients were carried out by crushing and dissolving a tablet of each formulation in 250 mL of transport buffer and drug.

An orbital environmental shaker at constant temperature (37 °C) and at agitation rate of 50 rpm was used to conduct the transport studies, which were performed in apical-to-basal (A-to-B) direction and pH = 7 in both chambers. At 15, 30, 45, and 90 min, samples were taken from the receiver side and replaced each time with fresh buffer. Permeability assays have been carried out in metoprolol (100 μM), telmisartan (API), and four pharmaceutical formulations (micardis, X1, X2, and Y1).

The apparent permeability coefficient was calculated according to the following equation: where $C_{receiver,t}$ is the drug concentration in the receiver chamber at time t, Q_{total} is the total amount of drug, $V_{receiver}$ and V_{donor} are the volumes in receiver and donor chamber, $C_{receiver,t-1}$ is the drug concentration in the receiver chamber at the previous time, f is the sample replacement dilution factor, P_{eff} is the permeability coefficient, S is the surface area, and Δt is the time interval as described by Mangas-Sanjuan et al.[7]

Disintegration. The tablet disintegration rate was measured with a tablet disintegration tester (PharmaTest) in water at 37 °C, and the experiment was done six times (Ph. Eur. Method 2.9.1). Mean of values and standard deviation were reported.

Dissolution Assays. Dissolution Tests. Media Composition. The media used for dissolution assays were the standard buffers described in the European Pharmacopeia.[10−12] The same media at pH 4.5 and 6.8 but containing Tween 80 at 0.05% and fasted state simulating media, FaSSIF, were also tested.[10−12]

An apparatus 2 (paddle method) (Pharma-Test PT-DT70) with 900 mL of different media (Table 2) at 37 ± 0.5 °C and 50 rpm was used to perform the dissolution assays. Samples (5 mL) at different times were taken and filtered in line through a 10 μM (Pharma test) filter. The same volume of buffer was replaced in order to keep the test volume constant throughout the entire test. These experiments were performed in six replicates.

DOI: 10.1021/acs.molpharmaceut.8b00153
Mol. Pharmaceutics 2018, 15, 2307−2315

Table 2. Dissolution Media, Paddle Rotational Speed, and Sampling Times Used for Dissolution Studies in Apparatus 2 (Paddle Model)

dissolution media	rotation speed (rpm)	sampling times (min)
Ph. Eur. media		
pH 1.2	50	5, 10, 15, 20, 30, 45, 60
pH 4.5	50	5, 10, 15, 20, 30, 45, 60
pH 6.8	50	5, 10, 15, 20, 30, 45, 60
different buffer capacity		
pH 6.0, 10 mM	50	5, 10, 15, 20, 30, 45, 60
pH 6.5, 10 mM	50	5, 10, 15, 20, 30, 45, 60
pH 6.8, 10 mM	50	5, 10, 15, 20, 30, 45, 60
Ph. Eur. media with surfactant		
pH 4.5 with Tween 80 (0.05%)	50	5, 10, 15, 20, 30, 45, 60
pH 6.8 with Tween 80 (0.05%)	50	5, 10, 15, 20, 30, 45, 60
biorelevant media		
FaSSIF	50	5, 10, 15, 20, 30, 45, 60

Telmisartan dissolution profiles were assayed also using a USP IV apparatus. These dissolution profiles were obtained with an automated flow-through cell system, USP IV apparatus (Erweka, Germany) with 22.6 mm cells (i.d.) and a piston pump (Erweka, Germany).

The experiments using USP IV apparatus (without recycling) were performed at 37.0 ± 0.5 °C and with a flow rate of 8 mL/min. Sequential sampling using 0.45 μm nitrocellulose membranes (Millipore) were taken at different intervals of time using six replicates. Tables 2 and 3 show, respectively, the

Table 3. Dissolution Media, Flow Rate, and Sampling Times Used for Dissolution Studies in Apparatus 4

dissolution media	flow rate (mL/min)	sampling times (min)
Ph. Eur. media		
pH 1.2 (during 15 min)	8	5, 10, 15
pH 4.5 (during 15 min)	8	20, 30
pH 6.8 (during 90 min)	8	45, 60, 90, 120
Ph. Eur. media with surfactant		
pH 1.2 with Tween 80 (0.05%) (15′)	8	5, 10, 15
pH 4.5 with Tween 80 (0.05%) (15′)	8	20, 30
pH 6.8 with Tween 80 (0.05%) (15′)	8	45, 60, 90, 120
Biorelevant media		
FaSSIF	8	5, 10, 15, 20, 30, 45, 60, 90, 120

experimental conditions using Apparatus 2 (paddle model) and Apparatus 4 (automated flow-through cell system). The amount of dissolved telmisartan was determined by HPLC.

To compare the dissolution profiles between test and reference formulations in each condition, the F_2 similarity factor was used. F_2 values greater than or equal to 50 indicates that the dissolution profiles are similar. When more than 85% of the drug is dissolved within 15 min, dissolution profiles may be accepted as similar without further mathematical evaluation.[10,13]

4. Analysis of the Samples. Samples were analyzed by HPLC with a fluorescence detector (excitation wavelength = 305 nm and emission wavelength = 365 nm) in following conditions: the column was a Nova-Pak C18 column (4 μM, 3.9 × 150 mm); the mobile phase was 6.5 mM trifluoroacetic acid solution and acetonitrile (54:1:45) and had a flow-rate of 1.0 mL/min. The accuracy of the method was calculated using five standards and analyzed in triplicate. Precision was calculated as the coefficient of variation of five determinations over the same standards (values less than 5%). Linearity was established over the range of concentrations present in the samples ($r^2 > 0.999$). The limit of quantification for telmisartan was 3.4 μg/mL.

Statistical Analysis. Two-tailed Student's *t* tests were used to compare mean values of two groups, and analysis of variance (ANOVA) and Scheffé post hoc test were used to compare mean values of more than two groups with a significance level of 0.05. The statistical analyses were made with the statistical package SPSS, V.20.00.

In Vitro–in Vivo Correlation. To develop the IVIVC with three formulations of different release rates, the data from both BE studies were combined. Test formulation plasma profiles were normalized based on the reference's ratios.[14] Normalization was carried out using the average concentration time profiles from each reference formulation. At each sampling time, reference ratios were calculated to obtain the normalization factor for correcting the test average concentration profiles of the second study. So finally, the reference profile, test 1, and test 2 normalized curves were used for the deconvolution analysis. As sampling times differed slightly in both BE studies for the nonmatching sampling times, the normalization factor was estimated as the average of the previous and next matching sampling time.

Telmisartan intravenous pharmacokinetic parameters were obtained by curve fitting a two compartment open model to the plasma profiles of a 40 mg intravenous 30 min infusion.[15] Data was captured from Figure 1 of the reference with the aid of a

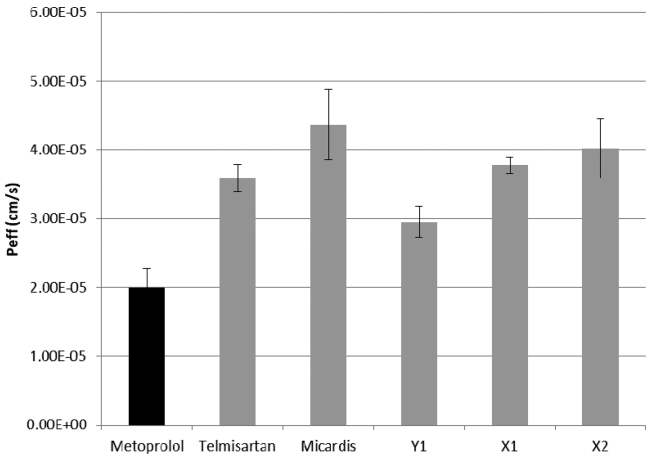


Figure 1. Telmisartan permeability as active substance, metoprolol, and different telmisartan formulations (reference product, nonbioequivalent formulation (X1, Y1), bioequivalent formulation (X2), and metoprolol).

GetData Graph Digitizer V.2.26. Curve fitting was performed in Microsoft Excel (Redmond, WA) with the PKsolver add-in macro.[16] Distribution microconstants (k12 and k21) and the elimination rate constant from the central compartment (k13) were estimated from alpha and beta disposition rate constants. In vivo oral fractions absorbed were estimated by the Loo-Riegelmann deconvolution method from the average plasma profiles. In vitro fractions dissolved and in vivo fractions absorbed profiles were made superimposable by a time scale

DOI: 10.1021/acs.molpharmaceut.8b00153
Mol. Pharmaceutics 2018, 15, 2307–2315

correction based on a previously constructed Levy plot. A Levy plot consists of a relationship between the time required in vivo and in vitro for the dissolution/absorption of a given fraction. An extent correction factor was also necessary to account for the incomplete in vitro release of telmisartan. In vitro profiles were scaled in extent, and the scaled in vitro dissolved fraction was used for construction of the Levy plot. In order to estimate the time needed for in vitro dissolution of a particular fraction, a first order dissolution model was previously fitted to the experimental values of each formulation.

The internal validation was done by estimating the prediction error percentage (PE%) on C_{max} and AUC values with the three formulations used for developing the correlation, i.e., Reference, X1, and X2. The external validation was done by calculating the prediction error of C_{max} of Y1, the nonbioequivalent formulation not included in the IVIVC development.

RESULTS

The solubility values of telmisartan were 0.229 ± 0.009, $1.05 \times 10^{-3} \pm 3.55 \times 10^{-5}$, and $1.56 \times 10^{-3} \pm 1.18 \times 10^{-4}$ mg/mL, at pH 1.2, 4.5, and 6.8, respectively; therefore, the lowest solubility of telmisartan was $1.05 \times 10^{-3} \pm 3.55 \times 10^{-5}$ at pH 4.5. The dose numbers (Do = 80 mg/250 mL/solubility mg/mL) were 1.40, 304.76, and 205.13, respectively, at pH 1.2, 4.5, and 6.8. In all of the conditions, Do is higher than 1, confirming the low solubility classification for telmisartan.

The permeability of telmisartan was studied at the clinical concentration (80 mg administered with 250 mL of water: 185.7 μM), and to determine BCS classification, the telmisartan permeability value was compared with the metoprolol permeability value (Figure 1). In this Figure, the permeability of different formulations of telmisartan was shown too.

Mean values of telmisartan and metoprolol were compared with two-tailed Student's t tests, differences among both compounds were detected ($p < 0.05$), and ANOVA analysis detected statistically significant differences ($p = 0.004$) between products. The results indicated statistically significant differences ($p = 0.42$ and $p = 0.02$) between the nonbioequivalent formulation (Y1) and the other formulations, but the test did not show differences between other formulations (Micardis, BE (X2), NBE (X1)).

Figure 2 summarizes the disintegration times of telmisartan products.

Dissolution profiles of all the formulations in the USP II apparatus in different conditions are summarized in Figure 3. Dissolution profiles obtained in the USP IV apparatus are depicted in Figure 4.

If the dissolution medium can predict the in vivo results, the dissolution profiles should be ordered this way: Micardis, X2, X1, and Y1; the f_2 value should be more than or equal to 50 for the formulation X2 and less than 50 for the others. The values of f_2 comparing the Micardis profile versus X1, X2, and Y1 obtained in

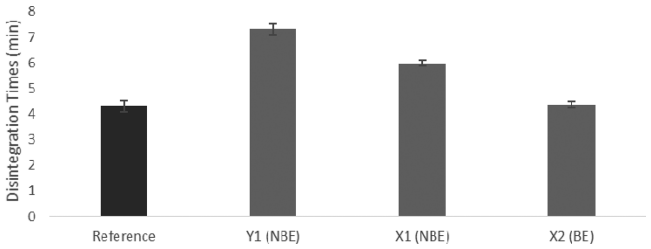


Figure 2. Disintegration times of telmisartan products.

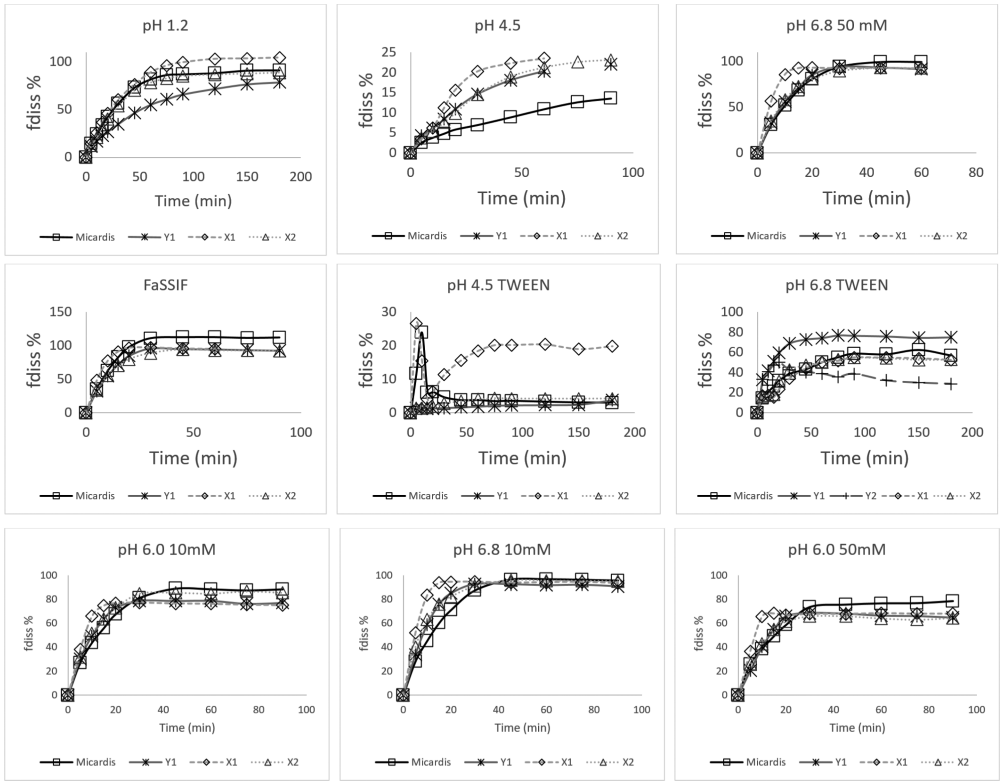


Figure 3. Dissolution profiles of telmisartan formulations (reference product, nonbioequivalent formulations (X1, Y1), and bioequivalent formulation (X2)) in the Apparatus 2 (paddle method 50 rpm) in different media conditions.

DOI: 10.1021/acs.molpharmaceut.8b00153
Mol. Pharmaceutics 2018, 15, 2307−2315

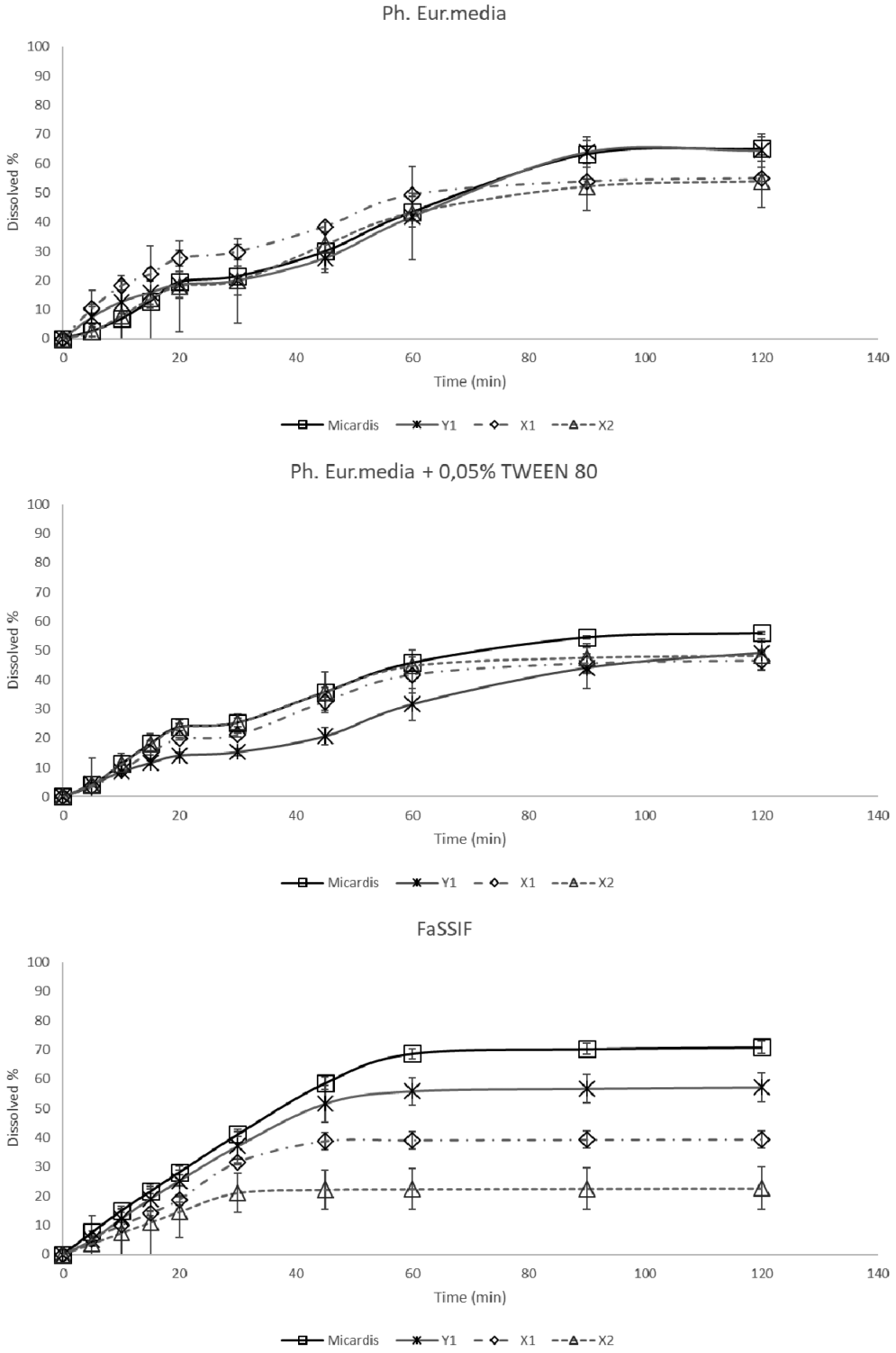


Figure 4. Dissolution profiles of telmisartan formulations (reference product, nonbioequivalent formulations (X1, Y1), and bioequivalent formulation (X2)) in the Apparatus 4 (automated flow-through cell system) in different conditions.

the USP IV apparatus with media change, and in the presence of surfactant, they were 59.08, 73.99, and 43.04, respectively.

Figure 5 shows the absorption profiles of the three formulations studied obtained by the Loo-Riegelman deconvolution method. In addition, Figure 6 shows all of the absorption and dissolution profiles (obtained with USP IV apparatus) in the same graph to highlight the time scale and extent difference between in vitro and in vivo.

In order to correct for the time scale difference, a Levy plot was constructed. As a change in slope was observed, a preliminary Levy plot was constructed with two linear relationships, and finally, a second order equation was applied to avoid time discontinuities in the estimations of the equivalent in vitro times. Both Levy plots are shown in Figure 7.

After Levy Plot adjustment, absorption and dissolution profiles were made superimposable (Figure 8), and a linear correlation

DOI: 10.1021/acs.molpharmaceut.8b00153
Mol. Pharmaceutics 2018, 15, 2307−2315

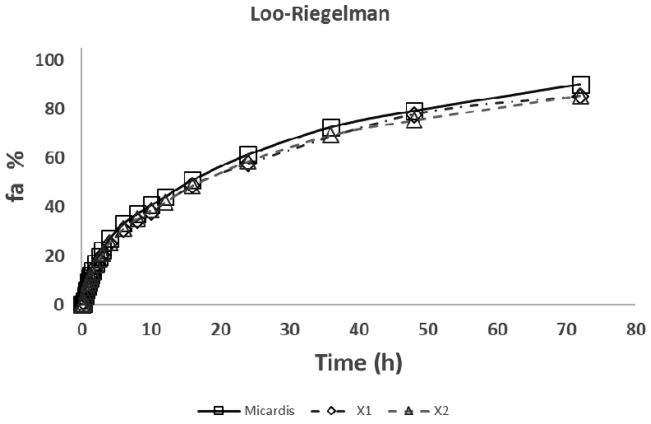


Figure 5. In vivo absorption profiles (oral fraction absorbed (f_a) versus time) of telmisartan formulations (reference, X1, and X2) obtained by Loo-Riegelman method.

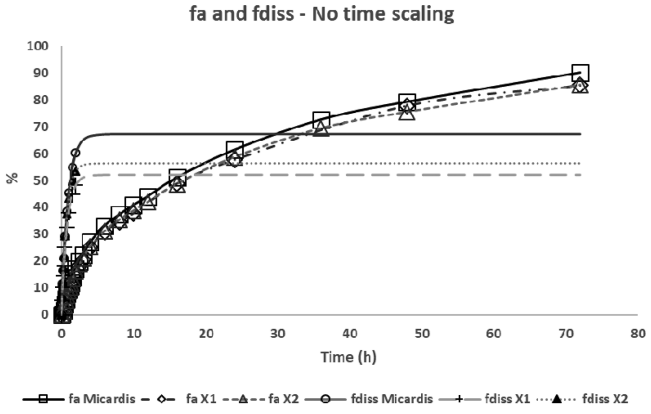


Figure 6. Fractions absorbed and fractions dissolved represented together in the real scale to show the extent and time scale difference in vitro versus in vivo. In vitro release was not complete after 2 h, while 100% f_a was reached in vivo.

was established between the in vitro fraction dissolved and oral fraction absorbed, which is represented in Figure 9.

Table 4 summarizes the internal and external validation. Prediction error percentages (PE%) for C_{max} and AUC were obtained comparing the experimental values and the predicted values. Experimental and predicted plasma profiles are represented in Figure 10.

To predict C_{max} and AUC values, the fractions absorbed were estimated from the experimental fractions dissolved through the IVIVC relationship, and then, the predicted fractions absorbed were reconvoluted with the disposition parameters to obtain the predicted plasma profiles.[14] Plasma profiles of the scaled test formulation were rescaled to the original scale with the original scaling factors at each time point.

DISCUSSION

Both solubility and permeability experimental results confirm the BCS class II nature of telmisartan. In vivo dissolution from its formulations is the limiting step for its absorption, and in this work, an in vivo predictive−in vitro dissolution method has been successfully developed.

The telmisartan permeability value ($3.59 \times 10^{-5} \pm 2.03 \times 10^{-6}$) was higher than the metoprolol permeability value ($2.00 \times 10^{-5} \pm 2.85 \times 10^{-6}$). Telmisartan permeability in the presence of the formulations' excipients was also higher than the metoprolol

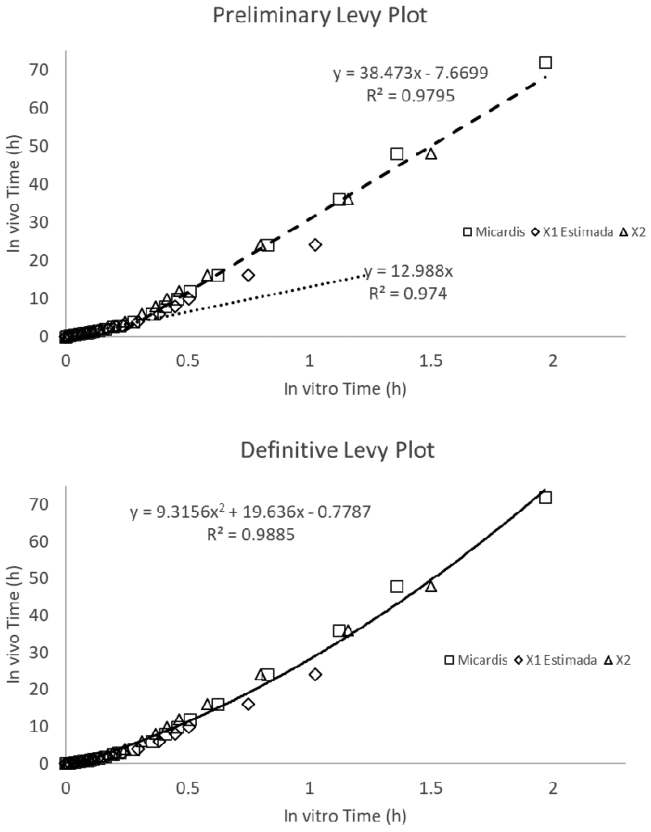


Figure 7. Levy plot.

permeability, confirming that the high permeability characteristics of the drug are not affected by the excipients of these formulations. Telmisartan permeability in the presence of the excipients' Y1 formulation was statistically significant lower compared to the other formulations. Nevertheless, that value is still higher than the metoprolol value; thus, in principle, it would not be expected that this fact affects the telmisartan rate and extent of absorption. Nevertheless, a negative effect on C_{max} due to the excipients affecting the permeation rate cannot be ruled out. Borbas et al.[17] recently reported the effect of some excipients on telmisartan flux across membranes, and the in vitro dissolution profiles obtained in a dissolution−permeation system were well correlated with the human in vivo BE studies. The authors used for the predictions the early phase of the dissolution-permeation test, which showed the combined effect of both processes, as it is difficult to ascertain the true limiting step. One of the formulations showing a reduced telmisartan flux presented a lower boundary of the 90% confidence interval of C_{max} outside the acceptance limit (80%), so some effect of the altered permeability rate in the obtained results with the Y1 formulation cannot be discarded.

The disintegration time of Micardis was 4.30 min. This time is very similar to that obtained with the BE formulation X2 (4.37 min). Both nonbioequivalent formulations obtained disintegration times higher than that of the reference, indicating that this process was clearly slower. In this case, the disintegration test has proven to be indicative of the in vivo observed differences. Although this test is used only for quality control purposes, much more research is needed to evaluate its potential and reliability as a biopredictive tool.[18−21]

Regarding the several dissolution conditions assayed, only the USP IV apparatus with media change and in the presence of

DOI: 10.1021/acs.molpharmaceut.8b00153
Mol. Pharmaceutics 2018, 15, 2307−2315

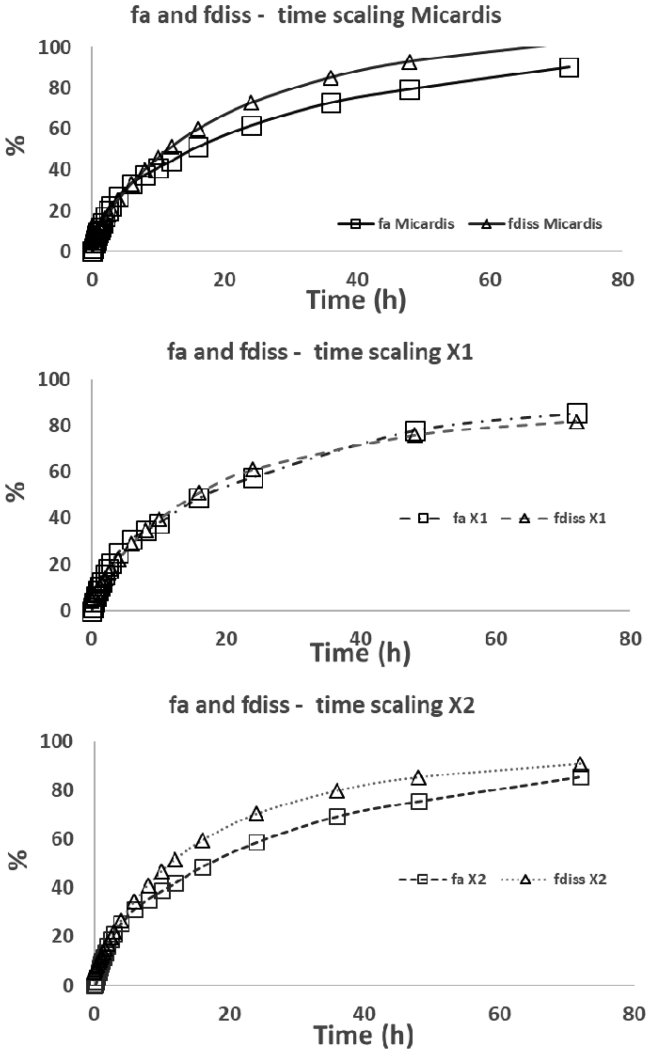


Figure 8. Absorption and dissolution processes after time and extent scaling.

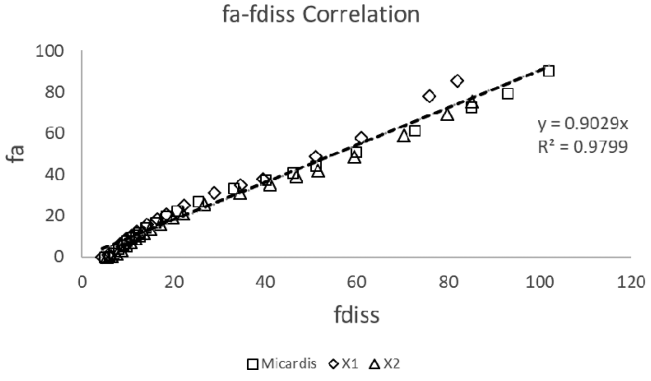


Figure 9. In vitro−in vivo correlation.

0.05% of tween 80 was able to order the formulations in vitro in the same sequence as obtained in vivo. F_2 values of the dissolution profiles in this condition confirm the similarity of the bioequivalent formulations and the dissimilarity of the non-bioequivalent ones.

In vitro dissolution in the selected conditions is not complete, which probably reflects in vivo dissolution and leads to an incomplete oral bioavailability. The fraction absorbed was

Table 4. Prediction Errors of C_{max} and AUC Values from the Developed in Vitro−in Vivo Correlation

	C_{max}			AUC		
	exp	pred	PE%	exp	pred	PE%
Micardis	456.1	439.5	3.6	4445.5	4522.5	1.7
X1 scaled	400.2	390.9	2.3	4187.3	3562.1	14.9
X2	445.2	474.8	6.6	4208.9	3884.0	7.7
Y1	369.5[a]	231.5	37.3	4312.2	4285.1	0.6

[a]Estimated experimental value from the point estimate of the ratio and Micardis value.

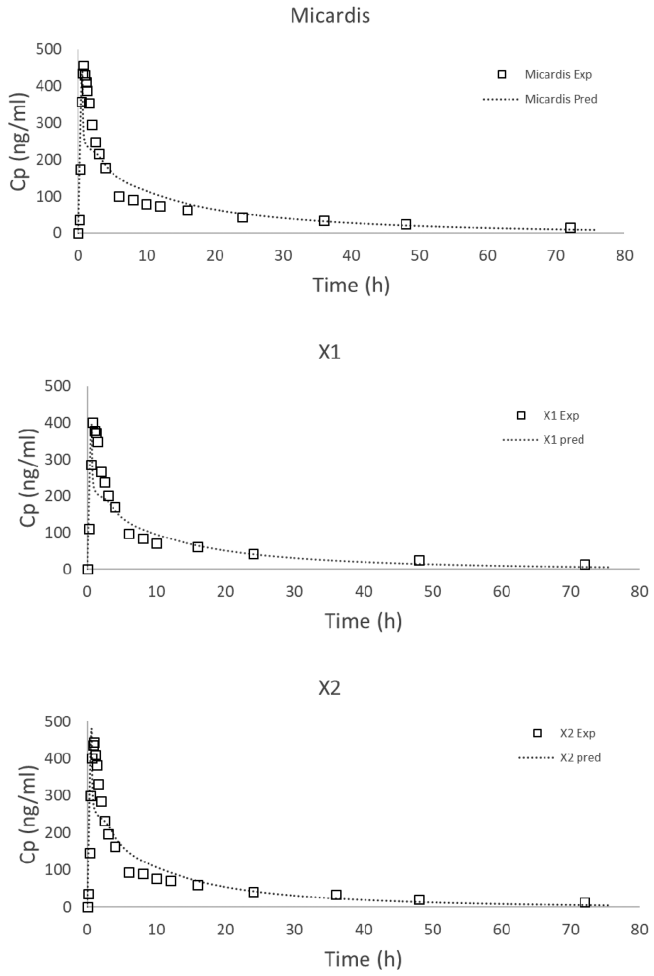


Figure 10. Experimental and predicted telmisartan plasma profiles for the three studied formulations.

determined in humans to be 50%, while absolute bioavailability is 43%.[22] As permeability is high, this fact indicates that in vivo dissolution is not complete, and it is the limiting factor for telmisartan absorption. Nevertheless, in the two-step approach, the incomplete in vitro dissolution required a correction of the extent to correlate the fractions dissolved with fractions absorbed that was obtained with the Loo-Riegelman method, which always reached 100%. A time scale correction was also necessary, as in vitro dissolution was faster than the in vivo dissolution process, or in other words, the in vitro dissolution and in vivo input curves were not directly superimposable, and they were made superimposable by the use of a scaling factor (Levy Plot). The Levy plot was not a single linear correlation, and there is a clear slope change around 4 h (in vivo time). This fact points out a

DOI: 10.1021/acs.molpharmaceut.8b00153
Mol. Pharmaceutics 2018, 15, 2307−2315

change in in vivo dissolution conditions, which could be explained by the transit from small intestine to colon with a more alkaline pH and less fluid available. The higher value of this second slope in the in vivo versus in vitro times plot means a further reduction in the dissolution rate for later time points. Finally, with the adequate scaling of time and extent, the method used to establish a Level A IVIVC was successful and allowed an IVIVC with a determination coefficient (r^2) of 0.92.

The prediction errors were within the accepted limits for X1 and X2 formulations (15% for the individual formulations and 10% for the average), but the PE were higher than accepted in regulatory guidelines for Y1, so it would not be adequate for a biowaiver claim. This is not surprising, because Y1 is a different formulation from a different company with different excipients and a different manufacturing process, outside of the design space of the manufacturer of X1 and X2. Nevertheless, thanks to the correlation, the proposed dissolution method obtained in this study could be useful as a development tool for selecting the most promising formulation for further in vivo studies.

In summary from a regulatory point of view, Y1 would have never been acceptable for a formal IVIVC; however, this IVIVC would predict that formulation X1 would not be bioequivalent to the reference prior to the in vivo study. This is highly valuable for the formulation developer and could save valuable resources and avoid BE failures, by selecting with the in vitro method the formulation with highest probability of success in the human BE trial.

CONCLUSION

Telmisartan is a pH-dependent low solubility drug, for which in vivo dissolution seems to be the limiting factor for absorption. The in vivo dissolution of telmisartan formulations is determined by physiological variables, as pH changes during intestinal transit and the presence of natural surfactants.

In this work, a more physiological dissolution set up in the USP IV apparatus has been developed, allowing simulation of the pH gradient and the presence of surfactants in the gastrointestinal tract. This new model is able to predict the formulation differences in dissolution that were previously observed in vivo in one successful and one failed bioequivalence study.

The studies carried out during this investigation have allowed the development of a level A IVIVC with a good correlation coefficient, which could be used as a risk-analysis tool for formulation selection in future bioequivalence trials.

The dissolution conditions of the in vitro biopredictive test presented here would need further refinement to improve the IVIVC predictability and to fulfill regulatory requirements for a biowaiver claim.

AUTHOR INFORMATION

Corresponding Author

*E-mail: isabel.gonzalez@goumh.umh.es; Mailing Address: Isabel Gonzalez-Alvarez, Edificio Muhammad Al-Shafra, Facultad de Farmacia, UMH, Carretera Alicante Valencia km 87, 03550 San Juan de Alicante, Alicante, Spain; Phone +34 965 919217; Fax +34 963544911 (I.G.-A.)

ORCID

Isabel González-Álvarez: 0000-0002-1685-142X

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge partial financial support to projects: "Modelos in vitro de evaluación biofarmaceutica" SAF2016-78756(AEI/FEDER, EU) funded by Agencia Estatal Investigación and European Union, through FEDER (Fondo Europeo de Desarrollo Regional) and FA-426-08-07-C, Comparación de los perfiles de disolución de distintos principios activos, from CDCHT-ULA. S.C.U. received a grant from DAP, University of Los Andes, Venezuela, to develop this project.

ABBREVIATIONS

BE, bioequivalent; N-BE, nonbioequivalent; BCS, Biopharmaceutics Classification System; H, high; L, low

REFERENCES

(1) Tsume, Y.; Mudie, D. M.; Langguth, P.; Amidon, G. E.; Amidon, G. L. The Biopharmaceutics Classification System: subclasses for in vivo predictive dissolution (IPD) methodology and IVIVC. *Eur. J. Pharm. Sci.* **2014**, *57*, 152−63.

(2) Yang, L.; Shao, Y.; Han, H. K. Improved pH-dependent drug release and oral exposure of telmisartan, a poorly soluble drug through the formation of drug-aminoclay complex. *Int. J. Pharm.* **2014**, *471* (1−2), 258−63.

(3) Park, J.; Cho, W.; Cha, K. H.; Ahn, J.; Han, K.; Hwang, S. J. Solubilization of the poorly water soluble drug, telmisartan, using supercritical anti-solvent (SAS) process. *Int. J. Pharm.* **2013**, *441* (1−2), 50−5.

(4) Pubchem. Telmisartan: Chemical and Physical Properties.https://pubchem.ncbi.nlm.nih.gov/compound/65999#section=Chemical-and-Physical-Properties.

(5) Ruiz-Garcia, A.; Lin, H.; Pla-Delfina, J. M.; Hu, M. Kinetic characterization of secretory transport of a new ciprofloxacin derivative (CNV97100) across Caco-2 cell monolayers. *J. Pharm. Sci.* **2002**, *91* (12), 2511−9.

(6) Oltra-Noguera, D.; Mangas-Sanjuan, V.; Centelles-Sangüeesa, A.; Gonzalez-Garcia, I.; Sanchez-Castano, G.; Gonzalez-Alvarez, M.; Casabo, V.; Merino, V.; Gonzalez-Alvarez, I.; Bermejo, M. Variability of permeability estimation from different protocols of subculture and transport experiments in cell monolayers. *J. Pharmacol. Toxicol. Methods* **2015**, *71*, 21−32.

(7) Mangas-Sanjuan, V.; Gonzalez-Alvarez, I.; Gonzalez-Alvarez, M.; Casabo, V. G.; Bermejo, M. Modified nonsink equation for permeability estimation in cell monolayers: comparison with standard methods. *Mol. Pharmaceutics* **2014**, *11* (5), 1403−14.

(8) Gundogdu, E.; Mangas-Sanjuan, V.; Gonzalez-Alvarez, I.; Bermejo, M.; Karasulu, E. In vitro-in situ permeability and dissolution of fexofenadine with kinetic modeling in the presence of sodium dodecyl sulfate. *Eur. J. Drug Metab. Pharmacokinet.* **2012**, *37* (1), 65−75.

(9) Gonzalez-Alvarez, I.; Fernandez-Teruel, C.; Garrigues, T. M.; Casabo, V. G.; Ruiz-Garcia, A.; Bermejo, M. Kinetic modelling of passive transport and active efflux of a fluoroquinolone across Caco-2 cells using a compartmental approach in NONMEM. *Xenobiotica* **2005**, *35* (12), 1067−88.

(10) European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP). *Guideline on the Investigation of Bioequivalence*; CPMP/EWP/QWP/1401/98 Rev. 1; 2010.

(11) US Department of Health and Human Services, US FDA, Center for Drug Evaluation and Research. *Guidance for industry: bioavailability and bioequivalence studies for orally administered drug products—general considerations*; 2002.

(12) WHO Expert Committee on Specifications for Pharmaceutical Preparations. *Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability*; WHO Technical Report Series, No. 992; 2006.

(13) US Department of Health and Human Services, US FDA, Center for Drug Evaluation and Research. *Guidance for industry: dissolution testing of immediate release solid oral dosage forms*; 1997.

DOI: 10.1021/acs.molpharmaceut.8b00153
Mol. Pharmaceutics 2018, 15, 2307−2315

(14) Humbert, H.; Cabiac, M. D.; Bosshardt, H. In vitro-in vivo correlation of a modified-release oral form of ketotifen: in vitro dissolution rate specification. *J. Pharm. Sci.* **1994**, *83* (2), 131−6.
(15) Stangier, J.; Su, C. A.; Roth, W. Pharmacokinetics of orally and intravenously administered telmisartan in healthy young and elderly volunteers and in hypertensive patients. *J. Int. Med. Res.* **2000**, *28* (4), 149−67.
(16) Zhang, Y.; Huo, M.; Zhou, J.; Xie, S. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Comput. Methods Programs Biomed* **2010**, *99* (3), 306−14.
(17) Borbas, E.; Nagy, Z. K.; Nagy, B.; Balogh, A.; Farkas, B.; Tsinman, O.; Tsinman, K.; Sinko, B. The effect of formulation additives on in vitro dissolution-absorption profile and in vivo bioavailability of telmisartan from brand and generic formulations. *Eur. J. Pharm. Sci.* **2018**, *114*, 310−317.
(18) Nickerson, B.; Kong, A.; Gerst, P.; Kao, S. Correlation of dissolution and disintegration results for an immediate-release tablet. *J. Pharm. Biomed. Anal.* **2018**, *150*, 333−340.
(19) Kindgen, S.; Rach, R.; Nawroth, T.; Abrahamsson, B.; Langguth, P. A Novel Disintegration Tester for Solid Dosage Forms Enabling Adjustable Hydrodynamics. *J. Pharm. Sci.* **2016**, *105* (8), 2402−9.
(20) Kindgen, S.; Wachtel, H.; Abrahamsson, B.; Langguth, P. Computational Fluid Dynamics Simulation of Hydrodynamics and Stresses in the PhEur/USP Disintegration Tester Under Fed and Fasted Fluid Characteristics. *J. Pharm. Sci.* **2015**, *104* (9), 2956−68.
(21) Al-Gousous, J.; Langguth, P. Oral Solid Dosage Form Disintegration Testing - The Forgotten Test. *J. Pharm. Sci.* **2015**, *104* (9), 2664−75.
(22) Stangier, J.; Schmid, J.; Turck, D.; Switek, H.; Verhagen, A.; Peeters, P. A.; van Marle, S. P.; Tamminga, W. J.; Sollie, F. A.; Jonkman, J. H. Absorption, metabolism, and excretion of intravenously and orally administered [14C]telmisartan in healthy volunteers. *J. Clin Pharmacol* **2000**, *40* (12 Pt 1), 1312−1322.

DOI: 10.1021/acs.molpharmaceut.8b00153
Mol. Pharmaceutics 2018, 15, 2307−2315