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Level A IVIVC for immediate release tablets confirms *in vivo* predictive dissolution testing for ibuprofen☆

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ABSTRACT

A bioequivalence study comparing two fixed dose combination tablets containing 200 mg ibuprofen and 30 mg pseudoephedrine hydrochloride showed bioequivalence for pseudoephedrine AUC and C_{max}, but the reference product showed higher C_{max} than the test product in fasted conditions. The main difference between products was the presence of tribasic calcium phosphate in the reference tablet, resulting in an increased surface pH of the dissolving ibuprofen particles under gastric and intestinal conditions and, consequently, higher solubility of ibuprofen. A mechanistic model based on mass balance and ionization equilibria was used to calculate the pH of the particle surface under different buffer conditions. The discrepancies in surface pH between test and reference tablet were pronounced in 0.1 M and 0.01 M hydrochloric acid and in diluted maleate 7 mM pH 6.5 and phosphate 5 mM pH 6.7 buffers (but negligible in compendial phosphate buffer pH 6.8. Only those dissolution tests using pre-treatment in acidic conditions could be used to build a one-step *in vitro-in vivo* correlation (IVIVC). This work shows the potential of these discriminatory and *in vivo* predictive dissolution methods to obtain IVIVCs for BCS class IIa drugs and for extending BCS biowaivers to BCS class IIa drugs.

1. Introduction

The Biopharmaceutics Classification System (BCS) classifies drugs according to their solubility and intestinal permeability into four classes. High solubility is concluded when the highest dose completely dissolves in 250 mL of water in a pH range from 1.2 to 6.8 and high permeability is claimed when > 85 % of the dose is absorbed after oral administration (Amidon et al., 1995; ICH, 2019). BCS-based biowaiver approaches can be a resource-saving alternative to the *in vivo* human bioequivalence studies that are, in principle, mandatory to demonstrate bioequivalence (BE) between two products containing the same drug if certain conditions are met. One prerequisite for a BCS-based biowaiver is that the drug belongs to the BCS class I or III (i.e., highly soluble with high a low permeability, respectively). In such cases, BE can then be demonstrated *in vitro* using relatively simple dissolution tests. BE studies can also be waived when a level A *in vitro-in vivo* correlation (IVIVC) is established if

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the product to be waived is within the limits (i.e. design space) defined by the products tested to establish the IVIVC.

Extending biowaivers to drugs that belong to the BCS class II subclasses (Tsume et al., 2014) has been discussed in literature (Gonzalez-Alvarez et al., 2021; Kovaĉević et al., 2009; Tubic-Grozdanis et al., 2008; Yu et al., 2002). BCS class II drugs are highly permeable and poorly soluble and, therefore, the *in vivo* dissolution is the main influencing factor on oral bioavailability for this class. For weakly acidic drugs of the BCS class II (BCS IIa subclass), with supposedly increased solubility in intestinal fluid due to ionization, an extension of the biowaiver application has been proposed since the extent of absorption is expected to be complete once the drug is dissolved in the intestine. For ibuprofen, this BCS-biowaiver extension was proposed in a FIP monograph (Potthast et al., 2005). However, it was shown that the current *in vitro* dissolution conditions are not able to predict the equivalence in rate of absorption (Álvarez et al., 2011), which was attributed to the ionic strength of the phosphate buffer (Cristofoletti & Dressman, 2016a, 2016b, 2017; Krieg, 2015; Tsume et al., 2012). The evidence showing the lack of the discriminative power of the 50 mM phosphate buffer used for BCS biowaivers was questioned and it was claimed that the C_{max} differences had no clinical relevance (Cristofoletti and Dressman, 2014; Shohin et al., 2012). However, these claims were contested and it was stressed that the rate of absorption of ibuprofen has clinical impact on the onset of action (García-Arieta et al., 2015), which have been finally acknowledged and confirmed (Cristofoletti and Dressman, 2017, 2016a, 2016b, 2016c). In addition, the rate of absorption may also be critical when the first-pass effect is saturated or close to saturation since AUC might be altered in those cases.

Since the bioavailability of these class II drugs is mainly determined by the *in vivo* dissolution, the use of discriminatory *in vitro* test methods is essential for a correct BE prediction. Yet it is known that the compendial *in vitro* dissolution methods are not appropriately reflecting the *in vivo* situation and are thus little biopredictive and discriminative (Mudie et al., 2020, 2010). An *in vitro* dissolution method with low discriminatory power increases the risk for a false positive bioequivalence decision (Kubbinga et al., 2013). A simple method to increase the bio-predictivity of the dissolution method of ionizable drugs is to reduce the buffer molarity of the dissolution media to better account for the reduced buffer molarity in the intestinal fluid *in vivo* (Hens et al., 2017; Krieg et al., 2015; Tsume et al., 2012). This can be explained by the dissolution model proposed by Mooney et al. where a stagnant film layer is formed on the surface on of a dissolving solid. In a more diluted buffer the surface pH cannot be controlled as well as in concentrated one owing to the lower buffer capacity (Mooney et al., 1981). The model was further improved by accounting for the chemical nature of the *in vivo* buffering species bicarbonate in the reversible non-equilibrium (RNE) model proposed by Al-Gousous et al (Al-Gousous et al., 2019). Hofmann et al. used ibuprofen, a weak acid (pKa 4.41) with low solubility in acidic conditions and increased solubility in near neutral media (Potthast et al., 2005), as a model drug from BCS IIa and developed, based on the Mooney dissolution model and RNE-model, a phosphate based buffer that predicted accurately the *in vivo* dissolution of ibuprofen suspensions in *in vitro* experiments (Hofmann et al., 2020).

In this work, the bioavailability of two products containing a combination of 200 mg ibuprofen and 30 mg pseudoephedrine hydrochloride were compared. The pseudoephedrine plasma-profiles were bioequivalent (BE) while the ibuprofen profiles were not bioequivalent since the lower limit of the 90% confidence interval (CI) of the C_{max} Test/Reference ratio for the active S-Ibuprofen isomer was below the 80% BE limit and the CI did not include the 100% value for S and R isomers, which is indicative of a statistically significant difference between products. A larger study size would have likely concluded bioequivalence, but due to the statistically significant different in Cmax (mean difference of 13–15%) this study offers the possibility to explore if such difference can be detected by a dissolution test that consequently could be useful to establish specifications with clinical relevance or to waive *in vivo* bioequivalence studies under certain conditions.

In a recent study, Silva et al. identified calcium monohydrogen phosphate as pH-active excipient that could affect the dissolution of ibuprofen from different tablet formulations by formation of a microclimate surrounding the dissolving particles (Silva et al., 2020). Similar observations were also made by Bermejo et al. (Bermejo et al., 2019) who investigated the bioequivalence of three dexketoprofen trometamol products (reference, bioequivalent test and non-bioequivalent test) using the Gastrointestinal Simulator (GIS). The reason for the non-bioequivalent product exhibiting higher C_{max} and AUC than the two other products was identified as calcium monohydrogen phosphate in the non-bioequivalent product (Bermejo et al., 2019). Starting from this, the hypothesis was built that the tricalcium phosphate (TCP) in the reference product could be the reason for the different rate of absorption of the two products. The aim of this work was to explore the reasons for differences in rate of absorption of Ibuprofen using a mechanistic physico-chemical model to predict the microclimate pH and solubility of ibuprofen and to build a computational model to correlate the *in vitro* dissolution using biopredictive methods and the *in vivo* plasma concentration.

2. Materials and methods

2.1. Materials

All used reagents were of at least analytical grade. Sodium chloride, hydroxy napthole blue disodium salt, EDTA disodium salt solution 0.05 mol/L, triethanolamine and potassium dihydrogen phosphate were purchased from Carl-Roth GmbH & Co. KG (Karlsruhe, Germany). Acetonitrile HPLC Grade, concentrated hydrochloric acid (37 %) and sodium hydroxide were obtained at VWR Chemicals S.A.S. (Fontenay-sous-Bois, France).

Calcium phosphate was bought from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Phosphoric acid for HPLC (85–90 %) and ammonium chloride were purchased from Fluka TM (Seelze, Germany) and Fluka Chemie AG (Buchs, Switzerland). Sodium dihydrogen phosphate monohydrate was bought from Merck (Darmstadt, Germany). ratioGrippal® (200 mg Ibuprofen/30 mg pseudoephedrine hydrochloride; ratiopharm GmbH (Ulm, Germany)) was brought from a local German pharmacy and ibuprofen from BASF SE (Ludwigshafen, Germany). The reference product (Nurofen® cold and flu from Reckitt Benckiser Healthcare (UK) Ltd.) and the test product were supplied by Farmalider. The qualitative compositions are summarized in Table 1

Table 1
Composition of reference and test product.

Reference product (Nurofen)	Test product
Ibuprofen	Ibuprofen
Pseudoephedrine HCl	Pseudoephedrine HCl
Tricalcium phosphate (TCP)118	Lactose monohydrate
Microcrystalline cellulose	Microcrystalline cellulose
Polyvidone	Pregelatinised maize starch
Croscarmellose sodium	Croscarmellose sodium
	Silica colloidal anhydrous
Magnesium stearate	Magnesium stearate
Methylhydroxypropyl cellulose	Hypromellose
Talc	Talc
Opaspray Yellow M−1F−6168	Titanium dioxide
Mastercote Yellow FA 0156	Sunset yellow FCF
Black printing ink (shellac, iron oxide black and propylene glycol)	Quinoline yellow

2.2. Bioequivalence study

Data obtained from a BE 2 × 2 cross-over study in healthy subjects were available for comparison with *in vitro* data. The summary of the outcome of the study is shown in Table 2. Results of the BE study are indicated for both ibuprofen isomers. For the *in vitro-in vivo* correlation average plasma concentrations were used.

A clinical bioequivalence study was performed in the Phase I Clinical Trial Unit of the Hospital Clínico San Carlos, Madrid, Spain following the updated Declaration of Helsinki, with the approval of the Ethical Committee for Clinical Research and the Spanish Agency for Medicines and Health Care Products (EudraCT: 2017–002884-17). The study was randomized, open-label and single dose and it included 24 volunteers. All volunteers were non-smokers, healthy males or females. The subjects were determined to be in good health by physical examination, complete blood count, urinalysis, and serum test on hepatic and renal function. The average age and body weight were: 23.91 years and 65.65 kg. The volunteers were asked to abstain from taking any drug including OTC products for at least 1 week prior to or during the study. Written informed consent was obtained from subjects after explaining the nature and purpose of the study. After fasting overnight for minimum 10 h, a dose of test or reference product was orally administered with 200 mL of water. Blood was drawn before dosing and at 0.33, 0.66, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 6, 8, 10,12, 14 and 24 h after dosing through an indwelling catheter placed in an antecubital vein in the forearm. The blood samples were centrifuged and the plasma was collected and stored at −20 °C until assayed. Volunteers were allowed to take water ad libitum except for one hour before and two hours after drug administration. The first meal was served 4 h after dosing. Beverages and food containing caffeine were not permitted during the entire course of the study. Wash out period was of minimum one-week.

The primary pharmacokinetic parameters, C_{max} and AUC_{0-t}, were estimated from the plasma concentration time data using Phoenix WinNonlin 6.4. Cmax and Tmax were obtained directly from the data, and the AUC_{0-t} was calculated with the linear trapezoidal rule.

The elimination rate constant (k_e) was calculated using the data points from 4 to 12 h by linear regression of the ln-transformed average concentration data. AUC was calculated using the linear trapezoidal method (Eq. (1)):

$$AUC_{0\to\infty} = \frac{1}{2}\sum_{i=1}^{n}(C_{i-1}+C_i)(t_i - t_{i-1}) + \frac{C_n}{k_e} \quad (1)$$

Apparent Volume of distribution (V_d/F) was calculated by Eq. (2)

$$Vd/F = \frac{Dose}{AUC_{0\to\infty} * k_e} \quad (2)$$

2.3. Calcium titration

The titration was performed according to NF Monograph of tribasic calcium phosphate (United States Pharmacopœial Convention., 2019). Three tablets of the reference product were dissolved in 10 mL hydrochloric acid (5:3 mixture of hydrochloric acid 37 % and water). After 2 h of stirring, 125 mL of water was added. Afterwards, 0.5 mL of triethanolamine and 3 mg of hydroxy naphthol blue were added (the NF prescribes 300 mg of hydroxy naphthol blue, but in preliminary experiments this concentration turned out to be too high). Next, 10 mL of the EDTA-solution were added. The pH of the solution was adjusted to 12.3–12.5 using a 45 % sodium hydroxide solution. Then, the titration with EDTA was continued until the solution turned from violet to clear blue.

2.4. pH measurements

The effect of 200 mg ibuprofen as well as 47 mg of calcium phosphate and 7.96 mg of ammonium chloride (pKa (NH_4Cl) = 9.25 ("Ammonia | NH3 - PubChem," n.d.) compared to pKa (pseudoephedrine hydrochloride) = 9.22 (Benezra and McRae, 1979), equal to 30 mg pseudoephedrine hydrochloride) on the pH of 1 mL of a USP phosphate buffer pH 6.8, 5 mM phosphate buffer pH 6.7 (Cristofoletti and Dressman, 2016a) and 7 mM maleate buffer pH 6.5 was measured using a WTW pH Meter pH 538.

In addition, surface pH and ibuprofen solubility were calculated based on a mechanistical model that is based on ionization equilibria and mass balances (See Supplement). The model assumes that all solids are in equilibrium with dissolved molecules (which are also at equilibrium with each other).

2.5. Dissolution test

The test and reference tablets were tested in a USP II dissolution apparatus in 500 mL fluid volume at 50 rpm and 37 ± 0.5 °C. In addition to the 50 mM USP phosphate buffer pH 6.8, the tablets were tested in 5 mM phosphate buffer pH 6.7 and 7 mM maleate buffer pH 6.5 (see Table 3). pH was measured and corrected (with diluted NaOH or HCl) during the experiments. To mimic the *in vivo* situation dissolution tests were also performed with pretreating the tablets for 15 min in 20 mL of hydrochloric acid at pH 1.2 and pH 2.0 followed by the buffer stage. At predefined time points 1 mL samples were withdrawn and filtered through a 0.45 μm filters. Sample volume was replaced with fresh buffer. Analysis of ibuprofen and pseudoephedrine was performed using HPLC-UV. In the method to detect ibuprofen (λ = 229), the stationary phase was a Nova (C18 4Microm, 3.9X150mm) and the mobile phase was a mixture H20: ACN in a 30:70 ratio. In the method to detect pseudoephedrine (λ = 257), the mobile phase was a MeOH: ACN mixture in a 60:40 ratio and the stationary phase was the same column as with ibuprofen.

Dissolution profiles of test and reference formulation in each condition were compared with f_2 similarity factor calculated with the samples up to the fastest profile reaches 85% dissolved (ICH, 2019).

Table 3
Buffer composition for dissolution testing. All values are concentrations given in mM.

	50 mM phosphate buffer	5 mM phosphate buffer	7 mM phosphate buffer
NaOH	12	1.2	12.3
NaCl	45.5	101.3	97.7
$NaH_2PO_4 \times H_20$	50	5	–
Maleic acid	–	–	7

Table 2
Outcome of the BE study. C.I. Confidence interval. R: Reference; T: test.

Parameter	R-ibuprofen		S-Ibuprofen		Pseudoephedrine	
	Ratio T/R	90% C.I.	Ratio T/R	90% C.I.	Ratio T/R	90% C.I.
Cmax	86.61	80.48–93.21	85.24	**79.69–91.18**	100.16	95.17–105.41
AUC0-24 h	96.58	92.08–101.30	98.49	95.08–102.02	100.68	96.05–105.53

2.6. IVIVC

2.6.1. Dissolution model

Four different dissolution model were used to fit the experimental dissolution data using Berkeley Madonna (Version 10.2.8). Table 4 summarizes the equations used. The Biphasic dissolution model assumes two different fractions (f) of ibuprofen that dissolve with different first order rate constants. The diphasic dissolution model (DPDM) was previously described by Hofmann et al. (Hofmann et al., 2020) where the solid ibuprofen undergoes a change from state S1 to S2 with a first order rate constant (k_{elk}) and the dissolution rate constants from S1 and S2 differ from each other. The model with the smallest root mean square (RMS) which is directly calculated by the software was chosen for the *in vivo-in vitro* correlation (IVIVC).

2.6.2. One-Step IVIVC

Berkeley Madonna (Version 10.2.8) was used to build a model for a one-step IVIVC (see supplement). A better fraction absorbed (fa)-time profile was obtained from the plasma concentrations- time profiles (Concentration-time) using the Wagner-Nelson method instead of the Loo-Riegelman method (data not shown). As concentration–time profiles did not show clear two-disposition phases a one-compartment disposition model was used. For Weibull dissolution and DPDM the model in Fig. 1A and B were used, respectively. For both models values of k_{emp} and k_a were set to 2.77 h^{-1} (corresponding to a gastric emptying half-life time of 15 min) and 9.75 h^{-1} (taken from Hofmann et al. (Hofmann et al., 2020)), respectively. k_e and V_d calculated from *in vivo* data were used (k_e = 0.606 h^{-1} and V_d = 10.081 L). Values of the dissolution parameters were set to the results in Section 3.5.1. The internal predictability of the model was evaluated calculating the % prediction error (% PE) of C_{max} and $AUC_{0\rightarrow t}$ using Eq. (3):

$$\%PE = \frac{P_{experimental} - P_{predicted}}{P_{experimental}} \quad (3)$$

where P are the observed and predicted values for C_{max} and AUC. The model is valid when the individual %PE of C_{max} and $AUC_{0\rightarrow t}$ for each product is not more than 15 % and the average %PE of C_{max} and $AUC_{0\rightarrow t}$ is less than 10 %. Different time-scaling functions were tested (see Eqs. (4)–(6)) and the best fit was chosen based on the least RMS provided by the software.

XS	Amount ibuprofen stomach
XE	Amount ibuprofen solid in early intestinal phase
XL	Amount ibuprofen solid in late intestinal phase
XI	Amount ibuprofen solid intestine
Xd	Amount ibuprofen dissolved intestine
Xc	Amount ibuprofen in central compartment
k_{emp}	1st order rate constant for gastric emptying
k_{elk}	1st order rate constant for change from early to late kinetic

(*continued on next column*)

Table 4
Dissolution models tested.

Model	Function
1st order kinetic	$Q_{diss} = Dose \times (1 - e^{-k_d * t})$
Weibull function	$Q_{diss} = Dose \times \left(1 - e^{-\frac{t^a}{b}}\right)$
Biphasic dissolution model	$Q_{diss} = f \times Dose \times (1 - e^{-k_{b1} * t}) + (1 - f) \times Dose \times (1 - e^{-k_{b2} * t})$
Diphasic dissolution model (DPDM)	$Q_{diss} = Dose \times [1 - e^{(k_{diss1} + k_{elk}) \times t} - \frac{k_{elk} \times e^{-k_{diss2} \times t}}{(k_{elk} + k_{diss1} - k_{diss2})} - \frac{k_{elk} \times e^{-(k_{diss1} + k_{elk}) \times t}}{(k_{diss2} - k_{diss1} - k_{elk})}]$
Q_{diss}	Quantity dissolved
k	1st order rate constants
a	Shape-parameter of Weibull function
b	Scaling-parameter of Weibull function

(*continued*)

k_{d1}	1st order rate constant for early intestinal dissolution
k_{d2}	1st order rate constant for late intestinal dissolution
k_a	1st order absorption rate constant
k_e	1st order elimination rate constant from central compartment
a	Shape-parameter Weibull function
b	Scaling-parameter Weibull function

$$t\_scaled = a \times t + b \quad (4)$$

$$t\_scaled = a \times t^2 + b \times t + c \quad (5)$$

$$t\_scaled = a \times t^b \quad (6)$$

3. Results

3.1. Bioequivalence study

The plasma concentration Cp-time profiles of ibuprofen (average R- and S) and pseudoephedrine from the reference and test formulation are depicted in Fig. 2A and B, respectively. Values for $C_{max,\ ibuprofen}$ of 15.39 mg* L^{-1} and 13.93 mg* L^{-1} were reached after 1 h and 2.5 h for the reference and test product, respectively. Maximum concentrations of pseudoephedrine were $C_{max,\ reference}$ = 113.36 ng*mL^{-1} and $C_{max,\ test}$ = 113.74 ng*mL^{-1}. Ibuprofen $AUC_{0\rightarrow t}$ of reference and test formulation where 70.279 mg*h*L^{-1} and 68.849 mg*h*L^{-1}, respectively. Pseudoephedrine $AUC_{0\rightarrow t}$ of reference and test formulation where 1034.11 ng*h*mL^{-1} and 1039.31 ng*h*mL^{-1}, respectively. k_e of ibuprofen calculated from the terminal data points was 0.377 h^{-1} (reference) and 0.379 h^{-1} (test) and k_e of pseudoephedrine was 0.134 h^{-1} (reference) and 0.133 h^{-1} (test). V_d of ibuprofen was calculated to be 7.28 L and 7.35 L for in the reference and test formulation.

3.2. Calcium titration

A total of 27.2 mL of the 0.05 M EDTA-solution was used. This corresponds to 0.00136 mol EDTA (=n(Ca^{2+})). One mole of $Ca_3(PO_4)_2$ is equal to 3 mol of Ca^{2+}. The molecular weight of $Ca_3(PO_4)_2$ is 310.18 g/mol. Therefore, the total amount of $Ca_3(PO_4)_2$ in the sample is 140.61 mg and one tablet contains the equivalent of 46.87 mg of $Ca_3(PO_4)_2$.

3.3. pH measurements

The surface pH and solubility of ibuprofen were calculated according to the model presented in the supplement information and the results are given in Table 5. The basic nature of $Ca_3(PO_4)_2$ increases the pH significantly from initial acidic conditions thus increasing the solubility of ibuprofen. At 0.1 M HCl the solubility of the drug from the reference product is more than two times higher than the solubility of the test product. This difference becomes more pronounced in 0.01 M HCl where the solubilities differ around 11-fold. In the USP buffer there is almost no difference between the two products due to the high buffer capacity, indicating that the buffer is not capable of discriminating between the products during dissolution test. On the other hand, the surface pH and solubility in 5 mM phosphate and 7 mM maleate buffers differed notably between reference and test product. This is also in line with the dissolution experiments (Section 3.4), where these two low molar buffers were able to discriminate between the products.

Furthermore, the effect of ibuprofen and ammonium chloride (surrogate for pseudoephedrine hydrochloride) and ibuprofen, ammonium chloride and calcium phosphate (representing the test and reference product, respectively) on the pH of 1 mL buffered solutions was investigated. The results in Table 6 are close to the calculated values in Table 5.

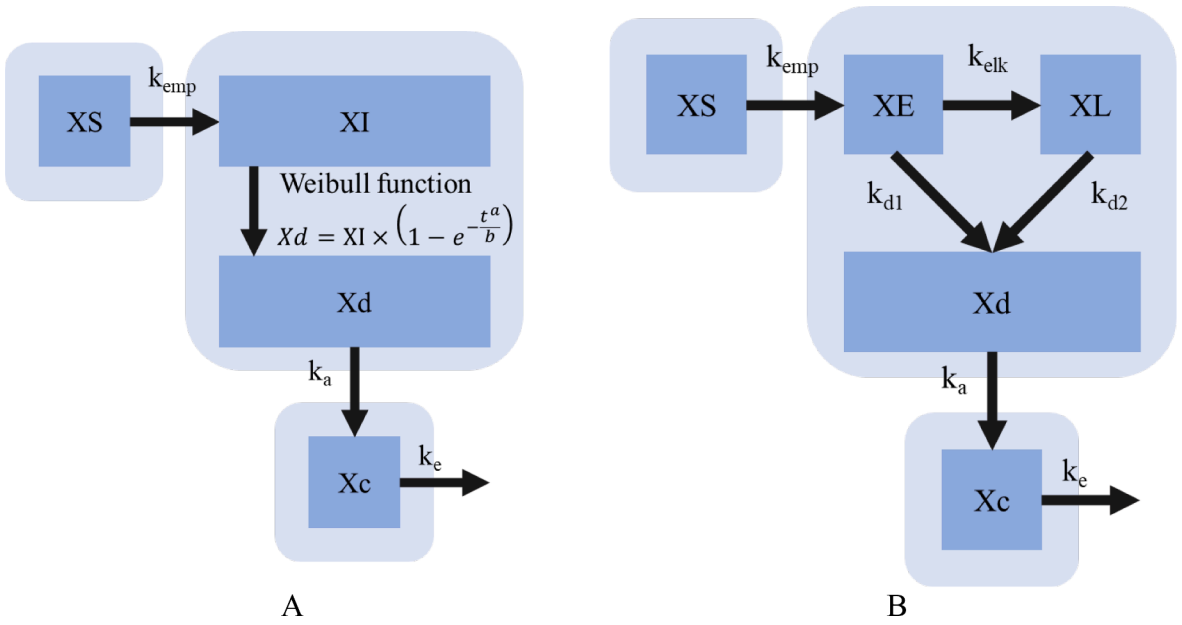

Fig. 1. Model used to build an IVIVC. (A) Using the Weibull function for dissolution in the intestine. (B) Using the DPDM proposed by Hofmann et al. [24].

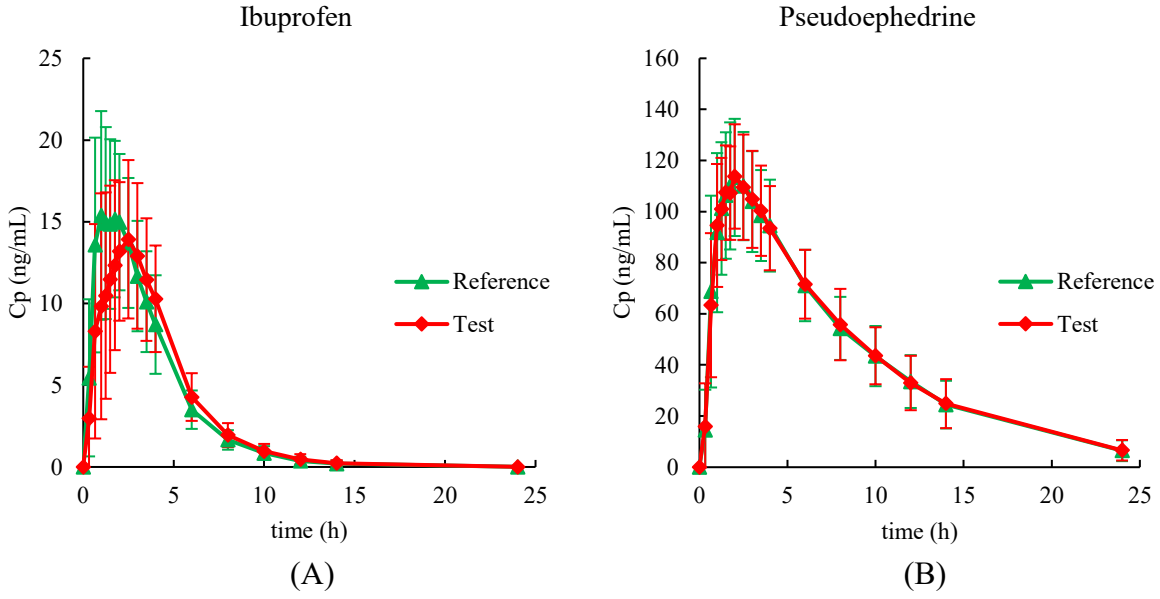

Fig. 2. (A) ibuprofen and (B) pseudoephedrine average plasma concentration (Cp)-time profiles of reference (green triangles) and test (red diamonds) formulation ± standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table5
Calculation of surface pH and solubility.

Buffer	0.1 M HCl		0.01 M HCl		USP		5 mM phosphate pH 6.7		7 mM maleate pH 6.5	
Product	Ref	Test	Ref	Test	Ref	Test	Ref	Test	Ref	Test
pH	4.50	1.00	5.41	2.00	6.04	6.01	5.66	5.08	5.81	5.36
Solubility (mg/L)	130.7	57.7	643.9	57.9	2556.6	2426.7	1108.13	336.59	1525.89	581.71

3.4. In vitro dissolution under buffered conditions

Fig. 3 shows the fraction dissolved (f_{diss}) in different dissolution conditions. Table 7 summarizes the f_2 values. Among all conditions tested the pseudoephedrine release was similar between the two products (f_2-value > 50) which is in line with the findings from the bioequivalence study where the pseudoephedrine was not only bioequivalent between both products, but the mean difference between product was less than 1%. In the 50 mM phosphate buffer no differences in the ibuprofen release could be observed (f_2-value > 50). This is due to the high buffer molarity of the buffer resulting in low discriminatory power of the test (Cristofoletti and Dressman, 2017) . Therefore, the

Table 6
pH of buffers before and after addition of ibuprofen, ammonium chloride and calcium phosphate. Mean values ± standard deviation are given (n = 3).

Buffer with	50 mM Phosphate pH 6.8	5 mM Phosphate pH 6.5	7 mM Maleate pH 6.8
+ Ibuprofen + NH_4Cl	6.04 ± 0.03	5.30 ± 0.08	5.43 ± 0.03
+ Ibuprofen + NH_4Cl + $Ca_3(PO_4)_2$	6.10 ± 0.01	5.81 ± 0.03	5.85 ± 0.03

dissolution data in 50 mM buffer were not used for further evaluation. In 5 mM phosphate and 7 mM maleate buffer a difference between the ibuprofen release from both products was detected (f_2-value less than 50). With the lower buffer molarity, the difference in drug release due to the TCP can be detected. A pre-treatment of the tablets in hydrochloric acid (pH 1.2 or pH 2.0) for 15 min increases the differences in ibuprofen release. Similar results were also observed in 7 mM maleate buffer where the f_2-value was less than 50 in all three test conditions.

3.5. IVIVC

3.5.1. Dissolution model

To find an *in vivo* predictive dissolution test and model for the IVIVC only the dissolution data in the buffered media, without the pre-

Table 7
f2 values for ibuprofen profiles in each experimental condition.

	No pre-treatment	Pre-treatment pH 1.2	Pre-treatment pH 2.0
Phosphate 5 mM	34.08	26.06	22.04
Phosphate 50 mM	56.14	61.80	61.85
Maleate	31.25	31.67	34.18

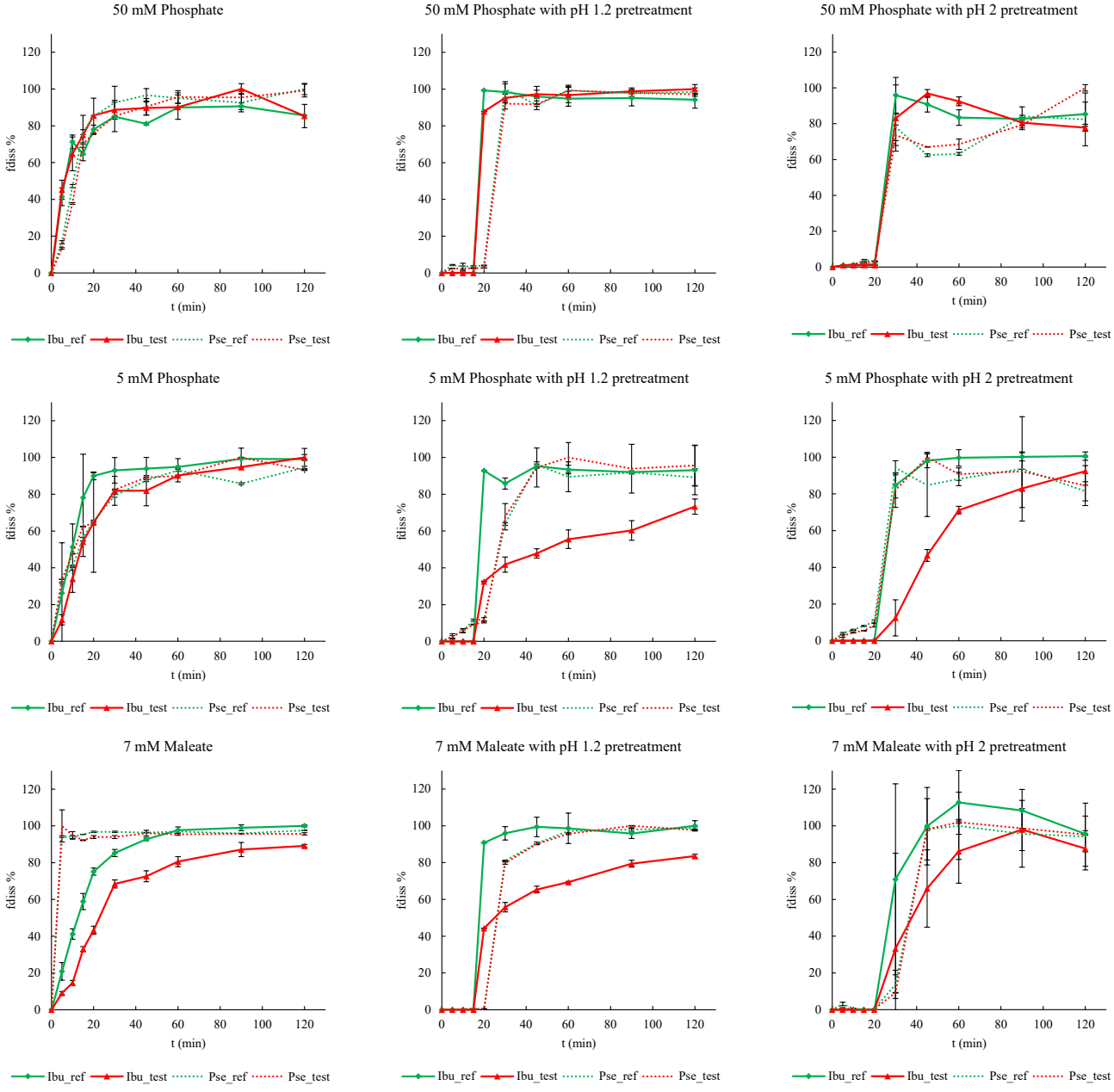

Fig. 3. *In vitro* mean dissolution profiles of pseudoephedrine (Pse) and ibuprofen (Ibu) in reference and test product in different dissolution conditions (n = 5).

treatment phase, were used. The calculated and experimental dissolution profiles are depicted in Fig. 4. For dissolution in buffers without acid pre-treatment and for dissolution in 7 mM maleate buffer with pre-treatment in pH 2.0, the Weibull function fitted the *in vitro* data the best, while the dissolution in 5 mM phosphate buffer with pre-treatment in pH 1.2 and pH 2.0 as well as in 7 mM maleate buffer with pre-treatment in pH 1.2 was better reflected by the DPDM (see Fig. 5). Accordingly, these dissolution models were used for obtaining a correlation between *in vitro* dissolution profiles and *in vivo* Concentration-time profiles.

3.5.2. IVIVC-One-compartment model

A one step IVIVC model was build according to Fig. 1. With the *in vitro* dissolution in 5 mM phosphate buffer with pretreatment in HCl pH 1.2 and in 7 mM maleate buffer with pretreatment in HCl pH 2 no valid models could be obtained since the %PE exceeded the criteria given in Section 2.6.2. On the other hand, valid models were obtained for the *in vitro* dissolution in 5 mM phosphate buffer without pretreatment and with pretreatment in HCl pH 2.0 and in 7 mM maleate buffer without pretreatment and with pretreatment in HCl pH 1.2. The predicted plasma concentration Cp-time profiles are given in Fig. 5 and the validation parameters of the IVIVC models are given Table 8. Interestingly, dissolution data in phosphate buffer (without pretreatment) could not reflect the experimentally obtained difference between the two formulations *in vivo*, even though a valid model had been obtained. This is in line with the *in vitro* dissolution experiments in Fig. 5 where the difference between the formulations is clearly visible in e.g., 5 mM phosphate buffer with pretreatment in pH 2 but becomes less pronounced in 5 mM phosphate buffer without pretreatment. Although the f_2-value calculated in 5 mM phosphate buffer without pretreatment is 34.08 and hence the *in vitro* dissolution is not similar, this difference cannot be observed in the predicted plasma concentration Cp-time profile (see Fig. 5A).

4. Discussion

One-step level A IVIVCs for immediate release ibuprofen has been established by means of a first stage pre-treatment in pH 1.2 dissolution media for 15 min and a second stage in 7 mM maleate buffer in the paddle apparatus at 50 rpm as well as with a first stage pre-treatment in pH 2.0 dissolution media for 15 min and a second stage in 5 mM phosphate buffer in the paddle apparatus at 50 rpm. The achievement of these Level A IVIVCs is an additional step in the search of *in vivo* predictive dissolution test to finally justify the acceptability of BCS-based biowaivers for products containing BCS class IIa drugs (García-Arieta et al., 2015). Additional evidence with many more drugs, salt forms and dosage forms would be necessary to support the possibility of a BCS-

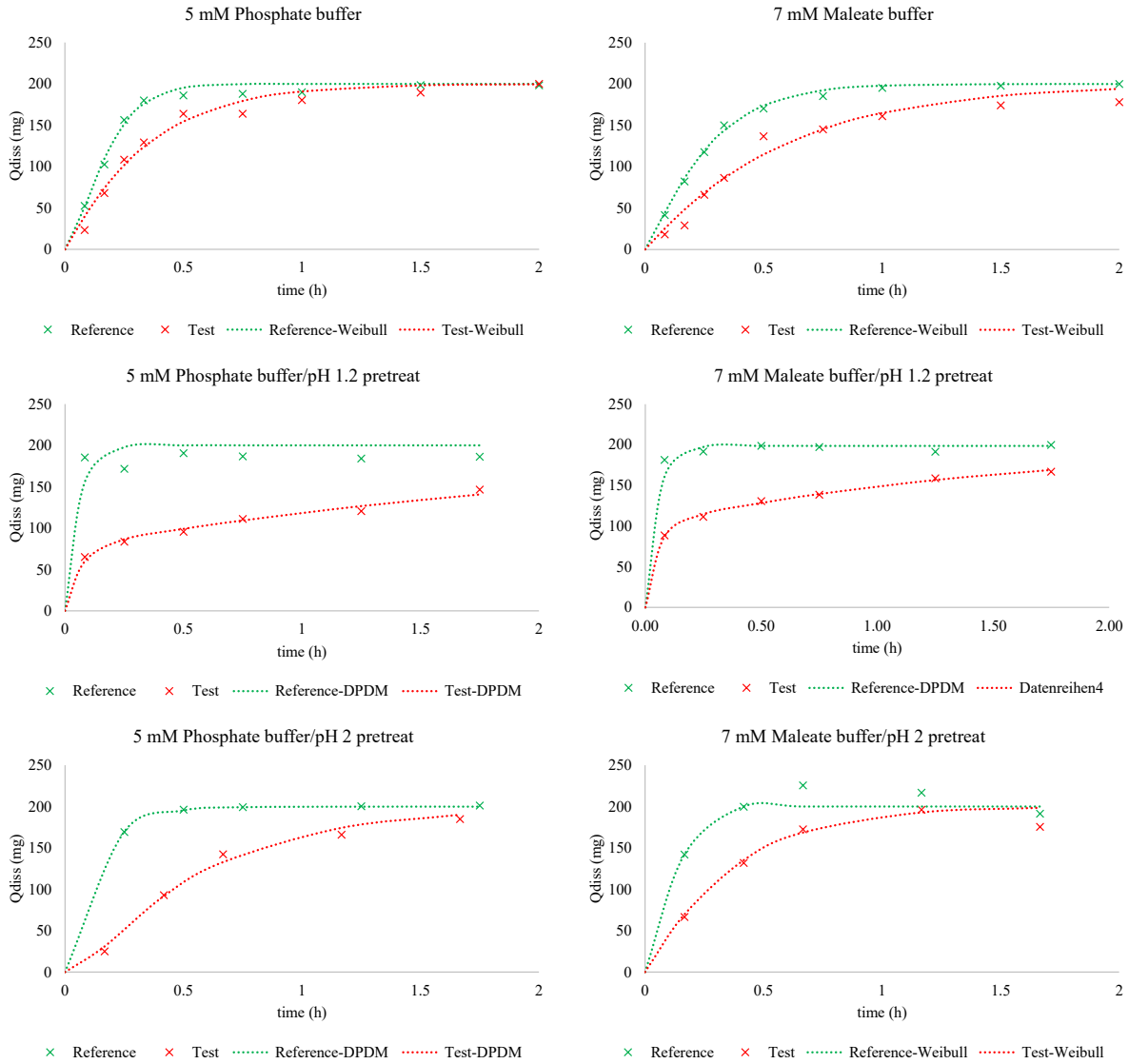

Fig. 4. Dissolution profiles of ibuprofen excluding the pre-treatment phase in hydrochloric acid.

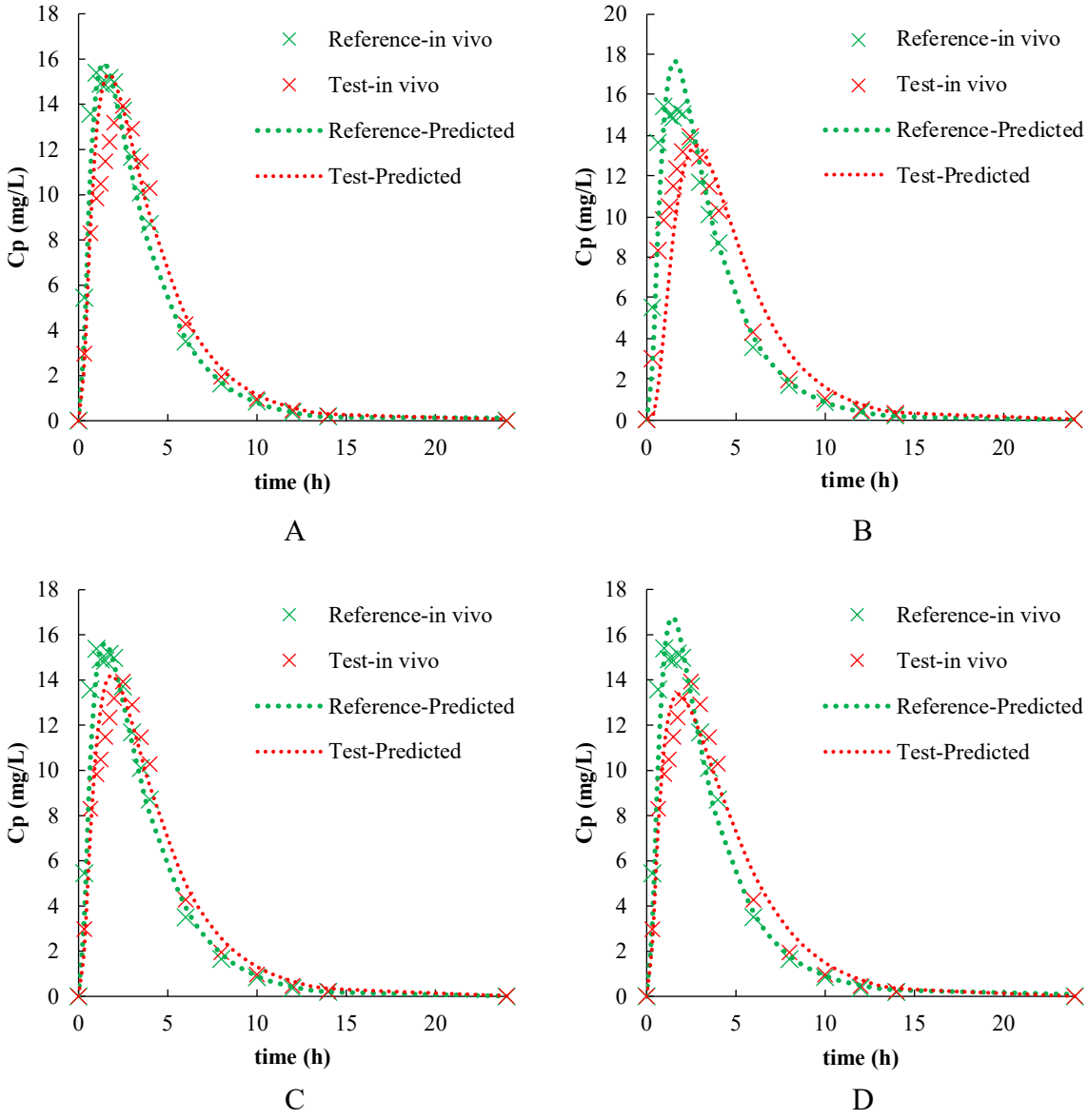

Fig. 5. Predicted Concentration (Cp)-time profiles. A 5 mM phosphate buffer without pretreatment, B 5 mM phosphate buffer with pretreatment in pH 2, C 7 mM maleate buffer without pretreatment and D 7 mM maleate buffer with pretreatment in pH 1.2.

Table 8
% RMS and Prediction error (% PE) of simulated $AUC_{0\rightarrow t}$ and C_{max}.

Scaling-function	RMS		% PE		
			Reference	Test	Average
5 mM phosphate					
$t\_scaled = a \times t^2 + b \times t + c$	2.62	$AUC_{0\rightarrow t}$	4.49	6.84	5.67
		C_{max}	2.54	9.95	6.25
5 mM phosphate / pH 2					
$t\_scaled = a \times t^b$	3.74	$AUC_{0\rightarrow t}$	5.06	7.31	6.18
		C_{max}	14.84	4.06	9.45
7 mM maleate					
$t\_scaled = a \times t^b$	2.09	$AUC_{0\rightarrow t}$	2.64	5.64	4.14
		C_{max}	1.54	1.66	1.60
7 mM maleate / pH 1.2					
$t\_scaled = a \times t^b$	2.12	$AUC_{0\rightarrow t}$	0.44	7.26	3.85
		C_{max}	8.96	4.09	6.52

based biowaiver for products containing BCS class IIa drugs since the current evidence seems to be limited to propionic acid derivatives (ibuprofen and naproxen (Loisios-Konstantinidis et al., 2020) in its different salt forms (acid, sodium, lysine and arginine) and dosage forms (suspensions, granulates, tablets and orodispersible tablets), where the bioavailability differences seem to be caused by particle size of the suspensions (Cristofoletti & Dressman, 2016a, 2016b. 2016c) or the different salt form and dosage forms (Cristofoletti & Dressman, 2017). Consequently, it is necessary to confirm these conclusions in other research groups and with other BCS class IIa drugs from other therapeutic areas (e.g. sartan antihypertensives and oral antidiabetics) or drugs with different structure within the same therapeutic group (e.g. derivatives of acetic acid and oxicams), with their corresponding salts and dosage forms, as well as to investigate other potential causes for bioavailability differences (e.g. differences in excipients causing differences in porosity and wettability or even differences in surfactants) defining an ambitious design space to generalize these conclusions to all BCS class IIa drugs and possible scenarios.

Although pseudoephedrine is highly soluble, only a minor portion of its dose is dissolved during the pre-treatment phase in HCl at pH 1.2 or pH 2.0 (see Fig. 4). The disintegration of the tablet and the de-aggregation of the clumps arising from disintegration seem to be hindered due to the relatively high fraction (approx. 53 % of the tablet

weight) of the poorly soluble ibuprofen in the reference formulation. These results suggests that the intimate contact between formulation ingredients is not disrupted by gastric disintegration. Consequently, when arriving into the intestine a microclimate allowing TCP to influence ibuprofen solubility still exists. This influence (see Tables 5 and 6) is a result of the basicity of the phosphate ion.

The microclimate raising effect of TCP seems to be the cause behind the observed supra-bioavailability of ibuprofen in the reference product. A parallel situation with a basic excipient was reported by Valizadeh et al (Valizadeh et al., 2004) for indomethacin and Eudragit E 100, where the basic Eudragit E promoted indomethacin (an acid) dissolution under acidic conditions (pH 1.2 medium). However, in our case there is little dissolution during acid pre-treatment. This discrepancy could be explained by the lack of solid dispersion formation with calcium phosphate in contrast to the situation with Eudragit E. This higher microclimate pH results in higher effective ibuprofen solubility and, accordingly, faster dissolution and by extension faster absorption. However, compendial phosphate buffer fails to detect this difference, incorrectly predicting similar performance of the test and reference products (Fig. 4). The reason behind this lies in the high buffer capacity of the USP SIF. A high-capacity buffer will be able to drive the surface pH close to the bulk pH regardless of the excipient composition. As a result, differences between products will be small. Moreover, in order to appropriately discriminate between the products, lower capacity buffers are needed as shown in Tables 6 and 7 and as other authors have already published (Cristofoletti and Dressman, 2017, 2016a, 2016b; Krieg et al., 2015; Tsume et al., 2012). As expected from these tables, dissolution in 5 mM phosphate buffer pH 6.7 and 7 mM maleate buffer pH 6.5 were more discriminative.

The identification of *in vitro* differences in ibuprofen release based on the f_2 similarity, where *in vivo* differences have been previously described, has also been achieved by Cristofoletti and Dressman (Cristofoletti and Dressman, 2017) with a 20 min pre-treatment stage and 75 rpm in the paddle apparatus in 500 mL. However, it is important to take into account that as soon as one of the product releases 85% or more, the sampling times for the f_2 similarity factor should be truncated (ICH 2019). It is not convenient to continue until 85% is reached in both products (Cristofoletti and Dressman, 2017, 2016a, 2016b). Considering the inherent variability of the migrating motor complex and gastric emptying of solid/particles, it would be difficult to stablish if a 15 min pre-disintegration step is more adequate than 20 min pre-treatment, but in this study, good predictive results have been obtained with a shorter time. In our case, the pre-treatment was reduced to 15 min, which may be indicative that the pre-treatment does not need to be prolonged since the half gastric emptying time has been described to be 13 $\pm$ 1 min (Mudie et al., 2014). Importantly, the use of an agitation rate of 50 rpm may have contributed to the achievement of level A IVIVCs, since it is known that the paddle apparatus is more discriminative at 50 rpm (Cristofoletti and Dressman, 2017). Importantly, in this study *in vitro* differences have been detected by the f_2 similarity factor when the mean C_{max} differences between products containing ibuprofen are only 15% approximately, whereas the C_{max} differences between products containing ibuprofen and its salts are usually larger. In fact, usefulness of low phosphate buffer concentration media to detect not only differences between ibuprofen acid and its salts, but also between bioequivalent and non-bioequivalent formulations have already been reported elsewhere (Krieg, 2015). Therefore, the discriminatory power of this *in vitro* dissolution test in two stages seems to be adequate, although a 13% difference in C_{max} was not detected by the f_2 similarity factor reported by Critofoletti and Dressman (2016b). These authors were able to discriminate with the f_2 similarity factor when the C_{max} difference between products was around 20%. This difference in discriminatory power might be caused by the different pre-treatment duration.

The results obtained for pseudoephedrine exhibited a difference between products less than 1% for both C_{max} and AUC, which gives internal validity to the study since it is demonstrative that the study was correctly conducted. If S-ibuprofen C_{max} has failed in the active enantiomer it is because the products exhibit different *in vivo* dissolution and absorption rates. As the 95% CI of C_{max} for both enantiomers (78.58 – 92.46% for S-ibuprofen and 79.26 – 94.64% for R-ibuprofen) did not include the 100% value, it can be concluded that there is a statistically significant difference between these products (p less than 0.05). Although a larger study size would have likely concluded bioequivalence since the lower boundary of the 90% CI of test/reference for Cmax of S-ibuprofen was 79.69%, the relevant aspect is not if bioequivalence was demonstrated, but if the *in vitro* dissolution methodology is able to detect a mean C_{max} difference of 15% between products. If the difference in C_{max} had been larger (e.g. 25%), it would not have possible to know if the dissolution methodology is sufficiently discriminative to ensure bioequivalence. In order to grant biowaivers it is essential to confirm that the *in vitro* methodology is as discriminative or more than the *in vivo* bioequivalence study. Similarly, it is expected that AUC of ibuprofen will be bioequivalent and in the present study the mean difference in ibuprofen AUC was less than 5%. In contrast, if the ibuprofen AUC would have failed to show equivalence or exhibited a significant difference, the validity of the study could have been questioned (Cristofoletti and Dressman, 2016b).

In any case, it is important to highlight that differences in the quantitative composition of critical excipients like sorbitol and Tween 80 would preclude the application of a BCS biowaiver (Cristofoletti and Dressman, 2016b; ICH, 2019; García-Arieta et al., 2015) and that BCS-based biowaivers can only be applied between the same dosage forms, but not between e.g. orodispersible tablets and film coated tablets (Cristofoletti and Dressman, 2017, 2016a; ICH, 2019).

The appropriateness of these *in vitro* dissolution methods was shown not only through f_2-values matching the *in vivo* rank order, but also through a one-step Level A IVIVC as well. The one step-IVIVC results presented in Fig. 5 and Table 7 provide a good case of immediate release product performance being predicted *in vitro* in a way that could obviate the need for *in vivo* BE studies in the future if an appropriate design space is investigated for the establishment of the IVIVC, since BCS-based waivers are not the only type of biowaivers. Importantly, for obtaining valid IVIVC models with correct predictions of the concentration–time profiles for ionizable BCS II drugs and discriminatory power, it is often not only important to use biopredictive dissolution media, but also take into account the exposure of the product to gastric media as already reported by Gonzalez-Alvarez et al. (Gonzalez-Alvarez et al., 2021), who tested three etoricoxib (basic BCS II drug or class IIb drug) products in the Gastro Intestinal Simulator (GIS) and in the USP II dissolution apparatus. The compendial method was not able to detect a difference between the tested products, whereas the GIS was able to account for the differences (Gonzalez-Alvarez et al., 2021). Even though resulting in a valid IVIVC model according to the %PE parameter, the difference in *in vitro* dissolution in 5 mM phosphate buffer without pretreatment (Fig. 4) could not predict a difference in concentration–time profiles between the two products (Fig. 5A). This raises the question if the validation parameter %PE is sufficient to ensure a correct prediction of the concentration–time profiles, yet further studies are needed to answer this point considering that in the present study the %PE is within the range of the observed differences, thus larger formulation differences are necessary to discriminate better between models.

5. Conclusion

Reducing buffer molarity of dissolution media to match physiological buffer capacities is a relatively simple tool to increase the discriminatory power of dissolution methods for drug products containing poorly soluble ionizable drugs. However, while sufficient to predict the rank order *in vivo*, it is not necessarily sufficient to predict pharmacokinetic profiles at a point-to-point IVIVC level. Addition of a gastric phase to the dissolution method has shown to be a valuable strategy to become more physiologically relevant and discriminative to detect

differences in the *in vivo* release rate. With an appropriate dissolution method, good predictions of the *in vivo* behavior could be obtained supporting the idea of extending biowaivers to BCS class IIa on IVIVC basis or to waive BE studies based on a level A IVIVC with a properly defined design space.

CRediT authorship contribution statement

I. Cámara-Martinez: Investigation, Methodology, Writing – original draft. **J.A. Blechar:** Formal analysis, Investigation, Software, Writing – original draft. **A. Ruiz-Picazo:** Investigation, Methodology, Validation. **A. Garcia-Arieta:** Conceptualization, Supervision, Visualization, Writing – review & editing. **C. Calandria:** Conceptualization, Data curation, Project administration. **V. Merino-Sanjuan:** Methodology, Supervision, Writing – review & editing. **P. Langguth:** Resources, Software, Supervision, Writing – review & editing. **M. Gonzalez-Alvarez:** Data curation, Resources, Software, Supervision. **M. Bermejo:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Visualization, Writing – review & editing. **J. Al-Gousous:** Software, Supervision, Validation, Writing – review & editing. **I. Gonzalez-Alvarez:** Conceptualization, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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