SIMULATION OF THE *IN VIVO* EXPOSURE TO IBUPROFEN BASED ON *IN VITRO* DISSOLUTION PROFILES FROM SOLID DOSAGE FORMS

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Abstract

Four ibuprofen products in two or three concentrations of active pharmaceutical ingredient were evaluated *in vitro*, using the conditions recommended for the upper pH-value of the three stage dissolution testing. The experimental data were included in the *in silico* prediction of the plasma concentration profiles, with consecutive analysis of the main pharmacokinetic parameters and assessment of the bioequivalence with the official reference drug product for the intermediate, 400 mg concentration. The results indicated an apparent feasibility of the biowaiver procedures, based on the dissolution similarity. Even when significantly different release profiles were obtained *in vitro*, the *in vivo* bioequivalence was concluded. The high permeability characteristic seems to be the key factor controlling the absorption profile of ibuprofen.

Rezumat

În acest studiu au fost evaluate *in vitro*, patru produse cu ibuprofen în două sau trei nivele de concentrație, utilizând condițiile recomandate pentru nivelul superior de pH, din cadrul testării în trei medii. Datele experimentale au fost incluse în predicții ale profilelor de concentrații plasmatice, cu analiza consecutivă a principalilor parametri farmacocinetici și evaluarea bioechivalenței cu produsul de referință, pentru doza intermediară de 400 mg. Rezultatele au indicat fezabilitatea aparentă a procedurilor de exceptare de la necesitatea efectuării studiilor *in vivo*, pe baza similarității dizolvării. Chiar și în cazul unor diferențe semnificative obținute între profilele de cedare, a fost concluzionată bioechivalența. Permeabilitatea mare pare să fie factorul cheie care controlează profilul de absorbție a ibuprofenului.

Keywords: ibuprofen, *in vitro* dissolution, simulation, bioequivalence.

Introduction

Discriminative *in vitro* methodology is essential for the adequate evaluation of the quality attributes for a given dosage form, when the active pharmaceutical ingredient must undergo a dissolution process prior to its absorption across the biological barriers. The information collected during these procedures serves as total quality tests, confirms the batch to batch consistency, but also may serve for prediction of possible changes in the *in vivo* performance [1]. The correlated biowaiver procedures rely on the accurate knowledge and strict definitions of the solubility and permeability values of the drug, as well as of its dissolution behaviour from the dosage forms. The applicability related to the Biopharmaceutical Classification Systems (BCS) differs between the main regulatory guidance. Nevertheless, the *in vitro* dissolution tests are currently accepted as adequate alternative tools for waived registration of immediate release oral solid dosage forms, without increased risks it concerns the safety and efficacy profiles [2]. The scale-up post-approval changes and extrapolation of the *in vivo* results from the highest to lower concentrations represent other important cases where dissolution methods could be used for both conventional and modified release formulations.

For high solubility - low permeability drugs, one of the major concerns limiting the extension of biowaiver approaches is the affinity for transport systems and their complex modulation phenomenon [3]. On the other hand, the compounds belonging to the BCS class II, display low solubility characteristics in one or more regions of the gastrointestinal tract. Some reports suggest the possible strictness of the current threshold values used in the official definitions of the biopharmaceutical properties [4]. It was emphasized that complete dissolution of the highest strength occurs in the distal segment of the small intestine, the major site of absorption, and this was further evoked as a key argument in the extension of the current limits in biowaiver application. The non-steroidal anti-inflammatory drugs, with weak acidic properties are of particular interest, many being included in the so called intermediate solubility class II [4]. Ibuprofen is to be remarked, based on its proven safety, wide therapeutic and concentrations ranges, as well as on the multitude of dosage forms used in therapy (immediate and modified release, oral, rectal and topical semisolids etc.) [5]. Preliminary reports have suggested that the compendial dissolution monograph is able to discriminate the kinetic differences in the release of the active ingredient, triggered by pharmaceutical non-equivalence. One should also consider that the current recommendations of the main regulatory agencies include the test *versus* reference comparison in three

aqueous media, with pH of 1.2, 4.5 and 6.8. For ibuprofen, only the highest pH values assure full compliance with the sink conditions requirement. Despite the initial assumption that the compendial methodology, i.e. *in vitro* release evaluation at pH=7.4 could be able to signal the potential in vivo non-similarity [5], a recent study stated that waiving the biostudy based on *in vitro* similarity is not feasible, but rather "illogical" [6]. The conclusion was reached after the review of several bioequivalence studies performed on solid oral dosage forms containing 600 mg ibuprofen, with supplementary three stages dissolution testing. The predictive power of the *in vitro* evaluation procedure was questioned relative to the peak exposure [7]. Therefore, the adjustment of the testing parameters, combined with adequate *in silico* simulation starting from the available physico-chemical and pharmacokinetic properties, were suggested for development of relevant evaluation standards [8].

The aim of the paper was to analyse and integrate the dissolution profiles of various oral solid dosage forms containing 200 - 600 mg ibuprofen into a simulation platform able to predict the pharmacokinetic profiles. The similarity based on *in vitro* release at the highest pH value, according to current approaches, was correlated with the conclusion on bioequivalence of the intermediate concentration (400 mg), in an attempt to evaluate the relevance of the dissolution testing and the feasibility of waived registration procedures.

Materials and Methods

Four ibuprofen products in two or three concentrations were included in the study. All the formulations are registered on the local market and, with one exception, the different concentrations of the same product presented a qualitative similarity in terms of qualitative composition (Table I). P200 and P400 were pharmaceutical non-equivalent (capsules and film coated tablets).

The *in vitro* testing was performed on a PharmaTest PTWS100 dissolution bath, PharmaTest GmbH, Germany, equipped with DFC-100RP semi-automated system, using paddle apparatus at 50 rpm. The capsule formulation was included in standard stainless steel 23 x 12 mm sinkers. The evaluations (n=6) were performed at 37°C, using 900 mL phosphate buffer pH=6.8, prepared according to the USP formula. Samples of 5 mL were collected at 5, 10, 15, 20, 30, 45 and 60 min, through immersed canulla filters (10 μm mean pore size). The quantitative analysis was performed as described previously [9, 16].

Table I

The qualitative composition of the *in vitro* evaluated oral solid dosage forms

Code of the formulation	**A200**	**A400**	**C200**	**C400**	**C600**	**I200**	**I400**	**P200**	**P400**
Ibuprofen (mg) conc.	**200**	**400**	**200**	**400**	**600**	**200**	**400**	**200**	**400**
hydroxypropyl cellulose	V	V	-	-	-	-	-	-	-
microcrystalline cellulose	V	V	-	-	-	-	-	-	-
methylcellulose	-	-	V	V	V	-	-	-	-
sodium starch glycolate	-	-	V	V	V	V	V	-	-
povidone K30	-	-	-	-	-	-	-	V	V
sodium croscarmellose	V	V	-	-	-	-	-	-	V
sodium lauryl sulfate	V	V	-	-	-	-	-	-	-
starch	-	-	V	V	V	V	V	V	V
pregelatinized starch	-	-				V	V		
lactose	-	-	V	V	V	-	-	V	V
magnesium stearate	-	-	V	V	V	-	-	V	V
stearic acid	-	-	-	-	-	V	V	-	-
anhydrous silicium dioxide colloidal	V	V	-	-	-			V	V
talcum	V	V	-	-	-	V	V	V	V
anhydrous silicium dioxide colloidal			V	V	V			-	V
hydroxypropyl-methylcellulose	V	V	V	V	V	V	V	-	-
macrogol 400	V	V	-	-	-			-	-
macrogol 6000	-	-	-	-	-	V	V	-	-
talcum	V	V	V	V	V	V	V	-	V
titanium dioxide	-	-	V	V	V	V	V	-	-
propylene glycol	-	-	V	V	V	-	-	-	-
carnauba wax	-	-	-	-	-	-	-	-	V
beeswax	-	-	-	-	-	-	-	-	V
simethicone emulsion	-	-	-	-	-	V	V	-	-
erythrosine	-	-	-	-	-	V	V	-	-
Erythrosine Supra dye	-	-	V	V	V	-	-	-	-
Batch	11AF003A-2	12AG027A-1	K21068	K20966	K30125	3090812	3320812	6124157	U1301661

The mean *in vitro* profiles were imported and fitted with predefined Z-factor model using GastroPlus platform, Simulation Plus Inc., version 8.5.0002 (CVODE 1.0.3.0, pHSol 1.4.3.0, APModule 6.1.1.6, RWFProg1

5.4.2.33). The predictions of the plasma concentrations of ibuprofen were based on the Advanced Compartmental Absorption and Transit (ACAT) model. The *in vivo* pharmacokinetic profile of the reference product (Nurofen® Forte, Boots Healthcare Intl.) was obtained from a previous open label, analytic blinded, single dose, randomized, fast state, crossover bioequivalence study, performed on 24 healthy subjects. The simulation procedures were conducted on the general *Human - Physiological - Fasted* physiological model, with *Opt logD* SA/V 6.1 absorption scale factor (ASF) model, as previously reported [8,10]. The intercompartmental transfer constants were obtained by analysis of the mean *in vivo* plasma concentration profile, using the *PKPlus*^{TM} module. The following biopharmaceutical characteristics were collected from existing literature data [5]: the Caco2 apparent permeability $53 \cdot 10^{-6}$ cm/s [11] (corresponding to $5.5697 \cdot 10^{-4}$ cm/s in humans); solubility in phosphate buffer pH=6.8 at 37°C, 3.37 mg/mL [5]. The prospected *in vivo* exposure after oral administration of each *in vitro* tested formulation was obtained based on virtual trials (population of 24 subjects, 240 data points for each simulation of 12 hours), using 10 to 20% log-normal distribution for the values of the main physiological parameters, respectively 3% for the dose. The results were used for the analysis of the main pharmacokinetic parameters (fraction absorbed, maximum plasma concentration, time of maximum plasma concentration, area under curve from 0 to 12 hours and extrapolated to infinity), as well as for the evaluation of bioequivalence with the reference profile.

Results and Discussion

All the evaluated formulations displayed a rapid dissolution profile in the implemented test conditions, the fraction released being higher than the official threshold of 85% in 30 min (Figure 1). Moreover, for the lower concentrations, the products are very rapidly dissolving (86.15 - 93.59% in 15 min), except for I200 (80.59%). The main differences were observed during the initial 10 min interval, corresponding to the particularities in the disintegration of the coating films. An obvious non-similarity was noticed between the pharmaceutical non-equivalent formulations, most probably due to the mechanism of release. Surprisingly, despite the qualitative equivalence of products C, the calculation of compendial metrics suggested distinct *in vitro* behavior, with values of the similarity factor lower than 50 (47.71 for C200 and, respectively, 30.01 for C400, using the highest concentration as reference).

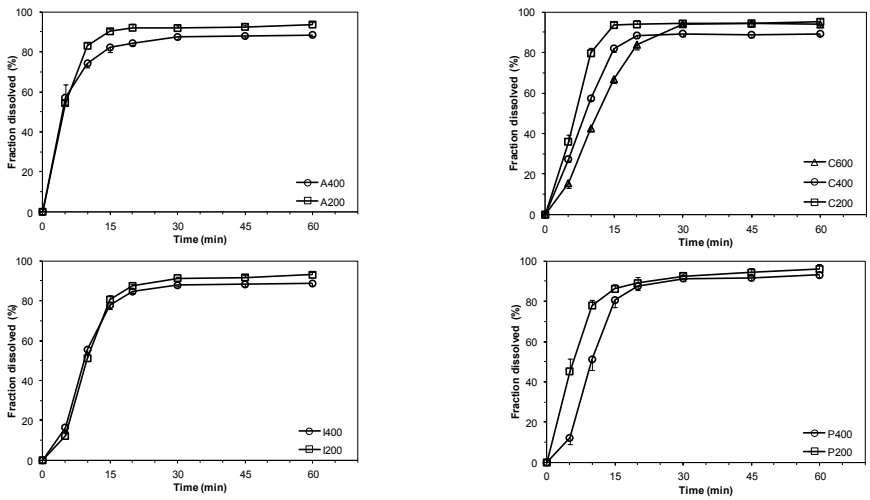
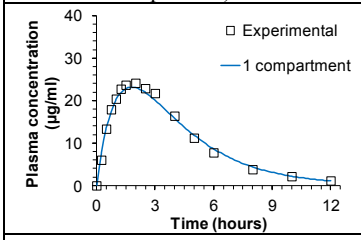
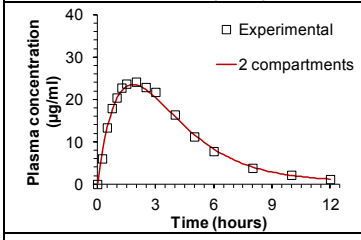
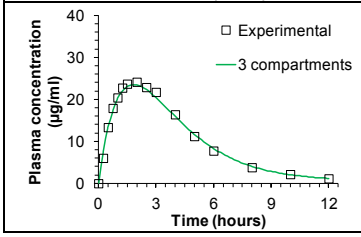


Figure 1

In vitro dissolution profiles of ibuprofen from solid dosage forms with different concentrations (mean values ± standard deviation, n=6)

Table II

The results of compartmental modeling procedures, performed on mean plasma concentration profile of the reference product (400 mg, n=24 healthy subjects)

Model (experimental and estimated profiles)	**Akaike Information Criterion**	**Schwarz Criterion**	**R^2**	**Model parameters** (1/h)	**CV** (%)
	-74.84	-72.52	0.9864	K_a = 0.813 K_{10} = 0.357	36.26 25.57
	-78.11	-74.25	0.9921	K_a = 0.553 K_{10} = 0.442 K_{12} = 0.099 K_{21} = 0.035	11.64 7.69 45.44 45.44
	-74.13	-68.73	0.9921	K_a = 0.547 K_{10} = 0.417 K_{12} = 0.051 K_{21} = 0.027 K_{13} = 0.078 K_{31} = 0.024	11.53 9.25 58.51 58.51 32.99 32.99

Concerning the *in vivo* profile of the reference product, the bicompartmental pharmacokinetic model fitted adequately the mean experimental data (Table II), being in acordance with previous reports [12]. The corresponding intercompartmental transfer constants (K_{12} = 0.099 h^{-1}; K_{21} = 0.035 h^{-1}) were further used for the prediction of the absorption profiles for the immediate release formulations.

Based on the *in silico* simulation, the fraction absorbed was higher than 99.9%, independent on the release characteristics of the pharmaceutical formulations (Figure 2).

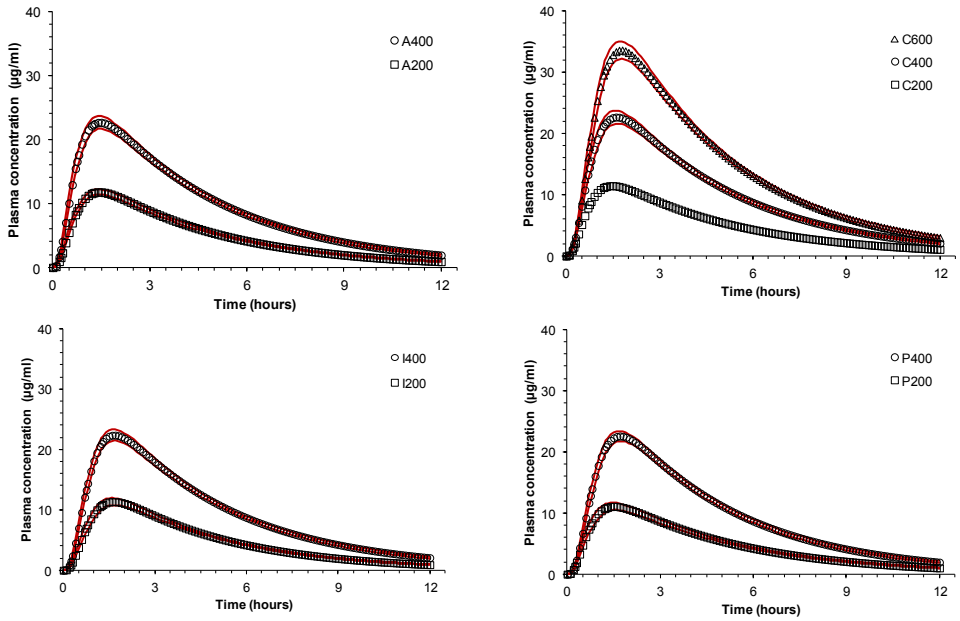


Figure 2

Estimated *in vivo* plasma concentration profiles (simulations on 24 subjects, mean values and intervals of variation)

The rapid dissolution profiles, combined with the estimated physico-chemical properties suggested a dose-proportionality of the global exposure, as expressed by the area under curve (Table III) for the 12 hours after oral administration or extrapolated to infinity. The previously mentioned *in vitro* non-similarities were transferred mainly into differences in terms of time to maximum plasma concentration (approximately 18 minutes, between P200 and P400), and, to a lesser extent, to peak levels.

Table III

The estimated values of the main pharmacokinetic parameters

Formulation	F_a (%)	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-12} (µg·h/ml)	$AUC_{0-\infty}$ (µg·h/ml)
A200	99.97 (0.01)	11.87 (9.43)	1.44 (10.19)	58.23 (7.24)	61.97 (6.8)
A400	99.97 (0.01)	22.83 (12.62)	1.46 (10.59)	113.64 (7.4)	121.77 (6.86)
C200	99.96 (0.04)	11.27 (15.03)	1.54 (16.23)	57.34 (8.48)	61.84 (7.83)
C400	99.96 (0.02)	22.85 (13.38)	1.63 (11.65)	116.54 (8.61)	125.27 (7.89)
C600	99.96 (0.01)	33.78 (12.17)	1.77 (8.29)	171.83 (7.45)	184.19 (6.81)
I200	99.96 (0.03)	11.5 (14.27)	1.66 (13.42)	56.86 (7.05)	60.61 (6.46)
I400	99.96 (0.02)	22.52 (11.54)	1.68 (9.19)	115.14 (5.95)	124.15 (5.51)
P200	99.97 (0.01)	12.17 (12.71)	1.41 (8.69)	58.65 (7.4)	62.37 (6.87)
P400	99.96 (0.01)	22.72 (10.22)	1.71 (9.79)	114.93 (8.54)	123.00 (7.97)

Note: F_a - fraction absorbed; C_{max} - maximum plasma concentration; T_{max} - time of maximum plasma concentration; AUC_{0-t} - Area under curve from 0 to 12 hours; $AUC_{0-\infty}$ - area under curve extrapolated to infinity. The coefficient of variations (%) is mentioned in parenthesis.

The simulated *in vivo* profiles suggested that the drug properties, especially the high permeability across cellular membranes, and not the formulation factors are the key parameters controlling the *in vivo* absorption pattern. A rapid release and a fast, non-saturable uptake process, mainly through passive diffusion, could account for the remarkable similarity of the pharmacokinetic profiles. On the other hand, it should be mentioned that the input values for the physiological data is the potential source for the low variation of the *in vivo* exposure parameters and, consequently, the narrow 90% confidence intervals (Table IV). The bioequivalence with the reference product was concluded in all the cases for the 400 mg dose.

Table IV

Calculation of the 90% confidence intervals for the generic products containing 400 mg ibuprofen (reference product: Nurofen® Forte, Boots Healthcare Intl.)

Test product	Parameter	90% CI	Geometric mean ratio
A400	C_{max}	89.68 – 97.971	93.73
	AUC_{0-12}	94.876 – 99.556	97.19
	$AUC_{0-\infty}$	91.043 – 95.896	93.44
C400	C_{max}	89.52 – 98.182	93.75
	AUC_{0-12}	97.325 – 102.6	99.93
	$AUC_{0-\infty}$	93.016 – 98.565	95.75
I400	C_{max}	89.005 – 96.322	92.59
	AUC_{0-12}	97.286 – 101.09	99.17
	$AUC_{0-\infty}$	92.833 – 96.74	94.77
P400	C_{max}	90.384 – 96.767	93.52
	AUC_{0-12}	95.469 – 100.8	98.10
	$AUC_{0-\infty}$	92.098 – 96.956	94.50

The results indicated an apparent feasibility of the biowaiver procedures, based on the dissolution similarity. Moreover, even when significantly different release profiles were obtained *in vitro*, the *in vivo*

bioequivalence was concluded. The behavior in non-sink conditions, i.e. hydrochloric acid pH=1.2 and acetate buffer pH=4.5 was not evaluated. Prospectively, even though these procedures could generate supplementary information on the role of the formulation factors and are mandatory from the regulatory perspective, they mainly expressed the solubility of the active ingredient. For example, the available reports on the three stages evaluation of ibuprofen formulation indicated a fraction dissolved lower than 10% in acidic conditions, making impossible the calculation of the similarity factor [6]. Also, the value of the metric depended upon the number of data points included in the analysis, a plateau region being observed after 15-20 min (below the 85% cut-off).

There are several formulation factors that could induce a change of the absorption profile of ibuprofen, by either physico-chemical or physiological and biochemical interactions [13]. The most significant impact was reported for pH-regulating excipients [14], although the nature of the cation can trigger complexation phenomenon with decreased apparent solubility and further decreased dissolution rates [15]. Sodium bicarbonate not only changes the microenvironment around the disintegrating oral solid dosage forms, but it can alter the physiological parameters, e.g. increasing the rate of gastric emptying and reducing the barrier properties of the gastric mucus [13, 15]. Other excipients, such as tensioactive agents or macromolecular compounds were also mentioned in the context of faster release rates and lower lag-times, but without a proven *in vivo* consequence [5]. Special precautions must be adopted when particular interactions are foreseen between the capsule shell and the composition of the *in vitro* media [17].

There are considerable differences in the acceptance of the biowaiver application for ibuprofen in several European regions. Interestingly, the current data supports the idea of predictable absorption profiles. Careful selection of the composition could benefit from the high permeability profile of the drug. Moreover, the pharmaceutical non-equivalence with the official reference product or the use of various salts of ibuprofen with faster dissolution were considered by the regulatory body for waived, *in vitro* based approval [18]. The present report suggested that the *in vivo* impact of dissolution non-similarity is not significant in terms of peak and global exposure. The dissolution methodologies at high pH values (6.8 or 7.2) could be considered as biorelevant, despite the reported low predictability [6] of this routine quality control procedure.

Conclusions

Various oral solid dosage forms containing ibuprofen in 200 to 600 mg concentrations were evaluated *in vitro* and the dissolution profiles were used for *in silico* predictions of the plasma concentration profiles. The experimental data suggest that, despite the considerable differences in terms of their qualitative and quantitative composition, transposed to a variable degree of *in vitro* similarity, proportionality between maximum or global exposure and doze can be observed. For the 400 mg dose, bioequivalence was concluded with the previously evaluated *in vivo* pattern of the official reference drug product. The results indicate that the biopharmaceutical properties of the drug, mainly the high permeability characteristic, are the key factors controlling the absorption profile of ibuprofen. The dissolution testing in media simulating the pH value in the distal regions of the intestine, providing adequate sink conditions, could be biorelevant.

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