ORIGINAL ARTICLE

IN VITRO DISSOLUTION METHODOLOGY AND ESTIMATED CONSEQUENCES OF BIOWAIVER EXTENSION FOR IMMEDIATE RELEASE SOLID ORAL DOSAGE FORMS WITH METFORMIN HYDROCHLORIDE

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Abstract

The *in vitro* dissolution methodologies are frequently used as quality control tools for the assessment of solid oral dosage forms. The current bioequivalence guidance issued by the European Medicine Agency indicates that biowaiver extension for high solubility and low permeability drugs can be granted based on a very rapid *in vitro* release profile (more than 85% of the label claimed content in 15 minutes or less). The paper presents the results of dissolution testing for four commercially available immediate-release solid dosage forms containing 500 mg metformin hydrochloride. The *in vitro* evaluation was performed according to compendial monograph and by implementing non-compendial devices (Palmieri baskets, Peak vessels). The most discriminative profiles were further used for the estimation of the absorption processes, based on build-in GastroPlus™ models, considering the permeability and the pharmacokinetic characteristics of metformin. Theoretical, the *in vivo* exposure patterns were generated for 85% fraction released in 15 and 30 minutes and considered as reference in the evaluation of bioequivalence. Despite the *in vitro* difference in the release rate, presumably related to formulation variables, the estimated pharmacokinetic profiles were similar in terms of rate and extent of absorption. It can be assumed that, in the absence of factors interfering with the gastro-intestinal volume of fluid, transit times or active transporters, compliance with compendial standards could be used as a basis of biowaiver granting for metformin hydrochloride.

Rezumat

Metodologiile de evaluare a profilelor de dizolvare *in vitro* sunt utilizate frecvent ca teste de control al calității pentru analiza formelor farmaceutice solide orale. Ghidul actual de evaluare a bioechivalenței emis de Agenția Europeană a Medicamentului indică faptul că o extensie a conceptului de tip *biowaiver* poate fi acordată pentru substanțele cu solubilitate mare și permeabilitate redusă pe baza profilelor de dizolvare foarte rapidă *in vitro* (mai mult de 85% din conținutul declarat în cel mult 15 minute). Lucrarea prezintă rezultatele testelor de dizolvare aplicate în cazul a patru forme farmaceutice solide orale cu cedare imediată autorizate, conținând 500 mg metformin clorhidrat. Evaluarea *in vitro* a fost realizată conform monografiilor compendiale și prin adoptarea unor aparate necompendiale (*Palmieri baskets*, *Peak vessels*). Cele mai discriminatorii profile au fost utilizate ulterior pentru estimarea proceselor de absorbție, pe baza modelelor GastroPlus™, considerând permeabilitatea și proprietățile farmacocinetice ale metforminului. Valorile expunerii teoretice *in vivo* au fost generate pentru valori ale fracției dizolvate de 85% în 15, respectiv 30 de minute, fiind considerate ca referință în evaluarea bioechivalenței. În pofida diferenței dintre vitezele de dizolvare *in vitro*, posibil corelate cu variabilele de formulare, profilele farmacocinetice estimate au fost similare în privința vitezei de absorbție și a cantității absorbite. Se poate afirma că, în absența unor factori care să modifice volumul de fluide gastro-intestinale, timpii de tranzit sau sistemele transport, conformitatea cu standardele compendiale poate fi utilizată pentru aplicarea procedurilor de tip *biowaver* în cazul metforminului clorhidrat.

Keywords: *in vitro* release, BCS class 3, *in silico* absorption models, bioequivalence, ACAT, metformin

Introduction

The *in vitro* dissolution testing of the drug dosage forms is frequently described as a total quality control tool, with extensive applications in the development of pharmaceutical formulations, routine evaluation of batch-to-batch consistency and reproducibility, but also in monitoring the impact of compositional and manufacturing variables on the *in vivo* performance [1].

In specific conditions, the mathematical relationships have been developed and validated between the fraction released *in vitro*, using compendial testing apparatus and specific conditions, and *in vivo* absorption parameters or global pharmacokinetic profile [2]. A key step is to adequately consider the particularities of the biopharmaceutical profile of active drug substance(s) and the specific features of the *in vitro* testing conditions. *In silico* modelling procedures have been successfully implemented for prediction impact of the release rates on the absorption profile for several high solubility drugs, furthermore analysed from the perspective of specific dissolution requirements on which biowaiver granting is based [3, 4].

Metformin is an anti-hyperglycaemic biguanide with considerable clinical experience, available as a variety multi-source, immediate and modified release oral solid dosage forms. It is a Biopharmaceutical Classification System (BCS) class 3 drug, with low permeability [5, 6] and high, almost pH-independent solubility in the physiological conditions [7]. Intestinal permeability is the rate limiting step of the oral absorption, frequently described as a concentration and site dependent process [6]. Available reports suggest a decrease of absolute bioavailability by increasing the dose-strength after oral administration [8]. Metformin hydrochloride has been described as a candidate for biowaiver procedures [9]. Due to the pH-independence of the solubility profile [7], the limiting step of the overall *in vitro* release process is the disintegration, greatly affected by the hydrodynamics imposed by the testing apparatus and operating parameters. It concerns the specific compendial methodologies for the oral solid dosage forms containing metformin hydrochloride, the United States Pharmacopeia (USP) describes several strength-dependent *in vitro* testing procedures. For various combination drug products, the dissolution test seems to be determined by the physico-chemical properties of the associated active pharmaceutical ingredient.

For 500 mg dose strength, two dissolution tests can be conducted, based on the specific USP monograph. Noteworthy, the testing parameters are highly different, i.e. test 1 indicates the use of apparatus 1 at the highest recommended rotational speed (100 rpm), whereas test 2 specifies apparatus 2 at lower limit (50 rpm). Several reports on the relationship between the *in vitro* release and the *in vivo* exposure profile selected the test 1 conditions for the comparative evaluation of solid oral dosage forms in three dissolution media, simulating the passage through the gastro-intestinal tract by means of aqueous buffer systems, with a pH of 1.2, 4.5 and 6.8. Frequently, a rapid release was observed, in many cases the fraction dissolved being higher than 85% of the labelled amount during the first 10 or 15 minutes [9-11]. From the biowaiver perspective [12], this indicates that the pharmaceutical formulation undergoes a very rapid dissolution and the associated biopharmaceutical profile could be similar to oral solutions. The testing parameters, especially the rotational speed of apparatus 1, could lead to a decreased discriminatory character of the methodology in terms of both formulation and process variables, but also for the prospected impact on the *in vivo* performance.

The solid oral dosage forms also contain considerable quantities of excipients. When apparatus 2 is selected, cone effect is presumably observed by disintegration of the dosage form, triggering a variable amount of active pharmaceutical ingredient dissolved in the first moments of *in vitro* dissolution testing [13]. The alternatives are to further increase the rotational speed or to use the basket apparatus. In the latter case, the particles generated can stick to the mesh, therefore limiting the access of the release media to the particles of the active pharmaceutical ingredient. For special dosage forms such as suppositories, a particular design of the USP apparatus 1 is available, known as the Palmieri basket. The 12 rectangular slots, 2.5 mm wide, offer a considerable open surface, with 52% porosity, and therefore prevent the incidence of blocking particles [14]. Evaluations of the hydro-dynamic pattern and application in the *in vitro* testing of solid dosage forms have not been reported.

Other non-compendial adaptations of the currently used dissolution apparatus are available from various manufacturers and can be implemented only with proper justification. Peak vessels from former Varian Corporation, mentioned in two USP monographs, are particularly designed to prevent the accumulation of particles in the low share-rate zone located below the paddle [15]. The available literature data suggest a lower variability compared to the compendial round bottom 1000 mL vessels and higher release rates, almost independent on the rotation speed, due to the altered hydrodynamic profile [16]. Despite the minimization of the effects of various internal and external factors of variations, concerns on their discriminatory character have been raised.

The current paper presents the results of implementation of the two USP methodologies and two non-compendial variations of the testing apparatus, in the assessment of the *in vitro* dissolution profiles for four commercially available immediate release solid oral dosage forms containing 500 mg metformin hydro-chloride. One of the goals was to identify which testing conditions could display a more discriminatory character for the impact of composition differences. Moreover, based on the current requirements of very rapid dissolution pattern for grating the biowaiver, the extreme release rate drug products were integrated into simulation trials procedures according to recently reported mechanistic *in silico* procedures, in order to assess the impact on the prospected *in vivo* performance and finally, on the bioequivalence conclusion.

Materials and Methods

In vitro dissolutions methodologies

Four commercially available immediate release oral solid dosage forms containing 500 mg metformin hydrochloride were subject to *in vitro* dissolution testing. The qualitative composition, according to the labelling information, is provided in Table I.

Table I

Qualitative composition of the four immediate release oral solid dosage forms containing 500 mg metformin hydrochloride (according to the labelling information)

Excipients	Product	A	B	C	D
	Dosage form	Film coated tablet			Tablet
Tablet	Crosspovidone			**V**	
	Sodium starch glycolate	**V**			
	Microcrystalline cellulose		**V**		**V** (PH 101)
	Corn starch	**V**	**V**		**V**
	Anhydrous colloidal silicon dioxide	**V**			**V**
	Povidone	**V**	**V** (K30)	**V**	**V** (K30)
	Prosolv HD 90 (silicified microcrystalline cellulose)			**V**	
	Magnesium stearate	**V**	**V**	**V**	**V**
	Sodium lauryl sulfate				**V**
	Talc				**V**
Film	Opadry 33 G 28707			**V**	
	Opadry II High Performance 85 F18378 White		**V**		
	Hydroxypropyl-methylcellulose	**V**		**V** (6 cP)	
	Titan dioxide (E 171)	**V**		**V**	
	Lactose monohydrate			**V**	
	Macrogol	**V** (6000)		**V**	
	Glycerol triacetate			**V**	
	Talc	**V**			

Note: **V** indicates the presence of the mentioned excipient in the composition, the type being mentioned in parenthesis, if available.

The tests were conducted on a VanKel^{TM} VK 7000 Dissolution System, Varian Inc, USA. 1000 mL phosphate buffer pH = 6.8 was used as the release media. The evaluation procedure included apparatus 1 (40 mesh basket, test 1) at 100 rpm and apparatus 2 (paddle, test 2) at 50 rpm. Additional tests were performed as variation to the standard, compendial methodology. As an alternative to test 1 conditions, Palmieri baskets were used in the same testing conditions, whereas for test 2, Peak^{TM} vessels replaced the hemispherical, compendial 1000 mL vessels (Figure 1).

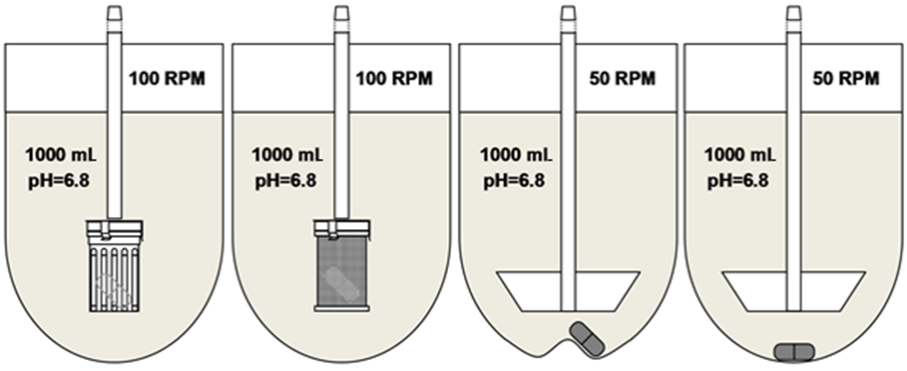

Figure 1.

Apparatus and testing conditions used for the *in vitro* dissolution of immediate release solid oral dosage forms containing 500 mg metformin hydrochloride

Samples of 5 mL were collected at 5, 10, 15, 20, 30, 45 and 60 minutes after test debut through 10 µm cannula filters and replaced with an equal volume of fresh media. Each test was performed at 37°C, on 6 units. The quantitative evaluation of the released amount of metformin hydrochloride was performed spectrophotometrically, at 233 nm, using a Jasco UV-Vis V-530 spectrophotometer (equipped with Spectra Manager software for Windows 95/NT, version 1.54.03).

Sodium phosphate dibasic dihydrate, sodium phosphate monobasic monohydrate and metformin hydrochloride were purchased Sigma Aldrich, Germany, and were of

analytical grade. A SGW Ultraclear UV Plus™ system was used for water purification.

Model parameters for in silico evaluations

The procedure used for simulating the *in vivo* exposure after oral administration using the GastroPlus™ software, Simulation Plus, Inc., USA, was presented previously [17]. This approach is described in various reports focused on metformin [3], but also on three other compounds representative for BCS class 3 characteristics, i.e. cimetidine, atenolol and amoxicillin [4]. Although leading to the same general conclusions by applying similar methodology, i.e. inclusion of personal (bioequivalence studies) or literature based biopharmaceutically relevant properties, the two reports differ mainly by the parameters of the Advanced Compartmental Absorption and Transit (ACAT) model [18], especially the absorption scale factors. The applied model parameters are detailed in Table II and represent literature data or estimated, using software-specific routines. After scaling the permeability values from single-pass intestinal perfusion in rats [6], the contribution of paracellular transport to the overall absorption process was included, as studies performed on Caco-2 cell monolayers emphasized the fact that this pathway accounts for almost 90% of the permeated amounts [19].

Based on the most discriminatory *in vitro* dissolution methodology, able to distinguish the previously presented difference in the qualitative composition, the products A and B with significant non-similarity of the release pattern were to be included in similar simulation trials, with a sample size of 24. For bioequivalence assessment, the 90% confidence intervals for the geometric mean ratio were calculated for the three main pharmacokinetic parameters [20, 21]: maximum concentration (C_{max}), area under data (AUC_{0-t}) and area under curve ($AUC_{0-\infty}$).

Table II

In silico model parameters for metformin hydrochloride

Parameter	Value
Molecular weight (g/mol)	129.17
Dose (mg)	500
Dose volume (ml) [4]	200
Gastric emptying time (h) [4]	0.28
Solubility (mg/ml, pH = 6.8)*	442.8
LogD (pH = 7.4) [5]	-1.228
pKa*	10.17
Mean precipitation time (sec) [4]	5
Diffusion coefficient ($cm^2/sec, 10^{-5}$)†	0.75
Particle density (g/mL)†	1.2
Effective permeability, duodenum (cm/sec, 10^{-4}) [6]	0.382
Effective permeability, jejunum (cm/sec, 10^{-4}) [6]	0.327
Effective permeability, ileum (cm/sec, 10^{-4}) [6]	0.314
Body weight†	70
Volume of the central compartment (l/kg)‡	1.42
Total clearance (l/h/kg)‡	0.425
k_{12} (1/h)‡	0.078
K_{21} (1/h)‡	0.111
k_{13} (1/h)‡	0.039
K_{31} (1/h)‡	0.00623

* - estimated using GastroPlus™; † - model defined; ‡ - Personal data from single dose, fast state, cross-over bioequivalence study performed on 12 healthy Caucasian subjects; the modelling of the mean pharmacokinetic profile was performed using PKPlus™ module of GastroPlus™

Results and Discussion

In-vitro dissolution tests

The results indicate that all products fulfil both test 1 and 2 requirements (not less than 70% of the labelled amount released in 45 minute, respectively not less than 80% released in 30 minutes). However, the two compendial methodologies generated different release rates, corresponding to the distinct hydrodynamic profile imposed by the two apparatus and their stirring rate. For the test 1 conditions, the fraction release was higher than 90%, without marked difference between the four products, conventional or film-coated tablets (Figure 2a). The most discriminatory character for the obvious differences in the qualitative composition was generated by the apparatus 2 (Figure 3a). It is to note that only this procedure permits the calculation of official difference and similarity factors, metrics routinely implemented for *in vitro* dissolution profile comparison. The fraction released in 15 minutes complied in two cases with biowaiver requirements of very rapid dissolution.

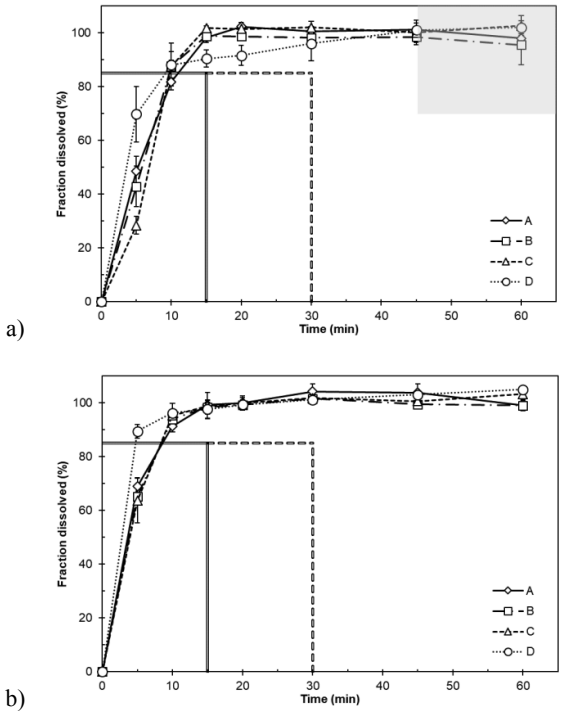

Figure 2.
Dissolution profiles (mean ± SD; N = 6) for the four pharmaceutical formulations
(◊, product A; □, product B; △, product C; ○, product D) using a) USP Apparatus 1 (test 1 from the specific monograph) and b) Palmieri basket. Grey region indicates USP acceptance criteria. Double straight line corresponds to very rapidly dissolving criterion, double dotted line to rapidly dissolving criterion.

The two non-compendial *in vitro* methodologies aimed to investigate the impact of two *in vitro* potentially interfering processes: clogging of baskets mesh by the dense particles resulting from the disintegration of the pharmaceutical formulations and appearance of the cone effects in the central region below the paddle element, both associated with variable and/or decreased release rates. Similar to apparatus 1, the use of Palmieri baskets induced similar release patterns for all four products, with slightly higher fraction released, in the case of non-coated product D (Figure 2b). The variability was considerably lower (for the 5 minutes sampling point, the coefficient of variation was 2.83%, compared to 14.7% for 40 mesh baskets). The Peak^{TM} vessels generated high rate, low-variability release patterns, without significant differences, probably due to accelerated disintegration of the pharmaceutical formulation and subsequent rapid dissolution in the increased share region surrounding the inverted cone (Figure 3b).

The most sensitive methodology seems to be the compendial test 2 (paddle apparatus, at 50 rpm), since it generates the highest difference between the four evaluated products. Notably, two of the mean dissolution profiles, corresponding to products A and B, were of particular interest and were selected as dissolution rate inputs for *in silico* modelling procedure. Product A didn't comply with either rapid release or very rapid release criterion, the fraction dissolved being 73.35% in 15 minutes and 83.88% in 30 minutes. For product B, showing the fastest dissolution, the fraction released was 89.52% in 15 minutes.

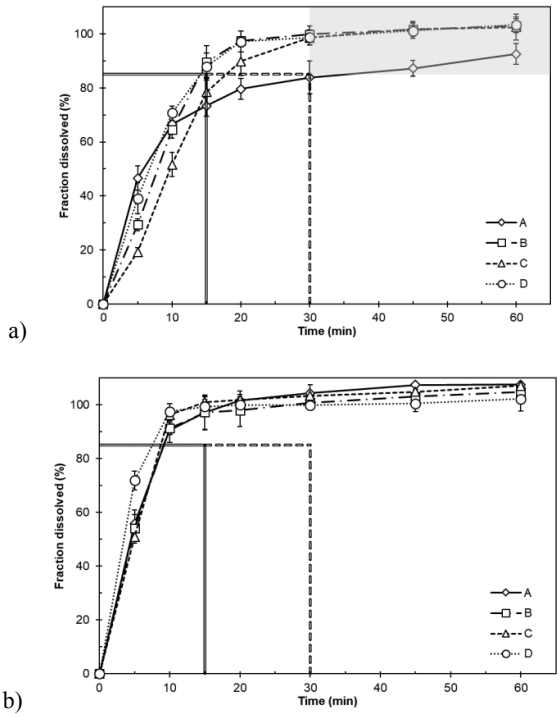

Figure 3.
Dissolution profiles (mean ± SD; N = 6) for the four pharmaceutical formulations
(◊, product A; □, product B; △, product C; ○, product D) using a) USP Apparatus 2 (test 2 from the specific monograph) and b) Peak^{TM} vessels. Grey region indicates USP acceptance criteria. Double straight line corresponds to very rapidly dissolving criterion, double dotted line to rapidly dissolving criterion.

In silico modelling of the pharmacokinetic profile

The model included the estimated profile of pH dependence of solubility and the distribution coefficient (Figure 4).

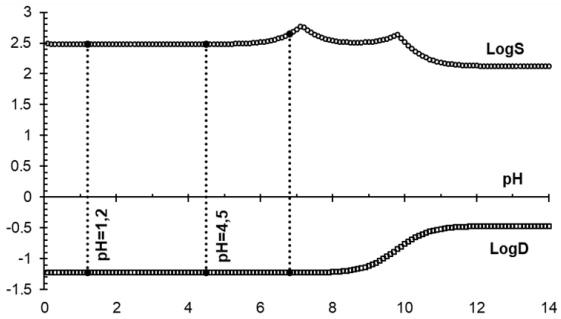

Figure 4.
The pH-dependence of the solubility (LogS, ○) and distribution coefficient (logD, □) of metformin
(estimated values, GastroPlus^{TM}, version 8.0.0002, Simulation Plus, Inc.)

Their values suggest that the considered dose of metformin hydrochloride is completely dissolved throughout the physiological range of gastro-intestinal pH. No precipitation phenomena are susceptible during the inter-compartmental transfers, in contrast to weak basic drugs [22]. The hydrophilic character is determined mainly by the high polarity, with a minimum solubility of 300 mg/ml (Figure 4). These estimates were in accordance with current BCS classification of metformin hydrochloride, based on its high-solubility, low permeability characteristics [4, 9].

In silico estimated profiles of plasma concentrations presented a mean maximum of 0.8476 µg/mL (27.94% CV) for the reference product with a theoretical fraction dissolved of 85% in 15 minutes (Figure 5).

The mean fraction absorbed was approximately 58% (19.67% CV), with no significant dependence on the theoretical *in vitro* release profile, in the considered interval of variation. The permeability across the intestinal membrane, by paracellular or transcellular transfer processes, seems to be the key factor for the slow and incomplete absorption of metformin hydrochloride, determining the observed flip-flop kinetics [19]. The time to maximum concentration varied between 2 and 5.67 h for the very rapid dissolving reference profile and between 2.30 and 5.48 h for the rapidly dissolving profile. The pharmacokinetic profiles confirm that the estimated absorption is controlled by the physiological factors, rather than the formulation variable or correlated *in vitro* performance. Based on previous reports [23], it can be assumed that the estimated inter-individual variability is determined by the model-generated variability in physiological parameters of the gastro-intestinal tract, especially the transit times (in this case randomly generated, 20% log-normal distribution).

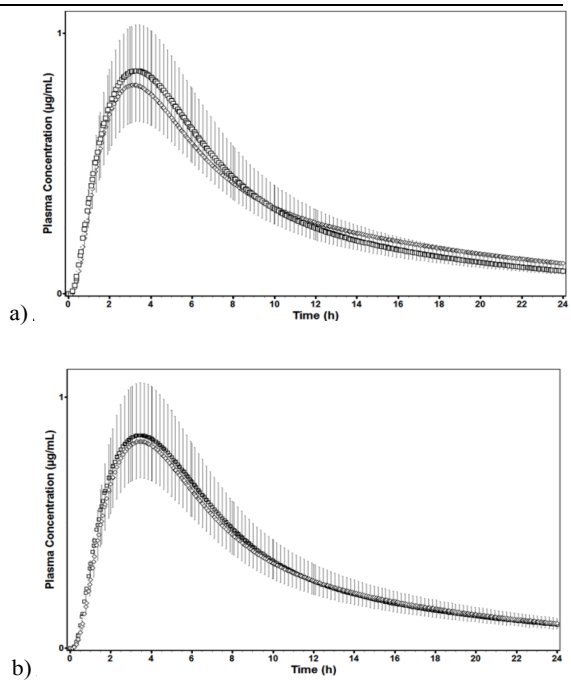

Figure 5.

In vivo simulated plasma concentration profiles (n = 24) For product A (◊) and B (□) using the fraction released *in vitro* according to the USP test 2 methodology at a) 15 minutes and b) 30 minutes. Bars indicate the 90% confidence interval. The reference profiles were generated based on mechanistically based *in silico* absorption models, including a theoretically 85% *in vitro* release at 15 and, respectively, 30 minutes (n = 500).

The impact of dissolution rate on the bioequivalence conclusion

Based on the calculation of the 90% confidence interval for the geometric mean ratios of test to reference product, for each of the *in silico* predicted pharmacokinetic profiles, bioequivalence was concluded. Table III presents the values for the peak and global exposure metrics, maximum concentration, respectively area under curve for the theoretical sampling interval 0 - 24 h and from 0 to infinity.

Table III

Results of the statistical comparison of the estimated *in vivo* profiles, based on fraction released *in vitro* at 15 and 30 minutes, using the USP test 2 methodology

Reference (n = 500)	Test (n = 24)	Parameter	GMR (%)*	90% CI†
Very rapidly dissolving (85% in 15 minute)	A	C_{max}‡	95.79	87.797 - 104.51
		AUC_{0-24}¶	96.22	86.643 - 106.85
		$AUC_{0-\infty}$§	100.2	89.046 - 112.67
	B	C_{max}	102.2	92.903 - 112.47
		AUC_{0-24}	96.32	87.287 - 106.30
		$AUC_{0-\infty}$	94.02	84.247 - 104.92
Rapidly dissolving (85% in 30 minute)	A	C_{max}	92.43	83.297 - 102.56
		AUC_{0-24}	93.34	82.999 - 104.96
		$AUC_{0-\infty}$	94.01	83.107 - 106.34
	B	C_{max}	96.65	86.024 - 108.59
		AUC_{0-24}	93.45	81.37 - 107.32
		$AUC_{0-\infty}$	93.62	81.275 - 107.85

* geometric mean ratio; † confidence interval, ln transformed; ‡ maximum concentration; ¶ area under plasma concentration from 0 to 24 hours; § area under area under plasma concentration from 0 to infinity ($AUC_{0-\infty}$).

The release rate, although not leading to fractions of metformin hydrochloride dissolved in 15 or 30 minutes above the biowaiver-requested limits, didn't have a significant impact on these pharmacokinetic parameters for product A. The most sensitive point estimator for the difference of 16% for the fraction dissolved *in vitro* after 15 minutes time is reflected by the point estimator of maximum concentration, varying between 95.79% and 102.2%.

Previous studies using *in silico* modelling of the absorption profiles for BCS class 3 drugs assessed the biowaiver extension included in the EMA guidelines [3, 4]. The *in vitro* dissolution profiles were either simulated or generated in three pseudo-luminal conditions, using the compendial recommendations. The validation of the model was conducted using either available clinical data [3] or by integrating BCS class I standard drugs, such as metoprolol and propranolol [4]. In the latter case, these two drugs are part of the build-in GastroPlus^{TM} database. We have used the same model, implementing the same adjustments and level of variation for key, physiological parameters, but we have included experimental dissolution profiles that proved the most discriminatory character for the formulation variables. The use of a single aqueous buffer system with a pH of 6.8 was justified by the data suggesting the biorelevance of these conditions, leading to level A *in vitro*/*in vivo* correlations [2, 10]. Compared to the other two acidic media, it provides the highest solubility for the active pharmaceutical ingredient, thus a formulation controlled *in vitro* release profile. Nevertheless, the same aqueous media is recommended by the compendial monograph, so it can be presumed that in this particular case, a quality control tool can also provide relevant information for the *in vivo* performance of the formulation.

The input values for several characteristics of metformin hydrochloride have been selected based on the available literature data. The jejunal permeability value of 2.2 x 10^{-4} cm/s used by Crison JR [3] is considerably higher than previously reported [5]. Instead, we have included the segmental experimental data generated in rat single-pass perfusion study [6]. Nevertheless, we have further included the paracellular transfer mechanism as the major contributor to the absorption process. This was supported by available studies on Caco-2 cell monolayer, suggesting that at 50 µm donor concentration, the basolateral efflux accounts for 7% of the total permeated amounts of metformin [19]. Furthermore, the inter-compartmental rate constants were generated by analysis of personal data generated in a single dose, fast state, cross-over bioequivalence study performed on 12 healthy Caucasian subjects. The roles of transporter systems and metabolic transformations were not included in the model [9].

As previously concluded, the very rapid release seems to be a restrictive requirement for immediate-release oral formulations of drugs with high solubility and permeability-limited absorption. Tsume Y [4] did not include metformin hydrochloride in the models. Supporting the extension of biowaiver criteria for profiles with 85% release in 45 to 60 minutes. Moreover, particularly for metformin hydrochloride, similar studies have demonstrated bioequivalence based on the peak and global exposure estimators for times to complete *in vitro* dissolution up to 120 minutes [3]. The current studies further confirms that, independently on the time point considered for 85% release, 15 or 30 minutes, the compliance with the compendial dissolution requirements generate *in vivo* pharmacokinetic profiles equivalent to the biowaiver-based estimated reference. The absorption process has been associated with the pharmacological effect of metformin hydrochloride, considering the high concentrations generated throughout the small intestine, the saturable transfer across biological membranes and a consecutive inhibition of glucose transport [24]. The dominant paracellular permeability and the contribution of "OCT-like" transporters have been used for description of "sponge hypothesis" [19]. Due to the high dose strength of the currently available oral formulation containing metformin hydrochloride, 500, 850 or 1000 mg, it can be assumed that, within large limits of *in vitro* release rates, the fraction of drug available in the intestine is able to saturate its own mechanism of absorption. The applicability of the provisional biowaiver extension will be limited to oral solid dosage forms without excipients interfering with transit time, local liquid volume and active transporters [25].

Conclusions

The *in vitro* methodology applied to four immediate-release oral solid dosage forms containing 500 mg metformin hydrochloride generated distinct dissolution profiles, dependent on the hydrodynamic conditions. The most discriminatory conditions were obtained using the USP test 2 methodology. The experimental fractions dissolved were used for *in silico* modelling of the absorption profiles. The results confirmed that for the considered BCS class 3 drug, different *in vitro* release rates generated similar *in vivo* pharmacokinetic patterns. The reference plasma concentration - time curves were simulated using either 15 or 30 minutes time interval for the release of 85% active pharmaceutical ingredient. The decision on bioequivalence was not affected by the compliance with current biowaiver requirements. This could suggest that, in the absence of factors interfering with the gastro-intestinal volume of fluid, transit times or active transporters, compendial standards could be used as the basis of biowaiver granting.

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References

1. Anand O, Yu LX, Conner DP, Davit BM, Dissolution testing for generic drugs: An FDA perspective. *AAPS J.*, 2011; 13: 328-335.
2. Balan G, Timmins P, Greene DS, Marathe PH, *In vitro-in vivo* correlation (IVIVC) models for metformin after administration of modified-release (MR) oral dosage forms to healthy human volunteers. *J Pharm Sci.*, 2001; 90: 1176-1185.
3. Crison JR, Timmins P, Keung A, Upreti VV, Boulton DW, Scheer BJ, Biowaiver approach for biopharmaceutics classification system class 3 compound metformin hydrochloride using *in silico* modeling. *J Pharm Sci.*, 2012; 101: 1773-1782.
4. Tsume Y, Amidon GL, The biowaiver extension for BCS class III drugs: The effect of dissolution rate on the bioequivalence of BCS class III immediate-release drugs predicted by computer simulation. *Mol Pharm.*, 2010; 7: 1235-1243.
5. Nicklin P, Keates AC, Page T, Bailey CJ, Transfer of metformin across monolayers of human intestinal caco-2 cells and across rat intestine. *Int J Pharm.*, 1996; 128: 155-162.
6. Song NN, Li QS, Liu CX, Intestinal permeability of metformin using single-pass intestinal perfusion in rats. *World J Gastroenterol.*, 2006; 12: 4064-4070.
7. Bretnall AE, Clarke GS, Metformin hydrochloride. In: Brittain HG, ed. Analytical profiles of drug substances and excipients. San Diego, California: Academic Press, 1998: 243-293.
8. Tucker GT, Casey C, Phillips PJ, Connor H, Ward JD, Woods HF, Metformin kinetics in healthy subjects and in patients with diabetes mellitus. *Br J Clin Pharmacol.*, 1981; 12: 235-246.
9. Cheng CL, Yu LX, Lee HL, Yang CY, Lue CS, Chou CH, Biowaiver extension potential to BCS class III high solubility-low permeability drugs: Bridging evidence for metformin immediate-release tablet. *Eur J Pharm Sci.*, 2004; 22: 297-304.
10. Homšek I, Parojčić J, Dačević M, Petrović L, Jovanović D., Justification of metformin hydrochloride biowaiver criteria based on bioequivalence study. *Arzneimittel-forschung*, 2010; 60: 553-559.
11. Oyetunde OO, Tayo F, Akinleye MO, *In vitro* equivalence studies of generic metformin hydrochloride tablets and propranolol hydrochloride tablets under biowaiver conditions in Lagos state. *Nigeria Diss Tech.*, 2012; 11: 51-55.
12. Verbeeck RK, Musuamba FT, The revised EMA guideline for the investigation of bioequivalence for immediate release oral formulations with systemic action. *J Pharm Pharm Sci.*, 2012; 15: 376-388.
13. Collins CC, Nair RR, Comparative evaluation of mixing dynamics in USP apparatus 2 using standard USP vessels and PeakTM vessels. *Diss Tech.*, 1998; 5: 7-21.
14. Palmieri A, Suppository dissolution testing: Apparatus design and release of aspirin. *Drug Dev Ind Phar.*, 1981; 7: 2472-2459.
15. Baxter JL, Kukura J, Muzzio FJ, Shear-induced variability in the United States Pharmacopeia apparatus 2: Modifications to the existing system. *AAPS J.*, 2005; 7: E857-864.
16. Beckett AH, Quach TT, Kurs GS, Improved hydro-dynamics for USP apparatus 2. *Diss Tech.*, 1996; 5: 7-18.
17. Popa DE, Lupuliasa D, Stănescu AA, Bârcă M, Burcea-Dragomiroiu GTA, Miron DS, Rădulescu FȘ, Simulation of the *in vivo* exposure to ibuprofen based on *in vitro* dissolution profiles from solid dosage forms. *Farmacia*, 2014; 62(3): 475-485.
18. Huang W, Lee SL, Yu LX, Mechanistic approaches to predicting oral drug absorption. *AAPS J.*, 2009; 11: 217-224.
19. Proctor WR, Bourdet DL, Thakker DR, Mechanisms underlying saturable intestinal absorption of metformin. *Drug Metab Dispos.*, 2008; 36: 1650-1658.
20. European Medicine Agency, Committee for Medicinal Products for Human Use. Guideline on the investigation of bioequivalence. 2010. www.emea. europa.eu.
21. U.S. Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for industry: Waiver of *in vivo* bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a Biopharmaceutics Classification System. 2000. www.fda.gov.
22. Aqel NN, Lupuleasa D, Mitu MA, Stănescu AA, Donea C, Miron DS, Rădulescu FȘ, The impact of differences between regulatory guidance for in vitro testing on the dissolution profiles of mycophenolate mofetil. *Farmacia,* 2014; 62(5): 942-949.
23. Marathe PH, Wen Y, Norton J, Greene DS, Barbhaiya RH, Wilding IR, Effect of altered gastric emptying and gastrointestinal motility on metformin absorption. *Br J Clin Pharmacol.*, 2000; 50: 325-332.
24. Vidon N, Chaussade S, Noel M, Franchisseur C, Huchet B, Bernier JJ, Metformin in the digestive tract. *Diabetes Res Clin Pract.*, 1988; 4: 223-229.
25. Blume HH, Schug BS, The biopharmaceutics classification system (BCS): Class III drugs - better candidates for BA/BE waiver?. *Eur J Pharm Sci.*, 1999; 9: 117-121.