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An *in vitro - in silico - in vivo* approach in biopharmaceutical drug characterization: metformin hydrochloride IR tablets

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Received October 28, 2014, accepted February 3, 2015

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Pharmazie 70: 458–465 (2015) *doi: 10.1691/ph.2015.4168*

The integrated *in vitro - in silico - in vivo* approach has emerged into a biopharmaceutical toolkit that could accelerate drug development and improve drug product clinical performance in patients. In the present study, the influence of physiologically based media and dynamic dissolution testing on drug release from two metformin hydrochloride immediate release products with proven bioequivalence was tested. Metformin-specific physiologically based pharmacokinetic (PBPK) model was developed based on a range of literature or *in silico* predicted data using gastrointestinal simulation technology implemented in the Simcyp® software package. Various approaches were employed in order to estimate the human effective permeability which was used as input for metformin plasma profile simulation. Influence of the rate and extent of metformin dissolution on drug absorption was evaluated. Both convolution and deconvolution approaches were used in order to establish a correlation between the *in vitro* and *in vivo* data. The results obtained indicate that physiologically based dissolution media and glass bead dissolution device exhibit certain advantages over the compendial dissolution apparatus and simple buffers which tended to be over-discriminative. Gastrointestinal simulation technology implemented in the Simcyp® Simulator was successfully used in developing drug-specific PBPK model for metformin. Simulations indicate that *in vitro* dissolution kinetics has no significant effect on metformin absorption, if more than 65% of drug is released in 1 hour. Level A *in vitro-in vivo* correlation was obtained using both convolution and deconvolution approaches.

1. Introduction

In vitro dissolution is the most commonly used drug product performance test aimed to assess its *in vivo* behaviour. It is, therefore, important to establish the *in vitro* methodology that would be predictive of drug product clinical performance and, if possible, establish a quantitative *in vitro-in vivo* correlation. An important feature of the dissolution test is its discriminative power. For a dissolution test to be discriminative, the results obtained should reflect differences in the product characteristics reflective of their *in vivo* release and absorption (Quareshi 2006). While simple dissolution media are preferred for quality control purposes, the use of more complex, physiologically based media that contain physiological surfactants has been proposed for product bioperformance assessment (Dressman et al. 1998, 2007; Galia et al. 1998; Jantartid et al. 2009; Sunsen et al. 2005). Besides the effect of surfactants, the influence of increased media viscosity, especially in the postprandial conditions has been emphasized (Braub and Parrott 1972; Cvijić et al. 2014; Parojčić et al. 2008; Smidt et al. 1991), as well as potential advantages of dynamic dissolution testing which should be further explored (Dickinson et al. 2012; Fang et al. 2010; McAllister 2010).

Prediction of *in vivo* drug performance from dissolution test results can be challenging since various physiological conditions, such as gastric emptying, pH, permeability and fluid volume in different segments of the gastrointestinal (GI) tract can also play a role in drug absorption. With the evolution of *in silico* PBPK models, it became possible to estimate the extent and rate of drug absorption based on the relevant physicochemical, physiological and formulation parameters (Jamei et al. 2009; Okumu et al. 2009; Shono et al. 2009; Willmann et al. 2004; Yu and Amidon 1999). It is anticipated that the use of *in silico* prediction tools could contribute to reduce the number of human *in vivo* studies in different phases of drug product development, their regulatory approval, as well as evaluation of certain post-approval changes (Leve et al. 2007).

Metformin hydrochloride is an oral antihyperglycemic drug that has long been used in the treatment of non-insulin-dependent diabetes mellitus. Due to high solubility and low permeability in the gastrointestinal tract (Balimane et al. 2006; Bertnall and Clarke 1998; Song et al. 2006), it is considered to be a Biopharmaceutics Classification System (BCS) class 3 drug. The pK_a values of metformin are 2.8 and 11.5, so the drug is mostly present in the ionised form along the intestine (Jack 1992). Tucker et al. (1981), reported that the fraction of metformin dose absorbed is in the range between 65 and 80%, with 20 to 35% of the dose recovered in feces. Vidon et al. (1988), studied metformin regional absorption in the gastrointestinal tract, and found that metformin administration did not affect gastric emp-

Table 1: Characteristics of the investigated products

	Product A	Product B
Tablet weight* (mg)	890	1085
Size (mm x mm)	13.4 x 13.4	21.2 x 10.1
Shape	round	ellipsoidal
Hardness* (N)	295	184
Disintegration time (min)	11.4	10.0
Dissolution (USP method)	85% in 20 min	85% in 8 min

* Mean values
USP – United States Pharmacopeia

tying and the drug is poorly absorbed across the gastric mucosa. Approximately 20% of the dose was absorbed in duodenum with further 60% absorbed across the jejunum and ileum, and about 20% recovered in feces (Vidon et al. 1988). Metformin absorption appears to be dose-dependent (Tucker et al. 1981; Noel 1979; Sambol et al. 1996a, 1996b). It was reported that absolute bioavailability of 850 mg metformin hydrochloride dose was 14% lower than bioavailability of 500 mg dose in healthy volunteers (Sambol et al. 1996a). The results of intestinal perfusion studies in rat indicate that metformin effective permeability in jejunum and ileum is significantly lower than in duodenum, at the same concentration. In addition, the effective permeability at high concentrations was significantly lower than at low and medium concentrations (Song et al. 2006).

The aim of the present study was to: (1) investigate the influence of physiologically based media and dynamic dissolution testing on drug dissolution from two metformin immediate release products with proven bioequivalence, and to identify discriminative dissolution test conditions; (2) develop a drug-specific PBPK model using gastrointestinal simulation technology (GST) implemented in the Simcyp® Simulator and (3) establish quantitative correlation between the *in vitro* and *in vivo* data. Considering the limited and variable metformin permeability data, various approaches were employed in order to estimate the human effective permeability ($P_{eff,man}$) which was subsequently used for metformin plasma profile simulation.

2. Investigations and results

2.1. In vitro study

Characteristics of the investigated tablets are presented in Table 1. It was noticed during most of the dissolution studies that product A does not disintegrate, while product B disintegrates rapidly. However, there were no pronounced differences in the disintegration times recorded in the tablet disintegration tester. Different drug dissolution rates were observed under the compendially recommended dissolution test conditions.

In vitro dissolution test results, obtained under different experimental conditions, are presented in Figs. 1 and 2. It can be noted that drug dissolution from product B was faster and, generally, no pronounced influence of dissolution media composition on drug release rate from this product was observed. In the case of product A, drug dissolution was faster in FaSSIF and FeSSIF than in compendial buffers as dissolution media. One of the proposed mechanisms that is used to explain food effect, is the influence of elevated intraluminal viscosity. In order to perform a more inclusive analysis of the elevated medium viscosity on drug dissolution and consequently absorption, *in vitro* studies in blank FeSSIF with 0.5% HPMC were performed. Somewhat slower dissolution was observed in viscous medium, for both products.

In the second set of experiments, drug dissolution was studied in the flow through cell apparatus using blank FaSSGF/blank FaSSIF as dissolution media and different media flow rates. The obtained results are presented in Fig. 2. In the case of product A, metformin dissolution was relatively slow and was not affected by the difference in media flow rate. On the other hand, drug dissolution from product B was faster and significantly affected by the increase in media flow rate, leading to more than 80% metformin dissolved after 20 min at 8 ml/min. Similarity factors between drug release rates from the two products were 27.5 and 19.9 for 4 ml/min and 8 ml/min flow rates, respectively.

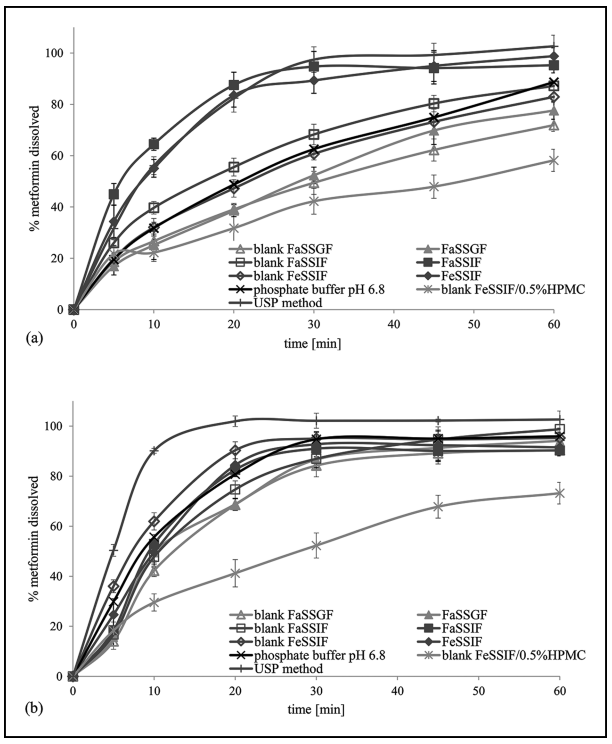

Fig. 1: Dissolution profiles obtained in the rotating paddle apparatus using various dissolution media: (a) product A; (b) product B.

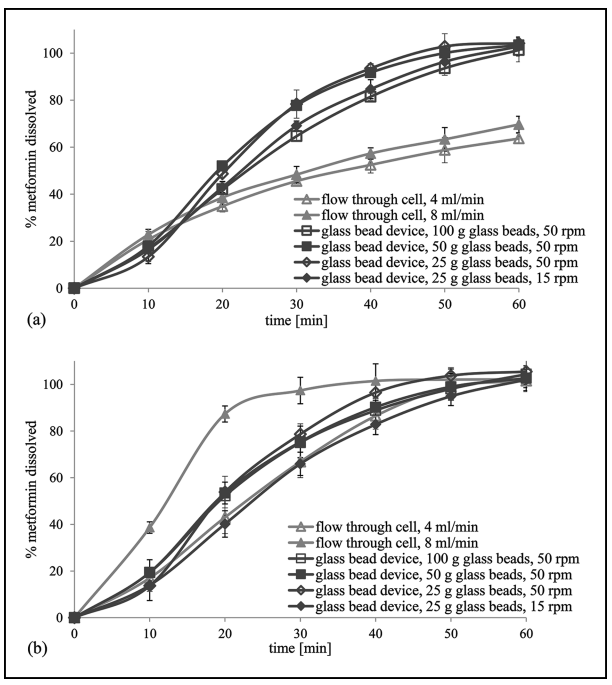

Fig. 2: Dissolution profiles in dynamic dissolution apparatuses (flow through cell and flow through system with glass bead dissolution device with different setups): (a) product A; (b) product B.

Table 2: Summary of the metformin input parameters employed for the gastrointestinal simulation

Parameter	Value
Dose	663 mg [a]
Molecular weight	129.16 g/mol
Log $P_{o:w}$	-1.43 [b]
Compound type	Monoprotic base [b]
pKa_1	2.8[c]
pKa_2	11.5[c]
Fraction unbound in plasma	100 % [d]
Absorption	
Model	ADAM
Human jejunal permeability, $P_{eff,man}$	see Table 3
Distribution	
Model	Full PBPK
Volume of distribution at steady state, V_{ss}	0.976 L/kg [e]
Elimination	
Renal clearance, CL_R	31 L/h [d]

ADAM model – Advanced Dissolution, Absorption and Metabolism model
Full PBPK model– Full Physiologically Based Pharmacokinetic model
[a]Equivalent to 850 mg metformin hydrochloride
[b] Literature data taken from Graham et al. 2011
[c] Literature values taken from Jack 1992
[d] Literature values taken from Tucker et al. 1981
[e] Simcyp® predicted value

The results obtained in the glass bead dissolution device are presented in Fig. 2. It can be noted that increase in the amount of glass beads, at constant speed of stirring bar rotation (i.e. 50 rpm), resulted in a slightly decreased drug dissolution rate. For both tested products, somewhat slower drug dissolution was observed with lower speed of stirring bar rotation. The calculated f_2 values between drug dissolution profiles from products A and B were 78.3, 87.0 and 57.3 for experimental setup with 25, 50 and 100 g of glass beads, respectively, and the stirring bar rotation speed of 50 rpm. The calculated f_2 value was 80.3 in the case where 25 g of glass beads was used and stirring bar rotation speed was set to 15 rpm.

2.2. In silico study

Simcyp® simulations were conducted using the input parameters shown in Table 2. The effective permeability values, estimated on the basis of the relevant *in silico*, *in vitro* or animal study results are presented in Table 3. The predicted metformin plasma concentration profiles, based on input $P_{eff,man}$ values predicted using different approaches, are shown in Fig. 3. Profiles a-e and a_t-d_t represent metformin plasma concentration profiles simulated on the basis of the respective permability data. Effective permeability values, estimated on the basis of molecular descriptors and PAMPA results were low, resulting in low plasma concentrations (Fig. 3, profiles a and b, dotted lines). Similar results were observed with the input data generated from the cell based systems, i.e. Caco-2 and MDCK II (Fig. 3, profiles c and d, dotted lines). The best match between the *in vivo* observed and *in silico* predicted absorption profiles was obtained with the $P_{eff,\ man}$ value estimated on the basis of metformin permeability in rats (Fig. 3, profile e), as demonstrated by low PE% values for the relevant pharmacokinetic parameters (Table 4). Colon permeability ($0.05 \cdot 10^{-4}$ cm/s) was estimated using Simcyp® Parameter Estimation module.

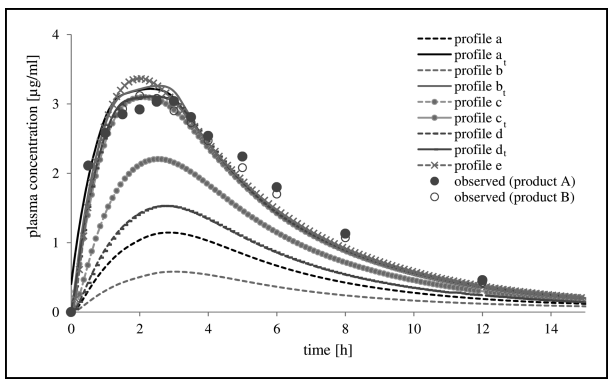

Fig. 3: *In vivo* observed and *in silico* predicted metformin plasma concentration profiles based on different human effective permeability values (without transporters (dotted lines) and with transporters (solid lines)).

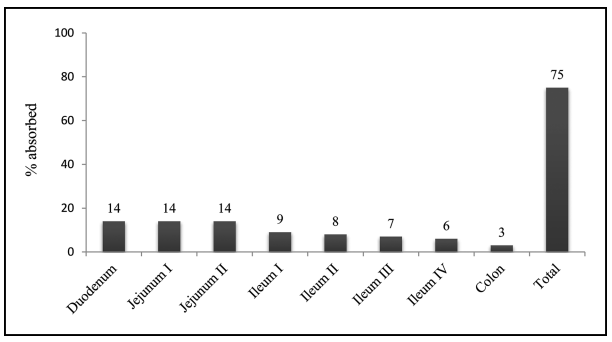

Fig. 4: Regional absorption of metformin in different segments of the gastrointestinal tract.

The predicted plasma concentration-time profiles, corresponding to different input dissolution data, are shown in Fig. 5. Statistical parameters of the linear regression analysis of the pooled data for products A and B, representing predicted metformin plasma concentration-time profiles versus data observed in the actual *in vivo* study are given in Table 5.

2.3. In vitro – in vivo correlation

2.3.1. Convolution approach

2.3.2. Deconvolution approach

Hypothetical iDDPs estimated using different deconvolution methods, are presented in Fig. 6. The profile obtained by DEC, using intravenous dose as a reference, gave a plateau pointing out on 70% of drug dose absorbed after eight hours. The profile obtained by GST gave a plateau on 74% of drug dose absorbed after three hours.

Table 3: Effective permeability values and transporter relative abundances used for the simulations

	$P_{eff,\ man}$ ($\cdot 10^{-4}$ cm/s)			Relative transporter abundances along GI tract compared to Jejunum I						
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum II	Ileum I	Ileum II	Ileum III	Ileum IV	Colon
Approach a		0.231		35	3	2	1.5	1	1	0
Approach b		0.106		40	4	3	1.5	1	1	0
Approach c		0.535		20	1.2	0.5	0.5	0.5	0.5	0
Approach d		0.328		30	3	2	1.5	1	1	0
Approach e	1.6	1.1	1.0	–	–	–	–	–	–	0

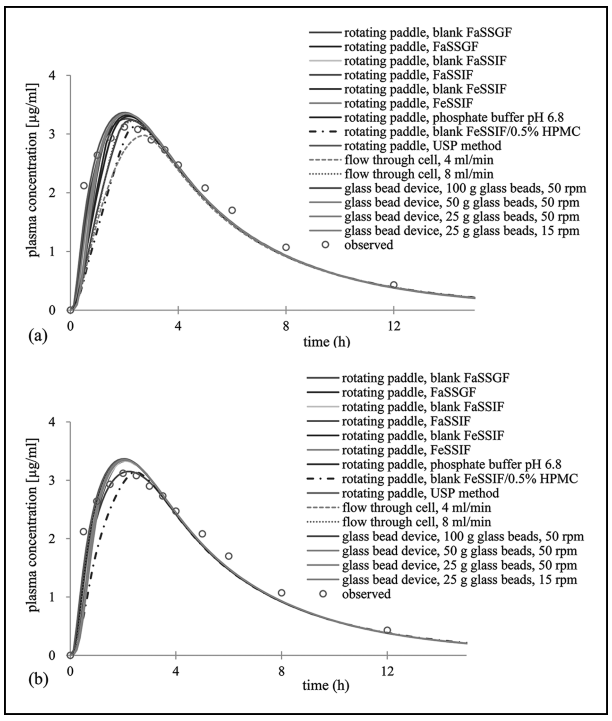

Fig. 5: Predicted *in vivo* plasma profiles corresponding to respective *in vitro* dissolution profiles: (a) product A; (b) product B.

Table 4: Predicted and observed pharmacokinetic parameters for metformin IR tablets

	C_{max} (µg/ml)	PE%	t_{max} (h)	PE%	AUC_{0-t} (µgh/ml)	PE%
Predicted						
Profile a_t	3.22	5.32	2.40	5.73	20.86	2.57
Profile b_t	3.26	4.11	2.50	10.1	20.81	2.80
Profile c_t	3.10	8.82	2.16	4.84	20.00	6.58
Profile d_t	3.11	8.53	2.40	5.73	20.39	4.76
Profile e	3.36	1.18	2.05	9.69	20.90	2.38
Observed						
product A	3.40	-	2.27	-	21.41	-
product B	3.38	-	2.40	-	21.81	-

%PE values shown in the table are calculated *based on the in vivo* data *observed for the reference product A; PE – prediction error values; AUC – area under the curve*

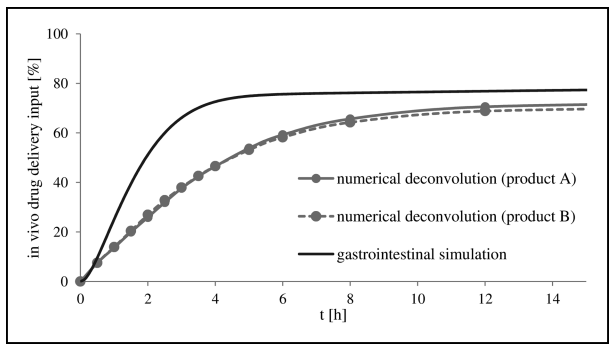

Fig. 6: *In vivo* drug delivery profiles obtained using different deconvolution methods.

Table 5: Statistical parameters of the obtained IVIVC

In vitro	Convolution approach		Deconvolution approach			
			DEC		GST	
	a	r	a	r	a	r
Rotating paddle, blank FaSSGF	1.008	0.934	1.104	0.878	0.905	0.884
Rotating paddle, FaSSGF	1.057	0.943	1.187	0.890	0.960	0.894
Rotating paddle, blank FaSSIF	1.064	0.951	1.239	0.954	1.130	0.954
Rotating paddle, FaSSIF	1.085	0.968	1.328	0.953	1.075	0.955
Rotating paddle, blank FeSSIF	1.071	0.939	1.943	0.775	0.853	0.831
Rotating paddle, FeSSIF	1.096	0.962	1.352	0.987	1.086	0.991
Rotating paddle, phosphate buffer pH 6.8	1.079	0.948	1.068	0.860	0.874	0.864
Rotating paddle, blank FeSSIF/0.5% HPMC	0.928	0.895	0.770	0.909	0.629	0.903
Rotating paddle, USP method	1.102	0.942	1.121	0.888	0.961	0.896
Flow trough cell, 4 ml/min	0.936	0.906	1.389	0.826	1.144	0.838
Flow trough cell, 8 ml/min	1.045	0.933	0.906	0.573	0.766	0.597
Glass bead device, 100 g glass beads, 50 rpm	1.082	0.935	1.808	0.992	1.444	0.992
Glass beads device, 50 g glass beads, 50 rpm	1.087	0.941	1.885	0.999	1.470	0.999
Glass beads device, 25 g glass beads, 50 rpm	1.091	0.939	2.044	0.997	1.659	0.999
Glass bead device, 25 g glass beads, 15 rpm	1.080	0.930	1.901	0.997	1.517	0.996

a - slope of the regression line; r - coefficient of correlation; DEC – numerical deconvolution; GST – gastrointestinal simulation

Table 6: Weibull parameter of the *in vitro* drug dissolution profiles and the relevant time scaling factors

In vitro	τ_d [h]r	TS	
		DEC	GST
Rotating paddle, blank FaSSGF	0.38	10.4	5.3
Rotating paddle, FaSSGF	0.41	9.6	4.9
Rotating paddle, blank FaSSIF	0.29	13.6	7.0
Rotating paddle, FaSSIF	0.22	17.9	9.2
Rotating paddle, blank FeSSIF	0.36	11.0	5.6
Rotating paddle, FeSSIF	0.25	15.8	8.0
Rotating paddle, phosphate buffer pH 6.8	0.38	10.4	5.3
Rotating paddle, blank FeSSIF/0.5% HPMC	0.35	11.3	5.8
Rotating paddle, USP method	0.23	17.2	8.8
Flow through cell, 4 ml/min	0.40	9.9	5.0
Flow through cell, 8 ml/min	0.40	9.9	5.0
Glass bead device, 100 g glass beads, 50 rpm	0.49	8.0	4.1
Glass beads device, 50 g glass beads, 50 rpm	0.42	9.4	4.8
Glass beads device, 25 g glass beads, 50 rpm	0.42	9.4	4.8
Glass bead device, 25 g glass beads, 15 rpm	0.47	8.4	4.3

TS – time scaling factor; DEC – numerical deconvolution; GST – gastrointestinal simulation

Time scaling factors (TS) were calculated as the ratio of respective time parameters obtained when hypothetical *in vivo* drug delivery profiles and *in vitro* dissolution profiles observed under various experimental conditions were fitted to the Weibull function (Table 6).

Statistical parameters of the linear regression analysis of the pooled data sets representing percent metformin dissolved under different experimental conditions *in vitro* (after rescaling of the time axis) versus the percent dissolved/absorbed *in vivo* at the same time points are presented in Table 5.

3. Discussion

Although there were no pronounced differences between the disintegration times recorded in the tablet disintegration tester, the differences in formulation between the two products, resulted in different drug dissolution rates, with the generic product exhibiting "very rapid dissolution" (more than 85% of drug dissolved in less than 15 min) under the compendially recommended dissolution conditions.

Generally, drug dissolution from the generic product was faster and less susceptible to the influence of the experimental conditions, in particular, the type of media employed. In the paddle apparatus, the effect of surfactants was observed only for the reference product, as illustrated by faster drug dissolution (Fig. 1). Considering high metformin solubility, the observed effect could be attributed to increased tablet wetting in the presence of lecithin and taurocholate. This effect was not observed in FaSSGF, which contains low concentration of surfactants. In viscous medium, dissolution was retarded for both investigated products. It may be assumed that poor wetting and reduced hydrodynamic shear rate in viscous medium contributed to the phenomenon observed. Significant differences (characterised by the similarity factor values less than 50) between drug dissolution from the two products in the paddle apparatus were observed in the majority of the media investigated, including the viscous medium, as well as under compendially recommended dissolution conditions. Similar dissolution profiles in the paddle apparatus were obtained only when FaSSIF and FeSSIF were used as dissolution media (f_2 values higher than 50).

Pronounced differences between the two investigated products were also observed in the flow through cell apparatus, as indicated by f_2 values lower than 50. Increase in the media flow rate significantly affected drug dissolution from the generic product, while this was not the case with the reference product.

Interestingly, in the glass bead dissolution device, alteration of hydrodynamic conditions did not significantly affect dissolution rate of the investigated products. In all the cases studied, dissolution profiles of the two products obtained in the glass bead dissolution device were similar (f_2 values higher than 50).

It was not possible to obtain "rapid drug dissolution" (i.e. more than 85% dissolved in 30 min) by using dynamic dissolution models, except in the case of product B when flow rate of 8 ml/min was employed in the flow through cell apparatus. Interestingly, the only dissolution profile compliant with the "very rapid dissolution" criteria (i.e. more than 85% in 15 min), which would be requested for biowaiver justification of a BCS class 3 drug, was obtained for product B using compendially recommended dissolution conditions.

GST implemented in Simcyp® Simulator was used to build metformin-specific PBPK model. Taking into account that metformin is a BCS class 3 drug, it is expected that permeability would have major impact on its absorption. Data on the effective permeability of metformin in man are not available and data from different *in silico*, *in vitro* or animal studies were used for its estimation. Colon permeability was estimated using the Simcyp® Parameter Estimation module in order to fit the actual pharmacokinetic profiles observed in the *in vivo* study. It has been reported that metformin absorption from gastrointestinal tract includes both passive diffusion and active transport (Scheen 1996; Proctor et al. 2008). Considering that molecular descriptors and PAMPA assay do not take into account active transport, relatively low values of the $P_{eff,\ man}$ were obtained based on this data, resulting in low plasma concentrations. On the other hand, cell based systems (i.e. Caco-2 and MDCK II) have lower expression of the relevant transporters compared to the human intestine (Balimane and Chong 2005), and estimated $P_{eff,\ man}$ values were also low, resulting in low plasma concentrations. In order to investigate the effect of active transport on metformin absorption, the effect of transporters was included into the *in silico* models a_t-d_t. With the inclusion of transporters effect, the predicted plasma profiles were in good agreement with the actual profile observed in the *in vivo* study. Such approach is supported by the experimental results reporting on the effect of transporters on metformin absorption (Proctor et al. 2008; Zhou et al. 2007; Wang et al. 2003).

The Simcyp® generated regional absorption, presented in Fig. 4, demonstrates that metformin is absorbed along the whole intestine, with the majority of dose absorbed in the proximal part of the small intestine. The *in silico* generated data are in agreement with the results of compartmental metformin absorption (Vidon et al. 1988; Noel 1979). The fraction of drug unabsorbed is 25%, which is in accordance with the literature data stating that 20 to 30% of metformin dose is not absorbed and is excreted in feces (Quareshi 2006; Pentikäinen et al. 1979).

The results obtained when different dissolution data were used as input, indicate that differences in the *in vitro* drug release kinetics have no significant impact on its *in vivo* performance. This finding is in accordance with the results of Crison et al. (2012) who used GST implemented in the GastroPlus™ software package to study dependency of metformin *in vivo* disposition on the rate and extent of drug dissolution. They have shown that AUC and C_{max} are not significantly affected by drug dissolution if 100% of the dose is released in 2 h or less (Crison et al. 2012).

Linear regression analysis of the pooled data sets, representing metformin plasma concentration-time profiles predicted on the basis of different *in vitro* dissolution profiles versus data observed in the actual *in vivo* study for products A and B, indicate high level of point-to-point IVIVC. The highest level of correlation, decribed by the correlation coefficient higher than

0.95 and slope of the regression line close to unity, was obtained for the *in vitro* experimental settings in which physiologically based media (i.e. FaSSIF and FeSSIF) and rotating paddle apparatus were used. Slight deviations from point-to-point IVIVC were observed only in the cases where slow and incomplete drug dissolution was used as input for the simulation (dissolution data obtained in viscous medium and at low media flow rate in the flow through cell apparatus). These results support the idea that the absorption of BCS class 3 drugs, like metformin, will mainly depend on drug permeability if dissolution is fast, and drug concentration is high enough to enable maximal extent of drug absorption. But, if drug dissolution is slow and uncomplete, then dissolution can limit the absorption process, and bad IVIVC can be expected.

Differences between the hypothetical iDDPs profiles could be observed, depending on the deconvolution method employed (Fig. 6). Drug absorption profile estimated by GST reflects relatively fast absorption, with the plateau phase reached after approximately 3 h. The total fraction of metformin absorbed was estimated to be 75%. Such results are in accordance with the limited metformin absorption which predominately occurs in the proximal part of the small intestine. The iDDPs obtained by numerical deconvolution using intravenous dose as a reference were somewhat slower, indicating gradual drug absorption.

Considering the time discrepancies between *in vivo* drug delivery profiles and drug dissolution *in vitro*, relevant TS factors were taken into account. The TS values differed depending on the deconvolution method used to obtain the *in vivo* input profile and the *in vitro* dissolution methodology employed. Relevant range of TS values obtained for iDDPs estimated by GST and DEC were 4.1 - 9.2 and 8.0 - 17.9, respectively. Highest correlation coefficients between the *in vitro* and *in vivo* data were obtained for dissolution profiles observed in the glass bead dissolution device ($r > 0.99$). The relevant values of the slope of the regression line varied in the range 1.444 to 2.044. These results clearly indicate that the course of dissolution in the glass bead apparatus does coincidence the Simcyp® predicted absorption profile.

Interestingly, the highest level of point-to-point IVIVC (described by the slope of 1.086 and coefficient of correlation 0.991) was obtained for the hypothetical *in vivo* drug delivery profile estimated by GST and *in vitro* dissolution profile obtained in the rotating paddle apparatus using FeSSIF as dissolution media. This finding indicates that the faster the *in vitro* dissolution is, better IVIVC for BCS Class III drug metformin could be expected. Certain limitation introduced when using GST in the deconvolution approach is that the same iDDP, predicted on the basis of the input data shown in Table 2, was attributed to both formulations.

The application of deconvolution and convolution approaches to establish IVIVC indicates somewhat different conclusions. For example, the results of the convolution approach demonstrate low dispersion between the profiles predicted on the basis of *in vitro* data obtained under various experimental conditions, and the actual *in vivo* data. Gaynor et al. (2008), emphasized that drug absorption rate, predicted by the deconvolution approach, can vary significantly in response to the difference in the *in vitro* dissolution data used in the deconvolution operation. Therefore, the IVIVC results for metformin IR tablets, obtained by applying the convolution approach may be considered more reliable.

In conclusion, physiologically based media and glass bead dissolution device provide bioperformance dissolution in the case of the two metformin IR formulations studied, while the other experimental conditions employed tended to be over-discriminative, indicating differences between the formulations which were not observed *in vivo*. GST was successfully used to predict metformin plasma profile using different data on drug effective permeability value, and the performed simulations revealed that there is no significant impact of the *in vitro* drug release rate on the *in vivo* drug absorption profile if more than 65% of metformin is released in 1 hour. Level A IVIVC was established using both convolution and deconvolution approach, but it was noted that the impact of the differences between the investigated drug products observed *in vitro* under various dissolution conditions was less pronounced on the convoluted drug absorption profiles using GST. This finding supports the idea that BCS class 3 drug dissolution does not have to conform to "very fast" drug dissolution in order to ensure complete absorption, but a broader bioperformance drug dissolution specification can be set for metofrmin, and possibly, other BCS class 3 drugs.

4. Experimental

4.1. Drug products

Two commercially available metformin immediate release (IR) tablet formulations, with proven bioequivalence, were evaluated. Product A was the reference product containing 850 mg metformin hydrochloride, povidone and magnesium stearate in the tablet core and hypromellose as a coating agent. Product B was the generic product containing 850 mg metformin hydrochloride, povidone, magnesium stearate, propylene glycol, cellulose, microcrystalline and croscarmellose sodium in the tablet core and hypromellose, talc, titanium dioxide and macrogol 6000 in the coating layer.

4.2. In vitro study

The investigated products were characterised with regards to tablet weight, hardness, disintegration time, and drug dissolution. Tablet disintegration test was performed according to the USP method with disks, in a disintegration apparatus (Erweka ZT 52, Germany), at $37 \pm 0.5\,^{\circ}C$, using 800 ml of USP phosphate buffer pH 6.8. For each product, disintegration time of six tablets was recorded, and the mean value was calculated. Dissolution testing was performed using various dissolution apparatuses and physiologically based dissolution media:

- A first set of experiments was performed in a rotating paddle apparatus (Erweka DT 70, Germany) at $37\,^{\circ}C$ and rotational speed of 50 rpm, using 500 ml dissolution media. The dissolution media used were: Fasted State Simulated Gastric Fluid (FaSSGF) (Vertzoni et al. 2005), Fasted State Simulated Intestinal Fluid (FaSSIF) and Fed State Simulated Intestinal Fluid (FeSSIF) (Marques et al. 2004) as well as their corresponding blank buffers. Blank FeSSIF with the addition of 0.5% HPMC was used in order to examine the effect of increased medium viscosity. Dissolution was also performed using phosphate buffer pH 6.8 and as well as under compendially recommended dissolution test conditions for products labelled to contain 850 mg of metformin (i.e. 1000 ml of phosphate buffer pH 6.8 as dissolution media and 75 rpm rotation speed) (USP 2014).
- The second set of experiments was performed in the USP flow through cell apparatus (Sotax CE7 Smart, Switzerland) using an open-loop configuration and 22.6 mm cells. One 5-mm bead was placed into each cell and then 6.5 g of 1-mm-sized glass beads was added. The tablet was placed on the top of the glass beads prior to the start of the test. Dissolution study began with blank FaSSGF (Vertzoni et al. 2005), which was replaced with blank FaSSIF (Marques et al. 2004) after 15 min. The media flow rates were set to 4 or 8 ml/min. Dissolution media samples were collected at 10 min intervals during 1 h time period.
- The third set of experiments was performed in the flow through system with glass bead dissolution device developed by Bogataj et al. 2010. The system was composed of a flat bottomed 150 ml glass vessel with inner diameter of 56 mm, a peristaltic pump (IKA, Germany), a medium reservoir, and connecting tubings. The vessel was immersed in a thermostated water bath and set on a magnetic stirrer (IKA, Germany). A magnetic stirring bar and 40 ml of dissolution media were added into the vessel. Then, different amounts (i.e. 25, 50 or 100 g) of glass beads (diameter 1 mm) were placed over the stirring bar. The medium in the vessel was agitated by the magnetic stirring bar set to a speed of 50 rpm. The peristaltic pump flow rate was set to 2 ml/min. In a novel setup, upgraded system with four working stations (Merel, Slovenia) with magnetic stirrers set to a speed of 15 rpm and peristaltic pumps (IKA, Germany) was used. Prior to the start of the test, each tablet was put in the medium on the surface of the beads layer. The medium was preheated to a temperature of $37\,^{\circ}C$. The dissolution experiments were initiated with blank FaSSGF, which was replaced with blank FaSSIF after 15 min. Dissolution media samples were collected at 10 min intervals during 1 h time period.

Dissolution media samples were filtered through a 0.45 μm PDF filter (25 mm GD/X Whatman) and, after appropriate dilution, assayed

for metformin hydrochloride UV spectrophotometrically at 233 nm. Each experiment was conducted at least in triplicate. Similarity factor values (f_2) were used for dissolution profiles comparison (Moore and Flanner 1996).
The chemicals used for dissolution media preparation were: sodium chloride, hydrochloric acid, acetic acid, and egg lecithin (60% pure), all purchased from Sigma-Aldrich Chemie GmbH (Germany); sodium taurocholate (technical grade) purchased from Fluka Chemie GmbH (Germany); potassium dihydrogen phosphate and sodium hydroxide, supplied both by Carl Roth GmbH (Germany); and hydroxypropylmethylcellulose (HPMC K4 M, Methocel® K4 M) supplied by Colorcon Co. (UK).
FaSSGF and its corresponding blank buffer were prepared as described by Vertzoni et al. (2005). FaSSIF, FeSSIF and their corresponding blank buffers were prepared as described by Marques (2004). Briefly, blank buffers were prepared as stock solutions. On the day of the experiment, fresh FaSSGF, FaSSIF and FeSSIF were prepared by addition of appropriate amounts of lecithin and sodium taurocholate. The media were stirred until clear solutions were obtained. To prepare the viscous medium (blank FeSSIF with addition of 0.5% HPMC), HPMC K4 M was dispersed in one third of the amount of purified water needed for media preparation preheated to 80 °C, and allowed to cool. The rest of water, in which sodium hydroxide and acetic acid were dissolved, was then added to the HPMC dispersion under constant stirring.

4.3. In vivo study

The design of the bioequivalence clinical study, with the investigated reference and generic metformin products, has been described in detail by Homšek et al. (2010). Briefly, the study was performed in 24 healthy volunteers of both sexes. After an overnight fast of 10 h, each volunteer received, in a randomized order, single oral dose of 850 mg metformin hydrochloride (one tablet). The investigated products were administered at 7-day interval, with 240 ml of noncarbonated water. The serum metformin was quantified after liquid extraction, using HPLC method with an electrospray ionization mass spectrometer.

4.4. In silico study

Simcyp® Population-Based Simulator (version 13.1; Certara™, USA) was used to develop the drug-specific PBPK model. The required input parameters related to metformin physicochemical and pharmacokinetic properties were taken from literature and/or *in silico* estimated. Summary of the input parameters used is given in Table 2. Considering that concentrations in the biological fluids are expressed as the free base, the metformin dose employed for the simulations was also expressed as free base (molecular weight 129.16 g/mol). The Advanced Dissolution, Absorption and Metabolism model (ADAM) was used in the simulations. Characteristics of the virtual subjects in the *in silico* study were set to match the subjects participating in the actual *in vivo* study (i.e. age range, ratio of males and females). Simulations were performed for population representative subject for the defined population.
Taking into account that metformin is a BCS class 3 drug, it is expected that permeability would have major impact on its absorption. Various approaches that can be used to estimate metformin permeability for model development were employed:

approach a. molecular descriptors, i.e. polar surface area of 88.99 Å and the number of hydrogen bond donors 4 (Metformin (DB00331), 2013);
approach b. apparent permeability value obtained from the parallel artificial membrane permeability assay ($P_{app} = 0.5 \cdot 10^{-6}$ cm/s) (Balimane et al. 2006);
approach c. apparent permeability value obtained from Caco-2 cell culture ($P_{app} = 0.366 \cdot 10^{-6}$ cm/s) (Balimane et al. 2006);
approach d. apparent permeability value obtained from MDCK cell culture ($P_{app} = 1.2 \cdot 10^{-6}$ cm/s)[1] (MDCK_Fact_Sheet 2014);
approach e. rat effective permeability values obtained using the single-pass intestinal perfusion technique (relevant permeability values were $4.51 \cdot 10^{-5}$ cm/s for duodenum; $3.26 \cdot 10^{-5}$ cm/s for jejunum and $2.96 \cdot 10^{-5}$ cm/s for ileum) (Song et al. 2006). The relationship established by Fagerholm et al (Fagerholm et al. 1996) was used to predict human effective permeability values.

Furthermore, in order to investigate the effect of active transport on metformin absorption, the effect of relevant transporters was included into the models a_t-d_t.

[1] In the case of Caco-2 cells data, P_{app} values for verapamil, cimetidin, propranolol and metoprolol measured under the same experimental conditions as the P_{app} value for metformin were used for the calibration of the permeability conversion equation; in the case of MDCK cell data, calibration was performed with P_{app} values for cimetidin, atenolol, propranolol and verapamil as reference compounds.

The generated *in silico* models were evaluated based on the calculated percent prediction error (PE%) between the *in vivo* observed and *in silico* predicted pharmacokinetic parameters (Eq. 1).

$$\mathrm{PE\%} = \frac{PK_{predicted} - PK_{observed}}{PK_{observed}} \times 100 \quad (1)$$

where PK denotes the relevant pharmacokinetic parameter (i.e. maximum plasma concentration, C_{max} or area under the plasma concentration curve, AUC).

4.5. In vitro – in vivo correlation

In order to evaluate the relationship between the *in vitro* and *in vivo* data, both convolution and deconvolution approaches have been employed.

4.5.1. Convolution approach

In the convolution approach, experimentally obtained dissolution profiles were used as inputs in the *in silico* developed metformin absorption model. The predicted pharmacokinetic profiles were compared with the mean plasma concentration profiles observed in the *in vivo* study. Linear regression analysis, performed using MS Office Excel 2010 (Microsoft Corporation, Redmond, WA), was used to estimate the correlation between the *in vivo* obtained and *in silico* generated data.

4.5.2. Deconvolution approach

In the deconvolution approach, hypothetical *in vivo* drug delivery profiles (iDDPs) were compared with the experimentally obtained dissolution profiles. Hypothetical *in vivo* drug delivery profiles were calculated using: (1) numerical deconvolution (DEC) (Kinetica 5.0, Thermo Scientific Inc, USA) and (2) gastrointestinal simulation technology (GST).
When numerical deconvolution was applied, the iDDPs were calculated based on metformin plasma concentration profiles after oral administration, observed in the *in vivo* study (Homšek et al. 2010), and literature data on metformin pharmacokinetics following intravenous administration, reported by Tucker et al. (1981).
GST refers to *in silico* simulations using Simcyp®. Absorption using ADAM model implemented in Simcyp® is modelled by a system of coupled linear and nonlinear rate equations used to simulate the effect of physiological conditions on drug absorption as it transits through successive GI compartments. GI tract is devided into nine compartments from stomach through the intestine to the colon. Drug absorption from each segment is described as a function of release from the formulation, dissolution, precipitation, luminal degradation, metabolism, transport and transit from one segment to another. It is assumed that absorption from stomach is negligible, that movements of the solid and liquid drugs through each segment of the GI tract may be described by first-order kinetics, and that metabolism in the colon is negligible. The total amount of drug absorbed is summed over the integrated amounts being absorbed from each compartment (Simcyp® Simulator, 2014).
Hypothetical iDDPs generated using different methods, and experimentally obtained dissolution profiles were fitted to Weibull distribution (Eq. 2) (Lagenbucher 1972).

$$W_t = \mathrm{W_{max}} \left[1 - e^{-\left(\frac{t}{\tau_d}\right)^{\beta}} \right] \quad (2)$$

where W_t is fraction of drug dissolved/absorbed at time t, W_{max} is maximum cumulative fraction dissolved/absorbed, τ_d is a time parameter (provides information on the overall rate of the process) and β is a parameter that provides information on the shape of the cumulative curve. The profiles were fitted with the 'Sigmoidal–Weibull' distribution, with a slight modification, using OriginPro® software, version 8 (OriginLab®, USA). Considering the time discrepancies between the *in vitro* and *in vivo* profiles, relevant time scaling factors (TS) were calculated as the ratio between τ_{dvivo} and τ_{dvitro}.
The relationship between the *in vitro* and *in vivo* data was evaluated by linear regression analysis of the data sets representing percent metformin dissolved *in vitro* at specified time points (after rescaling the time axis) under different experimental conditions versus the percent metformin dissolved/absorbed *in vivo* at the same time points. In order to develop a meaningful level A IVIVC for two bioequivalent formulations, pooled data sets that include relevant (*in vitro* and *in vivo*) data for both formulations were assessed.

Acknowledgment: This work was done under the project TR 34007 supported by the Ministry of Education, Science and Technological Development, Republic of Serbia.

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