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Prediction of the human oral bioavailability by using *in vitro* and *in silico* drug related parameters in a physiologically based absorption model

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ABSTRACT

Estimates of the human oral absolute bioavailability were made by using a physiological-based pharmacokinetic model of absorption and the drug solubility at the gastrointestinal pH range 1.5–7.5, the apparent permeability (P_{app}) in Caco-2 cells and the intrinsic clearance (Cl_{int}) in human hepatocytes suspensions as major drug related parameters. The predictive ability of this approach was tested in 164 drugs divided in four levels of input data: (i) *in vitro* data for both P_{app} and Cl_{int}; (ii) *in vitro* data for Cl_{int} only; (iii) *in vitro* data for P_{app} only and (iv) *in silico* data for both P_{app} and Cl_{int}. In all scenarios, solubility was estimated *in silico*. Excellent predictive abilities were observed when *in vitro* data for both P_{app} and Cl_{int} were used, with 84% of drugs with oral bioavailability predictions within $a \pm 20\%$ interval of the correct value. This predictive ability is reduced with the introduction of the *in silico* estimated parameters, particularly when Cl_{int} is used. Performance of the model using only *in silico* data provided 53% of drugs with bioavailability predictions within $a \pm 20\%$ acceptance interval. However, 74% of drugs in the same scenario resulted in bioavailability predictions within $a \pm 35\%$ interval, which indicates that a qualitative prediction of the absolute bioavailability is still possible. This approach is a valuable way to estimate a fundamental pharmacokinetic parameter, using data typically collected in the drug discovery and early development phases, providing also mechanistic information of the limiting bioavailability steps of the drug.

1. Introduction

Oral administration, due to its ease and patient compliance, is the preferred route and a major goal in the development of new drug entities. It is also traditionally one of the reasons for either discontinuation or prolongation of the development time of compounds (Singh, 2006). In this context, and as a consequence of the large output of molecular synthesis due to combinatorial chemistry, initial screening of hits in a number of thousands is done typically by using *in silico* approaches. *In vitro* tests are then used to reduce the number of compounds from hundreds to dozens and *in vivo* animal models to 1–5 finally potential drugs that proceed to clinical trials (Venkatesh and Lipper, 2000). In this process, a large amount of data are typically produced, many of which never results in a new drug entity. However, this information, far from being discarded, is currently used to build many *in silico* models that may help in the early screening of new drugs.

Another currently performed effort is the Integration of data from all these development phases for the lead selection (Saxena et al., 2009). In this regard, physiologically based pharmacokinetic (PBPK) models are one of the most promising tools (Rowland et al., 2011), and some examples of their application in the drug development are already available (Lupfert and Reichel, 2005; Norris et al., 2000; Parrott and Lave, 2008; Parrott et al., 2005; Poulin and Theil, 2000; Theil et al., 2003).

Various physiological compartmental models of absorption are described in the literature (Agoram et al., 2001; Grass, 1997; Huang et al., 2009; Yu and Amidon, 1999; Yu et al., 1996b), but considering the basic structure and the importance of the gastrointestinal tract (GIT) transit time, it is fair to say that they are all implementations and optimizations over the Compartmental Absorption and Transit (CAT) model (Yu et al., 1996b). In its initial form, CAT model assumed passive absorption, instantaneous dissolution, linear transfer kinetics for each segment and minor absorption from the stomach and colon. Although simple assumptions were considered, this initial approach was able to predict in fair agreement the bioavailability of 10 passively diffused drugs (Yu and Amidon, 1999). By including Michaelis–Menten kinetics (Yu and Amidon, 1998), gastric emptying and dissolution (Yu, 1999), the model applicability was extended for other classes of drugs. Various

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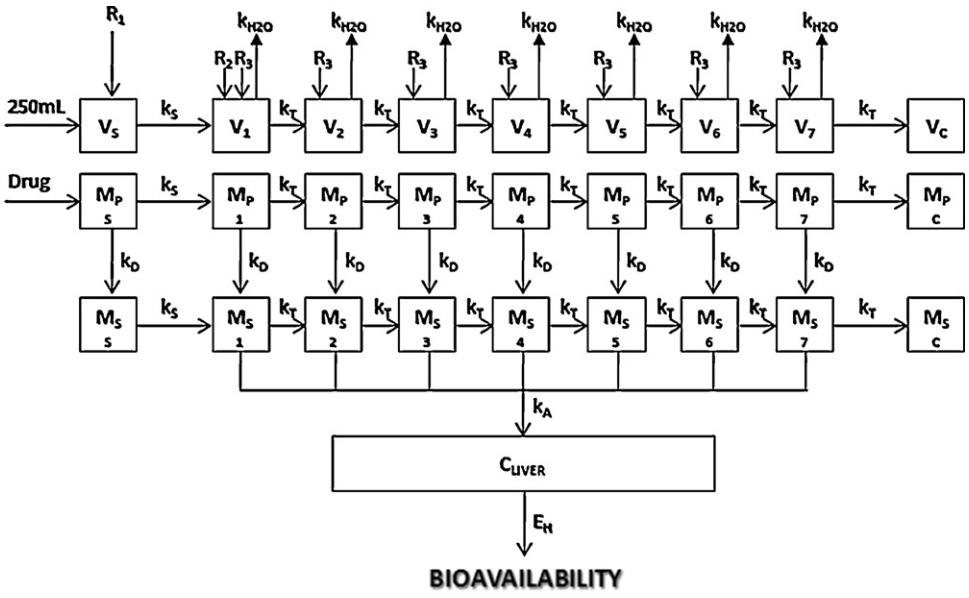

Fig. 1. Structure of the physiologic-based pharmacokinetic model of absorption.

subsequent optimizations were latter made in commercial packages by including first-pass metabolism and colon absorption – Gastroplus™ (Agoram et al., 2001), introducing direct physiologic meaning in the model compartments – iDEA™ (Grass, 1997) and considering the physiologic heterogeneity of the GIT – SimCyp™ (Di Fenza et al., 2007). If the ability to simulate, within the model assumptions, the effect of changes in the fundamental drug related parameters on the pharmacokinetic profile of the drug is unquestionable, some efforts have also been made in order to quantify the predictive ability of these same models in a new drug scenario with promising results (Cai et al., 2006; De Buck et al., 2007a,b; Parrott and Lave, 2002; Parrott et al., 2005). However, and specially when *in silico* drug parameters are required, due to the proprietary nature of these commercial packages and the use of internal training sets for model parameters optimizations, it is difficult to effectively test the models with "true" external validation data. Additionally, the complexity of these models may also constrain their applicability in the early development due to limitations on the available data.

The purpose of this work is to evaluate the use of a simple physiologically based absorption model that describes the fundamental steps involved in the human oral bioavailability aiming at the initial phases of drug development where only the fundamental biopharmaceutical characteristics of the molecules are known or predicted. Its performance was tested using *in vitro* data from Caco-2 cells and suspensions of human hepatocytes. Replacement of these data sources with *in silico* derived ones, in a PBPK-QSAR integrative approach, was tested by considering only drugs that were not previously used in the internal training and testing processes of the QSAR model building strategies.

2. Materials and methods

2.1. Model structure

The present model was built on the basis of CAT model (Yu and Amidon, 1998, 1999) in its integrated form (Yu, 1999) in order to consider permeability, dissolution and solubility limited absorption. A parallel model was included to establish the water volume changes in the GIT. Additionally, a liver compartment was included to quantify the 1st pass effect on absolute bioavailability (Rowland et al., 1973). The final structure of the physiologically based absorption model is presented in Fig. 1. The GIT is divided into three segments with a series of multiple compartments connected by linear transfer kinetics from one to the next. The first segment represents the stomach (subscript S) and consists of a single compartment, which is connected to the second segment, that defines the small intestine, consisting of a sequence of seven compartments with different volumes but equal residence times (subscripts 1–7). The final segment, with only one compartment, is related to the colon (subscript C). Drug, in an immediate release dosage form, is administered to the stomach with 250 ml of water where it may be dissolved. Both solid (M_p) and soluble drug (M_s) will then undergo similar gastric emptying rates (k_s) and move through the different intestinal segments with similar transit time characteristics (k_T). Drug dissolution rate (k_D) is defined by the Noyes–Whitney equation without "sink conditions". Due to this, and in order to determine the concentration of dissolved drug, the water content (V) of the GIT was also modeled. The same segment series were considered, with transfer kinetics between the compartments similar to the previously described. Water volume is considered dependent on the rate of salivary and gastric (R_1), duodenal (R_2) and intestinal mucous (R_3) secretions as well as the intestinal water reabsorption (k_{H_2O}) process. Only dissolved drug is assumed to be absorbable in the small intestine at a rate defined by k_A. All absorbed drug will pass by the liver where it will be metabolized according to the "well-stirred" model (Rowland et al., 1973). By calculating the mass of solid drug reaching the colon, being absorbed and escaping the liver, bioavailability limited by dissolution (F_d), absorption (F_{perm}) and metabolization (F_{met}) may be determined as well as absolute bioavailability (F_{oral}). The presented model is a typical case of an initial value problem of a system of differential equations and was numerically solved by the use of ADAPT II (D'Argenio and Schumitzky, 1979; D'Argenio and Schumitzky, 1997).

2.2. Physiological parameters

Physiological parameters of the model used in the present study are presented in Table 1. Gastric emptying is assumed to follow first-order kinetics with a mean residence time of 0.25 h (k_s = 1/0.25 h) (Yu and Amidon, 1998). The small intestine transit time was found to be 3.32 h and the 7 compartment model has shown to be the best compartmental model to depict the small

Table 1
Physiological parameters used in the pharmacokinetic model.

Transit rate constants	
$k_s = 4.00$	h^{-1}
$k_T = 2.11$	h^{-1}
Intestine radius	
$r_1 = 1.70$	Cm
$r_2 = 1.58$	Cm
$r_3 = 1.47$	Cm
$r_4 = 1.37$	Cm
$r_5 = 1.26$	Cm
$r_6 = 1.16$	Cm
$r_7 = 1.05$	Cm
Water model parameters	
$R_1 = 104.2$	ml h^{-1}
$R_2 = 91.70$	ml h^{-1}
$R_3 = 10.70$	ml h^{-1}
$V_s = 26.04$	ml
$V_1 = 73.47$	ml
$V_2 = 58.95$	ml
$V_3 = 48.05$	ml
$V_4 = 39.87$	ml
$V_5 = 33.73$	ml
$V_6 = 29.13$	ml
$V_7 = 25.67$	ml
$k_{H_2O} = 0.7015$	h^{-1}

References for the parameters are provided in Section 2.

intestine transit time distribution (k_T = 7/3.32 h) (Yu et al., 1996a). Water model was optimized by considering a 24 h secretion of 1000 ml of saliva, 1500 ml of gastric secretions (joint in R_1), 1000 ml of pancreatic secretions, 1200 ml of Bile secretion (Joint in R_2) and 1800 ml of intestinal mucus secretion (equally divided in the seven compartments and considered as R_3) (Guyton and Hall, 1996). The sum of water secretion in the GIT totals 6500 ml day^{-1} and k_{H_2O} was determined by non-linear regression in order to result in a steady-state amount of reabsorbed water of 5200 ml day^{-1}, corresponding to a 80% of water reabsorption in the intestine (Vander et al., 2001). Water volumes of the different compartments – stomach water volume (V_s) and individual small intestine water volumes (V_1–V_7) – are the steady-state volumes for the stated conditions and were thereafter considered as the initial condition volumes. With the administration of the drug, it is also administered 250 ml of water as a bolus directly to the stomach, which results in a temporal change of the amount of water throughout the GIT.

2.3. Drug related parameters

Specific parameters were introduced in order to describe the dissolution, absorption and metabolization characteristics of the different drugs. Although it has long been recognized that during dissolution a reduction of the particle size occurs (Costa and Sousa Lobo, 2001) and that this, as well as the shape of the drug particles (Fukunaka et al., 2006), may influence the rate of dissolution (R_D), for the sake of simplicity and according to Yu (Yu, 1999), we considered the Noyes–Whitney equation to described this process

$$R_D = \frac{3 \times D \times M_{(P,i)}}{\rho \times h \times r_p} \left(S_i - \frac{M_{(S,i)}}{V_i} \right) \quad (1)$$

where D is the diffusion coefficient with a value of 5×10^{-6} cm^2 s^{-1}, ρ is the density of drug with a value of 1200 mg cm^{-3}, h is the diffusion layer thickness that was set to be 30 μm and r_p the radius of the particles, considered constant over time with a value of 50 μm (Yu, 1999). These values were kept constant in all simulations. S_i is the solubility of the drug in the different compartments, taking into consideration the pH differences that the drug is exposed to when transiting across the GIT. It is assumed that in stomach pH 1.5. Duodenum (compartment 1 of the small intestine) presents a pH 4.6, jejunum (compartments 2 and 3) presents a pH 6.5 and ileum (compartments 4–7) presents a pH 7.5. Bile salts are known to play an important role in the emulsification and solubilization of drugs (Wiedmann and Kamel, 2002), and various studies evaluating the differences in solubility between pH 6.5 buffered solutions and FaSSIF media for low solubility drugs ($\log P > 2.5$) showed solubility increases up to 90 times (Fagerberg et al., 2010; Sugano, 2009). Based on this fact, it was considered that drugs with $\log P$ values above 2.5 would be 50 times more soluble in the duodenum than the drug aqueous solubility at pH 4.5. Additionally, it was also assumed that when, due to pH changes, the amount of drug dissolved enters a compartment with lower solubility, a supersaturated solution may be formed and no precipitation occurs.

Considering drug absorption in the model, the rate of absorption (R_A) (Yu and Amidon, 1999) was calculated by,

$$R_A = \frac{2 \times P_{eff}}{r_i} \times M_{(S,i)} \quad (2)$$

where P_{eff} is the drug effective human permeability, $M_{(S,i)}$ is the mass of dissolved drug in each of the seven individual small intestine compartments and r_i is the mean radius for each of the small intestine individual compartments (Table 1), which was determined by assuming a linear decrease from 1.75 cm at the proximal to 1.0 cm at the distal end as well as the described length of the GIT (Willmann et al., 2004).

The relative amount of drug metabolized in the liver (E_H), and according to the "well-stirred" model (Rowland et al., 1973), is defined as,

$$E_H = \frac{f_{u,B} \times Cl_{int}}{Q_H + f_{u,B} \times Cl_{int}} \quad (3)$$

where Q_H is the liver blood flow with a physiological value of 81 L h^{-1}; $f_{u,B}$ is the fraction of unbound drug in blood. For acids, this parameter was determined by the ratio between the free fraction of drug in plasma ($f_{u,P}$) and the drug blood-to-plasma concentration ratio (R_b). For basic, neutral and zwitterionic drugs $f_{u,B}$ was considered to be equal to 1, according to previous works (Paixao et al., 2010a; Sohlenius-Sternbeck et al., 2010; Wan et al., 2010). Finally, Cl_{int} is the hepatic intrinsic clearance of the drug.

2.4. S_i, P_{app} and Cl_{int} datasets

We based our evaluation of the model and its applicability in predicting human absolute bioavailability on the datasets previously provided by Paixao et al., 2010a,b. Combination of the two databases included 405 drugs and drug-like molecules. Within these molecules, a survey was performed in order to collect relevant pharmacokinetic properties and all drugs with the following characteristics were removed prior to analysis: CL/F determination, CL with high variability, liposomal formulations, isomers with different pharmacokinetics, known metabolization by multiple organs, CL data obtained in cancer patients, non-linear elimination pharmacokinetics, pro-drug data, re-conversion of the metabolite, endogenous substances, unreliable pharmacokinetic data or due to impossibility to calculate all the required molecular descriptors. A total of 164 drugs complied with the above procedures and were considered for further analysis. Absolute bioavailability, total plasma clearance, fraction of unchanged drug excreted in urine and plasma protein binding were recorded. Drugs were also classified by their chemical class based on the *in silico* pK_a obtained using the on-line ADME Boxes (http://www.pharma-algorithms.com/webboxes) and considering the most relevant species at pH 7.4 (Table 2).

Ideally, *in vivo* solubility, permeability and metabolic activity data would be used in Eqs. (1)–(3) in order to characterize the drug dependent process on oral bioavailability. In practice, and

Table 2
Pharmacokinetic data for the 164 drugs used to test the model applicability in predicting the human absolute bioavailability. F_{oral} is the drug oral bioavailability; f_{renal} is the percentage of parent drug eliminated in urine; f_p is the percentage of drug bound to plasma proteins; Cl_{plasma} is the drug total clearance determined in plasma; R_b is the blood to plasma concentration ratio of the drug; In drug class, A stands for acid, B: basic, N: neutral and Z: zwitterionic drug at pH 7.4; Cl_H is the drug blood hepatic clearance according to Eqs. (6) or (7); F_{abs} is the relative amount of drug absorbed according to Eq. (9) and F_{met} the relative amount of drug escaping the liver first-pass effect according to Eq. (8).

Drug	F_{oral}	f_{renal}	f_p	Cl_{plasm} (ml.min^{-1}.kg^{-1})	R_b	Drug class	Cl_H	F_{abs}	F_{met}	
Acebutolol	0.37	0.40	0.26	6.8	1.00	B	4.1	0.46	0.80	(A)
Acetaminophen	0.88	0.30	0.20	5.0	1.04	B	2.4	1.00	0.88	(B)
Acyclovir	0.30	0.75	0.15	6.2	1.08	N	1.4	0.32	0.93	(B)
Alendronate	0.02	0.45	0.78	1.1	1.70	Z	0.4	0.02	0.98	(B)
Allopurinol	0.90	0.12	0.01	9.9	1.09	N	1.9	1.00	0.90	(B) (C)
Alprazolam	0.88	0.20	0.71	0.7	0.78	N	0.8	0.91	0.96	(B)
Amiodarona	0.46	0.00	1.00	1.9	0.73	B	2.6	0.53	0.87	(B)
Amitriptyline	0.48	0.02	0.95	11.5	0.86	B	10.4	1.00	0.48	(B)
Amlodipine	0.74	0.10	0.93	5.9	1.20	B	4.4	0.95	0.78	(B)
Amoxicillin	0.50	0.86	0.18	2.6	1.04	A	0.4	0.51	0.98	(B)
Antipyrine	1.00	0.95	0.10	1.5	1.00	N	0.1	1.00	1.00	(D) (E)
Aprepitant	0.59	0.00	0.95	1.3	0.60	N	2.1	0.66	0.89	(B) (F)
Atenolol	0.58	0.94	0.05	2.4	1.07	B	0.1	0.58	0.99	(B)
Benzydamine	0.87	0.55	0.20	2.3	1.00	B	1.0	0.92	0.95	(G) (H) (I)
Bepridil	0.60	0.01	0.99	5.3	0.67	B	7.8	0.99	0.61	(A)
Betaxolol	0.89	0.15	0.55	4.7	1.00	B	2.2	1.00	0.89	(A)
Bisoprolol	0.90	0.63	0.35	3.7	1.00	B	1.4	0.97	0.93	(A)
Bosentan	0.50	0.01	0.98	2.2	1.00	A	2.2	0.56	0.89	(J)
Bromocriptine	0.05	0.02	0.93	5.0	1.00	B	4.9	0.07	0.76	(A)
Bufuralol	0.46	0.00	0.85	6.2	1.00	B	6.2	0.67	0.69	(K)
Buspirone	0.04	0.00	0.95	28.3	0.62	B	19.2	1.00	0.04	(B)
Caffeine	1.00	0.01	0.36	1.4	0.80	N	0.0	1.00	1.00	(A)
Calcitriol	0.61	0.10	1.00	0.4	0.55	N	0.7	0.63	0.96	(B)
Candesartan	0.42	0.52	1.00	0.4	0.55	A	0.3	0.43	0.98	(B)
Carbamazepine	0.70	0.01	0.74	0.9	1.06	N	0.9	0.73	0.96	(B)
Carvedilol	0.25	0.02	0.95	8.7	0.72	B	11.9	0.62	0.41	(B)
Cefixime	0.40	0.41	0.67	1.3	0.62	A	1.3	0.43	0.94	(B)
Cephalexin	0.90	0.91	0.14	4.3	1.02	A	0.4	0.92	0.98	(B)
Cetirizine	0.85	0.71	0.99	0.5	1.00	Z	0.2	0.86	0.99	(B)
Chlorpheniramine	0.59	0.10	0.70	1.7	1.34	B	1.1	0.63	0.94	(B)
Chlorpromazine	0.40	0.01	0.97	8.6	0.78	B	10.9	0.88	0.45	(B)
Chlorprothixene	0.41	0.00	0.99	12.4	0.81	B	11.8	1.00	0.41	(L)
Chlorthalidone	0.64	0.65	0.75	0.0	0.73	N	0.0	0.64	1.00	(B)
Cimetidine	0.60	0.62	0.19	8.3	0.97	B	3.3	0.72	0.84	(B)
Cinacalcet	0.25	0.00	0.95	18.0	0.64	B	15.0	1.00	0.25	(B)
Ciprofloxacin	0.60	0.50	0.40	7.6	1.07	Z	3.6	0.73	0.82	(B)
Clindamycin	0.53	0.13	0.94	4.7	0.76	B	2.6	0.61	0.87	(B) (M)
Clonidine	0.75	0.62	0.20	3.1	1.04	B	1.1	0.79	0.94	(B)
Clozapine	0.55	0.01	0.95	6.1	1.13	B	5.4	0.75	0.73	(B)
Cyclophosphamide	0.88	0.07	0.13	1.3	1.06	N	1.1	0.93	0.94	(B)
Dapsone	0.86	0.15	0.73	0.6	1.04	N	0.5	0.88	0.98	(B)
Desipramine	0.38	0.02	0.82	10.0	0.96	B	10.2	0.78	0.49	(A)
Diazepam	1.00	0.01	0.99	0.4	0.58	N	0.7	1.00	0.97	(B)
Diclofenac	0.64	0.01	1.00	4.2	0.56	A	7.2	1.00	0.64	(B) (N)
Dicloxacillin	0.49	0.60	0.96	1.6	0.55	A	1.2	0.52	0.94	(B)
Didanosine	0.38	0.36	0.05	16.0	1.08	N	9.5	0.72	0.52	(B)
Diltiazem	0.38	0.04	0.78	11.8	1.00	B	11.3	0.88	0.43	(B)
Diphenhydramine	0.72	0.02	0.78	6.2	0.65	B	5.6	1.00	0.72	(B)
Dofetilide	0.96	0.52	0.64	5.2	0.72	B	0.8	1.00	0.96	(B)
Doxycycline	0.93	0.41	0.88	0.5	1.70	Z	0.2	0.94	0.99	(B)
Entacapone	0.46	0.00	0.98	10.3	0.55	A	10.8	1.00	0.46	(B)
Ethambutol	0.77	0.79	0.18	8.6	0.96	B	1.9	0.85	0.91	(B)
Etoposide	0.52	0.35	0.96	0.7	0.55	N	0.8	0.54	0.96	(B)
Famotidine	0.45	0.67	0.17	7.1	1.00	N	2.3	0.51	0.88	(A)
Finasteride	0.63	0.01	0.90	2.3	0.56	N	4.1	0.79	0.80	(B)
Flecainide	0.74	0.43	0.61	5.6	0.89	B	3.6	0.90	0.82	(B)
Fluconazole	0.90	0.75	0.11	0.3	1.06	N	0.1	0.90	1.00	(B)
Flumazenil	0.16	0.00	0.40	9.9	1.00	N	9.9	0.32	0.51	(B)
Fluorouracil	0.28	0.10	0.10	16.0	1.09	N	13.2	0.82	0.34	(B)
Fluphenazine	0.03	0.00	0.92	10.0	0.69	B	14.6	0.10	0.27	(B)
Foscarnet	0.09	0.95	0.15	1.6	1.27	A	0.1	0.09	1.00	(B)
Furosemide	0.43	0.66	0.99	2.0	0.55	A	1.2	0.46	0.94	(B) (O)
Gabapentin	0.60	0.66	0.03	1.6	1.10	Z	0.5	0.62	0.98	(B)
Galantamine	0.95	0.20	0.18	5.7	1.04	B	1.0	1.00	0.95	(B)
Ganciclovir	0.09	0.91	0.01	3.4	1.08	N	0.3	0.09	0.99	(B)
Gemfibrozil	0.95	0.01	0.97	1.7	0.55	A	1.0	1.00	0.95	(B)
Glimepiride	1.00	0.01	1.00	0.6	0.55	A	0.0	1.00	1.00	(B)
Glyburide	0.73	0.00	1.00	1.3	0.56	A	2.3	0.83	0.88	(B) (P)
Granisetron	0.60	0.16	0.65	11.0	0.86	B	8.0	1.00	0.60	(B)
Hydrochlorothiazide	0.71	0.95	0.58	4.9	1.70	N	0.1	0.72	0.99	(B)
Hydromorphone	0.42	0.06	0.07	14.6	1.07	B	12.8	1.00	0.36	(B)
Ibuprofen	0.80	0.01	0.99	0.6	0.55	A	1.1	0.85	0.95	(B)

Table 2 (*Continued*)

Drug	F_{oral}	f_{renal}	f_p	Cl_{plasm} (ml.min^{-1}.kg^{-1})	R_b	Drug class	Cl_H	F_{abs}	F_{met}	
Imatinib	0.98	0.05	0.95	3.3	0.64	B	0.4	1.00	0.98	(B)
Imipramine	0.42	0.02	0.90	15.0	1.10	B	11.6	1.00	0.42	(B)
Irbesartan	0.70	0.02	0.90	2.1	0.64	Z	3.3	0.84	0.84	(B)
Isosorbide dinitrate	0.22	0.01	0.28	46.0	1.00	N	15.6	1.00	0.22	(B)
Isosorbide-5-mononitrate	0.93	0.05	0.00	1.8	1.08	N	1.6	1.00	0.92	(B)
Isradipine	0.17	0.00	0.97	10.0	0.55	N	16.2	0.89	0.19	(A)
Ketoprofen	1.00	0.01	0.99	1.2	1.00	A	0.0	1.00	1.00	(A)
Lamivudine	0.82	0.50	0.36	5.0	1.06	N	2.3	0.93	0.88	(B)
Lansoprazole	0.81	0.01	0.97	6.2	0.56	N	3.8	1.00	0.81	(B)
Letrozole	1.00	0.04	0.60	0.6	0.92	N	0.0	1.00	1.00	(B)
Levetiracetam	1.00	0.66	0.10	1.0	1.07	N	0.3	1.00	0.98	(B)
Levofloxacin	0.99	0.70	0.30	2.5	1.05	Z	0.2	1.00	0.99	(B)
Lidocaine	0.37	0.02	0.70	9.2	0.84	B	10.7	0.80	0.46	(B)
Linezolid	1.00	0.35	0.31	2.1	0.73	N	0.0	1.00	1.00	(B)
Lorazepam	0.93	0.01	0.91	1.1	1.05	N	1.0	0.98	0.95	(B)
Losartan	0.36	0.12	0.99	8.1	0.55	A	12.9	1.00	0.35	(B)
Meloxicam	0.97	0.01	0.99	0.2	1.22	A	0.1	0.98	0.99	(B)
Melphalan	0.56	0.12	0.90	5.2	0.96	Z	4.8	0.74	0.76	(B) (Q)
Meperidine	0.52	0.05	0.58	17.0	0.87	B	9.6	1.00	0.52	(B)
Mercaptopurine	0.12	0.22	0.19	11.0	1.20	N	7.2	0.19	0.64	(B)
Metformin	0.52	1.00	0.00	7.6	1.04	B	0.0	0.52	1.00	(B)
Methadone	0.86	0.24	0.89	1.7	0.75	B	1.6	0.93	0.92	(B) (R)
Methotrexate	0.70	0.81	0.46	2.1	0.71	A	0.6	0.72	0.97	(B)
Methylprednisolone	0.82	0.05	0.78	6.2	0.78	N	3.6	1.00	0.82	(B)
Metoclopramide	0.76	0.20	0.40	6.2	0.96	B	4.8	1.00	0.76	(B)
Metoprolol	0.38	0.10	0.11	15.0	1.00	B	12.4	1.00	0.38	(B)
Metronidazole	0.99	0.10	0.11	1.3	1.07	N	0.2	1.00	0.99	(B)
Midazolam	0.44	0.01	0.98	6.6	0.80	N	8.2	0.74	0.59	(B)
Montelukast	0.62	0.00	0.99	0.7	0.55	A	1.3	0.66	0.94	(B)
Morphine	0.24	0.04	0.35	24.0	0.95	B	15.2	1.00	0.24	(B)
Moxifloxacin	0.86	0.22	0.39	2.3	1.05	Z	1.7	0.94	0.92	(B)
Nadolol	0.34	0.73	0.20	2.9	1.00	B	0.8	0.35	0.96	(A)
Nalmefene	0.40	0.10	0.34	15.0	1.11	B	12.2	1.00	0.39	(B)
Naloxone	0.02	0.00	0.30	22.0	1.22	B	18.0	0.20	0.10	(B)
Naproxen	0.99	0.01	1.00	0.1	1.00	A	0.1	1.00	0.99	(B)
Nifedipine	0.50	0.00	0.96	7.0	1.63	N	4.3	0.64	0.79	(B)
Nitrendipine	0.23	0.01	0.98	21.0	0.70	N	15.5	1.00	0.23	(A) (S)
Nitrofurantoin	0.90	0.47	0.62	9.9	0.76	A	2.0	1.00	0.90	(B)
Nortriptyline	0.56	0.02	0.92	7.2	1.50	B	4.7	0.73	0.76	(B)
Omeprazole	0.71	0.00	0.95	7.5	0.58	N	5.8	1.00	0.71	(A)
Ondansetron	0.62	0.05	0.73	5.9	0.83	B	6.8	0.94	0.66	(B)
Oxazepam	0.97	0.01	0.99	1.1	0.66	N	0.6	1.00	0.97	(A)
Oxycodone	0.42	0.19	0.45	12.4	1.03	B	9.8	0.82	0.51	(B)
Phenacetin	0.37	0.40	0.33	20.0	1.01	N	11.9	0.91	0.41	(T)
Phenobarbital	1.00	0.24	0.51	0.1	0.86	N	0.1	1.00	1.00	(B)
Phenytoin	0.90	0.02	0.89	5.9	1.00	N	2.0	1.00	0.90	(B)
Pindolol	0.75	0.54	0.51	8.3	1.00	B	3.8	0.93	0.81	(A)
Pirenzepine	0.33	0.90	0.11	3.8	1.00	B	0.4	0.34	0.98	(U) (V)
Pravastatin	0.18	0.47	0.45	13.5	0.55	A	13.0	0.51	0.35	(B)
Prazosin	0.68	0.04	0.95	3.0	0.70	N	4.1	0.86	0.79	(B)
Prednisone	0.80	0.03	0.75	3.6	1.00	N	3.5	0.97	0.83	(B)
Procainamide	0.83	0.67	0.16	1.7	1.00	B	0.6	0.85	0.97	(B)
Propafenone	0.05	0.01	0.95	17.0	0.70	B	14.0	0.17	0.30	(A)
Propofol	0.00	0.00	0.98	27.0	1.25	N	20.0	1.00	0.00	(B)
Propranolol	0.26	0.01	0.87	16.0	0.89	B	14.8	1.00	0.26	(B)
Quetiapine	0.09	0.01	0.83	19.0	0.90	B	18.2	1.00	0.09	(B)
Quinidine	0.75	0.18	0.87	4.7	0.88	B	4.4	0.96	0.78	(B)
Quinine	0.76	0.16	0.90	0.9	0.91	B	0.8	0.79	0.96	(B)
Ranitidine	0.52	0.69	0.15	10.4	1.03	B	3.1	0.62	0.84	(B)
Repaglinide	0.56	0.01	0.97	9.3	0.55	A	8.8	1.00	0.56	(B)
Riluzole	0.60	0.01	0.98	5.5	1.70	N	3.2	0.71	0.84	(B)
Risedronate	0.01	0.87	0.24	1.5	1.07	A	0.2	0.01	0.99	(B)
Risperidone	0.66	0.03	0.89	5.4	0.67	B	6.8	1.00	0.66	(B)
Rizatriptan	0.47	0.28	0.14	12.3	1.04	B	8.5	0.82	0.57	(B)
Scopolamine	0.29	0.06	0.10	15.5	1.00	B	14.2	1.00	0.29	(W)
Sildenafil	0.40	0.00	0.96	6.0	0.99	N	6.0	0.57	0.70	(B)
Sulfamethoxazole	1.00	0.14	0.53	0.3	0.79	A	0.3	1.00	0.98	(B)
Sulpiride	0.27	0.74	0.00	5.9	1.00	B	1.5	0.29	0.92	(X)
Sumatriptan	0.14	0.22	0.18	22.0	1.03	B	16.7	0.84	0.17	(B)
Tamsulosin	1.00	0.13	0.99	0.6	0.55	B	1.0	1.00	0.95	(B)
Tegaserod	0.11	0.00	0.98	18.0	0.72	B	17.8	1.00	0.11	(B)
Tenoxicam	0.95	0.00	0.99	0.0	0.67	A	0.1	0.95	1.00	(Y)
Terazosin	0.82	0.13	0.92	1.2	0.84	N	1.2	0.87	0.94	(B)
Terbutaline	0.26	0.57	0.23	3.4	1.00	B	1.5	0.28	0.93	(Z) (AA)
Tetracycline	0.77	0.58	0.65	1.7	1.70	Z	0.4	0.79	0.98	(B)
Theophylline	0.96	0.18	0.56	0.7	1.33	N	0.4	0.98	0.98	(B)
Timolol	0.75	0.08	0.10	7.7	0.87	B	4.8	1.00	0.75	(B) (AB)

Table 2 (*Continued*)

Drug	F_{oral}	f_{renal}	f_p	Cl_{plasm} (ml.min^{-1}.kg^{-1})	R_b	Drug class	Cl_H	F_{abs}	F_{met}	
Tolbutamide	0.85	0.00	0.96	0.2	0.55	A	0.4	0.87	0.98	(B)
Tramadol	0.70	0.20	0.20	8.0	1.03	B	6.2	1.00	0.69	(B)
Trazodone	0.81	0.01	0.93	2.1	0.81	N	2.6	0.93	0.87	(B)
Triazolam	0.86	0.02	0.90	2.5	0.62	N	2.8	1.00	0.86	(A)
Trimethoprim	0.63	0.63	0.37	1.9	1.03	N	0.7	0.65	0.97	(B)
Valproic acid	1.00	0.02	0.93	0.1	0.64	A	0.2	1.00	0.99	(B)
Valsartan	0.23	0.29	0.95	0.5	0.55	A	0.6	0.24	0.97	(B)
Verapamil	0.22	0.03	0.90	15.0	0.77	B	15.6	1.00	0.22	(B)
Vinorelbine	0.27	0.11	0.87	21.0	0.58	B	14.6	1.00	0.27	(B)
Vinpocetine	0.57	0.00	0.66	5.2	0.57	B	8.7	1.00	0.57	(AC)
Warfarin	0.93	0.02	0.99	0.1	0.55	A	0.1	0.93	1.00	(B)
Zaleplon	0.31	0.01	0.60	15.7	0.99	N	13.8	1.00	0.31	(B)
Zidovudine	0.63	0.18	0.25	26.0	1.06	N	7.4	1.00	0.63	(B)
Ziprasidone	0.59	0.01	1.00	11.7	0.81	B	8.2	1.00	0.59	(B)
Zolpidem	0.72	0.01	0.92	4.5	0.76	N	5.6	1.00	0.72	(B)

References for the pharmacokinetic data are from (A) Goodman et al. (1996), (B) Goodman et al. (2006), (C) Breithaupt and Tittel (1982), (D) Rimmer et al. (1986), (E) Atiba et al. (1987), (F) Majumdar et al. (2006), (G) Koppel and Tenczer (1985), (H) Chasseaud and Catanese (1985), (I) Baldock et al. (1991), (J) Weber et al. (1999), (K) Balant et al. (1980), (L) Raaflaub (1975), (M) Gatti et al. (1993), (N) Hinz et al. (2005), (O) Smith et al. (1980), (P) Neugebauer et al. (1985), (Q) Alberts et al. (1979), (R) Dale et al. (2004), (S) Mikus et al. (1987), (T) Raaflaub and Dubach (1975), (U) Vergin et al. (1986), (V) Lee et al. (1986), (W) Putcha et al. (1989), (X) Wiesel et al. (1980), (Y) Heintz et al. (1984), (Z) Fagerstrom (1984), (AA) Nyberg (1984), (AB) Else et al. (1978), (AC) Vereczkey et al. (1979). R_b values are from Paixao et al. (2009).

especially in early phases of the lead development, *in vivo* data are not available and extrapolations or allometric scaling approaches are typically used. In our study, we used both *in silico* and *in vitro* based estimations of permeability and metabolic activity. Since a complete pH solubility profile was needed for each drug and these data were not available for the majority of the drugs, only *in silico* estimations of solubility were used. In all cases, and taking in consideration the well described solubility/dose effect on bioavailability (Benet et al., 2011; Rinaki et al., 2003; Takagi et al., 2006), the studied doses (Tables 3–6) were the largest that are described for clinical practice (Ritschel, 2000).

In silico estimated solubility values were obtained using the on-line ADME Boxes (http://www.pharma-algorithms.com/webboxes) considering the "In buffer solubility" option and the pH values of the stomach, duodenum, jejunum and ileum. In a recent paper testing the predictive performance of various *in silico* solubility models in a new data set of 122 drugs, ADME Boxes presented 59% of well predicted drugs within ±0.5 log unit of measured value and a standard error of 0.62 log values (Dearden, 2006).

In order to estimate the effective permeability of the different drugs in the GIT, both *in silico* and *in vitro* apparent permeabilities values based on the Caco-2 cell system were used. *In vitro* values were collected in the literature, under similar experimental conditions, namely, experimental pH values ranging from 6.8 to 7.4, with low to median passage numbers (28–46) and typically at a cell age close to 21 days. For drugs without *in vitro* data available, an *in silico* ANN model was used (Paixao et al., 2010b). This model was based on calculated molecular descriptors for a total of 296 *in vitro* Caco-2 apparent permeability (P_{app}) drug values also collected in the literature. The model presented correlations of 0.843 and 0.702 and a root mean square error (RMSE) of 0.546 and 0.791 for the train (N = 192) and test (N = 59) group respectively. An external validation step was also performed with an additional group of 45 drugs resulting in a correlation of 0.774 and RMSE of 0.601. P_{app} values were used to estimate the effective human permeability (P_{eff}) by performing a multiple linear regression, based on 29 reference drugs with known human effective permeability, resulting in the equation $\log(P_{eff})(\text{cm h}^{-1}) = 0.932 + 0.763 \times \log(P_{app})(\text{cm h}^{-1}) + 0.0324 \times \text{RBN}$ (RBN being the number of rotable bonds in the molecule) and presenting an r = 0.887 and RMSE = .301 (Fig. 2).

To estimate the *in vivo* hepatic intrinsic clearance both *in silico* and *in vitro* intrinsic clearance (Cl_{int}) values based on suspensions of human hepatocytes were used. *In vitro* Cl_{int} values were obtained from published studies on drug metabolism in human hepatocytes using the substrate depletion method in absence of added serum. For drugs without *in vitro* data available, an *in silico* ANN model was again used (Paixao et al., 2010a). This ANN model was built based only on calculated molecular descriptors and 89 *in vitro* Cl_{int} values. Data were divided into a train group of 71 drugs for network optimization and a test group of another 18 drugs for early-stop and internal validation resulting in correlations of 0.953 and 0.804 for the train and test group, respectively. The external validation was made with another 112 drugs by comparing the *in silico* predicted Cl_{int} with the *in vivo* Cl_{int} estimated by the "well-stirred" model based on the *in vivo* hepatic clearance (Cl_H). Acceptable correlations were observed with r values of 0.508 and 63% of drugs within a 10-fold difference when considering blood binding in acidic drugs only. In order to scale the *in silico* and *in vitro* Cl_{int} values to the *in vivo* Cl_{int}, hepatocellularity was considered to be 107×10^6 cell g^{-1} liver (Wilson et al., 2003) and it was also assumed that liver weighed 20 g kg^{-1} of body weight.

To test the applicability of the PBPK based absorption model, data from Table 2 were grouped in terms of P_{app} and Cl_{int} source. *In vitro* data for both P_{app} and Cl_{int} were available for 49 drugs (Table 3). *In vitro* data for Cl_{int} only were available for 25 drugs (Table 4). *In vitro* data for P_{app} only were available in another 22 drugs (Table 5). For the remaining 68 drugs, *in silico* based data were used (Table 6).

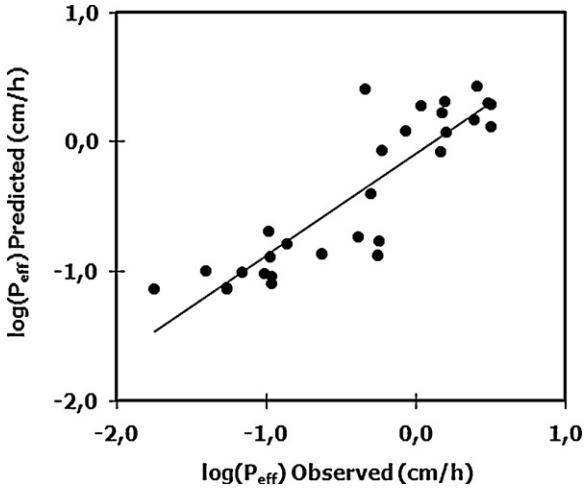

Fig. 2. Relationship between the predicted logarithm of the effective human jejunal permeability by the multiple linear regression and the *in vivo* observed values.

Table 3
Drug parameters and predicted bioavailability by the pharmacokinetic model for the 49 drugs with *In vitro* data for both P_{app} and Cl_{int}.

Drug	Data parameters							Observed	Predicted			
	In vitro Cl_{int} ($L\,h^{-1}$)	*In vitro* P_{eff} ($cm\,h^{-1}$)	F_{oral} (%)	Si pH 4.6 (mg/ml)	Si pH 6.5 (mg/ml)	Si pH 7.5 (mg/ml)	Dose max (mg)	F_{oral} (%)	F_d	F_{perm}	F_{met}	F_{oral} (%)
Acebutolol	16.18	0.21	106.40	106.40	106.40	21.23	200	37	1.00	0.62	0.83	51
Acetaminophen	6.97	1.96	7.58	7.58	7.58	7.58	2000	88	1.00	1.00	0.92	92
Antipyrine	4.22	1.87	19.71	14.95	14.95	14.95	600	100	1.00	1.00	0.95	95
Atenolol	0.90	0.12	923.64	923.64	923.64	473.70	50	58	1.00	0.43	0.99	42
Betaxolol	22.47	1.80	82.76	4138	82.76	15.77	40	89	1.00	1.00	0.78	78
Bosentan	13.48	0.19	0.00	0.02	0.01	0.06	600	50	0.08	0.43	0.99	3
Bromocriptine	332.56	0.20	17.22	860.98	17.22	2.17	5	5	1.00	0.61	0.20	12
Caffeine	21.12	1.70	31.50	31.50	31.50	31.50	350	100	1.00	1.00	0.79	79
Carbamazepine	12.58	2.03	0.04	0.04	0.04	0.04	200	70	0.62	0.94	0.86	51
Chlorpromazine	72.49	1.55	3.50	174.83	2.78	0.36	100	40	1.00	0.99	0.53	52
Cimetidine	10.79	0.24	390.91	390.91	390.91	310.51	400	60	1.00	0.66	0.88	58
Clozapine	53.93	1.73	179.62	1055	0.29	0.06	150	55	1.00	1.00	0.60	60
Desipramine	62.92	0.95	59.64	2982	59.64	40.32	50	38	1.00	0.97	0.56	55
Diazepam	10.08	1.92	2.26	0.07	0.07	0.07	15	100	1.00	1.00	0.89	89
Diclofenac	395.47	1.43	0.03	3.81	4.09	30.31	50	64	1.00	0.99	0.96	95
Diltiazem	116.84	2.60	16.89	16.89	0.87	0.13	120	38	1.00	1.00	0.41	41
Famotidine	0.90	0.15	1019	16.15	0.29	0.11	40	45	1.00	0.51	0.99	50
Furosemide	0.09	0.06	0.29	3.98	194.77	630.27	40	43	1.00	0.24	1.00	24
Ibuprofen	37.75	1.91	0.06	8.38	9.01	58.15	800	80	1.00	1.00	0.99	99
Imipramine	71.90	0.68	28.05	1402	23.87	3.01	200	42	1.00	0.94	0.53	50
Ketoprofen	22.47	2.27	0.02	3.19	3.35	25.43	200	100	1.00	1.00	0.99	99
Lidocaine	120.44	3.05	575.33	575.33	66.06	12.88	750	37	1.00	1.00	0.40	40
Methylprednisolone	87.18	0.89	0.08	0.08	0.08	0.08	24	82	0.99	0.94	0.48	45
Metoprolol	62.92	2.68	558.70	558.70	558.70	217.36	100	38	1.00	1.00	0.56	56
Morphine	215.71	0.60	220.28	11014	87.69	13.27	30	24	1.00	0.92	0.27	25
Nadolol	0.90	0.14	759.61	759.61	692.77	245.80	120	34	1.00	0.49	0.99	48
Naloxone	714.55	1.41	568.97	568.97	45.20	7.85	20	2	1.00	0.99	0.10	10
Naproxen	35.95	1.53	0.18	27.62	27.06	148.68	250	99	1.00	0.99	0.99	99
Nitrendipine	66.51	1.28	0.27	0.09	0.09	0.09	20	23	1.00	0.98	0.55	54
Ondansetron	12.58	2.52	40.50	2025	2.28	0.32	8	62	1.00	1.00	0.87	86
Oxazepam	17.98	2.87	0.03	0.81	0.02	0.02	25	97	0.97	1.00	0.82	79
Phenytoin	26.47	1.93	0.16	0.16	0.16	0.18	300	90	0.99	0.97	0.75	73
Pindolol	25.17	1.76	2213	2213	1567	988.74	20	75	1.00	1.00	0.76	76
Pirenzepine	0.90	0.07	1976	734.28	37.66	5.83	10	33	1.00	0.29	0.99	29
Prazosin	20.67	0.58	541.64	171.28	4.40	1.84	5	68	1.00	0.91	0.80	72
Propofol	961.72	1.18	0.21	10.47	0.21	0.21	1	0	1.00	0.98	0.08	8
Propranolol	116.84	2.04	482.99	24149	482.99	175.36	80	26	1.00	1.00	0.41	41
Quinidine	49.43	1.02	893.64	893.64	67.79	10.50	400	75	1.00	0.98	0.62	61
Ranitidine	8.99	0.14	1505	703.99	38.69	5.59	150	52	1.00	0.49	0.90	44
Scopolamine	62.92	1.11	1076	1076	152.06	35.65	0.5	29	1.00	0.98	0.56	55
Sildenafil	46.74	2.70	2795	368.45	9.47	2.86	50	40	1.00	1.00	0.63	63
Sulpiride	0.09	0.06	304.34	304.34	76.45	11.31	100	27	1.00	0.25	1.00	25
Terbutaline	0.09	0.12	1006	1006	528.20	196.25	5	26	1.00	0.43	1.00	43
Theophylline	4.49	1.34	17.61	17.61	18.02	18.02	400	96	1.00	0.99	0.95	94
Tolbutamide	9.71	3.50	0.19	0.24	4.00	28.31	500	85	1.00	1.00	0.99	99
Valproic acid	4.40	1.98	2.95	4.46	83.00	262.47	500	100	1.00	1.00	0.99	99
Verapamil	269.64	2.49	8.66	433.18	3.45	0.44	120	22	1.00	1.00	0.23	23
Warfarin	9.89	1.76	0.20	16.52	10.45	69.03	6	93	1.00	1.00	1.00	99
Zidovudine	28.77	0.76	15.74	15.74	15.74	15.74	350	63	1.00	0.95	0.74	70

2.5. Statistical and pharmacokinetic analysis

Since bioavailability values range from 0% and 100%, correlation between the predicted and observed values was determined by means of the Spearman rank correlation coefficient (r_s) for the four groups of data. In order to assess the precision and bias of the model, RMSE and mean error (ME), respectively, were also calculated by using the following equations.

$$\mathrm{RMSE} = \sqrt{\frac{\sum (F_{pred} - F_{obs})^2}{N}} \quad (4)$$

$$\mathrm{ME} = \frac{\sum (F_{pred} - F_{obs})}{N} \quad (5)$$

Percentage of correct values within an absolute ±20% margin error was determined in order to test the quantitative ability of the model to predict absolute bioavailability. Additionally, percentage of correct values within $a \pm 35\%$ error was also determined in order to test the qualitative predictive ability of the model. A multifactor ANOVA analysis was also performed, in order to establish the effect of data origin (*in silico vs in vitro*) for P_{app} and Cl_{int} as well as differences from the different drug classes (acidic, basic, neutral and zwitterionic) on the oral bioavailability prediction, assessed by the squared residuals between predicted and observed values.

In vivo Cl_H values from Table 2 drugs were determined by using Eq. (6) (Naritomi et al., 2003),

$$\mathrm{CL_H} = \frac{\mathrm{CL_{total}^{plasma}}}{R_b} \times (1 - f_{renal}) \quad (6)$$

This equation assumes that total blood clearance, determined by the ratio between the described total plasma clearance ($\mathrm{CL_{total}^{plasma}}$) to the drug blood-to-plasma concentration ratio (R_b), is the sum of Hepatic and Renal Clearance, the last being determined by using the fraction of drug eliminated by the kidneys (f_{renal}). Some drugs, however, may have other non renal elimination routes besides the

Table 4
Drug parameters and predicted bioavailability by the pharmacokinetic model for the 25 drugs with *In vitro* data for Cl_{int} only.

Drug	Data parameters							Observed	Predicted			
	In vitro Cl_{int} ($L\,h^{-1}$)	*In silico* P_{eff} ($cm\,h^{-1}$)	Si pH 1.5 (mg/ml)	Si pH 4.6 (mg/ml)	Si pH 6.5 (mg/ml)	Si pH 7.5 (mg/ml)	Dose max (mg)	F_{oral} (%)	F_d	F_{perm}	F_{met}	F_{oral} (%)
Benzydamine	184.40	0.92	269.52	13476	19.52	2.46	5	87	1.00	0.97	0.31	30
Bepridil	17.98	1.87	220.90	11045	6.67	0.88	200	60	1.00	1.00	0.82	82
Bisoprolol	14.38	0.87	527.90	527.90	527.90	178.88	10	90	1.00	0.96	0.85	82
Bufuralol	62.92	2.37	75.39	3769	41.43	5.85	60	46	1.00	1.00	0.56	56
Carvedilol	314.58	0.79	4.07	203.26	0.13	0.02	12.5	25	1.00	0.95	0.20	20
Cetirizine	0.90	0.39	42.65	0.42	0.37	0.47	10	85	1.00	0.81	0.99	81
Chlorpheniramine	25.17	1.39	1045	52242	315.54	73.97	4	59	1.00	0.99	0.76	76
Chlorprothixene	125.83	2.27	3.02	150.84	1.20	0.15	100	41	1.00	1.00	0.39	39
Diphenhydramine	53.93	1.56	286.55	14328	97.10	16.11	44	72	1.00	0.99	0.60	60
Gemfibrozil	197.74	1.02	0.08	6.42	3.46	25.62	600	95	1.00	0.98	0.88	86
Granisetron	80.89	1.45	1059	1059	431.31	110.86	1	60	1.00	0.99	0.50	50
Isradipine	161.78	3.36	0.14	0.05	0.05	0.05	5	17	0.98	1.00	0.33	33
Lorazepam	5.71	1.52	0.02	0.61	0.01	0.01	2	93	0.98	0.99	0.93	91
Midazolam	106.28	1.57	17.10	4.38	0.00	0.00	10	44	1.00	0.99	0.43	43
Nifedipine	59.77	1.51	0.62	0.21	0.21	0.21	10	50	1.00	0.99	0.58	57
Nortriptyline	24.63	1.08	13.20	660.09	13.20	6.77	125	56	1.00	0.98	0.77	75
Omeprazole	15.28	3.53	165.35	0.74	0.46	0.48	20	71	1.00	1.00	0.84	84
Phenacetin	67.41	1.72	1.36	1.36	1.36	1.36	1000	37	1.00	1.00	0.55	54
Prednisone	87.18	1.44	0.11	0.11	0.11	0.11	10	80	1.00	0.99	0.48	48
Procainamide	11.68	0.72	1076	1076	438.28	135.44	1000	83	1.00	0.94	0.87	82
Propafenone	517.71	0.51	121.17	6058	121.17	24.74	150	5	1.00	0.89	0.14	12
Tenoxicam	23.37	3.07	0.49	0.51	8.67	58.64	20	95	1.00	1.00	1.00	100
Triazolam	14.29	1.35	8.23	0.57	0.00	0.00	0.25	86	1.00	0.99	0.85	84
Vinpocetine	2336.88	0.37	78.47	3923	11.61	1.57	20	57	1.00	0.80	0.03	3
Zolpidem	32.27	2.45	172.88	1016	0.50	0.28	10	72	1.00	1.00	0.72	71

hepatic one. In these cases, it is expected that Cl_H determined by Eq. (6) would be over predicted. To minimize this, *in vivo* Cl_H was also determined by Eq. (7) (Iwatsubo et al., 1997),

$$CL_H = Q_H \times (1 - F_{oral}) \quad (7)$$

This equation assumes that oral bioavailability (F_{oral}) is only a result of the first pass-effect in the liver, allowing the determination of the liver extraction ratio (E_H) and the Cl_H by multiplying E_H with the hepatic blood flow rate (Q_H) with a value of $20\,ml\,min^{-1}\,kg^{-1}$. Although Eq. (7) could also provide over predicted values for Cl_H, in theory these would be the maximum possible values for this parameter. For this reason, when comparing Cl_H determined by Eq. (6) to the value obtained with Eq. (7), if the first is bigger the latter prevails. For these drugs, the relative amount of drug escaping the first-pass effect (F_{met}) is equal to F_{oral} resulting that the relative amount of drug absorbed (F_{abs}) presents a value of 1. For the remaining drugs, F_{met} was determined by using Eq. (8),

$$F_{met} = 1 - \left(\frac{Cl_H}{Q_H}\right) \quad (8)$$

And F_{abs}, including both the effect of *in vivo* solubility and *in vivo* permeability, was determined by using Eq. (9),

$$F_{abs} = \frac{F_{oral}}{F_{met}} \quad (9)$$

Table 5
Drug parameters and predicted bioavailability by the pharmacokinetic model for the 22 drugs with *In vitro* data for P_{app} only.

Drug	Data parameters							Observed	Predicted			
	In silico Cl_{int} ($L\,h^{-1}$)	*In vitro* P_{eff} ($cm\,h^{-1}$)	Si pH 1.5 (mg/ml)	Si pH 4.6 (mg/ml)	Si pH 6.5 (mg/ml)	Si pH 7.5 (mg/ml)	Dose max (mg)	F_{oral} (%)	F_d	F_{perm}	F_{met}	F_{oral} (%)
Acyclovir	30.79	0.14	23.05	5.79	5.79	5.92	400	30	1.00	0.48	0.72	35
Amoxicillin	7.77	0.09	2.65	0.30	0.34	6.65	3000	50	0.87	0.28	0.93	23
Cephalexin	16.81	0.08	1.03	0.14	0.16	0.32	500	90	0.66	0.30	0.85	17
Ciprofloxacin	3.83	0.17	208.80	9.39	1.56	1.35	500	60	1.00	0.55	0.95	53
Clonidine	191.67	1.51	148.57	7429	6.05	0.96	0.1	75	1.00	0.99	0.30	30
Doxycycline	0.03	0.86	46.54	1.25	1.20	1.22	100	93	1.00	0.96	1.00	96
Etoposide	333.91	0.24	0.07	0.07	0.07	0.07	100	52	0.51	0.60	0.20	6
Fluconazole	0.78	1.34	7.52	1.50	1.50	1.50	200	90	1.00	0.99	0.99	98
Foscarnet	0.01	0.01	457.51	1414	1414	1414	560	9	1.00	0.05	1.00	5
Gabapentin	3.89	0.00	241.93	7.48	6.51	6.51	600	60	1.00	0.01	0.95	1
Ganciclovir	0.94	0.09	26.12	9705	9705	9705	1000	9	1.00	0.35	0.99	34
Hydrochlorothiazide	13.15	0.11	0.94	0.94	0.96	0.99	12.5	71	1.00	0.42	0.86	36
Losartan	35.79	0.19	2.93	0.42	0.01	0.08	50	36	1.00	0.59	0.99	59
Meloxicam	4.52	1.31	7.18	0.73	41.29	226.91	15	97	1.00	0.99	1.00	99
Metformin	0.01	0.10	873.50	873.50	873.50	873.50	500	52	1.00	0.39	1.00	39
Methotrexate	0.01	0.19	0.50	0.01	0.13	0.97	50	70	1.00	0.57	1.00	57
Pravastatin	0.86	0.35	0.05	0.14	6.89	51.05	20	18	1.00	0.77	0.99	77
Sulfamethoxazole	1.00	1.04	1.42	0.68	4.61	29.76	1000	100	1.00	0.97	1.00	97
Sumatriptan	17.46	0.26	725.24	725.24	501.75	144.71	100	14	1.00	0.69	0.82	57
Tetracycline	0.05	0.23	137.36	4.34	4.15	4.24	250	77	1.00	0.65	1.00	65
Timolol	11.45	1.30	1231	1231	1149	549.98	20	76	1.00	0.99	0.88	87
Trimethoprim	26.44	2.35	290.36	187.47	4.20	1.03	160	63	1.00	1.00	0.75	75

Table 6
Drug parameters and predicted bioavailability by the pharmacokinetic model for the 68 drugs with only *in silico based* data.

Drug	Data parameters							Observed	Predicted			
	In silico Cl_{int} ($L\,h^{-1}$)	*In silico* P_{eff} ($cm\,h^{-1}$)	Si pH 1.5 (mg/ml)	Si pH 4.6 (mg/ml)	Si pH 6.5 (mg/ml)	Si pH 7.5 (mg/ml)	Dose max (mg)	F_{oral} (%)	F_d	F_{perm}	F_{met}	F_{oral} (%)
Alendronate	0.03	0.01	463.88	292.69	292.69	292.69	70	2	1.00	0.04	1.00	4
Allopurinol	2.89	2.96	3.05	3.05	3.05	3.05	300	90	1.00	1.00	0.97	97
Alprazolam	27.52	1.66	0.09	0.35	0.01	0.01	4.5	88	0.31	0.99	0.75	23
Amiodarona	109.31	9.10	0.33	16.55	0.05	0.01	400	46	1.00	1.00	0.43	43
Amitriptyline	76.65	0.84	5.41	270.48	2.15	0.28	100	48	1.00	0.96	0.51	49
Amlodipine	7.26	0.85	564.47	564.47	145.09	23.53	10	74	1.00	0.96	0.92	88
Aprepitant	114.69	2.75	10.18	1.31	0.00	0.00	125	59	1.00	1.00	0.41	41
Buspirone	20.15	1.54	1932	17182	8.63	1.64	20	4	1.00	0.99	0.80	80
Calcitriol	8.64	4.99	0.01	0.33	0.01	0.01	0.0042	61	0.90	1.00	0.90	81
Candesartan	30.67	2.62	0.07	0.04	0.02	0.14	16	42	1.00	0.99	1.00	98
Cefixime	0.05	0.02	1.19	2.22	130.79	557.93	200	40	1.00	0.23	1.00	23
Chlorthalidone	3.40	2.04	0.02	0.02	0.02	0.02	50	64	0.63	0.94	0.96	57
Cinacalcet	13.68	2.21	1.18	59.18	0.37	0.05	75	25	1.00	1.00	0.86	85
Clindamycin	114.78	0.73	996.41	996.41	75.59	15.43	150	53	1.00	0.95	0.41	39
Cyclophosphamide	7.20	0.73	32.87	32.87	32.87	32.87	50	88	1.00	0.95	0.92	87
Dapsone	0.01	0.42	0.08	0.05	0.05	0.05	100	86	0.51	0.73	1.00	37
Dicloxacillin	414.94	0.05	0.14	604.51	481.32	540.05	2000	49	1.00	0.23	0.73	17
Didanosine	12.28	2.00	15.25	11.57	11.57	11.84	400	38	1.00	1.00	0.87	87
Dofetilide	7.05	0.43	384.64	285.14	5.56	0.80	0.55	96	1.00	0.85	0.92	78
Entacapone	66.46	0.23	1.08	1.08	1.76	6.53	400	46	1.00	0.66	0.97	64
Ethambutol	18.25	0.56	1952	1952	1447	955.86	800	77	1.00	0.90	0.82	74
Finasteride	72.47	4.22	0.03	1.74	0.03	0.03	5	63	1.00	1.00	0.53	53
Flecainide	5.26	1.03	104.09	5204	70.37	9.94	100	74	1.00	0.98	0.94	92
Flumazenil	88.89	0.78	0.87	0.44	0.44	0.44	200	16	1.00	0.95	0.48	45
Fluorouracil	288.30	1.49	6.99	6.99	7.32	8.80	1050	28	1.00	0.99	0.22	22
Fluphenazine	41.20	3.35	234.99	2512	0.71	0.12	12	3	1.00	1.00	0.66	66
Galantamine	1.90	0.77	673.71	673.71	244.61	51.11	12	95	1.00	0.95	0.98	93
Glimepiride	75.25	0.20	0.05	3.16	1.05	7.96	3	100	1.00	0.60	0.99	60
Glyburide	40.59	0.13	0.01	0.88	0.29	2.21	3	73	1.00	0.47	0.99	47
Hydromorphone	137.61	0.85	668.97	668.97	211.55	42.21	4	42	1.00	0.96	0.37	36
Imatinib	139.77	1.20	2058	2412	0.65	0.12	400	98	1.00	0.99	0.37	36
Irbesartan	4.74	0.28	2.36	2.09	0.02	0.02	50	70	1.00	0.72	0.94	68
Isosorbide Dinitrate	88.48	0.37	0.92	0.92	0.92	0.92	20	22	1.00	0.80	0.48	38
Isosorbide-5-mononitrate	0.88	0.32	195.61	195.61	195.61	195.61	20	93	1.00	0.76	0.99	75
Lamivudine	23.00	0.22	741.97	22.93	14.14	14.14	100	82	1.00	0.63	0.78	49
Lansoprazole	8.30	5.01	97.16	19.34	0.24	0.25	15	81	1.00	1.00	0.91	91
Letrozole	11.77	3.45	0.46	4.62	0.09	0.09	2.5	100	1.00	1.00	0.87	87
Levetiracetam	2.10	0.86	60.40	60.40	60.40	60.40	500	100	1.00	0.96	0.97	94
Levofloxacin	206.33	0.56	610.17	19.38	1.66	1.44	500	99	1.00	0.90	0.28	25
Linezolid	9.29	0.31	35.96	1.50	1.44	1.44	600	100	1.00	0.75	0.90	67
Melphalan	82.59	0.58	14.28	4.12	4.12	4.21	25	56	1.00	0.91	0.50	45
Meperidine	56.04	2.58	566.69	28334	149.06	28.40	20	52	1.00	1.00	0.59	59
Mercaptopurine	0.02	4.77	0.57	0.57	0.61	0.92	100	12	1.00	1.00	1.00	100
Methadone	63.77	3.03	23.48	1174	2.96	0.39	10	86	1.00	1.00	0.56	56
Metoclopramide	82.87	0.62	788.66	788.66	193.59	36.05	20	76	1.00	0.92	0.49	46
Metronidazole	50.77	1.13	62.15	15.98	15.98	15.98	100	99	1.00	0.98	0.61	60
Montelukast	269.11	0.05	0.01	0.06	0.00	0.02	10	62	0.56	0.18	0.94	10
Moxifloxacin	51.35	2.27	139.21	0.58	0.02	0.02	400	86	1.00	1.00	0.61	61
Nalmefene	88.56	0.98	100.18	5009	7.96	1.10	50	40	1.00	0.97	0.48	47
Nitrofurantoin	147.73	0.40	0.10	0.10	0.11	0.19	50	90	1.00	0.78	0.35	27
Oxycodone	349.39	1.39	722.54	722.54	161.76	31.54	5	42	1.00	0.99	0.19	19
Phenobarbital	2.55	0.73	0.44	0.44	0.49	0.79	90	100	1.00	0.94	0.97	92
Quetiapine	124.58	4.53	1241	21028	12.41	2.53	250	9	1.00	1.00	0.39	39
Quinine	27.95	2.68	893.64	44682	67.79	10.50	700	76	1.00	1.00	0.74	74
Repaglinide	57.24	0.06	0.72	0.33	0.01	0.06	4	56	1.00	0.26	0.97	25
Riluzole	494.00	1.81	52.44	9.97	0.12	0.12	50	60	1.00	1.00	0.14	14
Risedronate	0.01	0.01	214.78	24.66	214.78	503.48	5	1	1.00	0.06	1.00	6
Risperidone	142.81	4.06	593.41	17471	8.19	1.16	3	66	1.00	1.00	0.36	36
Rizatriptan	15.73	0.90	1072	1072	309.30	72.51	10	47	1.00	0.97	0.84	81
Tamsulosin	67.17	0.67	219.42	219.42	46.91	6.63	0.4	100	1.00	0.93	0.55	51
Tegaserod	0.96	0.50	81.13	81.13	81.13	67.48	6	11	1.00	0.88	0.99	87
Terazosin	23.96	0.66	510.81	150.75	3.70	1.54	1	82	1.00	0.93	0.77	72
Tramadol	19.77	3.02	692.87	34643	692.87	479.35	100	70	1.00	1.00	0.80	80
Trazodone	5.22	2.21	1176	6448	3.17	1.32	100	81	1.00	1.00	0.94	94
Valsartan	23.60	0.06	0.03	82.80	106.92	477.60	80	39	1.00	0.26	0.97	26
Vinorelbine	1154.79	1.95	1004	14809	4.92	0.65	170	27	1.00	1.00	0.07	7
Zaleplon	31.82	3.40	0.35	0.20	0.20	0.20	10	31	1.00	1.00	0.72	72
Ziprasidone	168.26	0.79	180.27	1216	0.33	0.06	20	59	1.00	0.96	0.33	31

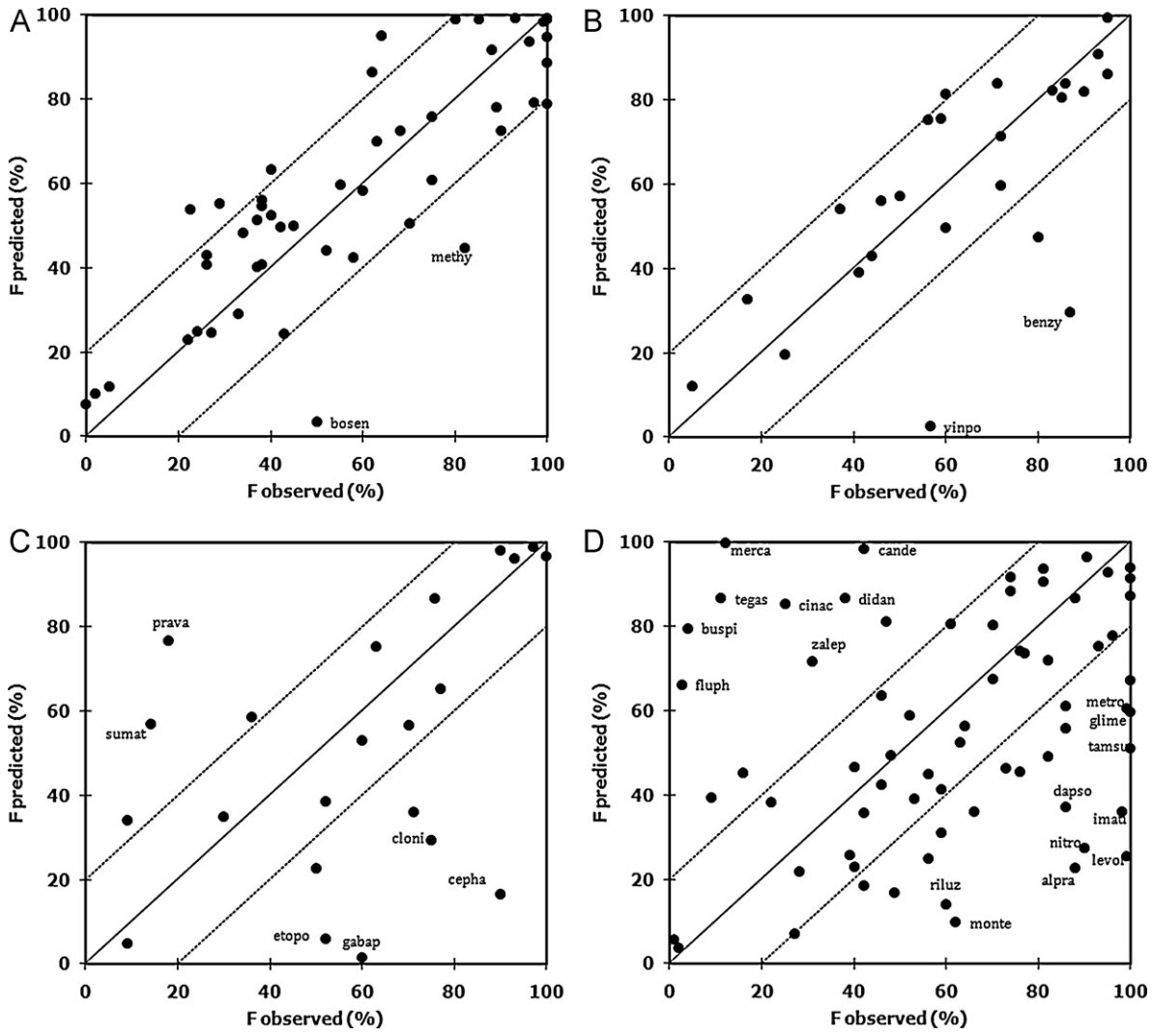

Fig. 3. Plot of the *in vivo* observed bioavailability *vs.* the model predicted bioavailability by the PBPK model of absorption using: (A) *in vitro* data for both P_{app} and Cl_{int}; (B) *in vitro* data for Cl_{int} only; (C) *in vitro* data for P_{app} only; (D) *in silico* data for both P_{app} and Cl_{int}. Solid line represents the line of unity and the dashed line the ±20% absolute tolerance. Drugs outside the ±35% absolute tolerance value are also labeled.

For drugs presenting a prediction error outside the ±20% threshold value, an evaluation of the probable cause of failure was made based on the comparison of the *in vivo* F_{abs} and F_{met} (Table 2) with the model predictions on the individual drugs F_d, F_{perm} and F_{met} values (Tables 3–6). Since *in vivo* F_d and F_{perm} could not be separated, when the predicted $F_d \times F_{perm}$ were significantly different (±20%) from the *in vivo* F_{abs}, an individual bibliographic survey was undertaken for the establishing of the probable cause for prediction failure within absorption.

3. Results

3.1. Prediction using in vitro data

The first evaluation of the presented methodology was made based on absorption and biotransformation data obtained from *in vitro* experiments for the drugs from Table 3. Fig. 3A) presents the relationship between the observed and the predicted oral bioavailabilities for the 49 drugs in this data set. A correlation of $r_s = 0.824$ (0.706–0.897 CI95%) was observed with a RMSE = 16.0% and a ME = 1.9%. The model was able to predict 84% of data within the accepted ±20% error interval and 96% of data lies within the ±35% error margin, thus showing excellent qualitative and quantitative prediction capabilities. When evaluating the possible reasons for predictions outside the accepted ±20% error interval, solubility was responsible for one case (Bosentan), P_{app} was responsible for another one case (Sildenafil) and Cl_{int} was responsible for the remaining 6 cases (Caffeine, Diclofenac, Methylprednisolone, Nitrendipine, Ondansetron and Scopolamine) of badly predicted drugs.

3.2. Prediction using in silico P_{app} Caco-2 values

When considering drugs from Table 4, for which only *in vitro* Cl_{int} data were available, the model was tested by including *in silico* predicted P_{app} values to characterize the drug absorption phase. Fig. 3B) presents the relationship between the observed and the predicted oral bioavailabilities for the 25 drugs in this data set. A correlation of $r_s = 0.718$ (0.450–0.867 CI95%) was observed with a RMSE = 19.8% and a ME = −2.7%. The model was able to predict 84% of data within the accepted ±20% error interval and 92% of data within the ±35% error interval, indicating again excellent qualitative and quantitative prediction capabilities. Again, the evaluation of the possible reasons for predictions outside the accepted ±20% error interval indicated that none of the drugs presented solubility or *in silico* P_{app} related estimation problems. *In vitro* Cl_{int} was

responsible for the observed four cases (Benzydamine, Bepredil, Prednisone and Vinpocetine) of badly predicted drugs.

3.3. Prediction using in silico Cl_{int} hepatocytes values

For the drugs from Table 5, only *in vitro* P_{app} data were available. In this case, the model was tested by including *in silico* predicted Cl_{int} values to characterize the drug metabolization in the liver. Fig. 3C) presents the relationship between the observed and the predicted oral bioavailability for the 22 drugs in this data set. A correlation of $r_s = 0.532$ (0.142–0.779 CI95%) was observed with a RMSE = 31.9% and a ME = −6.7%. The model resulted in 55% of data well predicted within the accepted ±20% error and 73% of data within the ±35% error margin, indicating acceptable quantitative but good qualitative prediction capabilities. Solubility was responsible for two cases (Cephalexin and Ganciclovir) of drugs outside the accepted ±20% error interval. *In vitro* P_{app} was responsible for 8 drugs (Amoxicillin, Cephalexim, Etoposide, Gabapentin, Ganciclovir, Hydrochlorothiazide, Losartan and Pravastatin) and *in silico* Cl_{int} was responsible for another five cases (Clonidine, Etoposide, Losartan, Pravastatin and Sumatriptan) of badly predicted drugs. As mentioned above, some drugs were badly predicted due to more than one drug related parameter.

3.4. Prediction using in silico data

For the remaining drugs, included in Table 6, only *in silico* P_{app} and Cl_{int} data were used. Fig. 3D) presents the relationship between the observed and the predicted oral bioavailability for the 68 drugs in this data set. As expected, due to the increase prediction variability of the added *in silico* models, a weak but statistically significant correlation of $r_s = 0.284$ (0.049–0.489 CI95%) was observed with a RMSE = 34.6% and a ME = −4.5%. The model resulted in 53% of data well predicted within the accepted ±20% error but still was able to predict 74% of data within the ±35% error. In this scenario, solubility was responsible for two drugs (Alprazolam and Dapsone) outside the accepted ±20% error interval. *In silico* P_{app} was responsible for 12 cases (Candesartan, Dapsone, Dicloxacillin, Flumazenil, Fluphenazine, Glimepiride, Glyburide, Lamivudine, Linezolid, Mercaptopurine, Montelukaste and Repaglinide) and Cl_{int} was responsible for another 22 drugs (Buspirone, Cinacalcet, Didanosine, Fluphenazine, Imatinib, Levofloxacin, Mercaptopurine, Methadone, Metoclopramide, Metronidazole, Moxifloxacin, Nitrofurantoin, Oxycodone, Quetiapine, Repaglinide, Riluzole, Risperidone, Rizatriptan, Tamsulosin, Tegaserod, Zaleplon and Ziprasidone with low prediction accuracy).

4. Discussion

4.1. Model structure

We used an oral bioavailability compartmental model, based on the CAT model, which considers gastric and intestinal transit time, solubility, permeability and hepatic metabolism, as primary conditionings of drug bioavailability. Although some important factors were not considered, namely the effect of the GIT drug transporters, drug degradation in the GIT lumen, enterocyte metabolization, to name a few, the considered drug characteristics are expectably the main factors to limit bioavailability for the majority of drugs. However, since Caco-2 cells present both metabolization and transport systems (Vogel, 2006) these mechanisms may be included in the permeability estimation by the Caco-2 cells, if P_{app} values were collected outside the saturation zone and the drugs present proportional dose absorption.

In order to characterize drug dissolution in the GIT, it is necessary to consider the water volume in each compartment. With this purpose, a water model was built based on the described daily rates of secretions in the different parts of the GIT as well as the described percentage of water reabsorption in the small intestine. The sum of the model steady-state values for the small intestine total a volume of 308 ml, which is in agreement with the *in vivo* experimental value of 165 ml (range 25–350 ml) (Marciani et al., 2007). Additionally, the calculated water absorption rate constant, with a value of 0.7015 h^{-1} ($P_{eff} = 1.7 \times 10^{-4}$ cm s^{-1}) is also consistent with the experimental water (D_2O) P_{eff} values of 1.4 and 2.4×10^{-4} cm s^{-1} under diffusion and convective conditions in humans (Fagerholm et al., 1999).

The absorption rate of the drugs was assumed to follow first order kinetics and dependent on the jejunal effective permeability (P_{eff}). Since Caco-2 apparent permeabilities (P_{app}) were used as estimators of P_{eff}, a multiple linear regression model, using Caco-2 P_{app} values and RBN, was built to relate these parameters. Previous authors had used simple linear relationships with variable success (Parrott and Lave, 2002, 2008; Sun et al., 2002). However, this may not reflect the fact that, if highly permeable drugs would most likely be absorbed in the upper part of the villus, low permeability drugs are likely to diffuse throughout the intervillous space and will have access to the majority of the absorptive area (Lennernas, 1998; Palm et al., 1996). The RBN descriptor, since it is also related to the molecular weight, can account for the difference in surface area that exists, due to the lack of villus on Caco-2 cell, between Caco-2 cells and the human intestine for low permeability drugs. Another described morphophysiological difference between Caco-2 and the intestinal epithelia is the larger density of tight junctions presented in Caco-2 (Collett et al., 1997). This fact implies that, for drugs with important paracellular absorption, Caco-2 would under predict the actual *in vivo* value. RBN, that counts the number of bonds in the molecule that allow a free rotation around themselves and is a measure of the flexibility of the molecule, can also compensate this effect.

Metabolization of the drug was assumed to occur primarily at the liver, and the "well-stirred" model was used to simulate that organ. The choice of liver model does not seem to significantly influence the Cl_H predictive capacity both when using rat isolated microsomes and hepatocytes suspensions (Ito and Houston, 2004). There is no significant difference for low metabolized drugs when using the "well-stirred", the "parallel tube" or the "dispersion" models and, for the sake of simplicity and minor differences in the prediction on *in vivo* Cl_H, the "Well-Stirred" model can be used for Cl_H prediction (Houston and Carlile, 1997; Ito and Houston, 2004, 2005). Use of f_B is also a question of debate, with various studies indicating that the non-inclusion of this parameter in basic, zwitterionic and neutral drugs resulted in improved Cl_H estimations both when using microsomes (Obach, 1999) or hepatocytes suspensions (Jacobson et al., 2007; Lau et al., 2002; McGinnity et al., 2004; Reddy et al., 2005). In a previous study, considering 30 drugs with *in vitro* Cl_{int} data from human hepatocytes suspensions, we also observed better *in vivo* Cl_{int} predictions with increased precision (RMSE 0.643 *vs* 1.042), less bias (ME −0.073 *vs* −0.838) and increased number of compounds predicted within 2-fold (52% *vs* 16%) when neglecting f_B for basic, neutral and zwitterionic drugs in comparison with the inclusion of f_B for all drug classes (Paixao et al., 2010a).

4.2. Drug parameters

An important drug specific parameter involved in dissolution is the drug solubility in the GIT medium. This may depend on a variety of factors, such as pH, surfactant, buffer capacity and ionic strength that are difficult to simulate *in vitro* (Takano et al., 2006).

As an approximation we used *in silico* intrinsic water solubility and the effect of pH in the drug ionization, and by consequence in its solubility, at the GIT pH values using ADME Boxes. Additionally, various authors have indicate a relationship between drug lipophilicity and its increased solubility in the presence of bile salts (Mithani et al., 1996; Wiedmann and Kamel, 2002; Wiedmann et al., 2002), and a linear relationship was shown between log *P* and the micelle/aqueous partition coefficient *in vitro* (Wiedmann et al., 2002). However different bile salts interact with drugs with different affinities (Atanackovic et al., 2009) and human intestinal fluids show large variations with regard to composition, which is known to influence the solubility of poorly soluble drugs in a log *P* independent manner (Kleberg et al., 2010). In this context, and for simplicity issues, the surfactant effect was introduced by empirically considering that drugs with log *P* values above 2.5 would be 50 times more soluble in the duodenum than the drug aqueous solubility at pH 4.5. Finally, there is the possibility of drug precipitation when the dissolved drug transits from one compartment to the next due to pH and water volume changes in the GIT. However, Box. (Box et al., 2006) identified that about 95% of the acids and 75% of the bases they studied by using a potentiometric procedure to establish the aqueous solubility, were capable to form supersaturated solutions. In this context, it was assumed that no precipitation occurs. Parrott et al. (2005) using Gastroplus™ without adjusted solubility for bile salt solubilization, observed that 27 of 29 compounds with low solubility, presented their bioavailability under predicted. In our case, although simple approximations were made, only 3.04% of drugs presented bad predictions due to solubility questions.

In order to quantify the drug absorption process, permeability data collected in Caco-2 cells were used. This cell system is a widely performed *in vitro* test with interesting properties when extrapolating results to bioavailability. Caco-2 cells, which are polarized epithelial cells, can form a differentiated monolayer that resembles the morphological and biochemical characteristics of the human intestinal epithelium (Vogel, 2006). Additionally, a sigmoid relationship between the P_{app} across Caco-2 cells and the fraction absorbed in humans has been shown for passively absorbed drugs (Stenberg et al., 2001). Although similar gene expression was observed between Caco-2 cells and the human duodenum, around 17% of gene sequences presented at least a 5-fold difference in expression (Sun et al., 2002). Due to this, extrapolating P_{app} Caco-2 values for drugs absorbed by carrier mediated mechanisms or subject to important metabolic degradation at the enterocyte is more difficult. For this reason, we used *in vitro* Caco-2 data describing mainly the passive diffusion mechanism of absorption (Paixao et al., 2010b) which resulted in around 86% of drugs with correct absorption predictions within the ±20% threshold value (combining Tables 3 and 5 data), although some of the badly predicted drugs, like Cephalexin or Gabapentin, were indeed substrates of transporters. These results were also observed with the *in silico* based P_{app} Caco-2 data. In this case (combining Tables 4 and 6 data), 86% of drugs presented correct absorption predictions and were not considered statistically different ($p < 0.4175$) from the *in vitro* derived ones by the ANOVA analysis, indicating that the used *in silico* model is a valid alternative to the *in vitro* model in the lead development phase when *in vitro* data are not available.

To characterize the first-pass effect at the liver, we used *in vitro* data obtained in suspensions of isolated human hepatocytes (Paixao et al., 2010a). Hepatocytes are intact cells with a complete set of phase I and II metabolizing enzymes that mimic the *in vivo* metabolization of drugs (Gomez-Lechon et al., 2003). Additionally, the presence of uptake and efflux transporters is also an important characteristic of this cell system with relevance in the drug metabolization process (Hewitt et al., 2007). With the optimization of the cryopreservation protocols, an increased pool of liver sources is now available with a minimal loss of metabolic activity (Blanchard et al., 2005; Griffin and Houston, 2004; McGinnity et al., 2004). Due to these facts, it appears to be the most promising tool to predict Cl_H in the development phase of new drug entities (Fagerholm, 2007). Our results confirm that using *in vitro* data from this model (combining Tables 3 and 4 data) is suitable to predict the first-pass effect, with around 88% of drugs with correct predictions. When using the *in silico* model (combining data from Tables 5 and 6), lower and a statistically significant difference ($p < 0.0011$) in the prediction ability was observed, with around 71% of drugs with correct predictions within the ±20% threshold value. This value improved, however, to around 81% of drugs with correct predictions when the ±35% threshold value was considered making this model still a valid tool in drug discovery and development. Overall, no statistically significant differences were observed between the different drug classes ($p < 0.8437$).

4.3. Model performance

The model statistical performance under the four studied scenarios is presented in Table 7. As expected, the model best performance in predicting human bioavailability was obtained when using only *in vitro* P_{app} and Cl_{int} data. In this scenario, 84% of good predictions within the ±20% acceptance range were observed with the lower RMSE of all the simulations. Parrott and Lave (2002) evaluated the performance of two commercial packages (Gastroplus™ and iDEA™) in predicting the absorbable fraction in 28 drugs. A RMSE of 22% was described for both models, larger than the RMSE value obtained in our data considering the complete bioavailability process. De Buck et al. (2007b) in a retrospective analysis of 16 clinically tested drugs and using Gastroplus™ with *in vitro* P_{app} Caco-2 ($n = 13$), *in silico* P_{app} Caco-2 ($n = 3$) and *in vitro* Cl_{int} determined in human and rat microsomes and hepatocytes suspensions obtained an average fold error of 1.06 and a RMSE of 15%, similar to the present results. Cai et al. (2006) evaluated an integrated *in vitro* – PBPK model to predict human bioavailability of another 16 drugs. Cl_{int} was determined in human hepatocytes suspensions, and the absorption data were obtained from the literature and in-house reports. Their method outperformed the commercial package iDEA™ with 69% *vs.* 63% of good predictions within the ±20% accepted range and RMSE = 19% *vs.* RMSE = 25%. Our work, as well as these reports, indicates that *in vitro* data obtained in Caco-2 and Human hepatocytes suspensions, when used in various PBPK models of absorption, are capable of providing statistically relevant predictions of the drug bioavailability in humans.

It is frequent in the lead development, that not all the *in vitro* data are available at some time of the discovery phase. Additionally,

Table 7
Statistical comparison of the performance of the pharmacokinetic model based on the introduction of the different data sources.

	In vitro P_{app} *In vitro* Cl_{int}	*In silico* P_{app} *In vitro* Cl_{int}	*In vitro* P_{app} *In silico* Cl_{int}	*In silico* P_{app} *In silico* Cl_{int}
r_s	0.824	0.718	0.532	0.284
RMSE (%*F*)	16.0	19.8	31.9	34.6
ME (%*F*)	1.9	−2.7	−6.7	−4.5
%Correct values within ± 20%	83.7	84.0	54.5	52.9
%Correct values within ± 35%	95.9	92.0	72.7	73.5

in the beginning of the new drug discovery process, pure *in silico* methods are frequently used (Venkatesh and Lipper, 2000). We tested the ability of the proposed method to predict human oral bioavailability in the typical discovery pipeline, when all data are not yet available.

The second situation explored considers that *in vitro* Cl_{int} in human hepatocytes suspensions data are available, but only *in silico* estimates of P_{app} in Caco-2 are possible to be used. In this case an ANN model, presenting a correlation of 0.774 and RMSE of 0.601 log values in a validation group of 45 drugs, was used (Paixao et al., 2010b). Using these inputs, the PBPK model presented 84% of good predictions within the ±20% accepted range and a RMSE of 19.8%. De Buck et al. (2007a), using *in vitro* Cl_{int} determined in rat microsomes, *in silico* P_{app} values and Gastroplus™, obtained a RMSE of 32.1 and 63.3% of values within a 2-fold error when predicting the rat oral bioavailability. Parrott et al. (Parrott et al., 2005) when evaluating the utility of PBPK models in early drug discovery, using *in vitro* P_{app} in PAMPA, *in vitro* Cl_{int} in rat hepatocytes and Gastroplus™, obtained a RMSE of 31% and a r of 0.40 when predicting the rat oral bioavailability. Although different species were considered, our *in silico* P_{app} model provided better prediction capabilities than theses models, even when *in vitro* PAMPA P_{app} data are considered.

The third situation explored considers that *in vitro* P_{app} Caco-2 data are available, but only *in silico* estimates of Cl_{int} in human hepatocytes suspensions are possible to be used. In this case an ANN method, able to predict 63% of *in vivo* Cl_{int} within a 10-fold error in a validation group of 112 drugs, was used (Paixao et al., 2010a). When introduced in the proposed PBPK Model, 55% of good predictions within the ±20% accepted range and a RMSE of 31.9% were obtained. Although some *in silico* approaches to predict metabolic clearances are emerging (Sheikh-Bahaei and Hunt, 2011; Yu, 2010), the used ANN model for Cl_{int} predictions is the only described *in silico* model to predict the metabolization of drugs in human hepatocytes suspensions, limiting the comparisons with other works.

The final situation explored considers that no *in vitro* data are available, simulating the initial situation in the drug development process. When introducing only *in silico* derived data in the proposed PBPK Model, 53% of good predictions within the ±20% accepted range and a RMSE of 34.6% was obtained. A low correlation of $r_s = 0.284$ was also obtained. This result was expected due to the increase of the predictive error presented in the *in silico* models, that combined in the PBPK model, resulted in a poor ability to quantitatively predict the Human oral bioavailability. Considering, however, that the model resulted also in 74% of correct values within $a \pm 35\%$ error, which is sufficient to the establishment of the qualitative class of absorption, indicates that this approach can still be used for the early candidate selection. Yoshida and Topliss (2000) developed a QSAR model for human oral bioavailability classification that was able to correctly predict the class of absorption of 24 in 40 drugs (60% success). Our model, with similar classification rates, provides in addition a mechanistic information concerning the reason for the drug limited absorption.

5. Conclusions

The presented methodology, a PBPK model of absorption considering drug dissolution and absorption in the GIT and drug metabolization in the liver, is a convenient approach to predict and characterize the human oral bioavailability in the early drug development process. When based on *in silico* drug solubility, and both *in vitro* absorption and metabolization data, it was able to correctly establish the oral bioavailability for the vast majority of the studied drugs. Inclusion of *in silico* permeability provided similar prediction abilities when compared with the *in vitro* derived data. However, the use of *in silico* metabolization data degraded the model performance. If the absorption process seems to be sufficiently predicted based only on the molecular structure of the drug, *in silico* prediction of the metabolization rate, in spite of the initial modeling efforts, is still prone to improvement. However, qualitative establishment of oral bioavailability was still statistically possible, which indicates that this modeling approach may be an important tool in the drug discovery pipeline, allowing the refinement of its predictions and indicating lines of investigation in order to improve the overall success rate of lead development.

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