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RESEARCH PAPER

Untangling Absorption Mechanisms and Variability in Bioequivalence Studies Using Population Analysis

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ABSTRACT

Purpose Both inter-individual (IIV) and inter-occasion (IOV) variabilities are observed in bioequivalence studies. High IOV may be a cause of problems on the demonstration of bioequivalence, despite strict measures are taken to control it. The objective of this study is to investigate further means of controlling IIV by optimizing study design of crossover studies.

Methods Data from 18 bioequivalence studies were used to develop population pharmacokinetics (popPK) models to characterize the absorption and disposition processes of 14 drugs, to estimate IOV for each drug substance and to evaluate possible correlations with biopharmaceutical properties of drug substances, classified in accordance to the Biopharmaceutics Drug Disposition Classification System (BDDCS).

Results Plasma-pharmacokinetics profiles for the 14 drugs analyzed were successfully described using popPK. The pharmacokinetic parameters that showed greater variability were first-order rate constant of absorption, duration of the zero-order absorption process, relative bioavailability and time of latency. ISCV% estimated for C_{max} seems to correlate with the log-Dose-Number for Class 1, 2 and 3, despite no direct correlation was observed between popPK model residual variability (RUV) and ISCV%. Nevertheless, higher RUV estimates were observed for Class 2 drugs in comparison to Class 1 and 3.

Conclusion Pharmacokinetic parameters related to drug absorption showed greater variability.
Ingestion of the IMP along with 240 mL of water showed to standardize gastric emptying.
Given the dependency between C_{max} variability and dose-solubility ratio, for classes 2 and 4, *ad libitum* water intake may increase C_{max} and AUC ISCV%. A water ingestion standardization until the expected T_{max} of the drug is suggested.

KEY WORDS BDDCS · bioequivalence · population pharmacokinetics

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INTRODUCTION

Since the initial FDA's regulations and guidelines that set forth procedures to establish bioequivalence requirements for approval of medicinal drug products (1), followed by the European Medicines Agency (EMA) in 1995, tremendous advances have occurred.

Bioavailability (BA) and bioequivalence (BE) became the cornerstones for the approval of brand-name and generic medicines globally. Nevertheless, there are continuing efforts by regulatory authorities and the scientific community, both nationally and internationally, to understand and develop more efficient and scientifically valid approaches to the assessment of BE of various pharmaceutical forms. An example of the development of a valid approach to reduce unnecessary costs and risks involved in conducting clinical trials to demonstrate BE, is the application of the scientific principles underlying the Biopharmaceutical Classification System (BSC) developed by Amidon *et al* (2) and the Biopharmaceutics Drug Disposition Classification System (BDDCS) developed by Wu and Benet (3) to exempt *in vivo* BE studies based on the assumption that equivalence in *in vivo* performance can be supported by satisfactory *in vitro* data (2).

BE is defined by EMA (4) as the absence of a significant difference in the BA (rate and extent) to which the active substance in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose, under similar conditions, in an appropriately designed study. BE intends to ensure comparable *in vivo* performance, i.e. similarity in terms of safety and efficacy between drug products (5).

BE studies are generally conducted in healthy male and female adults under standardized conditions. Most BE studies use a 2-way crossover design, in which subjects are administered the Test and the Reference formulations alternately, enabling to derive from the pharmacokinetic (PK) profiles in plasma/blood samples, the PK parameters area under the concentration *versus* time curve (AUC) and the maximal observed concentration (C_{max}). Usually, BE between Test and Reference formulations are inferred if the 90% confidence interval for the Test-to-Reference geometric mean ratio (GMR) calculated on the *ln*-transformed parameters C_{max} and AUC are all within the 80.00% to 125.00% acceptance interval (4).

Both inter-subject (or inter-individual as defined on popPK modeling) and intra-subject (or inter-occasion as defined on popPK modeling) variabilities are observed in BE studies. Albeit inter-subject variability is not pertinent to crossover study design, high intra-subject variability may be a root cause of problems on the demonstration of BE, despite the strict measures taken to control this variability at the time of BE study planning and conduct. Major causes of intra-subject variability are due to individual physiological variabilities associated to the complex kinetic processes related to the absorption, distribution, metabolism and excretion of a drug substance, as well as those associated to the *in vivo* performance of the product formulation.

Prediction of the extent of intra-subject variability is crucial to appropriately scale a human BE study. Prediction of intra-subject coefficient of variation (ISCV%) is generally achieved from BE study results for the primary PK parameters, by the use of the square root of $e^{s^2} - 1$, where s^2 is the mean square error obtained from the Analysis of Variance (ANOVA) of the *ln*-transformed parameters. In accordance with the EMA guideline on BE (4), the ANOVA is performed using a general linear model, with sequence, subject nested within sequence, period and formulation as fixed effects.

Considering the importance of population pharmacokinetic (popPK) models to provide a means of characterizing the extent of inter-subject and intra-subject variabilities, in this work data from 18 BE studies were used to develop popPK models to characterize the absorption and disposition processes of 14 drugs, to estimate inter-occasion variability for each drug substance and to evaluate possible correlations with biopharmaceutical properties of drug substances, classified in accordance to the Biopharmaceutics Drug Disposition Classification System (BDDCS). The objective of the current study is to investigate further means of controlling intra-subject variability, by refining inclusion/exclusion criteria for study participation and optimize study design of crossover studies.

MATERIAL AND METHODS

The current work, which consists of secondary use of study data, was reviewed and approved by the *National Ethics Committee for Clinical Research* (CEIC), and authorized by the sponsor of the studies. It comprises anonymized data from 18 crossover BE clinical trials involving 14 different orally administered drugs. All investigational medicinal products (IMPs) were immediate-release formulations, except clonidine, which was prolonged release tablets. For each study, data on study design, individual demographic characteristics and drug plasma concentrations along time were extracted from the Clinical Study Reports and included on project database.

All the BE studies were conducted after the respective study protocols have been reviewed and approved by CEIC and the *National Authority of Medicines and Health Products* (INFARMED, I.P.). All these BE studies were conducted according to the International Conference on Harmonization (ICH) Good Clinical Practices (GCP) (6), and to Declaration of Helsinki and have followed EMA and FDA current guidelines.

The study design characteristics are listed in Table 1. All studies were conducted at the Clinical Pharmacology Unit of BlueClinical Ltd (BlueClinical Phase I, Hospital da Prelada, Porto, Portugal), and were performed under fasting conditions. For all studies, bioequivalence between Test and Reference products has been demonstrated.

Population Characteristics

The clinical trials included in the project enrolled a total of 431 healthy male and non-pregnant female volunteers; 221 (51.3%) were male and 210 (48.7%) were female; among the female volunteers, 109 (51.9%) did not use hormonal contraception compared to 101 (48.1%) who used hormonal contraception during the study.

All BE studies included male and female participants. The use of hormonal contraceptives by female participants was allowed in all studies, but in the study with ibrutinib there were no female participants taking hormonal contraception.

The main inclusion criteria for participation in these trials included free written informed consent prior to any procedure required by each study; age between 18 and 55 years; body weight ≥48 kg and body mass index (BMI) of 18.0 to 31.0 kg/m^2; without clinically significant abnormalities on physical examination, 12-lead ECG and hematology, biochemistry, coagulation, and urinalysis parameters; negative results from viral serology (HIV, hepatitis B and C); non-smokers or ex-smokers (at least 3 months); and willing to accept and comply with study restrictions (e.g., alcohol consumption, diet, exercise, contraception, and medications). Female subjects were infertile or postmenopausal, and if fertile were willing to accept the use of a non-hormonal or hormonal contraceptive method from at least 4 weeks prior admission to the first treatment period of the study, in order to sufficiently minimize the risk of pregnancy. Hormonal contraceptives were used on a stable continuous regimen until the end of the study to ensure stable plasma hormonal levels during the whole study duration.

The main exclusion criteria for participation in these trials included at screening a known hypersensitivity/allergy reaction to the study drug substance or any of the excipients or severe hypersensitivity reaction to any other drug; any medical condition (e.g. gastrointestinal, renal or hepatic, including peptic ulcer, inflammatory bowel disease or pancreatitis) or surgical condition (e.g. cholecystectomy, gastrectomy) that could affect drug pharmacokinetics (absorption, distribution, metabolism or excretion) or subject safety; history of cardiac and psychiatric diseases, seizures, glaucoma, and presence of electrolytic disturbances. Additionally, subjects were excluded in case of history of regular alcohol, drugs of abuse and methylxanthines consumption or in the case of exposure to other drugs (except contraceptives).

Study Drugs

Tables 2 and 3 list the 14 drug substances integrating the current evaluation together with their main PK and biopharmaceutical characteristics. Drugs were also classified in accordance to the BDDCS (3), which considers not only the solubility of the molecule, but also the metabolism, including the elimination routes of drugs and transporters from the gastrointestinal tract for drugs administered orally. The work includes drugs belonging to the 4 classes: *Class 1* (high solubility / extensive metabolism): Alprazolam, Amlodipine, Fluoxetine, Sertraline, Sunitinib, and Tofacitinib; *Class 2* (low solubility / extensive metabolism): Etoricoxib, Febuxostat, Ibrutinib, and Zofenopril; *Class 3* (high solubility / poor metabolism): Clonidine, Hydrochlorothiazide, and Moxifloxacin; and *Class 4* (low solubility / poor metabolism): Chlorthalidone.

Dosing and Sampling

In all bioequivalence trials, the test and the reference products were administered in the corresponding study period as a single dose, followed by an extensive blood sampling that allowed to properly characterize the PK profiles after each IMP administration. The number of samples per subject varied from 17 (tofacitinib) to 22 (etoricoxib) (Table 1). Most samples were collected up to 72 hours post dose, except for alprazolam, ibrutinib, febuxostat, tofacitinib and zofenopril, with samples collected up to 48, 24, 16 and 6 hours post dosing, defined in accordance to the elimination half-life of the drug.

According to study protocols, subjects were randomly allocated to receive the IMPs orally with 240 mL of water in the morning, after an overnight fasting of at least 10 hours, and remain fasted for 4 hours, in a semi-reclined position. Between IMPs administration, a washout period ranging from 7 to 21 days was completed, depending on the elimination half-life of the drug.

Analytical Plasma Samples Analysis:

For all BE studies, drugs were quantified in plasma samples by the bioanalytical laboratory using previously validated bioanalytical methods based on liquid chromatography. The concentration range, accuracy and precision of the analytical methods are shown in

Table I Bioequivalence Clinical Trials Included in This Work

Drug	Study code	Dose (mg)	Type of study	Formulation	N° of subjects	Male	Female without HC	Female with HC	N° of samples per subject	Sampling period (h)	ISCV (%) C_{max}	ISCV (%) AUC_{o-t}
Alprazolam	BLCL- ALP	1	2x2x2	Tablets	20	10	10	0	20	0-48	14	4.8
Amlodipine	BLCL-AML	10	2x2x2	Tablets	25	17	2	6	18	0-72	10.8	6.6
Chlorthalidone	BLCL-CHL	25	2x2x2	Tablets	28	13	8	7	20	0-72	19.3	12.2
Chlortha-lidone	BLCL-CHL-B	50	2x2x2	Tablets	24	11	9	4	20	0-72	26.2	18.1
Clonidine	BLCL-CLO-A	0.1	2x2x2	PRT	11	6	2	3	18	0-72	10.1	8.5
Clonidine	BLCL-CLO-B	0.1	2x2x2	PRT	22	11	7	4	18	0-72	10.1	8.5
Etoricoxib	BLCL-ETO	120	2x2x2	Tablets	18	10	5	3	22	0-72	12.3	6.5
Febuxostat	BLCL-FEB	120	2x2x2	Tablets	47	21	7	19	18	0-24	33	12
Fluoxetine	BLCL-FLU	60	2x2x2	Tablets	28	16	7	5	20	0-72	11.7	3.8
Ibrutinib	BLCL-IBR-A	140	2x2x2	Capsules	17	8	9	0	20	0-48	55.6	39.9
Ibrutinib	BLCL-IBR-B	140	2x2x4	Capsules	32	21	11	0	20	0-48	55.6	39.9
Moxifloxacin	BLCL-MOX	400	2x2x2	Tablets	27	13	6	8	21	0-72	14.3	3.9
Sertraline	BLCL-SER	100	2x2x2	Tablets	24	13	2	9	18	0-72	15.6[(1)]	14.6[(1)]
Sunitinib	BLCL-SUN-A	50	2x2x2	Capsules	9	5	2	2	20	0-72	7.6	7.7
Sunitinib	BLCL-SUN-B	50	2x2x2	Capsules	20	9	6	5	20	0-72	7.6	7.7
Tofatitinib	BLCL-TOF-A	5	2x2x2	Tablets	31	17	8	6	18	0-16	21.9	5.7
Tofacitinib	BLCL-TOF-B	5	2x2x2	Tablets	10	1	3	6	17	0-12	21.9	5.7
Zofenopril	BLCL-ZOF	30	2x2x2	Tablets	38	19	5	14	18	0-6	31.9	20.8
HCTZ	BLCL-ZOF	12.5	2x2x2	Tablets	38	19	5	14	20	0-72	16.6	8.6

2x2x2 = two-treatment, two-sequence, two-period crossover study; 2x2x4 = two-treatment, two-sequence, full-replicate, four-period crossover study; PRT: Pronged release tablets; HC: Hormonal contraceptives; HCTZ:Hydrochlorothiazide; ISCV: Intra-Subject Coefficient of Variation

[(1)]EPAR DK/H/2631/001-002/DC http://mri.cts-mrp.eu/human/downloads/DK_H_2631_002_PAR.pdf

Table 2 Physical-Chemical Properties of Studied Drugs

BDDCS Class	Compound	Administered Salt	MW (g/mol)	pKa	Aqueous Solubility (mg/L)	Log P	Dose (mg)	D_0
1	Alprazolam		308.77 (7)	5.08 (8, 9)	73 (7)	2.12 (7)	1	0.055
	Amlodipine	Amlodipine besylate	408.89 (7)	9.45 (8, 9)	2930 (10)	3 (7)	10	0.014
	Fluoxetine	Fluoxetine hydrochloride	345.79 (11)	9.8 (8, 9)	14000 (11)	4.05	60	0.017
	Sertraline	Sertraline hydrochloride	306.24 (7)	9.16 (8, 9)	3800 (12)	5.51	100	0.105
	Sunitinib	Sunitinib malate	398.48 (7)	8.95 (8, 9)	25000 (13)	5.2 (13)	50	0.008
	Tofacitinib	Tofacitinib citrate	312.37 (14)	5.07 (14)	50 (14)	1.15 (14)	5	0.400
2	Etoricoxib		358.85 (7)	4.96 (15)	140 (8, 9)	2.79 (15)	120	3.429
	Febuxostat		316.38 (7)	3.08 (15)	13 (7)	3.52 (15)	120	36.923
	Ibrutinib		440.5 (16)	6.58 (15)	3 (16)	3.63 (15)	140	186.667
	Zofenopril	Zofenopril calcium	429.0 (17)	3.5 (15)	3.11 (15)	4.31 (15)	30	38.585
3	Clonidine	Clonidine hydrochloride	266.6 (18)	8.16 (15)	480 (19)	2.55 (19)	0.863	0.007
	HCTZ		297.74 (7)	9.09 (15)	600 (8, 9)	-0.07 (7)	12.5	0.083
	Moxifloxacin	Moxifloxacin hydrochloride	401.44 (7)	9.42 (15)	168 (19)	2.9	400	0.058
4	Chlorthalidone		338.77 (7)	8.76 (15)	270 (8, 9)	0.85 (7)	25 and 50	0.370 and 0.741

BDDCS: Biopharmaceutics Drug Disposition Classification System; HCTZ, Hydrochlorothiazide; LogP: Partition coefficient (octanol/water); MW: Molecular weight; pKa: Acid dissociation constant

Aqueous solubility corresponds to salt forms when these are administered

D_0: Dose Number, determined as $\boldsymbol{D_0 = \frac{\frac{Dose\ (mg)}{250\ mL}}{Solubility\ (mg/L)}}$

Table 3 Pharmacokinetic Properties of Studied Drugs

BDDCS Class	Compound	F (%)	% bound to plasma protein	% excreted unchanged in urine	t_{max} (h)	$t_{1/2}$ (h)
1	Alprazolam	84-92[(o)] (20)	80 (21)	20 (22)	1-2 (21)	6.3-26.9 (21)
	Amlodipine	64-90[(a)] (23)		10 (22)	6 - 12 (23)	30 - 50 (23)
	Fluoxetine	<90[(o)] (24)	94.5 (11)	1.25 (22)	6-8 (25)	24-72 (25)
	Sertraline	44[(a)] (26)	98 (27)	0.2 (22)	4.5-8.4 (27)	22-36 (26)
	Sunitinib	High (28, 29)	90-95 (13)	4 (22)	6-12 (13)	40-60 (13)
	Tofacitinib	74[(o)] (14)	39 (14)	29 (14)	0.5-1 (14)	3 (14)
2	Etoricoxib	100 (30)	92 (30)	0.5 (22)	1 (30)	22 (30)
	Febuxostat		99.2 (31)	3 (22, 31)	1-1.5 (31)	5-8 (31)
	Ibrutinib	2.9[(a)] (16)	97.3 (16)		1-2 (16)	4-6 (16)
	Zofenopril				1.5 (32)	
3	Clonidine	70-80 [(a)] (33)	20-40 (18)	40-60 (33)	1-3 (33)	12-16 (33)
	HCTZ	65-75[(o)] (35)	40-68 (35)	100 (22)	1-5 (35)	5.6-14.8 (34)
	Moxifloxacin	90 (36)	30-50 (37)	20 (37)	0.5-4 (36)	12 ± 1.3 (36)
4	Chlorthalidone	64[(o)] (38)	74.8 ± 1.2 (39)	65 (22)	8-12 (38)	40-50 (38)

F: Absolute bioavailability; $t_{max:}$ Time to reach maximum plasma concentration; $t_{1/2}$: Elimination half-life; [(a)]: Absolute bioavailabilty; [(o)]: Oral bioavailability

Appendix 1. All methods were considered to be validated in accordance to the applicable FDA or EMA guidances.

Data Analysis

Data from each drug were modeled separately using the nonlinear mixed-effects modeling population approach with the Monolix®software version 2018R2 (Lixoft, Antony, France) which implements a SAEM algorithm for nonlinear mixed-effects models without approximations for estimation of the population parameters and conditional distribution method for individual estimation. Data were logarithmically transformed during the analysis.

Inter-individual (IIV) and inter-occasion variability (IOV) were modeled exponentially providing

non-negative estimates for any of the individual parameters in the model. Covariance between random effects was tested for significance (see below). Residual variability was modeled using the additive error model in logarithmic scale. Inter-individual variability in the bioavailability factor (F) was also explored. Given that variability in clearance affects both area under the curve (AUC) and elimination half-life ($t_{1/2}$) parameters, and that variability in F affects AUC but not $t_{1/2}$, therefore based on the differences in the PK profiles across individuals, it is possible, when present, to estimate IIV on F as on other parameters as well.

Drug disposition was characterized using compartmental models parameterized in apparent volumes of distribution of the central and peripheral compartments (V_1 and V_2, respectively), distribution clearance (Cl_D) and total elimination clearance (Cl). Linear (concentration and time independent) pharmacokinetics was assumed, unless indicated by the data and/or model misspecifications. Absorption process was characterized using first or zero order kinetics characterized by the first order rate constant of absorption (k_a) or the duration of the absorption process (TK_0), respectively, considering the eventual presence of a lag time. Absolute bioavailability could not be estimated and arbitrary value of 1 was used instead, which could vary across individuals reflecting IIV and/or IOV. In cases where more than one dose level was administered (different studies pooled together) a relative bioavailability different from 1 was also explored.

Given that all BE trials in the current evaluation include participants subject to strict inclusion and exclusion criteria and were designed as crossover, i.e. the same participant completing the study is administered each IMP, no covariate model was included in the developed popPK models. Differences in the absorption processes between test and reference formulations were not evaluated given that for all drugs, IMPs were bioequivalent on both C_{max} and AUC, reflecting the rate and extent of absorption, respectively.

Model Selection

Selection between competitive models was performed according to the following different criteria: (i) Goodness of fit plots, where the absence of trends indicate absence of major model misspecifications, (ii) parameter precision expressed as relative standard error equal to the ratio between the standard error of the parameter divided by the corresponding parameter estimate, where RSE values lower than 50% suggest that the parameter is identifiable, and (iii) Akaike information criteria (AIC) (40) which informs whether the addition of model complexities (represented by the corresponding extra parameters) is associated with an improvement in the description of the data.

Fig. 1 Visual predictive checks (VPC). Simulation-based diagnostic plots for plasma concentration (mg/L) versus time (h). The observed (empirical) 10%, 50% and 90% percentiles are presented with blue lines, and summarize the distribution of the observations. Prediction intervals for each percentile estimated across all simulated data, computed with a level of 90% confidence interval (CI), are displayed as coloured areas (red for the 50th percentile, and blue for the 10th and 90th percentiles).

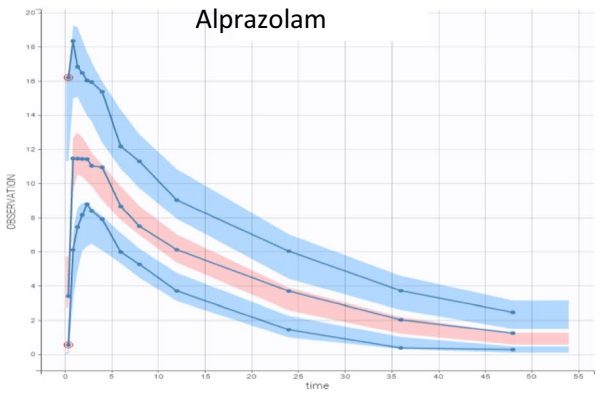
Model Evaluation

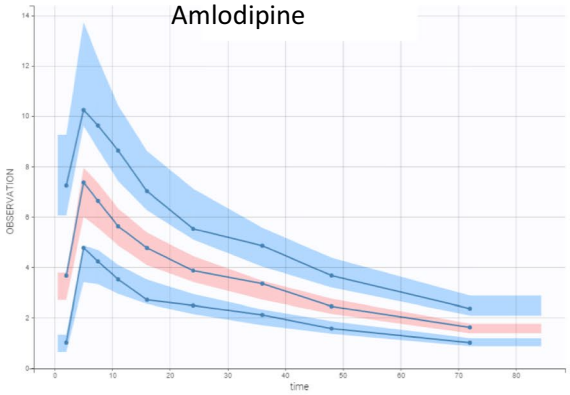
Once the population pharmacokinetic model is selected, further evaluation takes place. For the visual predictive checks (41, 42), a simulation-based diagnostics was used. One thousand virtual studies of the very same design characteristics as the original one were simulated using the selected model and parameter estimates. For each of the simulated studies and sampling time the 5th, 50th, and 95th percentiles of the simulated concentrations were calculated. Then the 90% prediction intervals of the above-mentioned percentiles were computed and represented graphically with the same percentiles obtained from the raw data.

RESULTS

Pharmacokinetics profiles in plasma for the 14 drugs analyzed in the current evaluation have been successfully described using the population pharmacokinetics as it can be observed from the simulation based diagnostics shown in Fig. 1. Both the median tendency of the data as well as their dispersion are in general well captured for all cases. However, it is observed in the case of febuxostat that C_{max} and a small part of the terminal phase shows some deviation in the 90th percentile. Also in etoricoxib, a slight deviation is observed after reaching C_{max} in the 50th and 10th percentiles.

Tables 4 and 5 list the estimates of, respectively, the fixed and random effects parameters, together with their corresponding precision. The values of RSE (%) indicated that all parameters listed in both tables were identifiable, as the values were lower than 25%, except for fluoxetine Cl_D (31.90%) and alprazolam Cl_D (49.00%), as presented in Table 4. In all popPK models, typical estimates of disposition parameters are in accordance to reported values in the literature, as shown in Table 4.


Alprazolam
OBSERVATION
time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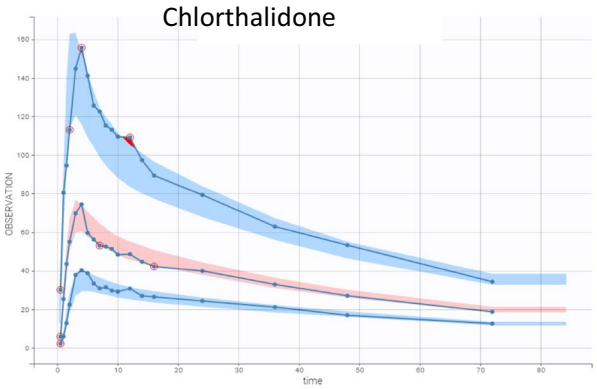
OBSERVATION
time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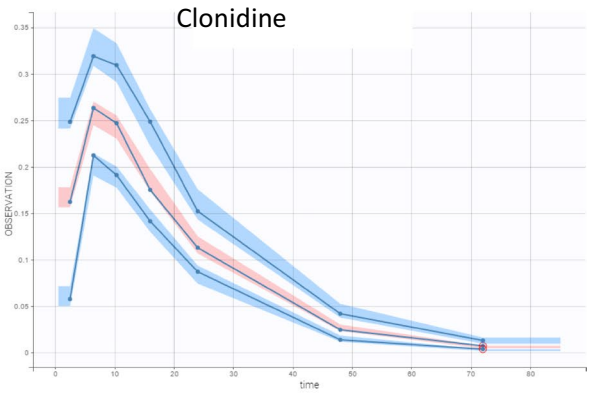
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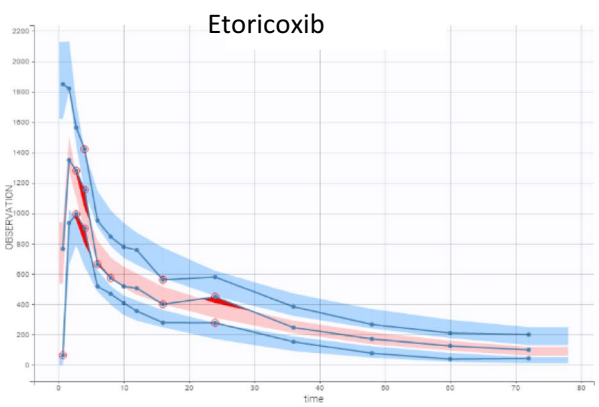
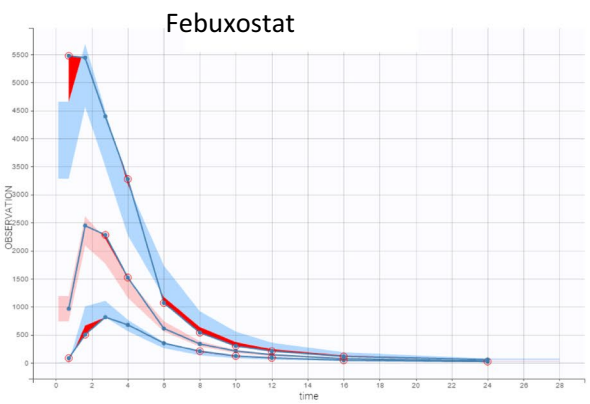
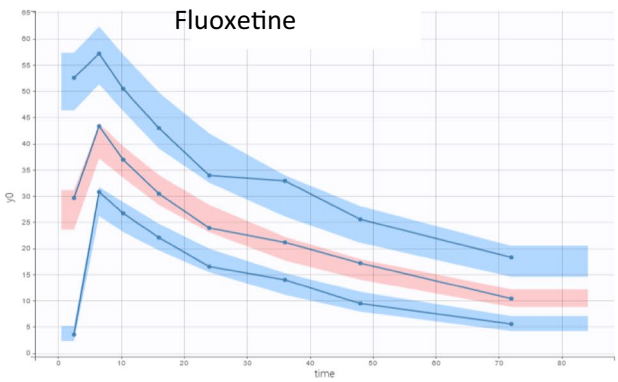
time


Etoricoxib
OBSERVATION
time


Febuxostat
OBSERVATION
time


Fluoxetine
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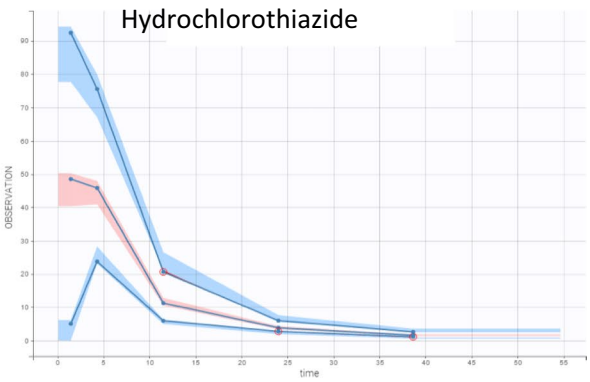
time


Hydrochlorothiazide
OBSERVATION
time

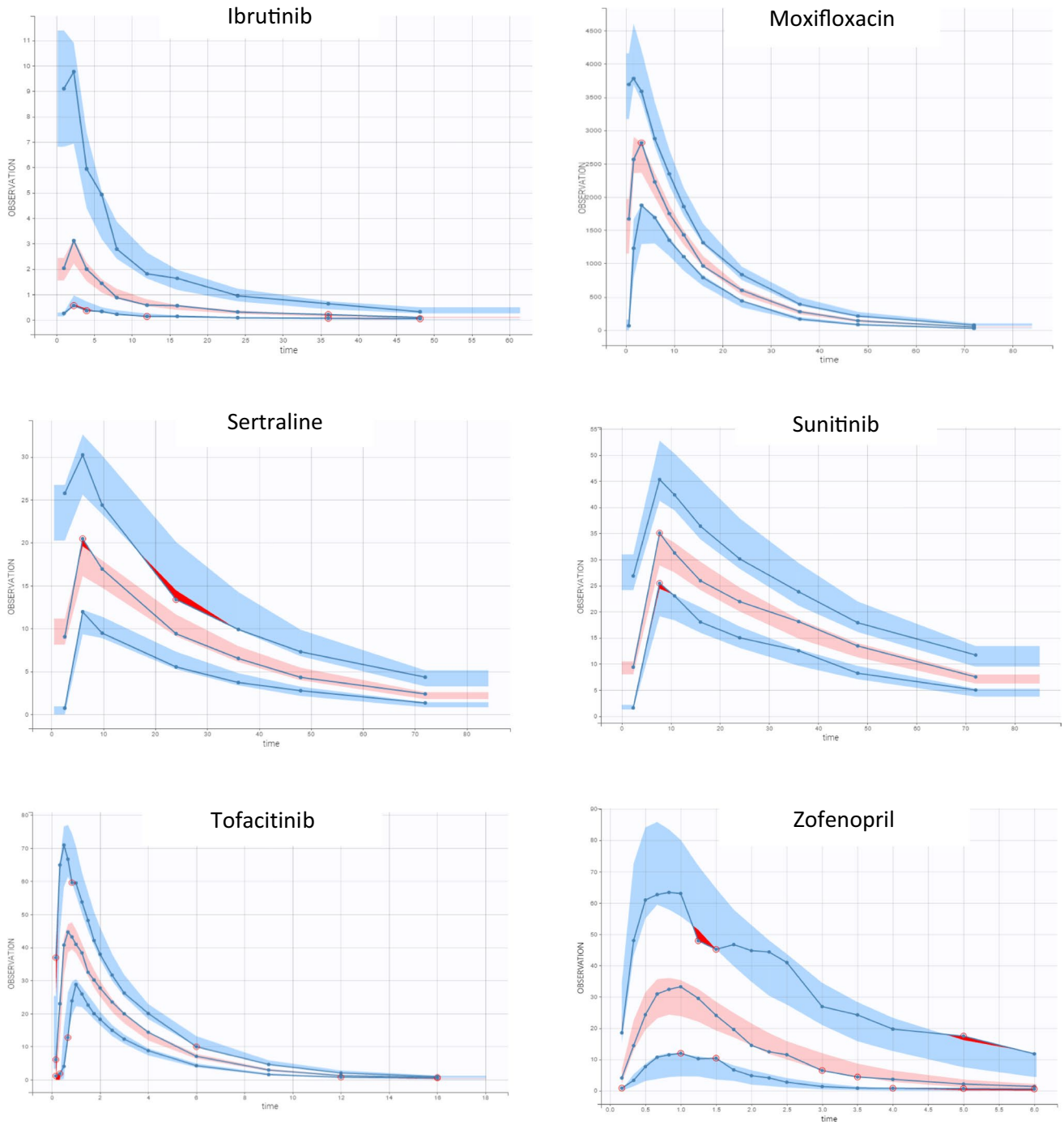


Fig. I (continued)

Also, mention that in the estimates of random effects parameters (Table 5), an RSE of 85.2% was observed in V_2/F of IOV for alprazolam, as well as in k_a of IIV for tofacitinib of 53.6%. However, removing those random effect parameters from the model increase the -2LL value significantly ($p<0.01$) suggesting that the 95% CI are not symmetric. In addition, the shrinkage values for each of the random effects parameters and RUV in no case exceeded 20%.

With the exception of clonidine, sertraline, sunitinib and zofenopril, where disposition was best characterized with a one compartment model, all the other drugs exhibited two compartmental kinetics. For almost half of the studied compounds the estimates of the apparent volumes of distribution largely exceed the physiological body volume, being the extreme cases represented by sertraline and ibrutinib, where the total apparent volume of distribution (V/F, derived by the sum of V_1/F

Table 4 Estimates of the Fixed Effects Model Parameters

								Literature	
BDDCS	**DRUG**	T_{lag} (h)	k_a (h^{-1}) or Tk_0 (h)	Cl/F (L/h)	Cl_D (L/h)	V_1/F (L)	V_2/F (L)	Cl/F (L/h)[b]	V/F (L) [L](b)
1	Alprazolam	0.225 (3.57)	3.24 (24.00)	4.55 (9.00)	9.08 (49.00)	63.9 (4.40)	16.8 (17.80)	2.94-6.3[c] (20)	56-91[c] (20)
	Amlodipine	0.207 (24.1)	4.01 (5.33) [a]	30.6 (6.11)	58.8 (15.10)	1120 (6.95)	446 (8.38)	42±9.4[d] (43)	2100±470[d] (44)
	Fluoxetine	0.702 (5.55)	2.62 (8.08) [a]	26.2 (6.67)	13.5 (31.90)	1250 (2.62)	452 (13.30)	36-50 (25)	840-3010[e] (25)
	Sertraline	0.875 (5.43)	2.97 (8.03) [a]	157 (14.2)	NA	4810 (10.2)	NA	121±44.6[f] (27)	3063±1329[f] (5)
	Sunitinib	0.619 (4.28)	5.20 (4.48) [a]	32.5 (5.10)	NA	1380 (4.37)	NA	34-62 (13)	2230 (13)
	Tofacitinib	0.225 (6.05)	4.03 (11.30)	34.2 (2.05)	27.1 (12.90)	67 (2.56)	31.9 (6.30)	34.9[g] (45)	87 (45)
2	Etoricoxib	0.306 (9.49)	0.75 (12.60) [a]	4.35 (7.40)	13.4 (4.91)	62.5 (5.18)	68.2 (12.60)	2.90[h] (46)	120[h] (30)
	Febuxostat	0.316 (5.57)	1.34 (9.86) [a]	9.97 (4.08)	1.81 (7.95)	26.1 (5.24)	14.9 (7.43)	8.20±2.30 [i] (47)	33±12 [i] (47)
	Ibrutinib	0.320 (3.13)	1.00 (8.35) [a]	4070 (3.83)	5570 (11.8)	27300 (8.52)	44500 (13.6)	2000 (16)	10000 (16)
	Zofenopril	0.100 (10.00)	1.04 (13.10)	346 (6.47)	NA	264.42 (4.00)	NA	323±183[j] (17)	419[j] (17)
3	Clonidine	0.270 (14.50)	0.300 (3.24)	15.5 (2.83)	NA	251 (1.45)	NA	15.4±6.4[k] (33)	147±28[k] (18)
	HCTZ	0.436 (5.46)	0.787 (7.92)	24.8 (8.77)	13.4 (5.79)	96.4 (6.00)	121 (5.59)	11.46-22.6[l] (34)	50.5-210.9[l] (34)
	Moxifloxacin	0.225 (7.10)	2.16 (22.20)	8.64 (1.73)	1.12 (12.20)	116 (3.62)	24.8 (6.47)	11.1±2.8[m] (36)	208±52.4 [m] (36)
4	Chlorthalidone	0.431 (4.70)	1.88 (6.71) [a]	8.07 (1.56)	39.7 (6.42)	371 (5.58)	179 (4.63)	3.67-8.67[n] (39)	364-903[n] (39)

Model parameters: T_{lag} (lag time); k_a (first order absorption rate constant); Cl (total plasma clearance); Cl_D (inter-compartment distribution clearance); V_1 (apparent volume of distribution of the central compartment); V_2 (apparent volume of distribution of the peripheral compartment). Results are expressed as parameter estimates with RSE(%) in parenthesis. NA, not applicable (i.e., one compartmental model).

[a]Parameter corresponding to the duration of the absorption process (Tk_0).

[b]For Cl/F and V/F estimates from the literature presented as normalized by body weight, a 70 kg subject is assumed. Estimates are presented as Mean±SD if available;

[c]Alprazolam: Estimates for Cl/F and V/F are 0.7-1.5 mL/min/kg and 0.8-1.3 L/kg, respectively;

[d]Amlodipine: Cl/F was estimated as Dose/AUC for 2x5 mg capsules, based on data from a comparative bioavailability trial (n=12). From the same study, V/F was derived as the ratio of Cl/F by k_e estimate (0.020 h^{-1}) described for the same data;

[e]Fluoxetine: Estimate for V/F estimate is 12-43 L/Kg;

[f]Sertraline: Estimate Cl/F was obtained as Dose/AUC for the oral solution of Zoloft, based on data from a comparative bioavailability trial (n=20). From the same study, V/F was derived as the ratio of Cl/F by k_e estimate (0.0336 h^{-1}) described for the same oral solution data;

[g]Tofacitinib: Estimate for Cl/F based on a meta-analysis of non-compartmental PK parameters from healthy subjects across 16 Phase 1 studies, and presented as the pooled geometric mean estimate;

[h]Etoricoxib: Estimate Cl/F was obtained as Dose/AUC for the single dose oral administration, based on data from a PK study (n=12). From the same study, V/F was derived as the ratio of Cl/F by k_e estimate (0.0224 h^{-1}) described for the same data;

[i]Febuxostat: Estimates for Cl/F and V/F were obtained from literature reference summarizing PK results following a single 120 mg dose on fasting conditions, in healthy subjects;

[j]Zofenopril: Estimate for Cl/F was obtained as Dose/AUC for an oral dose of 60 mg zofenopril calcium corresponding to 57.4 mg zofenopril (Tablet form), based on data from a single dose pharmacokinetic study (n=18). From the same study, V/F was derived as the ratio of Cl/F by β estimate (0.770 h^{-1}) described for the same oral data;

[k]Clonidine: Estimates for Cl/F and V/F were derived from estimates for Cl and V based on intravenous dosing (219±92 mL/min and 2.1±0.4 L/kg, respectively) and assuming an estimate for the absolute bioavailability as 0.75 (described as 0.7 to 0.8) (48)

[l]Hydrochlorothiazide: Estimate for total Cl/F corresponds to the estimate of Cl_R/F, considering that hydrochlorothiazide is not metabolized. Estimate range of Cl_R/F was obtained from healthy subjects (Cl_{CR} >100 mL/min, age 23-71 years)(n=6) cohort included in a renal impairment PK study, with mean of 285 mL/min and range of 191-377 mL/min; From the same study, V/F was derived as the ratio of Cl_R/F by β estimate described for the same oral data, showing a mean of 154.2 L and a range of 50.5-210.9 L;

[m]Moxifloxacin: Estimate for Cl/F was obtained as Dose/AUC for oral single doses of 400 mg (n=372). From the same study, V/F was derived as the ratio of Cl/F by k_e estimate (0.0.0533 h^{-1}) derived from an assumed elimination $t_{1/2}$ of 13h (range 11.5-15.6 h) described for the same oral data;

[n]Chlorthalidone: Estimate range of Cl/F was obtained from healthy subjects (n=6) included in a PK study, with mean of 92.8 mL/min and range of 61.2-144.5 mL/min; From the same study, V/F was estimated with a mean of 7.6 L/kg and a range of 5.2-12.9 L/kg;

Table 5 Estimates of Random Effect Parameters

BDDCS	DRUG	IIV							IOV							RUV
		T_{lag}	ka /Tk0	Cl /F	Cl_D/F	V_1/F	V_2/F	F	T_{slag}	ka/Tk0	Cl /F	Cl_D/F	V_1/F	V_2/F	F	
1	ALP	N.e.	0.75 (32.0)	0.33 (17.2)	N.e.	0.07 (41.0)	N.e.	0.18 (20.0)	0.19 (20.8)	0.87 (19.3)	N.e.	0.83 (29.5)	N.e.	0.11 (85.2)	N.e.	0.16 (2.91)
	AML	0.71 (38.7)	[(1)] 0.19 (24.4)	N.e.	N.e.	0.24 (16.1)	N.e.	0.31 (15.0)	0.69 (25.2)	[(1)] 0.14 (21.5)	N.e.	N.e.	N.e.	N.e.	0.05 (18.4)	0.13 (2.77)
	FLU	0.20 (31.1)	[(1)] 0.34 (21.5)	0.27 (17.6)	1.17 (34.1)	N.e.	N.e.	0.22 (15.4)	0.26 (16.3)	[(1)] 0.31 (16.3)	0.12 (19.7)	N.e.	N.e.	N.e.	0.05 (21)	0.11 (2.48)
	SER	N.e.	N.e.	0.16 (15.9)	N.e.	N.e.	N.e.	0.22 (23.9)	0.36 (11.0)	[(1)] 0.52 (11.8)	N.e.	N.e.	N.e.	N.e.	0.22 (16.9)	0.20 (2.76)
	SUN	N.e.	[(1)] 0.21 (16.8)	0.10 (19.2)	N.e	N.e.	N.e.	0.27 (13.5)	0.29 (12.6)	[(1)] 0.12 (21.5)	0.07 (22.1)	N.e.	N.e.	N.e.	0.03 (31.7)	0.12 (2.38)
	TOF	0.22 (44.1)	0.35 (53.6)	N.e.	0.37 (26.9)	N.e.	N.e.	0.24 (11.9)	0.45 (12.1)	0.80 (11.7)	0.12 (8.92)	0.21 (39.3)	0.04 (25.7)	N.e.	N.e.	0.14 (2.44)
2	ETO	0.29 (39.0)	N.e.	0.31 (17.8)	N.e.	0.15 (33.6)	0.43 (18.9)	N.e.	0.36 (18)	[(1)] 0.72 (13.2)	0.05 (30.5)	N.e.	0.18 (21.1)	N.e.	N.e.	0.16 (2.98)
	FEB	N.e.	N.e.	N.e.	N.e.	0.17 (14.1)	N.e.	0.22 (14.5)	0.49 (8.64)	[(1)] 0.88 (8.53)	N.e	N.e.	N.e.	N.e.	0.15 (14.7)	0.40 (2.08)
	IBR	N.e.	[(1)] 0.35 (28.1)	N.e	0.42 (36)	0.41 (16.5)	0.74 (17.3)	0.59 (12.8)	0.34 (7.11)	[(1)] 0.68 (8.89)	N.e.	0.95 (9.7)	0.52 (8.29)	0.71 (12.3)	0.44 (7.29)	0.31 (1.67)
	ZOF	0.48 (18.1)	0.44 (45.2)	0.30 (22.2)	N.e.	N.e.	N.e.	0.27 (18.9)	N.e.	0.87 (13.7)	0.33 (15.5)	N.e.	N.e.	N.e.	N.e.	0.49 (2.64)
3	CLO	0.54 (30.3)	0.15 (18.1)	0.14 (13.8)	N.e.	N.e.	N.e.	0.14 (14)	0.65 (17.5)	0.07 (31.4)	0.04 (16.1)	N.e.	N.e.	N.e.	0.04 (19.7)	0.10 (2.41)
	HCTZ	0.20 (37.6)	0.33 (25.1)	0.15 (14.5)	N.e.	N.e.	N.e.	0.23 (15.4)	0.37 (11.8)	0.41 (13.6)	N.e.	N.e.	N.e.	N.e.	0.06 (18.7)	0.20 (2.35)
	MOX	0.30 (23.0)	0.88 (23.9)	N.e.	N.e.	0.19 (14.9)	N.e.	0.14 (14.9)	0.26 (17.4)	0.96 (15.8)	0.04 (23.6)	N.e.	N.e.	N.e	N.e.	0.20 (2A0)
4	CHL	N.e.	[(1)] 0.26 (37.8)	N.e.	N.e.	0.34 (13.0)	N.e.	0.15 (10.8)	0.45 (8.3)	[(1)] 0.55 (10.7)	N.e.	0.22 (27.8)	0.22 (9.08)	0.35 (9.29)	N.e.	0.13 (1.86)

Estimates of variability are expresses as coefficient of variation calculated as $CV(\%) = \left(\sqrt{e^{\omega^2} - 1}\right) \times 100$, were ω^2 corresponds to the estimate of the variance, and RSE (%) in parenthesis.

[(1)]Parameter correspond to a zero-order absorption rate constant defined as Tk_0.

BDDCS: Biopharmaceutics Drug disposition classification system; IIV: Intra-individual variability; IOV: Intra-occasion variability; RUV: Residual variability [log(ng/mL)]; N.e.: not estimated; ALP: Alprazolam; AML: Amlodipine; CHL: Chlorthalidone; CLO: Clonidine; ETO: Etoricoxib; FEB: Febuxostat; FLU: Fluoxetine; IBR: Ibrutinib; MOX: Moxifloxacin; SER: Sertraline; SUN: Sunitinib; TOF: Tofacitinib; ZOF: Zofenopril; HCTZ: Hydrochlorothiazide.

and V_2/F in case of two compartment models) was estimated up to 4810 and 71800 L, respectively. In general, it is observed in Table 4 that this volume is higher in the population models of drugs belonging to Class 1 and Class 2, as previously referenced by Wu and Benet (3). Remarkably, for ibrutinib, total plasma elimination clearance (Cl/F) was estimated as 4070 L/h, suggesting that oral bioavailability for this particular compound is very low. This is confirmed in Tables 2 and 3, which shows that the bioavailability of ibrutinib is 2.9% due to extensive first pass metabolism. The corresponding clearance for sertraline was 157 L/h a value not as high as the previous one suggesting extensive body distribution as well. Also note the high Cl/F of zofenopril, 346 L/h. Apart of the values of Cl/F mentioned above, the rest ranged between 1.12 and 128 L/h.

The zero and first order rate mechanisms of absorption occurred evenly in the set of studied drugs. For each BDDCS class, the values of either the zero order (presented in Table 4 as duration of absorption process (Tk_0), as obtained from Monolix) or first order absorption rate constants were quite consistent. For class 1 drugs, the residual standard error (RSE) ranged from 4.48% to 8.08% for Tk_0 estimates and from 11.30% to 24.00% for k_a estimates; for class 2 drugs, RSE ranged from 8.35% to 12.60% for Tk_0 estimates and was determined as 13.10% for k_a estimate of zofenopril; for class 3 drugs, RSE ranged from 3.24% to 22.20% for k_a estimates; and for the class 4 chlorthalidone, RSE was determined as 6.71% for Tk_0 estimate.

For BDDCS class 1, four out of six drugs (amlodipine, fluoxetine, sertraline and sunitinib) were absorbed through a zero-order mechanism, with Tk_0 ranging from 2.62 to 5.20 h. For these drugs, T_{max} estimates are described in the literature to occur between 4.5 and 12 h (Tables 2 and 3). Two drugs (alprazolam and tofacitinib) were absorbed through a first order kinetics, with k_a of 3.24 and 4.03 h^{-1}, respectively. For these drugs T_{max} occur between 0.5 and 2 h (Table 3).

For BDDCS class 2, three out of four drugs (etoricoxib, febuxostat and ibrutinib) were absorbed also through a zero-order mechanism, with Tk_0 ranging from 0.75 to 1.34 h. One drug (zofenopril) was absorbed through a first order kinetics, with k_a of 1.04 h^{-1}. For all BDDCS class 2 drugs, T_{max} estimates are described in the literature to range between 1 and 2 h (Table 3).

For BDDCS class 3, all the three drugs (clonidine, hydrochlorothiazide and moxifloxacin) presented a first order absorption kinetics, with k_a ranging from 0.300 to 2.16 h^{-1}. For these drugs, T_{max} estimates are described to range between 0.5 and 5 h. For BDDCS class 4, chlorthalidone was absorbed through a zero-order kinetics, with Tk_0 estimated as 1.88 h (same magnitude of class 2 drugs) and a described T_{max} of 8-12 h.

For all drugs, estimates for T_{lag} ranged from 0.100 to 0.875 h, which is within the time of gastric emptying.

The pharmacokinetic parameters for which IIV could be estimated for the vast majority of the drugs were relative F, and k_a/Tk_0 followed by T_{lag}, V_1/F, Cl/F, and Cl_D. The magnitude of IIV in relative bioavailability in general appears to be lower than the values obtained from k_a/Tk_0. No apparent correlation between the magnitude of IIV and BDDCS classes is seen.

As it was seen for IIV, the parameters that showed, for almost all drugs, significant IOV were those related to absorption. Interestingly the magnitude of IOV associated to T_{lag}, and k_a/Tk_0 was in several cases greater than the corresponding estimate of IIV. On the other hand, the estimates of IOV obtained for relative bioavailability were markedly lower than those of IIV. No apparent correlation between the magnitude of IOV and BDDCS classes is seen. The fact that the magnitude of IOV is higher than IIV will hamper the dose adjustment to achieve a desired therapeutic outcome, although drugs associated with values of IOV comparable or even higher tan IIV are part of successful therapeutic drug monitoring programs (49).

The estimation of an IIV and IOV for the absorption parameters is supported by the complex nature of the gastrointestinal drug absorption phenomena, gradually unveiled in the last twenty years, involving drug/formulation-dependent factors (e.g. drug solubilization, supersaturation, drug dissolution, drug precipitation) and system dependent factors (e.g. selective permeability and drug ionization changes along the gastrointestinal lumen affecting all above processes, among other processes) (50, 51).

With respect to residual error and when drugs were clustered based on their BDDCS class, a very similar magnitude was showed. For BDDCS class 1 and 3 values ranged between 0.11 and 0.2 ranging. For BDDCS class II the residual variability resulted higher.

In order to validate Cl/F and V/F estimates as derived from the developed popPK models, a comparison was performed with literature values for the same parameters. Both estimates were correlated by means of Fig. 2, representing the plot of observed versus predicted for the Log (Cl/F) and Log (V/F). Predicted V/F estimates for drugs following 2 compartment disposition correspond to V_{ss}/F as determined by the sum of V_1/F and V_2/F.

Additionally, a correlation between ISCV (%) and the Log of Dose Number (D_0) was investigated for C_{max} and $AUC_{0\text{-}t}$ in Fig. 3. D_0 is a dimensionless parameter

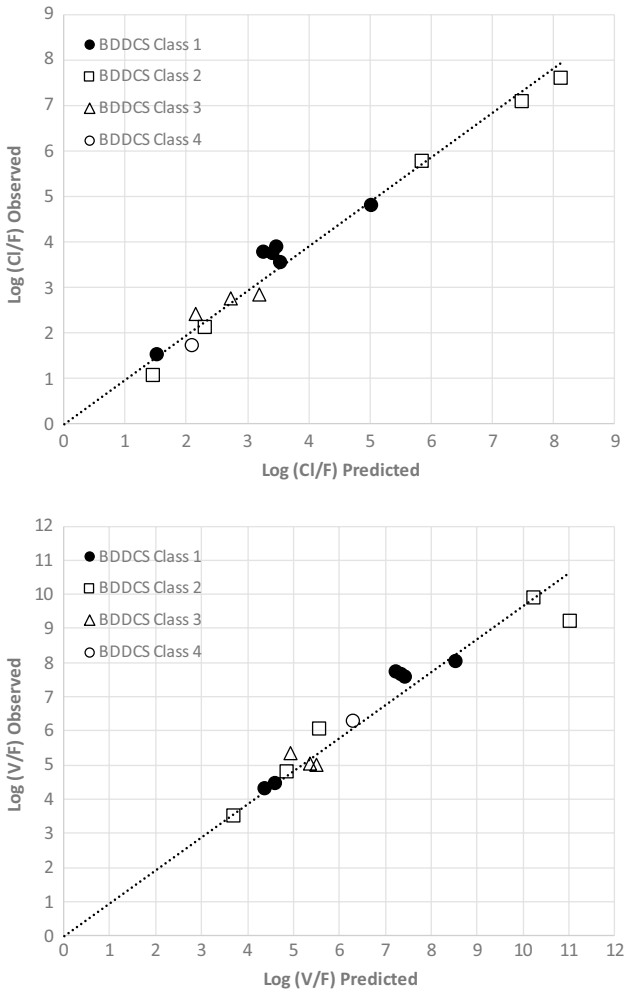


Fig. 2 Plot of observed versus predicted for the Log (Cl/F), Upper panel, and Log (V/F), Lower panel. Predicted V/F estimates correspond to V_{ss}/F as determined by $V_1/F+V_2/F$. Line represents the line of unity.

initially used by Dressman *et al* (48) as part of the Absorption Potential concept. D_0 is determined as

$$D_0 = \frac{\frac{Dose\ (mg)}{250\ mL}}{Solubility\ (mg/L)} \quad (1)$$

where 250 mL is the assumed volume of water taken with the dose. The importance of dose/ solubility ratio was investigated by several authors, unveiling its central role not only for the dynamics of dissolution process but also for the extent of a drug absorption from the GI tract and in consequence for the Biopharmaceutical classification system (52–56).

DISCUSSION

In the current evaluation, we have analyzed the PK profiles obtained from BE studies from a variety of drugs covering different BDDCS classes. All models showed good performance as suggested by the simulation-based diagnostics and provided estimates of the typical population parameters in accordance where those previously reported in literature and listed in Table 4 and as shown in Fig. 2. For all models and drugs, estimates for population Cl/F and V/F were within 2-fold from the estimates described in the literature with the exception for ibrutinib, fluoxetine, and sunitinib. For ibrutinib, the discrepancy might be explained by its low bioavailability being estimated as 2.9% (90% CI: 2.1, 3.9) in fasted condition (16). Slightly different experimental conditions might affect bioavailability provoking an impact in model parameters. Fluoxetine and sunitinib have high bioavailability (24, 28, 29), and are the drugs showing the largest values of terminal half-life (13, 25). In those circumstances expanding short sampling periods could hamper proper characterization of terminal disposition phase providing rationale for the discrepancies found.

As presented in the Results section, the typical estimates of T_{lag} were within the physiological range of gastric emptying and similar across the different classes for all drugs, given the similarity in the PK sampling strategy defined for all the 18 BE studies testing immediate release formulations only. Once the administration of the drug is carried out on an empty stomach, under these conditions, one of the possible triggers of IIV and IOV is the Motor Myoelectric Complex (MMC), also known as Migrating Motor Complex. MMC is a contraction pattern of the gastrointestinal muscle that travels from the stomach to the ileocecal valve and is interrupted by the entry of food into the stomach. Each cycle is divided into 4 phases each with different characteristics and duration (57–59). In a BE trial we cannot ensure that all the subjects are in the same phase of the MMC, so to try to minimize this fact the administration of the drug is accompanied with a sufficient amount of water, 240 mL in the 18 studies, in order to try to make a synchronization between all the subjects and between all the occasions, and to minimize this triggering factor of variability.

The ISCV% estimated for C_{max} and $AUC_{0\text{-}t}$, as derived from the ANOVA performed for bioequivalence trials, was generally lower for BDDCS class 1 and 3 drugs (high solubility drugs), in comparison to class 2 and 4 (low solubility drugs). No direct correlation was obtained between model residual variability (RUV) derived from the developed popPK models (Table 5) and ISCV%. Nevertheless, a higher variability in the PK profiles is expected for low solubility drugs (i.e., BDDCS class 2 and 4). For these drugs, a high ISCV% is usually expected for the PK metrics C_{max} and AUC used in bioequivalence statistical analysis. The same

trend is observed for RUV. For BDDCS class 2, and despite a RUV of 0.16 was derived for the model fitted to etoricoxib data, all the other drugs from this class have shown RUV estimates of 0.31 to 0.49. For BDDCS class 4, no conclusions can be taken, as data from only one drug (chlorthalidone) is available. Opposite, for high solubility drugs (i.e., BDDCS class 1 and 3) a low variability is anticipated. Similarly, obtained RUV from the PopPK modeling for BDDCS class 1 and 3 drugs was ≤0.20.

Interestingly, the ISCV% estimated for C_{max} seems to correlate with the log of Dose Number for BDDCS Class 1, 2 and 3 (Fig. 3), turning the variability associated to C_{max} dependent on the ratio between dose and solubility. For Class 4 only two observations are available (for chlorthalidone) and therefore no reliable conclusions can be obtained.

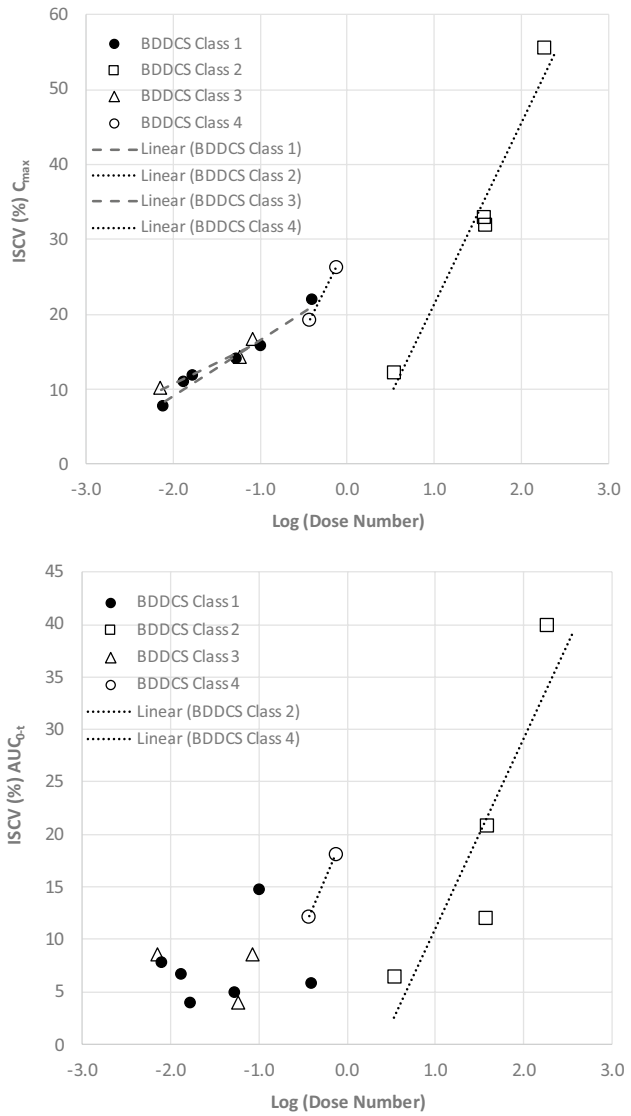


Fig. 3 Intra-Subject Coefficient of Variation (ISCV %) for C_{max} and AUC_{0-t} versus the Log (Dose Number) relationship.

From the same approach, ISCV% estimated for AUC seems to correlate with the log of Dose Number only for BDDCS Class 2 drugs (low solubility) (Fig. 3), turning the variability associated to this parameter independent from the ratio between dose and solubility for high solubility drugs (class 1 and 3).

Variations in the volume of solvent result in variations in the Dose Number, in an inverse manner. These data may have implications for conducting crossover BE studies, as higher volume of fluids may correspond to lower ISCV%. According to current practice (which reflects the regulatory guidances (60)), in bioequivalence studies under fasting conditions IMPs are usually administered with 240 mL of water, and fluid ingestion is prohibited for 1 h before and 1 hour after IMP administration; thereafter, fluids are allowed as desired. To minimize the ISCV%, fluid ingestion should be standardized at least until C_{max} is achieved, for each participant, in the different treatment periods. This is particularly relevant for BDDCS class 2 drugs, where an increase in the Dose Number was associated with a dramatic increase in ISCV%, both for C_{max} and AUC, but especially for C_{max} (Fig. 3).

Interestingly, our analyses show that four of the six BDDCS class 1 drugs followed zero order absorption processes. Due to the high solubility that characterize this class of drugs, this fact would not be anticipated, as solubility is not expected to represent the kinetic limiting step in drug absorption. In fact, it is assumed that, regardless of the formulation administered, class 1 drugs do not exhibit either dissolution or permeability limited absorption, which, coupled with the high surface area of the small intestine, lead to a rapid and extensive absorption. Based on these assumptions, in a recent publication (51) the authors made an approximation of this rapid absorption with a constant rate of drug penetration, as defined by Eq. 2 below, which is in accordance with our findings.

$$(Rate\ of\ Penetration)_{Class\ 1} = P \bullet (SA)_{si} \bullet \left(C_{GI}\right) = k_{Class\ 1} = \frac{Dose}{\tau_i} \quad (2)$$

where P is the permeability of drug expressed in velocity units (length/time), $(SA)_{si}$ is the surface area of small intestine and τ_i is the duration of the initial absorption phase (within the residence time in the stomach and small intestine).

For the other two class 1 drugs (alprazolam and tofacitinib), absorption kinetics followed a first-order population pattern. In both cases, no information on membrane transporters linked to these molecules was

found in the literature. Interestingly, in accordance to what is described for the other four drugs (amlodipine (61, 62), fluoxetine (63–65), sertraline (64, 66–68) and sunitinib (69–73)), these drugs are all substrates of the P-Glycoprotein, in addition to other membrane transporters. This evidence is however not in accordance with Wu and Benet (3), who states that transport effects are minimal in the gut and liver for this class 1 drugs. Therefore, the mechanism that supports zero-order absorption kinetics in this work, in line with other authors (51) should be further investigated.

Class 1 drugs showing zero-order absorption kinetics were characterized by a duration of the absorption process from 2.62 h to 5.20 h and class 1 drugs showing first-order absorption kinetics were characterized by a duration of the absorption process as 2.14 h (alprazolam) and as 1.72 h (tofacitinib), estimated by 10 times the absorption half-life values (0.214 h and 0.172 h, respectively). The duration of the absorption process was shorter than 4.86h (representing the sum of gastric and small intestine transit times), for all the drugs except for sunitinib which has shown a value a near value (5.20 h). Therefore, these drugs are characterized by a rapid absorption, with the small intestine being plausibly the major site of absorption (51).

On the other hand, for BDDCS class 2 drugs, the low solubility (as confirmed in Table 2) and the predominant effect of efflux transporters in gut (74) that characterize this class of drugs, could justify that three of the four drugs belonging to this class present population models with zero-order absorption kinetics. In this case, the low solubility may be the main kinetic limiting step in the absorption process, as absorption of these drugs is mainly through passive diffusion, despite it may also occur through influx transporters, for which saturation is not expected. As mentioned in the Results section, a greater residual variability (RUV) was observed for these drugs, which can also be explained by the low solubility that characterize this class of drugs.

Our results are also in accordance with the mentioned publication (51). For class 2 drugs, a low rate of absorption is expected since the maximum value of the term C_{GI} in Eq. 3 cannot be higher than the low saturation solubility (C_S) of the drug in the gastrointestinal fluids, which can be assumed to be constant. The rate of gastric and small intestine penetration for class 2 drugs was approximated by the authors (51) as Eq. 3

$$(Rate\ of\ Penetration)_{Class\ 2} = P \bullet (SA)_{si} \bullet \left(C_S\right) = k_{Class\ 2} = \frac{F_{abs} \bullet Dose}{\tau_i} \tag{3}$$

where F_{abs} represents the fraction of dose absorbed in the stomach and small intestine. Interestingly, the duration of the absorption process was estimated to be in the range of 0.75 h to 1.34 h for drugs following zero-order absorption kinetics, which is considerably lower in comparison the range of zero-order drugs from class 1, and as 6.66 h for the drug following first- order absorption (zofenopril).

Regarding BDDCS class 3 drugs, all drugs showed a first-order absorption kinetics. These drugs are characterized by a high solubility (as confirmed in Table 2), and by a low permeability, with membrane transporters having a predominant effect in the absorption process (74) With the exception of moxifloxacin, for which no membrane transporters related to this drug were found in the literature, the absorption of the other drugs is related to several transporters, such as OCT3, OCTN2, and OCTN1 for clonidine (75, 76), and OAT1, OAT3, OAT4, or MRP4 for hydrochlorothiazide (77, 78). Macheras *et al* (51) mathematically suggest a zero-order absorption kinetics for class 3 drugs due to the rate limiting low permeability (Eq. 4), taking only into account the passive diffusion absorption and not the effect of influx transporters. Differently, our observations show a first-order absorption kinetics for class 3 drugs.

$$(Rate\ of\ Penetration)_{Class\ 3} = P \bullet (SA)_{si} \bullet \left(C_{GI}\right) = k_{Class\ 3} = \frac{F_{abs} \bullet Dose}{\tau_i} \tag{4}$$

For class 3 drugs, the duration of the absorption process was estimated to be 23.10 h for clonidine, 8.81 h for hydrochlorothiazide and 3.21 h for moxifloxacin. The duration of the absorption process is therefore highly dependent on the influx transporters effect.

Regarding class 4 drugs, and despite only one drug belonging to this class was tested (chlorthalidone), a zero-order absorption kinetics was observed, with a duration of the absorption process estimated as 1.88 h. These drugs are characterized by a low solubility (as confirmed in Table 2), and by a low permeability. Moreover, according to Benet *et al* (74), absorptive and efflux transporter effects can be important, depending on the drug. Nevertheless, no information on membrane transporters linked to chlorthalidone was found in the literature. For chlorthalidone, a delayed T_{max} of 8-12 h is described. Given that absorption process is expected to end at approximately 1.88 h, the delayed T_{max} is due to the prolonged elimination half-life, averaging 50 h as described in Hylaton 50 mg Prescribing Information (38).

Our results for chlorthalidone are in accordance with Macheras *et al* (51), where a zero-order absorption kinetics is expected similarly as given in Eq. 3 for drugs not depending on transporters.

CONCLUSION

Both in the case of IIV and IOV, the pharmacokinetic parameters that showed greater variability were k_a / Tk_0, F and T_{lag}, which are the parameters more related to drug absorption, being also higher in the case of IOV than for IIV.

Ingestion of the IMP along with 240 mL of water showed to standardize MMC phase and consequently gastric emptying, since all the T_{lag} estimates were close for the different drugs.

Interestingly, the ISCV% estimated for C_{max} seems to correlate with the log of Dose Number for BDDCS Class 1, 2 and 3 (Fig. 3), turning the variability associated to C_{max} dependent on the ratio between dose and solubility. For class 4 as only two observations are available (for chlorthalidone), no reliable conclusions can be obtained.

Therefore, for IMPs belonging to BDDCS classes 2 and 4, it is hypothesized that allowing *ad libitum* water intake after one hour post administration may increase the intra-subject variability related to C_{max} and AUC. Therefore, it is suggested to standardized water ingestion until the expected T_{max} of the drug.

The tendency observed among IMPs belonging to BDDCS class 1 to follow a zero-order absorption kinetics should be further investigated.

Conflict of Interest statement

We have no conflicts of interest to disclose.

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