Journal of Pharmacokinetics and Pharmacodynamics (2019) 46:27–42
https://doi.org/10.1007/s10928-018-9615-8

ORIGINAL PAPER

# Variance based global sensitivity analysis of physiologically based pharmacokinetic absorption models for BCS I–IV drugs

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Received: 5 September 2018 / Accepted: 10 December 2018 / Published online: 14 December 2018


## Abstract

Regulatory agencies have a strong interest in sensitivity analysis for the evaluation of physiologically-based pharmacokinetic (PBPK) models used in pharmaceutical research and drug development and regulatory submissions. One of the applications of PBPK is the prediction of fraction absorbed and bioavailability for drugs following oral administration. In this context, we performed a variance based global sensitivity analysis (GSA) on in-house PBPK models for drug absorption, with the aim of identifying key parameters that influence the predictions of the fraction absorbed and the bioavailability for neutral, acidic and basic compounds. This analysis was done for four different classes of drugs, defined according to the Biopharmaceutics Classification System, differentiating compounds by permeability and solubility. For class I compounds (highly permeable, highly soluble), the parameters that mainly influence the fraction absorbed are related to the formulation properties, for class II compounds (highly permeable, lowly soluble) to the dissolution process, for class III (lowly permeable, highly soluble) to both absorption process and formulation properties and for class IV (lowly permeable, lowly soluble) to both absorption and dissolution processes. Considering the bioavailability, the results are similar to those for the fraction absorbed, with the addition that parameters related to gut wall and liver clearance influence as well the predictions. This work aimed to give a demonstration of the GSA methodology and highlight its importance in improving our understanding of PBPK absorption models and in guiding the choice of parameters that can safely be assumed, estimated or require data generation to allow informed model prediction.

**Keywords** Drug absorption · Oral bioavailability · Global sensitivity analysis · Variance based GSA · PBPK · BCS

## Introduction

The oral route is the preferred method of drug administration, mainly because of its convenience and minimal invasiveness. However, the bioavailability of drugs (i.e., the fraction that reaches the systemic circulation unchanged) is limited by several processes such as dissolution and absorption in the gut lumen, metabolism in the gut wall and liver [1].

In order to facilitate the development of oral formulations the Biopharmaceutics Classification System (BCS) was created [2]. The BCS uses physicochemical and physiological parameters to classify drugs into four different classes based on their permeability and solubility characteristics: class I (highly permeable, highly soluble); class II (highly permeable, lowly soluble); class III (lowly permeable, highly soluble); and class IV (lowly permeable, lowly soluble). The BCS is widely used by the European

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s10928-018-9615-8) contains supplementary material, which is available to authorized users.

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Medicines Agency (EMA) and United States Food and Drug Administration (US FDA) for developing guidance on formulation development and by the pharmaceutical industry during drug discovery and development [3]. Although considered an oversimplification of complex drug and formulation characteristics, the BCS is useful for informing experimental and clinical design, especially for class I compounds [1, 4].

Considerable efforts have been carried out to combine in silico mathematical modelling with the design and evaluation of experimental studies to reduce the number of in vivo bioequivalence studies needed, therefore reducing time and cost of biopharmaceutical development [5]. Among various types of in silico modelling techniques, physiologically based pharmacokinetic (PBPK) models have been used to investigate complex biopharmaceutical problems [1].

Several PBPK absorption models have been developed over the last decades and integrated into bespoke PBPK software (such as: GastroPlus, PK-Sim and Simcyp Simulator) or more general modeling platforms, such as MATLAB [6–11]. In general, these represent drug transit through the small intestine, release from formulation, dissolution/precipitation and absorption in the gastrointestinal tract, gut wall metabolism and active efflux/uptake transport. PBPK absorption models are used from lead optimization through phase 2 studies. For example, during lead optimization physiological models can be used to predict absorption from in vitro data. Moreover, these models are used to predict drug absorption in humans in combination with animal data obtained during pre-clinical development. Such predictions are possible because of the incorporation of physiological and biochemical differences between species. During clinical development, physiological models can be used to mechanistically interpret clinical data, to explore hypotheses and to guide formulation development [12]. The use of PBPK models has the potential to reduce the number of animal studies and replace or supplement clinical trials [13, 14].

The OrBiTo (Oral Biopharmaceutics Tools) project (Innovative Medicines Initiative), started in 2012 and aimed to address the gaps in gastrointestinal drug absorption knowledge and support a rational use of predictive tools for oral drug delivery. This was done by refining existing tools and defining new methodologies for oral drug delivery [15]. One of the various objectives of OrBiTo was to perform a large scale evaluation of PBPK models for oral drug absorption, to identify strengths and weaknesses of these models. The results of the analysis showed high variability in the performance [16, 17].

We believe that a better comprehension of the relationship between the model input parameters (e.g., drug/formulation-specific and physiological parameters) and outputs (e.g., drug exposure and secondary pharmacokinetic parameters) would be useful for the development and refinement of PBPK models. Performing a sensitivity analysis is useful for understanding how the uncertainty in input parameters translates to uncertainty in the outputs and, by this, identifying the most important parameters for a given output [18]. One definition of sensitivity analysis is: "The study of how uncertainty in the output of a model (numerical or otherwise) can be apportioned to different sources of uncertainty in the model input" [19].

There are different methodologies that could be used to perform sensitivity analysis, see for example [20–23]. It is possible to group sensitivity analysis methods into two classes: local and global. Local sensitivity analysis is performed when output uncertainty is obtained through small input variation around a nominal value. This analysis could be done studying the outputs when input parameters are varied one-at-a-time (OAT) or by using derivative based methods. Instead, global sensitivity analysis (GSA) is used in case of uncertain model inputs. In this case, a probability distribution is assigned to each model input and a multivariate variation of the parameters is performed to explore the impact of this uncertainty on some output measure [20, 23].

PBPK models have a complex structure and, usually, a significant variability in input parameters, for example, the variability that occurs in a given population, for parameters such as the gastric emptying time, the intestinal transit time and the enzymatic liver expression. Often there is a significant uncertainty in the estimation of some of these inputs, where the parameters are typically fixed to mean values or fitted to experimental data. Depending on the knowledge and information available, these parameters could vary within a certain defined range of values [24]. Thus, for these types of models, it is appropriate to perform GSA.

Furthermore, there is currently a strong regulatory interest from EMA and FDA in the use of sensitivity analysis to evaluate PBPK models in pharmaceutical research and drug development and in regulatory submissions [25, 26].

In this context, the aim of our work was to give a demonstration of the GSA methodology, by applying it on compartmental PBPK models that describe drug absorption, dissolution and transit in the gastrointestinal tract. This was done in order to identify what are the most important physiological and drug related parameters in determining the variability of the fraction absorbed ($f_a$) and bioavailability ($F_{oral}$) within each BCS class, for acidic, basic and neutral drugs, after an oral administration. Between various methods for GSA we choose the variance based method because it is model independent, considers each parameter in its full range of variation and allows

estimation of the interaction effects between input parameters [23]. The analysis was firstly done for neutral compounds on a mixing tank derived model [27] and then on a compartmental absorption and transit (CAT) derived model [28] for acidic, basic and neutral compounds. The GSA was performed separately for each BCS class because we expected that the order of importance of the parameters (e.g., relative to dissolution and absorption) could vary among classes.

## Methods

### Variance based GSA

Let us consider a generic model

$$Y = f(\boldsymbol{X}) = f(X_1, X_2, \ldots, X_k), \tag{1}$$

where $Y$ is a scalar output, $X_i$, $i = 1 \ldots k$, a scalar input and $\boldsymbol{X}$ a vector containing $X_{1\ldots k}$. A common method to perform the sensitivity analysis of $Y$, with respect to a factor $X_i$, is to calculate the derivative $\partial Y/\partial X_i$ at a given point $\bar{\boldsymbol{X}}$ of the parameter space. One characteristic of this method is that it is informative only at the point $\bar{\boldsymbol{X}}$ in which the derivative is computed, so it can be viewed as a local method. This could be a limitation if the input of the model is uncertain [23].

GSA methods deal with the presence of uncertainty in model input and, in this work, variance based GSA was used. The variance based GSA sees $Y$ and $\boldsymbol{X}$ in Eq. (1) as random variables. All the input factors are considered independent of each other. $Y$ is obtained by model evaluation after sampling each $X_i$ from its distribution. Two sensitivity indices are derived by decomposing the variance of $Y$, $V(Y)$ [29, 30]. These indices are known as the main effect, or first order effect, and total effect. They are related, respectively, with the part of $V(Y)$ explained by the variation of each $X_i$ taken singularly and the interaction of multiple input factors [29]. One of the objectives of the sensitivity analysis is to rank the input factors in order of importance. In the variance based GSA the most important factor is the one that, if fixed, causes the greatest reduction in $V(Y)$ (i.e., the factor that, with its variation, most explains $V(Y)$). Similarly, it is possible to define the second most important factor and so on, thus obtaining a ranking [19].

Given the generic model in Eq. (1), let $V_{\sim X_i}\left(Y|X_i = x_i^*\right)$ be the variance of $Y$ based on all factors except $X_i$ ($\sim \boldsymbol{X}_i$), with $X_i$ fixed to $x_i^*$. The smaller $V_{\sim X_i}\left(Y|X_i = x_i^*\right)$ is with respect to $V(Y)$, the more important $X_i$ will be [23]. However, $V_{\sim X_i}\left(Y|X_i = x_i^*\right)$ depends on $x_i^*$, a given value of $X_i$. In order to remove the dependency on $x_i^*$, the expected value ($E$) of that measure over all the possible values of $X_i$ can be computed, in Eq. (2).

$$E_{X_i}(V_{\sim \boldsymbol{X}_i}(Y|X_i)) \tag{2}$$

Moreover, it can be shown that [23]

$$V(Y) = E_{X_i}(V_{\sim \boldsymbol{X}_i}(Y|X_i)) + V_{X_i}(E_{\sim \boldsymbol{X}_i}(Y|X_i)). \tag{3}$$

So, the smaller $E_{X_i}(V_{\sim \boldsymbol{X}_i}(Y|X_i))$ is, or the larger $V_{X_i}(E_{\sim \boldsymbol{X}_i}(Y|X_i))$ is, the more important $X_i$ will be. The main effect, or first order effect, is defined as in Eq. (4).

$$S_i = \frac{V_{X_i}(E_{\sim \boldsymbol{X}_i}(Y|X_i))}{V(Y)} \tag{4}$$

A high value of $S_i$ denotes that $X_i$ is an important factor of $V(Y)$. However, it is not always valid that a low value of $S_i$ denotes that $X_i$ is uninfluential as there may be interactions between the input factors [23]. Interactions between model inputs could occur when more than one factor is allowed to vary at the same time. It is not possible to assess the effect of the interactions considering only $S_i$ or performing a sensitivity analysis with commonly used methods, such as the OAT methods, that study the model outputs varying one input factor at a time [31].[1]

$V(Y)$ can be decomposed with Sobol's scheme, as in Eq. (5) [29, 30].

$$V(Y) = \sum_i V_i + \sum_i \sum_{j>i} V_{ij} + \cdots + V_{12\ldots k} \tag{5}$$

where $V_i = V_{X_i}(E_{\sim \boldsymbol{X}_i}(Y|X_i))$ is a first order term, as in Eq. (3), $V_{ij} = V_{X_{ij}}\left(E_{\sim \boldsymbol{X}_{ij}}\left(Y|X_i, X_j\right)\right)$ a second order term and so on. Dividing all the members of Eq. (5) by $V(Y)$ the relation in Eq. (6) is obtained [29].

$$1 = \sum_i S_i + \sum_i \sum_{j>i} S_{ij} + \cdots + S_{12\ldots k} \tag{6}$$

It now becomes clear that the main effect is only related to the first order terms and does not consider the interactions between the parameters.

To take into account the interactions let us consider the value $V_{\sim X_i}(E_{X_i}(Y|\sim \boldsymbol{X}_i))$. This term includes the effect of any order for any factor, except $X_i$ [23] (in fact the dependency of $Y$ on $X_i$ is removed by using the expected value). So, the larger $V_{\sim X_i}(E_{X_i}(Y|\sim \boldsymbol{X}_i))$ becomes, the smaller the overall effect of $X_i$ (first order plus all the interactions) is. Considering the relation in Eq. (7), a sensitivity index can be defined as in Eq. (8) [23].

[1] Let us consider the model $Y = X_1 \cdot X_2$, with $X_1$ distributed normally with mean equal to 1 and variance equal to 1 and $X_2$ distributed normally with mean equal to 0 and variance equal to 1. The main effect of $X_1$ is equal to 0, because $X_2$ has mean 0. Thus, by limiting the analysis on the main effect, one may conclude that $X_1$ has no impact on $V(Y)$. Intuitively, this conclusion is wrong. In fact, $X_1$ impact can be observed if $X_2$ is allowed to vary from its mean value. Thus, $X_1$ impact on $V(Y)$ is due to interaction effect with $X_2$.

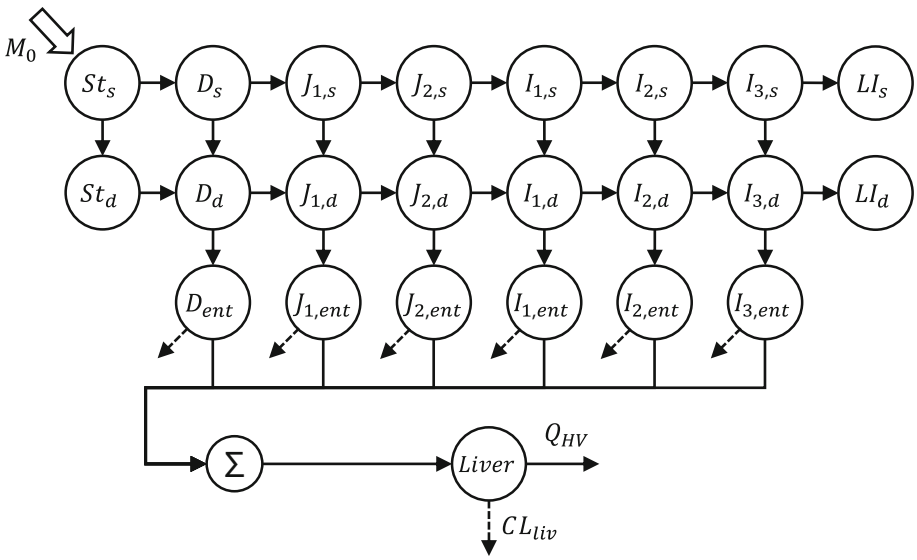


**Fig. 1** CAT derived model. *St* stands for stomach, *D* for duodenum, *J* for jejunum, *I* for ileum and *LI* for large intestine. Subscripts *s*, *d* and *ent* stand for solid, dissolved and enterocytes. Continuous and dashed arrows represent mass transfer and clearance processes, respectively. Drug is administered solid in the stomach compartment, then is subject to dissolution, transit, absorption in the small intestine and metabolism in gut wall and liver

$$V(Y) = E_{\sim \boldsymbol{X}_i}(V_{X_i}(Y|\sim \boldsymbol{X}_i)) + V_{\sim \boldsymbol{X}_i}(E_{X_i}(Y|\sim \boldsymbol{X}_i)) \quad (7)$$

$$S_{Ti} = 1 - \frac{V_{\sim \boldsymbol{X}_i}(E_{X_i}(Y|\sim \boldsymbol{X}_i))}{V(Y)} = \frac{E_{\sim \boldsymbol{X}_i}(V_{X_i}(Y|\sim \boldsymbol{X}_i))}{V(Y)} \quad (8)$$

$S_{Ti}$ is the total effect for the factor $X_i$ and contains any term of any order that include $X_i$, therefore $S_{Ti} \geq S_i$. The larger $S_{Ti}$ is, the more important $X_i$ is, $S_{Ti} = 0$ is a necessary and sufficient condition for the factor $X_i$ to be considered noninfluential [23]. The difference between $S_{Ti}$ and $S_i$ gives information about the extent of the interaction involving the $X_i$.

Some of the advantages of the variance based GSA are that it is possible to estimate the interaction effects among input parameters, that each parameter is considered in its full range of variability and that the technique is model independent. The latter property refers to the fact that it is possible to apply GSA methodology independently on the linearity, monotonicity and additivity of the model [19].

A program was developed to perform variance based GSA in MATLAB [32]. Briefly, the program extracts, following suitable rules [23], samples from the parameter distribution using a Latin Hypercube sampling method and the inverse cumulative distribution function of each parameter. Then, for each of the extracted values, the model is evaluated to compute the outputs. All the output values are then used to calculate the sensitivity indices [23, 29]. With the same set of input samples it is then possible to compute the sensitivity analysis for multiple output parameters.

## Physiologically based absorption models

Two different compartmental PBPK absorption models with different levels of detail were implemented in MATLAB, both aiming to describe the oral absorption process. One model was based on the mixing-tank model [27], describing drug dissolution and absorption in the gastrointestinal tract, where the small intestine was represented by one well-stirred luminal segment. The other model was based on the CAT model [28] and described drug transit, dissolution and absorption in the gastrointestinal tract.

In the CAT based model, represented in Fig. 1, the gastrointestinal tract is subdivided into eight different sections: the stomach, six small intestine segments (one for the duodenum, two for the jejunum, three for the ileum) and one for the large intestine. In the gut lumen, drug can be present in two states: solid and dissolved. It is supposed that absorption occurs only of dissolved drug in the small intestine. Drug is absorbed from the small intestine into the enterocytes where it can be metabolised or transported to the liver via the blood flow. Once in the liver the drug can be metabolised or reach the systemic circulation via the hepatic vein. The mixing-tank based model, represented in Fig. 2, is substantially similar to the CAT based model, where the gastrointestinal system is subdivided into three sections: the stomach, the small intestine and large intestine.

Models equations are presented in Online Resource 1, section 1, and references to them are indicated with an "S", followed by an Arabic number. All the models parameters are presented in Tables 1, 2 and 3.

The model outputs $f_a$ and $F_{oral}$ are defined as the values of $A_a$ and $A_{oral}$, in equations system (S3), at the steady state, both normalised with respect to the dose.

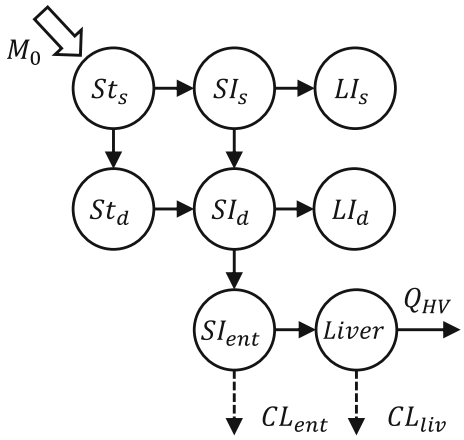


**Fig. 2** Mixing tank model derived model. *St* stands for stomach, *SI* for small intestine and *LI* for large intestine. Subscripts *s*, *d* and *ent* stand for solid, dissolved and enterocytes. Continuous and dashed arrows represent mass transfer and clearance processes, respectively

## Definition of the BCS classes and GSA

To perform the GSA, a probability distribution has to be defined for each input parameter of the model (see Table 1). In order to simplify the analysis, a number of physiological parameters were fixed to their mean values for a fasted state, including: all volumes, luminal pH values, blood flows and each small intestine segment radii and length (see Tables 2 and 3). The GSA algorithm extracts samples from the parameter spaces and, for each of them, evaluates the model and computes the outputs. Then, a drug is defined as a sample extracted from the joint space of the parameters (such as $P_{eff}$, *mw*…). The solubility relative to dose (dose number) and permeability were the only parameters that were assumed to differ between the BCS classes in this analysis (Fig. 3).

BCS classes I and II are characterised by high absorption, while classes III and IV by low absorption. The

**Table 1** Parameter distribution used for GSA

| Parameter name | Distribution parameters | Distribution type | Unit | Reference |
|---|---|---|---|---|
| $A_{3A4,ent}$: total enterocytes amount of CYP3A4 | 66.2 (60%) | Lognormal[a] | nmol | [38] |
| $C_{3A4,LM}$: CYP3A4 concentration in liver microsomes | 137 (41%) | Lognormal[a] | pmol/mg prot | [38] |
| *MPPGL*: microsomal protein per gram of liver | 39.79 (26.9%) | Lognormal[a] | mg prot/g | [38] |
| *GET*: gastric emptying time | 0.25 (38%) | Lognormal[a] | h | [10] |
| ln($CL_{int}$): intrinsic clearance | 2.0809, 2.4086 | Normal[b] | ml/(h pmol *CYP3A4*) | [39] |
| *SITT*: small intestine transit time | 4.04, 2.92 | Weibull[c] | *h* | [10] |
| $\rho$: density of the formulation | 1–1.8 | Uniform[d] | g/cm$^3$ | [40] |
| p$K_a$: acid dissociation constant | Acid: 2.5–13.5<br>Base: 0.5–12.5 | Uniform[d] | | [41] |
| *r*: formulation radius of the particle | 0.5–500 | Uniform[e] | µm | [40] |
| *mw*: molecular weight | 75.07–1202.64 | Uniform[e] | g/mol | [42] |
| $k_p$: precipitation time constant | 0.4–40 | Uniform[e,f] | h$^{-1}$ | [38] |
| $R_{ss}$: supersaturation ratio | 1–100 | Uniform[e,f] | | [38] |
| $D_0$: dose number | BCS I and III: 0.01–1<br>BCS II and IV: 1–100 | Uniform[e,g] | | [3] |
| $P_{eff}$: effective permeability | BCS I and II: 1.5–8.70<br>BCS III and IV: 0.03–1.5 | Uniform[d] | 10$^{-4}$ cm/s | [5] |

[a]For distribution parameters, *mean (coefficient of variation)* of the lognormal random variable

[b]For distribution parameters, *mean*, *standard deviation* of the natural logarithm of $CL_{int}$, estimated using the MATLAB *distribution fitter* toolbox

[c]For distribution parameters, *A*, *B* with *A* scale parameter and *B* shape parameter of the Weibull distribution (*Weibull Distribution object* of MATLAB). The distribution was truncated between 1.8 and 8 h [43] by using the MATLAB function '*truncate*'

[d]Uniform distribution between *minimum*, *maximum*

[e]For distribution parameters, *minimum*, *maximum* of the parameter. A uniform distribution of the natural logarithm (*ln*) of the parameter between *ln(minimum)* and *ln(maximum)* was used

[f]*Minimum* and *maximum* are, respectively, 1/10 and 10 times the mean value in Simcyp

[g]For doses of 100 mg and 1000 mg of BCS class I and III, $D_0$ limits were set to [0.1 1] in order to avoid too high solubilities and so a too stiff system

**Table 2** Physiological gastrointestinal parameters

| Compartment name | Lumen volume (ml)[a] | Length (cm)[b,d] | Diameter (cm)[b] | pH[a] | Volume enterocytes (l)[c,d] | Fraction CO to enterocytes[c,d] | CYP3A proportion[f] |
|---|---|---|---|---|---|---|---|
| Stomach | 48.92 (+ 250)[e] | – | – | 1.3 | – | – | – |
| Duodenum | 44.57 | 21 | 4.75 | 6.0 | 0.0262 | 0.0038 | 0.1376 |
| Jejunum 1 | 166.6 | 105/2 | 3.25 | 6.20 | 0.119/2 | 0.0178/2 | 0.5448/2 |
| Jejunum 2 | 131.0 | 105/2 | 3.25 | 6.40 | 0.119/2 | 0.0178/2 | 0.5448/2 |
| Ileum 1 | 102.0 | 156/3 | 2.9 | 6.60 | 0.079/3 | 0.0264/3 | 0.3176/3 |
| Ileum 2 | 75.35 | 156/3 | 2.9 | 6.90 | 0.079/3 | 0.0264/3 | 0.3176/3 |
| Ileum 3 | 53.57 | 156/3 | 2.9 | 7.40 | 0.079/3 | 0.0264/3 | 0.3176/3 |

[a][44]

[b][45]

[c][46]

[d]Measure relative to the total segment divided by the number of sections in which the segment is subdivided (for jejunum 2 and for ileum 3)

[e]Stomach volume (+ volume of water administered with the drug)

[f][38]

**Table 3** Constant parameters of the model

| Parameter name | Value | Unit | Reference |
|---|---|---|---|
| $BW$: body weight | 70 | kg | [47] |
| $CO$: cardiac output | 350.37 | l/h | [46] |
| $W_{liv}$: liver weight (percentage of $BW$) | 5.53 (0.079) | kg | [47] |
| $Q_{HV}$: hepatic vein blood flow (percentage of $CO$) | 89.34 (0.255) | l/h | [47] |
| $\rho_{liv}$: liver density[a] | 1.080 | kg/l | [48] |
| $T$: absolute body temperature | 310.15 (37) | K (°C) | |
| $k_b$: Boltzmann constant | 1.3806504 | $10^{-23}$ J/K | |
| $N_a$: Avogadro's number | 6.02214179 | $10^{23}$ mol$^{-1}$ | |

[a]Used to calculate $V_{liv}$ from $W_{liv}$: $V_{liv} = W_{liv}/\rho_{liv}$

parameter that controls the absorption in equations system (S2) is the absorption rate constant ($k_a$), defined as a function of the effective permeability ($P_{eff}$). The cut-off value for $P_{eff}$ that distinguish between high and low absorption was set to $1.5 \times 10^{-4}$ cm/s [5] and the ranges of its variation were taken from the same publication.

The parameter that was used to distinguish between high and low solubility (between classes I and II and between III and IV) was the dose number [2, 3],

$$D_0 = \frac{M_0/V_{in}}{C_s} \tag{9}$$

where $V_{in}$ is the volume of water taken with the drug (250 ml [2]) and $M_0$ the drug dose. If $D_0 \leq 1$ a compound is highly soluble, while if $D_0 > 1$ it is solubility limited. The ranges for this parameter were arbitrarily set from $10^{-2}$ to 1 for classes I, III and from 1 to $10^2$ for classes II and IV. However, the solubility ($C_s$), and not $D_0$, is present in systems of equations (S1) and (S2). So, once the dose, $M_0$, is fixed the algorithm computing the sensitivity indices extract a value for $D_0$ and calculates $C_s$ (supposed for a pH equal to 6). By doing this, extracting $D_0$ was equivalent to extracting $C_s$ once dose was fixed. Then $C_s$ results to be depended on the dose. For this reason, different dose levels were tested (0.1 mg, 1 mg, 10 mg, 100 mg and 1000 mg).

To perform GSA the number of samples, $n$, has to be chosen. Some authors suggest to set $n$ to 500 or 1000 [23], however, this may be insufficient. We decided to fix $n = 5000$ in order to have reasonable precise estimates, taking into account also the required computational time. $n$ samples correspond to $n(k + 2)$ model evaluations, where $k$ is the number of variable parameters [29]. Samples were extracted from a $k$-dimensional unit hypercube by using the latin hypercube sampling method ('*lhsdesign*' function in MATLAB). Each sample was converted to the parameter distribution using the inverse cumulative distribution function ('*icdf*' function in MATLAB). Outputs were calculated for each model evaluation and the main and total effect for $f_a$ and $F_{oral}$ were derived. The analysis was first carried out for the simple model, derived from the mixing tank model, for neutral drugs, then GSA was performed on the CAT derived model for acidic, basic and

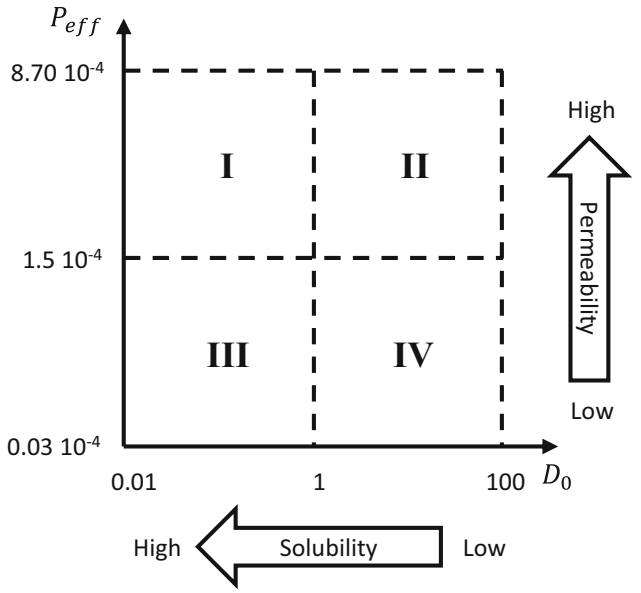


**Fig. 3** Model parameter space following to the definition of the BCS classes. A drug is defined as highly soluble if $D_0$ is between 0.01 and 0.1 and lowly soluble if it is between 1 and 100. A drug is defined highly permeable if $P_{eff}$ is between $1.5 \times 10^{-4}$ and $8.7 \times 10^{-4}$ cm/s, meanwhile is lowly permeable if it is between $0.03 \times 10^{-4}$ and $1.5 \times 10^{-4}$ cm/s. Roman numbers represent the BCS classes

neutral compounds. For basic compounds the GSA was also performed in the presence of precipitation. Uncertainty of GSA results was estimated using 1000 bootstrap samples [33]. Coefficient of variation (CV) for the most sensitive parameter, given a certain BCS class and a certain dose, are shown in Online Resource 1, Section 3.

Differential equations were solved for a time span of 0 (dose administration) to 100 h, to assure of reaching the steady state, using the '*ode23s*' MATLAB solver. The analysis was performed using MATLAB R2017b on a 64-bit computer configured with Intel® Core™ i7-7000 @ 3.60 GHz × 8 processor, running Ubuntu 16.04 LTS.[2] The computational time required to perform the sensitivity analysis for all the BCS classes and all the dosages of, for example, a neutral compound, was approximately 18 h.

## Results

A variance based GSA was performed on the two PBPK absorption models described above with the aim of identifying the relative importance of each parameter (both physiological and drug related), considered over its range of variation, in determining the variability of the predicted $f_a$ and $F_{oral}$. The analysis was performed for acidic, basic and neutral drugs from each BCS class. Figures 4 and 5 summarise the results of the analysis for $f_a$ and $F_{oral}$, respectively. References to figures present in the Online Resource 1 are indicated with an "S", followed by an Arabic number.

[2] The codes used to perform the analysis are available at the following link: http://aimed11.unipv.it/JPKPDMelillo18/.

### $f_a$, CAT based model

Main and total effect indices for $f_a$ values were calculated for neutral, acidic and basic drugs in each BCS class. Figures 6 and 7 represent the main and total effect for neutral compounds, Figures S7 and S8 the main and total effect for acidic compounds and Figures S13 and S14 represent the main and total effect for basic compounds. Each figure shows four heatmaps, one for each BCS class. Each heatmap shows the input parameters on the vertical axis and the different dose levels on the horizontal axis.

Considering the neutral case, in Figs. 6 and 7, for drugs of class I given at low doses, the most important parameter is the particle radius of the formulation, $r$. This means that the variance of $f_a$, among drug belonging form this class, is mainly explained by the variation of $r$. For drugs administered at higher doses the importance of $r$ is reduced and an increased importance of interactions can be seen, observable through the difference between the total and the main effect. For $r$ the difference between the total and the main effect increases from 0.0640 at a dose of 0.1 mg, to 0.3278 for a dose of 100 mg. For doses of 10 mg and 100 mg the importance of $D_0$ increases and $D_0$ becomes the second most important parameter, this is mainly due to interaction effects. Thus, limiting the analysis to the main effect or using sensitivity analysis approaches that cannot detect the interactions (e.g. OAT methods), could lead to an underestimation of parameters influence on the output variance. At a dose of 1000 mg there is an increase in the importance of the small intestinal transit time and the most important parameter becomes the effective permeability, $P_{eff}$. Within a given class, drugs administered at higher dose levels typically have higher solubility. This can be seen in Eq. (9), where if $D_0$ is fixed, higher values of $M_0$ imply higher values of $C_s$. Drugs of class I administered at 1000 mg will most likely have high solubility values and therefore the dissolution process will generally become fast with respect to the absorption process, independent of the value of $r$, even if the drug is highly absorbed. Therefore, $P_{eff}$ becomes the rate-limiting parameter, and therefore the most important parameter in determining $V(Y)$. The main and total effect indices are normalised with respect to the total variance of the output $V(Y)$. So, for doses of 1000 mg, $P_{eff}$ will become the most important parameter, but the variability of $f_a$, as can be seen in Fig. 8, is lower with respect to the lower dose levels. As explained before, higher dose levels imply higher values of $C_s$ and a reduction in the influence of $r$ variation on $f_a$ variability. This

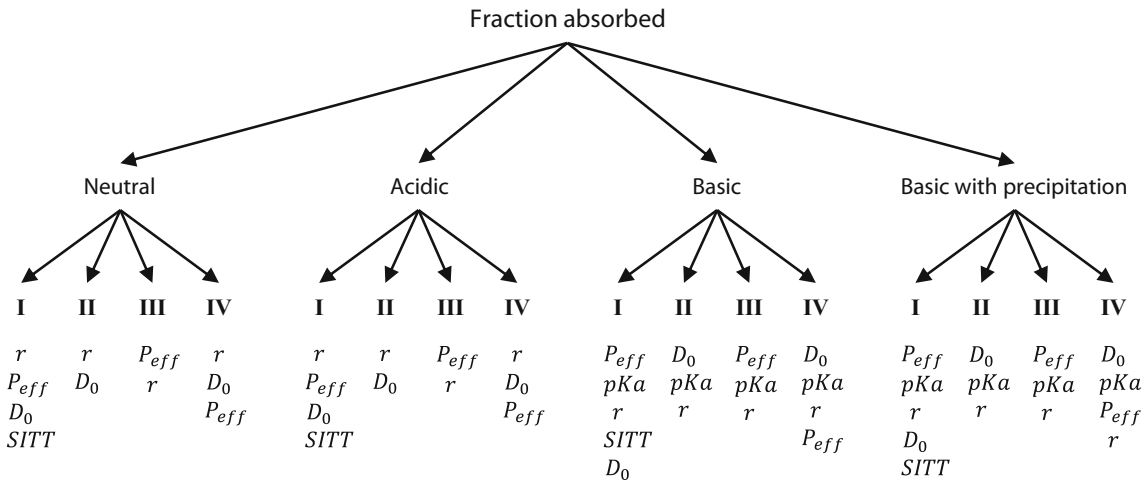


**Fig. 4** Summary of the CAT derived model results. This tree shows the parameters that mostly impact on the variance of the fraction absorbed for each BCS class, for neutral, acidic and basic compounds with and without the precipitation. The reported parameters have the total effect higher than 0.25. The parameters are written from up to down in descending order of their maximum total effect value through all the dose levels

causes a faster dissolution, resulting in an increase in $f_a$ and a reduction in $f_a$ variability for higher dose levels.

Moving from BCS class I to class II, there is an increase in the values of $D_0$ and therefore a reduction in the solubility for a given dose. Then, for class II compounds the most important parameters result to be $r$ and $D_0$, both related to the dissolution process. This is a consequence of dissolution rate being the limiting step of BCS class II drugs, in accordance with the definition of the class. When considering higher dose levels, and by as a consequence higher solubilities, $D_0$ becomes more sensitive than $r$. It can be seen that the interaction effect of $D_0$ decreases as the dose increases, in fact the difference between total and main effect is reduced from a value of 0.1997 for a dose of 0.1 mg to 0.0680 for a dose of 1000 mg.

In BCS class III, we start to consider compounds with low absorption properties. For drugs administered at low doses a similar situation as class I can be seen (with slightly more importance on $P_{eff}$). This is most likely because $C_s$ is not high enough and therefore $r$ is the more influential parameter with regards to the dissolution process, making it the limiting step. When examining compounds administered at higher doses, a progressive reduction of importance of $r$ and $D_0$ is observable and an increase of importance of $k_a$ can be seen. This effect is due by an increase in $C_s$ and so the limiting step is no longer dissolution but absorption.

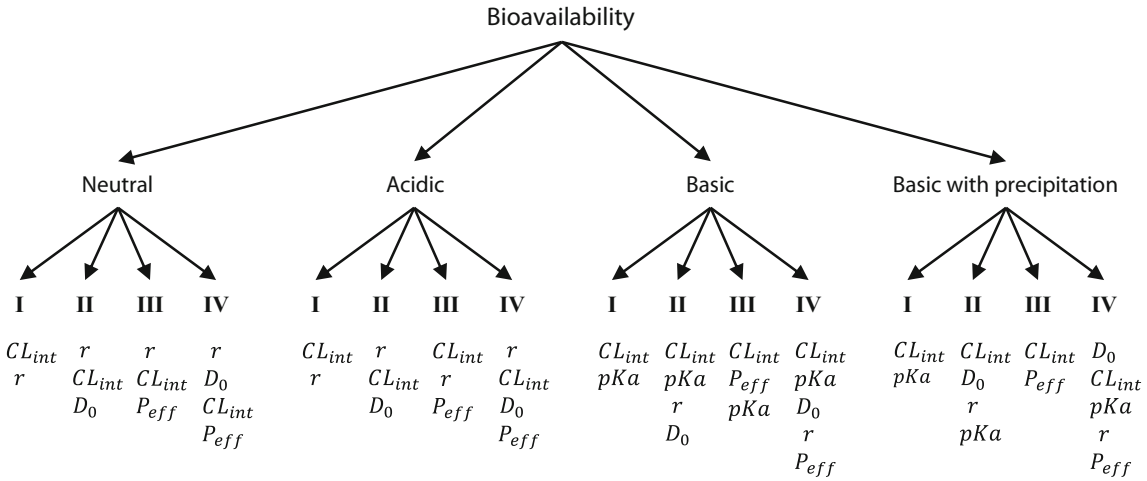


**Fig. 5** Summary of the CAT derived model results. This tree shows the parameters that mostly impact on the variance of the bioavailability for each BCS class, for neutral, acidic and basic compounds with and without the precipitation. The reported parameters have the total effect higher than 0.25. The parameters are written from up to down in descending order of their maximum total effect value through all the dose levels

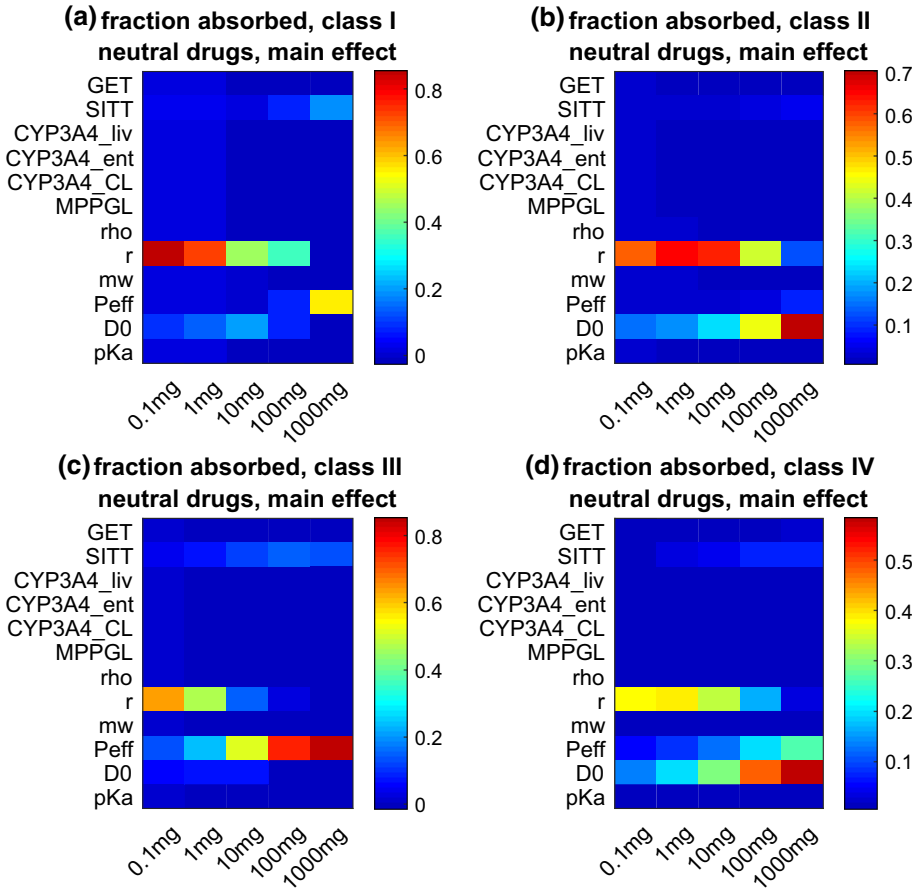


**Fig. 6** Fraction absorbed main effect of the CAT based model for neutral compounds. **a**–**d** are relative to BCS class I–IV compounds. Each panel contains a heatmap that has the input parameters on the vertical axis and the different dose levels on the horizontal axis. Each heatmap cell contains the value of the main effect relative to a particular parameter and dose level. Colour legends are shown to the right of each heatmap. *CYP3A4_liv*, *CYP3A4_ent* and *CYP3A4_CL* stand to the microsomal concentration of CYP3A4 in the liver, the total amount of CYP3A4 in the enterocytes and the intrinsic clearance (Color figure online)

A more complex situation can be seen for BCS class IV compounds, where parameters related to both dissolution and absorption remain important across the simulated dose levels. This happens because in class IV both solubility and permeability are low, and therefore both could act as the limiting step.

Considering the case of acidic drugs, in Figures S7 and S8, the results are similar to the case of a neutral drugs as in the stomach $\alpha_{st}$ is low compared to $\alpha_{ref}$ in (S1) due to the low pH in the gastric lumen ($pH_{stomach} = 1.3$), and so the drug dissolves to a lesser extent. The pH of the small intestine is around 6, which is the value used to calculate $\alpha_{ref}$, and so, in equations system (S2), $\alpha_i \simeq \alpha_{ref}$ and therefore the solubility is similar to the neutral case. The fact that the drug dissolves less in the stomach does not change the importance of the variables with respect to the case of a neutral drug.

For basic compounds, in Figures S13 and S14, results differ compared to the previous cases. For class I compounds, up till doses of 10 mg, the dissolution appears to be the limiting step, where $pK_a$ is the most influential parameter. This is probably because $\alpha_{st}$ in the stomach could reach higher levels depending on the $pK_a$ of the compound compared to $\alpha_{ref}$ and therefore the solubility in this compartment is enhanced. For compounds administered at higher doses the conclusions are similar to that of the neutral case. With respect to the neutral case, a stronger interaction effect can be seen, especially for $r$, $D_0$ and $pK_a$ for doses of 10 mg and 100 mg.

For BCS class II compounds all parameters related to dissolution, including $pK_a$, are of importance at all the simulated dose levels, similarly to the neutral case. Also $r$ is associated with a strong interaction effect. Concerning class III, the $P_{eff}$ is the most important parameter at all the dosage levels. This is probably due to the enhancing of dissolution in the stomach, meaning that the drug is already dissolved when reaching the intestine and therefore the absorption process becomes the rate limiting step. As for neutral and acidic drugs, BCS class IV presents a more complicated situation, in fact, both parameters related to dissolution and absorption remain important throughout the simulations. Interaction effects can be observed, especially for $r$ and $pK_a$ from doses of 1 mg up to 100 mg.

For neutral and acidic compounds, the interaction effects seem to occur to a lesser extent for class I compounds administered at low dosages and for classes III at high dosages. For basic compounds, interactions occur to a lesser extent only for class III drugs administered at high

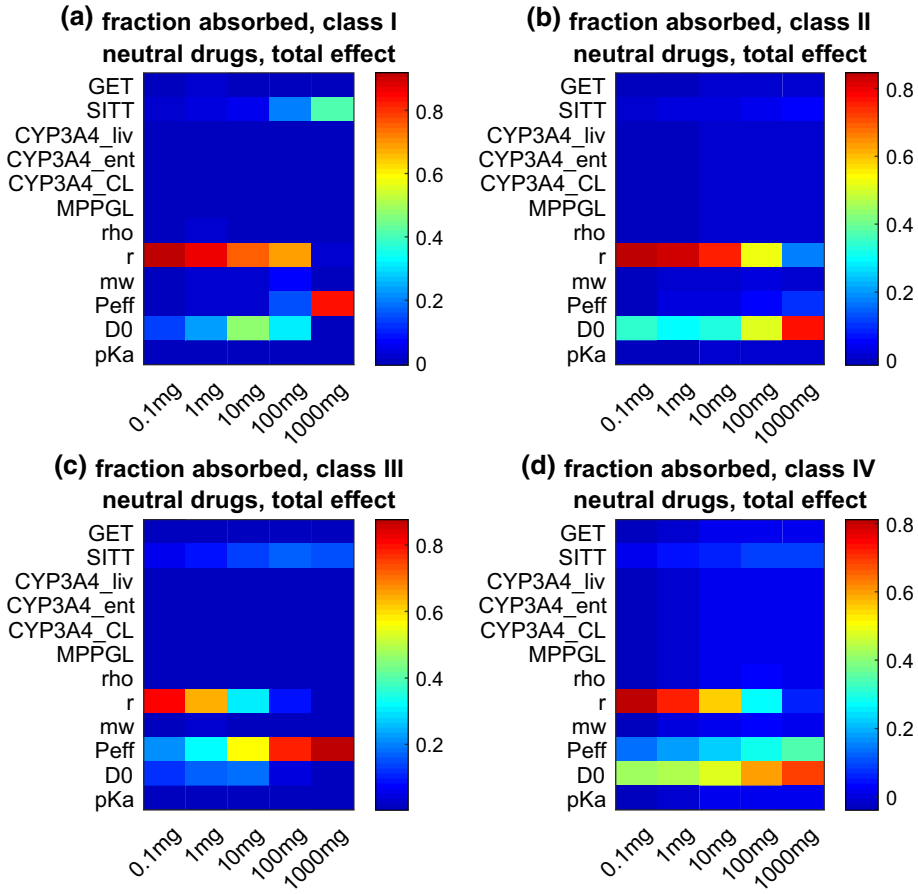


**Fig. 7** Fraction absorbed total effect of the CAT based model for neutral compounds. **a**–**d** are relative to BCS class I–IV compounds. Each panel contains a heatmap that has the input parameters on the vertical axis and the different dose levels on the horizontal axis. Each heatmap cell contains the value of the total effect relative to a particular parameter and dose level (Color figure online)

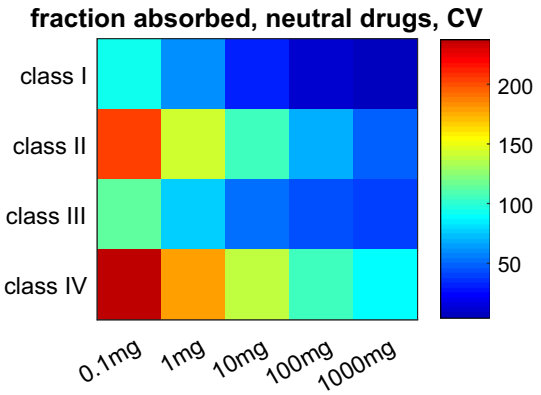


**Fig. 8** Fraction absorbed coefficient of variation (CV) in percentage, predicted using the CAT based model for neutral compounds. The heatmap vertical axis represents the BCS classes and the horizontal axis represents the dose levels. Each cell contains the value of fraction absorbed CV for a specific BCS class and dose level. Each CV was calculated from the samples used to calculate the main and total effect of the variance based GSA (Color figure online)

dosages. This happens probably because these cases represent extreme situations, in which the variation of only one parameter seems to determine the variability of the $f_a$. In all the other cases, the variance of $f_a$ can be affected by the variation of multiple parameters, so, the effect of one factor may depend on the values of other factors and interaction effects may arise.

## $F_{oral}$, CAT based model

Main and total effect indices for $F_{oral}$ values were calculated for neutral, acidic and basic drugs in each BCS class. Figures 9 and 10 represent the main and total effect for neutral compounds, Figures S9 and S10 the main and total effect for acidic compounds and Figure S15 and S16 represent the main and total effect for basic compounds.

Considering the neutral case (Figs. 9, 10) for class I compounds given at a low dose, the most important parameter is $r$, while for doses up to 10 mg the intrinsic clearance $CL_{int}$ becomes the most influential parameter. This is most likely because, as explained for $f_a$, for low dosages $r$ is more important for determining dissolution as $C_s$ is not high enough to become as influential, by making it the limiting step. Moving towards higher doses an increasing importance of $C_s$ can be seen. Given the high permeability, the rate limiting step becomes the clearance. Amongst all the parameters involved in the clearance process (e.g. liver enzymatic concentration, *MPPGL*) the

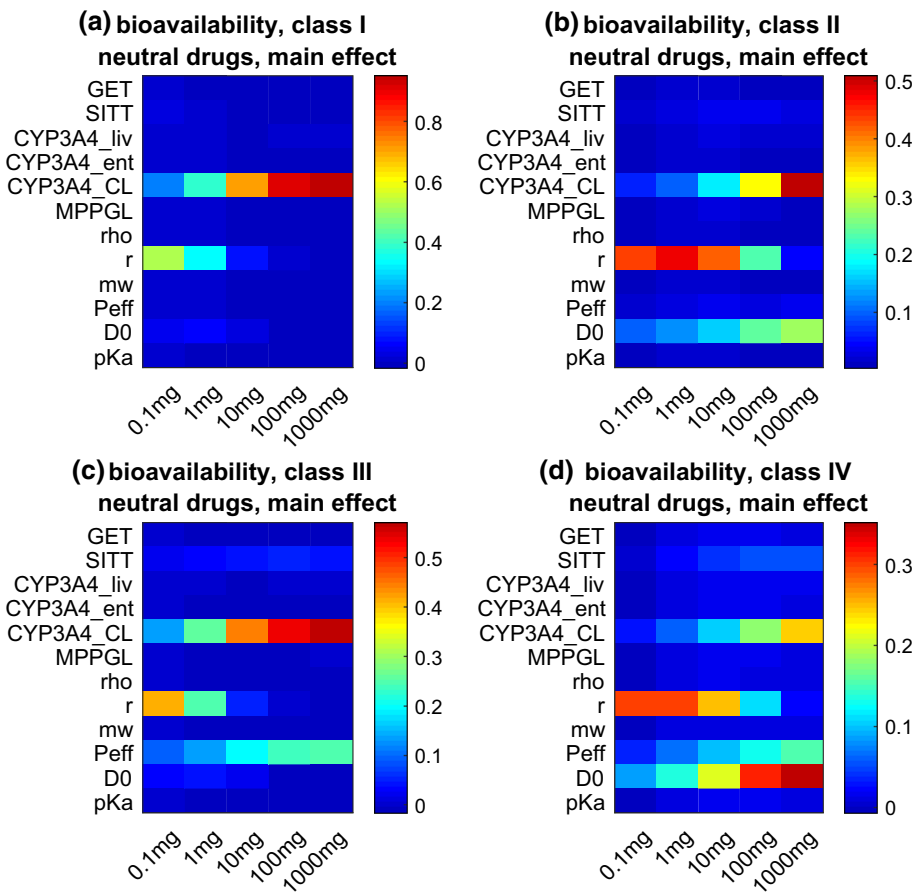


**Fig. 9** Bioavailability main effect of the CAT based model for neutral compounds. **a**–**d** are relative to BCS class I–IV compounds. Each panel contains a heatmap that has the input parameters on the vertical axis and the different dose levels on the horizontal axis. Each heatmap cell contains the value of the main effect relative to a particular parameter and dose level (Color figure online)

consistently most important parameter is $CL_{int}$, this is probably because the parameter was defined with a larger range of variation and because it appears at two sites in the model (gut wall and liver).

For compounds belonging to BCS class II the most important parameters are related to both dissolution and metabolism. At lower dose levels there is a higher importance of formulation related parameters, $r$, meanwhile moving towards higher dose levels, $CL_{int}$ and $D_0$ become the most important parameters. As seen for $f_a$, this is in accordance with the definition of the class properties. Notable interaction effects can be seen for $CL_{int}$, $r$ and $D_0$, especially for doses of 0.1 and 1 mg.

Moving to class III, for low dosages, $r$ is the most important parameter in determining variation in $F_{oral}$ followed by $CL_{int}$. At higher dose levels clearance and absorption become the rate limiting steps. The reasoning around the differing importance of $r$ and $P_{eff}$ throughout the dose levels follows the same argument as for class III and $f_a$. $CL_{int}$ is more influential at higher doses as compared to $P_{eff}$, which is probably due to its higher range of variation.

For BCS class IV compounds, dissolution, absorption and clearance parameters remain important across the simulated dose levels, with a reduction of importance of $r$ and an increase of importance of $CL_{int}$, $P_{eff}$ and $D_0$ when moving towards higher doses. Notable interaction effects can be consistently observed across dose levels for parameters $r$, $CL_{int}$, $P_{eff}$ and $D_0$.

Considering the case of an acidic compound, as for $f_a$, the results are similar to the case of the neutral one. As explained for $f_a$ the fact that an acidic compound dissolves to a lesser extent in the stomach, does not change the importance of the variables found in the case of a neutral drug.

The situation for basic compounds (Figures S15 and S16) is slightly different. For class I compounds the most important parameter for all the doses is $CL_{int}$. This is because for a base, as explained for $f_a$, the solubility could be highly enhanced in the stomach and so the drug could dissolve completely prior to reaching the small intestine. Given that the absorption is high, metabolic clearance becomes the rate limiting step. For class II compounds, as in the neutral case, dissolution and clearance are important determinants of variation in the output. Interaction can be mainly seen for the dissolution related parameters. For class III the most important parameters are $CL_{int}$ and $P_{eff}$ across all doses, with p$K_a$ being relevant at a dose of 0.1 mg, but mainly due to interaction effects. Like in the

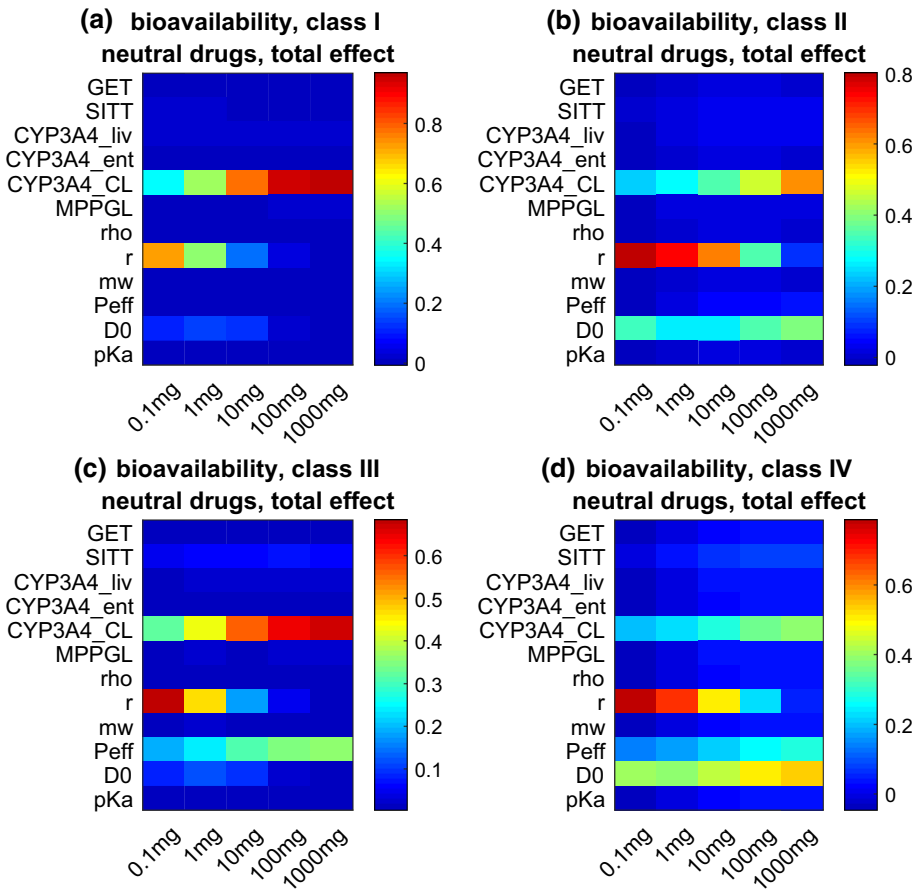


**Fig. 10** Bioavailability total effect of the CAT based model for neutral compounds. **a**–**d** are relative to BCS class I–IV compounds. Each panel contains a heatmap that has the input parameters on the vertical axis and the different dose levels on the horizontal axis. Each heatmap cell contains the value of the total effect relative to a particular parameter and dose level (Color figure online)

previous cases, BCS class IV compounds present a more complicated situation, where dissolution, absorption and clearance parameters remain important at all the studied dose levels. Interaction effects can be observed especially for the dissolution related parameters.

For all the compounds, the interaction effects seem to occur to a lesser extent especially for class I drugs administered at dosages higher than 10 mg. Similarly to what was explained for $f_a$, these are situation in which the output variance can be addressed almost uniquely to the variation of one parameter and consequently, limited interaction effects arise.

It is possible to observe that there is an apparent discrepancy between $f_a$ and $F_{oral}$ results for BCS class I compounds administered at high dosages. In fact, for $f_a$ the only sensitive parameters are $P_{eff}$ and *SITT*, while for $F_{oral}$ it is $CL_{int}$. In this case, both solubility and permeability are high, thus, practically all the drug gets absorbed. From Figure S5 and S6, it can be observed that the variability of class I compounds administered at 1000 mg is much higher for $F_{oral}$ than for $f_a$. So, the clearance processes explain almost all the $F_{oral}$ variability.

## $f_a$ and $F_{oral}$, CAT based model, for basic compounds with precipitation

GSA was carried out for basic compounds using the CAT based model with the additional consideration of precipitation. Main and total effect for $f_a$, in Figure S19 and S20, and for $F_{oral}$, in Figure S21 and S22, were calculated.

Concerning both $f_a$ and $F_{oral}$, for compounds belonging to BCS classes I and III, the results in presence of precipitation resemble the case of absence of precipitation. It can be seen that the variability of the supersaturation ratio $R_{ss}$ and of the precipitation time constant $k_p$ are not important in determining the variance of the output. In the stomach, the maximal concentration that a drug could achieve is equal to $M_0/V_{st}$, with $M_0$ the drug dose and $V_{st}$ equal to 298.92 ml (see Table 2). For BCS classes I and III, the drug solubility, calculated by using Eq. (9), results higher or equal to $M_0/V_{in}$, with $V_{in}$ equal to 250 ml (this happens because in this case $D_0$ was considered between $10^{-2}$ and 1). Moreover, in the stomach $\alpha_{st} > \alpha_{ref}$, so, the solubility is enhanced with respect to the neutral case. It follows that the maximal concentration that the drug could reach in the stomach is lower with respect to the solubility in that compartment. Consequently, the precipitation does

not occur in the stomach. It can be observed that even if the precipitation occurs in the small intestine this does not make $R_{ss}$ and $k_p$ important in determining output variability.

Concerning BCS classes II and IV, for both $f_a$ and $F_{oral}$, in case of presence of precipitation, $D_0$ is slightly more important in determining output variance with respect to the case of absence of precipitation. With respect to BCS classes I and III, in this case the maximal concentration that a drug could reach in the stomach can be lower than the solubility in that compartment. This happens because $D_0$ was considered between 1 and $10^2$. Therefore, precipitation could occur in the stomach. Probably, in this case $D_0$ acquires importance because its value it is used to determine the threshold at which the precipitation starts to occur. Moreover, when a drug once dissolved could precipitate, an additional dissolution step is required to allow absorption and $D_0$ is a parameter involved in the process of dissolution.

### $f_a$ and $F_{oral}$, mixing-tank derived model

Main and total effect for $f_a$, in Figures S25 and S26, and for $F_{oral}$, in Figures S27 and S28, were calculated for neutral compounds for each BCS class, using the mixing tank derived model.

For both $f_a$ and $F_{oral}$ the results are consistent with the CAT derived model for neutral compounds. This fact does not mean that the outputs of the CAT derived model are similar to that of the mixing-tank derived model, instead in both the models the variability in the output is explained to a similar extent by the same parameters.

## General discussion

We performed a variance based GSA on PBPK models describing drug dissolution, transit and absorption in the gastrointestinal tract, with the aim of finding the most important parameters that determine the variability of the predicted $f_a$ and $F_{oral}$ for acidic, basic and neutral drugs within each BCS class. In Figs. 4 and 5 the results of the analysis are summarised.

Performing a GSA could help in identifying limiting steps and bottlenecks, in different situations, and in understanding the behaviour of the model as a function of the variation of different parameters. This kind of information is difficult to obtain during performance evaluation exercises, such as OrBiTo [1, 16, 17], where the model predictions are affected by the quality of the data informing the values of compound specific parameters. In fact, in the OrBiTo compound database a high level of missingness for parameters such as particle size of solid formulation and solubility vs pH profiles was observed [1]. In this case, for example, following the analysis here reported, it is possible to conclude that a performance evaluation of PBPK absorption models where the radius of the formulation-specific particle size is fixed at an assumed or mean value could result in an incomplete interpretation, especially for compounds administered at low dose levels where the particle radius explains the majority of the output variation.

However, it must be considered that the validity of the GSA results is limited to the specific model and to the specific ranges of parameters investigated. This means that the level of importance of each parameter is relative to the model and to all parameter distributions, and thus it would be incorrect to attribute the results presented here to different absorption models or parameter distributions. Nevertheless, we could consider the theoretical case of a model that describes the absorption of a class III drug, administered at a dose of 100 mg, that exhibits uncertainty, or variability, in the $P_{eff}$ within a range that is wide with respect to the $P_{eff}$ range tested in this analysis.[3] Considering the results of the GSA presented in this study we could conclude that the variability of the $P_{eff}$ for that particular drug should be further investigated, otherwise, by fixing the value of $P_{eff}$ to the mean, it is possible that we ignore an important source of variability in estimating $f_a$ and $F_{oral}$. Anyway, if one wants to assess the impact of the variability of each parameter on the model outputs for a specific drug (and not to a class of compounds, as done in this study), one should perform a GSA adapting the parameters distributions to that particular case [35, 36].

One limitation of this analysis is that the classification between highly and lowly soluble drugs was defined using only $D_0$, as in [3], and does not take into account the effect of the formulation properties. Thus, it is possible that this will result in an overestimation of the importance of formulation related parameters (e.g., $r$) for BCS classes I and III. Another limitation is in the derivation of $F_{oral}$, we hypothesised that the metabolism in the gut wall and in the liver was due to CYP3A4 abundance and so, the results are limited to that particular case. Finally, we chose to use the variance based GSA method as per [29], but is also possible to use, for example, moment independent methods, or regression based methods [20, 22, 37].

In mathematical modelling sensitivity analysis should be performed to gain insight into the structure of the model and understand its behaviour in the parameter space of

[3] Atenolol is a BCS class III drug with a mean in vivo $P_{eff}$ of $0.5 \times 10^{-4}$ cm/s and a standard deviation of $0.2 \times 10^{-4}$ cm/s [5, 34]. Supposing that $P_{eff}$ is distributed log-normally, the 95th percentile is equal to 0.47 and the 5th percentile is below the inferior limit in Table 1. So, for Atenolol the range of variability of $P_{eff}$, from the lower limit in Table 1 to its 95th percentile, represents around 30% of the whole range of variation considered for class III drugs.

uncertainty and/or variability [20, 23]. In general, PBPK models include many parameters that are uncertain or variable at a population or individual level and whose impact on the outputs of interest is not trivial to predict. Performing GSA could help in identifying the few key parameters amongst many [23] that are mainly responsible for the variation in output.

Typically, if a model is well constructed, understood and characterized, the results of a sensitivity analysis should reflect the qualitative expectations of the model behaviour and thus, may appear to be obvious. However, especially if a model is involved in regulatory decisions, a sensitivity analysis should be performed to objectively confirm these expectations, as highlighted by regulatory agencies [25, 26]. In fact, sensitivity analysis can quantitatively assess the impact of each parameter variation on the variability of some output metrics. Understanding "how much" the input parameters influence the model outputs (so, the magnitude of the sensitivity indices) is crucial information that helps to understand if a given parameter can be assumed, fixed or require further investigation in order to allow informed model prediction. That information would be difficult to obtain without performing a sensitivity analysis.

## Conclusions

In mechanistic physiological models we often encounter uncertainty in the parameters values. Typically, when these models are used, there is a tendency to fix some uncertain parameters (e.g., radius of the particle, solubility) to an assumed value, mean value or to use in silico methods, such as quantitative structure–activity relationship (QSAR) models, to predict parameter values, without sufficiently exploring the impact on model development and predictions.

This work aimed to identify the importance of different parameters for different types of drugs, to improve our understanding of PBPK absorption models and guide the choice of parameters that can safely be assumed, estimated or require data generation to allow informed model prediction. Pharmaceutical regulators have identified the importance of sensitivity analysis in PBPK model qualification [25, 26]. Here we give a demonstration of the GSA methodology and highlight its utility by using a generalised example, spanning across a number of hypothetical compounds and showing its importance in identifying the key parameters that may be targeted for further investigation during pharmaceutical research and drug development.

**Acknowledgements** This work is an in-kind contribution to the OrBiTo Project (http://www.imi.europa.eu/content/orbito).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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