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How to scale clearance from adults to children for drugs undergoing hepatic metabolism? Insights from advanced PBPK modelling and simulation

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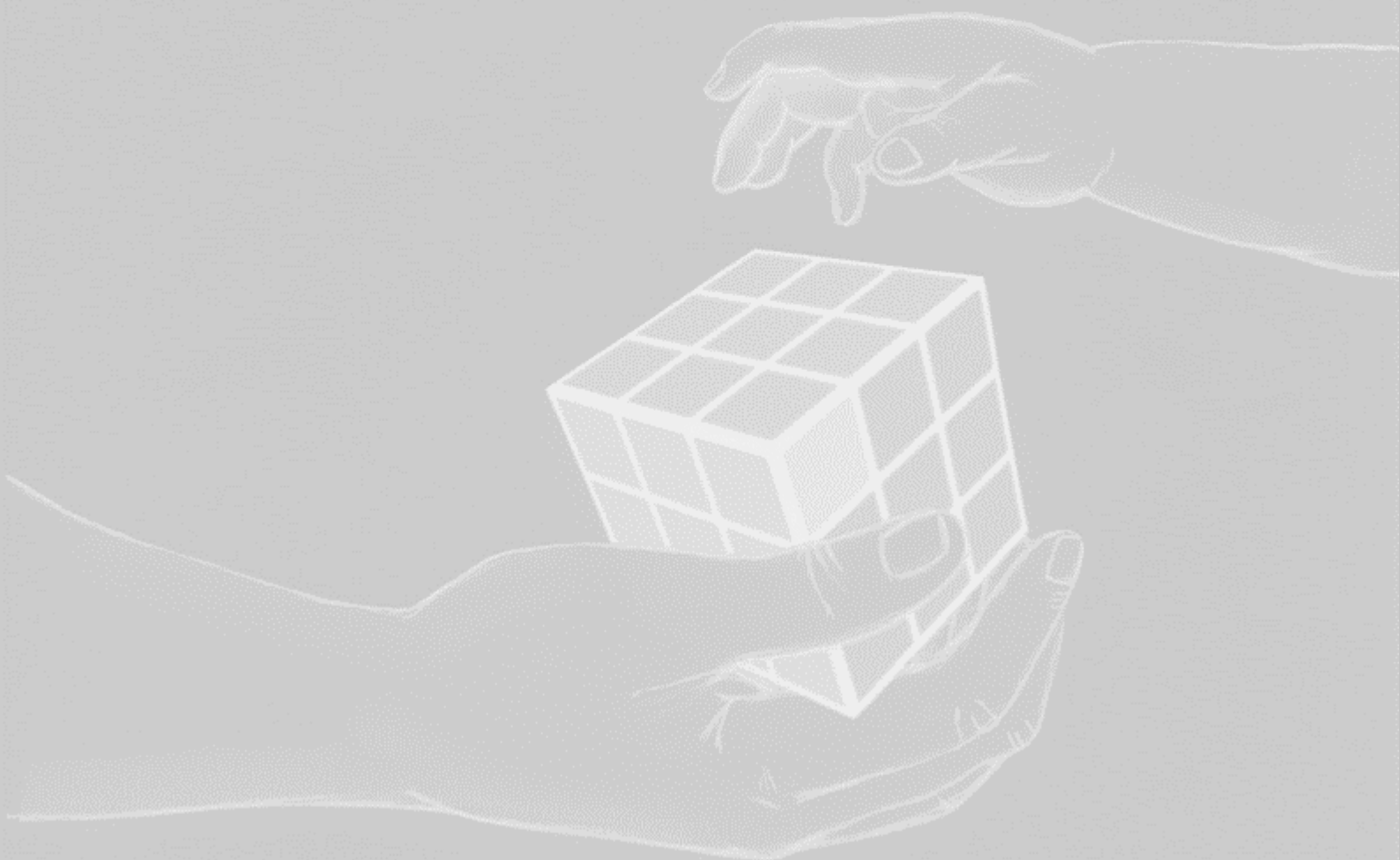
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Chapter 7

Can population modelling principles be used to identify key PBPK parameters for paediatric clearance predictions? An innovative application of optimal design theory

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Abstract

Purpose: Physiologically-based pharmacokinetic (PBPK) models are essential in drug development but require parameters that are not always obtainable. We developed a methodology to investigate the feasibility and requirements for precise and accurate estimation of PBPK parameters using population modelling of clinical data and illustrate this for two key PBPK parameters for hepatic metabolic clearance, namely whole liver unbound intrinsic clearance ($CL_{int,u,WL}$) and hepatic blood flow (Q_h) in children.

Methods: First, structural identifiability was enabled through re-parametrization and the definition of essential trial design components. Subsequently, requirements for the trial components to yield precise estimation of the PBPK parameters and their inter-individual variability were established using a novel application of population optimal design theory. Finally, the performance of the proposed trial design was assessed using stochastic simulation and estimation.

Results: Precise estimation of $CL_{int,u,WL}$ and Q_h and their inter-individual variability was found to require a trial with two drugs, of which one has an extraction ratio (ER) ≤ 0.27 and the other has an $ER \geq 0.93$. The proposed clinical trial design was found to lead to precise and accurate parameter estimates and was robust to parameter uncertainty.

Conclusion: The proposed framework can be applied to other PBPK parameters and facilitate the development of PBPK models.

7.1 Introduction

Physiologically-based pharmacokinetic (PBPK) models are an essential tool to predict the pharmacokinetics (PK) of new compounds in various human populations. PBPK models quantify how drug molecules, characterized by drug-specific parameters reflecting their properties, interact with organisms which are defined by system-specific parameters reflecting anatomical and physiological measures. Predictions are made by feeding drug-specific parameters into a PBPK model with the system-specific parameter values of the population of interest. This has been proven useful for instance to define first-in-child doses ¹⁻³ or support paediatric clinical trial design ^{2,4}.

System-specific parameter values for PBPK models can be either obtained experimentally by direct measurements or they can be derived from clinical PK data through model parameter estimation ⁵⁻⁷. The latter not only allows for coping with a lack of experimental data, which may be particularly relevant in special patient populations, but is also the most robust approach which has been well described by Tsamandoura *et al.* ⁸. For instance, it has been found that ontogeny functions estimated from clinical PK data performed markedly better than those developed from *in vitro* measurements ⁷. However, estimating parameter values is not always trivial due to structural identifiability issues. Structural identifiability refers to the possibility to uniquely estimate model parameters given a model and ideal, error-free data ^{9,10}. Without a guarantee of structural identifiability, parameter estimates will be either non obtainable or random and unreliable ¹¹. In such cases global structural identifiability, meaning that only one unique solution exists for each parameter, can be obtained by fixing or using priors for some model parameters, while estimating the others.

Once global structural identifiability of model parameters is achieved, population modelling of longitudinal data can be used for estimation of the PBPK parameters and their inter-individual variability. However, structural identifiability of a model does not guarantee precise and unbiased parameter estimates, as this depends on the information content of the data. Therefore, model parameters should also be numerically identifiable, meaning that accurate and precise estimates can be obtained given real, observed data. Numerical identifiability can in general terms be achieved by designing studies that yield sufficiently rich data ¹². Evaluation and optimization of trial designs for population PK analyses can be achieved without time-consuming clinical trial simulations using optimal design software ¹³. Optimal design software packages approximate the Fisher information matrix (FIM) of

population models and rely on the Rao and Cramer bound that states that the inverse of the FIM is the lower bound of the variance-covariance matrix of any unbiased estimator of the parameters. While to date population optimal design is routinely used in optimizing trials for the precise estimation of population PK parameters ¹⁴, it has never been applied to the estimation of population PBPK parameters.

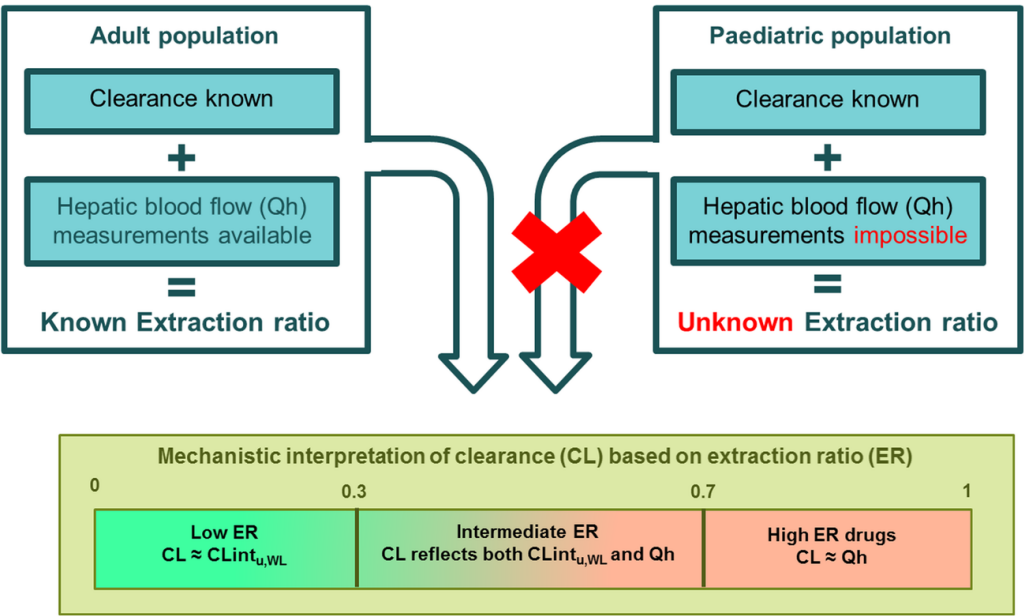


Figure 1 The relationships between extraction ratio (ER), whole liver unbound intrinsic clearance ($CL_{int,u,WL}$) and hepatic blood flow (Qh) for the adult and paediatric population. Without knowing Qh, the ER of a drug is unknown, making it mathematically impossible to disentangle Qh and $CL_{int,u,WL}$ from clinical concentration-time data of one drug. CL is the blood clearance of the unbound drug, see equations 1-6.

For drugs undergoing hepatic metabolism, the part of a paediatric PBPK model describing this clearance contains two key parameters that cannot be directly measured and that cannot be simultaneously estimated from the PK data of one drug, due to identifiability issues, namely whole liver unbound intrinsic clearance ($CL_{int,u,WL}$) and hepatic blood flow (Qh). As illustrated in Figure 1, both contribute to the overall hepatic metabolic clearance. The extraction ratio (ER) of a drug reflects the relative contribution of these two parameters to hepatic metabolic clearance. On one hand, clearance of high ER drugs reflects Qh, while clearance of low ER drugs reflects $CL_{int,u,WL}$. To achieve global structural identifiability, Qh is often fixed while $CL_{int,u,WL}$ is estimated based on PK data ^{5,15}. As Qh cannot be directly measured in very young children its value is often fixed to an assumed percentage of cardiac output ^{5,15}. When this assumed percentage is however not correct, the estimation of $CL_{int,u,WL}$

values and enzyme ontogeny functions may be biased. Therefore, there is a need for other methodologies that allow us to cope with the lack of experimental data on system-specific parameters. Even though paediatric PBPK models have been proven to predict clearance of many drugs with reasonable accuracy ^{15–17}, such methods would ultimately improve and accelerate the development and validation of these models in various paediatric populations.

The aim of this paper was to develop an analysis framework to investigate whether population modelling approach can be used to estimate PBPK model parameters from clinical PK data and establish the required criteria for such estimations. The developed analysis framework depends on firstly establishing the data requirements for structural identifiability of PBPK model parameters. Then on a subsequent application of innovative population optimal design theory to define a clinical trial design that yields precise estimates of the relevant model parameters. And lastly on the evaluation of the performance of the proposed trial design in terms of bias and imprecision of parameter estimates using stochastic simulation and estimation. The approach is illustrated using the simultaneous estimation of Q_h and $CL_{int,u,WL}$ in the paediatric population as an example.

7.2 Materials and Methods

An analytical workflow was developed, which is composed of the three following steps: structural identifiability, optimal design and evaluation of the optimized design performance. R version 3.3.1 was used for calculations, data management and visualizations ¹⁸. For optimal design procedures of step 2 of the analytical workflow, the PFIM program 4.0 running in R was used ¹⁹. PBPK simulations were performed with Simcyp software (Simcyp, Sheffield, UK) V15.R1 to derive PBPK model parameters value for optimal design procedures in step 2 and the stochastic simulations and estimations in step 3. Stochastic simulations and estimations were performed in step 3 using NONMEM version 7.3 ²⁰ and Perl-speaks-NONMEM software package version 4.6.0 ²¹.

7.2.1 Step 1: Structural identifiability

The dispersion model (equation 1 – 6) was used to describe hepatic plasma clearance based on PBPK-principles. This model was selected as it has been reported to better predict clearance than the well-stirred model for high clearance drugs, while both models lead to equivalent clearance predictions for other drugs^{22,23}.

$$CL_p = CL_b \times B:P \quad (1)$$

$$CL_b = Q_h \times ER \quad (2)$$

$$ER = 1 - F_H \quad (3)$$

$$F_H = \frac{4a}{(1+a)^2 \exp\{(a-1)/2D_N\} - (1-a)^2 \exp\{-(a+1)/2D_N\}} \quad (4)$$

$$a = (1 + 4R_N \times D_N)^{1/2} \quad (5)$$

$$R_N = (f_u/B:P) \times CL_{int,u,WL}/Q_h \quad (6)$$

In these equations CL_p is the total (bound and unbound) plasma clearance, $B:P$ is the blood to plasma ratio, CL_b is the whole blood clearance, Q_h is the hepatic blood flow, ER is the hepatic extraction ratio, F_H is the hepatic availability, f_u is the unbound drug fraction in plasma, $CL_{int,u,WL}$ is the whole liver unbound intrinsic clearance, R_N is the efficiency number and D_N is the dispersion number, which was taken to be 0.17²³.

In the dispersion model, CL_p values can be directly derived from PK data of intravenously administered drugs. In the paediatric population, the unbound drug fraction in plasma (f_u) and the blood to plasma ratio ($B:P$) of a drug can be obtained experimentally and, assuming these values were obtained precisely and accurately, they were fixed in the model (equation 1-6). Q_h and $CL_{int,u,WL}$ are thereafter the only two parameters that remain to be estimated. As in this case the dispersion model can be written as a single equation with two unknowns, clinical data on the PK of one drug will not yield structural identifiability. Therefore, clinical trial scenarios were explored based on obtaining PK profiles of two different drugs, in which case there would be two equations for clearance, each with two unknowns. Global structural identifiability can then be obtained if the unknowns are the same in both equations.

Using this approach, Q_h is a system-specific parameter that will be the same for two drugs administered to individuals from the same population, assuming the drugs do not alter Q_h . $CL_{int,u,WL}$ is however a parameter that combines system-specific properties (i.e., isoenzyme abundance) and drug-specific properties (i.e., isoenzyme activity measured in *in vitro* systems as intrinsic clearance ($\mu\text{l} / \text{min}$) per functional unit of system). This means that even when two drugs are metabolized by the same isoenzyme, their $CL_{int,u,WL}$ value is likely to be different. Therefore a re-parameterization of $CL_{int,u,WL}$ was performed to separate system-specific parameters from drug-specific parameters.

The ratio in $CL_{int,u,WL}$ (CL_{int_ratio}) of drug A and drug B that are metabolized by the same isoenzyme is equivalent to the ratio in the activity for the metabolizing isoenzyme of the two drugs according to equation 7. Therefore, this parameter is a drug-specific parameter.

$$CL_{int_ratio} = \frac{CL_{int,u,WL_B}}{CL_{int,u,WL_A}} = \frac{\text{isoenzyme activity B} \times \text{isoenzyme abundance}}{\text{isoenzyme activity A} \times \text{isoenzyme abundance}} = \frac{\text{isoenzyme activity B}}{\text{isoenzyme activity A}} \quad (7)$$

This ratio does not vary with age, as the isoenzyme activity towards a drug is believed to be unaffected by maturational processes. When fixing CL_{int_ratio} obtained for the two drugs, one unique $CL_{int,u,WL}$ value can be estimated in a patient population for the two drugs according to the re-parametrization in equations 8 and 9:

$$CL_{int,u,WL} = CL_{int,u,WL_A} \quad (8)$$

$$CL_{int,u,WL_B} = CL_{int,u,WL} \times CL_{int_ratio} \quad (9)$$

7.2.2 Step 2: Optimal design for precise population parameter estimates

Because population PK modelling disentangles inter-individual variability in PK parameters from residual unexplained variability, this method allows for the estimation of population PK parameter values Θ and their inter-individual variability ω^2 which is characterized as described in equation 10. In order to obtain precise estimates of Θ and ω^2 for $CL_{int,u,WL}$ and Q_h , a global sensitivity analysis of the uncertainty of these parameters with respect to the drugs ERs was undertaken using PFIM 4.0 (see under uncertainty section in methods). Defining the relationship between parameter uncertainty and the drugs ERs enables optimization of the trial design, by selecting drugs based on their ER. ER was chosen as the trial design parameter to optimize because it reflects the relative information content of drug clearance values regarding $CL_{int,u,WL}$ and Q_h (see Figure 1).

7.2.2.1 Clinical trial design

The clinical trial design is illustrated in Figure 2. To allow for structural identifiability, the clinical trial design was composed of two arms. The patients in each arm belonged to the same paediatric population, for which 1-year-olds were chosen, and the drugs administered in each arm were eliminated by the same isoenzyme and had different extraction ratios. In order to ensure that the sampling design was informative enough, while only focusing on the optimization of drug's ER, the number of patients and blood samples were chosen to be high, with 45 patients included in each arm and 7 blood samples drawn in each patient. This choice allowed us to assess the impact of the drug's ER on the performance of the trial design without a confounding impact of patient or sample number. Finally, in order to assess the impact of the drug's ER on the performance of the design without any confounding impact of sampling times, drug dosing, and number of patients and sampling times, these parameters were adapted to the drugs' properties. To do so, drugs were administered as a constant rate infusion with infusion rate and sampling times adapted to the drugs clearance and half-life respectively, and each arm of the study included the same number of patients and sampling times. The infusion rate was set to reach the same arbitrary steady state concentration of 70 mg/L. Sampling times were drawn every half-life, from the first half life after the start of the infusion up to seven half-lives.

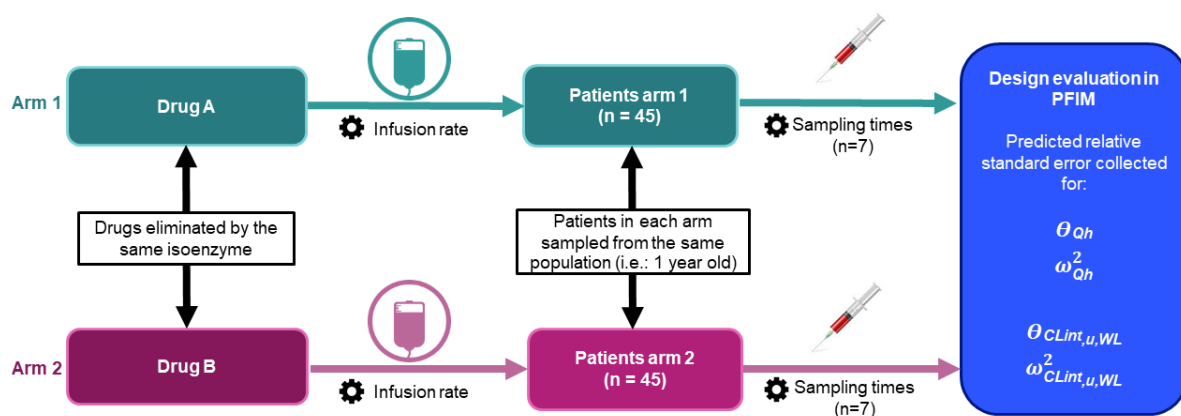


Figure 2 Overview of the clinical trial design. Q_h is the hepatic blood flow, $CL_{int,u,WL}$ is the whole liver unbound intrinsic clearance as define in equations 8 and 9. θ represents the fixed effect and ω^2 represents the inter-individual variance. Cogs indicate trial design variables which are adapted to the drug properties in order to prevent them from being confounding factors when assessing the impact of the drug's ER on the performance of the design.

7.2.2.2 Hypothetical drug combinations

A total of 99 hypothetical drugs were generated and each unique combination was tested in the design. In order to reduce computational costs and increase interpretability of the results, f_u and B:P were taken to be 1. Using the dispersion model, population values of $CL_{int,u,WL}$ ($\Theta_{CL_{int,u,WL}}$) were calculated to be such that ER values ranged from 0.01 to 0.99. This required population values of Q_h (Θ_{Q_h}) which was obtained by simulating 500 male and 500 female individuals of 1 year in Simcyp and estimating the population parameter value using the “fitdist” function from the fitdistrplus R package ²⁴ in R.

7.2.2.3 Studied models and parameters

For illustration, the proposed workflow is applied to identifying Q_h and $CL_{int,u,WL}$ in the paediatric population. As none of the parameters in the dispersion model are impacted by absorption or distribution processes, it is possible to study this PBPK sub-model in full while simplifying the remaining aspects of the PK model for the purpose of this work. Therefore, for each drug in the design (drug A and B), the structural model was a one compartment PK model with a volume of distribution V_A and V_B respectively and with constant intravenous infusion (see PFIM model code in Supplementary Material 2 and corresponding model equations in Supplementary Material 1). Clearance was parameterized as defined by the dispersion model (equation 1–6). $CL_{int,u,WL}$ for drug A and B was parameterized according to equations 8 and 9 respectively. The differential equations of the models, as implemented in PFIM can be found in Supplementary Material 2. Inter-individual variability was implemented on all fixed effects except on f_u , B:P, D_N and $CL_{int,ratio}$. Inter-individual variability on f_u and B:P can be measured and are therefore attainable parameters. D_N is a model specific parameter with a fixed value taken from literature. And as a drug-specific parameter, $CL_{int,ratio}$ is not subject to inter-individual variability. For the other parameters, inter-individual variability was assumed to follow a log normal distribution and implemented using equation 10, in which η is normally distributed with a mean of 0 and a variance of ω^2 (see eq.10).

$$P_i = \theta \times e^{\eta} \quad (10)$$

A proportional residual error model for each arm in the design with a standard deviation σ of 0.1 was assumed, to yield the same impact of the model error on the parameter uncertainty between study arms and study designs. Information on the values and estimation

of the fixed effects, inter-individual variability and residual errors implemented in the models can be found in Table 1.

Table 1 Estimated and fixed model parameters

Parameters	Values	Estimated and fixed
Fixed effects		
$\Theta_{CLint,u,WL}$	[0.10 – 156] L/h	Estimated
Θ_{Qh}	20.3 L/h	Estimated
Θ_{fu}	1	Fixed
$\Theta_{B:P}$	1	Fixed
Θ_{V_A}	4 L	Estimated
Θ_{V_B}	4 L	Estimated
Θ_{D_N}	0.17	Fixed
$\Theta_{CLintratio}$	[0.00065 – 0.97000]	Fixed
Inter-individual variability		
$\omega_{CLint,u,WL}^2$	[0.1 – 0.8]	Estimated
ω_{Qh}^2	[0.1 – 0.6]	Estimated
$\omega_{V_A}^2$	0.25	Estimated
$\omega_{V_B}^2$	0.25	Estimated
Residual error		
σ_A	0.1	Estimated
σ_B	0.1	Estimated

Θ fixed effect; ω^2 inter-individual variance. Θ_{V_A} , Θ_{V_B} , correspond to fixed effects for the volume of distribution of drug A and B respectively.

7.2.2.4 Design evaluation with PFIM 4.0

The models, parameters and designs described above were implemented in PFIM 4.0¹⁹. Concentration-time profiles obtained for both drug A and B were simultaneously analysed to enable for the estimation of Θ and ω^2 for $CLint,u,WL$ and Qh . The clinical design parameter to optimize for precise estimation of these parameters was the ER of each of the

two drugs in the design. To do so, the population Fisher information matrix (FIM) was evaluated for each drug combination in PFIM 4.0. The expected standard errors for each population parameter were calculated as the square root of the diagonal of the inverse of the FIM and reported in PFIM outputs with corresponding relative standard errors (rse). Parameter uncertainties were assessed using their rse with precise estimates being defined as having an $rse \leq 30\%$.

7.2.2.5 Uncertainty

ER, which is the design variable that was optimized, is dependent on the relative contribution of $CL_{int,u,WL}$ and Q_h to overall hepatic metabolic clearance, but not on their absolute values since there is an infinity of combinations of $CL_{int,u,WL}$ and Q_h values leading to a specific value of ER. Hence, combinations of $\Theta_{CL_{int,u,WL}}$ and Θ_{Q_h} that lead to similar ER values are estimated with the same rse%. Therefore, no uncertainty on the $\Theta_{CL_{int,u,WL}}$ and Θ_{Q_h} was implemented in the design evaluation procedure to avoid unnecessary computations, meaning that only one value for $\Theta_{CL_{int,u,WL}}$ and one value for Θ_{Q_h} as displayed in Table 1 was used in the PFIM runs.

For each combination of hypothetical drugs, the analysis was repeated for different values of $\omega_{CL_{int,u,WL}}^2$ and $\omega_{Q_h}^2$ in order to account for uncertainty in these parameters. The range in $\omega_{CL_{int,u,WL}}^2$ was set from 0.1 to 0.8, while the range in $\omega_{Q_h}^2$ was set from 0.1 to 0.6, both reflecting realistic ranges (see Supplementary Material 1). Then, design evaluations were repeated using each combination of the upper and lower uncertainty value of the defined range for $\omega_{CL_{int,u,WL}}^2$ and $\omega_{Q_h}^2$, running 4 possible inter-individual variance scenarios in total, yielding a global sensitivity analysis of the uncertainty of $CL_{int,u,WL}$ and Q_h . Only the lowest and highest values for $\omega_{CL_{int,u,WL}}^2$ and $\omega_{Q_h}^2$ were retained in the uncertainty analysis since this yields the extreme values for the rse values. Rse results of all variance scenarios were collapsed for each hypothetical drug combination into one single precision category reflecting the worst-case scenario of all variance scenarios. The PFIM model file, input file, and the R command to launch the PFIM evaluation runs for all combinations of hypothetical drugs for one uncertainty scenario are provided in Supplementary Material 2 to 4. The table containing the drug properties used for the PFIM runs are provided in Supplementary Table 1.

7.2.3 Step 3: Investigation of performance of the proposed design

In step 2, the requirements for ER of the two drugs yielding numerical identifiability of fixed effect Θ and inter individual variability ω^2 of $CL_{int,u,WL}$ and Q_h were identified. In the last step of the developed approach, the performance of the proposed design was investigated in terms of bias and imprecision using stochastic simulation and estimation (sse).

First, two drugs meeting the ER requirements defined in step 2 were selected and their ER was converted to expected plasma clearance (CLp) values in a one year old using the expected age-appropriate Θ_{Q_h} value derived from Simcyp in step 2. The volume of distribution of each drug was used together with their expected CLp to derive their expected half-life. Since the clinical trial design depends on the half-life and CLp of the two drugs for establishing sampling times and infusion rate respectively these parameters were used for its implementation.

Because the true value of Θ_{Q_h} might differ from the value derived from Simcyp in step 2, the selected drugs might have an ER differing from their expected ER. Therefore, uncertainty in both Θ and ω^2 values of $CL_{int,u,WL}$ and Q_h , were accounted for in the sse. This was performed by sampling each of these parameters 1000 times from a uniform distribution. For the population parameters the range of the distribution entailed the typical values from step 2 $\pm 50\%$. For the variance parameters the range of the distribution entailed the range defined for the parameter uncertainty in step 2. Sampling of population parameter values Θ_{Q_h} and $\Theta_{CL_{int,u,WL}}$ was restricted, such that their corresponding CLp values yielded a variation of $\pm 30\%$ of the drugs' CLps value derived from the ERs of the selected drug A and drug B. This mimics the accepted 30% uncertainty of the reported CLp value in literature. The performance of the design settings were assessed using relative estimation error re (equation 11), mean relative estimation error mre (equation 12), and relative root mean square error rmse (equation 13) for Θ and ω^2 $CL_{int,u,WL}$ and Q_h , using only the runs for which minimization was successful.

$$\text{Relative estimation error (\%)} = \frac{est_i - true_i}{true_i} \times 100 \quad (11)$$

$$\text{Mean relative estimation error (\%)} = \frac{1}{N} \sum_{i=1}^N \frac{est_i - true_i}{true_i} \times 100 \quad (12)$$

$$\text{Relative root mean square error (\%)} = \sqrt{\frac{1}{N} \sum_{i=1}^N \frac{(est_i - true_i)^2}{true_i^2}} \times 100 \quad (13)$$

In these equations, *true* is the true parameter value used in the simulation step, *est* is the estimated parameter value from the estimation step, and *i* is the run index, ranging from 1 to N, the total number of runs for which minimization was successful.

7.3 Results

7.3.1 Step 1: Structural identifiability

Q_h and $CL_{int,u,WL}$ were found to be structurally identifiable when the concentration-time profiles from a clinical trial with 2 arms were simultaneously analysed. Each arm of the trial required patients from the same population and administration of different drugs metabolized by the same isoenzyme for which the ratio in intrinsic clearance ($CL_{int, ratio}$) is known.

7.3.2 Step 2: Optimal design for precise population parameter and variance estimates

Precision of fixed effects and variance values for $CL_{int,u,WL}$ and Q_h obtained with combinations of two drugs in the described clinical trial design are summarized in Figure 3. Except for drug combinations with similar extraction ratio ER (i.e., drug combinations near the line of unity), most drug combinations will lead to a precise $\Theta_{CL_{int,u,WL}}$ estimate. However, for the estimate of $\omega_{CL_{int,u,WL}}^2$ to be precise, at least one of the drugs should have a low extraction ratio. For Θ_{Q_h} , most drug combinations leading to precise estimation include one drug with a high ER, while for precise estimation of $\omega_{Q_h}^2$, at least one drug with an $ER \geq 0.93$ is required. Finally, precise estimation of all parameter estimates ($rse \leq 30\%$) requires one drug with an $ER \geq 0.93$ and one drug with an $ER \leq 0.27$. Parameter estimation with $rse \leq 50\%$ (corresponding to the blue and green areas in Figure 3) for all parameters, requires one drug with an $ER \geq 0.85$ and one drug with an $ER \leq 0.41$.

Supplemental Figure 1 shows the results presented in Figure 3, but separated for the four variance scenarios. As shown by both Figure 3 and Supplementary Figure 1, $\omega_{CL_{int,u,WL}}^2$ and $\omega_{Q_h}^2$ drive the threshold for the highest and lowest ER value required in the drug combination for precise estimation of all parameters respectively. Supplementary Figure 1 also shows that the value of each of these thresholds are driven by both $\omega_{CL_{int,u,WL}}^2$ and

ω_{Qh}^2 . For precise parameter estimates, an increase in ω_{Qh}^2 from 0.1 to 0.6 leads on one hand to a decrease in the threshold for the highest ER value required in the drug combination from 0.79 to 0.56 when $\omega_{CLint,u,WL}^2$ is equal to 0.1 and from 0.93 to 0.80 when $\omega_{CLint,u,WL}^2$ is equal to 0.8. On the other hand, such an increase in ω_{Qh}^2 also leads to a decrease in the threshold for the lowest ER value required in the drug combination from 0.48 to 0.27 when $\omega_{CLint,u,WL}^2$ is equal to 0.1 and from 0.76 to 0.55 when $\omega_{CLint,u,WL}^2$ is equal to 0.8. The reverse is observed regarding $\omega_{CLint,u,WL}^2$, with an increase in $\omega_{CLint,u,WL}^2$ leading to an increasing threshold for the highest ER value required in the drug combination for precise estimates of ω_{Qh}^2 and Θ_{Qh} , and an increasing threshold for the lowest ER value required in the drug combination for precise $\omega_{CLint,u,WL}^2$ estimates.

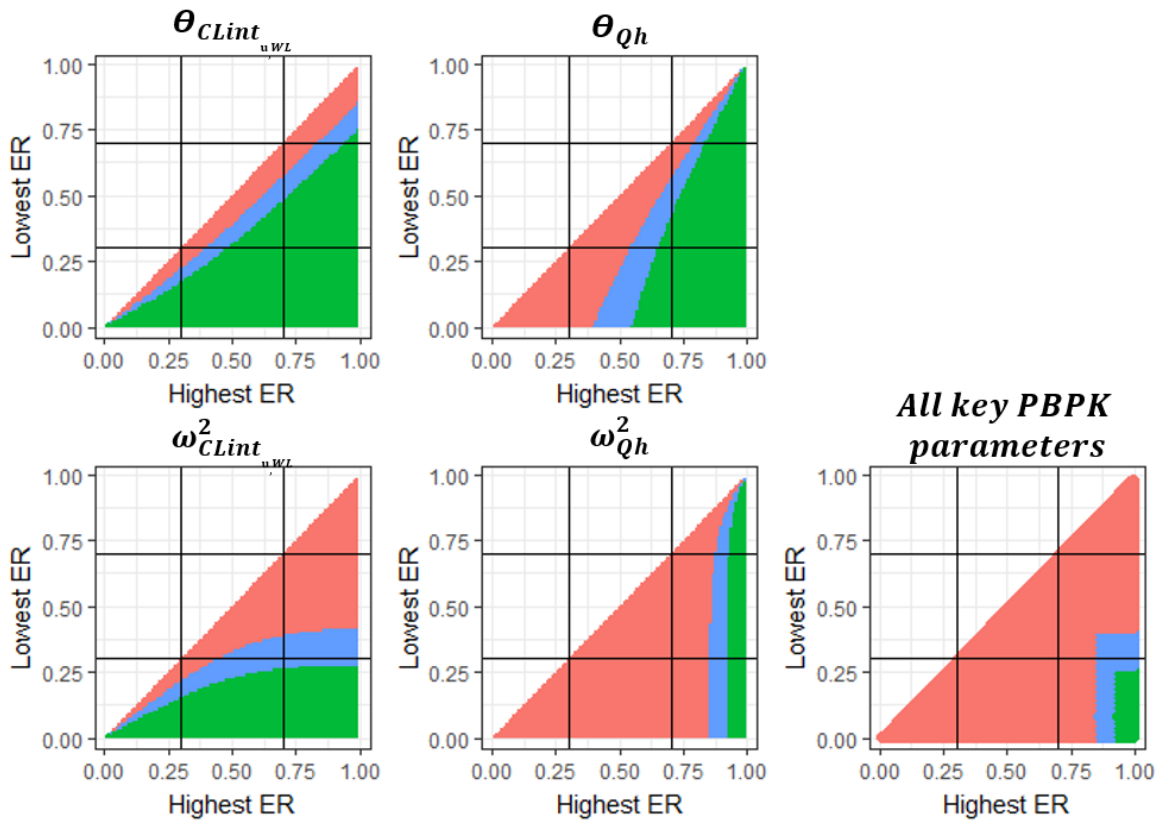


Figure 3 Parameter precision as a function of the extraction ratios (ER) of the two drugs studied in the clinical trial. Θ represents the fixed effect, ω^2 the inter-individual variance in the parameter. $CLint_{u,WL}$ is the whole liver unbound intrinsic clearance as defined in equations 8 and 9, Qh is the hepatic blood flow. For each tested drug combination, the precision of the parameter estimates are summarized, with each pixel representing the results for all four variance scenarios (i.e., $\omega_{CLint_{u,WL}}^2$ and ω_{Qh}^2 of 0.1 and 0.1, 0.1 and 0.6, 0.8 and 0.1 or 0.8 and 0.6 respectively) according to the following colour scheme: green indicates relative standard errors ($rse\%$) $\leq 30\%$ for all scenarios, blue indicates $rse\%$ is between 30 and 50% for at least one variance scenario, and red indicates $rse\% > 50\%$ for at least one variance scenario.

The results on ER requirements for drug A and B are independent of the age of children as the ER does not depend on absolute values of Θ_{Qh} and $\Theta_{CL_{int,u,WL}}$. However, the ERs of drugs are often unknown, especially in special patient populations, because as presented in Figure 1, ER in children changes due to maturation of both $CL_{int,u,WL}$ and Qh and the impact of these changes are isoenzyme-dependent due to different maturation patterns.

To facilitate the identification of model drugs for which clinical data could be obtained to precisely estimate population values and variance of $CL_{int,u,WL}$ and Qh at different ages, maturation patterns of different isoenzymes were used to identify isoenzymes and ages for which drugs with the lowest and highest ER required for such estimation are likely to exist. To do so, the dispersion model in combination with maturation patterns for $CL_{int,u,WL}$ of various isoenzymes as implemented in Simcyp V15 (see Supplementary Material 1) were used to calculate expected ER values for the hypothetical drugs that are substrates of these isoenzymes in children of various ages. We found that drugs with an $ER \leq 0.27$ are likely to exist for all investigated isoenzymes at all ages (results not shown). However, drugs with an $ER \geq 0.93$ are likely to exist only for specific isoenzyme pathways and ages, as shown in Table 2 by the increased blue and red boxes with younger ages for most isoenzyme pathways. This is, because low enzyme maturation will reduce the overall ER of drugs. As a result, drugs metabolized by slowly maturing isoenzymes such as CYP2E1 and UGT2B7, are unlikely to have a high ER at young ages. On the other hand, drugs metabolized by fast maturing isoenzymes, such as CYP1A2 or UGT1A4, with a very high ER over a wide range of paediatric ages are likely to exist and could be used as model drugs.

For convenience, to identify drugs with desired ER values, the ER values have also been translated into the required total plasma clearance values (CL_p) for various paediatric ages. This is presented in Table 3, which shows for instance that in a one-year old child, $ER \leq 0.27$ and $ER \geq 0.93$ translate into a $CL_p \leq 5.5L/h$ and a $CL_p \geq 18.9 L/h$, respectively.

Table 2 Identification of isoenzyme pathways and ages for which combinations of two drugs leading to precise Q_h and $CL_{int,u,WL}$ fixed effects and inter-individual variability estimates is theoretically possible

Isoenzyme	Uncertainty	1 day	1 month	6 months	1 year	2 years	5 years	15 years	25 years
CYP1A2	Qh - 50%								
	CL _{int,u,WL} +50%								
	none								
	Qh + 50%								
	CL _{int,u,WL} - 50%								
CYP2C18_19	Qh - 50%								
	CL _{int,u,WL} +50%								
	none								
	Qh + 50%								
	CL _{int,u,WL} - 50%								
CYP2D6	Qh - 50%								
	CL _{int,u,WL} +50%								
	none								
	Qh + 50%								
	CL _{int,u,WL} - 50%								
CYP2E1	Qh - 50%								
	CL _{int,u,WL} +50%								
	none								
	Qh + 50%								
	CL _{int,u,WL} - 50%								
CYP3A4_5	Qh - 50%								
	CL _{int,u,WL} +50%								
	none								
	Qh + 50%								
	CL _{int,u,WL} - 50%								
UGT1A1	Qh - 50%								
	CL _{int,u,WL} +50%								
	none								
	Qh + 50%								
	CL _{int,u,WL} - 50%								
UGT1A4	Qh - 50%								
	CL _{int,u,WL} +50%								
	none								
	Qh + 50%								
	CL _{int,u,WL} - 50%								
UGT1A6	Qh - 50%								
	CL _{int,u,WL} +50%								
	none								
	Qh + 50%								
	CL _{int,u,WL} - 50%								
UGT2B7	Qh - 50%								
	CL _{int,u,WL} +50%								
	none								
	Qh + 50%								
	CL _{int,u,WL} - 50%								

The cells indicate whether within the set of hypothetical drugs (see Supplementary Material 1) a combination of two drugs exist with which Q_h and $CL_{int,u,WL}$ fixed effects and inter-individual variability estimates can be obtained with $rse \leq 30\%$ (green), $30\% < rse \leq 50\%$ (blue), or $rse > 50\%$ (red). Calculations are performed for a situation in which Θ_{Q_h} and isoenzyme maturation are set to values as implemented in Simcyp V15 or to a value that is 50% higher or lower reflecting extreme scenarios of uncertainty on Θ_{Q_h} and $\Theta_{CL_{int,u,WL}}$.

Table 3 Conversion table to convert the extraction ratio (ER) values of 0.27 and 0.93 to total plasma clearance (CLp, equation 1) in various postnatal ages using expected hepatic blood flow (Qh) values and fu and B:P values taken to be 1

	Expected Θ_{Qh} (L/h)	CLp (L/h) corresponding to ER = 0.27	CLp (L/h) corresponding to ER = 0.93
25 years	87.0	23.5	80.9
15 years	89.2	24.1	83.0
5 years	41.5	11.2	38.6
2 years	30.0	8.1	27.9
1 year	20.3	5.5	18.9
6 months	13.0	3.5	12.1
1 month	7.5	2.0	7.0
1 day	6.4	1.7	6.0

Expected Θ_{Qh} is the fixed effect for the hepatic blood flow derived from Simcyp simulations.

7.3.3 Step 3: Investigation of bias

The performance of a clinical trial design resulting from step 1 was evaluated using sse with one drug with an ER of 0.94 and one drug with an ER of 0.2 in one-year old children. The uncertainty on $\Theta_{CLint_{u,WL}}$ and Θ_{Qh} included in the sse, lead to the simulation of 1000 combinations of $\Theta_{CLint_{u,WL}}$ and Θ_{Qh} with a range of ER from 0.09 to 0.38 and from 0.76 to 0.99 for the drug with the lowest and the highest ER respectively. Minimization was successful in 78.3% of the sse runs. The mre was $\leq 7.5\%$ for all parameters (Table 4), and the rmse was below 31%. The confidence interval (5th and 95th percentile) of the re was below or around 20% for $\Theta_{CLint_{u,WL}}$ and Θ_{Qh} . Higher values were found for $\omega_{CLint_{u,WL}}^2$ and ω_{Qh}^2 , with values below or around 47%.

Table 4 Assessment of the clinical trial performance based on the precision and accuracy of the parameter estimates of the sse with uncertainty

	$\Theta_{\text{CLint}_{u,\text{WL}}}$	Θ_{Qh}	$\omega_{\text{CLint}_{u,\text{WL}}}^2$	ω_{Qh}^2
re 5 th percentile	-16.2935	-18.356	-44.4268	-47.0063
re 50 th percentile	0.715404	-1.6823	-9.7909	-10.7792
re 95 th percentile	23.68881	16.63842	41.03665	46.80308
mre	1.548568	-1.19669	-7.38877	-7.24315
rrmse	12.21483	10.9555	26.24813	30.54991

Re, relative estimation error; *mre*, mean relative estimation error; *rrmse*, relative root mean square error; Θ , fixed effect, ω^2 , inter-individual variance. $\text{CLint}_{u,\text{WL}}$ is the whole liver unbound intrinsic clearance as define in equations 8 and 9 and Qh is the hepatic blood flow.

7.4 Discussion

The aim of this paper was to develop an analysis framework to investigate the feasibility and clinical trial requirements for the estimation on clinical data of PBPK parameters with a population PK approach. This work represents, to our knowledge, the first application of population optimal design principles for the estimation of PBPK parameters. Being able to *a priori* define trial requirements that yield sufficiently informative data (i.e., numerical identifiability), is essential for a decision-making process when costs and benefits of performing a study need to be weighed. The complex design requirements derived in our example for instance, would not be easy to define and would likely not have been met using conventional study design approaches. The execution of clinical trials that yield uninformative data that do yield numerical identifiability is both unethical, especially in vulnerable populations, and cost-inefficient.

While we focused on $\text{CLint}_{u,\text{WL}}$ and Qh in a paediatric population, the analysis workflow herein developed can be applied to other PBPK parameters, a different number of parameters, and for other populations. In these cases, the workflow would contain the same steps as outlined here: structural identifiability, optimal design and evaluation of the optimized design performance.

In our example, in the first step of the workflow, structural identifiability for the estimation of both $\text{CLint}_{u,\text{WL}}$ and Qh was found possible when PK data of a minimum of two drugs were simultaneously analysed. In addition, both drugs must be administered in patients groups from the same population, metabolized by the same isoenzyme, and their $\text{CLint}_{\text{ratio}}$

should be known. $CL_{int_{ratio}}$ is the ratio of the *in vitro* measured intrinsic clearance of the drugs and is a drug-specific parameter (equation 7).

In the second step, the use of optimal design not only allowed to optimize the characteristics of drugs to include in the clinical trial design in order to solve numerical identifiability issues, but also acts as a safeguard ensuring global identifiability of the model parameters. Indeed, optimal design identifies models that are either globally or locally structurally non identifiable since their Fisher information matrix is non invertible²⁵. We found that given the described trial design, the two drugs included in the trial should have an $ER \geq 0.93$ and an $ER \leq 0.27$, in order to precisely ($rse \leq 30\%$) estimate Θ and ω^2 for $CL_{int_{u,WL}}$ and Q_h . These requirements might not be easy to meet since drugs with $ER \geq 0.93$ are rare, especially in very young children whose isoenzyme maturation can lead to a decrease in ER with decreasing age²⁶. This is shown in Table 2, displaying isoenzymes and ages for which drugs with ER values that meet these criteria exist within the set of hypothetical drugs tested. These results show that characterization of isoenzyme specific ontogeny is challenging in very young children not only due to the sparsity and lack of available data in this population, but also due to intrinsic characteristics of the system which leads to a decrease in ER with decreasing age. Drugs metabolized by fast maturing isoenzymes were found to be the best model drugs to estimate Q_h and $CL_{int_{u,WL}}$ simultaneously. For instance, CYP1A2 substrates are likely to have the required ERs in children as young as 6 months even in situations where $\Theta_{CL_{int_{u,WL}}}$ and Θ_{Q_h} deviate up to $\pm 50\%$ of their expected values. UGT1A4 substrates are likely to have the required ERs in children as young as term neonates of one day, but only in situations where $\Theta_{CL_{int_{u,WL}}}$ and Θ_{Q_h} values correspond to their expected value or are up to $+50\%$ and -50% of their expected value respectively. To support the selection of such model drugs from existing drugs, the requirements in ER were translated into CLp values in the conversion table (see Table 3) using reported hepatic blood flow values. Overall, these results do highlight the importance of investigating the clinical trial requirements *a priori*, as otherwise the chances of successfully estimating PBPK model parameters from clinical PK data using population approach will be very low.

In the last step of our analysis workflow, stochastic simulations and estimations were used to assess the performance of the optimized study design, since optimal design only addresses parameter precision. While step 2 defined the ER of the two drugs required in the clinical trial, in practice the selected drugs might have an ER deviating from their expected values, which was accounted for in the sse. Uncertainty in fixed effects and inter-individual

variability for $CL_{int,u,WL}$ and Q_h was also accounted for in the sse. This step allowed for the assessment of whether further investigation to optimize the clinical trial design would be needed to ensure unbiased parameter estimates. Absolute mean ree was below 7.5% for all parameters and the rmsee was below 31%, meaning that on average the estimates both are accurate and precise. The confidence interval (5th and 95th percentile) of the relative estimation error was below or around 20% for $\Theta_{CL_{int,u,WL}}$ and Θ_{Q_h} . Higher values were found for $\omega_{CL_{int,u,WL}}^2$ and $\omega_{Q_h}^2$ which was expected due to parameter uncertainty and the general difficulty in estimating inter-individual variability. Overall, the sse showed that the proposed clinical trial design is robust to parameter uncertainty.

Once existing model drugs with the required ER are identified, clinical data to estimate Θ and ω^2 for $CL_{int,u,WL}$ and Q_h can be sought. The source of these data could either be new clinical trials or historical data from studies meeting the defined design requirements.

An advantage of PBPK models is that quantification of system-specific model parameters, like Q_h and the maturation profile of $CL_{int,u,WL}$, for each isoenzyme, needs to be performed only once. Once Q_h has been quantified for instance using substrates for the CYP1A2 or UGT1A4 isoenzymes, this can be implemented in the PBPK model either as fixed value or as prior in order to estimate Θ and ω^2 for $CL_{int,u,WL}$, for any other drug or other isoenzyme pathway in the same population. This is important because both $CL_{int,u,WL}$ and Q_h cannot be directly measured and because identifiability is important for the estimation of PBPK model parameters²⁷.

While in this work we focus on popPBPK parameter estimation for a specific age, the scalability of the rse results with regard to ER allows for the applicability of design requirements across the entire age range. Since most paediatric PK studies include patients of a range of ages, estimation of a maturation function would be preferable over the estimation of parameter values for each specific paediatric age. In the future this work could be extended to the design of studies for the estimation of such maturation functions. This will require the *a priori* definition of the most suitable covariate for maturation function and optimization of study designs for the estimation of parameters in continuous covariate functions, the latter of which is not yet available in the PFIM software.

In the developed analytical workflow, optimal design principles were applied in an innovative manner. The clinical design parameter optimized was the ER of the drugs included in the clinical trial. In a classical optimal design setting, optimized clinical design parameters

include the number of patients and samples, sampling times, and drug dose. In this work, in order to assess the impact of the drug's ER on the performance of the design without any confounding impact of these design parameters, these parameters were set such as to have the same impact for each hypothetical drug in the study. To do so, drugs were administered as a constant rate infusion with infusion rate and sampling times adapted to the drugs clearance and half-life respectively, and each arm of the study included the same number of patients and number of blood samples. Practically, such design adaptation on half-life and clearance means that when normalizing sampling times over the drug's half-life, sampling times and measured concentrations are identical between different drugs (between each arm of the design and between different drug combinations tested), which in turn allows for the same impact of sampling times and dosing regimen on the design performance. Another innovative feature of the workflow is that the optimal design is not given as a unique solution as classically performed, but as a range of solutions. This was done by investigating the parameter space of the design variable to optimize (ER) and categorization of the results by level of precision.

The number of patients and samples in the assessed trial designs in step 2 of the workflow were selected to be relatively high, so that these variables would not be limiting to the optimization of trial requirements regarding the definition of ER values. Once the important design features relevant for the research question of interest have been defined in the proposed workflow, traditional optimal design procedures could be applied to further optimize the trial design regarding these variables.

In the developed workflow, computational cost was tremendously reduced through the optimization of a scalable variable and the use of extreme variance scenarios to account for parameter uncertainty. The ER of a drug is a scalable variable, as it can be converted to clearance values in any paediatric age by using the expected hepatic blood flow in the corresponding age which represents the scaling factor. Indeed, ER reflects the relative contributions of Q_h and $CL_{int,u,WL}$ to the hepatic metabolic clearance, but is independent of the absolute value of these two parameters. Therefore, the results of the optimal design analysis obtained for one age, one-year-olds in our example, can be extended to any other ages, allowing to reduce the computational cost and to facilitate results interpretation. Moreover, the ER summarizes the influence of all drug-specific parameters (e.g., drug binding to plasma proteins, drug distribution in red blood cells, etc.), and therefore, accounting for uncertainty in $\Theta_{CL_{int,u,WL}}$ and Θ_{Q_h} becomes unnecessary in the optimal design phase and the result obtained in our example for drugs with an unbound drug fraction in plasma or f_u of 1 and blood to

plasma ratio or B:P of 1 can be translated to drugs for which these parameters take different values. Testing all combinations of extreme ω_{Qh}^2 and $\omega_{CLint_{u,WL}}^2$ values lead to results reflecting the best- and worst-case scenarios in terms of parameter precision. Because we defined precise parameter estimates as $rse < 30\%$ in all tested variance scenarios, the final results reflect the worst-case scenarios and account for all untested intermediate variance scenarios.

In conclusion, this work presents an analysis framework that allows for the *a priori* identification of clinical trial requirements that would allow for the estimation of PBPK model parameters from clinical data using population modelling. The example on $CLint_{u,WL}$ and Qh shows that it may be unlikely to design an adequate clinical trial, without the knowledge obtained by the application of this analysis framework. Being able to identify PBPK parameters that cannot be obtained by direct experimental measurements in a time and cost-efficient manner would greatly improve the development of PBPK models and their predictive performance.

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Supplementary Material 1

Realistic extraction ratio ranges for various isoenzymes and ages

To facilitate the identification of existing compounds that would be suitable model drugs, the maximum ranges in extraction ratio that are expected for hypothetical drugs that are substrates for various isoenzymes in children of various ages were calculated. This was done using the dispersion model in combination with maturation patterns of various isoenzymes as implemented in Simcyp V15. Θ_{Qh} values were used as obtained for each age as displayed in table III and $\Theta_{CLint,u,WL}$ values were computed as described in equation 1.

$$CL_{int,u,WL} = CL_{int,mic} \times MPPGL \times \text{liver weight} \times IO \quad (1)$$

$CL_{int,mic}$ stands for intrinsic microsomal clearance for which values between $0.56 \cdot 10^{-6}$ and $0.209 \cdot 10^{-3} \text{ L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ microsomal protein were used according to literature ¹. The population mean of the product of MPPGL (microsomal protein per gram of liver) and liver weight was derived from the Simcyp simulation similarly as for the computation of Θ_{Qh} . Isoenzyme ontogeny (IO) values for the different ages and isoenzymes were taken from Simcyp V15 and are displayed in the table below. IO is expressed as percentage of adult isoenzyme activity.

	1 day	1 month	6 months	1 year	2 years	5 years	15 years	25 years
IO CYP1A2 (%)	24	35	118	150	164	161	126	100
IO CYP2C18-19	30	33	84	95	97	98	100	100
IO CYP2D6 (%)	6	47	84	91	95	98	100	100
IO CYP2E1 (%)	10	37	59	67	74	82	88	100
IO CYP3A4_5 (%)	11	13	48	78	96	104	106	100
IO UGT1A1 (%)	0.2	23	98	104	100	100	100	100
IO UGT1A4 (%)	74	74	74	75	77	81	97	100
IO UGT1A6 (%)	15	30	63	76	87	95	100	100
IO UGT2B7 (%)	8	9	11	13	18	32	79	100

In order to account for uncertainty on Qh and $CL_{int,u,WL}$, extraction ratio ranges were also computed with Θ_{Qh} values of $\pm 50\%$ and with $\Theta_{CLint,u,WL}$ values of $\pm 50\%$ of the values described above. For each isoenzyme, the age range was identified in which drugs with realistic extraction ratio values exist that are required for the precise estimation of Qh and $CL_{int,u,WL}$.

Definition of realistic ranges for $\omega_{\text{CLint,u,WL}}^2$ and ω_{Qh}^2 for the implementation of parameter uncertainty in PFIM

In step 2 and 3 of the analytical workflow, uncertainty on $\omega_{\text{CLint,u,WL}}^2$ and ω_{Qh}^2 were implemented using realistic ranges for these parameters.

For $\omega_{\text{CLint,u,WL}}^2$, the range of variance was set to 0.1-0.8, including the minimum and maximum hepatic $\omega_{\text{CLint,u,WL}}^2$ estimates derived using Simcyp (0.46 and 0.77 respectively) and reported clearance variance of low extraction ratio drugs ². For populations of 1 day, 1 month, 6 months, 1, 2, 5, 15 and 25 year-olds, 1000 Simcyp simulations of hepatic CL_{int,u,WL} for each population were performed, with 50% females and 50% males, assuming drug metabolism by different isoenzymes. These isoenzymes were isoenzymes for which model drugs have been reported (drugs mainly metabolized by the isoenzyme), namely CYP1A2, CYP2C19, CYP2D6, CYP2E1, CYP3A4-5, UGT1A1, UGT1A4, UGT1A6 and UGT2B7 ³⁴. For each population and isoenzyme, $\omega_{\text{CLint,u,WL}}^2$ was estimated using the fitdist function from the fitdistrplus R package assuming a log normal distribution.

For Qh a range of variance of 0.1-0.6 was selected, which includes recently reported portal vein flow variance values in healthy adults ($\omega_{\text{Qh}}^2 = 0.13$) and in cirrhotic adults (omega =0.38) using phase contrast MRI sequence with compressed sensing acceleration and high spatial resolution ⁵. The chosen range also included higher values (0.6 against the highest reported value of 0.38 in adults) in order to account for potentially higher hepatic blood flow variability in paediatric (sub)populations.

Model equations

The equations of the pharmacokinetic model (structural model) for drug A and drug B implemented in PFIM in the step 2 of the analytical workflow are given below:

Plasma clearance of drug A

$$RnA = (fuA/BPA) \times CL_{int,u,WL}A / Qh$$

$$aA = \sqrt{(1 + 4RnA \times Dn)}$$

$$CLA = Qh \times BPA \times \left(1 - \frac{4aA}{(1 + aA)^2 \exp\{(aA - 1)/2D_N\} - (1 - aA)^2 \exp\{-(aA + 1)/2D_N\}}\right)$$

Plasma clearance of drug B

$$RnB = (fuB/BPB) \times CL_{int,u,WL}A \times CL_{int, ratio} / Qh$$

$$aB = \sqrt{(1 + 4RnB \times Dn)}$$

$$CLB = Qh \times BPB \times \left(1 - \frac{4aB}{(1 + aB)^2 \times \exp\{(aB - 1)/2D_N\} - (1 - aB)^2 \times \exp\{-(aB + 1)/2D_N\}}\right)$$

Plasma concentration time profile of drug A

$$\frac{dAA}{dt} = K0A - \frac{CLA}{VA} \times AA$$

$$CA(t) = \frac{AA(t)}{VA}$$

$$\frac{dAB}{dt} = K0B - \frac{CLB}{VB} \times AB$$

$$CB(t) = \frac{AB(t)}{VB}$$

With estimated parameters VA (volume of distribution for drug A), $CL_{int,u,WL}$ (whole liver intrinsic unbound clearance of drug A), Qh (hepatic blood flow) and VB (volume of distribution for drug B) and fixed parameters K0A (infusion rate of drug A), K0B (infusion rate of drug B), $CL_{int, ratio}$ (ratio $CL_{int,u,WL}B / CL_{int,u,WL}A$), equivalent to the ratio of microsomal intrinsic clearance as could be measured in vitro, fuA (fraction unbound of drug A), fuB (fraction unbound of drug B), BPA (blood to plasma ratio of drug A), BPB (blood to plasma ratio of drug B) and Dn (Dispersion number).

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Supplementary Material 2 R code PFIM model file

```
# PFIM model code
formED<-function(t,y,p){
# Estimated parameters
VA <- p[1]          # 4 L = Volume of distribution for drug A
CLINTA <- p[2]       # Whole liver intrinsic clearance of drug A
QH <- p[3]          # Hepatic blood flow
VB <- p[4]          # 4 L = Volume of distribution for drug B

# Fixed parameters
K0A <- p[5]         # Infusion rate of drug A
K0B <- p[6]         # Infusion rate of drug B
CLINTRATIO <- p[7]  # Ratio CLINTB/CLINTA

FuA <- 1 # Fraction unbound of drug A (fixed to 1)
FuB <- 1 # Fraction unbound of drug B (fixed to 1)
BPA <- 1 # Blood to plasma ration of drug A (fixed to 1)
BPB <- 1 # Blood to plasma ration of drug B (fixed to 1)

Dn <- 0.17 # Dispersion number

# Plasma clearance of drug A
RnA <- FuA*CLINTA/BPA/QH
aA <- sqrt(1+4*RnA*Dn)
CLA <- (QH*(1-(4*aA/((1+aA)**2*exp((aA-1)/(2*Dn))-(1-aA)**2*exp(-(aA+1)/(2*Dn))))))*BPA

# Plasma clearance of drug B
RnB <- FuB*CLINTA*CLINTRATIO/BPB/QH
aB <- sqrt(1+4*RnB*Dn)
CLB <- (QH*(1-(4*aB/((1+aB)**2*exp((aB-1)/(2*Dn))-(1-aB)**2*exp(-(aB+1)/(2*Dn))))))*BPB

# Plasma concentration time profile of drug A
yd1 <- K0A-CLA/VA*y[1]
# Plasma concentration time profile of drug B
yd2 <- K0B-CLB/VB*y[2]

list(c(yd1,yd2),c(y[1]/VA,y[2]/VB))

}
```

Supplementary Material 3 R code PFIM input file

```
#####
####          INPUT FILE FOR PFIM 4.0          ####
#####

#Name of the project
#-----
project<-"OMEGA QH = 0.1 & OMEGA CLINT = 0.1"

#Name of the file containing the PK or PD model
#-----
file.model<-"model.R"

#Name of the output file for the results and for the Fisher information
matrix: names after the drug combination
#-----
output<-paste("StdoutA",DrugA,"B",DrugB,".r",sep="");
outputFIM<-paste("FIMA",DrugA,"B",DrugB,".txt",sep="");

#FIM: Population (P) or Individual (I) or Bayesian (B) Fisher
information matrix
#-----
FIM<-"P"

#Previous information for population design (FIM<-"P") only:
#If previous information is available, please specify below the file
name;
#otherwise leave it as the default
#-----
previous.FIM<""

#RUN:  Evaluation (EVAL) or Optimisation (OPT)
#-----
run<-"EVAL"

#To display only graphs of models and/or sensitivity functions before
evaluating the Fisher Information matrix
graph.only<-F

#Block diagonal Fisher information matrix (option<-1) or complete
Fisher information matrix (option<-2)
#-----
option<-1

#Number of responses
#-----
nr<-2
```

```
##### MODEL OPTION #####

#Model form: Differential equations (DE) or analytical form (AF)
#-----
modelform<-"DE"

##### DIFFERENTIAL EQUATION OPTION #####

#Initial time for which initial conditions are given
#-----
time.condinit<-0
#Identical initial conditions in each elementary design (Yes=T, No=F)
#-----
condinit.identical<-T

# If 'Yes', enter once the expression of the initial values of the
system at the initial time
# else, enter the vectors of the initial conditions for each elementary
design
# If initial values depend on the parameters to be estimated,
# enter this parameter into the expression without any quotation marks
#-----
condinit<-expression(c(0,0))

# Error tolerance for solving differential equations
#-----
RtolEQ<-1e-10
AtolEQ<-1e-10
Hmax<-Inf

##### END DIFFERENTIAL EQUATION OPTION #####

#Name of the fixed effects parameters
#-----
parameters<-c("VA", "CLINTA", "QH", "VB", "K0A", "K0B", "CLINTRATIO")

#Fixed effects parameters values
#-----
beta<-
c(4,param_Drug[DrugA,1],0.338351,4,param_Drug[DrugA,3],param_Drug[DrugB
,3],(param_Drug[DrugB,1]/param_Drug[DrugA,1]))

#Some parameters may not be estimated (not estimated = T, estimated =
F)
#-----
beta.fixed<-c(F,F,F,F,T,T,T)

#Number of occasions
#-----
n_occ<-1

#Random effect model (1) = additive (2) = exponential
#-----
Trand<-2;
```

```

#Diagonal Matrix of variance for inter-subject random effects:
#-----
omega<-diag(c(0.25,0.1,0.1,0.25,0,0,0))

#Diagonal Matrix of variance for inter-occasion random effects:
#-----
gamma<-diag(c(0,0,0,0,0,0,0))

#Standard deviation of residual error (sig.inter+sig.slope*f)^2:
#-----
sig.interA<-0
sig.slopeA<-0.1
sig.interB<-0
sig.slopeB<-0.1

#List of the vectors of sampling times for each elementary design
#You can specify that a group has no sampling time by writing NULL
#(ONLY if you have several response)
#-----
# vector of sampling times:sampling every half lifes, from 1st to 7th
half life
Vi <- 4 # Volume of distribution of drug A and drug B
CLAi <- param_Drug[DrugA,2] # CLearance of drug A
CLBi <- param_Drug[DrugB,2] # Clearance of drug B

protA<-list(seq(log(2)/(CLAi/Vi),log(2)/(CLAi/Vi)*7,length.out
=7),NULL)
protB<-list(NULL,seq(log(2)/(CLBi/Vi),log(2)/(CLBi/Vi)*7,length.out
=7))

#Vector of initial proportions or numbers of subjects for each
elementary design
#-----
subjects<-c(45,45)

#Subjects input: (1) for number of subjects (2) for proportions of
subjects
#-----
subjects.input<-1

#####
#
#               Covariate model
#
#####

#####
# Covariates not changing with occasion #
#####

#Add covariate to the model (Yes==T No==F)
#-----
covariate.model<-F

```

```
#####
# Covariates changing with occasion #
#####

#Add covariate to the model      (Yes==T No==F)
#-----
covariate_occ.model<-F

##### GRAPH SPECIFICATION OPTION #####

#graphical representation of the model (Yes=T, No=F)
#-----
graph.logical<-F

#graphical representation of sensitivity functions (Yes=T, No=F)
#-----
graphsensi.logical<-F

##### END OF GRAPH SPECIFICATION OPTION #####
```

Supplementary material 4 R command to launch the PFIM evaluation runs for all combinations of hypothetical drugs for one uncertainty scenario

```
# Set working directory
# open PFIM.r in R studio and clic "source"

# Table containing drug properties for all hypothetical drugs.
# Each row contains the drug properties for one specific drug.
# 1st column = Whole liver intrinsic clearance, 2nd column = CL, 3rd
# column = infusion rate, 4th column = ER.

param_Drug <- read.csv("EC05_PBPkOD_ODPFIM_HDproperties.csv") #
# Table corresponding to supplementary table 1

combAB <- combn(nrow(param_Drug),2) # Generation of all possible
# combinations of drugs from the pool of hypothetical drugs in
# param_Drug (without repetitions)

# Run PFIM evaluation for each hypothetical drug combination
for (i in 1:ncol(combAB)){
  DrugA <- combAB[1,i] # Row index in param_Drug containing the drug
  # parameter values for the selected drug A
  DrugB <- combAB[2,i] # Row index in param_Drug containing the drug
  # parameter values for the selected drug B

  tryCatch({

    PFIM()
  }, error=function(e){})
}
```

Supplementary Table 1 excel table containing the drug properties used for the PFIM runs

CLint	CL	k0	ER
0.001697	0.001691	0.118403	0.004999
0.003405	0.003383	0.236814	0.009999
0.006855	0.006767	0.473678	0.019999
0.01035	0.01015	0.710515	0.029999
0.013892	0.013534	0.94739	0.04
0.017481	0.016918	1.18423	0.05
0.021119	0.020301	1.421094	0.060001
0.024806	0.023684	1.657906	0.069999
0.028545	0.027068	1.894782	0.080001
0.032336	0.030452	2.131643	0.090001
0.03618	0.033835	2.368471	0.1
0.040079	0.037219	2.605308	0.11
0.044034	0.040602	2.842132	0.119999
0.048047	0.043985	3.078976	0.129999
0.05212	0.04737	3.31587	0.140001
0.056252	0.050752	3.552667	0.149999
0.060448	0.054137	3.789561	0.160001
0.064707	0.05752	4.026403	0.170001
0.069031	0.060903	4.263208	0.179999
0.073424	0.064287	4.500099	0.190001
0.077885	0.06767	4.736918	0.2
0.082418	0.071054	4.973778	0.21
0.087024	0.074437	5.210625	0.22
0.091705	0.077821	5.447453	0.23
0.096464	0.081204	5.684305	0.24
0.101303	0.084588	5.921165	0.250001
0.106224	0.087972	6.158015	0.260001
0.111229	0.091355	6.394834	0.27
0.116322	0.094738	6.631687	0.28
0.121505	0.098122	6.868542	0.29
0.12678	0.101505	7.105361	0.299999
0.132152	0.104889	7.342235	0.31
0.137622	0.108272	7.579072	0.32
0.143194	0.111656	7.815906	0.329999
0.148872	0.115039	8.052761	0.34
0.154659	0.118423	8.289614	0.35
0.160559	0.121807	8.526475	0.360001
0.166575	0.12519	8.763307	0.37
0.172712	0.128574	9.000146	0.38
0.178974	0.131957	9.236978	0.389999
0.185367	0.135341	9.473856	0.400001

CLint	CL	k0	ER
0.191893	0.138724	9.710675	0.409999
0.19856	0.142108	9.947543	0.42
0.205371	0.145491	10.18437	0.43
0.212333	0.148875	10.42122	0.44
0.219452	0.152258	10.65807	0.45
0.226734	0.155642	10.89492	0.46
0.234185	0.159025	11.13175	0.469999
0.241814	0.162409	11.36862	0.48
0.249627	0.165792	11.60547	0.49
0.257632	0.169176	11.8423	0.5
0.265838	0.172559	12.07913	0.509999
0.274255	0.175943	12.31599	0.52
0.282892	0.179326	12.55285	0.53
0.291758	0.18271	12.78967	0.539999
0.300867	0.186093	13.02654	0.55
0.310229	0.189477	13.26339	0.56
0.319857	0.19286	13.50023	0.57
0.329765	0.196244	13.73708	0.58
0.339967	0.199627	13.97391	0.59
0.350481	0.203011	14.21076	0.6
0.361323	0.206394	14.44761	0.61
0.372511	0.209778	14.68445	0.62
0.384067	0.213161	14.9213	0.63
0.396012	0.216545	15.15815	0.64
0.40837	0.219929	15.395	0.65
0.421167	0.223312	15.63183	0.66
0.434433	0.226695	15.86868	0.67
0.448199	0.230079	16.10554	0.68
0.462499	0.233463	16.34238	0.69
0.477372	0.236846	16.57923	0.7
0.49286	0.24023	16.81607	0.71
0.509011	0.243613	17.05291	0.72
0.525878	0.246997	17.28976	0.73
0.543519	0.25038	17.52661	0.74
0.562	0.253764	17.76345	0.75
0.581398	0.257147	18.0003	0.76
0.601796	0.260531	18.23714	0.77
0.623292	0.263914	18.47399	0.78
0.645998	0.267298	18.71084	0.79
0.670042	0.270681	18.94768	0.8
0.695574	0.274065	19.18453	0.81
0.722768	0.277448	19.42137	0.82
0.751833	0.280832	19.65822	0.83

CLint	CL	k0	ER
0.783016	0.284215	19.89507	0.84
0.816612	0.287599	20.13191	0.85
0.852986	0.290982	20.36876	0.86
0.892586	0.294366	20.60561	0.87
0.935971	0.297749	20.84245	0.88
0.98386	0.301133	21.0793	0.89
1.037185	0.304516	21.31614	0.9
1.097192	0.3079	21.55299	0.91
1.165593	0.311283	21.78984	0.92
1.244824	0.314667	22.02668	0.93
1.338523	0.31805	22.26353	0.94
1.452439	0.321434	22.50037	0.95
1.596435	0.324817	22.73722	0.96
1.789531	0.328201	22.97407	0.97
2.075989	0.331584	23.21091	0.98
2.604656	0.334968	23.44776	0.99

Supplementary Figure 1

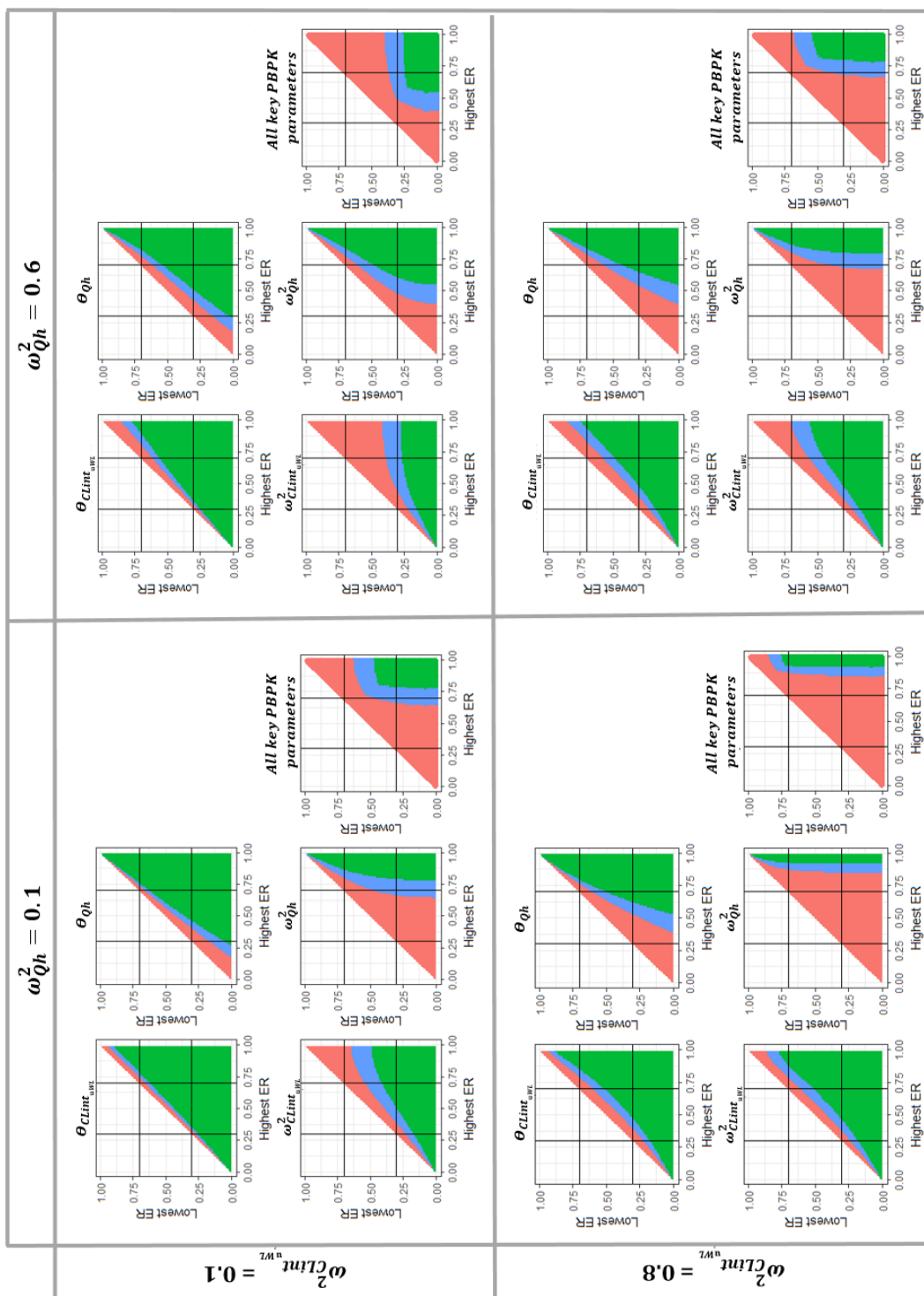


Figure S1 Parameter precision as a function of the extraction ratios (ER) of the two drugs included in the clinical trial design, separated by variance scenario. Each pixel represents a tested drug combination, with the precision of the parameters being summarized as $rse\% \leq 30$ (green), $30\% < rse\% \leq 50\%$ (blue), and $rse\% > 50\%$ (red).

