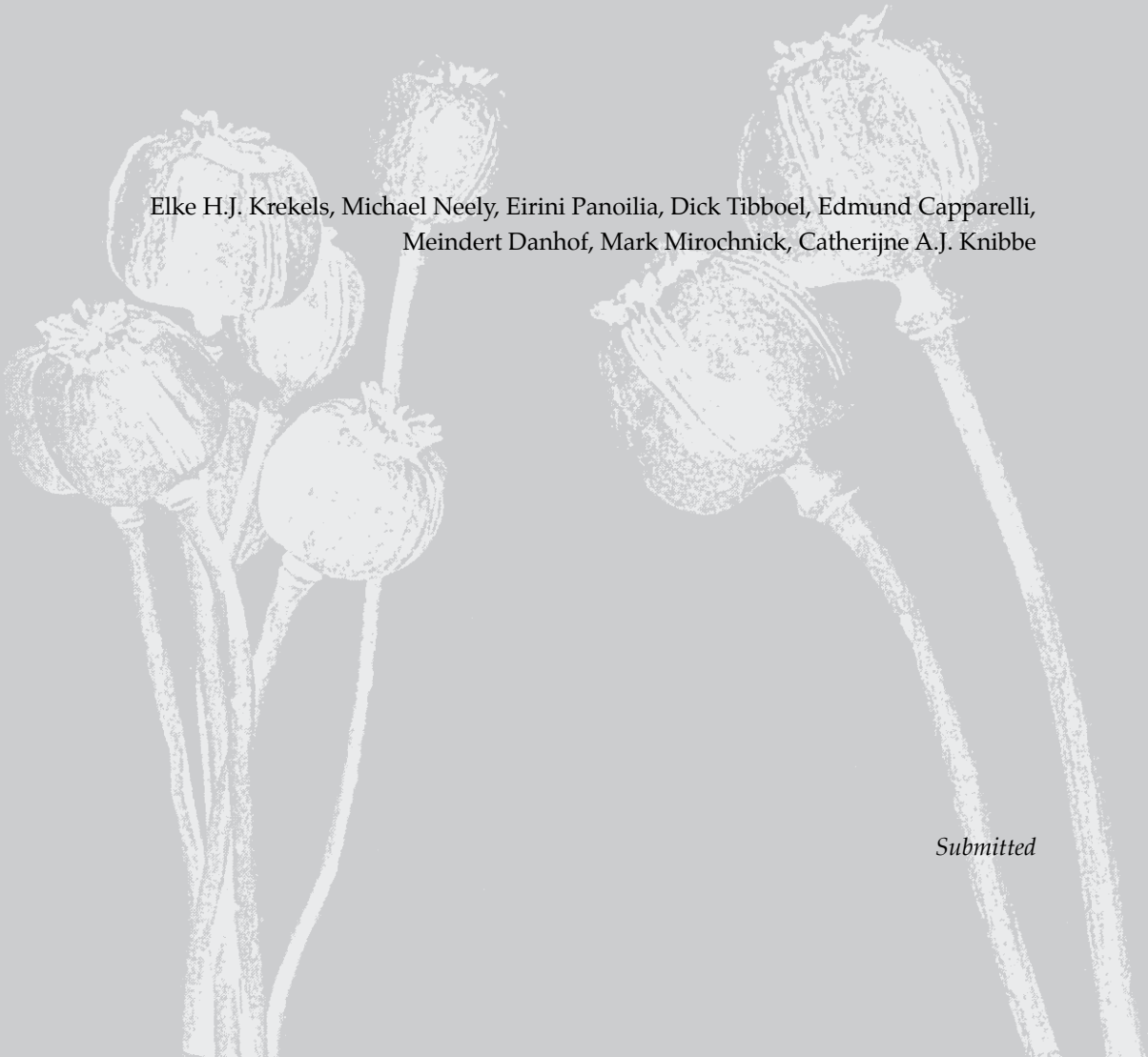
Section III

Semi-Physiological Covariate Model for Paediatric Glucuronidation

Chapter 6

Semi-Physiological Model for Glucuronidation in Neonates and Infants; Application to Zidovudine

Elke H.J. Krekels, Michael Neely, Eirini Panoilia, Dick Tibboel, Edmund Capparelli, Meindert Danhof, Mark Mirochnick, Catherijne A.J. Knibbe



Submitted

Abstract

Aim. New approaches to expedite the development of safe and effective paediatric dosing regimens are necessary. We test the hypothesis that paediatric pharmacokinetic covariate models describe developmental changes in the physiological system and can therefore be extrapolated between drugs that share elimination pathways. Morphine and zidovudine, both primarily eliminated through UGT2B7-mediated glucuronidation, were used as paradigm compounds.

Method. Two population pharmacokinetic models were developed for a dataset of zidovudine and zidovudine-glucuronide in neonates and infants. One model was based on a comprehensive covariate analysis and served as a reference model. In the second model, a validated covariate model for morphine glucuronidation was directly incorporated. The performance of this system-specific model was compared to the reference model.

Results. In the reference model, developmental changes in glucuronidation clearance were best described by postnatal age in a sigmoidal function, while the system-specific model used a bodyweight-based exponential equation. Nevertheless, both models predicted similar population clearance values for the individuals in the dataset. The descriptive performances of both models were good and similar between the models, as expressed by a difference in objective function of only 13 points and similar goodness-of-fit plots. The predictive performance assessed by normalized prediction distribution errors, were good and similar as well for both models.

Conclusion. This proof-of-concept study supports our hypothesis that paediatric covariate models describe the physiological system quantitatively and can be considered to be semi-physiological. This approach may benefit paediatric pharmacokinetic analyses, the development of paediatric dosing algorithms and first-in-child studies.

6.1 Introduction

It is currently well established that the clearance of many drugs differ between adults and children and between children of different ages, and that these differences are a major cause of age-dependent differences in dose requirements [1]. However, the pharmacological properties of many drugs that are commonly prescribed for children have often not been properly investigated in this vulnerable population [2]. Therefore evidence-based dosing recommendations are often lacking for this patient group.

Pharmacokinetic studies in the paediatric population are complicated by ethical, practical and legal constraints. These constraints can be addressed by the application of novel data-analysis approaches [3]. Population pharmacokinetic modelling approaches are based on the simultaneous analysis of data from an entire population, while still taking into account that different observations come from different patients. They allow for the simultaneous analysis of sparse and/or dense data or unbalanced data. Population models not only yield pharmacokinetic parameter values for the population as a whole, but also quantify and differentiate sources of variability in the population. By identifying which patient characteristics (e.g. bodyweight, age, gender, race, genetics, disease status etc.) are predictors of the variability in model parameters, trends in the population can be described. Such predictors are called covariates; the equations describing the relationship between a covariate and a model parameter are called covariate relationships; and a set of covariate relationships in a population model is referred to as a covariate model. Pharmacokinetic covariate relationships can serve as a basis for evidence-based dosing guidelines, as drug doses should be adjusted according to changes in pharmacokinetic parameters.

It would require tremendous resources to develop and thoroughly validate pharmacokinetic covariate models for every new and existing drug prescribed for the paediatric population. Therefore smarter and more efficient approaches to expedite the development of safe and effective paediatric dosing regimens are necessary. We have hypothesized before that validated paediatric covariate models contain quantitative information about the developmental changes in the underlying physiological system in children. This implies that covariate relationships describing the developmental changes in the clearance of a specific drug can be extrapolated to another drug that is cleared through the same pathway [4]. The extrapolation of covariate models between drugs would expedite the development of paediatric population models, which could serve in optimizing drug dosing in first-in-child studies and in facilitating the development of evidence-based paediatric dosing recommendations.

In this analysis, morphine and zidovudine are used as paradigm compounds, as morphine and zidovudine are both prescribed for children of all ages and are predominantly metabolized through glucuronidation by the UGT2B7 isoenzyme [5–8]. The current proof-of-concept study shows that paediatric pharmacokinetic covariate models for a given metabolic pathway are semi-physiological and can therefore be extrapolated from one drug to another.

6.2 Methods

6.2.1 Study Design

To test the between-drug extrapolation potential of paediatric pharmacokinetic covariate models, two population pharmacokinetic models were developed for a single dataset of zidovudine (also known as 3'-azido-3'-deoxythimidine or azidothymidine) and its glucuronide metabolite:

A. Reference Model: For this model a comprehensive covariate analysis was performed yielding a pharmacokinetic model with a set of covariate relationships that best described the current data according to statistical criteria. This model will be referred to as the 'reference model'.
B. System-Specific Model: In this model the internally and externally validated covariate model from a population pharmacokinetic model for morphine glucuronidation in patients under the age of three years (Chapters 3 and 4) was directly incorporated. This semi-physiological covariate model will be referred to as the 'developmental covariate model' and the full population model that is based on the developmental covariate model will be referred to as the 'system-specific model'.

The descriptive and predictive properties of the system-specific model (B) were assessed by comparing them to the descriptive and predictive properties of the fully optimized reference model (A).

6.2.2 Patients and Data

Zidovudine

The current analysis is based on 473 zidovudine concentrations and 173 zidovudine-glucuronide concentrations collected on 68 occasions from 29 individuals varying from term neonates to infants up to five months of age (PACTG 049 [9]). These data were obtained from a multicenter study to evaluate the safety, tolerability and pharmacokinetics of zidovudine as a prophylaxis to prevent mother-to-child HIV transmission in healthy neonates and infants born to HIV infected women. The study protocol was approved

by institutional review boards of the participating institutions and written informed consent was obtained from the parents or legal guardians of each patient.

For each patient dense data were available from multiple occasions that were days or weeks apart. Zidovudine was administered both intravenously and orally to each patient. Data were obtained after single dose administrations on separate occasions and for eight patients data from administrations that were part of a long-term oral dosing regimen were available as well. Dosing started at 2 mg/kg but could be increased to 4 mg/kg during the course of the study as deemed appropriate by the treating physician.

Morphine

A dataset of morphine and its glucuronides in 248 preterm and term neonates to three year old infants was used to obtain the developmental covariate model used in the system-specific model (Chapter 3). In table I study and patient characteristics for the zidovudine dataset used for both models in the current analysis and the morphine datasets used to obtain the developmental covariate model are shown for comparison.

Table I. *Patient and study characteristics of the zidovudine dataset that was the basis for the reference model and the system-specific model in the current analysis and the morphine dataset that was the basis for the developmental covariate relationships applied in the system-specific model.*

Characteristic	**Zidovudine dataset [9]**	**Morphine dataset (Chapter 3)**
Number of patients	29	248
Number of samples of parent compound	473	792
Number of samples of glucuronide	173 (G-ZDV)	664 (M3G) 722 (M6G)
Administration route	oral and short-term iv	short-term and continuous iv
Duration	multiple occasions days or weeks apart	single occasion of up to 5 days
Sampling	dense	sparse
Population	healthy patients	ventilated and post-operative (non-cardiac surgery) patients
Postnatal age (range, days)	2 – 145	0 – 1071
Postmenstrual age (range, weeks)	36 – 57	25 – 193
Bodyweight (range, kg)	1.9 – 6	0.5 – 16.8
Sex (M/F)	18 / 11 (62% / 38%)	144 / 104 (58% / 42%)

G-ZDV = zidovudine glucuronide, M3G = morphine-3-glucuronide, M6G = morphine-6-glucuronide

6.2.3 Model Development

NONMEM VI (ICON, Ellicott City, MD, USA) was used to perform the data analysis, with PLT Tools version 3.0.0 [10] in combination with R version 2.10.0 for the visualization of the data. All parameter estimates were obtained with the first-order conditional estimation method with interaction (FOCE-I).

Model development for the reference model and the system-specific model was performed in three steps:

1. choice of structural model
2. choice of error model
3. choice of the covariate model

For the reference model and the system-specific model, the first two steps in the model development process (i.e. the choice of the structural and error model) were the same. One- and two-compartment models were tested for the structural model. For the error model inter-individual variability on the model parameters was tested assuming a log-normal distribution described by an exponential distribution model depicted in equation 1. For bioavailability (F) inter-individual variability was described using equation 2 to avoid individual bioavailability estimates of more than 100%.

$$P_i = \theta * \exp(\eta_i) \qquad \text{(Equation 1)}$$

$$P_i = \frac{e^{\theta+\eta_i}}{1+e^{\theta+\eta_i}} \qquad \text{(Equation 2)}$$

In these equations P_i is the individual parameter estimate for the ith individual, θ represents the population parameter estimate for parameter P, and η_i is a random variable for the ith individual from a normal distribution with a mean of zero and estimated variance of ω^2. For the intra-individual variability and residual error in the observed zidovudine and zidovudine-glucuronide concentrations proportional (equation 3), additive (equation 4), and combination (equation 5) error models were tested:

$$C_{obs,ij} = C_{pred,ij}(1+\varepsilon_{ij}) \qquad \text{(equation 3)}$$

$$C_{obs,ij} = C_{pred,ij} + \varepsilon_{ij} \qquad \text{(equation 4)}$$

$$C_{obs,ij} = C_{pred,ij}(1+\varepsilon_{1,ij}) + \varepsilon_{2,ij} \qquad \text{(equation 5)}$$

where $C_{obs,ij}$ is the jth observation in the ith individual, $C_{pred,ij}$ is the predicted value of that observation and ε_{ij} is a random variable from a normal distribution with a mean of zero and estimated variance of σ^2.

The Likelihood Ratio, which was assumed to be χ^2 distributed, was used to assess whether the difference between (sub)models was statistically significant. A decrease in the objective function corresponding to $p < 0.01$ was considered to be significant. In addition, the following basic goodness-of-fit plots were used for diagnostic purposes: (a) observed *versus* individually predicted concentrations, (b) observed *versus* population predicted concentrations, (c) conditional weighted residuals *versus* time, and (d) conditional weighted residuals *versus* population predicted concentrations. Furthermore, the 95% confidence intervals of the model parameters and the correlation matrix were assessed.

The third and final step of the model development process (i.e. choice of the covariate model) was different for the reference model and the system-specific model:

A. *Reference model:* A comprehensive covariate analysis with forward inclusion and backward deletion of covariates was performed to obtain a covariate model with the best description of the current zidovudine data according to statistical criteria. The following covariates were tested for significance: postnatal age, postmenstrual age, gestational age at birth, bodyweight, sex, and creatinine clearance. The continuous covariates were tested in linear equations, exponential equations with estimated exponents, or sigmoidal equations. A decrease in the objective function corresponding to $p < 0.01$ for the forward inclusion of covariates was considered to be significant. Additionally, the aforementioned diagnostic criteria were used. When more than 1 significant covariate was identified, the most significant covariate was included in the model and the resulting model served as the basis for the subsequent exploration of additional covariate effects. For the backward deletion of covariates an increase in objective function corresponding to $p < 0.001$ was considered to be significant.

B. *System-specific model:* The previously obtained and internally and externally validated covariate model for morphine glucuronidation in children younger than three years (Chapters 3 and 4) was directly incorporated into the model for zidovudine. Specifically, a bodyweight-based exponential equation with an exponent of 1.44 for the formation and elimination of zidovudine-glucuronide with a reduced formation clearance of zidovudine-glucuronide in neonates younger than ten days was included, as was a linear correlation for distribution volume of the parent compound and metabolite (see figure 1 for equations). While this developmental covariate model describes the rate of developmental changes in clearance and distribution volume, the population values that describe the absolute values of these parameters for zidovudine were still estimated by NONMEM.

6.2.4 Model Evaluation

Model performance of the reference model and the system-specific model were evaluated and compared. Although the reference model and system-specific model are not nested, they are based on the exact same patients and data. Therefore the -2 log likelihood, by means of the NONMEM objective function, was used as a measure to statistically compare the description of the zidovudine data by the system-specific model to the description of the zidovudine data by the reference model. To directly compare clearance predictions between the two models, population clearance predictions from the reference model were plotted *versus* population clearance predictions from the system-specific model. As age and bodyweight change rapidly in this young population, estimated parameter values did not remain constant between the occasions, yielding one prediction per patient per occasion.

Furthermore, the descriptive properties of the models were assessed and compared by inspecting the basic goodness-of-fit plots of the models. These plots were stratified by age into a group that was younger and a group that was older than 38 days (the median age of the individuals at the different occasions) to ascertain that the entire age-range was described equally well. In addition, the covariate relationships describing the population predicted zidovudine clearances and the individual *post hoc* clearance estimates of each individual at each separate study occasion were plotted in one graph for each model, to visually assess the description of the individual zidovudine glucuronidation clearances by the covariate relationships. Finally, bias and precision of the individual zidovudine glucuronidation clearance values compared to the population predicted clearances described by the covariate relationships were quantified by calculating the percentage mean prediction error (%MPE, equation 6) and the root mean square error (RMSE, equation 7) respectively.

$$\%MPE = \frac{\sum \frac{(populationCL - individualCL)}{individualCL}}{n} \cdot 100 \qquad \text{(equation 6)}$$

$$RMSE = \sqrt{\frac{\sum (populationCL - individualCL)^2}{n}} \qquad \text{(equation 7)}$$

To compare the predictive properties of both models, a normalized prediction distribution error (NPDE) analysis [11] which is a simulation-based diagnostic, was used. The entire dataset was simulated 1000 times in NONMEM and subsequently each observed concentration was compared to the reference distribution of the simulated data points using the NPDE add-on package in R [12].

6.3 Results

6.3.1 Model Development

In the first step of model development (i.e. choice of structural model) a two-compartment model was found to best describe the time-course of zidovudine, and a one-compartment model was used to describe the time-course of the zidovudine-glucuronide, as is depicted in figure 1. Zidovudine absorption from the oral depot compartment was described by first-order absorption (k_a) and the oral bioavailability (F) was estimated. Zidovudine clearance through pathways other than glucuronidation was found to be not significantly different from 0. When estimated, the values of the distribution volume of the central (V_1) and peripheral (V_2) compartment of zidovudine were not significantly different from each other, these values were therefore fixed to be equal. The distribution volume of the glucuronide (V_3) was estimated as a fraction of the central compartment of zidovudine (θ_{V3}).

In the second step (i.e. choice of error model) significant inter-individual variability could be identified for the absorption rate constant (k_a), the formation (Cl_1) and elimination (Cl_2) clearance of zidovudine-glucuronide, the distribution volume of the central compartment (V_1), and the bioavailability (F). Additionally, in the reference model a correlation between the inter-individual variability of the distribution volume of the central compartment (V_1) and the formation clearance of zidovudine-glucuronide (Cl_1) was identified. The inter-individual variability and residual error for the reference model and the system-specific model were best described by a proportional error model (equation 3).

The third step (i.e. choice of covariate model) was different for the reference model and the system-specific model.

A. *Reference model*: In the comprehensive covariate analysis, age (either postnatal or postmenstrual age) and bodyweight were readily identified as predictive and statistically significant covariates for the clearance parameters. Due to the relatively small range in bodyweight and age of the patients in the current zidovudine dataset (see table I), only small differences in objective function and diagnostics between models using either of the three covariates or between models using these covariates in different equations (i.e. linear, exponential or sigmoidal) were obtained. Based on the objective function postnatal age was found to be the slightly superior covariate for the formation clearance (Cl_1) and the elimination clearance (Cl_2) of zidovudine-glucuronide. The inclusion of this covariate in the reference model was most optimal in a sigmoidal relationship on Cl_1 and in a linear relationship with estimated y-intercept on Cl_2.

Based on the statistical criteria no covariates were identified for the distribution volumes.

B. *System-specific model*: as mentioned in the methods section, the developmental covariate model included in the system-specific model consisted of bodyweight-based exponential equations with an exponent of 1.44 for the formation and elimination of zidovudine-glucuronide with a reduced formation clearance of zidovudine-glucuronide in neonates younger than ten days, and linear relationships between bodyweight and distribution volumes (Chapter 3).

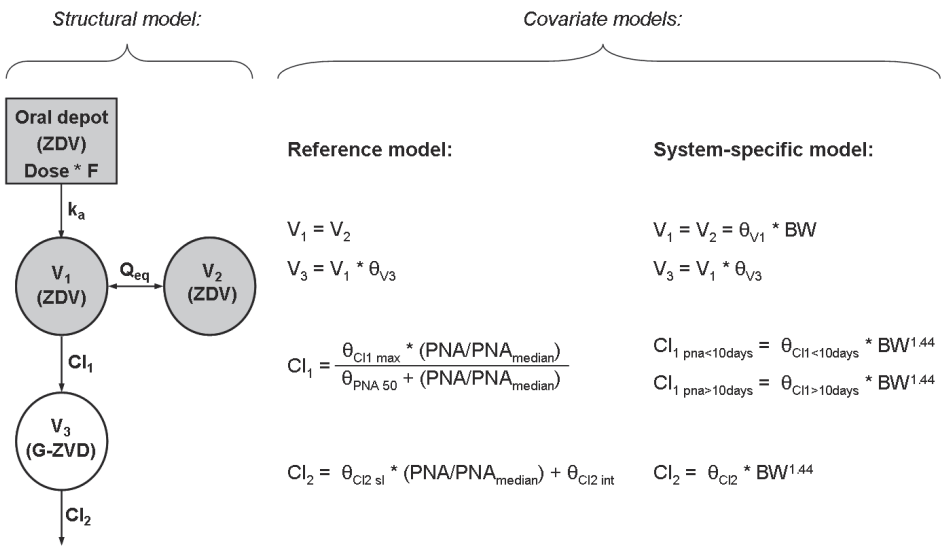


Figure 1. *Schematic representation of the structural model for the zidovudine models (left) and equations of the covariate relationships in the reference model (middle) and the system-specific model (right). ZDV = zidovudine, G-ZDV = zidovudine-glucuronide, F = bioavailability, k_a = absorption rate constant, V = distribution volume of designated compartment, Cl = clearance of designated route, Q= inter-compartmental clearance, θ_V = distribution volume of designated compartment as fraction of V_1, PNA = postnatal age with subscript 'median' indicating the median value of the individuals at the different occasions, θ_{Cl1} max = maximum value of the zidovudine glucuronidation clearance, $\theta_{PNA\ 50}$ = postnatal age at which half the maximum value of zidovudine glucuronidation clearance is reached, $\theta_{Cl2\ sl}$ = slope of the line describing age-related changes in zidovudine glucuronide elimination clearance, $\theta_{Cl2\ int}$ = y-intercept of the line describing age-related changes in zidovudine glucuronide elimination clearance, BW = bodyweight, $\theta_{Cl1<10days}$ = population value of zidovudine glucuronidation clearance value in children younger than ten days of age, $\theta_{Cl1>10days}$ = population value of zidovudine glucuronidation clearance value in children older than ten days of age, θ_{Cl2} = population value of zidovudine glucuronide elimination clearance value.*

Figure 1 shows the equations of the covariate relationships in the reference model and the system-specific model, in addition to providing a schematic representation of the structural model for both models.

Table II. *Final parameter estimates of the reference model and the system-specific model for zidovudine glucuronidation.*

Pharmacokinetic parameter [unit]	**Reference model**		**System-specific model**	
	model parameter [unit]	**value (CV%)**	**model parameter [unit]**	**value (CV%)**
Fixed effects				
F	θ	1.56 (21.0)	θ	1.55 (30.6)
	F [%]	82.6	F [%]	82.5
k_a [min^{-1}]		0.0307 (0.9)		0.031 (17.7)
$V_1 = V_2$	[l]	4.02 (1.3)	[l/kg]	1.08 (11.4)
V_3 [fraction of V_1]		0.226 (20.4)		0.211 (18.3)
Cl_1	$Cl_{1\ max}$ [l/min]	0.116 (7.6)	$Cl_{1<10days}$ [$l/min/kg^{1.44}$]	0.00435 (12.1)
	$Cl_{PNA\ 50}$ [days]	1.63 (17.9)	$Cl_{1>10days}$ [$l/min/kg^{1.44}$]	0.00853 (11.7)
Cl_2	$Cl_{2\ sl}$ [l/min/day]	0.00257 (17.5)	Cl_2 [$l/min/kg^{1.44}$]	0.00231 (10.7)
	$Cl_{2\ int}$ [l/min]	0.00911 (11.64)		
Q_{eq} [l/min]		0.0275 (11.8)		0.0289 (11.7)
Inter-individual variability				
ω^2 (F)		2.78 (42.5)		2.82 (45.0)
ω^2 (k_a)		0.625 (36.8)		0.607 (39.9)
ω^2 (V_1)		0.443 (56.2)		0.366 (49.7)
ω^2 (Cl_1)		0.328 (38.7)		0.255 (54.51)
ω^2 (Cl_2)		0.142 (46.3)		0.112 (70.3)
ω^2 (V_1-Cl_1) interaction		0.312 (54.5)		-
Residual variance				
σ^2 (ZDV)		0.11 (7.2)		0.11 (11.5)
σ^2 (G-ZDV)		0.158 (13.3)		0.152 (15.5)

F = bioavailability presented as value of θ in eq. 2 and population value of F calculated with eq. 2, ω^2 = variance of the normal distribution that quantifies the inter-individual variability on the designated parameter according to eq. 1 or eq. 2 for bioavailability, σ^2 = variance of the normal distribution that quantifies the residual error of the designated observation according to eq. 3. See figure 1 for explanation of other symbols.

In table II the model parameter estimates obtained for the models are shown. The values for structural parameters as well as for parameters of the error model are similar between the reference model and the system-specific model. Additionally, for both models the coefficient of variation of the fixed effects remain well below 50%, indicating that 0 was not in the 95% confidence interval of the parameter estimates and that the parameters can therefore be considered significant and estimated with acceptable precision. The coefficient of variation of some of the variance estimates of the inter-individual variability did exceed 50% indicating that the information in the dataset was uninformative for precise estimation of these parameter values. Interestingly, as shown in figure 2, both models estimate similar population clearance values for each individual at each occasion, despite the differences in covariate models.

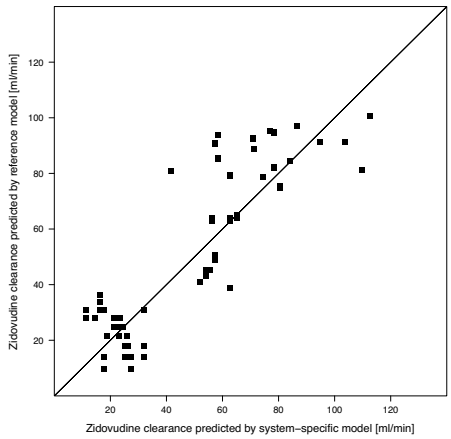


Figure 2. *Population predicted zidovudine clearances (Cl_1) for the reference model versus the system-specific model for each individual at each separate study occasion.*

6.3.2 Model Evaluation

The reference model was statistically superior over the system-specific model in describing the zidovudine data, as demonstrated by a difference in objective function of 13 points and a 2 point difference in degrees of freedom. Although statistically significant, this difference is small suggesting only a small difference in the description of the data between the two models. This is corroborated by the goodness-of-fit plots in figure 3. Visual inspection of the graphs shows that both models can describe the observed concentrations in children older and younger than the median age of children at the different occasions without bias and that the difference in the plots of the two models is negligible.

In addition to an unbiased description of the concentrations, the plots in figure 4 show that both models can also describe individual glucuronidation clearances for

zidovudine (Cl_1) in the population without bias, despite the use of different primary covariates as descriptors for the developmental changes. Accuracy of the individual zidovudine clearance values compared to the population values described by the covariate relationships was numerically quantified as mean percentage error and was 20.5% for the reference model compared to 11.3% for the system-specific model. The precision, numerically quantified as root mean square error, was 19.2 for both the reference model and the system-specific model.

In terms of predictive performance, the two models perform similar as well, as expressed by the results of the normalized prediction distribution error analysis shown in figure 5. The reference model and the system-specific model can accurately predict the median zidovudine concentrations, but they slightly over-estimate the variability in the observations. In addition, there is no bias in normalized prediction distribution errors in time or across the concentration range for any of the models.

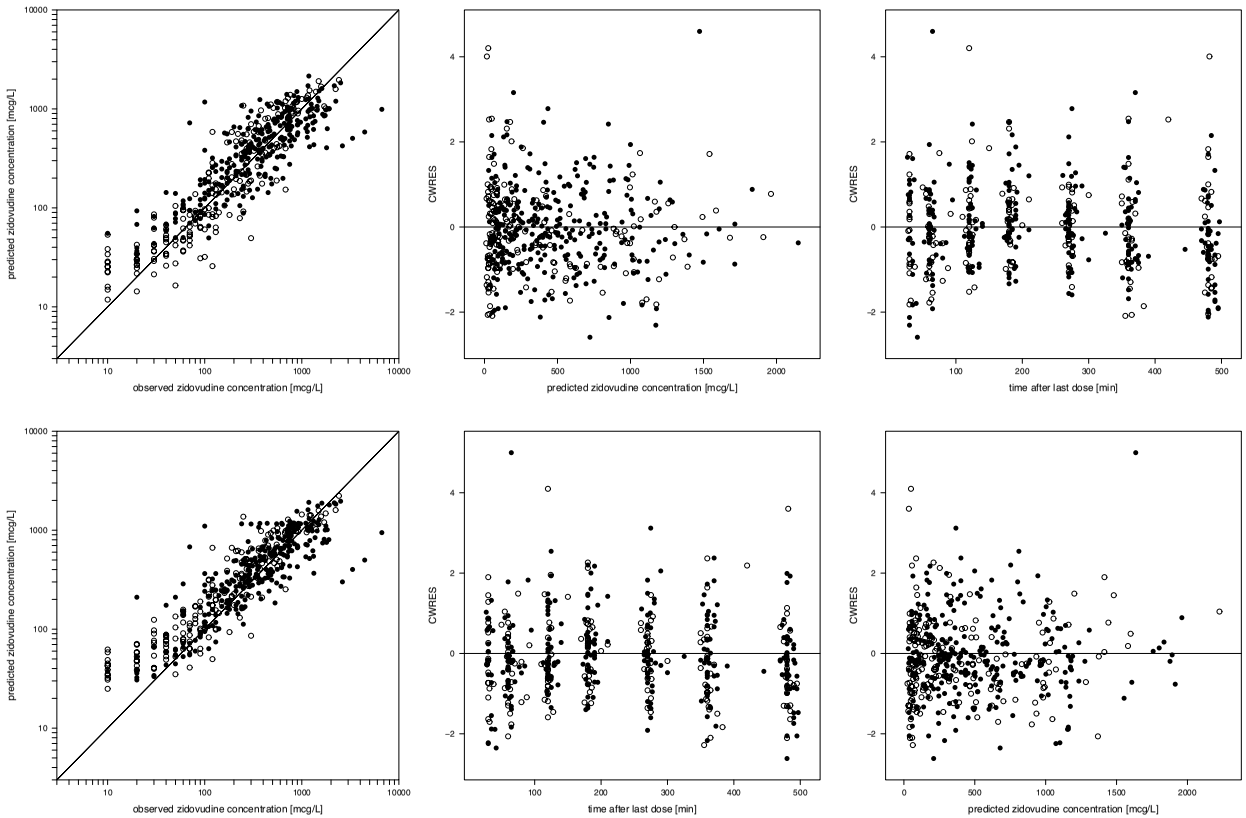


Figure 3. *Basic goodness-of-fit plots for zidovudine of the reference model (top) and the system-specific model (bottom). On the left are population predicted concentrations versus observed concentrations, in the middle are conditional weighted residuals (CWRES) versus time after last dose, and on the right are conditional weighted residuals (CWRES) versus population predicted concentrations. The plots are stratified by age, with solid circles indicating observations in children on occasions that they were younger than the median age of the individuals at the different occasions and open circles indicating observations in children on occasions that they were older than this median age.*

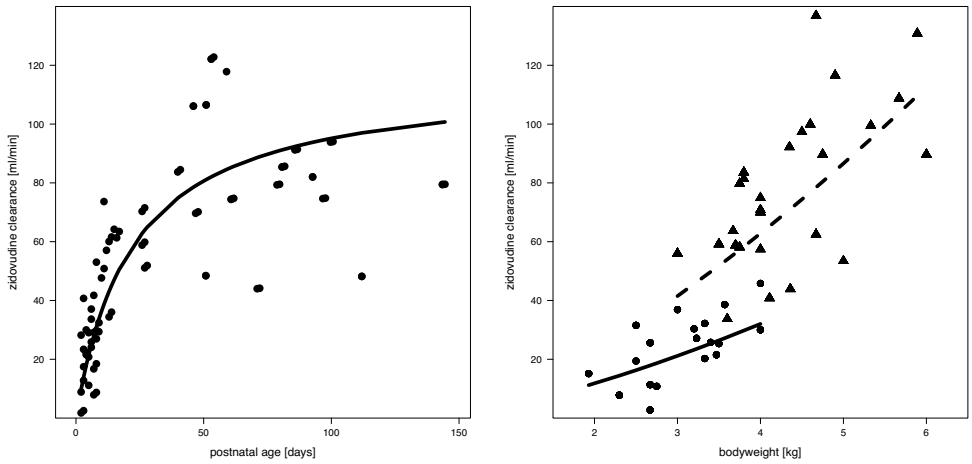


Figure 4. *Individual post hoc parameter values of the glucuronidation clearance to zidovudine-glucuronide (Cl_1) for each individual at each separate study occasion versus the most predictive covariate, which is postnatal age for the reference model (left) and bodyweight for the system-specific model (right). The covariate relationship describing the population clearance values are indicated with lines. For the plot of the system-specific model (right) individual post hoc parameter estimates and population estimates of children younger than ten days are indicated with circles and a solid line respectively, for children older than ten days triangles and a dotted line are used respectively.*

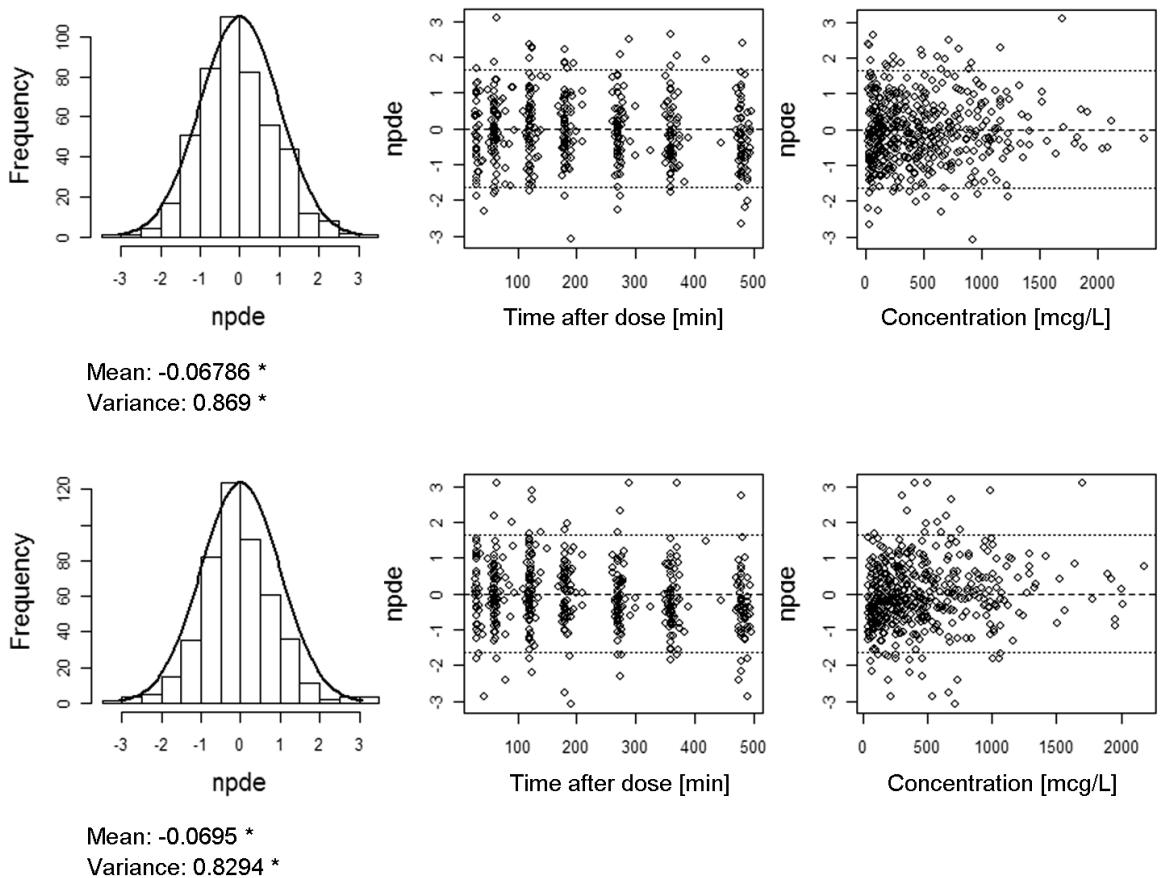


Figure 5. *Results of the NPDE analysis for zidovudine from the reference model (top) and the system-specific model (bottom). In the histograms the distributions of the NPDEs in the overall dataset are shown with the solid line depicting a normal distribution and the values below specifying the mean and variance of the npde distribution in the histogram. A significant ($p < 0.05$) deviation of the distribution from a mean of 0 and a variance of 1 is indicated with an asterisk (*). The NPDE distribution versus time after last dose (middle) and versus the observed concentrations (right) are also shown.*

6.4 Discussion

The current investigation is a proof-of-concept study to examine the hypothesis that paediatric covariate models for drug clearance describe changes in the underlying physiological system and can therefore be extrapolated from one drug to another drug that is eliminated through the same pathway. Our focus was on clearance in particular, because the ontogeny of clearance is considered to be the main driver of differences in pharmacological drug response in the paediatric population [1]. Covariate models that describe the developmental changes of clearance pathways in paediatric population pharmacokinetic models are crucial to determine first-in-child or evidence-based dosing regimen, as the covariate relationship describing these changes can be directly used in drug dosing algorithms.

Morphine and zidovudine were used as paradigm drugs in this investigation because they are both primarily eliminated through glucuronidation by the UGT2B7 isoenzyme [5–8] . The developmental glucuronidation model, an internally and externally validated paediatric covariate model for morphine glucuronidation (Chapters 3 and 4), was directly incorporated into the pharmacokinetic model for the glucuronidation of zidovudine. The descriptive and predictive properties of this system-specific model were compared to a reference model. The covariate model of the reference model was developed by a comprehensive covariate analysis of the same dataset to obtain a model that provided the best description of the data according to statistical criteria. The results of this analysis show these two models to have similar descriptive and predictive performances. Given that the difference in time it took to develop both models is measured in weeks, the system-specific model performed remarkably well.

Observed pharmacokinetic profiles are the result of the interaction between a drug and the physiological system. The parameters used to describe pharmacokinetic profiles therefore represent drug-specific and/or system-specific aspects of this interaction. The results from the current study suggest that developmental changes in drug glucuronidation are drug-independent and are therefore indeed likely to reflect changes in the underlying physiological system. Other studies suggest the same to be applicable to glomerular filtration as well [13]. Our group previously described and defined a distinction between drug-specific and system-specific parameters in population models for pharmacokinetic and pharmacodynamic processes [14]. Based on the current analysis the context of system-specific properties can be extended to not only include static descriptors of the physiological system, but to also include temporal changes in the physiological system as a result of developmental changes in the paediatric population. We therefore denote

the zidovudine model that was based on the developmental covariate model obtained with morphine as the 'system-specific model'.

With the incorporation of system-specific information into population models, the methodology proposed here is moving away from the empiricism of population modelling, towards the mechanistic approach of physiologically-based pharmacokinetic modelling. We envision that the developmental covariate models not necessarily only include the influence of age-related changes on drug pharmacokinetics, but that they may also include significant influences of other static and/or dynamic covariates (e.g. genetics, disease status etc.) on the underlying physiological system that is driving pharmacokinetics. It is however a prerequisite that these paediatric covariate models are extensively validated and that the population to which the covariate model applies is well defined in terms of other potentially important covariates, like for instance genetics or disease status.

Paediatric population pharmacokinetic models of hitherto unstudied drugs can be developed in a time-efficient manner and with limited resources, by the between-drug extrapolation of these semi-physiological paediatric covariate models. This methodology allows for the use of denser information or information from a wider age-range than may be available for the analysis of an unstudied drug. The developmental covariate model in the system-specific model of the current analysis was for instance based on the analysis of morphine glucuronidation in 248 patients ranging from preterm neonates to infants of three years, whereas in the current zidovudine analysis data from only 29 patients ranging from term neonates to infants of five months were available (see table I). Due to the small range in age and bodyweight in this zidovudine dataset, the difference in descriptive and predictive properties of models with different covariate relationships was small. In the comprehensive covariate analysis inclusion of postnatal age, postmenstrual age or bodyweight in either linear, exponential or sigmoidal relationships yielded models with similar objective functions and diagnostics, however based on statistical criteria postnatal age in a sigmoidal equation was selected as the final covariate model for the maturation of zidovudine glucuronidation. The bodyweight-based exponential covariate relationship identified for morphine glucuronidation was not identified for zidovudine in the comprehensive covariate analysis of the current zidovudine data. This is probably due to the indistinctive curvature of this relationship in the bodyweight-range of the zidovudine dataset. Nonetheless, direct incorporation of the developmental covariate model into the zidovudine model did provide a good description of the population and individual zidovudine clearance parameters as shown in figure 4. As such, information from one drug seems indeed to be of value for the analysis of a similar drug, which is especially important in the paediatric population where often only limited data are available.

The clinically observed developmental changes in drug pharmacokinetics represent the net result of the developmental changes in a number of processes in the underlying biological system. This may include changes in expression and function of drug metabolizing enzymes and active transporters, changes in body composition, changes in cardiac output and organ perfusion, changes in acid-base balance, and changes in the amount and composition of drug-binding plasma proteins and the presence of other blood components that may influence plasma protein binding [15]. The weight that each individual process has on the net observed changes in drug pharmacokinetics may be different for drugs with different molecular and pharmacokinetic properties. Morphine and zidovudine are quite similar with respect to these properties. Their molecular masses are 285 g/mol and 267 g/mol respectively. Plasma protein binding in adults ranges between 25% and 40% for both drugs [16,17] and their hepatic extraction ratios in adults range between 0.5 and 0.65 [18–20]. The pKa value for morphine is around 7.9 [21] and for zidovudine this value is around 9.5 [22]. Finally, $\log P_{octanol/water}$ values for these compounds were reported to be 0.75 [23] and 0.05 [24] respectively. It remains to be investigated how and to what extent differences in physicochemical and pharmacokinetic drug properties influence the between-drug extrapolation potential of the semi-physiological paediatric covariate models.

One of the drawbacks of the method applied in the current analysis is that model development for the new drug (zidovudine in this case) still relies on the availability of at least a limited amount of paediatric data to determine the population value of the clearance, which is mainly determined by the drug-specific parameters K_m and V_{max}. This does not pose a problem when a marketed drug that is unstudied in the paediatric population is already being used off-label in that population. However, when in drug development a drug has never been used in a paediatric age-range before, a methodology that does not rely on *in vivo* paediatric data of the drug under investigation is required. To date there is no suitable methodology based on population pharmacokinetic modelling available to extrapolate paediatric pharmacokinetic parameters from older to younger age-ranges in the drug development process [25]. With physiologically-based pharmacokinetic modelling the absolute value of drug clearance could be predicted without prior paediatric *in vivo* data. Unfortunately knowledge on all underlying physiological processes is currently incomplete especially for the paediatric population, which potentially impedes paediatric clearance predictions by physiologically-based pharmacokinetic modelling. Therefore the approach proposed here is combining the physiological insight from physiologically-based pharmacokinetic modelling with the descriptive approach of population modelling. If system-specific profiles on developmental changes in certain metabolic pathways were available over the entire paediatric age-range, these profiles could be used to design successive studies

in children of decreasing ages for unstudied drugs. These studies could then be of a confirmative rather than an explorative nature.

In conclusion, this proof-of-concept study supports our hypothesis that paediatric covariate models that describe the developmental changes in drug elimination pathways constitute system-specific rather than drug-specific information and can therefore be used for extrapolation between drugs that share an elimination pathway. This approach can be considered a semi-mechanistic hybrid between empirical population modelling and physiologically-based pharmacokinetic modelling. Between-drug extrapolation of semi-physiological covariate models can expedite the development of paediatric population pharmacokinetic models that can in turn be used to derive first-in-child and evidence-based dosing recommendations for this population.

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References

1. Alcorn J, McNamara PJ. Ontogeny of hepatic and renal systemic clearance pathways in infants: part I. *Clin.Pharmacokinet.* **41**, 959-998 (2002).
2. Cuzzolin L, Atzei A, Fanos V. Off-label and unlicensed prescribing for newborns and children in different settings: a review of the literature and a consideration about drug safety. *Expert.Opin.Drug Saf* **5**, 703-718 (2006).
3. De Cock RF, Piana C, Krekels EH, Danhof M, Allegaert K, Knibbe CA. The role of population PK-PD modelling in paediatric clinical research. *Eur.J.Clin.Pharmacol.* **67**, 5-16 (2011).
4. Knibbe CA, Krekels EH, Danhof M. Advances in paediatric pharmacokinetics. *Expert.Opin.Drug Metab Toxicol.* **7**, 1-8 (2011).
5. Court M *et al.* Evaluation of 3'-azido-3'-deoxythymidine, morphine, and codeine as probe substrates for UDP-glucuronosyltransferase 2B7 (UGT2B7) in human liver microsomes: specificity and influence of the UGT2B7*2 polymorphism. *Drug Metab Dispos.* **31**, 1125-1133 (2003).
6. Coffman BL, Rios GR, King CD, Tephly TR. Human UGT2B7 catalyzes morphine glucuronidation. *Drug Metab Dispos.* **25**, 1-4 (1997).
7. Barbier O *et al.* 3'-azido-3'-deoxythimidine (AZT) is glucuronidated by human UDP-glucuronosyltransferase 2B7 (UGT2B7). *Drug Metab Dispos.* **28**, 497-502 (2000).
8. Morrish GA, Foster DJR, Somogyi AA. Differential in vitro inhibition of M3G and M6G formation from morphine by (R)- and (S)-methadone and structurally related opioids. *Br.J.Clin.Pharmacol.* **61**, 326-335 (2006).
9. Boucher FD *et al.* Phase I evaluation of zidovudine administered to infants exposed at birth to the human immunodeficiency virus. *J.Pediatr.* **122**, 137-144 (1993).
10. Fisher DM. PLT Tools, available at http://www.pltsoft.com. (2011).
11. Brendel K, Comets E, Laffont C, Laveille C, Mentre F. Metrics for external model evaluation with an application to the population pharmacokinetics of gliclazide. *Pharm.Res.* **23**, 2036-2049 (2006).
12. Comets E, Brendel K, Mentre F. Computing normalised prediction distribution errors to evaluate nonlinear mixed-effect models: The npde add-on package for R. *Comput.Methods Programs Biomed.* **90**, 154-166 (2008).
13. De Cock RFW *et al.* Maturation of GFR in preterm and term neonates reflected by clearance of different antibiotics. *PAGE 20* **Abstr 2096**, (2011).
14. Danhof M, De Jongh J, De Lange EC, Della Pasqua OE, Ploeger BA, Voskuyl RA. Mechanism-based pharmacokinetic-pharmacodynamic modeling: biophase distribution, receptor theory, and dynamical systems analysis. *Annu.Rev.Pharmacol.Toxicol.* **47**, 357-400 (2007).
15. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology--drug disposition, action, and therapy in infants and children. *N.Engl.J.Med.* **349**, 1157-1167 (2003).
16. Olsen GD. Morphine binding to human plasma proteins. *Clin.Pharmacol.Ther.* **17**, 31-35 (1975).
17. Luzier A, Morse GD. Intravascular distribution of zidovudine: role of plasma proteins and whole blood components. *Antiviral Res.* **21**, 267-280 (1993).
18. Stanski DR, Greenblatt DJ, Lowenstein E. Kinetics of intravenous and intramuscular morphine. *Clin.Pharmacol.Ther.* **24**, 52-59 (1978).
19. Crotty B *et al.* Hepatic extraction of morphine is impaired in cirrhosis. *Eur.J.Clin.Pharmacol.* **36**, 501-506 (1989).

20. Naritomi Y, Terashita S, Kagayama A, Sugiyama Y. Utility of hepatocytes in predicting drug metabolism: comparison of hepatic intrinsic clearance in rats and humans in vivo and in vitro. *Drug Metab Dispos.* **31**, 580-588 (2003).
21. Kaufman JJ, Semo NM, Koski WS. Microelectrometric titration measurement of the pKa's and partition and drug distribution coefficients of narcotics and narcotic antagonists and their pH and temperature dependence. *J Med Chem* **18** , 647-655 (1975).
22. Raviolo MA, Brinon MC. Preformulation studies of Zidovudine derivatives: Acid dissociation constants, differential scanning calorimetry, thermogravimetry, x-ray powder diffractometry and aqueous stability studies. *Sci.Pharm.* **79**, 479-491 (2011).
23. Betschart HR, Jondorf WR, Bickel MH. Differences in adipose tissue distribution of basic lipophilic drugs between intraperitoneal and other routes of administration. *Xenobiotica* **18**, 113-121 (1988).
24. Teijeiro SA, Moroni GN, Motura MI, Brinon MC. Lipophilic character of pyrimidinic nucleoside derivatives: correlation between shake flask, chromatographic (RP-TLC and RP-HPLC) and theoretical methods. *J.Liq.Chrom.& Rel.Technol.* **23** , 855 -872 (2000).
25. Cella M, Zhao W, Jacqz-Aigrain E, Burger D, Danhof M, Della Pasqua OE. Paediatric drug development: Are population models predictive of pharmacokinetics across paediatric populations? *Br.J.Clin.Pharmacol.* **72**, 454-464 (2011).