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RESEARCH ARTICLE

On the population pharmacokinetics and the enterohepatic recirculation of total ezetimibe

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ABSTRACT

1. Ezetimibe is a potent cholesterol absorption inhibitor, with an erratic pharmacokinetic (PK) profile, attributed to an extensive enterohepatic recirculation (EHC).
2. The aim of this study was to develop a population PK model able to adequately characterize the complex distribution processes of total ezetimibe. The analysis was performed on the individual concentration-time data obtained from 28 healthy subjects who participated in a bioequivalence study comparing two oral ezetimibe formulations. The population PK analysis was performed using nonlinear mixed effect modeling, where different EHC models were developed and evaluated for their performance.
3. Total ezetimibe pharmacokinetics was best described by a four-compartment model featuring EHC through the inclusion of an additional gallbladder compartment, which was assumed to release drug at specific time-intervals consistent with food intake.
4. The final PK model was able to adequately estimate the population pharmacokinetic parameters and to allow for a formal characterization of the pharmacokinetic profile and the secondary peaks due to enterohepatic recirculation.

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Population pharmacokinetics; pharmacokinetic modeling; enterohepatic recirculation; ezetimibe; enterohepatic recirculation models

Introduction

Ezetimibe (EZE) is a widely used cholesterol absorption inhibitor indicated for the treatment of hypercholesterolemia (Kosoglou et al., 2005). The drug has been extensively studied in patients with primary hypercholesterolemia, homozygous familial hypercholesterolemia or homozygous familial sitosterolemia, both as monotherapy (Bays et al., 2001; Dujovne et al., 2002) and in combination with other lipid-lowering agents, such as statins (Ballantyne et al., 2003) and fibrates (Sweeney & Johnson, 2007). Clinical trials have shown that administration of ezetimibe at a dose of 10 mg once daily produces a marked inhibition of cholesterol absorption by 54–65%, which results in approximately 17–20% reduction of plasma low-density lipoprotein cholesterol in mild-to-moderate hypercholesterolemic subjects (Sudhop et al., 2002). The drug also shows a favorable safety profile, and has an adverse event profile similar to that of placebo, as demonstrated in numerous treated patients over the years (Patel et al., 2003).

Ezetimibe exerts its cholesterol-lowering activity by effectively blocking the intestinal uptake of dietary and biliary cholesterol, through the inhibition of apically localized sterol transporters in the small intestine (Jeu & Cheng, 2003; Nashimoto et al., 2017). Preclinical studies have shown that ezetimibe undergoes extensive glucuronidation via specific uridine glucuronosyltransferase isoenzymes to form an active glucuronide metabolite localized at the intestinal mucosa (van Heek et al., 1997, 2000). Therefore, the pharmacological activity of ezetimibe can be ascribed to both the parent drug and the metabolite, with conjugated ezetimibe being at least as potent an inhibitor of intestinal cholesterol absorption as the free drug (Kosoglou et al., 2005; van Heek et al., 2000).

Following oral administration, EZE is rapidly absorbed and undergoes extensive first-pass metabolism (>95% glucuronidation) in the intestinal wall to form the pharmacologically active metabolite, ezetimibe phenolic glucuronide (EZEG), which accounts for approximately 80–90% of the total drug in plasma (Patrick et al., 2002). Clearance of EZE and EZEG from blood is not a straightforward process, and both drugs exhibit multiple peaks in their plasma concentration-time profiles (Patrick et al., 2002). These multiple peaks are attributed to an extensive enterohepatic recirculation (EHC), in which both compounds are transported through portal vessels to the liver, where ezetimibe undergoes further glucuronidation and subsequent biliary secretion through the gallbladder back into the intestine (de Waart et al., 2009). In the intestinal lumen, EZE conjugates undergo enzymatic hydrolysis and are rapidly and completely reconverted to the

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parent drug which is reabsorbed into the systemic circulation (Kosoglou et al., 2005). EHC continues to happen until the drug is completely cleared from the body (Malik et al., 2016). Approximately 78 and 11% of an administered EZE dose are excreted in feces and urine, respectively, with a terminal elimination half-life for both EZE and EZEG of approximately 22 h (Jeu & Cheng, 2003; Patrick et al., 2002). Even though, the pharmacokinetics (PK) of ezetimibe are complex and associated with high inter- and intra-individual variability, the EHC process has the potential to enhance the residence time of the compound in the intestinal lumen, thereby potentiating its cholesterol-lowering activity and allowing for an once-daily dosing (Jeu & Cheng, 2003).

Generally speaking, an insight into the EHC of drugs with extensive bile excretion is of crucial importance, since it may significantly affect their pharmacokinetics and prolong their pharmacological effect (Roberts et al., 2002). Characterization of EHC through classical pharmacokinetic methodologies is proved inadequate for the in-depth description of such complicated concentration (C)–time (t) profiles. Unlike non-compartmental analysis, a model-based approach allows for a better approximation of drugs' kinetics and enables a further characterization of the influence of this re-distribution process on PK parameters such as absorption, distribution and clearance (Soulele et al., 2017). To date, most published PK studies for EZE focused mainly in the determination of model-independent PK parameters, such as the area under the plasma C–t curve (AUC), the maximal plasma concentration (C_{max}) and the elimination half-life ($t_{1/2}$) (Kosoglou et al., 2005; Patrick et al., 2002). To the best of our knowledge, only one compartmental model describing EZE PKs in humans is available in the literature (Ezzet et al., 2001), and thus data describing model-based parameters such as volumes of distribution, elimination and rate constants are sparse for ezetimibe. The limited use of population compartmental analysis in case of EZE could be attributed to the complex PK behavior of the drug and the high between-subject variability (BSV) caused by the EHC process.

In order to increase our understanding on the clinical pharmacokinetics of enterohepatic recycling, the primary objective of this study was to develop a novel population PK model for the description of EZE kinetics. Using data from a crossover bioequivalence (BE) study, nonlinear mixed effects modeling was employed to develop a model for total ezetimibe (parent and glucuronide metabolite) based on physiological considerations. Since conventional PK models were inadequate for capturing the multiple peaks observed in the obtained C–t profiles, additional models were developed to incorporate an EHC component. As a secondary aim of this study, the potential contribution of several covariates was examined in order to extract any useful information for EZE pharmacotherapy.

Materials and methods

Subjects and study design

The plasma concentration data used for model building were obtained from a BE study which employed a standard open-label, single-dose, randomized crossover design, in healthy adult subjects under fasting conditions. The study was in compliance with the Good Clinical Practice guidelines issued by the International Conference on Harmonization and was conducted according to the principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the National (Anveshhan) Independent Ethics Committee, and a written informed consent was obtained from each participant prior to enrollment in the study.

Thirty-six healthy, adult subjects were enrolled in the study. All participants underwent a physical examination, electrocardiogram (ECG) evaluation and laboratory tests, and a thorough medical history review to ensure their health status. The inclusion criteria referred to male or female subjects aged between 18 and 45 years, within the normal weight range and body mass index (BMI) from 18.5 to 30 kg/m^2, absence of intolerance or hypersensitivity to the study drug or any of the excipients, non-pregnant and non-lactating women, subjects having negative urine screen for drugs of abuse and negative alcohol, HIV or hepatitis B/C tests. Participants were excluded from study if they had a history, or presence, of significant cardiovascular or any other disease, any treatment which could alter hepatic enzyme function or any other prescribed medication during the last 1 month prior to study initiation, history or presence of significant alcoholism or drug abuse, smoking, asthma, urticaria or other significant allergic reaction. Volunteers were also excluded if they have donated $\geq$450 mL of blood within 3 months prior to study initiation or had a significant blood loss or other major illness.

In the treatment days, after at least 10 h of overnight fasting, subjects were randomly allocated in two groups receiving either one dose of Ezetimibe 10 mg Tablets (Rafarm S.A. Athens, Greece) or Ezetrol® 10 mg Tablets (Merck Sharp & Dohme S.A., Attica, Greece), administered orally with water. Fasting continued until 4 h after the initiation of drug administration, at which a standardized meal was served. Similar meals were also given at 8, 12 and 24 h post-dose. For each subject, 26 blood samples (each of 6.0 mL) were collected before dose and at 0.33, 0.67, 1.00, 1.33, 1.67, 2, 2.5, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 10, 12, 14, 16, 24, 36, 48, 72 and 96 h post-dose. Samples were collected in pre-labeled vacutainer containing K_3-EDTA as anticoagulant and plasma was separated by centrifugation and stored at $-80\,^{\circ}$C until the quantitative analysis. After a 14-day washout period, subjects received the alternate formulation and the same procedures were followed as in the first study period.

All study participants were monitored closely for potential adverse events, while clinical examinations and vital signs measurements (sitting blood pressure, oral body temperature, radial pulse rate and respiratory rate) were performed before and after drug administration in each study period. Baseline and post-study laboratory measurements, including hematology and biochemical parameters (serum creatinine, ALT, AST, bilirubin, urea, hemoglobin and albumin) were also performed, and all reported adverse effects were recorded and evaluated.

Assay methodology

Plasma samples were assayed for EZE and EZEG using a validated liquid chromatography/tandem mass spectrometry (HPLC-MS/MS) method. Briefly, the analytes were extracted by a solid phase extraction process, which employed as internal standards ezetimibe D4 and ezetimibe phenoxy D4 glucurodine analogs, respectively. The separations were carried out using a BDS Hypersil analytical column (C18, 250 × 4.6 mm, 5 μ) with the isocratic elution system of 35:65% v/v of water containing 1 mM ammonium acetate and acetonitrile:methanol (75:25% v/v), and a flow rate of 1 mL/min. The analytic method had a lower limit of quantitation (LLOQ) of 0.1 ng/mL and calibration curve range of 0.1 ng/mL to 15.0 ng/mL. It presented adequate sensitivity, precision, accuracy, specificity and linearity, with the intra-day coefficient of variation for the assay being below 3.89% and 1.67% for EZE and EZEG, respectively.

Population pharmacokinetic analysis

Both EZE and EZEG exhibit similar cholesterol-lowering activity and multiple peaks in their pharmacokinetic profiles. In this vein, the current population PK analysis was performed using the total EZE concentration-time data (i.e. the sum of unchanged EZE and EZEG). Also, since the analyzed *C*–*t* data derived from a crossover BE study, data obtained from the different treatment periods and administered products (*T* and *R*) were pooled together setting "period" and "treatment" effects as potential covariates in the final dataset. Similar methodologies in the treatment of EZE data and PK analysis have been suggested in previously published works (Chu et al., 2012; Ezzet et al., 2001; Soulele et al., 2015, 2017).

A non-linear mixed effects modeling approach was applied to the obtained dataset using Monolix® 2016R1 (Lixoft, Orsay, France). The Stochastic Approximation Expectation Maximization (SAEM) algorithm implemented in Monolix software was used for the estimations, while the algorithm setting was left to the default values; the maximal numbers of stochastic (k_1) and cooling (k_2) iterations were set to 500 and 200, respectively, with the use of automatic stopping rules and one Markov chain.

The PK parameter estimates for total EZE concentrations were determined following sequential steps. In the first step, single- and multi-compartmental PK models with linear elimination were fitted to the obtained data to determine the best structural PK model. One-, two- and three-compartment models with or without the presence of EHC were tested for their ability to describe the distribution and elimination processes of total EZE. Since multiple secondary peaks, indicative of the significant EHC of the drug, were observed in the mean and individual *C*–*t* profiles of subjects (Figure 1(A,B)), conventional PK models were proved inadequate to describe the complex re-circulation kinetics of the drug. For this reason, more sophisticated user-defined models incorporating an EHC process were developed during model building. These models were encoded as ordinary differential equation

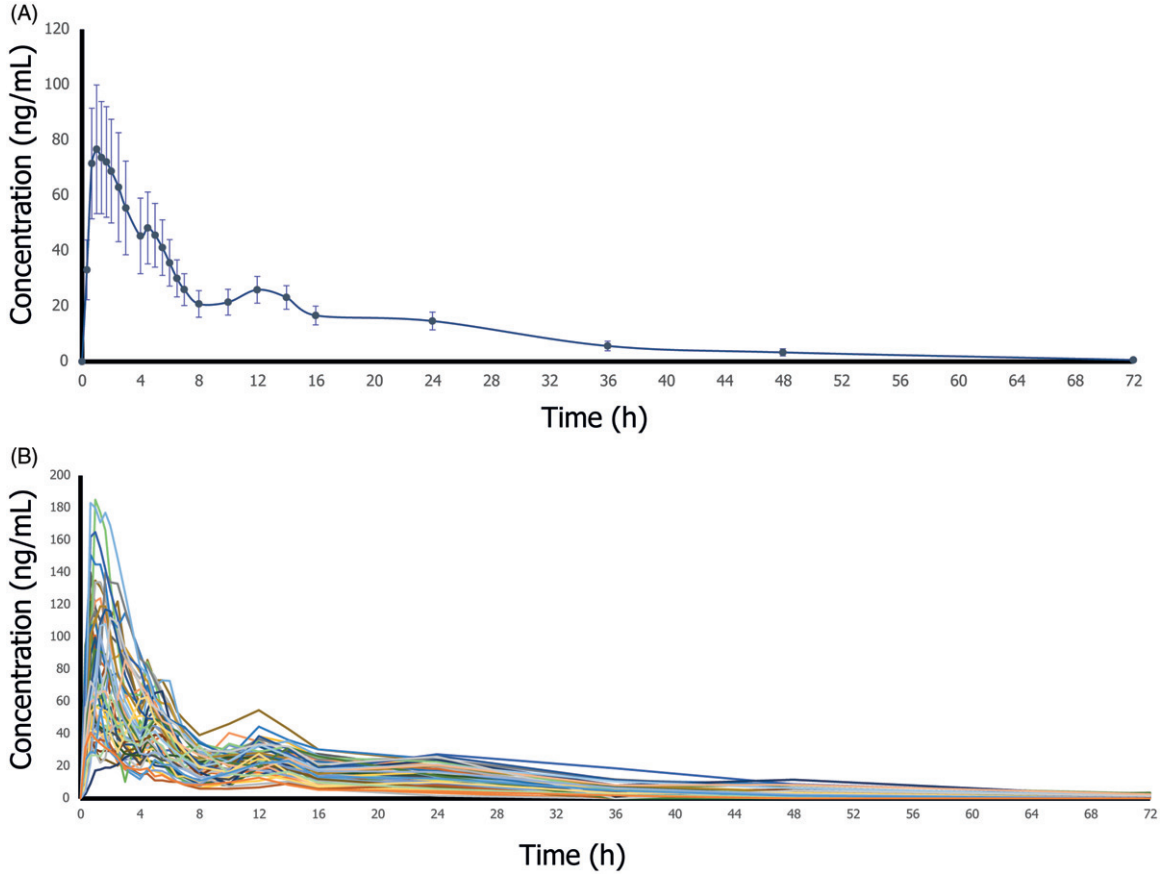

Figure 1. Mean (A) and individual (B) *C*–*t* plots of total ezetimibe following a single oral dose administration of 10 mg ezetimibe in 28 subjects. Data from both the two products (Rafarm S.A. & Merck Sharp Dohme S.A.) and both periods of administration are shown.

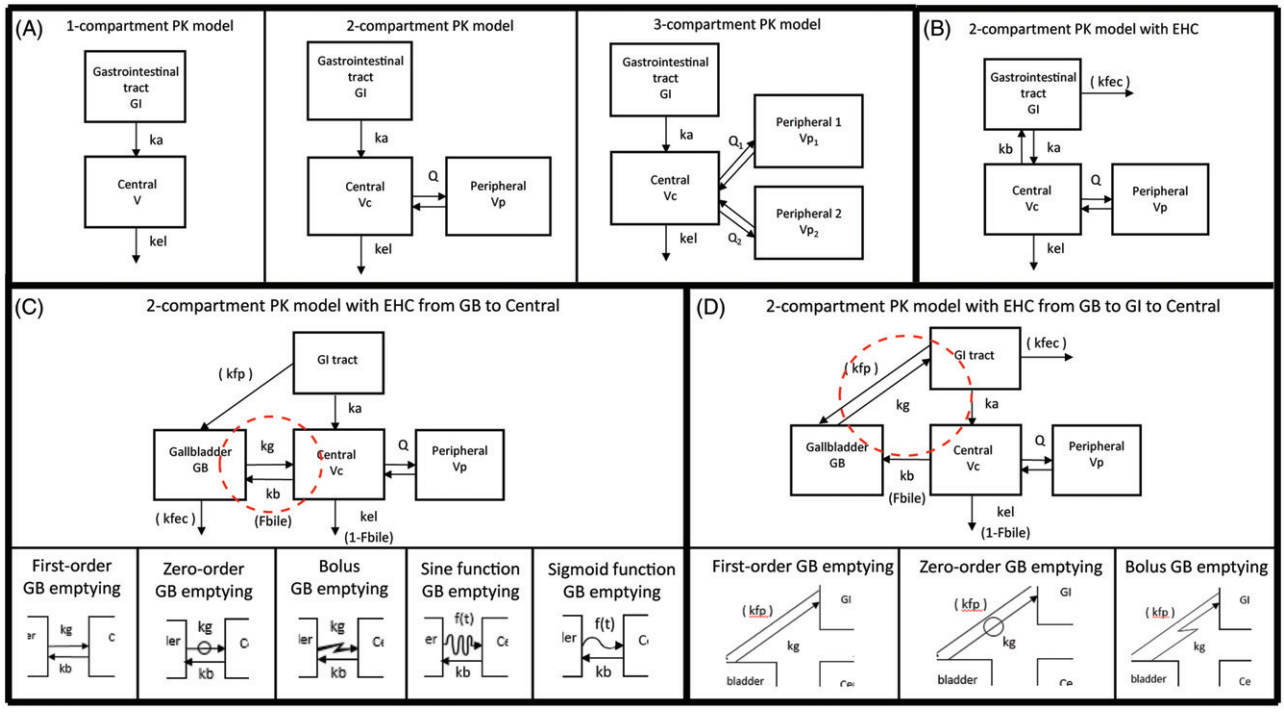

Figure 2. General forms of the structural models examined for total ezetimibe. The dashed circles highlight on the specific kinetics of the structural model and are described in the boxes below each model. k_a = first-order absorption rate constant; V_c/F: apparent volume of drug distribution of the central compartment; V_p/F: apparent volume of drug distribution of the peripheral compartment; Q/F: apparent inter-compartmental clearance of the drug; k_b: first-order transfer rate from the central compartment to the GB compartment; F_{bile}: fraction of dose transferred in the GB compartment; k_g: switch function release rate constant from the GB to the GI compartment; k_{fp}: first-order rate constant for first-pass metabolism; k_{el}: first-order elimination rate constant; k_{fec}: first-order rate constant for fecal elimination.

(ODE) systems using the custom-built model coding language MLXTRAN of Monolix® software.

Four different categories of models of increasing complexity were investigated. A schematic representation of the types of models investigated in the current analysis is shown in Figure 2. The first category (Figure 2(A)) included conventional one-, two- and three-compartment models without the inclusion of an EHC process. In the second category (Figure 2(B)), a three-compartment model incorporating the EHC process occurring between the GI and central compartment was evaluated. The third category (Figure 2(C)) included four-compartment models, where an additional gallbladder (GB) compartment was introduced into the system – in that case EHC was described by a direct bile release from the GB compartment to the central compartment using different release kinetics. Finally, the fourth category (Figure 2(D)) included a four-compartment model, as in case of Figure 2(C), with the difference that the GB compartment was assumed to release drug into the GI compartment, which was subsequently reabsorbed to the central compartment creating an EHC loop of three serial compartments.

In all tested PK models, the drug was presented into the GI compartment following oral administration, from which a fraction (F) of dose was absorbed by first-order process into the central compartment. The drug was either eliminated from the central compartment or transferred into the peripheral and redistribution compartments by first-order processes. All models were parameterized in terms of volumes of distribution for the central and peripheral compartments (V_c and V_p), inter-compartmental clearance (Q), and the first-order absorption and elimination rate constants (k_a and k_{el}). As bioavailability (F) could not be quantified, since only data from an oral administration were available, the term F, referring to the bioavailable fraction of dose, was included to the estimated PK parameters, i.e. V_c/F, V_p/F and Q/F, corresponding to their apparent values.

For the PK models with the EHC component, additional parameters describing the recirculation process had to be introduced. These parameters included a first-order rate constant (k_b) describing the drug transfer from the central compartment to the GB compartment and an excretion rate constant simulating the bile release from the gallbladder (k_g). The latter was also controlled by an intermittent switch function to account for the discontinuous GB emptying process. The choice of discontinuous bile release kinetics in the model was based on theoretical, as well as simulation studies which suggest that secondary peaks cannot be described by linear compartment systems with continuous cyclic transfer processes. For this reason, different scenarios were explored regarding the bile release kinetics, including bolus, first- or zero-order kinetics, time-dependent rate transfer, as well as sigmoid and sine function models, which were able to provide an oscillatory enterohepatic circulation. Several assumptions considering the time and duration (T_{GE}) of gallbladder emptying were also assessed for inclusion in the final model, along with the presence or absence of a smaller baseline bile release constant (kg*), a first-pass effect (k_{fp}) and the fraction of drug undergoing first-pass metabolism (F_p), fecal elimination (k_{fec}), or a parameter describing the fraction of drug undergoing EHC (F_b).

At first, the GB emptying times and durations were considered as parameters, but in order to increase the accuracy

of the other estimated PK parameters, these times were finally fixed. In most subjects, meal times were shown to trigger the timing of GB emptying, therefore, GB emptying times were standardized based on the intake of meals as defined in the study protocol (4, 8, 12 and 24 h after drug administration) and the appearance of the secondary peaks in the obtained *C–t* plot. Different durations of GB empting (0.01, 0.5, 0.75, 1 and 1.5 h) were also tested, based on physiological considerations and previously reported values (Berg et al., 2013; de Winter et al., 2009; Sam et al., 2009).

The statistical model accounting for variability in EZE pharmacokinetics included parameters for between-subject (BSV), inter-occasion (IOV) and residual unexplained variability. All parameters were assumed to follow log-normal distribution, while logit-transformation was implemented for the PK parameters constrained to be on the 0–1 scale. Correlations of the random effects of the PK parameters were also assessed for their contribution in the final model, while, the different error models for residual variability (constant, proportional, exponential, combined) were tested using the likelihood ratio test.

Along with the base model selection, the effect of certain covariates on model PK parameters was investigated. These included baseline demographic characteristics, such as body weight, age, height and BMI, as well as laboratory measurements obtained during screening tests, such as liver enzymes (AST and ALT), bilirubin (total, conjugated and free), serum creatinine, albumin, hemoglobin and urea. The impact of continuous covariates was tested according to allometric or linear relationships, either untransformed or centered around their "mean" value. Allometric scaling was applied to the apparent volume of distribution and clearance parameters, also as *a priori* and standardized to a body weight of 70 kg and fixed exponents (1 for the central and peripheral volumes of distribution and 0.75 for clearance). The variability in PK parameters between the different study periods (i.e. IOV) was also evaluated as a potential covariate, as well as the "treatment" effect accounting for differences on the PK parameters attributed to the different administered products (test or reference). Covariates were initially tested by univariate analysis and then by a combination of a stepwise forward addition and backward elimination process. Significance levels of 5% were considered in both procedures. The incorporation of covariates in the final model was also determined by the reduction in -2LL values, the parameter precision expressed as relative standard error, reductions in BSV values associated with parameters, and the physiological soundness of each covariate on the PK parameter.

Final model selection was based on goodness-of-fit criteria in addition to the plausibility and stability of the model. Models were compared using the commonly used numerical selection criteria of statistical significance: -2LL (-2 log likelihood), Akaike and Bayesian information criteria (AIC and BIC, respectively). To statistically distinguish between the nested models, the likelihood ratio test based on the reduction of -2LL value was used, while AIC and BIC were used to discriminate between nonhierarchical models. A reduction greater than 3.84 from the base or previous model to the current model was designated as statistically significant at $p < .05$. In addition, visual inspection of goodness-of-fit plots was also performed during model selection. PK parameter estimates were required to be physiologically plausible and a model had to remain stable when significant digits and initial parameter estimates were altered. Plots of residuals and weighted residuals, standard errors of parameter estimates and changes in estimates of inter-individual and residual variability were also examined. The following diagnostic plots were used to assess visually model fit: observed *versus* population predicted (PRED) or individual predicted (IPRED) values, individual and population weighted residuals (WRES) *versus* the predicted (PRED) concentrations or time, and the normalized prediction distribution errors (NPDE). Visual predictive checks plots (VPCs) were also performed to evaluate the predictability of the model, where the 95% confidence intervals around the 10th, 50th and 90th prediction percentiles from 500 simulated datasets were overlaid to the 10th, 50th and 90th percentiles of the observed data binned using the theoretical sampling times.

Results

Data from 28 male subjects were used in the population PK analysis. Demographic and biochemical parameters of the included population are presented in Table 1. The two ezetimibe oral formulations were found to be bioequivalent with both study medications being generally well tolerated. All volunteers completed the study procedures without any adverse effects. In total 1508 concentration–time values were analyzed. A high proportion (around 95%) of concentrations below the LLOQ were found for the 96 h time-point and for this reason the dataset was truncated up to 72 h. Figure 1 displays the mean and individual plasma *C–t* profiles of total EZE, which is indicative of the high variability encountered in the absorption and distribution processes among subjects. Plasma concentrations of total EZE reach C_{max} at around 1 h after dosing, with all participants demonstrating multiple secondary peaks at around meal-times, consistent with the known EHC of the drug. As this feature was apparent following administration of either formulation and was observed in all study participants, an EHC compartment was included *a priori* in the model structure. Additional prior information regarding the metabolism and disposition of EZE was considered during model development.

A population PK model, incorporating an intermittent GB emptying process able to simulate the EHC process of ezetimibe, was constructed (Figure 3). The structure of this model included four compartments: the central, peripheral, GI and GB compartments. The central compartment representing the blood and liver was reversibly connected to a peripheral tissue compartment. A hypothetical GB compartment was further introduced into the model and was linked to both the central and GI compartments creating the enterohepatic recirculation loop. The drug in the central compartment was either eliminated or transferred into the peripheral of GB compartment, following first-order kinetics. Following accumulation in the GB compartment, the drug was intermittently released from the GB to the GI compartment using a "very

Table 1. Demographic characteristics and some biochemical analytes of the studied population ($n = 28$).

Continuous characteristics	Mean	Range (Min–Max)
Age (y)	27	19–37
Body weight (kg)	59.6	47.3–76.7
Height (cm)	165.4	152–176.5
Body mass index (kg/m^2)	21.8	18.73–26.84
Serum creatinine (mg/dL)	0.7	0.59–0.93
Serum urea (mg/dL)	18.5	9.2–34.5
Serum albumin (gm/dL)	4.8	4.38–5.35
Aspartate aminotransferase (IU/L)	24.9	13.7–43.32
Alanine aminotransferase (IU/L)	23.8	8.2–45.1
Haemoglobin (g/dL)	13.7	12.2–16
Bilirubin total (mg/dL)	0.6	0.23–1.22
Bilirubin conjugated (mg/dL)	0.2	0.09–0.33
Bilirubin unconjugated (mg/dL)	0.4	0.14–0.9

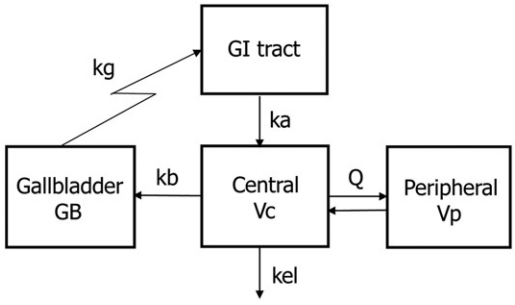

Figure 3. The final enterohepatic recirculation model for total ezetimibe. The lightning between the GB and GI compartments represent the bolus release of bile from the GB. k_a: first-order absorption rate constant; V_c: apparent volume of drug distribution of the central compartment; V_p: apparent volume of drug distribution of the peripheral compartment; Q: apparent inter-compartmental clearance of the drug; k_b: first-order transfer rate from the central compartment to the GB compartment; k_g: switch function release rate constant from the GB to the GI compartment; k_{el}: first-order elimination rate constant.

fast" first-order rate constant (like a bolus release), with fixed GB emptying times and duration. The drug was then re-absorbed from the GI tract to the central compartment with the same first-order rate constant as the administered oral dose. Elimination was considered to occur from the central compartment following first-order kinetics and accounted for both renal and fecal excretion.

Assuming all the kinetic processes, except bile release followed first-order kinetics, the set of ordinary differential equations for the PK model is described by Equations (1–6):

$$dA_1/dt = -\ ka \cdot A_1 + GBE \cdot k_{41} \cdot A_4 \quad (1)$$

$$dA_2/dt = ka \cdot A_1 - (k_{23} + k_{24} + kel) \cdot A_2 + k_{32} \cdot A_3 \quad (2)$$

$$dA_3/dt = k_{23} \cdot A_2 - k_{32} \cdot A_3 \quad (3)$$

$$dA_4/dt = k_{24} \cdot A_2 - GBE \cdot k_{41} \cdot A_4 \quad (4)$$

$$k_{23} = Q/Vc \quad (5)$$

$$k_{32} = Q/Vp \quad (6)$$

where, A_n represents the drug amount in the nth compartment: (1) the gastrointestinal tract compartment; (2) the central compartment; (3) the peripheral compartment; (4) the gallbladder compartment, and k_{ij} are the absorption, elimination or transfer rate constants among the compartments. The term GBE is a switching criterion with values 0 or 1, which refer to the situations in the absence of GB emptying (GBE = 0) or when GB emptying occurs (GBE = 1). The initial conditions of all compartments were set to zero except A_1, which is assumed to contain the entire EZE dose at zero time.

In this analysis, GB emptying was defined as a known intermittent process, with essentially complete emptying within each enterohepatic cycle. Based on the time of secondary peaks and the intake of food relative to dose, EHC was modeled to simulate three release times with the GB emptying described as a bolus impulse into the GI compartment. The duration of bile release was fixed at 0.75 h which approximates the mean GB emptying time in healthy individuals (Berg et al., 2013). Finally, the bile release rate constant (k_{41} or k_g) was assumed to either equal to zero or was fixed to a high positive value (Sam & Joy, 2010), i.e. arbitrarily set at 21 h $^{-1}$, which eventually was found to provide the best model performance.

Additional assumptions were also made to aid in producing a model that would successfully minimize:

- The rate constants associated with each compartment were not affected by the recycling.
- Liver, being a well perfused organ, was considered as part of the central compartment in the final model.
- GB emptied completely at each cycle and the three EHC were exactly the same.
- EZE glucuronide was totally and rapidly hydrolyzed to free EZE in the gut immediately after bile release, followed by complete reabsorption of EZE.
- No delay in the metabolism process of EZE to EZEG was considered.
- The recirculated drug was reabsorbed with the same first-order rate constant as the administered oral dose.

Fecal and renal elimination were incorporated in one drug elimination rate constant from the central compartment. The parameter estimates for k_a, V_c/F, V_p/F, Q/F, k_b (or k_{24}), and k_{el} that provided the best fit to the data set of each participant are summarized in Table 2. Between-subject variability exhibited moderate values, ranging from 22 to 56% with the exception of inter-compartmental clearance (Q/F) in which %BSV value was 86%. Model parameters were considered to be precisely estimated with relatively low RSE% values obtained for both PK parameters and BSV% estimates (Table 2).

A correlation of the random effects between V_c/F and Q/F (corr = 0.88) was also incorporated in the final model, as it significantly decreased the numerical criteria and improved goodness-of-fit plots. Residual variability was described by a combined error model consisting of an additive component α and multiplicative coefficient b:

$$C_{ij} = f_{ij} + (a + b\, f_{ij})\, \varepsilon_{ij} \quad (7)$$

where, C_{ij} is the jth observed concentration of EZE for the ith individual, a and b are the parameters of the residual error model, f_{ij} is the jth model predicted value for ith subject, and ε_{ij} is the random error which is assumed to be normally distributed with mean 0 and variance 1. The residual error parameters for the combined error model [(Equation (7)] were: $\alpha = 0.32$ and $b = 0.29$. No covariate effect including

Table 2. Population pharmacokinetic parameters for the final PK model applied to total ezetimibe data.

PK Parameter	Mean (RSE%)	BSV% (RSE%)
k_a (h^{-1})	0.86 (9)	39.75 (19)
V_c/F (L)	50 (8)	31.76 (17)
V_p/F (L)	146 (10)	56.58 (15)
Q/F (L/h)	30.5 (15)	86.59 (16)
k_b (h^{-1})	0.096 (12)	26.03 (88)
k_{el} (h^{-1})	0.208 (7)	22.17 (31)
PK random effects correlation		
V_c/F – Q/F	0.88 (11)	–
Residual error model		
a	0.32 (17)	–
B	0.29 (3)	–

PK: pharmacokinetic; k_a: first-order absorption rate constant (h^{-1}); V_c/F: apparent volume of drug distribution (L) of the central compartment; V_p/F: apparent volume of drug distribution (L) of the peripheral compartment; Q/F: apparent inter-compartmental clearance of the drug (L/h); k_b: first-order transfer rate from the central compartment to the gallbladder compartment (L/h); k_{el}: first-order elimination rate constant (h^{-1}); F: fraction of bioavailable dose; a and b: residual error parameters for the combined error model [Equation (7)]; RSE%: relative standard error of the calculation of the population pharmacokinetic estimate; BSV%: between subject variability.

age, body weight, height and BMI or any of the biochemical laboratory measurements tested was found significant for the estimated PK parameters. Inter-occasion variability and treatment effects were also not found to be statistically significant on any PK parameter.

Model comparison of the different types of models tested was done using mainly the AIC estimates. In all cases, AIC values were lower for the models including enterohepatic compartments compared to those derived from the same model without such compartments. In particular, introduction of the EHC process in the final PK model for total EZE decreased AIC by 1275 units relative to the relevant model (two-compartment) without enterohepatic recycling. Goodness-of-fit plots also demonstrate a desired performance of the final population PK model of total EZE (Figures 4–6). A good agreement between the individual predicted and the observed concentrations is depicted in Figure 4, showing an adequate degree of linearity. Moreover, a symmetrical distribution with no significant trends can be observed when the IWRES were plotted *versus* the population predictions (Figure 5). The bulk of residuals lie within the generally accepted range of ±2 units, indicating an acceptable description of the residual error by the combined error model. Finally, the VPC plot (Figure 6) demonstrated that the majority of the observed plasma concentrations lied within the 10th and 90th percentiles of the simulated drug concentrations, providing a good description of mean tendency of the *C–t* data and an acceptable predictive ability of the final model. Additional goodness-of-fit plots are presented in Appendix II (Figures A1–A3).

Discussion

Enterohepatic recirculation occurs when a significant amount of a drug or its conjugated metabolites are excreted into bile and then return back to the intestine, where they can serve as a secondary source for drug absorption (Gao et al., 2014; Roberts et al., 2002). This recirculation process has been shown to notably affect drug pharmacokinetics by prolonging drug elimination, increasing bioavailability and producing

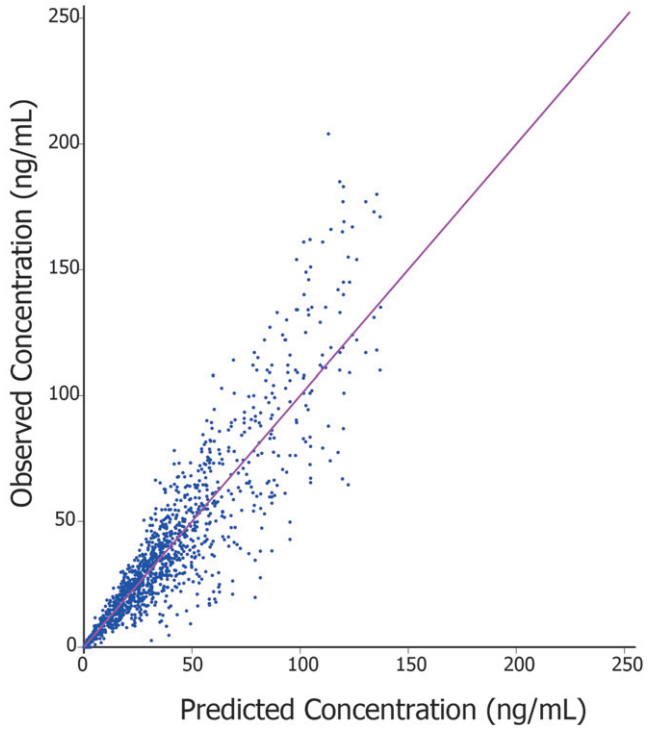

Figure 4. Observed plasma concentration values *versus* the individual predicted concentrations from the population pharmacokinetic model of total ezetimibe. The diagonal line represents the line of unity, namely, the ideal situation.

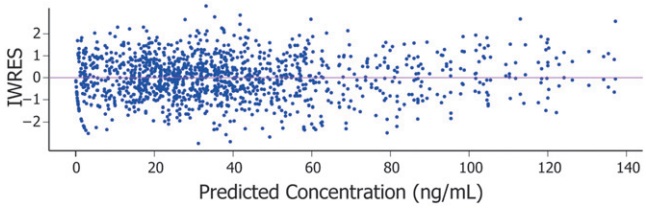

Figure 5. Graphical illustration of the individual weighted residuals (IWRES) *versus* the individual predicted concentrations (IPRED) for the final model of total ezetimibe.

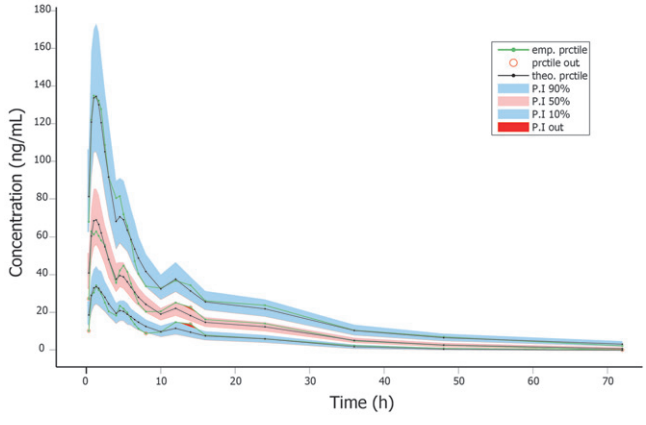

Figure 6. Visual predictive check plot of the final model of total ezetimibe. Light solid lines refer to the 10th, 50th and 90th percentiles of the empirical data; bold solid lines refer to the median of model predicted values in each percentile; shaded areas refer to the 95% prediction intervals around each theoretical percentile; circles and areas denote the outlier data.

complicated concentration-time profiles (Roberts et al., 2002). The physicochemical properties of the drug can further add variability and make the process even more perplexing. Excretion of drugs in bile depends mainly on their molecular weight and structure. A molecular weight threshold of >400 g/mole is associated with appreciable biliary excretion in humans (Malik et al., 2016; Roberts et al., 2002). Conjugation of drugs is also known to increase both the molecular weight and polarity, thus making these compounds even more pre-disposable to biliary excretion (Malik et al., 2016).

Following oral administration, EZE is rapidly absorbed by the intestine and extensively metabolized (>80%) to a pharmacologically active glucuronide conjugate. EZE alone has a molecular weight of over 400 g/mole and following glucuronidation, its molecular weight increases up to 580 g/mole, making both compounds prone to EHC. Previous studies have shown that EZE and EZEG are repeatedly delivered with bile into the intestinal lumen as a result of re-circulation, leading to an increased residence time of the drug to the site of action (Ezzet et al., 2001; Kosoglou et al., 2005). As evident in Figure 1, multiple secondary peaks are observed in the individual and mean *C–t* profiles of total EZE, with clear increases in plasma concentrations between 4–5, 11–13 and 23–25 h post-dose in almost all subjects. This timing is consistent with food intake stimulating the emptying of the gallbladder.

In this respect, and to further expand our current understanding on metabolism and disposition of EZE, we developed a population PK model for the description of total EZE concentrations (parent and active metabolite), that could describe the enterohepatic recirculation of the drug and adequately characterize its complex disposition kinetics. The analysis was performed on the *C–t* data of 28 healthy subjects who participated in a BE study comparing two solid oral dosage forms of ezetimibe: Ezetimibe 10 mg Tablets (Rafarm S.A., Athens, Greece) *versus* Ezetrol® 10 mg Tablets (Merck Sharp & Dohme S.A., Attica, Greece).

Modeling EHC has always been intricate and various models using different approaches have been investigated in our analysis to interpret the PK profile of total EZE (Okour & Brundage, 2017). The first models tested to describe EZE plasma profile were conventional models using variable number of compartments. One-, two- and three-compartment models were proven inadequate to capture the multiple concentration peaks observed in the *C–t* profile, as they did not consider for any recirculation process. Classical models were extended by a chain of compartments accounting for enterohepatic recirculation (Younis et al., 2009); however, these models did not consider the discontinuous pattern of bile release and the time elapsed during the recycling. Therefore, an appropriate time function had to be introduced in the PK model, accounting for the toggle nature of the GB emptying process.

In this sense, EHC models containing a variable number of compartments and different bile release kinetics, accommodating an irregular GB emptying were developed (Figure 2). Several treatments were applied for bile release kinetics, either directly to the central compartment or via the GI compartment. These included first-order (Berg et al., 2013), bolus (Jiao et al., 2007) or zero-order (Yau et al., 2009) release kinetics at *a priori* known times (e.g. time of food intake), or periodic bile release described by sine (Wajima et al., 2002) and sigmoid functions (Jain et al., 2011).

In the case of sine function, regular intervals of bile release were assumed which could not provide the required flexibility in terms of cycle's timing and duration in the model. Similar to the sine function, sigmoid function also led to poor model convergence and unsatisfactory goodness-of-fit criteria. As opposed to the sine or sigmoid functions, the use of switch on/off function for bile release provided more flexibility in terms of modeling the GB emptying process related to meal-times, providing the best physiological representation when compared to the rest of the modeling strategies (Okour & Brundage, 2017). In the switch function release models, the rate constant controlling the GB emptying process is described by a piece-wise function (Okour & Brundage, 2017). As reported previously, apart from bolus (instantaneous) release, zero- and first-order kinetics were tested to describe the GB emptying process. The inclusion of additional parameters, such as a baseline bile release rate constant (k_g*) during the fasting state (Shou et al., 2005), separate absorption (k_a) and reabsorption (k_a*) rate constants from the GI (Strandgården et al., 2000), a first-pass effect (k_{fp}) (Kim et al., 2015), or an additional parameter for the fraction of drug recycled in each EHC (F_{bile}) (Shou et al., 2005) were also evaluated during model development, but did not lead to significant improvements of fitting and were not included in the final model. More complex models containing liver as a separate compartment were also considered (Kim et al., 2015); however, such an attempt has resulted in model over-parameterization and poor convergence.

Following several trials and based on physiological considerations, the basic structural model for EZE consisted of a 4-compartment disposition model including an enterohepatic recirculation loop added onto the central compartment with instantaneous bile release at specific time intervals (Figure 3). The recirculation loop was incorporated between the central and the GI compartment via the inclusion of a gallbladder compartment accounting for the accumulation of the drug in bile. A similar PK model for the description of total EZE concentrations in healthy subjects has been previously described by Ezzet and coworkers (2001); however, the EHC component in that model was incorporated as a secondary input directly into the systemic circulation amounting to a percentage of the absorbed dose.

In the current model, EHC was simulated through multiple discharges of the GB compartment towards the intestine, and two circumstances were considered: (i) absence of GB emptying during the fasting period and (ii) presence of GB emptying around food intake. GB emptying was assumed to occur instantaneously at specific time intervals, where all the drug stored in the GB was released back to the intestine as a bolus impulse. This was achieved by using a first-order bile release constant fixed to a very large value ($k_g = 21\ h^{-1}$), in addition to assuming a relatively short GB emptying duration ($T_{GE} = 0.75$ h). Each cycle was followed by another cycle of

filling that proceeded until the next GB emptying triggered by the next meal and so on.

It is evident that models incorporating EHC can rapidly gain in complexity and may present parameter identifiability problems and numerical difficulties. Accordingly, in our case, to overcome such problems and keep a minimum model complexity, some parameters regarding EHC had to be fixed. Therefore, even though parameters, such as the bile release rate constant and the time and duration of GB emptying, were initially allowed to be estimated and vary within and between subjects, for the sake of parameter interpretation and covariate analysis, their values were finally fixed. It is acknowledged that fixing parameters for a recirculation process is not always an easy task, as it can dangerously distort and bias the choice of reabsorption kinetics and estimation of other PK parameters (Abi Khalil et al., 1993). However, in our case, based on a strict meal-time protocol and physiological considerations regarding GB emptying, as well as a thorough investigation on the most suitable bile release kinetics, we were able to develop a parsimonious model with the desired numerical stability and predictive power.

The estimated population parameter k_a suggests that EZE is relatively rapidly absorbed with a rate constant value indicative of the short T_{max} values observed in the PK profiles and similar to previously reported values (Ezzet et al., 2001; Kosoglou et al., 2005). Relatively high apparent volumes of distribution and low clearance were also obtained for EZE, consistent with the extensive recycling of the drug (Colburn, 1982; Roberts et al., 2002; Smith et al., 2015). The fraction of drug excreted into the bile within each EHC (%EHC) could also be indirectly estimated using a previously proposed method (Colom et al., 2014) (see Appendix I). The equation showed that about 30% of the amount absorbed is recirculated in each enterohepatic cycle, a fraction slightly higher than previously reported values of around 20% (Ezzet et al., 2001).

None of the tested covariates was found to be significant or to improve the numerical or graphical criteria of the final model. This may be partly attributed to the relatively homogenous population, which can constrain the ability of the model to unveil the signal of a covariate relationship. Enterohepatic recycling has been generally shown to be affected by several factors, such as patient characteristics and genetic variability, age- or gender-related effects, liver and kidney function, potential disease effects or co-medication (Roberts et al., 2002). However, our findings coincide with the literature evidence for EZE, where no significant effect of age, gender and race has been reported; the latter implies that no dosage adjustment is necessary for patients with mild renal or hepatic impairment (Jeu & Cheng, 2003; Kosoglou et al., 2005).

The semi-physiological PK model presented in the current investigation for the description of EHC of total ezetimibe was developed taking into account certain practical limitations and methodological issues. As reported above, specific assumptions had to be made regarding the description of EHC process. Thus, the onset of each enterohepatic cycle was fixed to certain time-points based on mealtimes, whereas GB emptying was modeled as a bolus release by fixing "k_g" to a very high value. However, since gallbladder emptying can be considered as a rather instantaneous process highly affected by food intake, fixing these parameters was found to be physiologically relevant and to significantly improve model fitting. Finally, given that data from an earlier bioequivalence study was used in this analysis, model development was based on a healthy and relatively homogenous population. Extension of the model to patients or other population groups could provide further information on the impact of hepatobiliary system physiology on enterohepatic recirculation of drugs.

Conclusion

The aim of this study was to apply population PK modeling in order to describe the *C–t* data of total EZE and furthermore to elaborate on the enterohepatic recirculation models. In order to achieve this, several different forms of EHC pharmacokinetic models were developed and evaluated. Eventually, total EZE was best described by a four-compartment model where EHC was modeled through the inclusion of an additional gallbladder compartment that released drug at specific time-intervals in agreement with food administration. This modeling approach led to the development of a novel population PK model for total EZE, which is sufficiently realistic from a PK and physiological point of view. This model was found to accurately estimate the relevant PK parameters of total EZE, as well as to adequately characterize the enterohepatic recycling process of the drug and accommodated all secondary peaks observed in the profile.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Appendix I

Equation for the fraction of drug excreted into the bile within each enterohepatic cycle:

$$EHC\% = (kb/kb + kel) \times 100 \quad (A1)$$

where, k_b is the rate constant for the transfer of drug from the central towards the theoretical bile compartment (GB) and k_{el} is the first-order elimination rate.

Appendix II

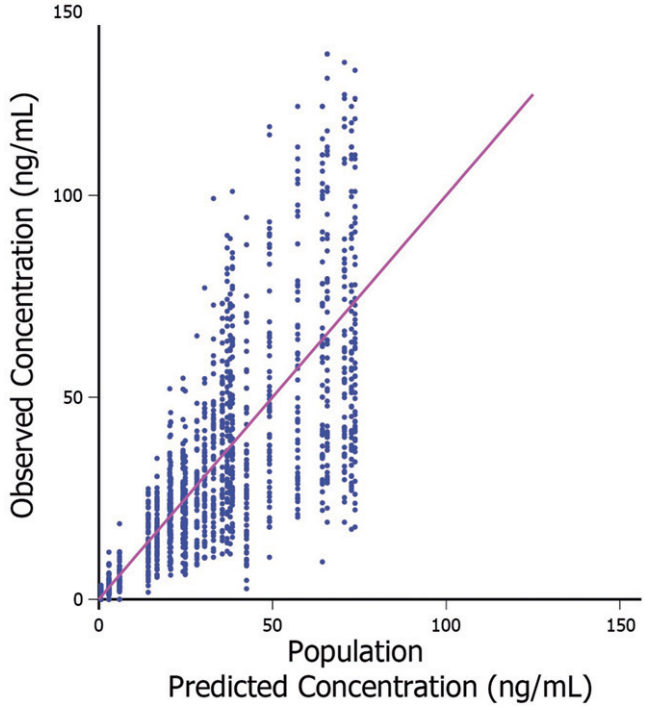

Figure A1. Population predicted concentrations from the population pharmacokinetic model *versus* the observed plasma concentration values of total ezetimibe. The diagonal line represents the line of unity, namely, the ideal situation.

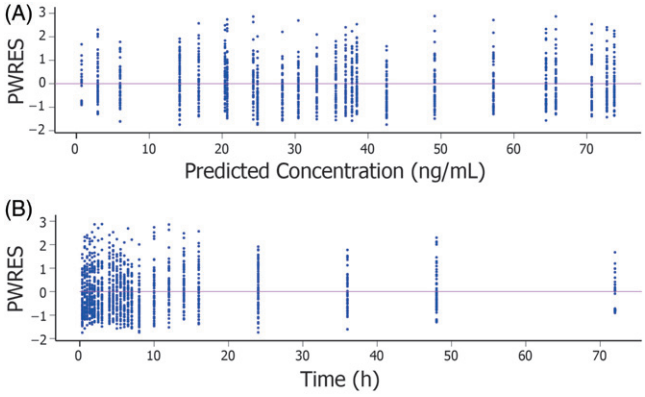

Figure A2. Graphical illustration of the population weighted residuals (PWRES) *versus* the population predicted concentrations (a) and time (b) for the final model of total ezetimibe.

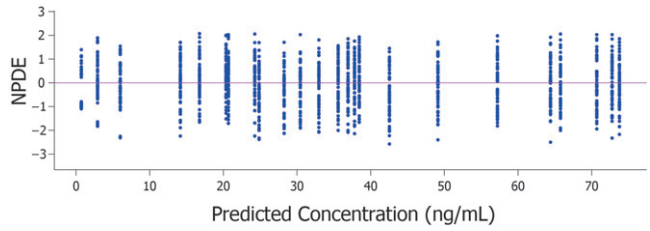

Figure A3. Graphical representation of the normalized prediction distribution errors (NPDE) *versus* the individual predicted concentrations (IPRED) for the final model of total ezetimibe.