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A new population pharmacokinetic model for dosing optimization of zonisamide in patients with refractory epilepsy

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ABSTRACT

Zonisamide exhibits significant pharmacokinetic variability, demanding for the development of population pharmacokinetic (PopPK) models to identify key factors influencing drug disposition.

This study aimed to develop and validate a PopPK model to optimize zonisamide posology in patients with refractory epilepsy.

A total of 114 plasma concentrations of zonisamide, obtained from 64 patients, were used for PopPK model development, employing the nonlinear mixed-effects modelling approach. The final model was evaluated by visually inspecting the goodness-of-fit plots and the visual predictive check plot and by the bootstrap resampling method. A one-compartment model with first-order elimination was the one that best described the pharmacokinetic profile of zonisamide. Between-patient variability (BPV) was included on clearance (CL/F), volume of distribution (Vd/F) and absorption rate constant (ka). The residual error (RE) was modeled as proportional. The final model estimates for CL/F, Vd/F and ka were 0.761 L/h, 48.10 L and 0.671 h^{-1}, respectively. The BPV associated with CL/F, Vd/F, and ka was 43.93%, 52.06%, and 91.27%, respectively, while the proportional RE was 7.18%. The concomitant administration of enzyme-inducing antiseizure drugs (EIASDs), included in the model as inducer drug load (INDDL), significantly accounted for BPV associated with CL/F and led to increased CL/F in patients receiving EIASDs compared to the others. Consequently, patients receiving EIASDs require higher daily doses of zonisamide to achieve therapeutic plasma concentrations compared to those not treated with EIASDs.

Model validation, using bootstrap and visual predictive checks, confirmed its stability and robustness, making it a valuable tool for individualized zonisamide dosing in adults with refractory epilepsy.

1. Introduction

Drug therapy stands as the main therapeutic approach for an effective management of seizures, and within this context, zonisamide emerges as a second-generation antiseizure drug. Its multi-target mechanism of action involves the blockage of pre-synaptic voltage-gated sodium channels and T-type calcium channels, as well as the enhancement of gamma-aminobutyric acid release and inhibition of glutamate release (Leppik, 2004; Biton, 2004; Biton, 2007; Wilfong and Willmore, 2006). Therefore, zonisamide is currently approved as adjunctive therapy in the management of focal seizures, whether they progress into bilateral tonic-clonic seizures or not, for patients diagnosed with epilepsy aged 6 years and above and as monotherapy in adults with newly diagnosed epilepsy (European Medicines Agency ZONEGRAN: Summary of product characteristics; Patsalos et al., 2018; Gidal et al., 2024). Off-label uses encompass the treatment of absence seizures, West syndrome, juvenile myoclonic epilepsy, Lennox-Gastaut syndrome, Doose syndrome, myoclonic seizures, and progressive

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myoclonic epilepsy (Patsalos et al., 2018). It is noteworthy, that the ability of zonisamide to target multiple pharmacodynamic pathways makes it a drug of choice for patients dealing with refractory epilepsy (Sills and Brodie, 2007; Brodie, 2006; Baulac and Leppik, 2007; Velizarova et al., 2014), which comprises approximately one-third of those undergoing antiseizure drug therapy (Kwan and Brodie, 2000; M.J. Brodie et al., 2012; Chen et al., 2018). In addition to the aforementioned mechanisms of action, zonisamide has demonstrated effects on dopaminergic and serotonergic neurotransmission, enhancing its clinical utility in addressing a spectrum of neurological and psychiatric disorders. Indeed, the therapeutic potential of zonisamide has demonstrated in Parkinson's disease (Goel et al., 2021; Matsunaga et al., 2017), parkinsonism in patients with dementia with levy bodies (Odawara et al., 2022; Tousi and Leverenz, 2021), essential tremor (Bruno et al., 2017; Sadeghi and Ondo, 2010), bipolar disorder (Buoli et al., 2017), binge-eating disorder (Buoli et al., 2017) and neuropathic pain (Moore et al., 2014).

Zonisamide exhibits rapid absorption following ingestion, reaching peak plasma concentrations within 2 to 5 h. With an oral bioavailability exceeding 90%, its absorption remains consistent regardless of food intake. However, the presence of food slightly prolongs the time to reach peak plasma concentration, extending it to 3 to 6 h. The volume of distribution for zonisamide ranges from 1.1 to 1.7 L/kg, probably owing its strong affinity to erythrocytes, leading to significant accumulation within these cells (M.J. Brodie et al., 2012). The concentration in erythrocytes can be up to 8-fold higher than that in plasma, but this relationship appears to exhibit linearity within therapeutic concentrations (Leppik, 2004). The metabolism of zonisamide include two main pathways. Firstly, it undergoes reduction to 2-sulfamoylacetylphenol, followed by glucuronidation. Reduction is predominantly mediated by the CYP3A4 isoform, although CYP2C19 and CYP3A5 seem also be involved. Secondly, zonisamide undergoes acetylation to form N-acetylzonisamide. The major route of excretion is through urine, with 35% of the dose excreted as zonisamide, 50% as the glucuronide, and 15% as N-acetyl zonisamide (Leppik, 2004; Holder and Wilfong, 2011). The elimination half-life of zonisamide is approximately 60 h, and steady state is achieved after approximately 14 days of stable dosing (M.J. Brodie et al., 2012). Its extended half-life allows for once-daily dosing schedules, contributing to enhanced patient adherence compared to many other antiseizure drugs that require twice or even three-daily dosing regimens (Gidal et al., 2024; M.J. Brodie et al., 2012; Holder and Wilfong, 2011; Mula, 2013).

The recommended therapeutic reference range for plasma concentrations of zonisamide is currently set at 10 – 40 mg/L (Patsalos et al., 2018; Patsalos et al., 2008). The relationship between doses and plasma concentrations of zonisamide is linear within the range of the recommended maintenance daily doses (300 to 500 mg/day). Importantly, this relationship significantly depends on age, body composition and organ function (Kimura et al., 1992; Perucca and Bialer, 1996; Italiano and Perucca, 2013), as well the drugs administered concomitantly (Sills and Brodie, 2007; Holder and Wilfong, 2011; Ragueneau-Majlessi et al., 2004; Shinoda et al., 1996; Levy et al., 2004; Fukuoka et al., 2004). However, the impact of each factor (or combination of factors) had not yet been quantitatively described, hindering to foresee the optimal dosing for each patient or specific population.

To overcome such variability, pharmacokinetic drug monitoring is advised to guide the individualization and adjustment of zonisamide posology (Patsalos et al., 2018; Patsalos et al., 2008). Model-informed precision dosing is emerging as a contemporary and useful tool for optimizing drug posology, especially for drugs with high pharmacokinetic variability and that take several days to attain the steady state. Effective implementation of this dosing strategy requires population pharmacokinetic (PopPK) models tailored to specific patient populations, preferably reflecting real-world scenarios. PopPK models describe mathematically the typical pharmacokinetic behaviour of a drug in a target population and variability within that population. Moreover, these models allow to identify the main factors responsible for such variability (Pérez-Blanco and Lanao, 2022; Darwich et al., 2017).

To the best of our knowledge, there are currently only three published PopPK models for zonisamide, all of which were developed enrolling Asiatic populations (Hashimoto et al., 1994; Okada et al., 2008; Qiu et al., 2016). Specifically, two of these models enrolled adult and pediatric epileptic patients (Hashimoto et al., 1994; Okada et al., 2008), while the third comprised healthy subjects (Qiu et al., 2016). Acknowledging the need for PopPK models tailored to the unique characteristics of European and Caucasian epileptic patients, particularly those diagnosed with refractory epilepsy, the present study aimed at developing and evaluating the first PopPK model to be further applied in the precise guidance of dose optimization for zonisamide in European adults diagnosed with refractory epilepsy.

2. Methods

2.1. Study design and clinical data collection

A retrospective observational study was conducted including Portuguese refractory epileptic patients admitted to the Refractory Epilepsy Centre of the Centro Hospitalar e Universitário de Coimbra, EPE (CHUC, EPE, Coimbra, Portugal) between January 2018 and April 2022. The study enrolled patients aged at least 18 years, who were undergoing zonisamide treatment for seizure control and underwent pharmacokinetic drug monitoring as part of their routine clinical management.

Patient-related data were collected, encompassing sex, age (years), body weight (BW, kg), height (HT, cm), and comprehensive details regarding the prescribed antiseizure drug regimen, including specific drugs and their respective dosages. In addition to this, calculations were made for ideal body weight (IBW, kg), body surface area (BSA, m^2), and body mass index (BMI, kg/m^2). Furthermore, the study gathered analytical data, comprising serum creatinine (Cr, mg/dL), serum albumin (ALB, g/dL), total proteins (TP, g/dL), red blood cells count (RBC, x 10^{12}/L), and haematocrit (HTC, %). The glomerular filtration rate (GFR, mL/min/1.73 m2) was estimated using the Chronic Kidney Disease Epidemiology Collaboration equations (Palacio-Lacambra et al., 2018).

To assess the drug burden on each patient, the antiseizure drug load (DL) was calculated as the sum of the ratios between the prescribed daily dose (DD) and the defined daily dose (DDD) (WHO ATC/DDD Index 2021 2023) of each antiseizure drug included in the patient's individual regimen (Deckers et al., 1997). Additionally, the inducer drug load (INDDL) was calculated as the sum of the ratios between the DD and the DDD of the enzyme-inducing antiseizure drugs (EIASDs), including carbamazepine (CBZ), oxcarbazepine (OXC), phenytoin (PHT), and phenobarbital (PB).

Ethical considerations were prioritized throughout the study, with approval obtained from the Ethics Committee of the Faculty of Medicine of the University of Coimbra, Coimbra, Portugal (CE-061/2018), and the Ethics Committee of CHUC, EPE (CHUC-144-18).

2.2. Blood sampling and drug quantification in plasma

Blood samples were collected into heparin-lithium tubes and subsequently centrifuged to extract plasma, following the established therapeutic drug monitoring protocol at the Refractory Epilepsy Centre of CHUC, EPE. A total of 114 plasma concentrations of zonisamide were obtained. To mitigate potential issues related to adherence, blood samples were collected on the last day of hospitalization approximately 1 hour after zonisamide intake (between 0.5 to 3.3 h post-drug intake, $n = 143$) and before the subsequent dose (between 8.6 to 14.4 h post-drug intake, $n = 173$). The date and time of each sample collection were recorded.

The quantification of zonisamide concentration in plasma samples was executed through a liquid–liquid extraction process followed by

high-performance liquid chromatography (HPLC) with diode array detection (DAD), as described and validated by Gonçalves et al. (Gonçalves et al., 2018). Briefly, 100 μL of plasma sample was mixed with 40 μL of methanol, 10 μL of internal standard (antipyrine) working solution, and 1 mL of ethyl acetate. Then, the sample was centrifuged at 12,045 × *g* for 3 min, to extract the zonisamide dissolved in the upper organic layer. Liquid extraction with ethyl acetate was repeated and the extract was evaporated at 45 °C under nitrogen stream and redissolved in 100 μL of a mixture of water and acetonitrile (90/10 v/v), which was then injected in the HPLC-DAD system (Shimadzu Corporation, Kyoto, Japan). The mobile phase was composed of water and acetonitrile and pumped at 1.0 mL/min, 40 °C, and the detection set at 220 nm. The lower limit of quantification for zonisamide was experimentally defined as 0.5 mg/L. The method demonstrated linearity in the concentration range from 0.5 to 50.0 mg/L ($r^2 \geq 0.998$) and exhibited accuracy (relative error ranging from −3.307% to 4.853%) and precision (coefficient of variation ranging from 2.420 to 6.288%).

2.3. PopPK modelling and model evaluation

The PopPK analysis was conducted using the nonlinear mixed effects modelling with the NONMEM® version 7.4 software (ICON Development Solutions in Ellicott City, MD, USA). The model-building process used the first-order conditional estimation method with interaction. The xpose4 package version 4.7.1 integrated with R software version 4.1.3 (The R Foundation for Statistical Computing) was used for graphical diagnostics to guide the model building process.

2.3.1. Base model

One-compartment and two-compartment models with first order absorption and linear elimination, parameterised in terms of absorption rate constant (ka, h^{-1}), apparent volume of distribution (Vd/F, L) and apparent clearance (CL/F, L/h), were investigated to identify the one that best describe the plasma concentration-time profiles of zonisamide. The Michaelis-Menten equation was also used to test a capacity limited elimination process in which case, the maximum elimination rate (Vmax, mg/day) and the concentration required to achieve the half value of Vmax (K_M, mg/L) were estimated. Between-patient variability (BPV) was evaluated for all pharmacokinetic parameters and modelled exponentially, assuming a log-normal distribution. Residual variability was tested as additive, proportional, and combined additive/proportional error. Additionally, the influence of zonisamide binding to red blood cells prompted an examination of residual error correction through haematocrit and red blood cells count.

The log-likelihood ratio test was employed to compare the models under investigation, with a significance level set at $p < 0.005$ for nested models with 1 degree of freedom, corresponding to a minimum objective function value (MOFV) difference of 7.88. The models were also evaluated in terms of physiological plausibility of the pharmacokinetic parameters, assessed by comparing them with the previously reported values, and their precision of estimation, expressed as the relative standard error (RSE, %).

2.3.2. Covariate model

The covariates considered for inclusion in the model encompassed a range of factors: gender, age, BW, IBW, BSA, BMI, GFR, haematocrit and red blood cells. Each concomitant antiseizure drug underwent individual testing, followed by categorization into EIASDs (carbamazepine, oxcarbazepine, phenobarbital, and phenytoin) or non-enzyme-inducing antiseizure drugs (clobazam, clonazepam, diazepam, eslicarbazepine acetate, lacosamide, lamotrigine, levetiracetam, perampanel, topiramate, and valproic acid). For antiseizure drugs identified as significant, the potential impact of their dose was also investigated. Likewise, for the group of EIASDs, INDDL was also tested. Continuous variables were examined using allometric, linear, exponential, and power relationships, normalized by their median values as appropriate. Categorical variables were tested as power functions.

The potential effect of each covariate on the pharmacokinetic parameters of zonisamide was explored through a comprehensive analysis. This involved an initial univariate assessment followed by a stepwise forward inclusion/backward elimination procedure. In the forward inclusion step, a covariate was retained in the model if resulted in a reduction of the MOFV by 3.84 units (equivalent to a significance level of $p < 0.05$ for 1 degree of freedom). However, a covariate remained in the final model only if its removal in the backward elimination step increased the MOFV by 10.83 units (corresponding to a significance level of $p < 0.001$ for 1 degree of freedom) (Yamaoka, 1978; Olofsen and Dahan, 2015).

Furthermore, covariate inclusion was also carried out taking into account the physiological plausibility of the pharmacokinetic parameters, changes in the standard error of parameter estimates (RSE, %), reduction of between-patient variability associated with each pharmacokinetic parameter, the condition number estimate (square root of the ratio between the major to the minor eigenvalue) indicative of the degree of collinearity between parameters,

η and Ɛ shrinkages to know to what extent the diagnostic plots based on individual-predicted concentrations or residuals are sufficiently informative of true covariate-Pk parameters relationships. (Savic and Karlsson, 2009), and the visual inspection of goodness-of-fit plots. These plots included observed concentrations (OBS) *versus* population (PRED) and individual (IPRED) predicted concentrations, individual weighted residuals (IWRES) *versus* IPRED, and conditional weighted residuals (CWRES) *versus* time (Ette and Ludden, 1995; Karlsson and Savic, 2007).

2.3.3. Model evaluation

The stability and robustness of the final model was assessed through a non-parametric bootstrap with resampling (Ette, 1997; Ette et al., 2003; Efron, 1979). Accordingly, 100 additional datasets were created by randomly resampling the original dataset. Then the final model was repeatedly fitted to each set of data and the medians and the medians and 95% confidence interval (2.5% and 97.5% percentiles) of the bootstrap pharmacokinetic parameters were calculated and compared with the PK parameters estimated from the final model. A prediction-corrected visual predictive check was also conducted as part of evaluation process (Bergstrand et al., 2011). Thus, 1000 concentration-time data sets were simulated from the final model. Then the medians and the 2.5^{th} and 97.5^{th} percentiles of the normalised observed and simulated data based on the typical population prediction for the median independent variable of several bins taken into account, were compared.

2.3.4. Model-based simulations

The final covariate model was applied to assess the impact of the previously identified covariates on the relationship between dosing and plasma concentrations of zonisamide. Appropriately, Monte Carlo simulations were conducted to generate concentration-time profiles for various dosing regimens of zonisamide, including 100, 200, 300, 400, 500 and 600 mg administered once-daily (q24 h) and divided in two daily doses (q12 h). These doses and posology intervals were defined owing to the current dosing recommendations (5; Gidal et al., 2024).

Herein, INDDL was identified as influencing the CL/F of zonisamide. Consequently, for each zonisamide dosing regimen simulated the effect of various cut-offs of INDDL (0, 0.5, 1, 1.5, 2 and 2.5).

From each dosing regimen/INDDL cutoff, 1000 trough concentrations at steady state were simulated, considering the estimates of the final covariate model. Then the percentage of values as falling under, within, or above the therapeutic range (10 to 40 mg/L) established for zonisamide were calculated. Median values of trough concentrations for each combination of covariates were also calculated. Simulation results were displayed using boxplots.

2.4. Statistical analysis

The statistical analysis was conducted using R software version 4.1.3 (The R Foundation for Statistical Computing). Descriptive statistics were presented as absolute and relative frequencies for categorical variables, while continuous variables were summarized as medians and the corresponding ranges (minimum – maximum).

3. Results

3.1. Population characterization

A population pharmacokinetic model was developed using 114 plasma concentrations of zonisamide from 64 patients diagnosed with refractory epilepsy. The characteristics of the patients are detailed in Table 1. The cohort included 28 males (43.75%) and 36 females (56.25%) with a median age of 31.50 years (ranging from 18 to 74). The median BMI was 24.74 kg/m^2, with values ranging from 17.21 to 41.40 kg/m^2, covering categories from underweight to obesity (WHO Body Mass Index 2023). For haematocrit, the median for men was 43.55% (ranging from 36.90% to 50.80%) and for women, it was 39.15% (ranging from 32.70% to 43.90%). Approximately 90% of all patients had haematocrit levels within the normal range. The red blood cell count for men was a median of 4.91 $\times$ 10^{12}/L (ranging from 3.52 to 6.39 $\times$ 10^{12}/L) and for women, it was 4.46 $\times$ 10^{12}/L (ranging from 3.74 to 5.30 $\times$ 10^{12}/L), with over 90% of patients having counts within the normal range. Notably, most patients (92.19%) were on polytherapy, taking between one and five additional antiseizure drugs alongside zonisamide. The most frequently co-prescribed antiseizure drugs were levetiracetam (35.94%), carbamazepine (28.13%), clobazam (26.56%), eslicarbazepine acetate (23.44%), and valproic acid (23.44%).

Table 1
Demographic, clinical, and pharmacological characteristics of the patients included in the study. Results are presented as absolute and relative frequencies for categorical variables, and as median (range) for continuous variables.

Study feature	Property value
Number of patients	64
Number of concentrations	114
Sex: males/females, n (%)	28 (43.75%)/36 (56.25%)
Age (years)	31.50 (18.00 - 74.00)
Body weight (kg)	71.00 (45.00 - 110.00)
Height (cm)	165.00 (150.00 - 194.00)
Ideal body weight (kg)	58.27 (43.33 – 87.67)
Body surface area (m^2)	1.81 (1.40 – 2.30)
Body mass index (kg/m^2)	24.74 (17.21 – 41.40)
Glomerular filtration rate (mL/min/1.73 m^2)	109.44 (76.81 – 143.71)
Haematocrit (%)	40.80 (32.70 – 50.80)
Red blood cells (x 10^{12}/L)	4.60 (3.52 – 6.39)
Daily dose of zonisamide, n (%)	
25 mg	1 (1.56%)
50 mg	1 (1.56%)
100 mg	4 (6.26%)
200 mg	21 (32.81%)
300 mg	17 (26.56%)
400 mg	17 (26.56%)
500 mg	2 (3.13%)
600 mg	1 (1.56%)
Co-administered antiseizure drugs per patient, n (%)	
0	5 (7.81%)
1	15 (23.44%)
2	24 (37.50%)
3	13 (20.31%)
4	6 (9.38%)
5	1 (1.56%)
Concomitant antiseizure drugs, n (%)	
Carbamazepine	18 (28.13%)
Clobazam	17 (26.56%)
Clonazepam	9 (14.06%)
Diazepam	3 (4.69%)
Eslicarbazepine acetate	15 (23.44%)
Lacosamide	2 (3.13%)
Lamotrigine	5 (7.81%)
Levetiracetam	23 (35.94%)
Oxcarbazepine	6 (9.38%)
Perampanel	12 (18.75%)
Phenobarbital	2 (3.13%)
Phenytoin	1 (1.56%)
Pregabaline	2 (3.13%)
Topiramate	1 (1.56%)
Valproic acid	15 (23.44%)

3.2. PopPK modelling and model evaluation

Concentration-time profiles of zonisamide were better described by a one-compartment model with first order absorption and elimination than by a two-compartment with first order absorption and elimination and a one-compartment with Michaelis-Menten elimination models. BPV was included on ka, Vd/F and CL/F, and the residual variability was best modelled as a proportional error.

The univariate analysis of the anthropometric covariates suggested a statistically significant effect of BW and BMI on the CL/F. BMI resulted in a higher reduction of MOFV than BW (Supplementary materials: Table S1). Regarding pharmacotherapeutic-related covariates, the univariate analysis also identified a statistically significant influence of CBZ, LEV, OXC, PHT, PB, the group of EIASDs (IND), as well as CBZ DD and INDDL on the CL/F of zonisamide. The inclusion of INDDL had a greater decrease in the MOFV and BPV of CL/F than the other inducer-related covariates (CBZ, OXC, PHT, PB, IND and CBZ DD) (Supplementary materials: Table S1). Fig. 1 displays the effect of INDDL on zonisamide CL/F individual values estimated from the base model. Accordingly, the CL/F of zonisamide is positively related with the INDDL. None of the covariates tested revealed to have a significant impact on the Vd/F of zonisamide. Accordingly, INDDL, LEV and BMI were selected to be included as covariates on CL/F of zonisamide in the final model. After the procedures of stepwise forward inclusion and backward elimination, of the selected covariates, only INDDL was retained in the final model (Supplementary materials: Table S2).

The goodness-of-fit plots for the final model are depicted in Fig. 2. The PRED concentration *versus* OBS concentrations spread randomly around the identity line (Fig. 2a) while CWRES *versus* time uniformly spread around the zero line (Fig. 2d), suggesting no model misspecification. In addition, IPRED concentrations *versus* OBS concentrations scattered around the identity line (Fig. 2b) as well as IWRES *versus*

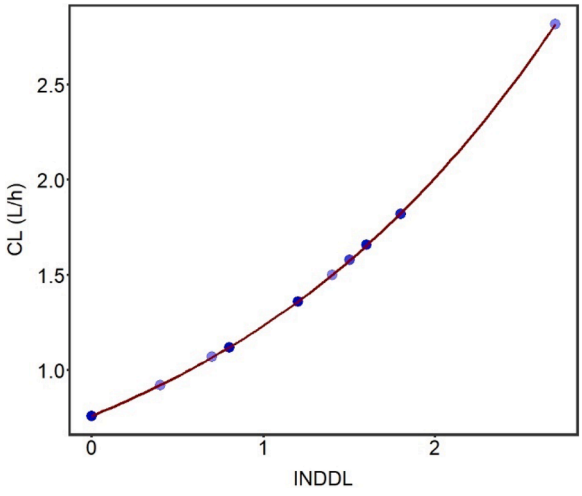

Fig. 1. Effect of inducer drug load on the clearance of zonisamide. CL/F, clearance (L/h); INDDL, inducer drug load. The red line represents a smoothed trend of the data.

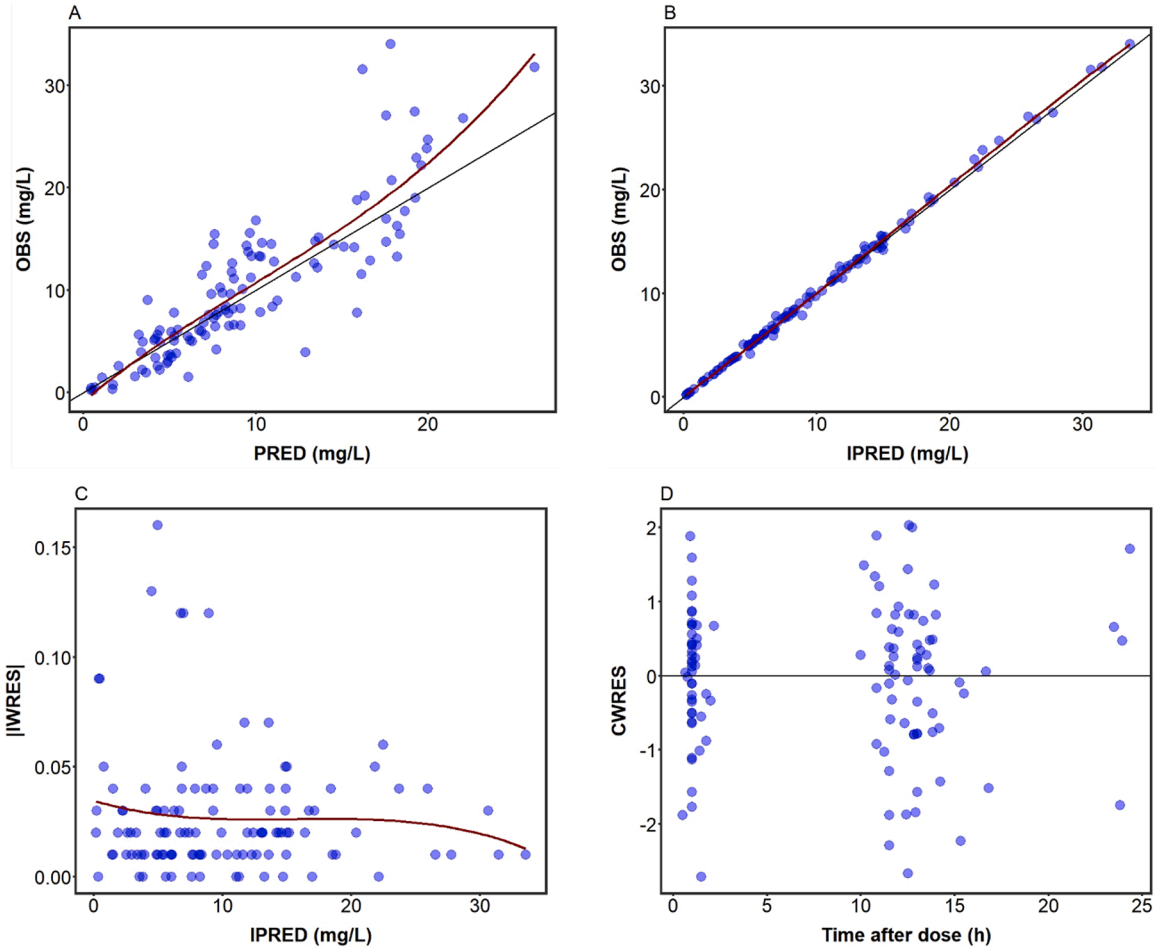

Fig. 2. Goodness-of fit plots of the final model: (A) population predicted concentrations *versus* observed concentrations, (B) individual predicted concentrations *versus* observed concentrations, (C) absolute individual weighted residuals *versus* individual predicted concentrations, and (D) conditional weighted residuals *versus* time after dose. CWRES, conditional weighted residuals; IPRED, individual predicted concentrations (mg/L); OBS, observed concentrations (mg/L); PRED, population predicted concentrations (mg/L); |IWRES|, absolute individual weighted residuals. The black line represents the line of identity, and the red line is a data smoother.

IPRED uniformly spread around zero (Fig. 2c), suggesting an adequate description of residual error. The value of the observed Ɛ-shrinkage was 49.49% and η-shrinkage associated to the CL/F, Vd/F and ka were 13.63%, 37.48% and 55.06%, respectively.

The parameter estimates of the base and final models as well as the bootstrap results are summarized in Table 2. The inclusion of the covariate explained the 16% of BPV associated with CL/F. The condition number of the model was 6.29, suggesting no notable collinearity.

All parameter estimates of the final model were found within the 95% confidence intervals estimated by the bootstrap method. The relative deviation between the estimated population value and the median value provided by bootstrap was less than 12% for all final parameters (Table 2). Prediction-corrected VPC showed that the 2.5%, 50%, and 97.5% percentiles of the observed concentrations were within the 95% prediction intervals of the corresponding percentiles estimated from simulated data, suggesting that the final model can acceptably predict the distribution of the observed plasma concentrations of zonisamide (Fig. 3).

Table 2
Population parameter estimates for the structural base and final models, along with bootstrap results.

Parameter	Base model		Final model		Bootstrap		Relative deviation (%)
	Estimate	RSE (%)	Estimate	RSE (%)	Median	95% CI	
TVCL/F (L/h)	1.01	8.24	0.761	12.48	0.756	0.449–0.919	0.66
TVVd/F (L)	44.30	21.22	48.10	23.28	49.04	31.14–77.02	−1.95
TVka (h^{-1})	0.599	30.72	0.671	30.40	0.636	0.272–5.866	5.22
INDDL$_{CL}$	–	–	1.63	11.90	1.65	1.33–2.27	−1.23
BPV$_{CL/F}$ (%)	52.25	54.20	43.93	54.16	43.01	26.27–58.99	2.09
BPV$_{Vd/F}$ (%)	58.40	63.38	52.06	76.35	50.00	16.43–75.10	3.96
BPV$_{ka}$ (%)	85.73	72.38	91.27	72.93	90.44	31.78–213.03	0.91
RE$_{proportional}$ (%)	7.77	64.61	7.18	86.64	8.00	3.66–12.88	−11.42

BPV_{CL}, between patient variability associated with clearance; BPV_{ka}, between patient variability associated with absorption rate constant; $BPV_{Vd/F}$, between patient variability of volume of distribution; INDDL, effect of inducer drug load on clearance; $RE_{proportional}$, proportional residual error; RSE, relative standard error; TVCL/F, typical value of clearance; TVka, typical value of absorption rate constant; TVVd/F, typical value of volume of distribution; 95% CI; 95% confidence interval. Relative deviation = [(Typical value of the parameter – Median of the parameter obtained by bootstrap)/ Typical value of the parameter)] x 100.

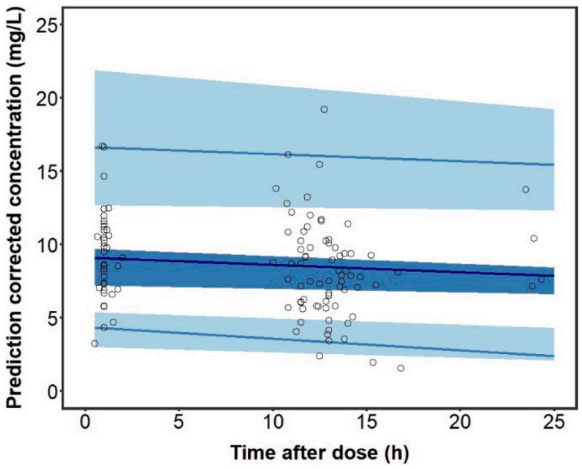

Fig. 3. Prediction-corrected visual predictive check of the final model. The lines represent, from bottom to top, the 2.5th, 50th and 97.5th percentiles of zonisamide plasma concentrations observed. Dark blue shading displays the simulated based 95% confidence intervals for the 50th percentile and light blue shading the 2.5th and 97.5th percentiles. The dots represent the prediction-corrected plasma concentrations of zonisamide (mg/L) at the respective time after dose administration.

3.3. Model-based simulations

Figs. 4 and 5 show the distribution of simulated trough plasma concentrations of zonisamide for once-daily and twice-daily regimens, respectively. Tables 3 and 4 display the percentages of trough plasma concentrations within the established therapeutic range for zonisamide (10 – 40 mg/L) and the corresponding median value of trough plasma concentrations, respectively. As expected, median trough concentration values increased with the dose and decreased with the INDDL value. In addition, trough concentration values were higher for twice daily administration compared to once daily at the same dose and INDDL value.

For patients not taking EIASDs (INDDL = 0) and under the once daily regimen, the percentage of target achievement increased from 69.8% to 80.2% for doses of 300 to 500 mg. At the dose of 600 mg some values exceeded the upper limit of the therapeutic range this leading to a lower percentage of target achievement (71.9%). Similarly occurred with the twice daily regimen, but achieved percentages were slightly higher than in the once daily administration (Table 3). Moreover, doses of 100 and 200 mg once daily provided median trough concentration values under the therapeutic range (4.84 and 9.40 mg/L, respectively) meanwhile, in the case of the twice daily administration only the median value of trough concentrations at the dose of 100 mg was under the lower limit of the therapeutic range (Table 4).

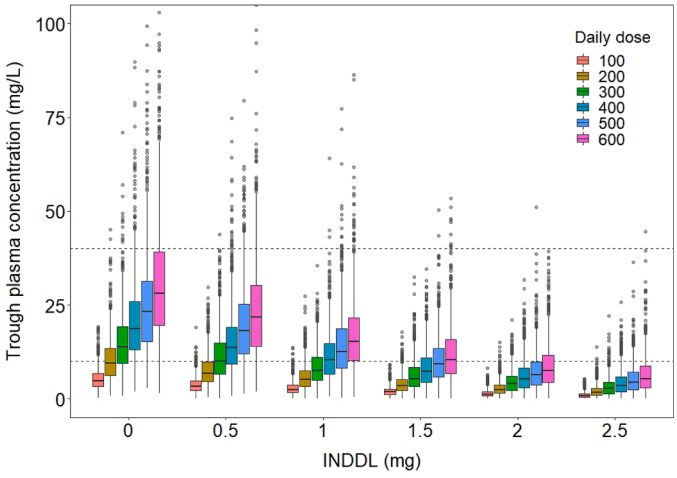

Fig. 4. Simulated plasma concentrations (mg/L) for once-daily (q24 h) zonisamide doses of 100, 200, 300, 400, 500, and 600 mg, considering various inducer drug load cut-offs (0, 0.5, 1, 1.5, 2, and 2.5). INDDL, inducer drug load. The horizontal dashed lines delimit the therapeutic range for zonisamide (10–40 mg/L).

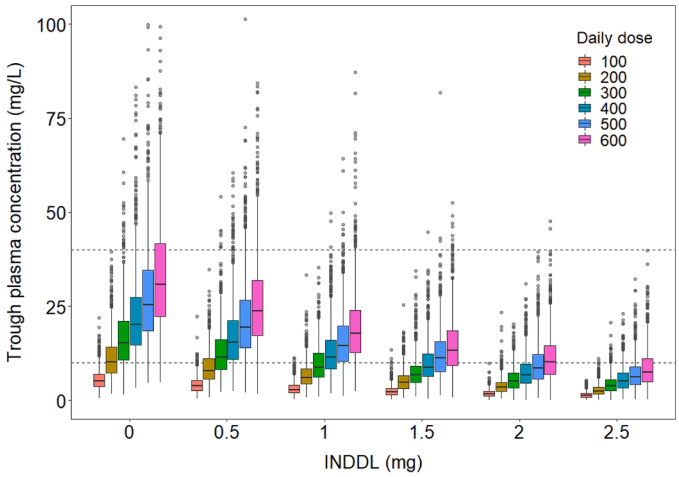

Fig. 5. Simulated plasma concentrations (mg/L) for daily zonisamide doses of 100, 200, 300, 400, 500, and 600 mg, administered in two daily doses (q12 h), considering various inducer drug load cut-offs (0, 0.5, 1, 1.5, 2, and 2.5). INDDL, inducer drug load. The horizontal dashed lines delimit the therapeutic range for zonisamide (10–40 mg/L).

Table 3
Percentages of simulated steady-state trough plasma concentrations of zonisamide within the therapeutic range (10–40 mg/L) for various daily doses (100, 200, 300, 400, 500, and 600 mg) at different inducer drug load (INDDL) levels. Values in bold indicate percentages ofsteady-state trough plasma concentrations that fall within the therapeutic range higher than 80%.

Posology	INDDL	Total daily dose (mg)					
		100	200	300	400	500	600
Once daily (q24h)	0	7.0	45.1	69.8	**81.5**	**80.2**	71.9
	0.5	1.3	23.6	51.0	68.6	77.5	79.2
	1	0.7	10.5	31.8	51.9	63.3	73.1
	1.5	0	3.0	15.1	31.0	44.9	51.4
	2	0	0.6	5.5	16.3	24.4	34.0
	2.5	0	0.3	1.9	6.0	11.2	18.1
Twice daily (q12h)	0	6.8	51.2	76.3	**84.4**	79.8	70.0
	0.5	1.6	32.1	60.2	76.8	**82.3**	**81.5**
	1	0.3	14.4	42.2	62.3	76.0	**82.2**
	1.5	0.3	5.5	21.8	41.1	58.0	68.9
	2	0	1.7	9.0	23.2	38.3	50.9
	2.5	0	0.2	3.1	10.3	19.1	30.4

INDDL, inducer drug load; q12 h, *quaque* 12 h (administered every 12 h); q24 h, *quaque* 24 h (administered every 24 h).

For patients taking EIASDs (INDDL > 0), regardless of the INNDL, the percentage of trough plasma concentrations within the therapeutic range (Table 3) and the corresponding median trough plasma concentrations (Table 4) increased with daily doses from 100 to 600 mg for both once- and twice-daily regimens. However, within the dose regimen, the percentages decreased as INNDL increased. The dosing regimens that provided more than 80% of trough plasma concentrations were 250 mg twice daily for patients with INNDL = 0.5, and 300 mg twice daily for patients with INDDL = 1 and 1.5.

It is worthy to note that for patients of the once daily regimen not taking EIASDs (INDDL = 0), a less increase with doses (from 200 to 400 mg) was observed in the percentages of trough plasma concentrations within the therapeutic range, compared to patients of the twice daily

Table 4
Median steady-state trough plasma concentrations of zonisamide simulated for various daily doses (100, 200, 300, 400, 500, and 600 mg) at different inducer drug load (INDDL) levels. Values in bold indicate median steady-state trough plasma concentrations that fall within the therapeutic range (10–40 mg/L).

Posology	INDDL	Total daily dose (mg)					
		100	200	300	400	500	600
Once daily (q24h)	0	4.84	9.40	**13.74**	**18.62**	**23.19**	**28.10**
	0.5	3.25	6.82	**10.08**	**13.62**	**18.11**	**21.80**
	1	2.50	5.17	7.50	**10.41**	**12.59**	**15.30**
	1.5	1.83	3.47	5.4	7.34	9.28	**10.40**
	2	1.22	2.45	3.99	5.36	6.35	7.55
	2.5	0.88	1.77	2.70	3.57	4.46	3.35
Twice daily (q12h)	0	5.18	**10.14**	**15.25**	**20.11**	**25.41**	**30.80**
	0.5	3.86	7.87	**11.50**	**15.47**	**19.51**	**23.80**
	1	2.86	6.11	8.84	**11.54**	**14.62**	**17.85**
	1.5	2.26	4.75	6.72	8.83	**11.28**	**13.25**
	2	1.67	3.47	5.07	6.73	8.58	**10.15**
	2.5	1.29	2.49	3.84	5.09	6.20	7.45

INDDL, inducer drug load; q12 h, *quaque* 12 h (administered every 12 h); q24 h, *quaque* 24 h (administered every 24 h).

regimen. Similarly occurred with median through concentration values. Indeed, a more marked increase occurred in the last case both in the percentages and median trough concentrations (Table 3).

4. Discussion

Zonisamide exhibits significant pharmacokinetic variability, leading to unpredictable circulating drug concentrations following a prescribed dosing regimen (Kimura et al., 1992; Shinoda et al., 1996; Fukuoka et al., 2004; Hashimoto et al., 1994; Okada et al., 2008; Qiu et al., 2016). The findings from the present study confirm this high variability (Table 2), with notable BPV observed in CL/F (≈ 44%), Vd/F (≈ 52%), and ka (≈ 91%). To address this issue, it is advisable to implement dosing strategies that personalize treatment by tailoring drug posology to the specific characteristics of each patient (Patsalos et al., 2018; Landmark et al., 2016).

In this study, a PopPK model for zonisamide was developed and validated in a cohort of adult European patients with refractory epilepsy. The concentration-time profiles of zonisamide of the population herein analysed were best described by a one-compartment model with first-order absorption and elimination. This finding aligns with the study by Okada et al. (Okada et al., 2008), which also employed a one-compartment model to describe the pharmacokinetics of zonisamide. However, it contrasts with the study by Qiu et al. (Qiu et al., 2016), which described the pharmacokinetics of zonisamide using a two-compartment model with first-order absorption and elimination. This discrepancy may be attributed to differences in sampling patterns. The present study used sparse concentration-time data from routine TDM, obtained approximately at one hour after zonisamide intake and immediately before the following administration. In contrast, Qiu et al. (Qiu et al., 2016) developed their model from intensive sampling pharmacokinetic profiles, which allowed a more detailed characterization of the distribution phase.

In paediatric patients, the pharmacokinetics of zonisamide was better described using a one-compartment model with Michaelis-Menten elimination, based on the assumption that the clearance of zonisamide is dose-dependent (Hashimoto et al., 1994). Accordingly, daily doses of zonisamide normalized by weight and the corresponding plasma concentrations follow a linear relationship until approximately 12 mg/kg. In contrast, our study focused on adult patients receiving daily doses ranging from 0.4 to 9.1 mg/kg, which fall within the linear pharmacokinetic range and do not suggest a transition to a Michaelis-Menten saturable elimination model.

Zonisamide exhibits a dose-proportional increase of areas under the concentration-time curve within the usually employed multiple daily doses of 100 to 400 mg. However, at doses exceeding 800 mg, zonisamide pharmacokinetics becomes nonlinear, possible due to the saturation of red blood cells (Leppik, 2004; Holder and Wilfong, 2011). In the present study, patients received daily doses ranging from 25 to 600 mg, with 61 (95.31%) patients receiving daily doses up to 400 mg, 2 (3.13%) patients receiving 500 mg, and 1 (1.56%) patient receiving 600 mg (Table 1). Consequently, all patients remained within the linear pharmacokinetic range for zonisamide, preventing the evaluation of non-linear models, such as the Michaelis-Menten model. Moreover, to verify the lack of dose-dependent pharmacokinetics of zonisamide, the daily dose of the drug was also tested as a covariate for CL/F and Vd/F. No significant effect was observed, confirming that all patients remained within the linear pharmacokinetic range for zonisamide.

The typical values for the CL/F and Vd/F of zonisamide estimated in this study were 0.761 L/h (12.68 mL/min) and 48.10 L (0.69 L/kg) for a typical 71-kg adult patient, respectively. Variations between the findings of this study and those reported in previous research can be attributed to differences in the populations studied, sampling strategies, and structural pharmacokinetic models employed, which limit direct comparability.

Regarding CL/F, the value observed in this study was lower than the typical value reported by Okada *et al.* (Okada et al., 2008) (1.22 L/h) in a population of Japanese patients with epilepsy, as well as the value reported by Qiu *et al.* (23.25 mL/min) in a population of healthy Chinese volunteers. These discrepancies may be attributed to differences in the studied populations, including factors such as ethnicity, health status, and other demographic or clinical characteristics.

For Vd/F, the value observed in this study was higher than that reported by Hashimoto *et al.* (Hashimoto et al., 1994) (1.27 L/kg for a typical 33-kg patient) in a population of pediatric patients with epilepsy. This difference is likely due to physiological distinctions between pediatric and adult populations, such as variations in body composition, organ development, and metabolic capacity, all of which influence pharmacokinetics. Similarly, Qiu *et al.* (Qiu et al., 2016) reported a central Vd/F of 34.50 L and a peripheral Vd/F of 12.19 L in a study of healthy Chinese volunteers, using dense sampling and a two-compartment model. The differences in demographic characteristics (*e.g.*, ethnicity and health status), as well as the use of a different structural model and sampling strategy, likely contribute to the observed discrepancies in Vd/F values.

In the model building workflow, several covariates with the potential to affect zonisamide pharmacokinetics, including INDDL, LEV, and BW, were evaluated for inclusion in the final model (Supplementary materials: Tables S1 – S2). The co-administration of EIASDs, represented by INDDL, was identified as a primary covariate influencing BPV associated with CL/F. The inclusion of this covariate resulted in a 16% reduction in BPV associated with the CL/F of zonisamide (Table 2).

The effect of EIASDs on the CL/F of zonisamide is supported by its CYP-mediated hepatic metabolism, involving CYP3A4, CYP2C19 and CYP3A5. Antiseizure drugs such as carbamazepine, oxcarbazepine, phenytoin, and phenobarbital are known inducers of these enzymes. Their co-administration increases the elimination of drugs like zonisamide, which are substrates of these enzymes, thereby reducing their plasma concentrations. Indeed, significant decreases in concentration/dose ratios have been reported in the presence of carbamazepine, phenytoin, and phenobarbital, indicating lower zonisamide concentrations at the same daily doses (Kimura et al., 1992; Shinoda et al., 1996; Fukuoka et al., 2004; Wallander et al., 2014). In this context, an increase of 13% in the maximum metabolic capacity was observed in patients receiving concomitant treatment with zonisamide and carbamazepine (Hashimoto et al., 1994). Complementarily, Okada *et al.* (Okada et al., 2008) found significant effects from the concomitant administration of carbamazepine, phenytoin, and phenobarbital, leading to their inclusion as covariates in their final PopPK model. These drugs increased the CL/F of zonisamide by 24%, 28%, and 29%, respectively.

In the present study, the effect of EIASDs was included as a

continuous covariate, represented by INDDL. Briefly, INDDL measures the combined effect of taking multiple EIASDs, considering both the presence and the dose of each drug. This helps to understand how these drugs, when co-administered at distinct doses, can affect zonisamide elimination and, consequently, the plasma concentrations of other drugs like zonisamide. The INDDL was calculated including carbamazepine, oxcarbazepine, phenytoin and phenobarbital. Although eslicarbazepine is structurally related to carbamazepine and oxcarbazepine and has been shown to reduce the exposure to simvastatin (a CYP3A4 substrate) (Falcão et al., 2013), its inducer effect on zonisamide elimination has not been established (Bialer and Soares-Da-Silva, 2012). The findings of this study suggest that eslicarbazepine does not exert an inducer effect on zonisamide elimination, despite the involvement of CYP3A4 in their metabolism. Specifically, in the univariate analysis, eslicarbazepine and other concomitant antiseizure drugs were evaluated as binary covariates (presence *vs*. absence). Unlike carbamazepine, oxcarbazepine, phenobarbital or phenytoin, which coadministration significantly reduced the MOFV (by 3.84 units), eslicarbazepine decreased MOFV only 0.98 units.

Herein, the inducer effect was found to be directly related to INDDL (Fig. 1). For instance, the CL/F of zonisamide enhanced approximately 28%, 108%, and 239% with INDDL values of 0.5, 1.5, and 2.5, respectively. These substantial increases in zonisamide CL/F corroborate the significant impact that EIASDs can have on its pharmacokinetics, emphasizing the need for careful dose adjustment in patients receiving these combinations to avoid suboptimal circulating drug levels.

Apart from INDDL, other covariates that might affect the pharmacokinetics of zonisamide were tested but did not show a significant impact and were therefore not included in the final model. However, specific attention should be given to BW/BMI, and haematocrit/red blood cell count. Although BW and BMI demonstrated a significant impact ($p < 0.05$) on the CL/F of zonisamide during the univariate analysis, this was not confirmed during the stepwise forward inclusion procedure, preventing their inclusion in the final model.

Zonisamide is highly bound to red blood cells, so variations in haematocrit and red blood cells are expected to influence its Vd/F and CL/F. A lower red blood cell count, or a low haematocrit would likely increase the free drug fraction, leading to a higher Vd/F referred to total drug concentrations. Conversely, given the high bioavailability (> 90%) of zonisamide and its reported clearance values (0.761 L/h in the present study), its extraction rate can be considered low. This implies minimal loss during each pass through the liver, consistent with the drug's long half-life (≈ 60 h). For drugs with low extraction rates or restrictive clearance, an increase in the free drug fraction typically leads to a higher CL/F when referred to total drug concentrations. However, the clearance of the free drug (which is directly related to the drug's pharmacodynamic effects) remains unchanged. In this study, when red blood cells count and haematocrit were tested as covariates for Vd/F and CL/F, no significant impact was observed. This lack of significant impact is probably because more than 90% of the enrolled patients presented values of haematocrit (median: 40.80%) and red blood cell count (median: 4.60×10^{12}/L) within the normal clinical range (32.70 – 50.80% and $3.52 – 6.39 \times 10^{12}$/L, respectively).

The internal evaluation results demonstrated stability and robustness of the model (Table 2 and Fig. 3), supporting its use in clinical practice for designing dose regimens that ensure effective and safe plasma concentrations of zonisamide in patients with refractory epilepsy. Consequently, this PopPK model can be applied for both *a priori* dosing regimen design through a model-informed precision dosing strategy and *a posteriori* adjustment of zonisamide dosing regimens using a Bayesian approach.

The final covariate model was employed to perform Monte-Carlo simulations and identify the most suitable dosing regimen of zonisamide for refractory epileptic patients, considering both dose and dosing interval. Concentration-time profiles of zonisamide were simulated for daily doses ranging from 100 to 600 mg, accounting for various inducer drug loads from 0 to 2.5, and both once (q24 h) and twice-daily (q12 h) regimens. Trough plasma concentrations were considered not only because they are recognized as predictive markers for zonisamide response but also because the efficacy and tolerability of zonisamide are directly related to its systemic exposure, with the established therapeutic range being 10 to 40 mg/L (Patsalos et al., 2018; Patsalos et al., 2008).

It is of note that none of the dosing regimens herein tested provided high rates (≥90%) of trough plasma concentrations of zonisamide within the therapeutic range. As can be observed in Table 3, Fig. 4 and Fig. 5, for every dosing regimen tested, at least 10% of patients exhibited plasma concentrations outside the therapeutic range. This can be attributed to the high variability in the pharmacokinetic parameters of zonisamide, underscoring the need for individualized dosing strategies that consider patient-specific characteristics.

The results of simulations demonstrated that 400 mg administered in one or two daily doses and 500 mg administered once daily provided the highest rates of trough plasma concentrations of zonisamide within the therapeutic range (Table 3), suggesting that they are the most indicated for patients not taking enzyme-inducing antiseizure drugs.

Dividing the dose into two daily doses slightly decreased the rates of plasma concentrations within the therapeutic range for daily doses of 100, 500, and 600 mg, and slightly increased the rates for daily doses of 200, 300, and 400 mg (Table 3). This can be explained by the observed half-life in this group of patients, which was approximately 44 h, indicating that dividing the dose does not significantly impact drug concentrations. Therefore, patients not taking EIASDs do not benefit from a twice daily regimen.

Regarding patients taking EIASDs, both the percentage of trough plasma concentrations within the therapeutic range (Table 3) and the corresponding median trough plasma concentrations of zonisamide (Table 4) decreased as INDDL increased, regardless the daily dose of zonisamide administered. Accordingly, as the INDDL increases, lower is the probability to attain the trough plasma concentrations of zonisamide within the therapeutic range. The highest rates of trough plasma concentrations within the therapeutic range were obtained for daily doses of 500 and 600 mg, administered in two daily doses (Table 3 and Fig. 5), indicating these are the most suitable dosing regimens for this group of patients.

Currently, there are no recommendations for adjusting the maintenance dosing regimens of zonisamide in patients who are co-prescribed with EIASDs (5; Gidal et al., 2024). However, the results of the simulations herein reported suggest that patients taking EIASDs are more likely to have zonisamide trough plasma concentrations outside the therapeutic range, particularly below the range, compared to those not taking these drugs. This difference can be attributed to the shorter half-lives observed in patients on EIASDs. As a shorter half-life leads to a faster decline in drug concentrations, patients on EIASDs may require higher daily doses of zonisamide and shorter dosing intervals than those not receiving EIASDs. Indeed, in the group of patients taking EIASDs, dividing the daily dose into two doses increased the rate of trough plasma concentrations of zonisamide within the therapeutic range compared to the group without EIASDs (Table 3). Therefore, patients co-administered with EIASDs are expected to benefit from higher doses and twice-daily dosing regimens.

Notably, whether on a once-daily or twice-daily regimen, some patients, will require daily doses of zonisamide of 600 mg or more, exceeding the recommended daily dose of 500 mg. Although these doses are higher than those indicated by the marketing authorization holder they are likely to achieve plasma concentrations within the therapeutic range and therefore, not associated with adverse effects such as dizziness, somnolence, confusion, weight loss, or even anorexia.

Regarding the use of high daily doses of zonisamide in refractory epileptic patients, Velizarova *et al.* (Velizarova et al., 2014) examined its clinical effects as adjunctive therapy in a group of 13 adult patients with refractory juvenile absence epilepsy. Accordingly, all patients experienced seizure reduction. While nine patients responded to daily doses

ranging from 150 to 400 mg, four patients achieved seizure reduction with daily doses of 550 to 600 mg, without reported adverse effects. Similarly, Miro *et al.* (Miro et al., 2016) explored the clinical effects of high doses of zonisamide (600 to 700 mg/day) as add-on therapy in a cohort of highly refractory epileptic patients. These high daily doses of zonisamide demonstrated to be effective and well-tolerated in approximately half of the patients. Notably, in the two patients receiving daily doses of 600 and 700 mg where plasma concentrations were measured, the levels were comfortably within the therapeutic range.

5. Conclusion

The present study demonstrated that the pharmacokinetics of zonisamide exhibit significant variability, resulting in unpredictable drug concentrations after a predefined prescribed dosing regimen. This variability is primarily driven by the concomitant administration of EIASDs, significantly enhancing the clearance of zonisamide. As a result, patients co-prescribed with EIASDs require higher doses of zonisamide to achieve therapeutic plasma concentrations compared to those not on such drugs.

To the best of our knowledge, this is the first PopPK model developed and validated in a population of adult European patients diagnosed with refractory epilepsy. The model demonstrated to accurately predict plasma concentrations of zonisamide and foresee the best posology for each patient. These findings underscore the potential clinical utility of the model in tailoring dosing regimens for patients with refractory epilepsy, thereby improving therapeutic outcomes.

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Ethics approval statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Faculty of Medicine of University of Coimbra, Coimbra, Portugal (CE-061/2018) on the 23 July 2018 and by the Ethics Committee of CHUC, EPE (CHUC-144-18) on the 3 July 2019.

Patient consent statement

Informed consent was obtained from all subjects involved in the study.

CRediT authorship contribution statement

Rui Silva: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Helena Colom:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Anabela Almeida:** Validation, Supervision, Methodology. **Joana Bicker:** Writing – review & editing, Investigation. **Andreia Carona:** Investigation. **Ana Silva:** Investigation. **Francisco Sales:** Resources, Investigation. **Isabel Santana:** Investigation. **Amílcar Falcão:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Formal analysis, Conceptualization. **Ana Fortuna:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2025.107023.

Data availability

The data that has been used is confidential.

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