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ORIGINAL ARTICLE

An innovative ethosuximide granule formulation designed for pediatric use: Comparative pharmacokinetics, safety, tolerability, and palatability profile versus reference syrup

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Abstract

Ethosuximide, the first-line therapy for childhood absence epilepsy, is currently formulated as a syrup (Zarontin®, Pfizer) with a bitter taste and high sugar content, poorly adapted to children, and a ketogenic diet. The collaborative European FP7 project KIEKIDS aimed at developing an innovative sugar-free, tasteless formulation convenient for pediatric use. This dual Phase-I study evaluated two granule formulations based on lipid multiparticulate (LMP) technology. Two panels of 6 healthy adult volunteers underwent a randomized, placebo-controlled, partly blinded, 3-way cross-over trial, comparing ethosuximide granules A or B with placebo granules and syrup at single 10 mg/kg doses. Corresponding plasma pharmacokinetic profiles of ethosuximide were compared, along with palatability, safety, and tolerability. The LMP granule A proved suboptimal due to bitterness and adherence to beaker walls, while the optimized granule B revealed excellent palatability, similar to placebo granules, and low adherence to glass. The relative bioavailability of granules A versus syrup, based on dose-normalized C_{max} and $AUC_{0-\infty}$ was 93.7% [90% CI: 76.3–115.1] and 96.1% [91.0–101.5], respectively. For granules B it was 87.6% [81.6–94.0] and 92.5% [88.5–96.6], respectively, with slightly delayed t_{max} of 0.75 h [0.5–4.05] compared to syrup 0.5 h [0.3–0.8]. Tolerability visual analog scales revealed a trend for statistically non-significant improvement versus syrup at peak (30 min) for transient dizziness (both granules), fatigue (granules A), and anxiety (granules B). The innovative ethosuximide granule formulation B achieves a suitable

Abbreviations: AUC, Area under the curve; CHUV, Centre Universitaire Hospitalier Vaudois; CL, clearance; CNS, central nervous system; EMA, European Medicines Agency; FDA, Food and Drug Administration; GI, gastrointestinal; LMP, lipid multiparticulate; LOQ, limit of quantification; PEG, polyethylene glycol; PIP, Pediatric Investigation Plan; UV, ultraviolet; VAS, visual analog scales.

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profile for pediatric use, being sugar-free, tasteless, bioequivalent, and well-tolerated while enabling precise adjustment to body weight.

KEYWORDS
bioequivalence, epilepsy, paediatrics, pharmacokinetics, phase I

1 | INTRODUCTION

Absence seizures account for approximately 10%–12% of seizures in children with epilepsy.[1] Ethosuximide, an antiepileptic belonging to the succinimide class, is a mainstay for initial monotherapy in childhood absence epilepsy. It proved to be better tolerated than valproate and more effective than lamotrigine, the other two alternatives as monotherapy in childhood absence epilepsy.[2–5] Interestingly, ethosuximide is devoid of the increased risk for hepatic toxicity, hair loss, and weight gain associated with valproate, while being associated with better behavioral outcomes.[6] It can also be used as monotherapy in juvenile absence epilepsy and as adjunctive therapy in other pediatric epilepsy syndromes with absence and/or myoclonic seizures, such as myoclonic absence epilepsy, Dravet syndrome, or Continuous Slow Waves during Sleep.

However, the currently available syrup formulation (Zarontin®, 50 mg/ml, Pfizer) is not adapted to the needs of pediatric epileptic patients. It contains high sugar concentrations (0.6 g saccharose per ml), which is in particular contra-indicated when setting up a ketogenic diet as an additional therapeutic approach.[7,8] The lack of alternative pediatric formulation results in restricted use of ethosuximide in clinical practice, which is in clear contradiction with expert consensus guidelines.[9] Furthermore, Zarontin® syrup has been withdrawn from several countries, including Switzerland, probably as a consequence of its very bitter persistent metallic taste and high sugar content limiting its use. Ethosuximide capsules (250 mg, Pfizer and generics) are available from various suppliers worldwide, but capsules cannot be used in children below 12 years and lack flexibility for adapting doses to body weight. Finally, marketed ethosuximide oral solutions with sweetener and glycerol, despite the addition of sucrose, exhibit a bitter and persistent taste, which affects compliance.

There was consequently a clear need to develop a sugar-free and age-adapted formulation of ethosuximide enabling flexible dose adaptation of ethosuximide to children between 2 and 17 years, considered to be a high priority for the treatment of absence seizures by the European Medicines Agency (EMA). It was under these auspices that the KIEKIDS project was funded by the European Union's Seventh Framework Programme (FP7) under grant agreement n°282 559.[10]

Bearing in mind the marked and persistent bitterness of ethosuximide, palatability was selected as the criterion of choice for a new sugar-free, flexible, and age-adapted formulation which should also enable to investigate accurately pharmacokinetic (PK) bioequivalence in adult volunteers. The best option in terms of pros and cons analysis was finally considered to be a tasteless granule formulation.

As part of the Pediatric Investigation Plan (PIP) validated by the EMA's Pediatric Committee (PDCO), this study compared the bioequivalence and palatability of two novel pediatric granule formulations of ethosuximide versus the already marketed Zarontin® syrup and placebo granules with a partially blinded study design.

2 | MATERIALS AND METHODS

2.1 | Subjects and study design

Eligible subjects for the clinical study were healthy adult male and female volunteers aged between 18 and 45 years, with a body mass index of 18–29 kg/m^2. Subjects with a history or evidence of clinically significant disease or conditions were excluded. Other exclusion criteria were: clinically significant laboratory abnormality including serology for hepatitis and HIV, relevant alcohol or drug abuse, pregnancy, recent acute illness, or use of medication the week prior to the study.

The study (EudraCT 2013-004687-61) was carried out in the Clinical Pharmacology Service at the University Hospital in Lausanne, Switzerland, according to the Declaration of Helsinki and current national regulations. The protocol and its amendments were approved by the local Independent Ethics Committee and the national regulatory authority (Swiss Agency for Therapeutic Products, Swissmedic, authorization number 2014DR1002) under the sponsorship of the Centre Universitaire Hospitalier Vaudois (CHUV).

The first panel (A) consisted of a three-way cross-over, randomized, 10 mg/kg single oral dose for the novel granule formulation A versus placebo (both double-blinded) and 10 mg/kg single oral dose for the reference drug Zarontin® syrup (open-label), to assess its PK profile and palatability, in 6 healthy adult volunteers. During each period, plasma samples were collected pre-dose, at 10, 20, 30, 45 min, 1, 2, 3, 4, 6, 9, 24, 48, 72, 96, 168, 336, and 504 h post-dose. Concomitant saliva samples were collected using Quantisal™ collection devices (Immunoanalysis) except at early time points (saliva collection starting 1 h post-dose).

A second panel (B) was performed to investigate carefully the optimized granule formulation B versus placebo granules and Zarontin® syrup to assess its PK profile and palatability, with the same three-way crossover design with 10 mg/kg doses and endpoints selected in panel A, however without collecting saliva samples.

Plasma and saliva samples in Nunc tubes were stored at −80 °C until analysis.

2.2 | Formulation development

A new granule formulation (ADV6770) based on lipid multiparticulate (LMP, a patented technology from Capsugel, France, now part of Lonza, Switzerland), was developed by Advicenne Pharma to

address the identified needs in a pediatric population treated with ethosuximide. The technology produces LMP using a high-shear mixer allowing a single pot pelletization process.

In view of developing the ethosuximide LMP, several lipid-based excipient families (i.e., vegetal oils, polyethylene glycol (PEG), hard fats, and glycerides) have been evaluated for their capacity to incorporate 20% w/w ethosuximide in an amorphous state. It was hypothesized that the amorphous state would provide an additional asset for long-term physical stability to granules by potentially preventing recrystallization of ethosuximide However, long-term stability does not seem to be an issue (at least up to 18 months, the longest time frame followed) although polymorphism did occur during granule storage, possibly driven by the low melting temperature of test drug (37°C). In terms of bioavailability, polymorphism is obviously not an issue since ethosuximide is very soluble in water.

Formulation A consisted of granules between 400 and 1400 μm diameter, in which ethosuximide was dispersed in a lipid matrix made of polyglycolized glycerides, glyceryl distearate, and microcrystalline cellulose.

Formulation B resulted from systematic optimization experiments for improving formulation A taste masking potential by delaying ethosuximide release after drug administration. Optimization was achieved by the addition of cellulose derivatives in the lipidic matrix to delay the ethosuximide diffusion and by further application of a uniform coating with a blend of lipid excipients presenting sufficient plastic properties to stick and cover the granule surface. The resulting granules (diameter range 800–1400 μm) have shown a delayed and slow release on an in-vitro dispersion method developed in-house. Finally, to avoid sticking, the addition of a flowing agent (talc 1%) and the use of desiccants in the primary packaging to limit moisture uptake were found to have a beneficial impact on physicochemical properties during long-term storage.

Granules were packed into sachets consisting of a multi-layer foil (PETP/ALU/LDPE). Stability studies were planned at 5°C and 25°C/60% RH for up to 24 months. For formulation A, compliant results were obtained at 3 months (stopped thereafter after study A completion) and for formulation B results were compliant up to 18 months (stopped thereafter). Thus, stability is considered satisfactory but would need to be investigated further.

Ethosuximide drug substance was provided by Katwijk Chemie (Katwijk aan Zee) and was controlled in compliance with the European Pharmacopeia current edition (Monograph 0764).

A placebo granule clinical batch was also produced using the same technology but devoid of the drug substance.

For drug administration the appropriate weight of granules according to study allocation (granules A, granules B, or placebo) was extracted from the sachets, weighted according to titer and body weight for achieving the theoretical 10 mg/kg dose of ethosuximide and suspended in 20 ml pure water by the unblinded pharmacist in charge operating under a secrecy agreement versus other team members, sponsor and volunteers. The suspension was administered to each subject (t = 0) and the beaker was rinsed with 2 × 20 ml water. The drug substance lost by adherence to beaker walls was calculated after evaporation (weight). The syrup was administered with a syringe of DEXA.

2.3 | Bioanalytical methods

In vitro samples were analyzed using LC (liquid chromatography) coupled with spectrometry (ultraviolet (UV)) wavelength at 203 nm for reference standards, quality control, and bench stability. Plasma and saliva samples were analyzed using HPLC (high-performance LC) with UV detection at 203 nm. The limit of quantification (LOQ) of ethosuximide was 0.1 μg/ml, with a linear range from 0.1 to 25 μg/ml to fit the range of concentrations measured in children in this Phase I study. The method was validated in plasma and qualified in saliva by INSERM U1129 (Institut National de la Santé et de la Recherche Médicale, Hôpital Necker Enfants-Malades) and found to be satisfactory for reproducibility, intermediate precision, selectivity, response linearity, and 3 freeze-and-thaw cycles. Mid-term stability for spiked plasma and spiked saliva samples was assessed at 3 temperatures (5°C for 5 days, −20°C, and −80°C for up to 6 to 8 months).

Each clinical sample generated in the study was analyzed in 2 separate runs and the mean was used if the variability was <15%. If variability was higher, a 3rd run was performed but less than 1% of the plasma samples actually required such a control.

2.4 | Pharmacokinetic analysis

Pharmacokinetic parameters were determined for ADV6770 formulations and for the marketed syrup using non-compartmental analysis, correcting for syrup titer, and precise dose administered. Maximal plasmatic concentration (C_{max}) and time of maximal plasmatic concentration (t_{max}) were obtained directly from the plasma concentration-time profiles. Area under the curve (AUC) values were calculated using the linear trapezoidal rule. $AUC_{0-\infty}$ calculations were used if the percentage of the $AUC_{0-\infty}$ was inferior to 10%, as recommended by the Food and Drug Administration (FDA). Otherwise, the AUC_{0-last} was used. The apparent clearance (CL) and the apparent volume of distribution (V) during the terminal elimination phase were calculated using the following formulae: $CL = Dose/AUC_{0-\infty}$ and $V = CL/\lambda$. The percentage bioavailability of ADV6770 relative to Zarontin® syrup (F_{rel}) following single oral administration was calculated based on geometric mean values of AUC_{0-last} and C_{max}. The descriptive PK parameters were compared between formulations using a mixed-effect ANOVA on log-transformed values. Pharmacokinetic calculations were performed with WinNonLIN by ClinBay using plasma (and saliva) concentrations measured by INSERM U1129. Formal statistical analysis was undertaken by ClinBay using SAS software (version 9.2).

2.5 | Palatability and safety

Palatability, safety, and tolerability were evaluated using visual analog scales (VAS) as well as reaction times to congruent and incongruent visual stimuli (computer-implemented Stroop test with color names). VAS applied a 100 mm scale from 0 (no complaint) to 100 mm (the worse possible complaint) at regular time intervals, except for overall palatability for which 50 mm was considered to be neutral, with values above 50 mm representing a good taste and values below 50 mm a deterioration (see Figure 1). VAS for palatability were performed over the first 10 min after intake to evaluate the intensity, sweetness, bitterness, and palatability. Typical adverse events with an emphasis on known gastrointestinal (GI) (nausea, abdominal pain) and central nervous system (CNS) symptoms (sedation/drowsiness, dizziness, anxiety, subjective concentration capacity, headache, and fatigue) were followed over 72 h and Stroop tests were recorded over 9 h post-dose. Mean changes from baseline were analyzed with a mixed-effect ANCOVA having the period baseline as a covariate. PK/PD correlations were identified graphically using scatter plots of PD values versus PK concentrations.

2.6 | Population pharmacokinetic modeling

An exploratory population PK analysis pooling data from panels A and B was performed to quantify the variability of absorption and disposition parameters (apparent *V* and CL) and to identify influential factors such as body weight, age, and sex applying a non-linear mixed-effect modeling approach using NONMEM® (version 7.1.0, ICON Development Solutions) and first-order conditional estimation with interaction (FOCEI). A stepwise procedure was applied to identify the model that best fitted the data comparing one and two-compartment models. Exponential errors were used for the description of between-subject variability (BSV) of PK parameters. Proportional, additive, and mixed error models were compared to describe the residual variability. Potentially influencing covariates (weight, age, gender) were included in the model following a sequential forward selection and backward elimination. Continuous covariates were implemented in the model using a linear (1), allometric (2), or exponential (3) equation. Continuous variables were centered on the median.

$$\theta = \theta_1 \cdot \left(1 + \theta_2 \cdot \left(\frac{\mathrm{COV} - \mathrm{COV}_{\mathrm{median}}}{\mathrm{COV}_{\mathrm{median}}}\right)\right) \quad (1)$$

$$\theta = \theta_1 \cdot \left(\frac{\mathrm{COV}}{\mathrm{COV}_{\mathrm{median}}}\right)^{\theta_2} \quad (2)$$

$$\theta = \theta_1 \cdot \exp\left(\theta_2 \cdot \left(\frac{\mathrm{COV} - \mathrm{COV}_{\mathrm{median}}}{\mathrm{COV}_{\mathrm{median}}}\right)\right) \quad (3)$$

Categorical covariates were implemented in the model according to the following equation:

$$\theta = \theta_1 \cdot (1 + \theta_2 \cdot \mathrm{COV}) \quad (4)$$

Differences in the objective function value (ΔOF) were used for model comparison. Since ΔOF between any two hierarchical models approximates a χ^2 distribution, it was considered statistically significant if it exceeded 3.8 ($p < .05$) and 6.6 ($p < .01$) points respectively, for one additional parameter during model building and backward deletion procedure.

The stability of the final model was assessed by means of the bootstrap method implemented in Perl speak to NONMEM (PsN, version 4.8.1).[11] Median parameter values with their 95% confidence interval (CI95%) were derived from 2000 replicates of the initial dataset and compared with the original estimates. Prediction-corrected visual predictive checks (pcVPC) were also performed using PsN-Toolkit and Xpose4 (version 4.3.5)[12] by simulations based on the final PK estimates using 1000 individuals to calculate median concentration-time profile and 95% prediction intervals (PI95%). The predictive performance of the pharmacokinetic model was evaluated by calculation of the normalized prediction distribution errors (NPDEs), simulating each original observation 3000 times. The NPDEs and their distributions were then computed. The accuracy and precision of the model were estimated through mean prediction error (MPE) and root mean squared error (RMSE) using log-transformed concentrations.[13]

3 | RESULTS

3.1 | Palatability of the granules A

Six subjects (four females and two males) completed the first panel (A). No drop-out occurred. Age (mean ± SD) was 26 ± 7 years (range 21–40 years) and weight was 67 ± 10 kg (range 60–87 kg).

The palatability of granules A was characterized by the persistence of an intense and bitter taste compared to placebo granules (Figure 1). In addition, the physicochemical properties of the granules A triggered adherence to the beaker walls after mixing with water, and drug administration was thus cumbersome. Loss of ethosuximide ranged between 1.0% and 12.8% of the dose to be administered, thus strongly supporting further optimization of the granule formulation for improving taste and limiting glass adherence.

3.2 | Palatability of granules B

Six different subjects (one female and five males) completed the second panel (B). No drop-out occurred. Age (mean ± SD) was 22 ± 2 years (range 19–26 years) and weight was 69 ± 7 kg (range 56–75 kg).

The optimized granule formulation (granules B) differed significantly for all four criteria evaluating taste when compared to syrup and they were similar to placebo granules: they lacked bitterness ($p < .001$), intensity ($p < .001$), and sweetness ($p < .01$), while improved palatability ($p < .001$) was recorded, with a well perceived neutral taste (Figure 1). When compared to placebo granules, granules B containing ethosuximide still adhered slightly to the glass

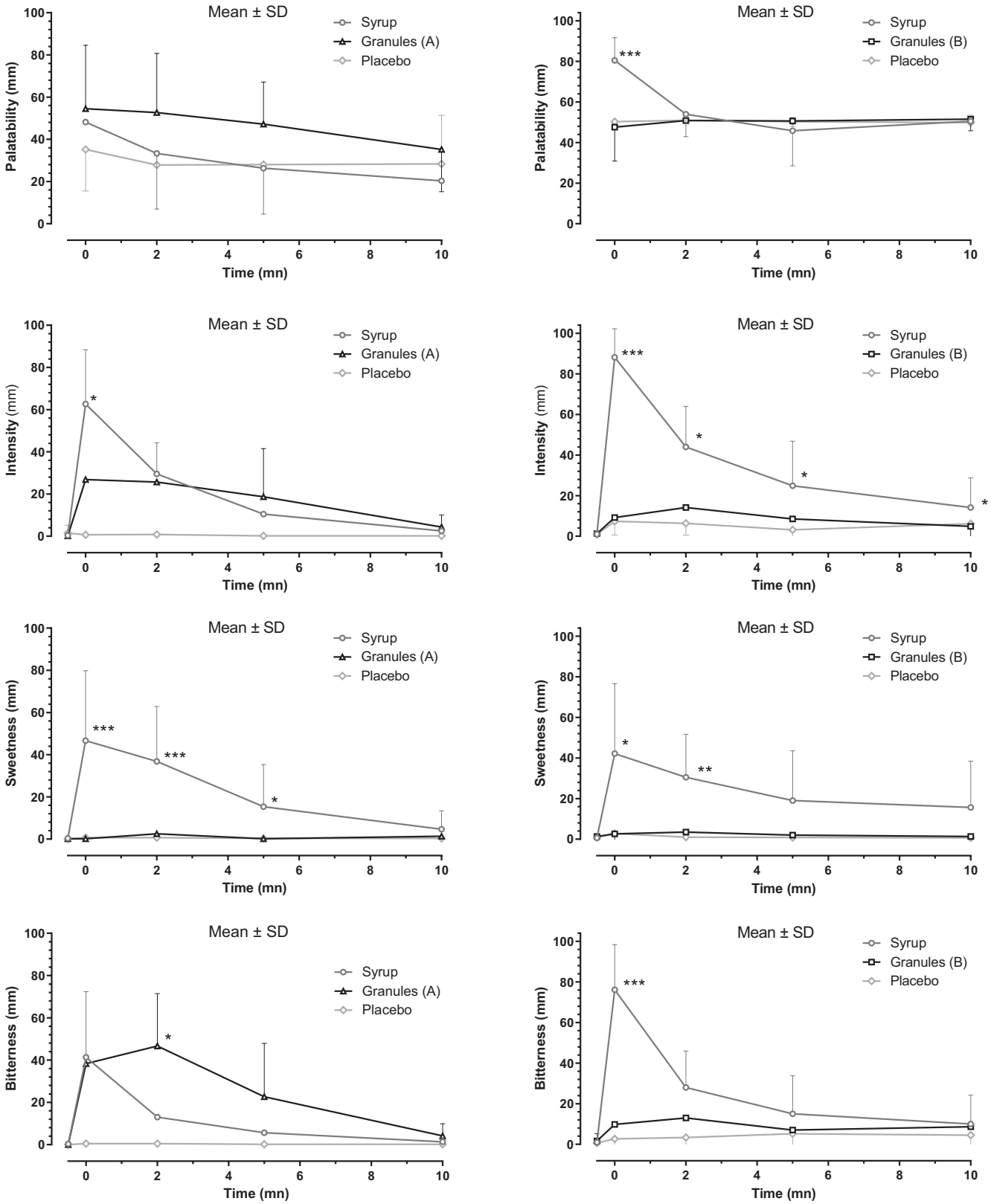

FIGURE 1 Evaluation of taste of different formulations of ethosuximide according to visual analog scales regarding intensity, sweetness, bitterness (all 3 items: 0 for absence and 100 mm for extreme intensity), and overall palatability (50 mm as neutral, perceived as pleasant if >50 mm and as poor if <50 mm). On the left: granules A (open triangles), syrup Zarontin® (open circles), and placebo (open diamonds) are represented. On the right: granules B (open squares), syrup Zarontin® (open circles), and placebo (open diamonds) are represented. ***$p < .001$, **$p < .01$, *$p < .05$ when statistically significant by ANCOVA (granules vs. syrup)

beaker when suspended in water, but administration loss was less marked than for granules A, ranging between 1.0 and 5.7%.

3.3 | Pharmacokinetics and bioequivalence

Ethosuximide plasma concentrations were determined after oral administration of both granule formulations (A and B) and the marketed syrup Zarontin®. The pharmacokinetic parameters of ethosuximide for each formulation are shown in Table 1 and plasma concentration-time profiles in Figure 2.

Median t_{max} values were 0.63, 0.75, and 0.5 h for granule A formulation, the optimized granule B formulation, and Zarontin® syrup, respectively. The t_{max} value was marginally delayed for granules A and more marked for granules B versus syrup. The C_{max} values are slightly lower with granules B compared to syrup, with a more blunted peak ($p < .02$). No other significant statistical differences were otherwise observed in PK parameters between syrup and granules.

The relative bioavailability of ethosuximide formulated as LMP granules in comparison to Zarontin® (F_{rel}) was calculated based on C_{max} and $AUC_{0-\infty}$ normalized to the 10 mg/kg dose. The mean log-transformed estimate of F_{rel} C_{max} was 93.7 (90% Confidence Interval (CI): 76.3–115.1) and F_{rel} $AUC_{0-\infty}$ was 96.1 (90% CI: 91.0–101.5) for the granule A formulation. The mean log-transformed estimate of F_{rel} C_{max} was 87.6 (90% CI: 81.6–94.0) and F_{rel} $AUC_{0-\infty}$ was 92.5 (90% CI: 88.5–96.6) for the optimized granules B formulation. Granule B formulation lay, therefore, entirely within the standard 80%–125% acceptance range for bioequivalence.

3.4 | Population PK model

A two-compartment PK model best-described ethosuximide disposition in terms of CL, inter-compartmental CL (Q), V of the central compartment (V_C), and peripheral compartment (V_P) (Figure 3). The decrease in objective function value (OFV) from the one-compartment model was −63.4 ($p < .001$). An improvement of the fit was observed while dissociating constants of the rate of absorption for the syrup (k_{a1}) and granules A and B together (k_{a2}) and by including between-subject variability (BSV) on CL, V_c, k_{a1}, and k_{a2}. Intra-subject variability was best described by a combined additive and proportional residual error model. The assignment of BW on V_C following a linear equation markedly improved the description of the data (ΔOF = −33.9, $p < .01$). The final population parameters with their BSV when included were a CL of 0.57 L/h (CV 18%), a Q of 10.2 L/h, a V_C of 31.3 L (CV 8%), a V_P of 13.9 L, a k_{a1} of 5.59/h (CV 110%), and a k_{a2} of 2.06 (CV 61%). Significant model improvement (ΔOF = −9.2, $p < .01$) was observed using distinct absorption rates for syrup versus both types of granules.

The parameter estimates of the final population PK model were within the bootstrap 95% CI and differed by less than 2% from the median bootstrap parameters, indicating the acceptability of the model. The model was unbiased with a mean prediction error of −0.01 (IC 95%: −0.04 to 0.01) and a precision of 14%. The final model parameters and bootstraps results are presented in Table 2, goodness of fit plots in Figure S6 (Appendix S1), results of pcVPC in Figure S7 (Appendix S1), and normalized prediction error (NPDE) in Figure S8 (Appendix S1).

3.5 | Safety profile

No major adverse reaction was reported throughout the study. Safety and tolerability assessments disclosed limited impact on GI and CNS using VAS, as well as Stroop visual reaction time. GI effects were isolated, short-lived, and not linked to a particular formulation with a maximum increase of 10% on VAS. One female subject reported nausea after administration of the optimized granule formulation B and a lag time to maximal plasmatic concentration (t_{max} 4 h) was observed in this treatment period, suggesting delayed gastric emptying. The fasting state is considered to have contributed to this observation.

CNS effects were isolated and only very modest increases were observed. Dizziness, fatigue, and concentration difficulties were reported following the administration of ethosuximide, whatever the formulation.

Differences for VAS intensity CNS scores were observed between syrup and granule formulations for dizziness (granules A and B), fatigue (granules A), and anxiety (granules B) with peak intensity at 30 min post-dose, but without reaching statistical significance. More specifically, dizziness was graphically more pronounced after the administration of the syrup formulation than ethosuximide granules B (Figure 4), although the mean difference (32.5 vs. 19.0 mm) was not statistically significant ($p = .2$). The mean difference for anxiety was 20.7 mm for syrup versus 10.0 mm for granules B and was not statistically significant ($p = .17$). This trend for improvement may

TABLE 1 Pharmacokinetic parameters of ethosuximide according to formulation (non-compartmental analysis, 6 volunteers per formulation)

Ethosuximide formulation	t_{max} [h] (range)	C_{max} [µg/ml]	k_a [h^{-1}]	$AUC_{(0,last)}$ [µg·h/ml]	$AUC_{(0,\infty)}$ [µg·h/ml]	$t_{1/2}$ [h]	CL_{Tot}/F [ml/h/kg]	V_z/F [ml/kg]
Granules (A)	0.63 (0.3–1.0)	18.2 ± 2.1	2.06 (CV 61%)	1340 ± 411	1350 ± 423	62.3 ± 15.3	7.6 ± 2.0	646 ± 83
Granules (B)	0.75 (0.5–4.05)	15.8 ± 1.9		1090 ± 100	1100 ± 100	54.4 ± 10.5	8.9 ± 0.8	695 ± 107
Syrup (Zarontin®) (B)	0.5 (0.3–0.8)	18.2 ± 1.0	5.59 (CV 110%)	1200 ± 125	1210 ± 125	57.1 ± 8.9	8.3 ± 0.8	678 ± 103

Note: t_{max} is a median (range). All other values are arithmetic mean ± SD. C_{max} and AUC are normalized to the administered dose.

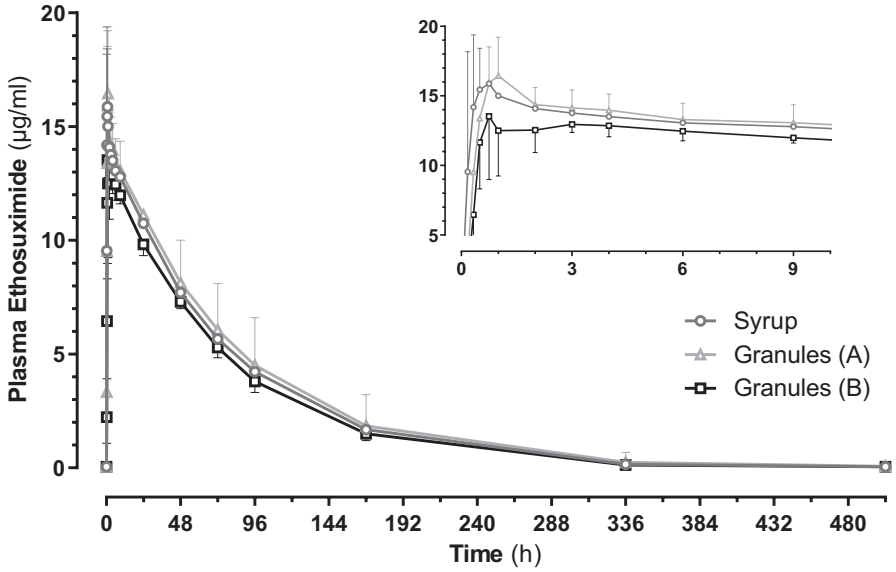

FIGURE 2 Time profile of geometric mean with log-SD plasmatic ethosuximide concentrations according to the formulation. The syrup is represented by open circles, granules A as open triangles, and optimized granules B as open squares. Details of the concentrations during the first 8 h are shown in the insert

be explained by lower C_{max} and more blunted peaks in plasma concentrations due to slower absorption of granules B.

Visual reaction times (Stroop test) showed no change linked to formulation, but they were not performed at the earlier time points when CNS symptoms were at the highest. The adverse event profile was marginally better for granules than for syrup.

Saliva and plasma ethosuximide levels were measured with the formulation of granules A. Assays of saliva samples indicated a very good correlation with plasma with a correlation ratio of 0.88–0.94 (Figure 5).

4 | DISCUSSION AND CONCLUSION

It has been demonstrated in this study that the innovative ethosuximide granule formulation B based on LMP technology has achieved the target profile identified for pediatric use, being sugar-free, tasteless, and bioavailable within the regulatory usual range when compared to the reference syrup, Zarontin®, and well-tolerated, while enabling flexible adjustment to body weight.

Indeed, while granules A displayed a bitter and intense taste, improved lipid matrix and coating process for granules B allowed to mask this persistent and unpleasant taste with a palatability profile shown to be similar to placebo granules in terms of intensity, sweetness, and bitterness. Considering that the poor palatability of the currently marketed syrup markedly influences compliance with ethosuximide therapy, the development of a palatable formulation is a major advantage, in particular for pediatric patients.

Furthermore, dizziness intensity according to plasma concentrations for syrup Zarontin® when compared to granules B (Figure 4) demonstrates a clockwise hysteresis, favoring granule formulation in terms of CNS tolerability. This observation is explained by a slight delay in t_{max} values (more prevalent for granules B) and lower values of k_a with both granule formulations, compared to a syrup which is very rapidly absorbed. The clinical meaning of this slight, statistically non-significant improvement, remains to be evaluated in the long-term therapeutic use of this granule formulation. Nonetheless, both granule formulations remained bioequivalent at C_{max} level. Granules A fulfilled better bioequivalence criteria than granules B, but it should be stressed that individual variability at C_{max} is least marked for granules B. Tolerability was otherwise similar to the marketed syrup formulation. Specific endpoints, i.e., Stroop test (reaction times), were unfortunately ineffective in translating more precisely neurological adverse reactions linked to ethosuximide intake. These results are attributed to the timing of testing: indeed, no Stroop tests could be performed between 0 and 2 h post-dose as a consequence of PK sampling workload, whereas most CNS adverse reactions occurred shortly after drug intake. The non-specific nature of symptoms following intake of ethosuximide in non-tolerant subjects has probably also contributed to difficulties in capturing them on specific neuropsychological tasks.

There are few studies published on the ethosuximide PK profile. It is consequently noteworthy to mention that the syrup is reported in the literature with a t_{max} between 1–4 h compared to 0.3–1 h measured in this study, suggesting that the evaluation of earlier time points has allowed improving the accuracy of t_{max} values for ethosuximide.

To our knowledge, this is the first ethosuximide population PK model described. A two-compartment model best described the PK disposition of ethosuximide. The addition of weight on V_C significantly improved the model objective function, while these covariates did not influence CL. Age did not impact CL, although this observation is of limited value since age ranged from 18 to 40 years. Absorption constants k_a were specified with the model and were lower for granule formulations (2.06 versus 5.59/h for syrup), confirming a decrease in the absorption rate of granules and probably explaining the improved CNS tolerability. This causes the absorption phase to interfere with the early distribution phase, explaining that the model describes the curve peak after granule intake.

A good correlation was observed between ethosuximide saliva and plasma concentrations despite the dilution factor of 4 introduced by the buffer in Quantisal tubes. The advantages of using saliva for assessing therapeutic drug monitoring and compliance include avoiding

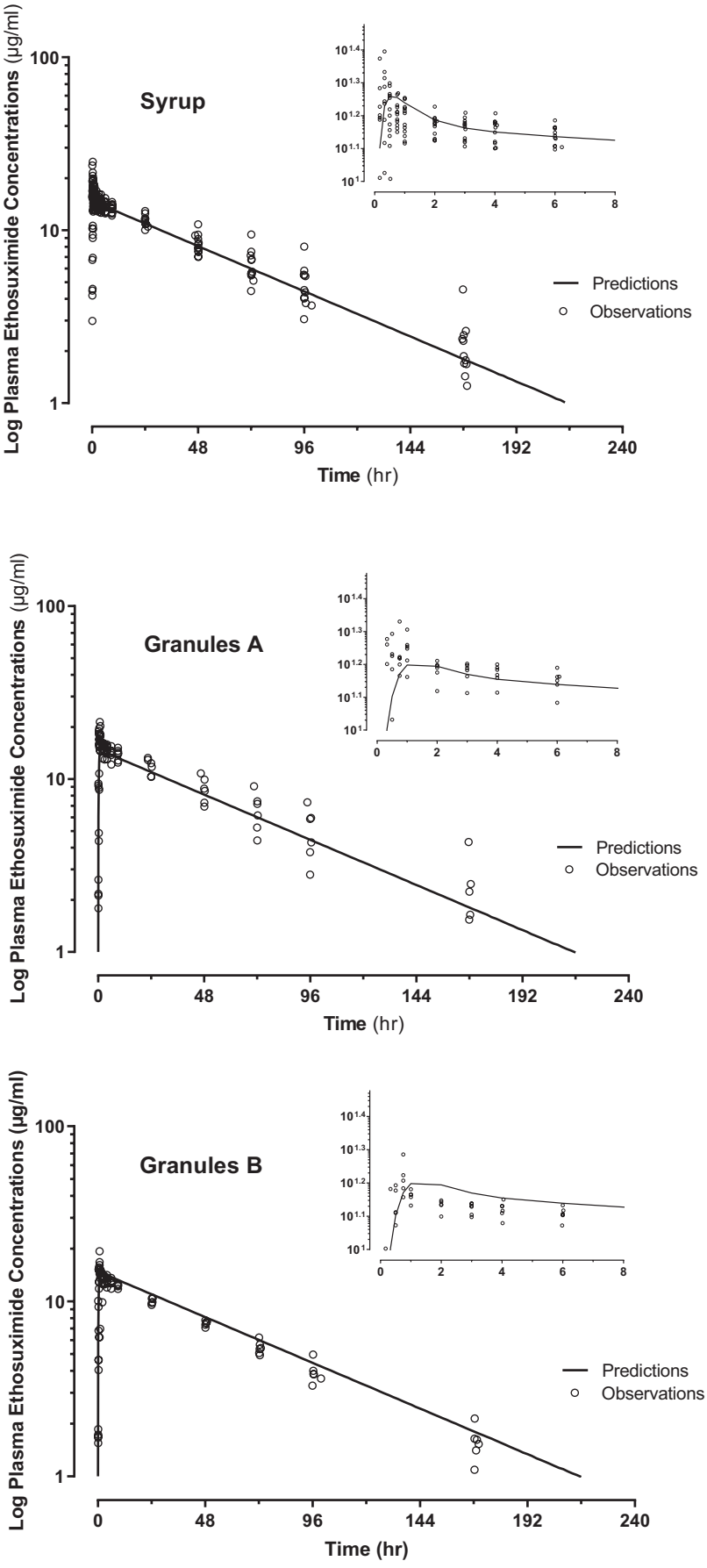

FIGURE 3 Two-compartment model describing ethosuximide concentrations according to time. Observations are represented by open circles and predictions by a continuous line. Concentrations during the absorption phase are represented in the inset. Detail of the concentrations during the first 8 h is shown in the inserts.

TABLE 2 Final model and bootstrap parameters

	Final model				Bootstrap			
Parameter	**Estimate**	**RSE(%)**	**BSV(%)**	**RSE (%)**	**Estimate**	**$CI_{95\%}$**	**BSV(%)**	**$CI_{95\%}$**
CL (L/h)	0.569	4	17.6	12	0.567	(0.53; 0.61)	17.2	(12.3; 20.9)
V_c (L)	31.3	4	8.3	21	31	(27.9–33.8)	7.9	(3.2; 11.5)
Q (L/h)	10.2	18	—	—	10.5	(7.7–15.6)	—	—
V_p (L)	13.9	9	—	—	14.2	(11.7–16.8)	—	—
ka_{syrup} (h^{-1})	5.59	36	153	16	5.6	(2.70; 11.8)	104.5	(66.3; 144.4)
$ka_{granulesA\&B}$ (h^{-1})	2.06	19	66	19	2.06	(1.40; 2.95)	57.2	(32.5; 78.3)
θ_{weight}	1.71	11	—	—	1.75	(1.38; 2.26)	—	—
σ_{prop} (CV%)	0.156	10	—	—	0.154	(0.12; 0.18)	—	—
σ_{add} (CV%)	0.027	11	—	—	0.027	(0.02; 0.04)	—	—

Note: Final model: $CL = CL_{pop} \cdot \eta CL$; $V_c = V_{cpop} \cdot (1 + \theta_{weight} \cdot (WT - WT_{median})/WT_{median}) \cdot \eta V_c$; $Q = Q_{pop}$; $V_p = V_{ppop} \cdot \eta V_p$.

Abbreviations: BSV, between-subject variability; CI 95%: 95% confidence interval; CL, clearance; Q, intercompartmental clearance; RSE, relative standard error of the estimate defined as SE estimate/estimate, expressed as a percentage, with SE estimate retrieved directly from the NONMEM output file; V_c, central volume of distribution; V_p, peripheral volume of distribution; θ_{WT}, effect of body weight expressed as $(1 + (WT - WT_{median}/WT_{median}))$ with $WT_{median} = 67.2$ kg; σ_{add}, additive residual error; σ_{prop}, exponential residual error.

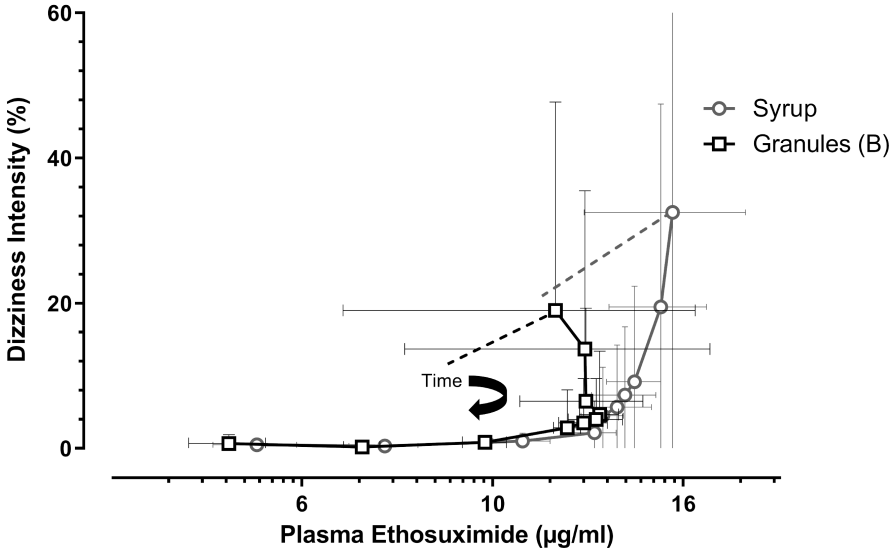

FIGURE 4 Dizziness intensity according to plasma concentrations for syrup Zarontin® compared to granules B. Clockwise hysteresis is observed

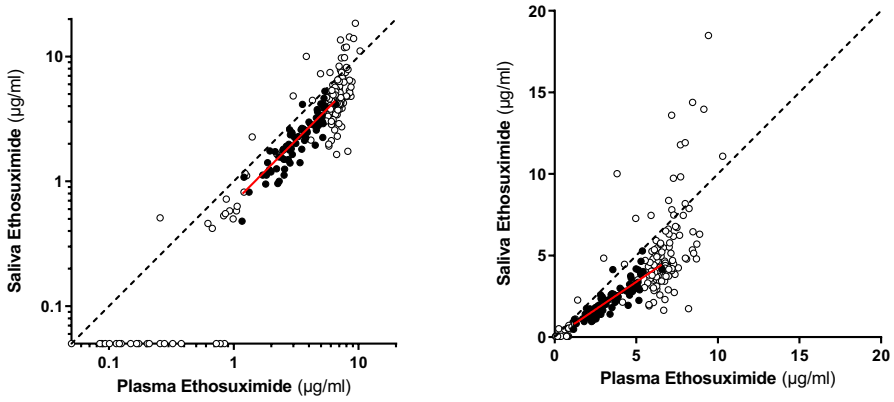

FIGURE 5 Linear correlation (forced through the origin) of plasma and salivary ethosuximide. Only the points from 24 to 96 h (black circles) are included in the linear regression model (red line); the early and late points are dropped (open circles). The estimated slope is 0.67 ± 0.016 (IC95: 0.6438 to 0.7095), the correlation coefficient is 0.87, and the RMSE (calculated on Logs) is 21.6%

phlebotomy, the reflection of free (active) concentrations, easy sampling, and good acceptance by parents and patients at reduced costs.[14,15]

In conclusion, the innovative ethosuximide granule formulation B has achieved the ideal target profile defined for pediatric use, being sugar-free, tasteless, bioequivalent to the reference syrup, and well-tolerated, while enabling precise adjustment to body weight. A two-compartment model was shown to best describe the plasma concentrations measured over time. Further controlled trials with

granules B need to be undertaken for confirming their efficacy and usefulness when treating pediatric patients with absence epilepsy.

AUTHOR CONTRIBUTIONS

The KIEKIDS Steering Committee met at least twice a year and was constituted by Catherine Chiron (Coordinator), Vincent Jullien (bioanalytics), FV (statistics), FBF, and by Advicenne members involved in strategy, formulation, validation of the investigation plan, clinical research, and project planning (LAG, CRM, CG, MMS). CRM was responsible for formulation selection and development, coordinated production and analytical methods development for granules as well as stability for preclinical and clinical batches. TB, FBF, CG, and LAG designed the study, FBF provided regulatory, monitoring, and technical support while LD, KD, TB, LR, HC, and CB, performed the study and interpreted data, and PA (operating under secrecy agreement) was in charge of clinical supplies (preparation, administration, calculation). VJ validated the bioassays and analyzed the plasma and saliva samples, IB provided data management, FV performed the statistical analysis, and LD, KD, TB, and FBF drafted the manuscript. All authors critically reviewed the manuscript.

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CONFLICT OF INTEREST

Catherine Guittet is an employee of Advicenne Pharma while Caroline Roussel-Maupetit, Maria A. Manso-Silvan, and Luc-André Granier are former employees. These four co-authors hold (or held) shares in the company. François Vandenhende and Françoise Brunner-Ferber were consultants to Advicenne Pharma (and to several other pharmaceutical entities) and hold (or held) shares in the company. Catherine Chiron organized training sessions for Advicenne Pharma staff (and for several other pharmaceutical entities). Other co-authors have no reported conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author at a reasonable request.

ETHICS APPROVAL

Obtained from local Independent Ethics Committee for study protocol, amendments, case report forms, and informed consents.

CONSENT TO PARTICIPATE

Obtained from all volunteers.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Supplemental material

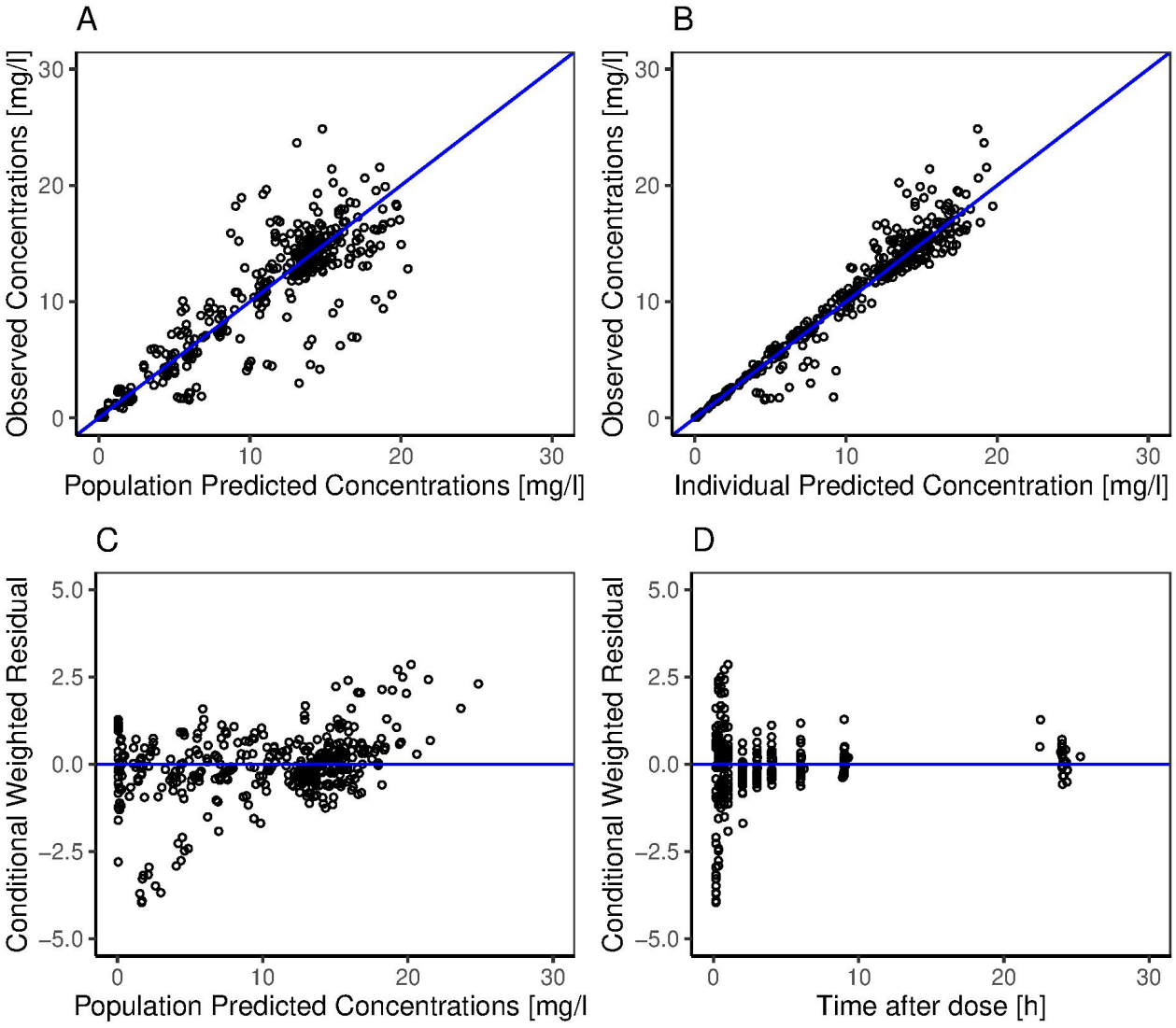

Figure 6: Goodness of fit plots of the final PK model: observed concentrations versus population predicted concentrations (A), versus individual predicted concentration (B), conditional weighted residual versus population predicted concentrations (C), versus time after dose (D)

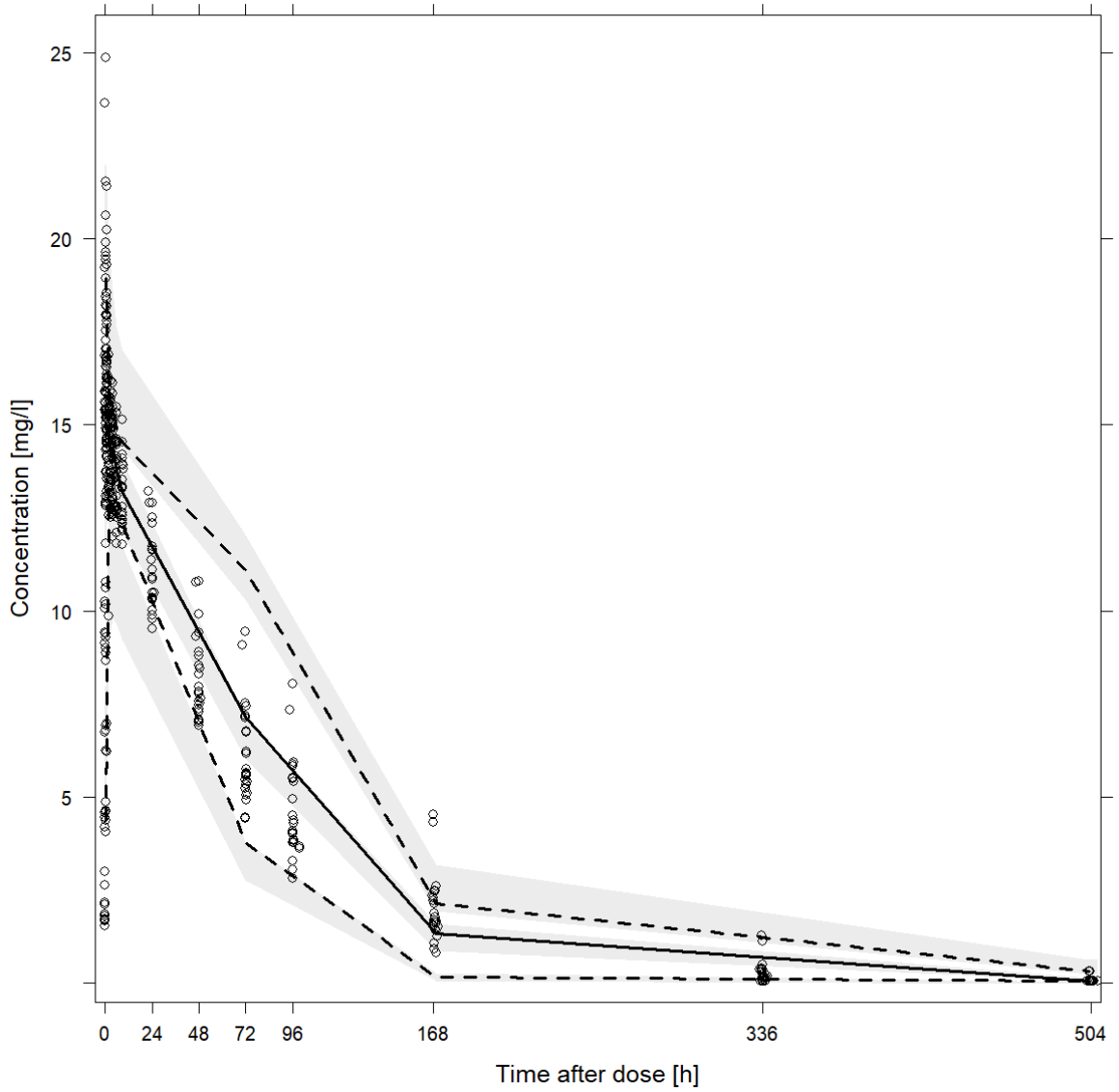

Figure 7: Prediction-corrected Visual Predictive Check of the final model with ethosuximide prediction-corrected concentrations (circles) and population prediction (solid line) and 95% prediction interval (semi-solid line). Grey fields represent the model-based percentile 95% confidence interval.

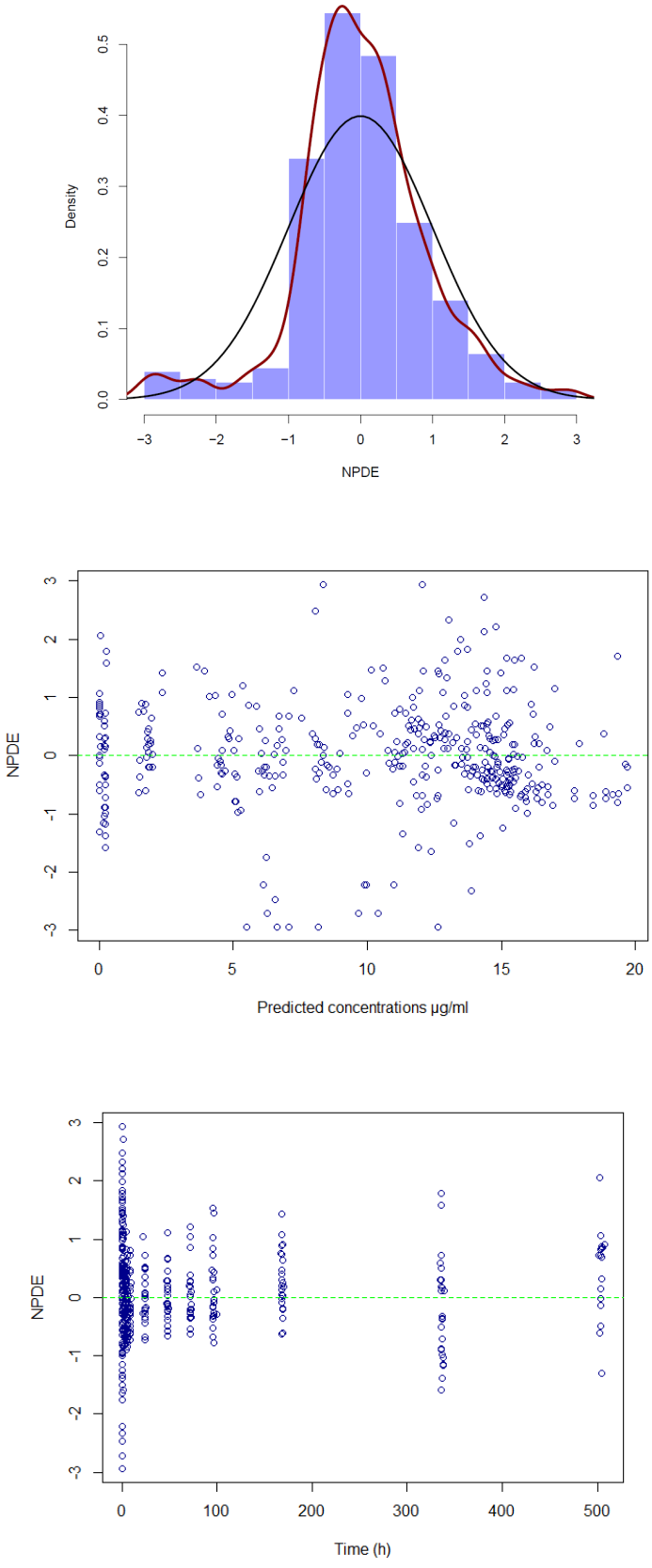

Figure 8: Normalized prediction error (NPDE): distribution of residues, NPDE versus time and NPDE versus predicted concentrations.