Pharmacokinetic analysis of modified-release metoprolol formulations: an interspecies comparison.

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Abstract

In the current study, we investigated the metoprolol absorption kinetics of an in-house produced oral sustained-release formulation, matrices manufactured via prilling, and two commercially available formulations, ZOK-ZID® (reservoir) and Slow-Lopresor® (matrix) in both New Zealand White rabbits and Beagle dogs, using a population pharmacokinetic analysis approach.

The aim of this study was to compare the in vivo pharmacokinetic (PK) profiles of different formulations based on metoprolol, a selective adrenergic β_1-receptor antagonist, in dogs and rabbits and to contrast the observed differences. To that end, metoprolol (50 to 200 mg) was administered to 6 Beagle dogs and 6 New Zealand White rabbits as a single intravenous (IV) bolus injection and to 8 dogs and 6 rabbits as an oral modified release formulation. To derive pharmacokinetic parameters from the data, a non-linear mixed-effects model was developed using NONMEM® where the contribution of observations below the limit of detection (BDL, below detection limit) to the parameter estimates was taken into account in the parameter estimation procedure.

In both species and for the three modified release formulations, different absorption models were tested to describe the PK of metoprolol following oral dosing. In Beagle dogs, plasma concentration-time profiles were best described using a sequential zero- and first-order absorption model. In rabbits though, the absorption phase was best described using a first-order process only.

In both species, the reservoir formulation ZOK-ZID® was behaving quite similarly. In contrast, the absorption properties of both matrix formulations were rather different between

45 species. This study indicates that the PK of the reservoir formulation is similar in both
46 species, even after accounting for the almost completely missed absorption phase in rabbits.
47 The insights gained further illustrate that rabbits are not very well suited to study the PK of
48 the current matrix formulations in view of their less optimal prolonged release
49 characteristics and the resulting fast decline in metoprolol plasma levels.

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51 KEYWORDS: Metoprolol, Pharmacokinetic model, Modified-release drug formulations,
52 Beagle dogs, New Zealand White rabbits

53

1. Introduction

The aim of the present study was to compare the absorption kinetics of metoprolol from three types of modified-release dosage forms, both in the frequently used preclinical animal models Beagle dogs and New Zealand White rabbits, and to evaluate the observed differences. Metoprolol is a β_1-selective adrenergic blocking agent, commonly used in the treatment of hypertension, angina pectoris and heart failure. Being a Class I compound, according to the Biopharmaceutics Classification System (BCS), it possesses both a high solubility and permeability. However, as metoprolol is characterized by a short half-life (3-4h) caused by extensive hepatic first-pass metabolism, frequent dosing during the day is required (Åblad et al., 1975; Regårdh et al., 2015). Hence, the drug is a suitable candidate for incorporation into a controlled release dosage form that delivers the drug over an extended period of time thereby significantly decreasing the frequency of dosing.

Metoprolol's bioavailability of an in-house developed multiparticulate sustained-release matrix system by means of prilling was compared with two commercially available modified-release formulations: Slow-Lopresor® (Daiichi Sankyo Belgium S.A., Louvain-la-Neuve, Belgium) and ZOK-ZID® (Pfizer S.A., Brussels, Belgium). While Slow-Lopresor® represents a controlled release matrix tablet, ZOK-ZID® is a tablet which immediately disintegrates into reservoir coated pellets.

Here, we present a model-based analysis to compare metoprolol pharmacokinetics between ZOK-ZID®, Slow-Lopresor® and the in-house produced prills in the selected preclinical species.

2. Materials and methods

2.1. Materials

Metoprolol tartrate was purchased from Esteve Quimica (Barcelona, Spain), while behenic acid (Radiacid 0560) was purchased from Oleon (Ertvelde, Belgium). Polyethylene glycol (PEG) 4000 was obtained from Fagron (Waregem, Belgium). All other chemicals were of analytical grade.

2.2. Prilling

An in-house developed multiparticulate sustained-release matrix system was prepared by means of prilling. This technique basically consists of converting a liquid melt into droplets that are subsequently cooled below their solidification temperature (Rahmanian et al., 2013). The process initially involves the solubilization or dispersion of a drug into a molten lipid base before extrusion through calibrated nozzles. The break-up of the liquid jet allows perfect calibration of the droplets and finally results in the production of narrow-sized spherically shaped particles, called prills (Pivette et al., 2009; Pivette et al., 2012). Due to the hydrophobic properties of the lipids, the process is able to produce diffusion-controlled matrix systems.

Prilling was performed using the PrillDrop® device developed by Peira (Turnhout, Belgium). Behenic acid and PEG 4000 were simultaneously molten and the drug was added under stirring. The mixture was heated to 100 °C before droplet formation was started. By applying air pressure, the mixture was fed towards the thermostated nozzle (90 °C) consisting of a valve and a needle (inner diameter: 0.33 mm). Using a drop time of 0.04 s (i.e. period during which the valve is open) and an air pressure of 0.5 bar, droplets were produced at the needle. Finally, these droplets were quench cooled in liquid nitrogen yielding solid spherical particles.

Thermogravimetric analysis indicated that metoprolol tartrate, behenic acid and PEG 4000 were stable at the process temperature (data not shown). The prills showed a mean particle size of 2.4 mm with narrow particle size distribution. Furthermore, the prills exhibited perfect sphericity with a mean aspect ratio of 1.1.

2.3. In Vitro and In Vivo Study

2.3.1 In Vitro Dissolution Profile Study

In vitro drug release was determined using the USP dissolution apparatus 1 (baskets). The equipment consisted of a VK 7010 system coupled with a VK 8000 automatic sampling station (Vankel, New Jersey, USA). In case of the prills, an amount of prills corresponding to 30 mg metoprolol tartrate was exposed to the dissolution medium, whereas 1 tablet was tested in case of Slow-Lopresor® (200 mg metoprolol tartrate) and ZOK-ZID® (95 mg metoprolol succinate). The dissolution medium consisted of 900 mL of demineralized water. Basket rotational speed was set at 100 rpm and the temperature of the dissolution medium was maintained at 37 ± 0.5 °C. Samples of 5 mL were withdrawn after 0.5, 1, 2, 4, 6, 8, 12, 16, 20 and 24 h and analyzed spectrophotometrically at 222 nm using a double beam spectrophotometer (UV-1650PC, Shimadzu, Antwerp, Belgium). Metoprolol concentrations were calculated from a calibration curve between 0 and 33 µg/mL.

2.3.2 In Vivo Animal Study

All procedures were performed in accordance with the guidelines and after approval by the local Ethics Committee. Each time, 8 dogs and 6 rabbits were orally dosed with (i) the prills containing 10% metoprolol tartrate, 5% PEG 4000 and 85% behenic acid (filled in hard-gelatin capsules), (ii) the commercial reservoir formulation, ZOK-ZID®, consisting of tablets containing

95 mg metoprolol succinate (equivalent to 100 mg metoprolol tartrate) and (iii) the commercial matrix formulation, Slow-Lopresor®, containing 200 mg metoprolol tartrate. The intravenous (IV) bolus injection was administered to 6 dogs and 6 rabbits. For the oral formulations, 200 mg metoprolol tartrate was administered to the dogs, while 100 mg metoprolol tartrate was administered to the rabbits. Beagle dogs were treated with two tablets of ZOK-ZID® and rabbits received one tablet. Slow-Lopresor® was dosed as one tablet to Beagle dogs and rabbits received half a tablet. In case of the intravenous injection, an isotonic solution was made based on metoprolol tartrate. The IV dose administered was 100 mg and 50 mg for the dogs and rabbits, respectively.

The formulations were administered in a cross-over fashion with a wash-out period of at least 7 days. All animals were fasted from 12 h prior till 12 h after dose administration, although water was available ad libitum. Before dose administration, a blank blood sample was collected. The oral formulations were administered with 20 mL water. Blood samples were collected in dry heparinized tubes at predetermined time points after drug administration, centrifuged at 1500 × g for 5 min and resulting plasma was stored at -20 °C until analysis.

2.4. HPLC Analysis

A validated HPLC method with fluorescence detection was used for the determination of metoprolol in plasma. We refer to the paper written by Vervaeck et al. (2013) for more details.

2.5 Population PK Analysis Methods

Due to the significant number of samples below the quantification limit (BQL) and the fact that the extrapolated area under the curve (AUC) is higher than 20% for all formulations, except for Slow-Lopresor® in dogs (10.36%), the data were analyzed using population pharmacokinetic analysis with implementation of the M3 method (Ahn et al., 2008; Jusko, 2012; Keizer et al., 2015), instead of non-compartmental analysis to avoid biased PK parameters.

A total of 258 and 205 ln-transformed metoprolol observations sampled from 30 Beagle dogs and 24 rabbits were available for population PK analysis. Population PK analysis was performed by means of non-linear mixed-effects modeling using NONMEM® (version 7.3.0, ICON, Hanover, MD,USA). All NONMEM runs were executed using Pearl-speaks-NONMEM (PsN) 4.2.0 (Beal et al., 2011; Lindbom et al., 2005). The statistical package R (version 3.1.2, R Development Core Team, 2011) was used during model development for a graphical assessment of the goodness-of-fit (GOF) of the different tested models.

In the first stage of model development, one- and two-compartment models with linear elimination from the central compartment were fitted to the IV data alone using the first-order conditional estimation (FOCE) method. Thereafter, oral data were combined with IV data and the absorption part of the model was optimized. During model development, a first order absorption process besides models assuming parallel or sequential zero- and first-order absorption pathways were tested.

Observations between the limit of detection (10.1 ng/mL) and the limit of quantification (30.6 ng/mL) were taken into account during the analysis, whereas BDL data were analysed using the M3 method (Ahn et al., 2008; Jusko, 2012; Keizer et al., 2015). The percentages of

BQL and BDL data were 9.5% and 6.2% in Beagles and 16.5% and 6% in rabbits, respectively, indicating that the BQL data need to be considered during the analysis in order to avoid bias in parameter estimates. The M3-method was suggested by Beal to handle data below the limit of quantification and is based on maximization of the likelihood for all the data. The M3 method includes simultaneous modeling of continuous and categorical data by treating the BQL observations as censored categorical data. We applied this method on our retained BDL observations in the dataset, using the indicator variable F_FLAG.

Population PK parameters including their inter-individual variability (IIV), and the residual unexplained variability (RUV) were estimated using the LAPLACIAN estimation method. Inter-individual variability around the typical PK parameters was estimated using an exponential model and was assumed to be normally distributed (in the logarithmic domain) with zero mean and variance ω^2. The residual variability was described with an additive error in the ln domain and was defined as being normally distributed with zero mean and variance σ^2.

Models were selected based on the objective function value (OFV), goodness-of-fit plots and a condition number below 1 000. The OFV is defined as minus twice the log-likelihood of the model. During the model building procedure, a p-value below 0.05, representing a decrease in OFV greater than 3.84 points after the addition of one single model parameter, was considered to represent a statistically significant model improvement (Bonate, 2011).

2.5.1 Validation of the Pharmacokinetic Model

In order to assess the final model's performance, the PsN-toolkit was used to produce a prediction-corrected visual predictive check (pcVPC) (Bergstrand et al., 2011; Bergstrand and Karlsson, 2009). The pcVPCs were based on 5000 new datasets, simulated using the parameter estimates of the final model. The 2.5^{th}, 50^{th} and 97.5^{th} percentiles were calculated

for each simulation run. A two panel type of pcVPC was chosen to evaluate the model with respect to both data above and below the limit of detection.

Stability of the final PK parameter estimates and their 95% confidence intervals were evaluated using log-likelihood profiling (LLP), performed using the PsN-toolkit. As an advantage over confidence intervals derived from the estimated variance-covariance matrix of the model, this method makes no assumptions regarding symmetry of the confidence intervals.

2.6 In Vitro and In Vivo Correlation

The relationship between in vitro release and in vivo absorption was evaluated using an in vitro-in vivo correlation (IVIVC) analysis (Emami, 2006), and more specifically a multiple level C one. The multiple level C IVIVC establishes a single point relationship between a dissolution parameter and a pharmacokinetic parameter of interest. Based on the in vivo data, partial areas under the mean plasma concentration-time curves ($AUC_{0\text{-}1\ h}$, $AUC_{0\text{-}2\ h}$, $AUC_{0\text{-}4\ h}$, $AUC_{0\text{-}6\ h}$ and $AUC_{0\text{-}8\ h}$), as obtained through non-compartmental analysis, and in vitro release at equivalent time points were evaluated through correlation analysis. The non-compartmental analysis was done using the free statistical software package R (version 3.1.2, R Development Core Team, 2011) using the PK package and function *nca.complete*. For each pair of values across the three different formulations, Pearson's correlation coefficients were calculated using R (version 3.1.2, R Development Core Team, 2011). For each formulation and species, the partial mean AUCs, as indicated before, mean C_{max} (referring to the peak plasma concentration) and mean t_{max} (referring to the time at which the C_{max} occurs) were correlated with the cumulative metoprolol amount dissolved at the

corresponding time points. In this case, the in vitro and in vivo data were treated as the independent (x) and dependent (y) variables, respectively.

3. Results

3.1. In Vitro Dissolution Profile Study

The in vitro drug release profiles are illustrated in Fig. 1. Slow-Lopresor® showed a complete drug release after 16 h, although 92.4% of the total drug amount was already released after 8 h. The prills and ZOK-ZID® showed a comparable drug release profile over a 24 h period. However, the prills initially showed a faster release since 44.1% metoprolol was released after 4 h, compared to only 28.0% for ZOK-ZID®. Previously, dissolution experiments using the reciprocating cylinder method (Vervaeck et al., 2014) also indicated that the prills were more sensitive to the hydrodynamic flow and stress conditions in the gastrointestinal tract. In contrast, release from ZOK-ZID® was not influenced by these conditions and a similar release profile was obtained for both methods (Vervaeck et al., 2014).

3.2 In Vivo Animal Study

For both preclinical animal species, it was possible to simultaneously describe the metoprolol plasma concentration versus time data after intravenous and oral administration of the different modified-release formulations using a population approach. For both species, a two-compartment model was preferred since the observed plasma concentration-time profiles of metoprolol decreased bi-exponentially over time. Metoprolol PK in Beagle dogs was best described using a sequential zero- and first-order absorption model, and linear elimination from the central compartment. In rabbits, metoprolol PK profiles were

242 best described using a conventional first-order absorption model and linear elimination from
243 the central compartment.

244 The included model parameters consisted of plasma clearance (CL), volume of distribution of
245 the central compartment (V2), inter-compartmental flow (Q), volume of distribution of the
246 peripheral compartment (V3), oral bioavailability (F1) and absorption rate constant (ka). In
247 addition to these parameters, the duration of the zero-order input (D1) was estimated for
248 the dogs. Inter-individual variability was included on CL, V2, V3 and Q in dogs and on CL and
249 F1 in rabbits. The final models for the preclinical species are illustrated in Figs. 2 and 3,
250 whereas the final population PK parameter estimates are shown in Tables 1 and 2. Because
251 of the small variability in body weight (11.9 ± 1.5 (mean ± standard deviation in kg, Beagles)
252 and 4.5 ± 0.2 (mean ± standard deviation in kg, rabbits)), the animals' weight was not
253 expected to affect the systemic exposure to metoprolol and consequently, no covariates
254 apart from formulation type were included in the model.

255 Besides the observed differences in model parameters CL, V2, V3 and Q, variability across
256 species was also observed for oral bioavailability (F1), the absorption rate constant (ka) and
257 duration of zero-order input (D1). Based on our available data, the model predicted a faster
258 and more extensive absorption of metoprolol in rabbits.

259 Tables 1 and 2 also illustrate differences between oral formulations within each of the
260 species. Based on the performed analysis, it can be concluded that ZOK-ZID® showed the
261 slowest absorption in both species. In dogs, we were not able to demonstrate a difference in
262 absorption behavior between the prills and Slow-Lopresor®. In contrast, a difference
263 between these two formulations could be observed in rabbits, in line with the in vitro
264 release findings. In this case, Slow-Lopresor® shows a faster absorption compared to prills

and ZOK-ZID®, respectively. Regarding the absolute oral bioavailability, it was not possible to distinguish between formulations in dogs, and no inter-individual variability in F1 could be estimated. In rabbits, the oral bioavailability for prills and Slow-Lopresor® was slightly higher (8.71%) as compared to ZOK-ZID® (5.88%). Based on the individual profiles (not shown here) and parameter estimates for ka and F1, the best slow-release profile (lowest peak-to-trough ratio) was obtained with the reservoir formulation ZOK-ZID® in both species.

The agreement between the observed and model-predicted metoprolol concentrations in the final model is illustrated in Fig. 4. This graphical evaluation confirms that our model is capable of describing the data quite well. A pcVPC of the final model further confirms that the developed population PK model adequately describes metoprolol PK data in Beagle dogs and rabbits (Fig. 5). The lower panels indicate that the predicted fractions of BDL data match the observed fractions of BDL data across time reasonably well.

3.3 In Vitro and In Vivo Correlation

Figs. 6 and 7 show the level C in vitro-in vivo correlation between partial mean AUC and percentage of metoprolol dissolved, for both dogs and rabbits. From the results, it is clear that a level C correlation is achieved across multiple time points, indicative of a multiple level C IVIVC. The Pearson's correlation coefficients (represented as r in Figs. 6 and 7) were higher for rabbits compared to dog data. The linear relationship between the dissolution kinetics and the mean t_{max} and C_{max} was also investigated (data not shown) and led to similar results, but was not explored further.

4. Discussion

In this article, for the first time, a pharmacokinetic model-based analysis is presented in which metoprolol pharmacokinetics were compared between the two commercially available modified-release formulations (ZOK-ZID® and Slow-Lopresor®) and the in-house produced prills across two pre-clinical species. Production of formulations via prilling represents a promising technology for the development of multiparticulate solid dosage forms. From a pharmacokinetic point of view, multiparticulate systems offer several advantages. Compared to a classic tablet (single-unit system), the smaller dimensions of the sub-units allow to immediately pass the pylorus, independent of the feeding state of the stomach. Since gastric emptying suffers from strong intra- and inter-subject variability, the administration of a drug via a multiparticulate system generally results in a higher reproducibility of the therapeutic effect. In addition, there is a lower risk of high local drug concentrations and gastro-intestinal side-effects as the dose is more uniformly distributed throughout the gastro-intestinal tract (Bechgaard and Nielsen, 1978; Bodmeier and Paeratakul, 1994; Gupta and Robinson, 1992; Krämer and Blume, 1994; Shukla et al., 2011). The flexible design of multiparticulate formulations also offers advantages for formulation scientists: the dose can be easily adjusted by increasing or decreasing the number of sub-units, without the need for formulation changes and without any effect on the drug release pattern. Also co-administration of different drugs (e.g. fixed-dose combinations) can be easily achieved (Bodmeier and Paeratakul, 1994; Shukla et al., 2011).

Besides the fact that multiparticulate systems are obtained via prilling, the technique also differentiates from other production methods via the use of lipid excipients. Characterized by a water-insoluble character, lipids represent an interesting platform for the development of controlled release formulations. Furthermore, lipid excipients are beneficial since they are

low-cost, physiologically non-toxic and biodegradable products (Reitz and Kleinebudde, 2007; Rosiaux et al., 2014).

In this study, plasma concentration-time data of administered metoprolol equivalently dosed to metoprolol tartrate over a dose range of 50 – 200 mg to Beagle dogs and New Zealand White rabbits were adequately described by a two-compartment model with linear elimination from the central compartment. In Beagles, the absorption rate constant was similar for Slow-Lopresor® and the prills. In contrast, Slow-Lopresor® was absorbed faster compared to the prills and ZOK-ZID® in rabbits. In both species, ZOK-ZID® resulted in the lowest absorption rate constant.

Overall, the pharmacokinetics of metoprolol in the study animals were highly variable. The pcVPC however indicates that the model performs reasonably well and predicts both the central tendency and the variability between animals in observed plasma concentration-time profiles for each preclinical species and for all formulations.

A rank-order relationship between in vitro dissolution and in vivo release and absorption at multiple time points, as derived from mean plasma concentration-time profiles, of the modified-release formulations could be observed in both species. In this respect, it seems that the rabbit is the preferred species to compare the in vitro results with. Based on the multiple level C IVIVC, the correlation coefficients for the rabbit data were higher than those for the dog data. A level A correlation is likely also achievable, based on the established multiple level C correlation, but wasn't explored further due to the focus on metoprolol absorption kinetics in vivo.

Following administration of the metoprolol modified-release formulations in rabbits, some concentration-time profiles exhibited quite erratic profiles, which were not always captured

well in the model, also because the number of animals per group was rather small. In all likelihood, this phenomenon will have contributed to a higher estimated inter-individual variability in absorption-related parameters.

During the population PK analysis, it could be confirmed that all modified-release formulations except for Slow-Lopresor® in rabbits, exhibited flip-flop pharmacokinetics, as expected. This phenomenon occurs when release of compound from the formulation is the slowest step in the process, as reflected in its absorption characteristics. This can only be derived if IV data are also available, which stresses the importance of the availability of an IV formulation and extended sampling schemes. The ultimate aim of a slow release formulation is to drive the in vivo PK profile, allowing an optimized plasma concentration-time profile including a lower peak-trough ratio. In addition, a more practical dosing regimen (once daily versus three times a day in this case) leading to increased adherence and a higher efficacy is what is strived for.

5. Conclusion

In summary, a metoprolol population pharmacokinetic model has been developed and validated in a group of Beagle dogs and New Zealand White rabbits, by applying the M3-method, to account for observations below the limit of detection. Following intravenous bolus dosing, plasma concentration-time profiles in both species were best described by a two-compartment model with first-order elimination. Plasma concentration-time curves in Beagle dogs following oral dosing of the different modified-release formulations were best described using a sequential zero- and first-order absorption model. The absorption phase in rabbits was best described using a first-order process. In addition, a multiple level C IVIVC

was constructed based on partial AUCs and the cumulative metoprolol amount dissolved for both Beagles and rabbits.

Plasma concentration-time profiles for the reservoir formulation ZOK-ZID® were comparable in both species, which was not the case for the matrix formulations. This study indicates that the PK of the reservoir formulation is similar in both species, even considering the almost completely missed absorption phase in rabbits. The insights gained from the current study further illustrate that, in view of their less optimal prolonged release characteristics and the resulting fast decline in metoprolol plasma levels, rabbits are not the species of choice to study PK of the matrix formulations in this study set-up.

Acknowledgements

The authors would like to thank Mr. Daniël Tensy for his assistance with the in vivo experiments. We thank Dr. Pieter Colin for his constructive comments during preparation of this manuscript. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Figures

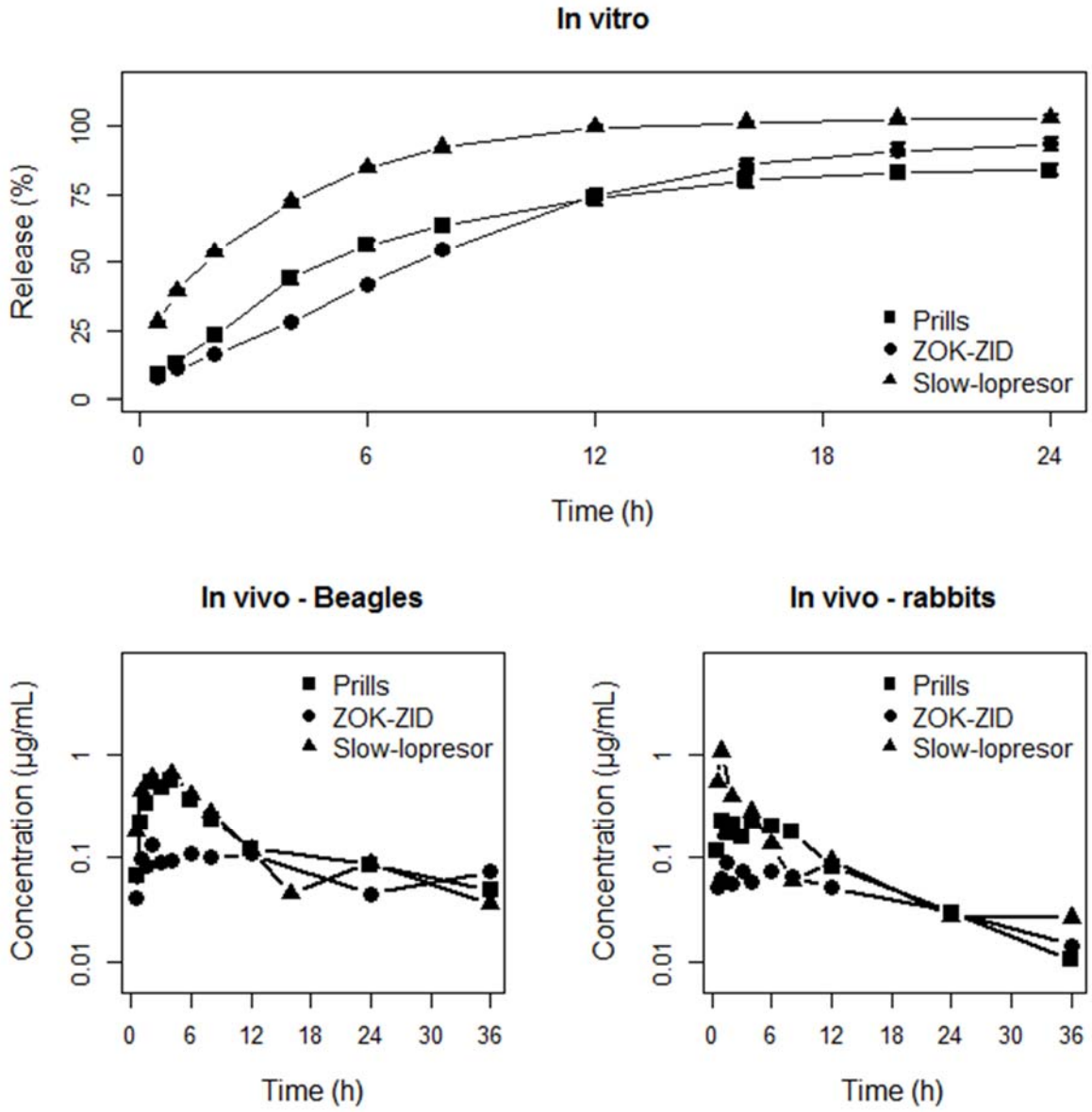


Fig. 1. In vitro dissolution profiles and mean plasma concentration-time profiles in dogs and rabbits

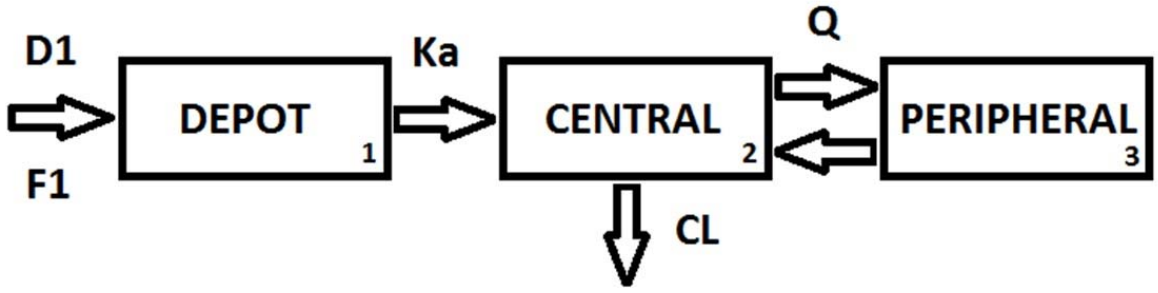


Fig. 2. Schematic representation of the PK model for metoprolol in Beagles

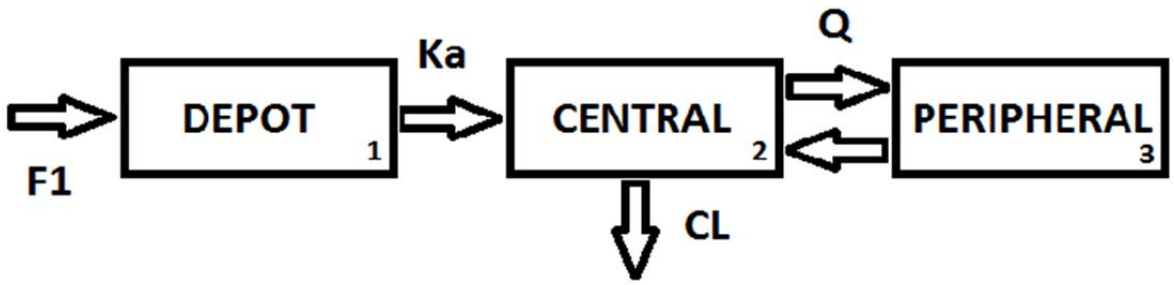


Fig. 3. Schematic representation of the PK model for metoprolol in rabbits

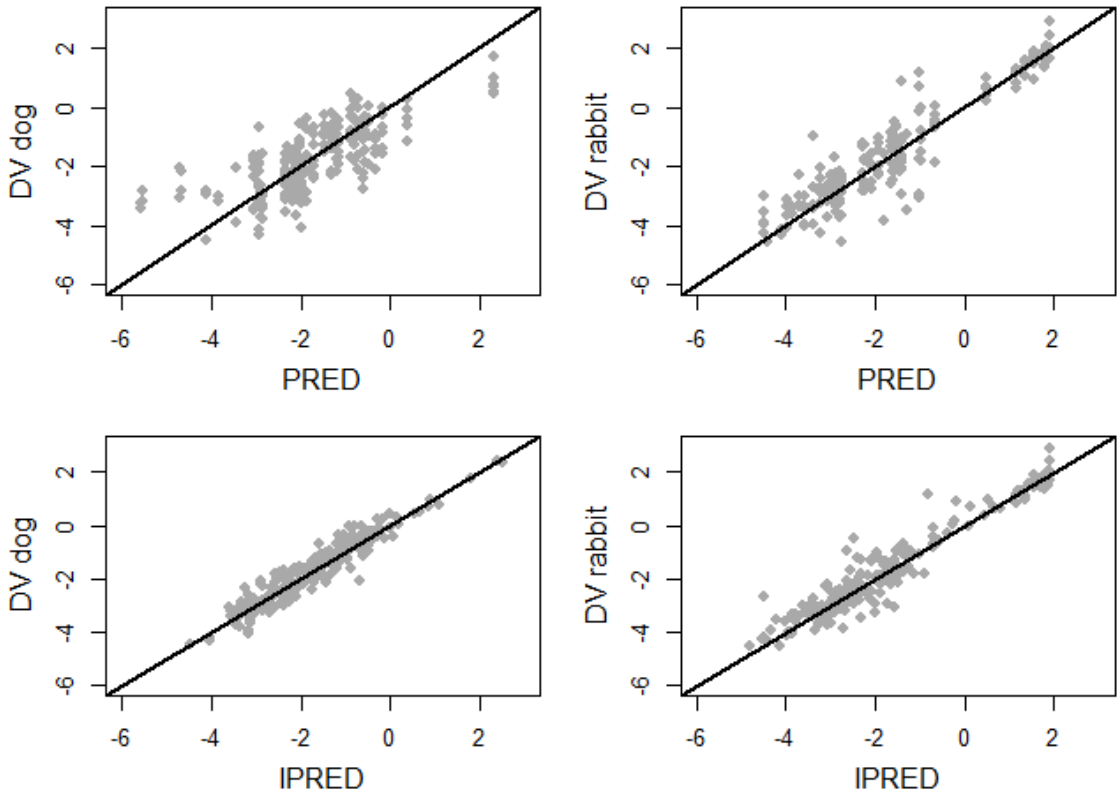


Fig. 4. DV vs PRED and IPRED for both dog and rabbit model

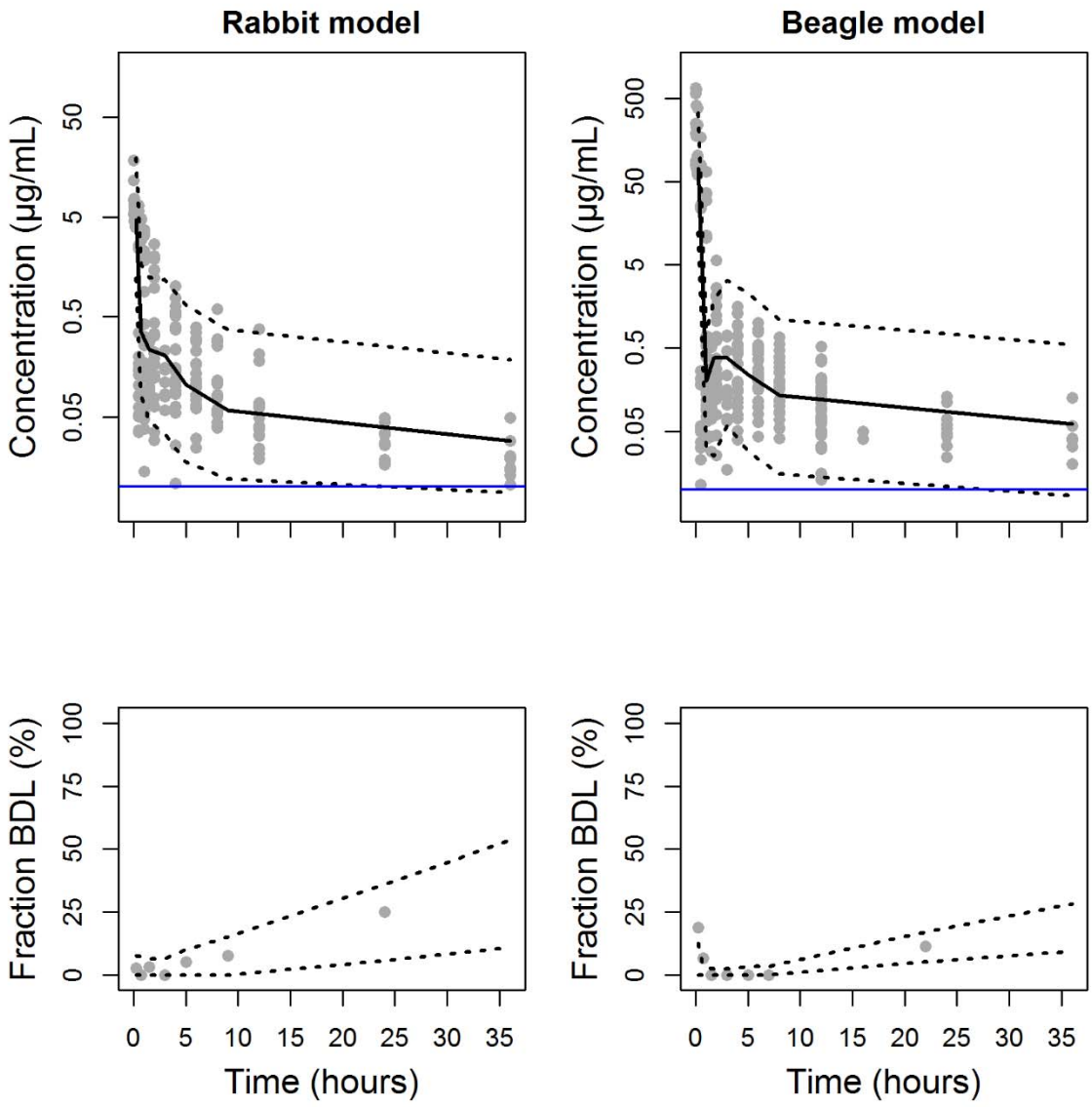
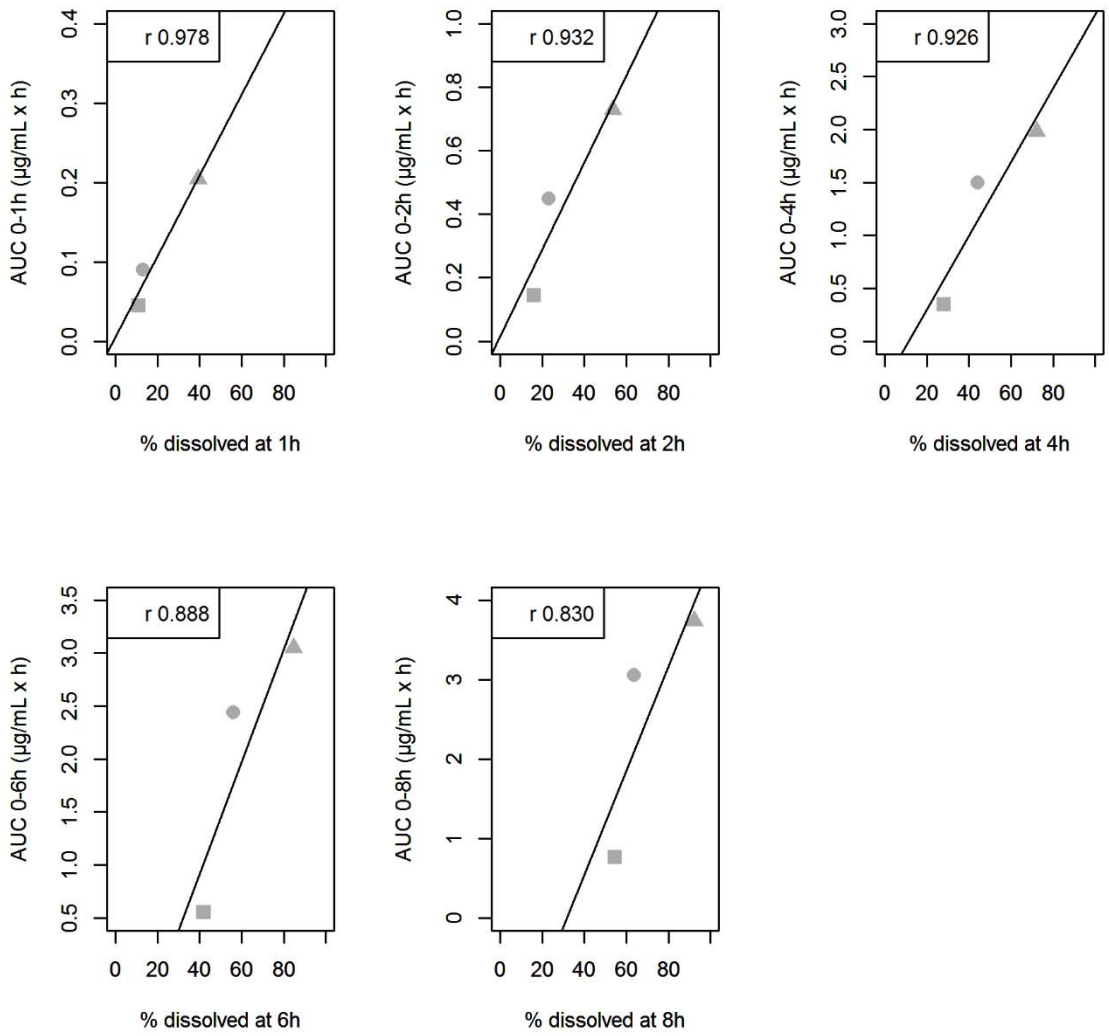


Fig. 5. Pred-corrected VPC in rabbits and Beagles (the horizontal line refers to the limit of detection, 10.1 ng/mL)



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393 **Fig. 6. IVIVC multiple level C for dogs (the squares refer to ZOK-ZID®, the triangles**
394 **to Slow-Lopresor® and the circles to the prills)**

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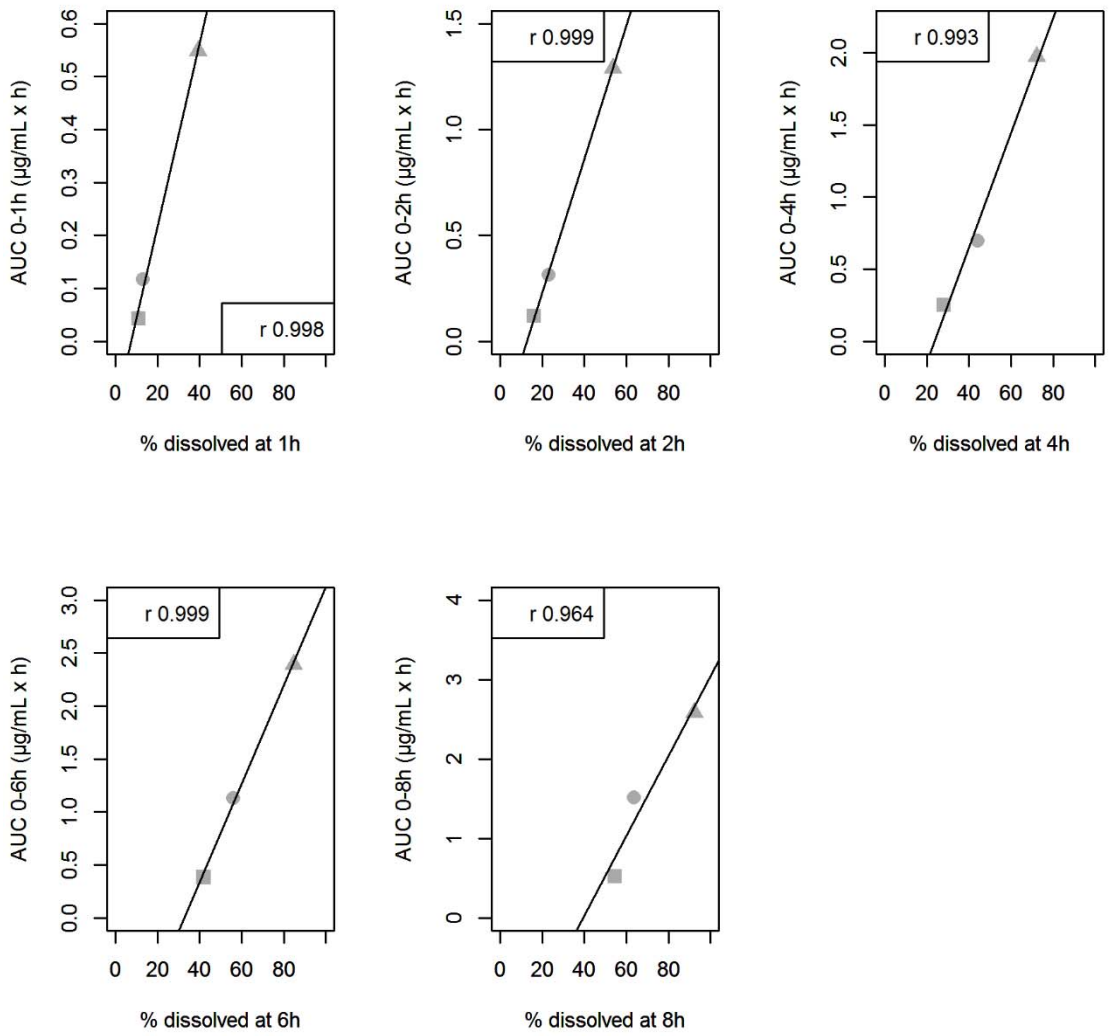

Fig. 7. IVIVC multiple level C for rabbits (the squares refer to ZOK-ZID®, the triangles to Slow-Lopresor® and the circles to the prills)

Tables

Table 1: Final population PK parameter estimates in Beagles

Parameter [units]	Final model estimate (%RSE[a])	95% Confidence interval (LLP)
CL [mL/h]	776 (25.4)	471 to 1 242
V2 [mL]	631 (24.0)	339 to 934
V3 [mL]	1 490 (134.1)	729 to 8 559
Q [mL/h]	116 (23.6)	62.5 to 170
Ka prills/slow-lopresor® [/h]	0.332 (9.9)	0.277 to 0.406
Ka ZOK-ZID® [/h]	0.0521 (16.2)	0.037 to 0.070
F1	0.0131 (25.3)	0.008 to 0.021
D1 prills [h]	2.26 (13.9)	1.701 to 2.932

D1 ZOK-ZID®/Slow-lopresor® [h]	1.29 (17.8)	0.978 to 1.879
IIV CL (% CV[b])	66.7 (15.5)	50.2 to 90.6
IIV V2 (% CV[b])	84.3 (22.0)	61.4 to 134
IIV V3 (% CV[b])	83.6 (38.0)	39.4 to 164
IIV Q (% CV[b])	59.1 (32.2)	38.3 to 113
Residual variability, ε (% CV[b])	67.6 (2.1)	65.3 to 70.9

[a] %RSE (percent relative standard error) was calculated based on the 95% confidence intervals (CI) obtained by LLP under the assumption of a symmetrical 95% confidence interval. The used formula to obtain the standard error was (upper limit CI – lower limit CI)/3.92, the %RSE was calculated by dividing the standard error by the parameter estimate and multiply by 100%.

[b] %CV (percent coefficient of variation) was calculated as sqrt(ω^2) x 100%; in case of LLP %CV was calculated by taking the square root of the lower and upper value of the confidence intervals given by PsN (Lindbom et al., 2005).

Table 2: Final population PK parameter estimates in rabbits

Parameter [units]	**Final model estimate (%RSE[a])**	**95% Confidence interval (LLP)**
CL [mL/h]	3 390 (19.9)	2 341 to 4 979
V2 [mL]	7 350 (11.6)	5 844 to 9 197
V3 [mL]	29 800 (66.1)	15 866 to 93 091
Q [mL/h]	2 180 (21.2)	1 424 to 3 237
Ka prills [/h]	0.27 (16.9)	0.197 to 0.376
Ka ZOK-ZID® [/h]	0.075 (29.0)	0.042 to 0.127
Ka Slow-lopresor® [/h]	0.59 (29.0)	0.358 to 1.029
F1 prills/Slow-lopresor®	0.0871 (29.3)	0.051 to 0.151
F1 ZOK-ZID®	0.0588 (56.4)	0.026 to 0.156
IIV CL (% CV[b])	51.6 (30.1)	28.8 to 89.6
IIV F1 (% CV[b])	64.3 (24.5)	40.3 to 102.1
Residual variability, ε (% CV[b])	75.4 (3.00)	71.3 to 80.1

[a] %RSE (percent relative standard error) was calculated based on the 95% confidence intervals (CI) obtained by LLP under the assumption of a symmetrical 95% confidence interval. The used formula to obtain the standard error was (upper limit CI – lower limit CI)/3.92, the %RSE was calculated by dividing the standard error by the parameter estimate and multiply by 100%.

[b] %CV (percent coefficient of variation) was calculated as sqrt(ω^2) x 100%; in case of LLP %CV was calculated by taking the square root of the lower and upper value of the confidence intervals given by PsN (Lindbom et al., 2005).

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