PKPD relationships and dose rationale in analgesic drug development : towards the prediction of target engagement

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SECTION II

MODEL BASED ANALYSIS OF BEHAVIOURAL PAIN RESPONSE

CHAPTER 4

Application of ED-optimality to screening experiments for analgesic compounds in an experimental model of neuropathic pain

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ABSTRACT

The high variability in the response to evoked pain prevents accurate ranking of compounds during the screening of drugs for inflammatory and neuropathic pain. In this study, we explore the feasibility of introducing optimality concepts to experimental protocols, enabling estimation of parameter and model uncertainty.

Pharmacokinetic and pharmacodynamic data from different experiments in rats were pooled and modelled using nonlinear mixed effects modelling. Pain data on gabapentin and placebo-treated animals were generated in the complete Freund's adjuvant (CFA) model of neuropathic pain. A logistic regression model was applied to optimise sampling times and dose levels to be used in an experimental protocol. Drug potency (EC_{50}) and inter individual variability (IIV) were considered the parameters of interest. Different experimental designs were tested and validated by SSE (stochastic simulation and estimation) taking into account relevant exposure ranges.

The pharmacokinetics of gabapentin was described by a two-compartment PK model with first order absorption (V2=0.118 l, V3=0.253 l, Cl=0.159 l/h, Ka=0.26 h^{-1}, Q=1.22 l/h). Drug potency (EC_{50}) for the anti-allodynic effects was estimated as 1400ng/ml. Protocol optimisation improved bias and precision of the EC_{50} improved by 6 and 11.9.%, respectively, whilst interindividual variability (IIV) estimates showed improvement of 31.89 and 14.91%, respectively.

Variability in behavioural models of evoked pain response leads to uncertainty in drug potency estimates and consequently to inaccurate ranking of compounds during screening. As illustrated for gabapentin, ED-optimality concepts enable analysis of discrete data taking into account experimental constraints.

INTRODUCTION

Despite the number of compounds entering early clinical development, neuropathic pain is an area of high attrition rate, with most treatments failing at proof-of-concept in patients. This situation is critical if one considers that currently available drugs for neuropathic pain show less than 50% efficacy in the overall target population [1]. Among other factors, the lack of efficacy in humans has been assigned to the poor correlation between evoked pain in pre-clinical models of disease and the differences in aetiology in humans [2]. On the other hand, another important point which has remained less evident is the fact that experimental protocols in pain research are often based on empirical criteria[3] and little or no attention is given to basic concepts such as accuracy and precision. Poor experimental designs often lead to biased and inaccurate parameter estimates [4], which consequently may influence the selection of suitable candidate molecules for progression into humans.

In early drug development, screening of compounds ought to rely on accurate ranking of their pharmacokinetic (PK) and pharmacodynamic (PD) properties, yielding evidence of their pharmacokinetic-pharmacodynamic (PKPD) relationships [5]. However, the use of a model-based approach for the analysis of such experiments, while desirable, is often precluded by practical constraints and resources [6]. Suitable designs entail the use of repeated measurements that describe the time course of drug concentrations and the pharmacological effects of interest. Feasibility considerations often limit the collection of repeated samples in individual animal and thus compromise the design of the experiment. Given the requirement for sparse sampling, appropriate sampling times become critical [7]. Therefore, accurate and precise model parameters estimates depend greatly on the experimental design.

In the current investigation, we use the CFA model, a well-known experimental animal model of inflammatory pain [8, 9], as paradigm to explore the feasibility of introducing optimality concepts in the screening of analgesic compounds. Dichotomisation of response is proposed as the basis for generalisation of a model-based approach in this phase of development. In optimal experimental design, D-optimality is by far the most used criterion in individual, and population modelling studies. Herein optimisation is carried out assuming there is no uncertainty (imprecision around the parameters of interest) i.e. there is no uncertainty distribution around this parameter. This assumption is also a disadvantage since for D-optimality to be applied, the true parameter value should be determined based on prior knowledge or model fitting in a previous exercise [10]. Although D-optimality has been considered the classic approach to designing an experiment optimally [11, 12], this method may not be suitable for prospective evaluation of the compounds during screening experiments when little data is available and prior knowledge about the pharmacokinetic and pharmacodynamic properties are limited.

Here we apply ED-optimality, an approach which has been applied in different areas of clinical research when the model parameters have uncertainty distributions [4]. The use of ED-optimality assumes a prior distribution around the parameters of interest [13]. While optimal design has been extensively used for optimizing different types of continuous repeated measurements, with non-linear mixed-effects modelling, little work has been done with discrete data [14]. We aimed at defining optimal design requirements for screening experiments, assuming EC_{50} and inter-individual variability as the parameters of interest for optimisation.

We anticipate that improved parameter precision and accuracy will contribute to better ranking of compounds and enhanced ability in discriminating false positives from false negatives during the screening of compounds for neuropathic pain.

MATERIALS AND METHODS

Experimental Procedures

In the CFA model, central sensitisation (NP) is induced following injection of an algogen. Allodynia or pain with a non-noxious stimulus is then measured as threshold to affected paw withdrawal with increasing diameter of von Frey filaments [15]. The experimental protocol was performed according to a double-blind, randomized, placebo controlled study with 9 animals per cohort. Sprague-Dawley rats received single doses of 0, 10, 30,100 mg/kg gabapentin orally. The study was approved by the Institutional ethics committee.

Pharmacodynamic measurements

The threshold to paw withdrawal to a normally non-noxious stimulus was measured as a marker of the anti-allodynic effect, whilst the change in threshold relative to baseline was selected as the PD endpoint. PD measurements were collected at hourly intervals for 4 h post-dose for each animal.

Pharmacokinetic experiments / measurements

PK data were obtained from 2 separate experiments in conscious rats. In the first, gabapentin was administered orally in the doses 0, 10, 100, 300 mg/kg in a formalin induced hypersensitivity experiment [16]. There were three rats per dose group, with each animal being sampled four times up to 6 hours post dose. In a second experiment, 63 animals received 50 mg/kg of gabapentin as an IV infusion. The rats were sampled 8 times up to 24 h post dose [17].

Data Analysis

Model parameterisation

The threshold for paw withdrawal was dichotomised as a binary response variable, with response being defined as changes in the threshold for paw withdrawal were >30% relative to baseline. The exposure-response relationship was modelled using a logistic regression (LR) model, using a parameterisation previously described by [18]:

$$p = \frac{e^{f(P,x)}}{1 + e^{f(P,x)}} \tag{1}$$

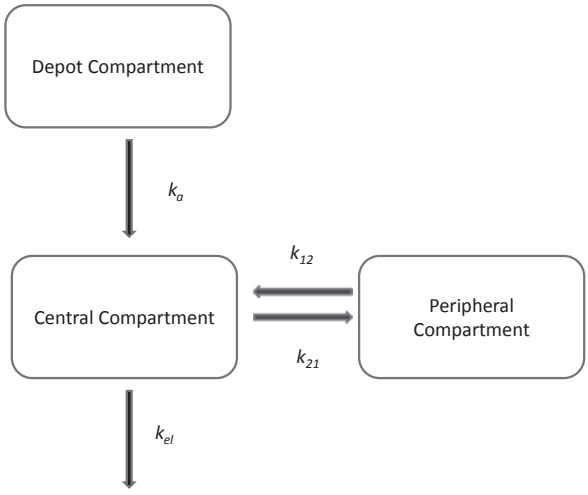
Where p is the probability of an event P, x are parameters and independent variables respectively. The odds of the event are therefore given by p/1−p and its logit may be expressed as:

$$logit(p) = ln\frac{p}{1-p} = f(P,X) \Leftrightarrow p = \frac{e^{f(P,X)}}{1+e^{f(P,X)}} \tag{2}$$

An E_{max} model was considered for the characterisation of the drug effect on the logit space. Random effects were denoted by η. This term represented both inter individual (IIV) as well as the random variability. Substituting for drug effects and p, the odds of an event may be represented by the following expression:

$$\frac{1}{1 + e^{\frac{Emax*CONC}{EC50+CONC}+plac+\eta}} \tag{3}$$

Drug concentrations at time points corresponding to PD measures were simulated using a two-compartment pharmacokinetic model with dose-limited absorption (see Figure 4.1). The model was built using a two-step approach. First, IV data from a previous experiment was modelled to obtain parameter estimates. Subsequently, using these parameters, bioavailability estimates were obtained for oral data. Details of the PK analysis are presented in the appendix (supplemental material).



$$K_{el} = \frac{CL}{V_2}, K_{12} = \frac{Q}{V_2}, K_{21} = \frac{Q}{V_3}$$

Figure 4.1: Two-compartment model used to describe the pharmacokinetics of gabapentin.

k_a=absorption rate constant, k_{el}=elimination rate constant, *Cl*=clearance,V_2 and V_3=volume of distribution in the central and peripheral compartments, respectively, *Q*=intercompartmental clearance.

ED-Optimal Design

Since drug potency is the parameter of interest, we focused on optimizing the experimental design in order to yield precise estimates of EC_{50} as well as the corresponding IIV. A summary of the model parameters used for the optimisation are given in Table 4.1. Non-linear mixed effects modelling based on the maximum likelihood estimation method was used for optimisation purposes. Theoretical aspects on the optimisation strategy are described in the appendix (see supplemental material).

We assumed drug exposure to be a determinant of response and thus optimised for sampling times and dose levels. Given that the bioavailable fraction of gabapentin is dose-dependent, the doses were optimised taking into account such differences. Other experimental variables were kept constant keeping in mind experimental constraints. These included, sample size (9 per cohort), number of measurements /individual (5), no of dose groups (4) and a maximum dose of 300mg/kg. Any predicted sampling times which were identified to occur within 30 minutes interval were moved apart from each other to ensure at least 30 min difference between two consecutive sampling times. A diagram of the general optimisation process is outlined in Figure 4.2. The empirical design was the benchmark design and the parameters of interest were EC_{50} and IIV.

Table 4.1: PK and PKPD model parameter estimates used in the optimisation process. RSE is shown between parentheses.

Model parameters	Values
Pharmacokinetics	
Central compartment volume *(V_1) (l)*	0.118 (9.8)
Peripheral volume *(V_2) (l)*	0.253 (4.2)
Clearance *(Cl) (l h^{-1})*	0.159 (4.1)
Intercompartmental clearance*(Q) (l h^{-1})*	1.22 (0.25)
Bioavailability fractions*(F)*	1, 0.75, 0.22, 0.087*
Absorption Rate constant *(K_a) (/h)*	0.26 (20)
Random error	0.30 (3.3)
Pharmacodynamics	
E_{max} (%)	97 (25)
Baseline/placebo effect (%)	2.81 (40)
EC_{50}*(ng ml^{-1})*	1400 (145)
IIV	3.14 (74)

** for doses 10, 30,100,300 mg/kg respectively*

Optimization Strategy

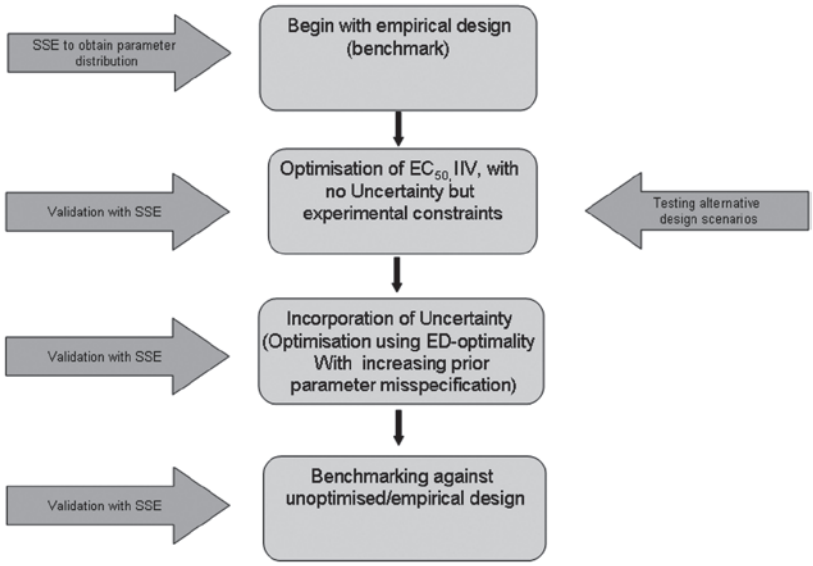


Figure 4.2: Overview of the general Optimisation procedures used during the analysis.

IIV= inter individual variability, SSE=stochastic simulation and estimation, EC_{50}=drug potency.

Prior parameter misspecification and uncertainty was incorporated into the optimisation by ED-optimality. A lognormal distribution was assumed for the parameters of interest, with mean values fixed as the true estimate. The maximum prior parameter misspecification was

predetermined to be 50%. An additional scenario was simulated and optimized to account for the effects of linear pharmacokinetics, assuming no change in the bioavailability across dose levels. The standard deviation of the priors was chosen so as to take into account expected parameter uncertainty and was defined as a distribution rather than a point value. The validation of the optimised design was carried out using stochastic simulation and estimation (SSE).

Stochastic simulation & estimation (SSE)

SSE was used to test the robustness of the optimal designs. In brief, during optimisation, gabapentin concentrations were simulated using the PK the model. The combined PKPD model was then used to simulate 'optimal sampling scenarios'. The initial values of the PD parameters in these models were considered the 'true estimates' and specified as the upper, lower and middle points of the distribution. The simulated optimal sampling datasets were then fitted to a pharmacodynamic model using nonlinear mixed effects modelling to assess the parameter estimates yielded by the proposed design. This two-step process was performed 500 times for each optimal design scenario. Estimation was considered successful when a normal flag (minimization successful) was obtained. Values of the 500 first successful estimations were recorded and summarised for each SSE run, along with their standard errors (SEs). To prevent numerical problems causing failure of the design, we began by first applying the ED-optimal design criteria with point values for priors (i.e., D-optimality) then increased prior breadth, until they reached the relevant uncertainty.

Concordance between optimised parameters and true values

Agreement between estimated and values used for simulations ('true values') were assessed using the mean of the estimation, the mean prediction error (MPE) and root mean square error (RMSE), which reflect precision and bias in model parameters [19]. Their calculation is as follows:

$$RMSE = \sqrt{\frac{mean((Est_i - True)^2)}{True^2}} \quad (4)$$

$$MPE = \frac{mean(Est_i - True)}{True} \quad (5)$$

where Est_i is the i^{th} parameter estimate and *True* is the simulation value (initial estimate) of the parameter. Matlab 7.9 (The Mathworks Inc., Natick, MA, 2008) and popED 2.11 (University of Uppsala, Sweden) were used for implementation of the optimization. PsN

3.12 (University of Uppsala, Sweden) and NONMEM 7.1 (ICON Development Solutions. Ellicott City, MD) were used for SSE. Data manipulation, graphical and statistical summaries were performed in R (www.r-project.org)

RESULTS

PK and PKPD model parameter estimation

An overview of the estimated PK and PKPD parameters are presented in Table 4.1. As can be seen from Figure 4.3(a), the simulated concentration profiles for all three doses of gabapentin are not significantly different from each other. When corrected for differences in bioavailability the observed exposures are equivalent to doses of 22.3, 22.5 and 27mg/kg respectively. Relative bioavailability was found to decrease nonlinearly from 100% at 10 mg/kg to 9% at 300 mg/ kg. The resulting logistic model for the response data is shown in Figure 4.3(b).

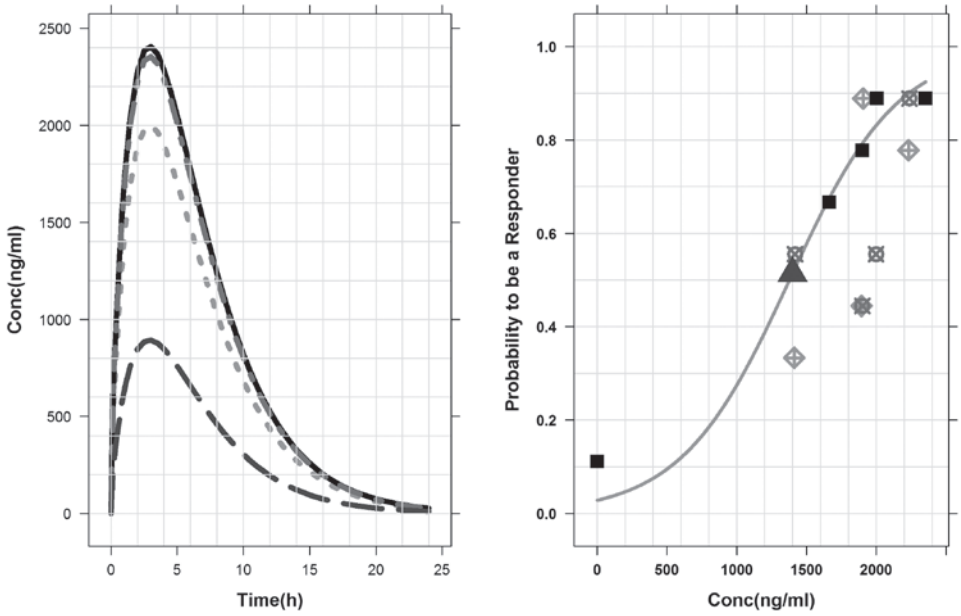


Figure 4.3: (a) Simulated plasma concentration *vs.* time profiles of gabapentin in rats after administration of 10(dashed line), 30(dotted line), 100(dash-dotted line), 300(solid line) mg/kg doses. (b) Logistic regression model showing the exposure-response curve (gabapentin concentration *vs.* probability of response). Symbols depict the proportion of observed responses after doses of 30(open diamond), 100(crossed circle), 300(filled square) mg/kg whereas the curve is the model-predicted probability.

Table 4.2: Priors used in the experimental protocol optimisation

Parameter	True Value	ED-optimal Design (0% variance)		ED-optimal Design (10% variance)		ED-optimal Design (50% variance)	
		Misspecification %	Mean	Misspecification %	Mean	Misspecification %	Mean
EC_{50} #	1400	0	1400	10%	1400	50%	1400
IIV (η)	3.14	0	3.14	10%	3.14	50%	3.14

 # values were applied to the logit space

DESIGN OPTIMISATION

As shown in Table 4.2, uncertainty in parameter estimation was explored with prior distributions of 10% (initial runs) and 50% (final run). As it can be seen in Table 4.3, whilst the same sampling scheme is used irrespective of dose in a typical empirical protocol, optimal designs require a different scheme for each dose. The optimised design performed equally irrespective of the uncertainty (i.e., 10 or 50% variance in parameter distribution).

Table 4.3: Comparison of dose levels and sampling times for the empirical and optimised protocol designs. Dose levels are shown in mg/kg, sampling times are in hours (h).

Design variables	Empirical design	ED-optimal (10% variance)	ED-optimal (50% variance)
Dose 1	0	0	0
Sampling times	0,1,2,3,4	0, 3.15, 4.72, 7.80, 9.93	0, 3.11, 4.23, 6.34, 10
Dose 2	30	100	100
Sampling times	0,1,2,3,4	0, 1, 1.5, 2, 4.9	0, 1.45, 2.49, 3.54, 4.54
Dose 3	100	150	150
Sampling times	0,1,2,3,4	0, 1.8, 2.80, 3.10, 4.10	0, 0.57, 1.7, 5.51, 6.01
Dose 4	300	300	300
Sampling times	0,1,2,3,4	0, 0.62, 1.12, 7.23, 8.23	0, 0.69, 1.19, 1.69, 5.67

Table 4.4: Impact of nonlinear absorption on protocol design optimisation.

Dose level (mg/kg) Sampling times (h)	Empirical design	Bioavailable dose level	ED-optimal (50% variance)
Dose 1	0	0	0
	0,1,2,3,4		0, 2.74, 3.45, 5.78, 8.45
Dose 2	30	29.5	100
	0,1,2,3,4		6.15, 6.65, 7.65, 8.15
Dose 3	100	41.7	150
	0,1,2,3,4		0, 1.34, 4.79, 5.29, 5.79
Dose 4	300	87.6	300
	0,1,2,3,4		0, 0.50, 1, 1.5, 2

Findings with gabapentin show that bioavailability decreases with increasing dose levels. Dose levels are shown in mg/kg, sampling times are in hours (h)

Comparison of parameter estimates & respective standard errors

Optimal estimates were closer to the true estimates and parameter standard errors decreased by more than half when sampling was based on ED-optimality concepts. Figure 4.4 reveals that improvements can be achieved not only in parameter estimates but also in SEs after design optimisation as compared to empirical protocol designs. Furthermore, we show that with varying bioavailability (Table 4.4), the difference between the optimised and empirical protocols is more marked. As shown in Table 4.5, parameter precision increases and bias is lower.

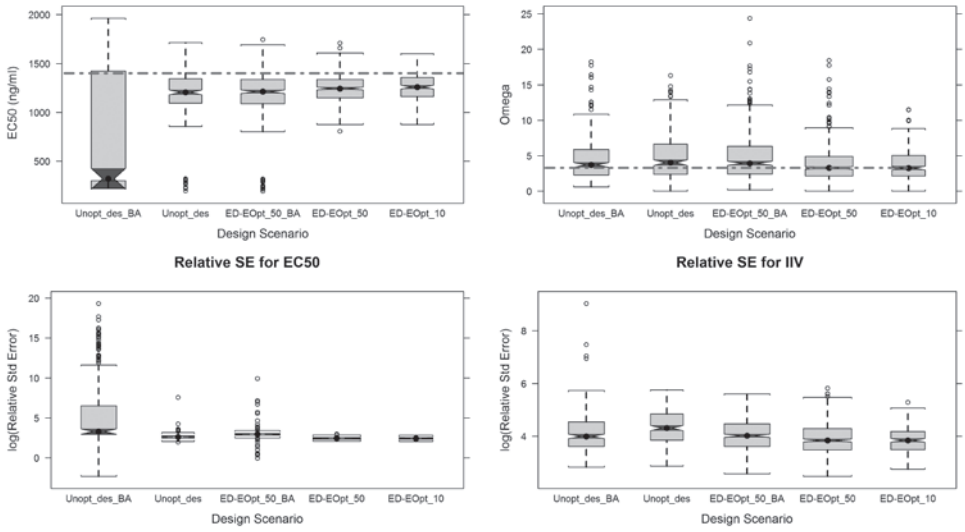


Figure 4.4: Comparison of model parameters (EC_{50} and IIV) and the corresponding estimates of bias and precision for various design scenarios described in Table 4.4. Dashed line indicates true estimate values.

Table 4.5: Comparison of parameter estimates for empirical and optimised experimental designs.

Design type	**EC_{50}* median (range)**	**IIV (omega) mean (SD)**
Empirical	1205.5 (200-1714)	4.82 (3.30)
ED-optimality (variance 10%)	1258 (876-1601)	3.70 (2.13)
ED-optimality (variance 50%)	1252 (806-1709)	3.96 (2.30)
Empirical with varying F	319 (223-1963)	4. 62 (3.25)
ED-optimality with varying F (variance 50%)	1211(191.7-1754.90)	4.78 (3.31)
True Value	1400	3.13

unit of measurement ng /ml

Bias and precision of parameter estimates

Uncertainty was introduced in a stepwise manner. RMSE is indicative of precision whilst MPE reflects bias in parameter estimates. In Table 4.6, the RMSE & MPE obtained after empirical and optimal designs are compared with each other. The implications of optimised sampling times in experimental protocols is shown graphically in Figure 4.5. As indicated by the different symbols response is sampled at time points where drug concentrations are informative of the expected drug potency. The sampling points for the empirical designs describe a monotonic pattern while those for the optimal designs are different for each dose and distribute around the EC_{50}.

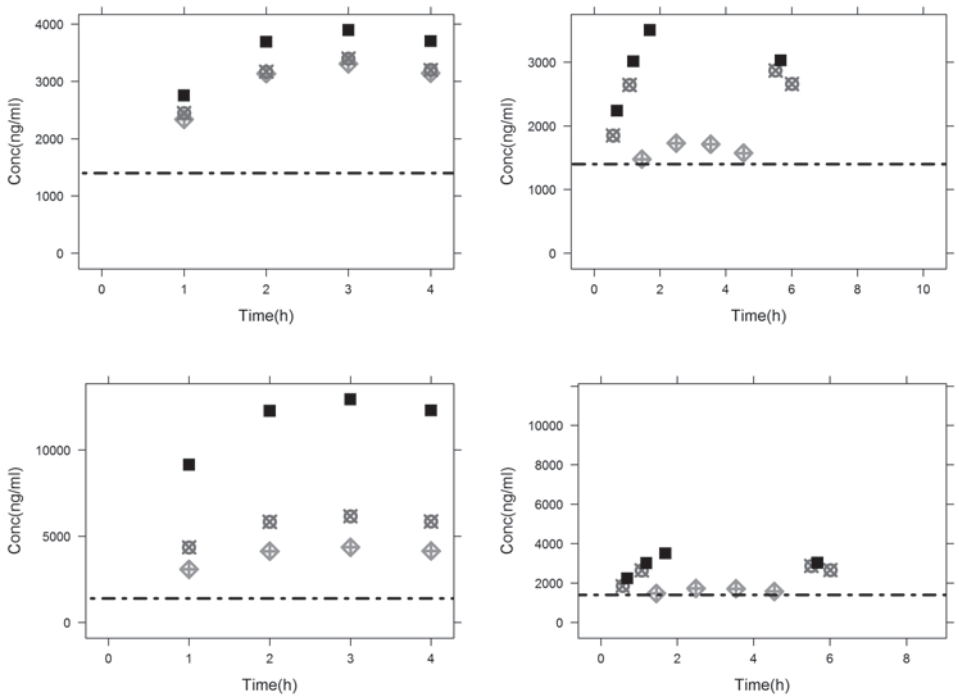


Figure 4.5: Selected sampling times relative to EC_{50} values (dashed line) based on a typical empirical protocol (left panels) and ED-optimal design (right panels). Based on theoretical principles, optimal sampling times should provide concentration values supporting the estimation of the parameter of interest. For gabapentin, our analysis show that variable bioavailability must be considered during optimisation to ensure accurate sampling times (lower panels). Symbols represent different dose levels namely; 30(open diamond), 100(crossed-circle), 300 (filled squares) mg/kg for all scenarios, except the top right panel where the optimal doses were, 100, 150 and 300mg/kg.

Table 4.6: Comparison of RMSE and MPE for the empirical and ED-optimal designs.

	EC50		Omega	
Design Type	RMSE	MPE	RMSE	MPE
Empirical	23.17	7.95	41.61	54.21
ED-optimal(50% variance)	11.27	2.37	26.70	22.32
Empirical with varying F	63.43	44.39	39.08	47.81
ED-optimality with varying F (variance 50%)	22.10	7.025	41.08	52.84

DISCUSSION AND CONCLUSIONS

The rat CFA model is typically classified as a model of inflammatory somatic pain. However the typical symptoms of allodynia and hyperalgesia are reproduced in this experimental model, which are considered indicative of central sensitization [20]. Hence this test is commonly employed as part of the battery of screening experiments when looking for analgesic/anti-neuropathic pain activity.

It is evident that challenges exist in the identification of suitable targets for the treatment of neuropathic pain [21]. Given the gaps in the understanding of the mechanisms underlying neuropathic pain disorders, drugs are tested pre-clinically without evidence as to which target will yield a clinically relevant response. However, this is further compounded by the paradigm currently used for the screening of compounds, which relies on evoked-pain response associated with general positive symptoms such as allodynia and hyperalgesia [22, 23]. Ongoing research strongly suggests that evidence of concentration-effect relationships is necessary for translational purposes and accurate ranking of compounds [24].

Although further advancement of the field will certainly depend on the identification of specific biomarkers of disease, the assessment of pharmacokinetic-pharmacodynamic relationships remains fundamental for characterising the properties of novel compounds and interpreting response across species. Unfortunately, screening procedures and protocols do not consider the implication of empirical designs, which can result in estimates of drug properties such as potency, which are often imprecise and extremely variable[25]. As a consequence, inaccurate ranking of compounds is likely to occur during the screening stage, which then progress into development. Our investigation illustrates the implications of optimality concepts to better design experimental protocols and obtain more precise and accurate parameter estimates.

There have been numerous attempts in the implementation of optimal designs for experimental protocols [26], but most designs were explored under the assumption of no parameter uncertainty [27], a condition which does not correspond to the screening phase of novel molecule candidates. Furthermore, application of optimality concepts imposes the availability of a model, which is unknown at the early stages of drug discovery and development, as is the case during the screening phase. These conceptual constraints are

further complicated by practical challenges during the experiments, such as the potential interference of blood sampling for pharmacokinetics between behavioural measurements and limited sampling frequency due to habituation and other possible effects on pharmacodynamics.

Here we have shown how the use of a binary response can overcome technical limitations associated with model building, by emphasising the assessment of drug- and system-specific parameters. The parameterisation of drug effects in terms of EC_{50} allows discrimination of drug properties, whereas baseline and maximum response E_{max} reflect experimental model characteristics and as such can be estimated in conjunction with historical data, which are incorporated as parameter priors. In addition, as shown in the different scenarios, ED-optimality also allows the inclusion of uncertainty in a formal manner.

Our analysis focused on the simultaneous optimisation of two design variables, namely dose and sampling times, in a similar way to what has been previously reported by Nyberg and collaborators [28]. In contrast to empirical designs, the results show that the use of optimality concepts yields sampling at time points around the expected parameter estimate (Figure 4.5), thereby maximizing the information obtained from the an experimental protocol. Even in the case parameter misspecification, ED-optimality appears to provide more informative data than designs based on 'best guess' estimates.

Since gabapentin exhibits carrier-mediated absorption, bioavailability was found to be nonlinear, decreasing with increasing doses [29]. This effect caused an unusual situation in which changes in the bioavailable fraction resulted in practically the same exposure across the different dose levels. In such circumstances, the 'best guess' estimates were not as biased as one would normally observe. To illustrate the effect of such nonlinearity we have therefore investigated an optimisation scenario in which bioavailability estimates decreased with increasing doses but yielding wide variation in plasma concentrations. The results reveal that empirical protocols perform much more poorly (as defined by the bias and precision of EC_{50}) than optimised designs.

Limitations

The optimisation procedures were constrained by the effect on nonlinear absorption and relatively sparse availability of oral pharmacokinetic data, which prevented accurate estimation of the pharmacokinetic parameters of interest. Therefore, drug concentrations from an IV experiment were used to support the estimation of clearance and volume of distribution. Whilst data were accurately fitted to the model, we did not attempt to describe the transporter-limited absorption of gabapentin in a mechanism-based manner. Instead, we applied a 'curve linearization' approach, under the assumption that bioavailability decreased linearly across the dose range.

In addition, it has been documented that optimal designs based on D-optimality have a tendency to cluster at parameter point estimates because model expectations is assumed to be the same for each individual. Apparently, this clustering effect may be minimised by the use of priors [30]. Regardless of the use of priors clustering was observed during the analysis, but this issue was resolved by imposing minimum interval between sampling times, as describe in the methods section. We have not performed a sensitivity analysis to explore the potential impact on the estimates of bias and precision obtained for the different optimisation scenarios. We anticipate however that the use of stepwise iterations during optimisation should minimise such issues, including failure due to numerical problems. Lastly, it should be noted that IIV estimates were higher with varying bioavailability. It is not clear if these findings may have been caused by inflated random residual variability in this specific scenario.

In conclusion, our study reveals that experimental requirements must be considered for the purposes of screening and ranking of compounds. Accurate estimation of drug potency (EC_{50}) entails modification to the protocol design, including specific changes to sampling procedures and dosing rationale, which cannot be guessed without applying ED-optimisation concepts. It is time for experimentalists to understand the implications of empirical protocols and make sure experiments are suitable for the evaluation of pharmacokinetic-pharmacodynamic properties of novel molecules.

APPENDIX 1: PK AND PKPD MODELLING DETAILS

The pharmacokinetics of gabapentin was described by macro-constants according to the following expression[31]:

$$C = \frac{K_a FD}{V_1}\left\{\frac{(k_{21}-\lambda_1)e^{-\lambda_1 t}}{(k_a-\lambda_1)(\lambda_2-\lambda_1)} + \frac{(k_{21}-\lambda_2)e^{-\lambda_2 t}}{(k_a-\lambda_2)(\lambda_1-\lambda_2)} + \left(\frac{(k_{21}-K_a)e^{-K_a t}}{(\lambda_1-k_a)(\lambda_2-k_a)}\right)\right\} \quad (6)$$

Where $k_{a=}$absorption rate constant, V_1=central volume of distribution , F=bioavailability of the administered dose, λ_1 and λ_2 correspond to the initial and terminal slopes representing bi-exponential decline respectively and K_{21} is a rate transfer microconstant between compartments 1 and 2.

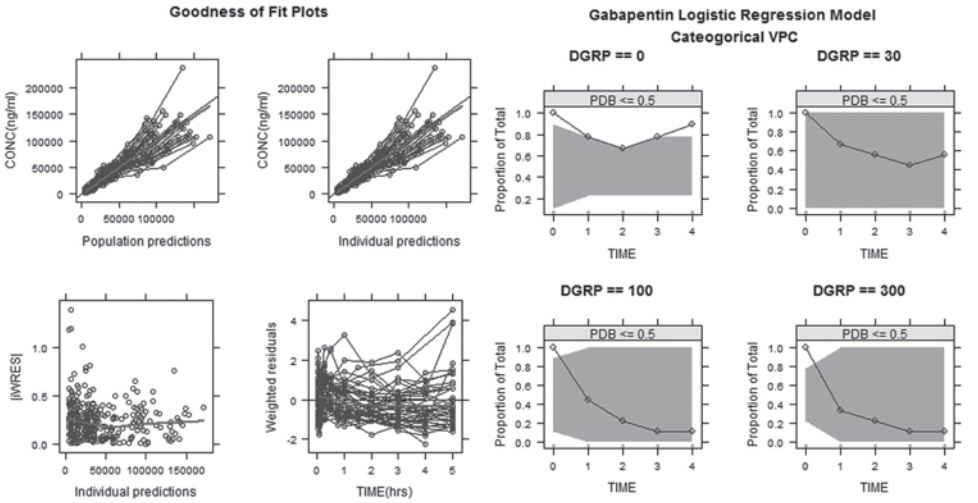


Figure 4.6: Goodness-of-fit plots for the pharmacokinetic (left panel) and PKPD (right panel) models. Lines represent the observed data, whereas the shaded area depicts the 90% confidence intervals

APPENDIX 2: - OPTIMISATION CONCEPTS FOR BINARY RESPONSE

In optimal design, the probability density $p(y_i|\theta)$ of the experimental observations y_i depends on a vector of likelihood parameters θ, where

$$\theta^T = [(\beta pop)^T, d^T] \tag{7}$$

The maximum likelihood estimate θ is the value that maximises the joint log-likelihood function:

$$L(\vartheta(= \sum_{i=1}^{m} logp(y_i \mid \vartheta) \tag{8}$$

$$Cov\vartheta \geq (FIM)^{-1} \tag{9}$$

As a result of a smaller covariance matrix, the lower the FIM^{-1} the greater is the precision, where FIM is defined as:

$$FIM = E_y[(\frac{\partial}{\partial\theta} L(\theta))^T \frac{\partial}{\partial\theta} L(\theta)] \tag{10}$$

By choosing the optimal design variables $\hat{X}$ that minimize FIM^{-1}, one obtains the design variables that yield the smallest possible lower bound for the covariance matrix of the population parameter estimates [4].

Using the ED-optimality criterion, the parameters of interest are assigned a prior distribution and an expectation. A design X^D is said to be ED-optimal if it minimises the negative expected (Eα) determinant of the FIM with respect to the parameter priors.

$$jED(x) = E\alpha[det[F(\alpha, x)]] \tag{11}$$

$$Int_{-inf} \int_{-¥}^{+¥} p(\alpha).det[F(\alpha, x)]dx \tag{12}$$

$$x^{ED} = argmax_n[j^{ED}(x)] \tag{13}$$

Laplace approximation was used for calculation of the FIM. The optimization criteria used was D-/ED optimal (i.e., optimizing the determinant of FIM). The Latin hypercube (LH) sampling was used in the MC calculation of the likelihood to speed up and stabilise the likelihood calculation. The number of LH samples differed between the different models but was between 40-200 individual samples. The FIM was calculated both using the

expectation of the gradient product of the first derivative of the log-likelihood with respect to the parameters as well as the expectation of the negative 2nd order derivative of the log-likelihood with respect to the parameters.

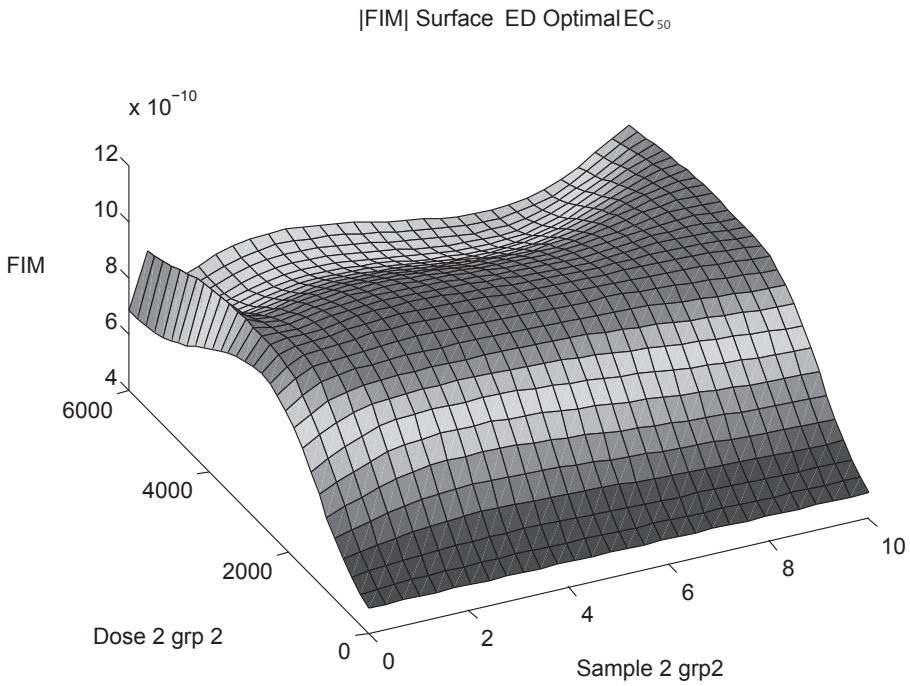


Figure 4.7: Fisher Information Matrix surface versus the highest dose (300mg/kg) and sample times combination for ED design with 50% variance in the expected parameter estimates. The dark red surface represents the optimal design for this dose and sampling time's combination.

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