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RESEARCH PAPER

Modeling Sleep Data for a New Drug in Development using Markov Mixed-Effects Models

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ABSTRACT

Purpose To characterize the time-course of sleep in insomnia patients as well as placebo and concentration-effect relationships of two hypnotic compounds, PD 0200390 and zolpidem, using an accelerated model-building strategy based on mixed-effects Markov models.

Methods Data were obtained in a phase II study with the drugs. Sleep stages were recorded during eight hours of sleep for two nights per treatment for the five treatments. First-order Markov models were developed for one transition at a time in a sequential manner; first a baseline model, followed by placebo and lastly the drug models. To accelerate the process, predefined models were selected based on *a priori* knowledge of sleep, including inter-subject and inter-occasion variability.

Results Baseline sleep was described using piece-wise linear models, depending on time of night and duration of sleep stage. Placebo affected light sleep stages; drugs also affected slow-wave sleep. Administering PD 0200390 30 min earlier than standard dosing was shown through simulations to reduce latency to persistent sleep by 40%.

Conclusion The proposed accelerated model-building strategy resulted in a model well describing sleep patterns of insomnia patients with and without treatments.

KEY WORDS Markov model · NONMEM · pharmacodynamics · polysomnography · population analysis · sleep · transition model · zolpidem

INTRODUCTION

Initial dose ranging studies conducted to evaluate new drugs developed for the treatment of insomnia typically include evaluation of a drug's effect in the sleep laboratory with polysomnography (PSG) conducted over an eight-hour period. The PSG provides objective measures of sleep, including sleep onset, maintenance of sleep, and sleep architecture. Based on the standard definition of sleep stages by Rechtschaffen and Kales (1), each sleep stage is determined over a 30-second interval, i.e. one epoch. Based on these sleep stages, quantitative endpoints describing sleep efficacy are calculated for each night of dosing.

Results of the PSG recordings are summarized over the eight-hour period but may also be summarized in shorter intervals over night, e.g. hourly. For each of these intervals, endpoints such as sleep time and time spent in each stage may be calculated. This helps define the time course of the

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different sleep stages overnight as well as provide an understanding of the time course of drug effect. A further refining of the time interval would be to examine the sleep stage for each epoch. A pharmacokinetic-pharmacodynamic (PK-PD) model that examines transitions to and from each stage based on the epochs would provide a granular description of the time course of sleep in the population and the effect of different drugs in terms of both their time course of response and their different effect on sleep architecture. Such an approach has previously been applied using Markov models to describe the probability of transitioning from each stage of sleep (2–4). Kemp *et al.* (2) used Markov models for analyzing and simulating the individual sleep profiles of healthy volunteers. One limitation of the model developed by Kemp *et al.* was to assume that transition probability from one stage to another is constant throughout the night, i.e. the transition probability did not vary over time. In the work by Karlsson *et al.* (3), the transition probability for all stages was investigated using mixed-effects analysis, and the transition probability was allowed to change during the night. In that study, the sleep pattern in an insomnia population was characterized for baseline sleep under the influence of placebo treatment. Karlsson *et al.* were also able to characterize the effects of a benzodiazepine, temazepam. Bizzotto *et al.* (4) expanded the model proposed by Karlsson *et al.* to include restrictions on the probability of transiting between stages to ensure that no probability exceeds one and applied the model to primary insomnia patients to characterize the baseline sleep-including placebo effects.

We have applied the mixed-effects Markov model to data from a proof-of-concept study of a new drug in development for the treatment of insomnia. The test drug, PD 0200390, is a calcium channel α2δ subunit-binding compound (5). The drug is rapidly and almost completely absorbed following oral administration with a mean T_{max} value of less than two hours. Following administration in the morning, peak PD response, measured using a sleepiness scale in healthy volunteers, is delayed relative to peak plasma concentrations and observed typically 3–4 h post-dose. A GABAa agonist, zolpidem, was also included in the study as active control. Administration of zolpidem results in a rapid onset of action. Its half-life is about 2.5 h, which results in a rapid offset of action as well; thus, the degree of somnolence observed following administration of zolpidem to healthy volunteers peaks 1 h post-dose and returns to baseline within 4–6 h post-dose (6).

A difficulty with development of Markov models, in particular for categorical data with many categories, is the extensive work that is needed for model building. In the case of sleep data with five categories, the total number of transitions is 20. Additionally, the frequent sampling of sleep contributes to the complexity of the model building as most software for mixed-effects modeling has an upper limit for the number of observations allowed per individual. In addition, the run-times of the models increase rapidly with an increasing number of observations. Thus, efforts were made to develop a model-building strategy to accelerate this process.

The purpose of this PK-PD analysis was to characterize the time course of sleep stages and the concentration-effect relationship of PD 0200390 on different sleep stages relative to placebo and zolpidem. This model was also used to assess the effects of different dosing regimens using simulations. This was done using an accelerated model-building process for sleep data using Markov models.

MATERIAL AND METHODS

Study Subjects

Healthy subjects of any race and either gender between the age of 18 and 63 years, with normal electrocardiogram and with a three-month history of primary insomnia, were eligible for the study based upon subjective average latency to sleep ≥30 min and subjective average total sleep time ≤6.5 h by history. These subjects were scheduled for two nights of screening PSG in the sleep lab.

Forty three subjects, who according to the screening PSG met all entry criteria, including a normal and stageable PSG, 4 h ≤mean total sleep time ≤7 h, mean latency to persistent ≥20 min, with the latency to persistent sleep ≥15 min on both nights, were randomly assigned to the treatments. The definitions of latency to persistent sleep and total sleep time are given in the following section (Sleep Measurement). Demographics for the patients are given in Table 1.

Sleep Measurement

Each study night (including the screening nights), the subjects went to bed at their habitual bedtime and underwent PSG evaluation for 8 h. The PSG recording started at the time the subject went to bed. PSG evaluation included standard measurements of central and occipital electroencephalogram, submental electromyogram, electro-oculogram, airflow, respiratory effort, anterior tibialis electromyogram and electrocardiogram.

PSG data were collected digitally and transferred directly to a central reader for scoring. The central reader was blinded to treatment conditions. Sleep stages were determined by the central reader according to Rechtschaffen and Kales criteria (1) for each epoch of the PSG as wakefulness, stage 1, stage 2, stage 3, stage 4, and REM sleep. As observations of stage 3 and stage 4 were few, these two were lumped into one stage called slow-wave sleep.

Table I Patient Demographics from All Patients in the Analysis

Subject characteristics	
Gender	
Male : Female	18 : 25
Hormonal Status	
Male : Pre-menopausal : Surgical sterile : post-menopausal	18 : 12 : 2 : 11
Race	
Caucasian : Black : Hispanic : Other	29 : 8 : 4 : 2
Smoking habits	
Non-smoker : former smoker : smoker	27 : 7 : 9
Age (years)	
Mean (SD)	45.2 (9.36)
Min, Max	26, 63
Weight (kg)	
Mean (SD)	77.2 (14.1)
Min, Max	45.5, 121
Height (cm)	
Mean (SD)	171 (9.36)
Min, Max	141, 190.5
Alcohol consumption during a month (cL of spirits)	
Mean (SD)	3.27 (1.00)
Min, Max	0, 14
Creatinine Clearance (mL/min)	
Mean (SD)	94.3 (19.4)
Min, Max	59.6, 141

Eighteen efficacy endpoints, measuring sleep quality and quantity, were calculated based on the scored sleep stages. These measurements, including their definitions and abbreviations, are listed in Table 2.

Study Design

This was a randomized, double-blind, active- and placebo-controlled, four-way crossover study, performed to assess the safety and efficacy of PD 0200390. All patients received all four treatments blocks: 25 mg and 75 mg of PD 0200390, 10 mg zolpidem and placebo. They were all assigned to one of four treatments according to a Williams design. In each treatment block, the study medication was administered 30 min prior to the subject's habitual bedtime for two consecutive days. A one-week washout window prior to the next treatment was established.

Blood samples were collected 15 to 30 min and 30 to 60 min after awakening. Samples were assayed for PD 0200930 concentrations only.

Dataset

The dataset was prepared with sleep stage as the dependent variable. The collected sleep stages are shown in Fig. 1 divided on previous observation for different time intervals during the night. Also included in the dataset were columns giving the observation from the previous measurement of sleep stage, the time elapsed since the last change of sleep stage (referred to as stage time), the time elapsed since going to bed divided by the total time spend asleep (referred to as relative bedtime), the predicted concentrations of zolpidem, and the individually predicted PK parameters of PD 0200390. Apart from these columns, standard columns, such as subject number and covariates, were included in the dataset.

A Markov process is a stochastic process in which the future states depend only on the present state. Thus, the probability of transitioning to a sleep stage is conditioned on the present sleep stage observation. In our study, sleep was divided into six stages: initial sleeplessness (IS), wakefulness (0), REM (5), stage 1 (1), stage 2 (2), and slow-wave sleep (3). The stage of initial sleeplessness was introduced to allow estimation of a separate transition probability for the first time the patients fell asleep, since this was different from falling asleep when sleep already has occurred. All patients started in the initial sleeplessness stage and the only occurring transition from this stage was to stage 1. Furthermore, patients were not allowed return to this stage once a transition occurred.

Since there were five stages plus initial sleeplessness, a total of 21 transitions from one stage to another were theoretically possible. However, not all transitions occur physiologically, and some transitions were very infrequent. To reduce the number

Table 2 Endpoint Measurements for Sleep Efficacy with Abbreviations and Definitions

Efficacy Endpoint	Abbreviation	Definition
Latency to persistent sleep	LPS	Time from start of PSG recording to sleep lasting at least 5 min
Total sleep time	TST	Time from sleep onset to final awakening subtracting time of wakefulness
Sleep efficiency	SE	Proportion of sleep, defined as TST divided by the time in bed
In period 1	SE1	SE for sleep period 0–2 h
In period 2	SE2	SE for sleep period 2–4 h
In period 3	SE3	SE for sleep period 4–6 h
In period 4	SE4	SE for sleep period 6–8 h
Wake after sleep onset	WASO	Time of wakefulness after sleep onset
Number of awakenings	NAW	Number of arousals to wakefulness during the time in bed
Number of arousals	NAR	Number of arousals to stage 1 and wakefulness during the time in bed
Stage shift	SHIFT	Number of shifts from one stage to another during the time in bed
Sleep onset latency	SOLAT	Time from start of the PGS recording to stage 1 is observed
REM sleep latency	RELAT	Time from start of the PGS recording to REM sleep is observed
Total time in stage 1	T1	Total time spend in stage 1 during the night
Total time in stage 2	T2	Total time spend in stage 2 during the night
Total time in slow-wave sleep	T3	Total time spend in stage 3 or stage 4 (slow-wave sleep) during the night
Total time in REM sleep	TREM	Total time spend in REM sleep during the night
Total time in non-REM	TnonREM	Total time spend in stage 1, stage2, stage 3 or stage 4 during the night

of transitions to model, three criteria were defined to identify the transitions of interest: (i) an average transition probability of at least 1%, (ii) a transition represented at least 10% of all transitions from a stage, and (iii) a transition represented at least 10% of all transitions to a stage. A transition was modeled if at least one of the criteria was fulfilled.

Data Analysis

Markov mixed-effects models, similar to the models used by Karlsson *et al.* (3), were used to analyze the sleep data. If $Y_{ij} = (Y_{ij1}, Y_{ij2}\ldots Y_{ijn})$ is the vector of observations for the *i*th subject at the *j*th occasion, then the probability that

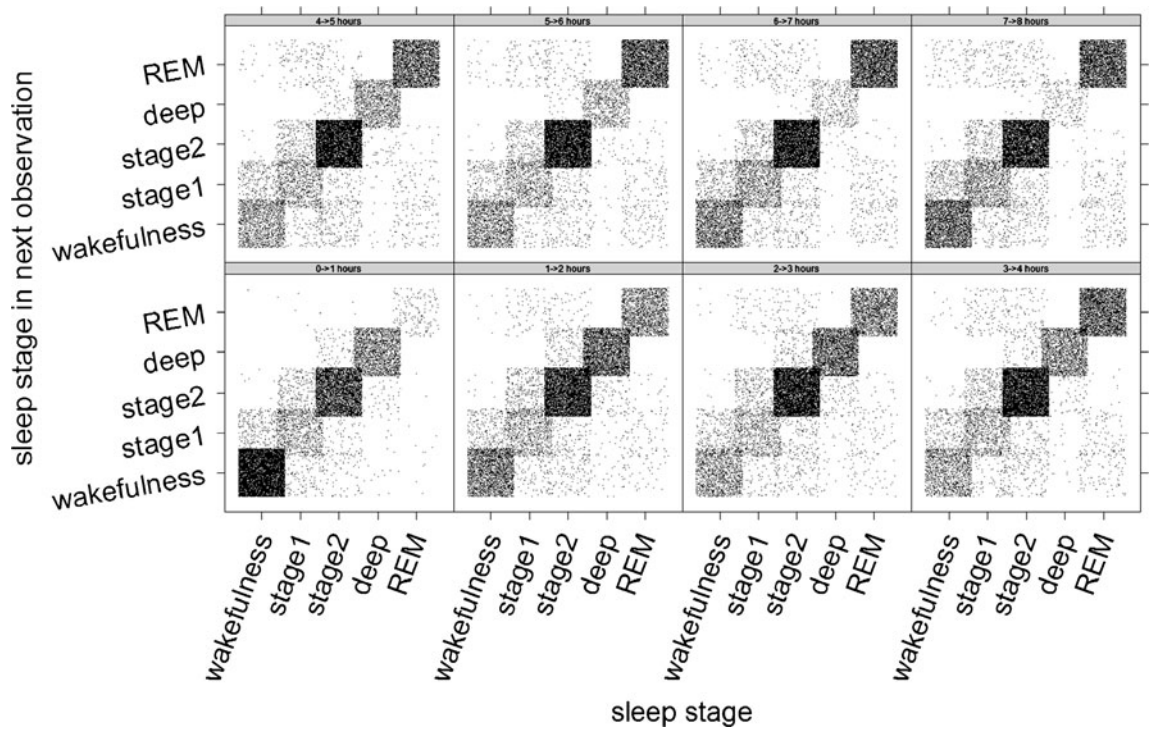

Fig. 1 Distribution of transitions in the observed sleep data divided into hourly intervals.

Y_{ijt} is equal to the stage m (m=IS, 0, 1, 2, 3, 5) at time = t, given that the preceding observation was k ($k \neq m$) has the following general structure:

$$logit\left(P(Y_t = m|Y_{t-1} = k)_{ij}\right) = logit\left(p_{ijm|k}\right) = g_{m|k} + \eta_i + \kappa_j \quad (1)$$

where

$$logit\left(p_{ijm|k}\right) - \ln\left(\frac{p_{ijm|k}}{1 - p_{ijm|k}}\right) \quad (2)$$

hence,

$$P(Y_t = m|Y_{t-1} = K)_{ij} = \frac{e^{g_{m|k}+\eta_i+\kappa_j}}{1 + e^{g_{m|k}+\eta_i+\kappa_j}} \quad (3)$$

The logit transform is used to ensure the probability to be between zero and one; $g_{m|k}$ defines the four sub-models and was implemented as a function of relative bedtime, stage time, drug and placebo effects, as defined in Eq. 4. The random effects η_i and κ_j are normally distributed with a mean of zero and variances ω^2 and π^2, respectively, describing between-subject and between-occasion variability. An occasion was defined as one visit, and since all four treatments and screening were measured at two consecutive nights, there were ten occasions for each subject.

$$\begin{aligned} g_{m|k} &= \ln\left(\frac{TP_{m|k}}{1-TP_{m|k}}\right) + Placebo + DrugExposure \\ TP_{m|k} &= f(relative\ bedtime) \cdot f(stage\ time) \end{aligned} \quad (4)$$

in which $TP_{m|k}$ describes the baseline sub-model, *Placebo* the placebo sub-model and *DrugExposure* the drug sub-models for PD 0200390 and zolpidem. $TP_{m|k}$ is the transition probability to m given previous observation of k as a function of relative bedtime and stage time.

For all sub-models, baseline sleep, placebo and exposure-response, a set of predefined models with increasing complexity, were chosen as part of the accelerated model-building process (Fig. 2 and Table 3). Three models were evaluated both for the relative bedtime effect, *f*(relative bedtime), and the stage time effect, *f*(stage time), a linear, a piece-wise linear (PWL) with fixed breakpoints and PWL with the internal breakpoint being estimated. The first and last breakpoint for the PWL models of relative bedtime were fixed at the first and last time of observation of that stage, respectively. Correspondingly, the first and last breakpoint for the PWL models of stage time were fixed at entry of the stage (stage time=0) and the longest stage time observed, respectively. In the PWL model with all breakpoints fixed, the positioning of the internal breakpoint was based on having approximately equal amount of data in the intervals between the breakpoints. For relative bedtime, a constant model was tried as the first choice, similar to the approach used by Kemp *et al.* (2) but using mixed-effects modeling.

Two placebo models were evaluated: a step and an exponential model with relative bedtime as driving force. Several different PK-PD models were tried to explore the exposure-response relationships.

For PD 0200390, dose, individual predicted plasma concentrations and individual predicted effect site concentrations were used as driving force into a step, a linear and an Emax model. Concentrations were predicted through Bayesian estimation using a previously developed population PK model with measured concentrations and covariates (age, weight, and creatinine clearance) as inputs (Fig. 3). A one-compartment model defined in terms of oral clearance (CL/F), oral volume of distribution (V/F), first-order rate of absorption (ka) and lag time for absorption (tlag) was used to describe the concentrations of PD 0200390 (7,8). Effect site concentrations were calculated assuming a value of 0.8 $hour^{-1}$ for k_{eo}, the rate constant describing the time until equilibration between systemic and effect site concentrations (9). This value was obtained from a previous PK-PD analysis in healthy volunteers using the Stanford Sleepiness Scale as the PD measurement (7).

For the active control, dose- and population-predicted concentrations were used as the driving force into a step, a linear and an Emax model. Concentrations of zolpidem were predicted from a population PK model developed using data obtained in 39 healthy subjects receiving zolpidem 10 mg alone as part of a drug-drug interaction study. The PK model consisted of a one-compartment model with lag time and covariate effect of weight on oral volume of distribution (V/F) using a power model with weight centered on a 70-kg individual. Mean parameters for CL/F, V/F, ka, and Tlag were 12.8 L/hour, 62.7 L, 3.03 $hour^{-1}$, 0.218 h, and the exponent of the power function of 0.945. This was similar to previously published values (10–13).

Introducing $TP_{m|k}$ into the model as the logit of the expression was done to allow estimation of parameters in the range of standard values of probabilities rather than logit transformed values. To ensure that the values of $TP_{m|k}$ did not exceed one, the upper limits of the parameters of *f*(stage time) were set to the reciprocal of the upper limits of the corresponding parameters of *f*(relative bedtime). If the parameters of both functions were estimated to the upper limits, $TP_{m|k}$ was one. Ensuring non-negative values of $TP_{m|k}$ was done by setting the lower limit of the parameters of *f*(relative bedtime) and *f*(stage time) to zero. Keeping the estimates of *f*(relative bedtime) in the same ranges as the probability ensures the estimates of *f*(stage time) to be interpreted as multiplicative factors. Thus, if the stage

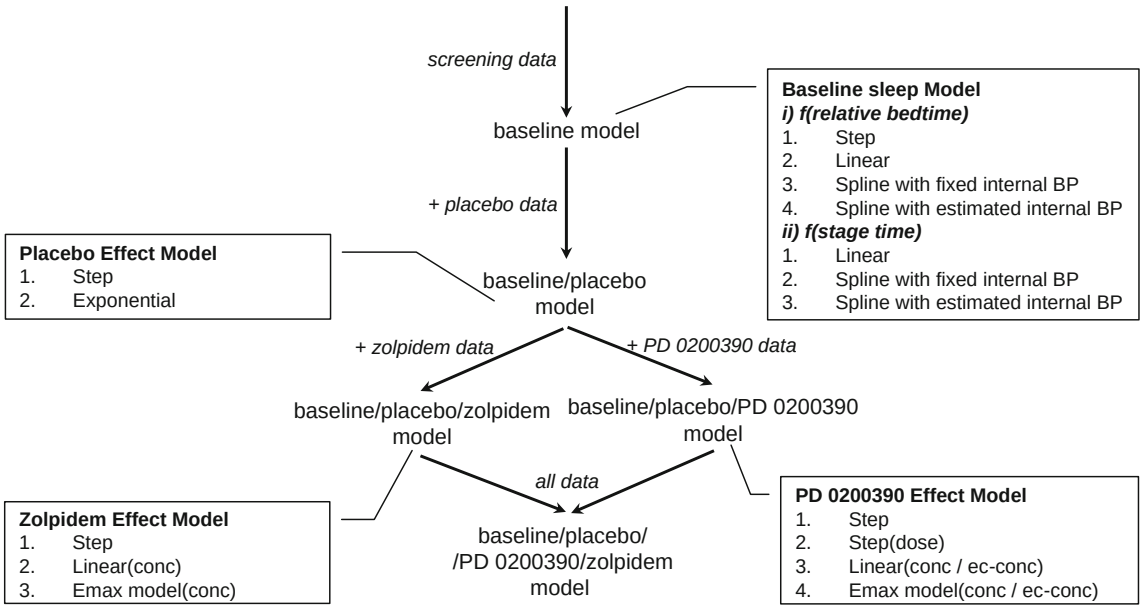

Fig. 2 The model-building strategy included predefined models for each of the four sub-models: baseline, placebo, PD 0200390 and zolpidem, where the baseline model included both effects of relative bedtime and stage time. This accelerated model-building procedure was performed for each transition. BP breakpoint, conc—predicted concentrations, ec-conc—predicted effect compartment concentrations.

time effect was estimated to 2, the transition probability was doubled, and, consequently, if the stage time effect was estimated to 0.5, the transition probability was reduced to half.

The accelerated model-building approach also included a strategy, illustrated in Fig. 2, which can be described by the following steps: i) define baseline model using screening sleep data; ii) add placebo data and explore the placebo model; iii) develop the sub-models of PD 0200390 and zolpidem in parallel, based on the data for each drug added to the placebo and screening data; and iv) re-estimate all parameters with the support of all data. In each step of adding data, the parameters of the previously developed model were fixed.

As no parameters were shared between the different transitions, all transitions were modeled separately. This was done to avoid any problems with the software's limits for maximum allowed number of observations per individual and to decrease the runtimes. In the last step of finalizing the joint model, covariance between the transition models' empirical Bayes estimates of etas were examined to verify that this assumption was in fact valid, given the shrinkage in the empirical Bayes estimates were not higher than 30% (14).

Model discrimination was based on simulation-based goodness-of-fit plots, scripted and executed in S-PLUS v.6 (Insightful Corp.) and R v.2.11.1 (15), changes in the objective function values (OFV) provided by NONMEM, and scientific plausibility. At each step when including new parameters or changing the shape of the model, goodness-of-fit plots depicting the observed and predicted transition probability *versus* relative bedtime/stage time were produced. Since subjects with high transition probability will exit the stage quicker than those with low, the plots will be downwards biased. However, they served the purpose of giving an indication on the shape of

Table 3 Predefined Models for Relative Bedtime with Stage Time, Placebo and Exposure-Response Models

Baseline sleep	Placebo	PD 0200390/zolpidem
$\theta_{const} \cdot 1$	$I_{0/1} \cdot \theta_{step}$	$I_{0/1} \cdot \theta_{step}$
$\theta_{BTlinear} \cdot BT_{\min(BT)-\max(BT)} \cdot \theta_{STlinear} \cdot ST_{0-\max(ST)}$	$\theta_{ICPT} \cdot e^{-BT}$	$I_{0/1} \cdot \theta_{Dose1} + I_{0/1} \cdot \theta_{Dose2}$
$\theta_{BT1} \cdot BT_{\min(BT)-\overline{BT}} + \theta_{BT2} \cdot BT_{\overline{BT}-\max(BT)} \cdot \theta_{ST1} \cdot ST_{0-\overline{ST}} + \theta_{ST2} \cdot ST_{\overline{ST}-\max(ST)}$		$conc \cdot \theta 1$
$\theta_{BT1} \cdot BT_{\min(BT)-\theta_{BTbp}} + \theta_{BT2} \cdot BT_{\theta_{BTbp}-\max(BT)} \cdot \theta_{ST1} \cdot ST_{0-\theta_{STbp}} + \theta_{ST2} \cdot ST_{\theta_{STbp}-\max(ST)}$		$\frac{conc \cdot \theta 1}{(conc+\theta 2)}$

θ—estimated parameter, BT—relative bedtime, ST—stage time, $\overline{BT}$—mean relative bedtime, $\overline{ST}$—mean stage time, $I_{0/1}$—indicator variable being 1 if drug/placebo is present and 0 otherwise, conc—concentration of drug, concentration may be exchanged for effect compartment concentration of drug

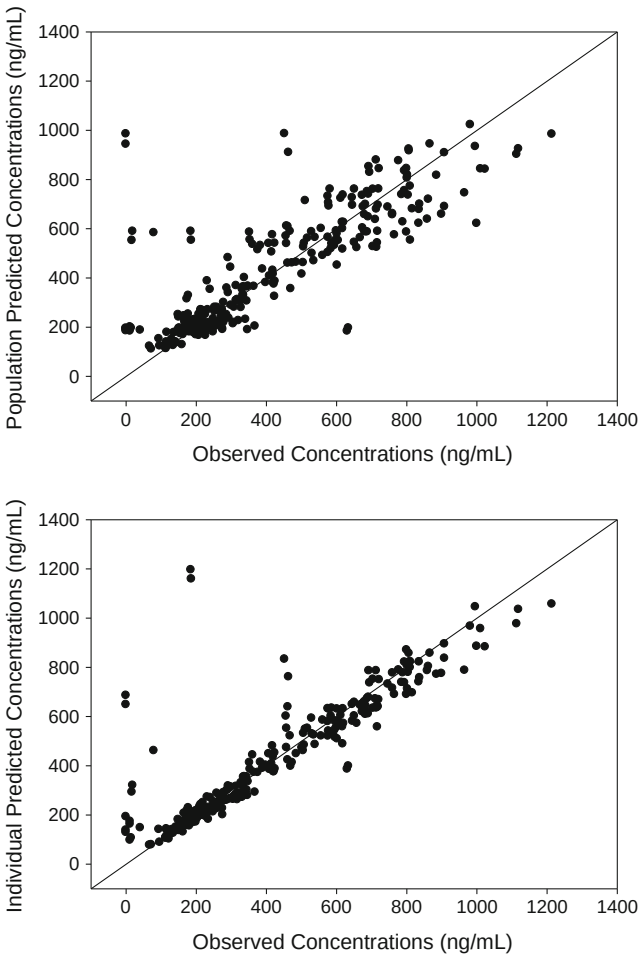

Fig. 3 Population (*top*) and individual (*bottom*) predictions for the pharmacokinetic model of PD 0200390 used in the PK-PD analysis *versus* the actual observations of concentrations. Observations below limit of quantifications were plotting at zero, while predictions from the model were plotted at the prediction value.

the relationship. Differences in OFV were used to discriminate between hierarchical models, and a decrease in OFV of 3.84 (χ^2-distributed) corresponds to a significance level $p<0.05$, with 1 degree of freedom.

The likelihood of L was maximized using the Laplacian method in NONMEM version V (Icon Dev. Solutions) (16) with the likelihood option as given in Eq. 5:

$$L = P(Y_t = m|Y_{t-1} = k)_{ij} \cdot I_{ijt} + (1 - P(Y_{t-1} = k)_{ij}) \cdot (1 - I_{ijt}) \quad (5)$$

in which I_{ijt} is an indicator variable taking the value one if Y_t is equal to m and zero if Y_t is not equal to m.

Model Evaluation

A joint simulation model was built from all transition models developed during the analysis, and 100 datasets were simulated using the realized design and the covariates as given in the observed dataset. The joint model was built using Perl. In order to assess the validity of the population model, a predictive check, related to the method suggested by Gelman *et al.* (17) and used by Girard *et al.* (18), was performed. The check involved comparing observed and simulated efficacy endpoints as defined in Table 2. For each efficacy endpoint, distributions of the median, maximum, and minimum values were generated from the simulated datasets and compared to the corresponding observed values. This was done for the first night following screening, placebo, both doses of PD 0200390, and zolpidem.

To assess the difference between the observed and the simulated, the relative deviation between the simulated and observed efficacy endpoints was plotted. The relative deviation was calculated according to Eq. 6:

$$relative\ deviation = \frac{endpoint_{simulated} - endpoint_{observed}}{endpoint_{observed}} \quad (6)$$

Another method used to visualize the predictive performance of the model was plotting the average time spent in a particular stage for the observed and simulated in hourly intervals of relative bedtime. To avoid memory allocation problems with S-PLUS, these plots were made using 25 simulations instead of 100 simulations.

Clinical Trials Simulations

Three different study designs were simulated based on the joint simulation model: 1) 50 mg of PD 0200390 given 30 min prior to habitual bedtime, 2) 25 mg and 75 mg of PD 0200390 administered 60 min prior to habitual bedtime, and 3) 25 mg and 75 mg of PD 0200390 administered 120 min prior to habitual bedtime. Each simulated designed was assessed by calculating the efficacy endpoints listen in Table 2.

Using the assumption of linear PK characteristics, the concentrations following a 50 mg dose of PD 0200390 were calculated as twice the concentrations following a 25 mg dose of PD 0200390. The dose-dependent magnitude of the step models for drug effects were calculated as a linear interpolation of the magnitudes estimated for 25 mg and 75 mg. The concentration time profile for the simulations of dosing 60 and 120 min before bedtime were assumed to have the same concentration time profile as was observed

for dosing 30 min before bedtime, but with a shift in time of 30 and 90 min, respectively. This was achieved by shifting the concentrations 30 or 90 min earlier relative to bedtime and extrapolating the concentrations for the last 30 or 90 min using the individual terminal half-lives.

RESULTS

Distribution of the transitions in the observed data divided into hourly intervals is shown in Fig. 1. The most frequent transitions were within a stage as seen by the darkness of the diagonal squares. The transitioning into REM increased with increasing hours of sleep. Also, the amount of slow-wave sleep increased with increasing hours of sleep.

The criteria defined for selection of important transitions resulted in a reduction of modeled transitions from 21 to 16. The ignored transitions were to slow-wave sleep from any stage but stage 2, to stage 2 from wakefulness, and to REM sleep from slow-wave sleep. The chosen transitions are shown in Fig. 4.

The baseline transition probabilities are shown relative to bedtime in Fig. 5. A majority of the transitions were described using PWL models with the internal break point being estimated. Only the transition from slow-wave sleep to wakefulness was described using a linear model. As the transition probability in the beginning of the night for several transitions was too low to be estimated, the corresponding parameter was fixed to low values. All models included between-occasion variability or between-subject variability except the model for transitions from stage 2 to REM sleep. All parameter estimates are given in the Appendix. No correlations between the empirical Bayes estimates of the IIVs for the different models were identified, and since the shrinkage ranged from 4.2% to 26.6%, this approach was judged valid.

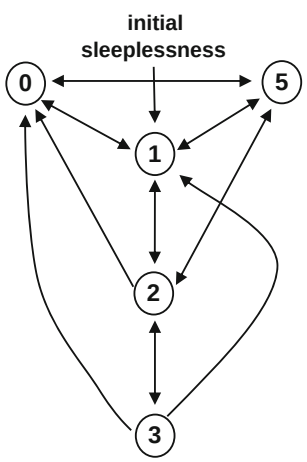

Fig. 4 Transitions chosen for modeling are shown, indicated with an arrow. 0—wakefulness, 5—REM, 1—stage 1, 2—stage 2 and 3—deep sleep.

Figure 5 also shows the effect of placebo relative to baseline, which was significant on four of the transitions. Two of the effects were described using an exponential placebo effect with bedtime as the driving force. Both effects promoted sleep by increasing the transition probability from initial sleeplessness to stage 1 and by decreasing the transition probability from stage 1 back to wakefulness. The two other placebo effects were described as a time-constant increase in transition probability, between stage 1 and REM sleep and *vice versa*. The result of these two opposing effects was an increased number of transitions to and from REM sleep.

All stage time effects on transition probability were described using PWL models as seen in Fig. 6. For the transition from initial sleeplessness, the bedtime is the same as the stage time, and thus stage time was not modeled for this transition. Most stage time effects were either monotonically increasing or decreasing the transition probability. If the stage time effect was monotonically increasing, this was interpreted as the longer a patient stayed in a stage, the more likely it would be that the patient transitions to another stage, and thus *vice versa* for the monotonically decreasing stage time. However, there were a few stage time effects that did not change monotonically, e.g. from REM sleep to stage 1 and from stage 2 to wakefulness.

Figure 7 illustrates the effect of both drugs on the transition probability as a function of bedtime. Two drug effects were described using a step model varying with dose of PD 0200390: wakefulness to REM sleep and REM sleep to stage 1. In all other cases where a drug effect was found, the effect was described using a linear model with predicted concentrations as the driving force for the exposure-response models.

In general, sleep was promoted by both drugs as seen in the decreased probability of transitioning to wakefulness and increased probability of transitioning from wakefulness. The effect of zolpidem was greater than the effect of PD 0200390 for increasing the transition probability from wakefulness to stage 1, resulting in a much lower latency to persistent sleep for zolpidem-treated patients. Four transitions were only affected by PD 0200390: from stage 2 to stage 1, from REM to stage 2, from stage 1 to REM, and REM to stage 1. The net result of these drug effects was a lower probability of being in stage 1 for patients on PD 0200390 treatment. However, the total time spent in REM sleep was not changed as the increased transition probability from stage 1 to REM sleep accounted for the decreased amount of stage 1 sleep. There were no effects of either the drugs or placebo on four of the transitions: from

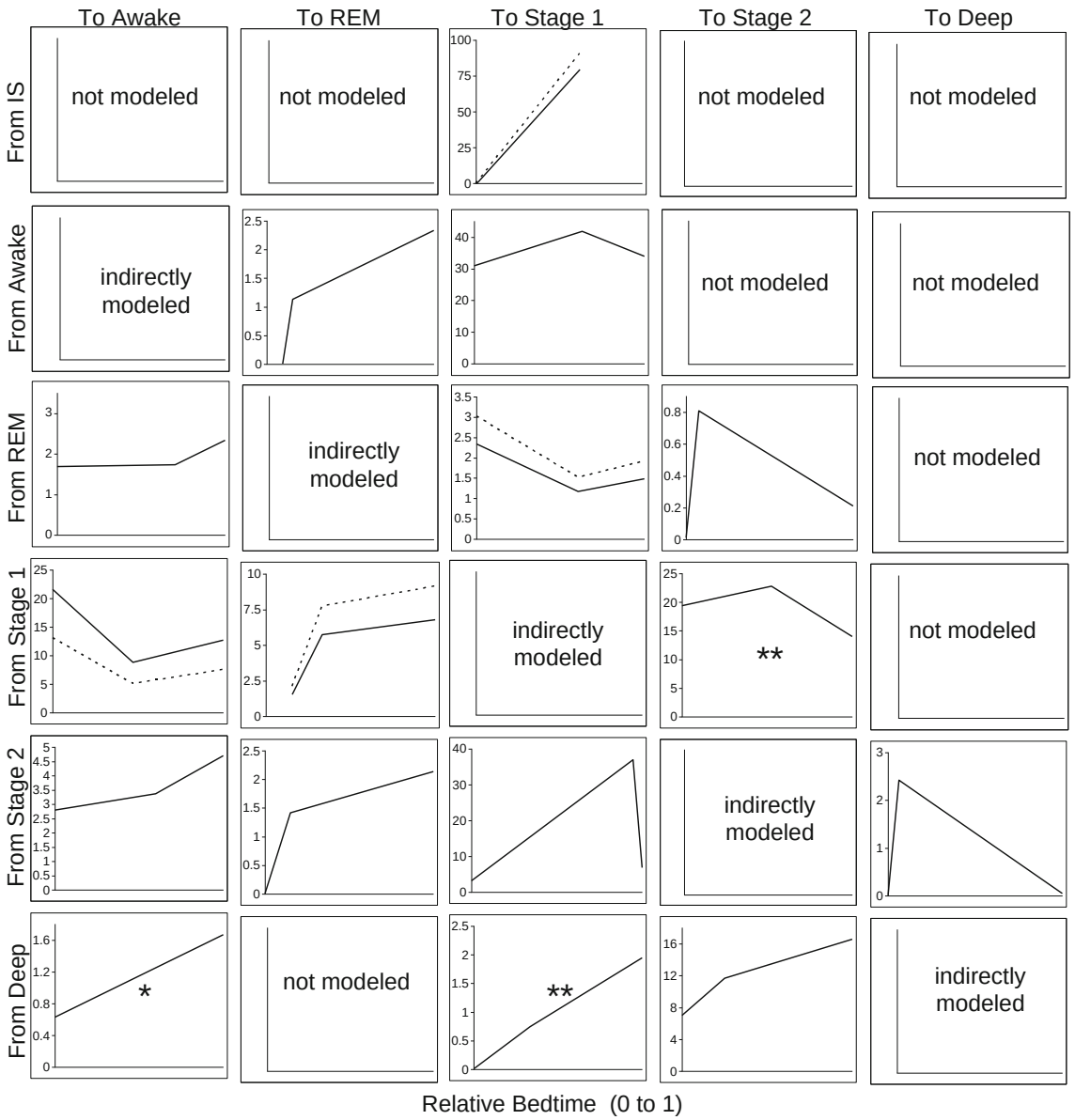

Fig. 5 The probability (given in percent) of making a transition during the next 30-second interval of sleep changing as a function of relative bedtime for baseline sleep (*solid line*) and placebo treatment (*dotted line*). The probabilities shown are for a typical individual that just entered a stage (i.e. stage time effect is 1). The x-axis shows relative bed time starting when the patients goes to bed, at zero and ends when the patient wakes up, at one. The x-axis is the same for all panels. The transition probability is described as *linear, **piece-wise linear with internal breakpoint fixed, or if nothing is indicated, piece-wise linear with internal breakpoint estimated. IS is an abbreviation of initial sleepless-ness which is a state of wakefulness.

stage 1 to stage 2, from stage 2 to deep sleep, from stage 2 to REM sleep, and from deep sleep to stage 2.

The simulations performed as part of the predictive check showed a good agreement with the observed efficacy endpoints (Fig. 8). Only 6 of the 90 observed efficacy endpoints were found outside the 90% prediction interval of the simulated data. These 6 parameters were evenly distributed among the 5 treatments and among the 18 different endpoints, being both over- and underpredicted, thus showing no trends.

The average time spent in a particular stage is presented in Fig. 9. This visual predicted check was performed on 25 simulations; hence, no prediction intervals were added to the graph. Twenty-five simulations are sufficient to judge the central tendency though. The early peak of stage 2 sleep for zolpidem-treated patients was not entirely captured; otherwise, the main trends seen in the observed data were captured by the simulations. The following was observed:

- The characteristic rapid decrease in the beginning of the night followed by the slower increase at the end of the night for time spent in wakefulness was well described with the model.

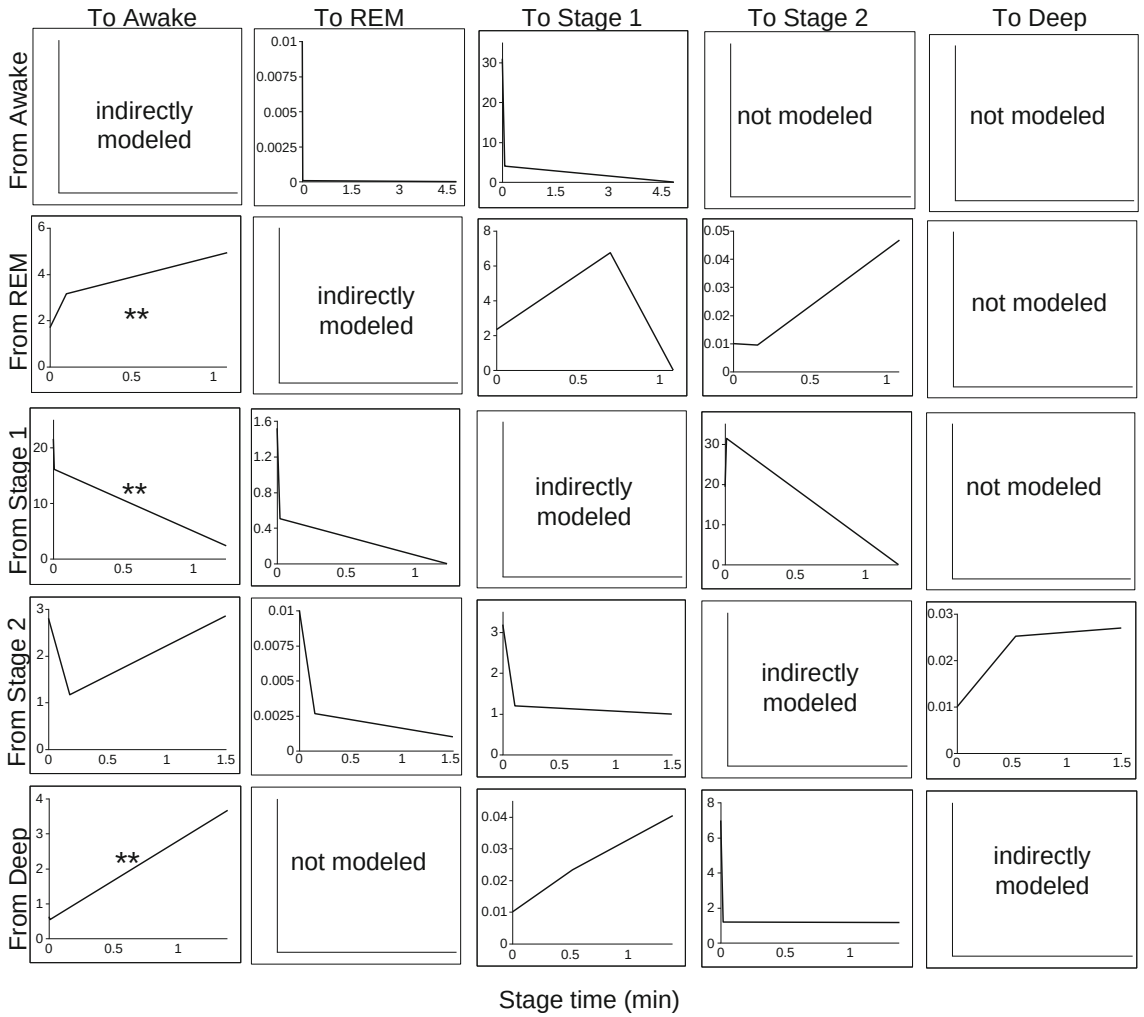

Fig. 6 The transition probability (given in percent) during the next 30-second interval of sleep changing as a function of the stage time for baseline sleep. The x-axis shows the stage time in hours. The probabilities shown are for a typical individual in the beginning of the night (i.e. relative bedtime is zero). The transition probability is described as *linear, **piece-wise linear with internal breakpoint fixed, or if nothing is indicated, piece-wise linear with internal breakpoint estimated.

- Stage 2 was present throughout the night but most abundant in the middle of the night in the observed as well as simulated data.
- Slow-wave sleep showed a rapid increase in the beginning of the night and a constant decrease from approximately two hours of sleep until the end of the night, which was well captured by simulations using the model.
- REM sleep increased constantly from the beginning of the night, peaking at the end of the night in both observed and simulated data.

As the model's central tendency over time and performance on efficacy endpoints was evaluated, these were chosen for the assessment of simulation results. Three study designs were simulated using the developed model: 50 mg PD 0200390 given 30 min prior to bedtime and 25 mg and 75 mg PD 200390 given 60 and 120 min prior to bedtime. Results of the 25 mg and 50 mg doses are given in Table 4. The efficacy endpoints most affected by changing the study design were all latency parameters and, consequently, sleep efficiency in period one and total sleep time. Changing the time of dosing to earlier had the largest impact.

DISCUSSION

Sleep is regulated by three processes: a homeostatic process determining the duration and intensity of the sleep, a circadian rhythm determining the timing of sleep, and an ultradian regulation keeping the amount of REM sleep *versus* non-REM sleep fairly constant within a

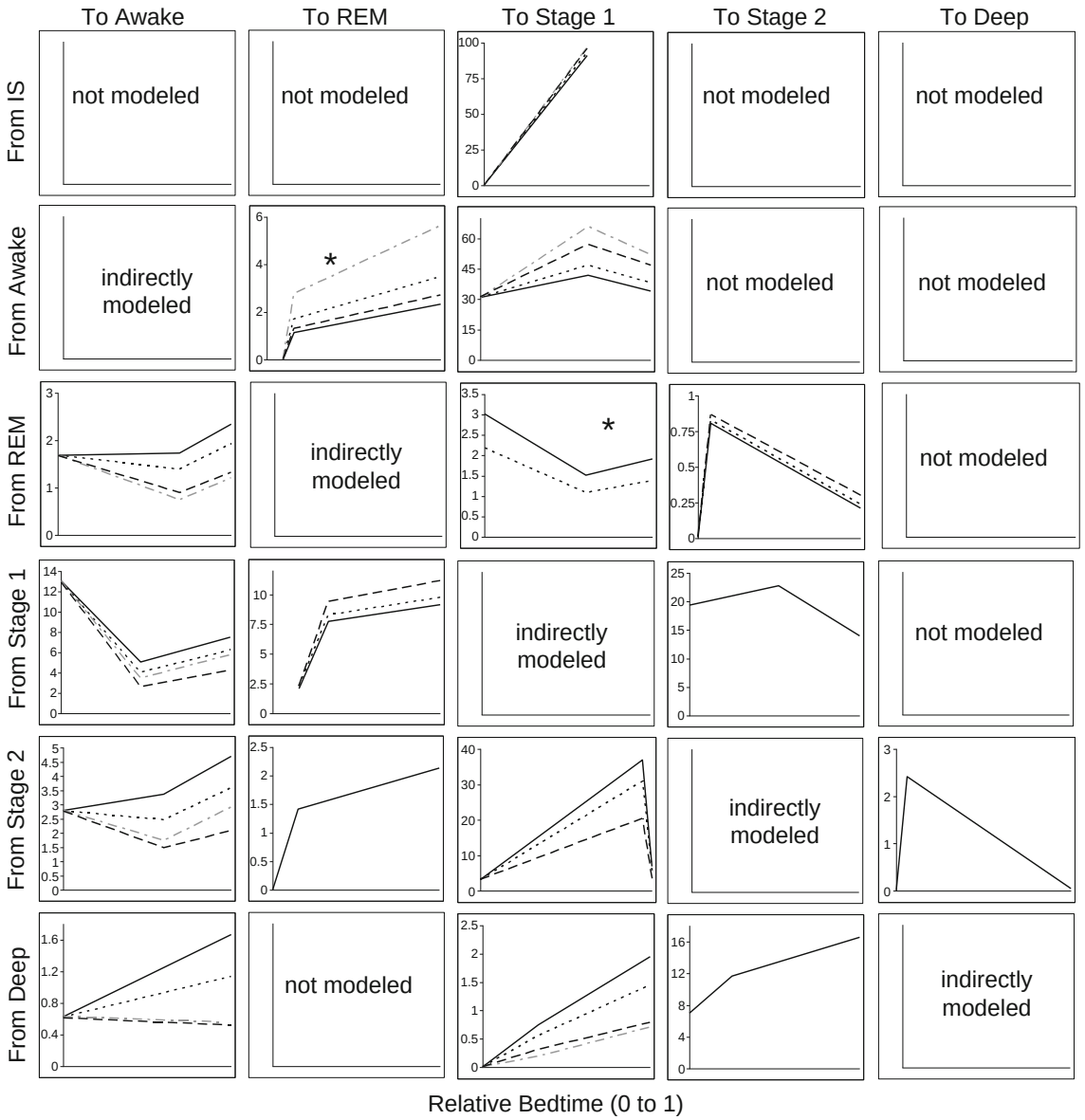

Fig. 7 The transition probability (given in percent) during the next 30-second interval of sleep changing as a function of bedtime for placebo (black, solid line) and drug treatment with 25 mg of PD 0200390 (black, dotted line), 75 mg of PD 0200390 (black, dashed line) and 10 mg zolpidem (grey, partly dotted partly dashed line). Probabilities shown are for a typical individual that just entered the stage (i.e. stage time effect is 1) with drug concentrations of a typical individual dosed 30 min prior to bedtime. Drug effect of PD 0200390 is described by *a step model varying with dose, or if nothing is indicated, a linear model varying with effect compartment concentrations. The drug effects of zolpidem are all described by a linear model varying with concentrations. The x-axis shows the relative bedtime starting when the patients goes to bed, at zero, and ends when the patients wakes up, at one. The x-axis is the same for all panels. IS is an abbreviation of initial sleeplessness which is a state of wakefulness.

night. None of these processes were explicitly modeled. The effect of the circadian rhythm on sleep pattern in this study was assumed to be negligible. As the study was performed around the same time every day and the patients were studied during a fairly short time period, i.e. 5 weeks, changes in sunrise/sunset were not contributing to changes in sleep pattern. The homeostatic process was implicitly modeled with the between-occasion variability, which allowed a patient with poor sleep one night, e.g. few hours of slow-wave sleep, to have better sleep the following night. As the homeostatic process was included in unexplained variability, the simulations would not necessarily include the behavior of better sleep the night following a night of poor sleep, but as simulations were assessed on a population level and not on an individual level, this would not influence the simulation results. Ultradian regulation was partly accounted for in the model by the inclusion of stage time as a driving force for baseline sleep. As the stage time of REM sleep

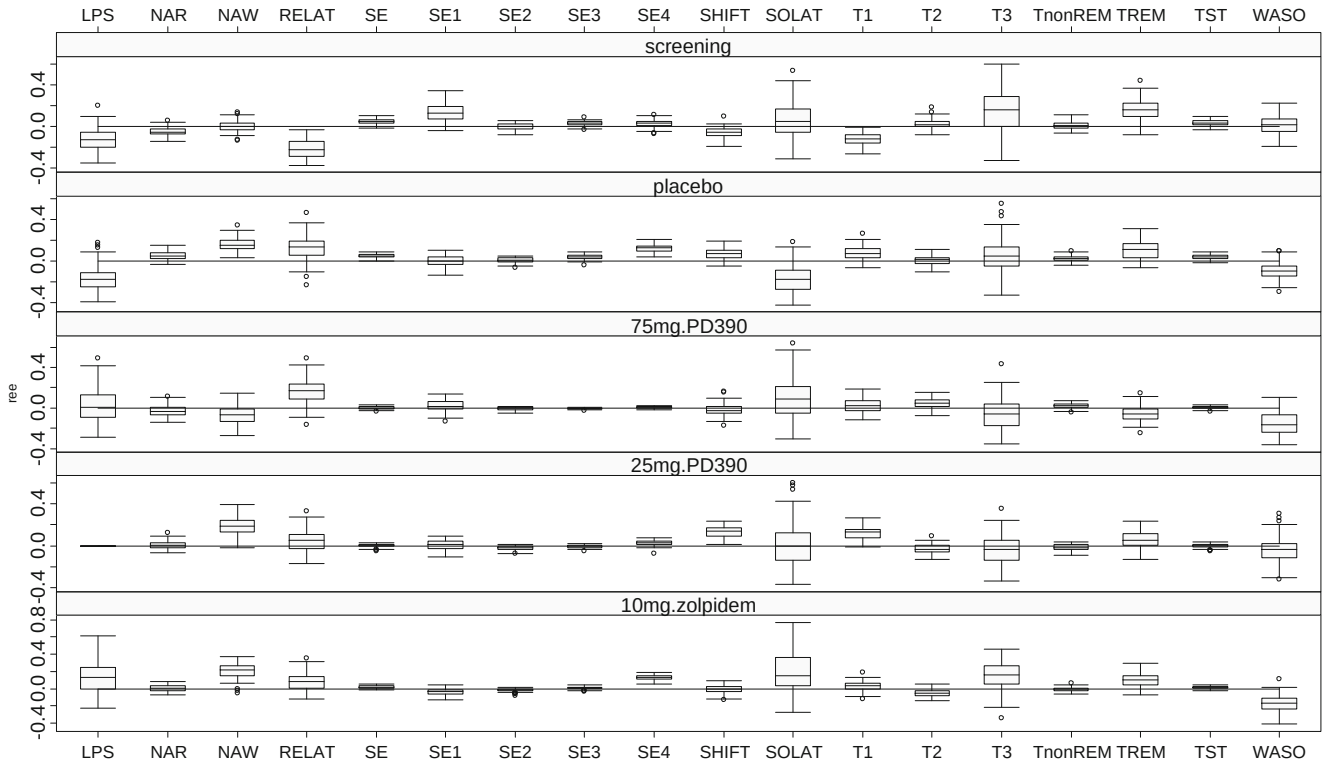

Fig. 8 The relative estimation error for all efficacy endpoints in the screening (top row), placebo (2nd row), 25 mg of PD 0200390 (3rd row), 75 mg PD 0200390 (4th row) and zolpidem (bottom row). LPS latency to persistent sleep, TST total sleep time, SE sleep efficiency, WASO wake after sleep onset, NAW number of awakenings, NAR number of arousals (to stage 1 and awake), SHIFT no. of shifts to lighter stages of sleep, T1 time in stage 1, T2 time in stage 2, T3 time in deep sleep, TREM time in REM sleep, TNonREM time in non-REM sleep, SOLAT sleep onset latency, RELAT REM sleep latency, SE1 sleep efficiency in period 1 (i.e. 0–2 h of bedtime), SE2 sleep efficiency in period 2 (i.e. 2–4 h of bedtime), SE3 sleep efficiency in period 3 (i.e. 4–6 h of bedtime), SE4 sleep efficiency in period 4 (i.e. 6–8 h of bedtime).

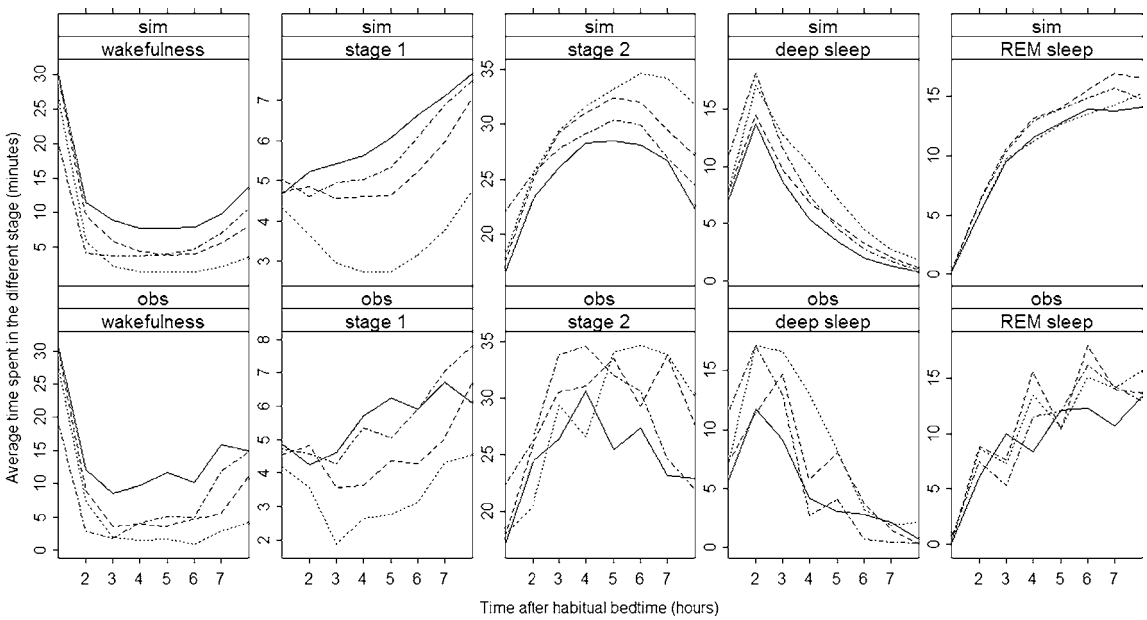

Fig. 9 Average time in minutes spent in the different stages, wakefulness (first column), stage 1 (second column), stage 2 (third column), deep sleep (fourth column) and REM sleep (last column) at different times after habitual bedtime in hours for different treatments; placebo (solid line), 25 mg of PD 0200390 (*dotted line*), 75 mg PD 0200390 (*dashed line*) and 10 mg of zolpidem (dot-dashed line) in the observed (lower row) and in 25 of the simulated datasets (upper row).

Table 4 Efficacy Endpoints for 25 mg and 50 mg Dose of PD 0200390, Calculated for Observed and Simulated Data. Simulated Values are Means of 100 Simulations. Definitions and Abbreviations of the Efficacy Endpoints are Given in Table 1. p.t.bt—prior to bedtime

Efficacy endpoint (mean)	Observed 25 mg, 30 min p.t.bt	Simulated 25 mg, 60 min p.t.bt	Simulated 25 mg, 120 min p.t.bt	Simulated 50 mg, 30 min p.t.bt
LPS (min)	40.3	23.4	22.4	36.7
TST (min)	407	421	423	422
SE (%)	85.2	88.3	88.5	88.2
WASO (min)	43.6	43.2	41.4	28.0
NAW	7.8	9.38	9.17	6.96
NAR	27.5	28.0	27.7	22.5
SHIFT	17.1	19.6	19.4	15.6
SOLAT (min)	30.5	16.2	16.6	30.9
RELAT (min)	93.8	110.3	112.8	101
T1 (min)	0.101	0.099	0.098	0.079
T2 (min)	0.547	0.548	0.552	0.555
T3 (min)	0.124	0.131	0.134	0.133
TREM (min)	0.230	0.223	0.217	0.229
TnonREM (min)	0.772	0.778	0.784	0.773
SE1 (%)	66.9	78.2	79.5	69.0
SE 2 (%)	92.3	93.5	93.8	95.7
SE 3 (%)	93.9	94.1	93.9	96.5
SE 4 (%)	90.5	89.9	89.7	94.4

increased, the model predicted a decreased transition probability, and *vice versa*, all in line with keeping the total REM sleep fairly constant. However, when sleep patterns change as a result of placebo and/or drug effects, the ultradian regulation may affect the sleep pattern as well. If a drug increases the transitioning to REM sleep, the ultradian regulation would as a consequence increase the transitioning from REM sleep in order to keep the total time in REM sleep fairly constant. Interpreting drug effects with this model was thus difficult, as effects related to sleep regulation would be attributed to drug effect. The drug responses found in the model, not related to concentration of PD 0200390, were transitions from REM sleep to stage 1 and from wakefulness to REM sleep. These drug effects could be a secondary effect of drug treatment related to ultradian regulation. An alternative approach would be to explicitly model the ultradian regulation also for drug effects by including a stage time effect.

In this study, the placebo effect on sleep could be separated from the natural time-course of sleep in insomnia patients, as data in this analysis were available both with and without placebo treatment. However, baseline recordings were for all patients made on the two first nights at the sleep laboratory, making separation between placebo effects and first night effect (19) impossible. The first night effect gives a changed EEG with worse sleep as the consequence when the sleep environment is changed. The veering off of the first night effect is likely to increase the transition probability in favor of slow-wave sleep as will the placebo effect. Thus, the four placebo effects found in this analysis were a combination of true placebo effects and patients being more accustomed to the sleep environment.

For PD 0200390, the PK model was developed using data from primary insomnia patients, and the predictions were so-called post hoc estimates, using measured concentrations and covariates in the study population to support the predictions. The assumptions associated with this approach are weaker than for the approach taken for zolpidem, where the predicted concentrations were population predictions using a PK model developed on a healthy population, adjusting for measured covariates in the study population. Changes in the PK of zolpidem has been reported for geriatric patients (age > 70) and patients with impaired renal or hepatic function (10), but since this study was performed in a population with generally young (26–63 years) and healthy insomnia patients, the extrapolation between the populations was reasonable. An alternative to using population-predicted concentrations would be to use a kinetic drug action model, i.e. KPD model. In this model, the drug effect with a delay is driving the effect. The population-predicted concentrations in this study were, however, in all models for zolpidem and in a majority of the models for PD 0200390 found to be a superior predictor of drug effects, compared to dose, and thus explained part of the variability in effect. Any unexplained variability or potential bias from PK was, however, carried over to the PD parameters.

Markov mixed-effects modeling used to analyze sleep has been previously published. Both Karlsson *et al.* (3) and

Bizzotto *et al.* (4) used more than three breakpoints at pre-fixed intervals for their PWL models, either equidistant in time or with equal amounts of data in the intervals. In the current work, only three breakpoints in the PWL models were used. Exploring fewer breakpoints reduced the number of runs, in line with the accelerated model-building approach, and computational efforts were instead focused on estimating the position of the internal breakpoint. Additionally, in the model by Karlsson *et al.*, no more than three breakpoints were supported in the final model, even though more were investigated. Stage time was included in the modeling of baseline sleep in both Karlsson *et al.* and the current analysis. Using the stage time in predicting baseline sleep was discussed in the paper by Bizzotto *et al.* but not included in the analysis. Including this effect on the baseline sleep might eradicate the need for more than three breakpoints.

Each transition model included a transformation to ensure that the probability could not exceed one within a transition. Additionally, with Markov models, the overall probability of transitioning from and within a state is not allowed to exceed one. The risk of estimating an overall transition probability in the current study was low, as the probability of transitioning within a stage was much higher than transitioning from a stage. However, to check the validity in this assumption, the overall transition probabilities from a stage at the highest stage time effect within an individual were calculated, and none of these sums exceeded one. Implementing the model using transformations to ensure that the overall transition probability was not estimated greater than one was not possible with our model, as the transitions were modeled separately. A transformation of this kind has, however, been suggested by Bizzotto *et al.*

Performing a covariate analysis on these data would be an obvious way to proceed with the analysis. Identifying candidate covariates could be done by plotting the empirical Bayes estimates of etas against all possible covariates and see if there are any trends. Caution should, however, always be taken, since shrinkage might disguise or enhance a covariate relationships (14). The shrinkage was, however, low in this analysis, making this approach a quicker option than the more computer-intensive automated SCM (20). This approach is, however, an exhaustive method to find covariate relationships. The easiest way to perform a covariate analysis for these data would be to first explore the relationships on each individual transition and then explore any similarities between the models that might reduce the covariate model. For this particular data, several potentially important covariates were available, such as smoking habits, weight, and alcohol habits.

The accelerated model-building procedure was designed to provide stable final models in a time- and run-efficient manner. This was accomplished by modeling the transitions as separate models and using sets of pre-defined models for each sub-model. This approach is similar to what is used in standard PK modeling where models with increased complexity are investigated, starting with a one-compartment model, then a two-compartment model, etc. The choice of pre-defined models was based on previous publications of Markov models for sleep (2–4), though further simplifications could have been made. The constant model included as the simplest models could have been omitted, as it is implausible that the probability of any transition is the same throughout a night. None of the final models did include the constant model, supporting this reasoning. Adding data first after a model was finalized and proceeding, keeping the parameters of previously finalized models fixed, was also part of the reduced model-building procedure. The observed drop in the objective function when including a new parameter in the model represented an overestimate of the drop expected had all parameters in the model been estimated. Keeping this in mind, fixed parameter estimates when going to the next step in the model-building process could be used to accelerate the model building.

Only with model-based analysis of sleep data can new dosing regimens be simulated and used for guidance in designing new studies. Two types of simulations were performed in this work: simulation of a new dose of PD 0200390 and simulation of a new dosing schedule. The different dosing schedules were selected to match the time needed to reach the maximum PD effect after dosing with PD 0200390 better, thus potentially providing better overall efficacy on sleep parameters. The performed simulations suggested that giving PD 0200390 earlier would largely improve the effect on LPS, an improvement that could not be achieved by only increasing the dose. These types of conclusions can sometimes only be drawn based on simulations, and they can act as a basis support for decisions made in later stages of drug development. Hence, building models for the test drug and the disease during drug development is of great benefit.

Using a model-based approach to analyzing sleep could also render additional benefits for follow-up compounds, compounds of a different mechanism of action, and sleep model validation for healthy volunteers. For a follow-up compound with a similar mechanism of action but different PK and/or PK-PD profile (e.g. new drug with different onset, or controlled-release formulation), the model could be used as it is to perform forecasts of the outcome, enabling an easy comparison between competing compounds before performing the studies of the follow-up compounds. For studies performed in a similar population but with a drug of a different mechanism of action, the baseline and placebo model could be re-used to re-investigate only the drug effects. This would greatly reduce the time needed for this type of analysis and would be a clever way to industrialize the use of Markov-models for sleep analysis in drug development. A model-based approach could also be used for validating the commonly used insomnia model for healthy volunteers, i.e. the phase-advanced population. A similar model for the phase-advanced population

could be compared with the insomnia population, and the differences between these populations—the surrogate healthy population and the patient population—could be readily identified and quantified and by that potentially validated as a good or poor substitution of the target population.

Conclusively, the proposed accelerated model-building process resulted in a robust sleep model where the parameters of baseline sleep, placebo, drug effects of PD 0200390, and drug effects of zolpidem could be well characterized.

ACKNOWLEDGMENTS

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APPENDIX

I. Final parameter estimates for baseline sub-model with the estimated variabilities. Transition probabilities at each breakpoint are given in percent with the positioning of the breakpoint in minutes. One transition is described with a linear model, and for this model neither an internal breakpoint nor a transition probability for the internal breakpoint is given, as none was estimated. The stage time effect is given at each breakpoint with the positioning of the breakpoints in minutes. Stage time effect for the first breakpoint was by definition 1. First stage time breakpoint was by definition 0. Variability was modeled as additive on the logit scale. Variability was allowed to be different at the different breakpoints if supported. IS—initial sleeplessness, 0—wakefulness, 1—stage 1, 2—stage 2, 3—slow—wave sleep, 5—REM sleep

Transition		Baseline sub-model										Variability model					
From stage	To stage	f(relative bedtime)						f(stage time)				Between Subject Variability			Between Occasion Variability		
		Probability (%)			Breakpoint (min)			Stage time effect		Breakpoint (min)							
		1st	2nd	3rd	1st	2nd	3rd	2nd	3rd	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
IS	1	0.01[a]	0.668	79.3	0[a]	5.23	300[a]	NA	NA	NA	NA	–	0.956	15.3	12.6	–	–
0	1	31.0	41.8	34.0	0[a]	304	480[a]	0.131	0.0001[a]	4.24	925[a]	0.138 × 0.134[b]	0.134	0.427	–	–	0.379
	5	0.01[a]	1.13	2.33	45[a]	74.9	480[a]	0.00615	0.0001[a]	1.22	295[a]	–	2.39	0.978 × 2.39[b]	–	0.501	–
1	0	21.6	8.79	12.7	0[a]	22.6	480[a]	0.745	0.108	0.500[a]	74[a]	0.202	–	0.128	–	0.143	–
	2	19.4	22.8	14.0	0[a]	252[a]	480[a]	1.62	0.0001[a]	0.596	74[a]	0.121[c]	0.121[c]	0.121[c]	0.113	0.304	−0.113[d]
	5	1.52	5.72	6.77	75[a]	159	480[a]	0.331	0.0001[a]	1.21	74[a]	–	–	0.283	3.98	0.643	–
2	0	2.80	3.37	4.7	0[a]	287	480[a]	0.417	1.02	11.0	91[a]	0.248	0.144	0.144[b]	–	–	–
	1	3.19	36.9	6.89	0[a]	454	480[a]	0.377	0.313	6.48	91[a]	–	0.192	–	0.316	–	–
	3	0.01[a]	2.42	0.0480	0[a]	29.4	480[a]	2.52	2.70	32.5	91[a]	1.38[c]	1.38[c]	1.38[c]	–	0.546	1.80
	5	0.01[a]	1.42	2.14	0[a]	73.0	480[a]	0.264	0.100[a]	9.30	91[a]	–	–	–	–	–	–
3	0[e]	0.627	NA	1.67	0[a]	NA	480[a]	0.854	5.85	0.500[a]	83[a]	–	1.18	–	–	–	–
	1	0.01[a]	0.747	1.95	0[a]	160[a]	480[a]	2.33	4.05	31.1	83[a]	16.7	0.0357 × 16.7[d]	–	–	–	–
	2	6.98	11.7	16.6	0[a]	120	480[a]	0.170	0.165	1.21	83[a]	1.09[b]	1.09	2.66 × 1.09[b]	–	–	–
5	0	1.69	1.73	2.34	0[a]	337	480[a]	1.87	2.92	6.00[a]	65[a]	0.451	0.190	0.190[b]	–	–	–
	1	2.34	1.17	1.48	0[a]	290	480[a]	2.89	0.0001[a]	41.9	65[a]	–	0.789	–	–	–	0.537
	2	0.01[a]	0.808	0.212	0[a]	37.0	480[a]	0.939	4.68	9.54	65[a]	0.438[c]	0.438[c]	0.438[c]	–	–	–

NA not applicable

[a] Fixed parameter

[b] No variance was estimated for this breakpoint, as the random effect of this breakpoint is equal to the random effect of the internal breakpoint. A fixed effect is estimated to give the width of the variability at this breakpoint.

[c] Same variability for all three breakpoints

[d] No variance was estimated for this breakpoint, as the random effect of this breakpoint is equal to the random effect of the first breakpoint. A fixed effect is estimated to give the width of this variability at this breakpoint.

[e] Linear model of relative bedtime

II. Final parameter estimates of the placebo, the PD 0200390 and zolpidem effect sub-models with the between-subject variability estimated for the effects of PD 0200390. Only one model included between-occasion variability

estimated instead of between-subject variability. Two placebo sub-models were exponential with the estimated parameters half-life and intercept, and two were step models. All sub-models describing the effect of PD 0200390 were linear with the parameter slope, but two that were described used step models with the estimated parameters constant low dose and constant high dose. All effects of zolpidem were linear, with slope being the only estimated parameter. IS—initial sleeplessness, 0—wakefulness, 1—stage 1, 2—stage 2, 3—slow-wave sleep, 5—REM sleep

Transition		Placebo			PD 0200390				Zolpidem
From stage	To stage	Half-life (h)	Intercept	Constant	Slope (ml/μg)	Step, low dose	Step, high dose	Variability	Slope
IS	1	1.09	1[a]	NA	1.01[b]	NA	NA	–	5.22
0	1				0.704	NA	NA	0.500[c]	4.45
	5				NA	0.415	0.156	0.586	5.53
1	0	2.10	−0.605	NA	−0.786	NA	NA	0.409	−1.66
	5	NA	NA	0.326	0.291	NA	NA	0.430	–
2	0				−1.11	NA	NA	–	−3.02
	1				−1.04	NA	NA	–	–
3	0				−1.54	NA	NA	–	−6.62
	1				−1.20	NA	NA	–	−6.13
5	0				−0.765	NA	NA	0.281	−4.03
	1	NA	NA	0.263	NA	0	−0.334	–	–
	2				0.454	NA	NA	–	–

NA not applicable

[a] Parameter not estimated

[b] Parameter included at a significance level of $p \leq 0.10$, instead of $p \leq 0.05$ as the other Parameters

[c] This variability estimate is between occasion variability

III. Example code for a transition.

```
$PROB MARKOV MODEL FOR TRANSITIONS FROM STAGE 2 TO AWAKEFULNESS
$DATA ...
$INPUT ID=L1 NMI2 NGHT TRT DOSE WGT TIME NN=DV PDV LENG CE

$PRED
; DATA DEPENDENT CONSTANTS
 NF  = 0     ;FIRST VALUE OF TIME AT WHICH AN OBSERVATION IS MADE
 NL  = 1     ;LAST  VALUE OF TIME AT WHICH AN OBSERVATION IS MADE
 SF  = 0     ;FIRST VALUE OF STAGE AT WHICH AN OBSERVATION IS MADE
 SL  = 1.508 ;LAST  VALUE OF STAGE AT WHICH AN OBSERVATION IS MADE
 TO  = 0     ;STAGE TO WHICH TRANSITION IS MADE
 FR  = 2     ;STAGE FROM WHICH TRANSITION IS MADE

; NIGHTTIME
 N1  = THETA(1)  ;PROBABILITY OF TRANSITING AT NF  AND S1
 N2  = THETA(2)  ;PROBABILITY OF TRANSITING AT N50 AND S1
 N3  = THETA(3)  ;PROBABILITY OF TRANSITING AT NL  AND S1
 N50 = THETA(4)  ;TIME OF BREAK POINT (ESTIMATED)

; PHARMACODYNAMICS - PD0200390
 SLPD = THETA(5) ;SLOPE OF DRUG EFFECT
 CPPD = CE/1000  ;SCALING EFFECT CMT CONC
 PD   = 0
 IF(TRT.EQ.2.OR.TRT.EQ.3) PD = SLPD*CPPD
```

```
; PHARMACODYNAMICS - ZOLPIDEM
 CL   = 12.8
 V    = 62.7*(WGT/70)**0.954
 K    = CL/V
 KA   = 3.03
 CPT  = TIME*8 + 0.5 - 0.218
 CPAC = 10*KA/V/(K-KA)*(EXP(-KA*CPT)-EXP(-K*CPT))
 SLAC = THETA(10) ;SLOPE OF DRUG EFFECT
 AC = 0
 IF(TRT.EQ.4) AC = SLAC*CPAC

; STAGETIME
 STAG= LENG/2/60     ;SCALING INTO MINUTES
 S1  = 1             ;REFERENCE STAGE FOR N
 S2  = THETA(6)      ;FRACTIONAL PROBABILITY AT S50 OF TRANSITING CF S1
 S3  = THETA(7)      ;FRACTIONAL PROBABILITY AT SL  OF TRANSITING CF S1
 S50 = THETA(8)      ;TIME OF BREAK POINT FOR STAGE (ESTIMATED)

 SA  = S1*(S50-STAG)/S50      + S2*STAG/S50           ;FIRST STAGE SLOPE
 SB  = S2*(SL-STAG) /(SL-S50) + S3*(STAG-S50)/(SL-S50);SECOND STAGE SLOPE
 SMX = STAG**50     /(S50**50 + STAG**50)
 ST  = (1-SMX)*SA + SMX*SB    ;COMBINED EQUATION W. BOTH STAGE SLOPES

; BSV + BOV
 BSV1=ETA(1)
 BSV2=ETA(2)
 BSV3=ETA(3)

 IF(TRT.EQ.0) THEN
   BOV1=ETA(3)
   BOV2=ETA(8)
   BOV3=ETA(13)
 ENDIF
 IF(TRT.EQ.1) THEN
   BOV1=ETA(4)
   BOV2=ETA(9)
   BOV3=ETA(14)
 ENDIF
...

; OVERALL LOGIT
 T1  = LOG(N1*ST/(1-N1*ST)) + BSV1 + BOV1 + PD + AC ;LOGIT 1ST BREAKPOINT
 T2  = LOG(N2*ST/(1-N2*ST)) + BSV2 + BOV2 + PD + AC ;LOGIT 2ND BREAKPOINT
 T3  = LOG(N3*ST/(1-N3*ST)) + BSV3 + BOV3 + PD + AC ;LOGIT 3RD BREAKPOINT

 O1  = T1*(N50-TIME)/(N50-NF) + T2*(TIME-NF)/(N50-NF) ;1ST SLOPE
 O2  = T2*(NL-TIME)/(NL-N50)  + T3*(TIME-N50)/(NL-N50);2ND SLOPE
 NMX = TIME**50/(TIME**50 + N50**50)

 LGT = (1-NMX)*O1 + NMX*O2  ;COMBINED EQUATION W. BOTH SLOPES

; CONDITIONAL PROBABILITIES
 P1  = EXP(LGT) / (1+EXP(LGT))  ;PROBABILITY OF NO TRANSITION
 P0  = 1 - P1                   ;PROBABILITY OF TRANSITION

 OBS = 0
 IF(DV.EQ.TO) OBS = 1
 Y   = P1*OBS + P0*(1-OBS) +.0001
 IF(TIME.LT.NF) Y = 1

$THETA ...
$OMEGA ...
$ESTIM PRINT=1 MAX=9990 METHOD=COND LAPLACE LIKE NUMERICAL
```

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