# Mind the time : 24-hour rhythms in drug exposure and effect

Kervezee, L.

# CHAPTER

# Levofloxacin-induced QTc prolongation depends on the time of drug administration

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## ABSTRACT

Understanding the factors influencing a drug's potential to prolong the QTc interval on an electrocardiogram is essential for the correct evaluation of its safety profile. To explore the effect of dosing time on drug-induced QTc prolongation, a randomized, cross-over, clinical trial was conducted in which 12 healthy male subjects received levofloxacin at 02:00, 06:00, 10:00, 14:00, 18:00 and 22:00. Using a pharmacokinetic-pharmacodynamic modelling approach to account for variations in pharmacokinetics, heart rate and daily variation in baseline QT, we find that the concentration-QT relationship shows a 24-hour sinusoidal rhythm. Simulations show that the extent of levofloxacin-induced QT prolongation depends on dosing time, with the largest effect at 14:00 (1.73 [95% prediction interval: 1.56-1.90] ms per mg/L) and the smallest effect at 06:00 (-0.04 [-0.19-0.12] ms per mg/L). These results suggest that 24-hour variation in the concentration-QT relationship could be a potentially confounding factor in the assessment of drug-induced QTc prolongation.


## STUDY HIGHLIGHTS

**What is the current knowledge on the topic?**

- The propensity of new drugs to prolong the QTc interval is typically assessed in a clinical trial in which drug administration occurs at a fixed time of the day
- Many factors, including time of day, may influence the relationship between the concentration of a drug and the extent of QTc prolongation

**What question this study addressed?**

- The objective of this study was to investigate the effect of dosing time on the extent of levofloxacin-induced QTc prolongation.

**What this study adds to our knowledge?**

- The relationship between the levofloxacin concentration and the extent of QTc prolongation varies systematically over the course of the day
- Dosing time is a potentially confounding factor in the assessment of drug-induced QTc prolongation.

**How this might change drug discovery, development and/or clinical therapeutics?**

- To accurately assess a drug's effect on the QTc interval, an approach is required that takes into account the time of drug administration

# INTRODUCTION

Over the past decades, several non-cardiac drugs have been withdrawn from the market or their use has been restricted due to their propensity to delay ventricular repolarization (Roden, 2004). This potentially serious side-effect is manifested as a prolonged heart rate-corrected QT (QTc) interval on the electrocardiogram (ECG). The most common mechanism by which drugs cause QTc prolongation is through blockade of the hERG channel, a potassium channel that underlies the rapid component of the delayed rectifier potassium current (IKr) in cardiomyocytes(Sanguinetti and Tristani-Firouzi, 2006). Reduced IKr delays cardiac repolarization and, often in combination with other predisposing factors such as genetic polymorphisms or hypokalemia, may lead to the occurrence of early afterdepolarizations and Torsades de Pointes (Kannankeril et al., 2010).

Much effort has been put into the identification of the different sources of variability that affect the extent of drug-induced QTc prolongation, including gender, age, ethnicity, co-morbidity and co-medication (Malik and Camm, 2001). Moreover, it is well known that the baseline QTc interval shows 24-hour variation (Bonnemeier et al., 2003; Browne et al., 1983a). Based on the 24-hour variations in various physiological processes, such as serum potassium levels (Schmidt et al., 2015) and cardiac ion channel expression (Jeyaraj et al., 2012; Schroder et al., 2013, 2015; Yamashita et al., 2003), it has also been suggested that the magnitude of the effect of a drug on the QTc interval may depend on the time of day (Dallmann et al., 2014; Watanabe et al., 2012). However, this hypothesis has not been investigated directly. In fact, current approaches to evaluate drug-induced QT prolongation during drug development include one time-point of drug administration and exclude night-time recordings (Malik et al., 2008), thereby relying on the implicit assumption that delayed ventricular repolarization does not depend on dosing time.

To test this assumption, we investigated whether the sensitivity to drug-induced QTc prolongation varies during the 24-hour period, using levofloxacin as a model compound. Levofloxacin is a fluoroquinolone antibiotic that blocks hERG channels (Alexandrou et al., 2006; Kang et al., 2001). Causing a slight but significant prolongation of the QTc interval l(Noel et al., 2003; Sugiyama et al., 2012; Taubel et al., 2010), it was shown previously that levofloxacin can be used as a positive comparator in thorough-QT (TQT) studies (Taubel et al., 2010). In this study, we used pharmacokinetic-pharmacodynamic modelling to characterize the relationship between levofloxacin concentration and the extent of QTc prolongation after oral administration to twelve healthy male subjects at six different time-points during the day and night.

# METHODS

### Study design

Data used for model development were obtained from a clinical trial that was described previously (Kervezee et al., 2016). Briefly, 67 occasions were completed by twelve healthy subjects (10 subjects completed 6 occasions, 1 subject completed 3 occasions, 1 subject

completed 4 occasions). In each occasion, the subjects received an oral dose of 1000mg levofloxacin (Aurobindo Pharma B.V., Zwijndrecht, the Netherlands) at a different time of day (t=0 at 02:00, 06:00, 10:00, 14:00, 18:00 and 22:00). The occasions were separated by at least one week and each subject was randomly allocated to a sequence of dosing times. Subjects fasted from t=-2h until t=6h. At t=6h and t=10h, subjects were allowed to eat a maximum of four slices of bread and a small snack, respectively. Twelve-lead ECGs were recorded at t=0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12h after dosing using a Marquette MAC 5500 (GE Healthcare, Milwaukee, Wisconsin, USA) and stored using the MUSE Cardiology Information System (GE Healthcare). The ECG parameters (RR and QT intervals) were calculated automatically and each ECG recording was manually reviewed by a physician. Blood samples to measure levofloxacin and potassium concentrations were drawn via an indwelling intravenous catheter immediately after each ECG recording and at t=5 and 10h. The concentration of levofloxacin in these samples was analyzed by an LCMS method (Kervezee et al., 2016). The study was approved by the Medical Ethics Committee of the Leiden University Medical Centre and registered in the European Clinical Trials Database (EudraCT Number: 2013-001976-39). All subjects gave written informed consent prior to the study.

**Data exploration**

For data exploration, drug concentrations and the change from the pre-dose QT interval, corrected for heart rate by the Fridericia formula (ΔQTcF), was stratified by time of drug administration and plotted over time as mean and 95% confidence intervals. The relationship between observed levofloxacin concentrations in plasma and ΔQTcF was also stratified by dosing time. Linear mixed effects modelling to assess this relationship was performed with the nlme package (v3.1.118) in R (v3.1.2 R Development Core Team, 2008), using ΔQTcF as the dependent variable, drug concentration and dosing time as fixed effects (including interaction) and subject as random effect.

**Pharmacodynamic modeling**

Population modelling was performed using NONMEM (v7.3 Icon plc, Dublin, Ireland(Beal et al., 2009)). R, Pirana (v2.8.2), PsN (v3.7.6) and Xpose (v4) were used for evaluation and graphical representation of the models (Keizer et al., 2013). First-order conditional estimation with interaction (FOCEI) was used throughout the analysis and interindividual variability (IIV) and interoccasion variability (IOV) in model parameters were assumed to be log-normally distributed. Additive and proportional error structures were considered to describe the residual error. A sequential modelling approach was used: firstly, a baseline model was developed based on the pre-dose QT data; secondly, the concentration-effect relationship was modelled using pre- and post-dose QT data.

**Baseline model**

To describe the relationship between the QT and RR interval as well as potential 24-hour variation in the QT interval in the absence of levofloxacin, a baseline model was developed

as described previously (Chain et al., 2011) using Equation 1.

$$QT_{baseline}(t) = QT_0 * RR^{\alpha} + \Sigma^{N}_{(n=1)}[A_n * \cos(2\pi * n * (t-\varphi_n) / 24) \quad \text{Equation 1}$$

where $QT_0$ represents the intercept of the QT-RR relationship in ms, RR is the observed RR interval in s, α is the correction factor for RR, N is the total number of harmonics included in the model, $A_n$ is the amplitude of the 24-hour variation of the $n^{th}$ harmonic in ms, $\varphi_n$ is the acrophase (time of peak) of the $n^{th}$ harmonic in hours after midnight and t is the time of the observation in hours after midnight. The number of harmonic terms was determined by the criteria for statistical significance explained below. Because sleep may affect the QT-RR relationship(Browne et al., 1983b; Extramiana et al., 1999), the use of a separate value of α during sleep (between 23:30 and 07:30) was investigated. A linear mixed effects model (as described above) was used to investigate whether the α estimated by the final baseline model adequately removed the dependency of the QTc interval on RR, using QTc as the dependent variable, RR as fixed effect and interaction between dosing time and RR, and subject as random effect.

**Drug effect model**

The concentration-dependency of QTc (PD) and temporal relationship between PK and PD effects (PK-PD) was modeled using the pre- and post-dose ECG recordings. Individual pharmacokinetic parameters were fixed to their estimates from a previously reported population pharmacokinetic model (Kervezee et al., 2016) and used to predict individual concentration-time profiles for PK-PD modeling. Briefly, the PK model was a one compartment model with one transit compartment to describe the absorption phase. The transit rate constant (Ktr) was equalized to the absorption rate constant (Ka), which both showed 24-hour variation that was modeled as a cosine function with a fixed period of 24 hours.

Throughout development of the drug-effect model, the fixed-effect estimates of $QT_0$, α, A and φ were fixed to the values obtained in the baseline model, while the concentration-effect parameters as well as IIV and IOV were estimated. Initially, a linear model was appended to the baseline model shown in Equation 1 to describe the concentration-effect relationship as follows:

$$QT(t) = QT_{baseline}(t) + Slope * C \quad \text{Equation 2}$$

where $QT_{baseline}(t)$ is the model described in equation 1, Slope is a linear term to describe the concentration-QT relationship (in ms per mg/L) and C is the levofloxacin concentration in plasma (in mg/L). Because it has been reported that a 1000mg oral dose of levofloxacin may transiently increase heart rate (Noel et al., 2004; Taubel et al., 2010), which could affect the relationship between the QT and RR interval, inclusion of a separate α for off- and on-drug data was considered as recommended previously (Garnett et al., 2012).

A sequential modeling strategy was applied to investigate whether the effect of levofloxacin on the QT interval is influenced by time of day. Firstly, IOV was included on

the slope parameter, with the different dosing times representing the different occasions. Secondly, it was investigated whether any bias in the distribution of the occasion-specific random effects could be reduced by estimating separate values for slope for each of the 24 hours or by describing slope as a cosine function with one or more harmonic terms and a principal period of 24 hours (Equation 3).

$$Slope = Slope\_Mesor + \Sigma_{(n=1)}^{N} [\, Slope\_A_n * \cos(\, 2\pi * n * (t\text{-}Slope\_\varphi_n)/24)] \qquad \text{Equation 3}$$

The use of these approaches was possible because the data were collected evenly across the 24-hour period with an average of 30 ECG recordings per hour (range: 24-36 observations).

Potassium levels in plasma were considered as a covariate on $QT_0$ or Slope as follows:

$$P_{i,j,t} = \theta_P + \theta_{POT} * (Potassium_{i,j,t} - Potassium_{Median}) \qquad \text{Equation 4}$$

With parameter $P_{i,j,t}$ as a function of $\theta_P$ (typical parameter value), $\theta_{POT}$ (linear change in P per unit of potassium) and the difference between the potassium concentration in the ith individual on the jth occasion at sampling time t and the median concentration of potassium in the population (4 mmol/L).

**Model evaluation**

Model selection was based on objective function value (OFV), plausibility and precision of the parameter estimates, and goodness-of-fit plots. The fit of nested models was compared using the likelihood ratio test with the significance level set at p=0.01, corresponding to a drop in OFV of at least 6.63 points upon inclusion of one additional parameter, assuming that the difference in OFV is $\chi^2$ distributed. The fit of non-nested models was compared using the Akaike information criterion (AIC) (Mould and Upton, 2013).

Because the baseline parameters were fixed to the values obtained with a limited pre-dose data set during development of the drug-effect model, we determined whether misspecification of the baseline model affects the estimated concentration-QT relationship in the final model by fixing the 24-hour variation in the baseline QTc to values reported literature (Chain et al., 2011; Dubois et al., 2016; Green et al., 2014; Smetana et al., 2002, 2003). Additionally, the bias and precision of the parameter estimates of the final model, with all (baseline and drug-effect) parameters estimated, were evaluated using a bootstrap analysis with 500 resampled datasets. The parameter estimates returned by the bootstrap were summarized as medians and 95% prediction intervals of the parameters.

**Clinical trial simulation**

The fixed and random parameter estimates of the PK-PD model were used to simulate clinical trials in which concentration and QTc profiles were obtained in 24 subjects receiving a placebo, therapeutic dose (500 mg) and supratherapeutic dose (1500mg) in a crossover design. Five hundred clinical trials with PK and PD sampling at t=0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 8, 12, 16, 20, 24h post-dose and additional PD sampling at t=-2 and -1h were simulated

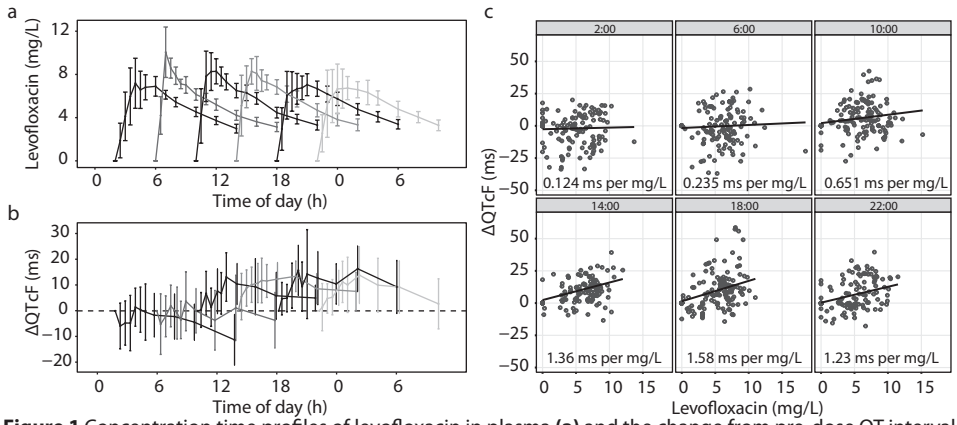


**Figure 1** Concentration time profiles of levofloxacin in plasma **(a)** and the change from pre-dose QT interval corrected for heart rate by the Fridericia formula (ΔQTcF) over time **(b)** after dosing at six different clock times. Data are presented as mean ± 95% confidence intervals. Concentration time profiles were published previously(Kervezee et al., 2016). **(c)** The relationship between levofloxacin concentration and ΔQTcF after dosing at six different clock times. Dots in c represent observed data points; lines and numbers show the estimated regression coefficients from a linear mixed effect model.

per dosing time (02:00, 06:00, 10:00, 14:00, 18:00 and 22:00) and were re-estimated with two alternative PK-PD models: (1) a model that did not include a concentration-effect relationship and (2) a model that did include a linear concentration-effect relationship. The pharmacokinetic component of the alternative models were simplified versions of the final pharmacokinetic model (no covariance between CL and V, no cosine and IIV on Ka, no transit compartment) to accommodate the simpler study design. A significant drug effect was observed if the OFV returned by alternative model 2 was more than 3.84 points (significance level α=0.05) lower than the OFV in alternative model 1. These simulations and re-estimations were performed using the stochastic simulation and estimation (SSE) tool in PsN. The output was used to compute 1) the slope of the drug effect; 2) the percentage of studies in which a significant drug effect was observed and 3) the percentage of studies in which the upper limit of the two sided 90% confidence interval of the ΔQTc at the population predicted $C_{max}$ exceeded 10ms in alternative model 2.

# RESULTS

### Data exploration

The concentration-time profiles of levofloxacin in plasma and the change from pre-dose QT interval corrected for heart rate by the Fridericia formula (ΔQTcF) after administration of a 1000mg oral dose at six different time-points are shown in Figure 1A and B. There was a significant interaction between the effect of levofloxacin concentration and the effect of dosing time (p=0.0319; linear mixed effects model), indicating that dosing time influences the relationship between levofloxacin concentration and ΔQTcF (Figure 1C).

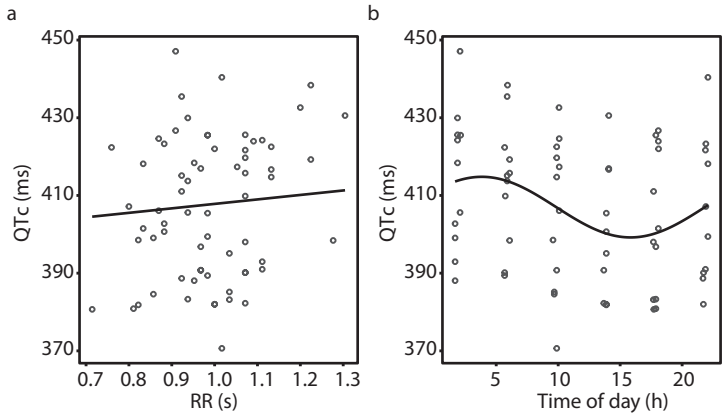


**Figure 2 (a)** The relationship between the RR interval and the QTc interval in pre-dose ECG recordings after correction for heart-rate with the coefficient estimated by the baseline model (α=0.216). The line shows the regression coefficient estimated by a linear mixed effect model **(b)** Variation in pre-dose QTc interval over the time of day. The line shows the shape of the cosine function estimated by the baseline model. The dots in (a and b) show observed data.

### Baseline QT model

To correct for potential 24-hour variation in the baseline QT interval and for study-specific dependency of the QT interval on heart rate, a baseline model was developed. The parameter estimates of this model are shown in Supplemental Table 1. A proportional error structure was used to describe the residual error. Interindividual and interoccasion variability were included on the intercept of the QT-RR relationship ($QT_0$). A one harmonic cosine function with a period of 24 hours best described the variation in the baseline QT interval over the course of the day (ΔOFV -15; $p<0.01$; 2df). Inclusion of an additional harmonic with a period of 12 hours did not further improve the fit of the model (ΔOFV -3.1; $p>0.05$; 2df vs model with 24-h cosine). Accounting for this 24-hour variation in the baseline QT interval decreased the interoccasion variability on $QT_0$ from 3% to 2.3% and removed a bias observed in the conditional weighted residuals (CWRESI) over time of day (Supplemental Figure 1). Estimation of a separate value of α during sleep did not significantly improve the fit of the model (ΔOFV -6.2, $p>0.01$, 1df). This baseline model adequately removed the dependency of the QTc interval on the RR, as evidenced by the non-significant effect of RR on QTc ($p=0.49$; linear mixed effects model), and described the 24-hour variation in the QTc intervals of the baseline data (Figure 2).There was no indication that the relationship between the QT and RR interval depends on time of day (Supplemental Figure 2).

### Drug effect model

The development process of the drug-effect model and corresponding changes in OFV are shown in Table 1. A linear function best described the relationship between drug concentration and the QT interval, but a bias was observed in CWRESI versus the time of day (Figure 3A). Additionally, the distribution of the interoccasion variability on the

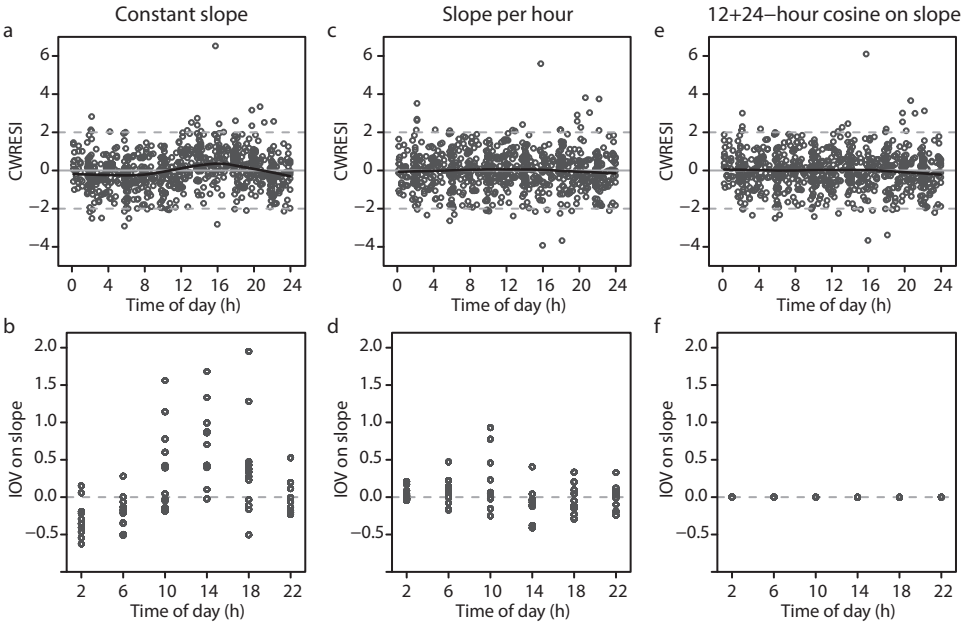


**Figure 3** Distribution of the conditional weighted residuals with interaction (CWRESI) and of interoccasion variability (IOV) on slope versus time of day in a model in which the linear concentration-effect relationship is constant over the 24 hours **(a-b)**, includes 24 estimates of slope depending on the time of the ECG recording **(c-d)**, and is described by a cosine function with two harmonics with periods of 24 and 12 hours **(e-f)**. Black line in panels a, c and e: non-parametric regression line (loess curve with span 0.6).

concentration-QT relationship depended on the time of drug administration (Figure 3B). These biases could be corrected by estimating a separate value for the concentration-QT relationship for each of the 24 hours (Figure 3C and D). Alternatively, describing the concentration-QT relationship by a cosine function with two harmonic terms with periods of 24 and 12 hours significantly improved the fit of the model and also corrected the bias in CWRESI over time of day and interoccasion variability (Figure 3E and F). Interoccasion variability was reduced to 0.3% and no longer affected the fit of the model.

Inclusion of potassium as a covariate on $QT_0$ significantly improved the model fit (see Table 1). It was found that for a 1mmol/L increase in potassium levels, $QT_0$ decreased by 5.7ms. However, the uncertainty in the parameter estimate was large (65%), while other parameter estimates were minimally affected. Potassium levels varied over the 24-hour period within a narrow physiological range (Supplemental Figure 3), so the observed effect of potassium on the QT interval is of limited clinical relevance in this study. Therefore, this parameter was not further included in the model.

### Model evaluation

The values of the concentration-QT relationship from the model in which this relationship was estimated independently for each of the 24 hours had a low level of precision, but followed a sinusoidal-like pattern over time, with higher values in the afternoon and lower

**Table 1** Changes in objective function values during model development. Models shown in bold were selected for subsequent modelling steps.

| Model no.[a] | Reference model | Description | d.f.[b] | OFV | ΔOFV |
|---|---|---|---|---|---|
| **01** | | Baseline model with linear C-QT | | 3910 | |
| 02 | 01 | C-QT as $E_{max}$ function | 1 | 3904 | -6 |
| 03 | 01 | Separate α for on-drug measurements | 1 | 3909 | -1 |
| 04 | 01 | IIV on C-QT | 1 | 3903 | -7 |
| **05** | **01** | **IOV on C-QT** | **1** | **3898** | **-12** |
| 06 | 05 | IIV and IOV on C-QT | 2 | 3898 | 0 |
| 07 | 05 | Estimation of C-QT per hour | 24 | 3754 | -144 |
| **08** | **05** | **C-QT as cosine with 24-hour period** | **3** | **3860** | **-38** |
| **09** | **08** | **C-QT with additional 12-hour cosine** | **5** | **3786** | **-74** |
| **10** | **09** | **No IOV on C-QT** | **4** | **3786** | **0** |
| 11 | 10 | Potassium as covariate on $QT_0$ | 5 | 3773 | -13 |
| 12 | 10 | Potassium as covariate on C-QT | 5 | 3779 | -6 |
| 13 | 11 | Potassium as covariate on $QT_0$ and C-QT | 6 | 3773 | 0 |
| **Final model** | **10** | **All parameters estimated** | **-** | 3783 | - |

OFV: objective function value; ΔOFV: change in OFV compared to reference model; d.f.: degrees of freedom; IOV: inter-occasion variability; IIV: inter-individual variability; C-QT: concentration-QT relationship

a. $QT_0$, α, $\varphi_{baseline}$ and $amplitude_{baseline}$ were fixed to the values of the baseline model; pre- and post-dose data included

b. Compared to Model01

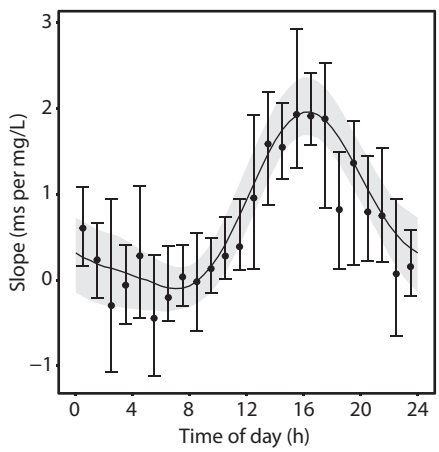


**Figure 4** 24-hour variation in slope. Dots: median ± 95 prediction intervals derived from 500 bootstrap runs of the model in which a separate value for slope was estimated for each of the 24 hours. Solid black line: estimated cosine function from the model with fixed baseline parameters, with the light grey area representing the 95% prediction interval derived from 500 bootstrap runs.

values in the early morning (Figure 4, dots). This pattern was closely matched by the model in which the concentration-QT relationship was described by a two harmonic cosine function (Figure 4, line). The cosine model was selected over the model with 24 separate estimates because of a lower AIC (ΔAIC = -6), indicating a better trade-off between model complexity (number of model parameters) and fit of the data. Additionally, providing a continuous description of the variation in the concentration-QT relationship over the 24-hour period, the cosine model has more predictive value than the other model.

Of note, comparable parameter estimates and a similar shape of the cosine function were obtained when $QT_0$, α, A and φ were estimated with the full (on- and off- drug) data set instead of fixed to the values of the baseline model (Table 2 and Supplemental Figure 4). Additionally, we found that, regardless of the baseline model used, the shape of the estimated 24-hour variation in the concentration-QT relationship was characterized by a peak in the late afternoon and a trough in the early morning (Supplemental Figure 4).

**Table 2** Parameter estimates of the final QT model with fixed baseline parameters and estimated baseline parameters.

| **Parameter** | | **Value (RSE)** (fixed baseline parameters) | **Value (RSE)[b]** (estimated baseline parameters) | **Bootstrap median (95% CI)** |
|---|---|---|---|---|
| OFV | | 3786 | 3783 | |
| $QT_0$ (ms) | | 407[a] | 409 (1%) | 409 (399-419) |
| α | | 0.216[a] | 0.211 (6%) | 0.210 (0.190-0.243) |
| A (ms) | | 7.8[a] | 6.27 (24%) | 6.28 (3.54-9.28) |
| φ (h from midnight) | | 3.84[a] | 4.11 (11%) | 4.05 (3.16-4.93) |
| | Mesor (ms per mg/L) | 0.73 (19%) | 0.73 (18%) | 0.73 (0.49-1.04) |
| | $A_1$ (ms per mg/L) | 0.977 (10%) | 0.763 (25%) | 0.772 (0.409-1.12) |
| Slope | $\varphi_1$ (h from midnight) | 16.7 (1%) | 17.3 (3%) | 17.3 (16.3-19.0) |
| | $A_2$ (ms per mg/L) | 0.274 (21%) | 0.269 (22%) | 0.285 (0.159-0.395) |
| | $\varphi_2$ (h from midnight) | 15.8 (3%) | 15.8 (4%) | 15.8 (14.7-16.9) |
| IIV $QT_0$ (CV%) | | 4.3% (23%) | 4.3% (22%) | 4.1% (2.3-5.9) |
| IOV $QT_0$ (CV%) | | 1.4% (10%) | 1.4% (10%) | 1.3% (1.1-1.6) |
| Proportional residual error (CV%) | | 1.8% (5.8%) | 1.8% (6%) | 1.8% (1.6-2.0) |

a. Values fixed to parameter estimates from the baseline QT model

b. All parameters were estimated simultaneously using the full pre- and post-dose dataset

OFV: Objective Function Value; $QT_0$: intercept of QT-RR relationship; α correction term for RR interval, A: amplitude of the 24-hour variation in QT; φ: acrophase (time of peak) of the 24-hour variation in QT; Slope: C-QT relationship; Slope_Mesor: rhythm-adjusted mean of the slope; Slope_$A_1$ and Slope_$A_2$: amplitude of the first and second harmonic of slope, respectively; Slope_ $\varphi_1$ and Slope_ $\varphi_2$: phase of the first and second harmonic of slope, respectively, IIV: interindividual variability; IOV interoccasion variability; RSE: relative standard error.

**Table 3** Results of clinical trial simulations in which oral doses of 0, 500 and 1500mg levofloxacin were administered to 24 subjects in a crossover design. 500 clinical trials were simulated per dosing time.

| **Dosing time** | **Slope (ms per mg/L)** (median [95% PI]) | **Trials with significant drug effect (%)** [95% CI][a] | **Trials with upper limit 90% CI of ΔQTc > 10ms** (%) [95% CI] |
|---|---|---|---|
| 02:00 | 0.27 [0.11-0.44] | 85 [82-88] | 0 [0-0.8] |
| 06:00 | -0.04 [-0.19-0.12] | 5.2 [3.6-7.5] | 0 [0-0.8] |
| 10:00 | 0.71 [0.55-0.88] | 100 [99-100] | 44 [40-49] |
| 14:00 | 1.73 [1.56-1.90] | 100 [99-100] | 100 [99-100] |
| 18:00 | 1.08 [0.88-1.29] | 99 [98-100] | 96 [94-97] |
| 22:00 | 0.50 [0.33-0.69] | 99 [98-100] | 0 [0-0.8] |

CI: Wilson confidence interval; PI: prediction interval; ΔQTc: change from the baseline QT interval corrected for heart rate

The parameter estimates of the final model, in which the baseline and concentration-effect parameters were simultaneously estimated, showed good precision (RSE values between 1 and 25%; Table 2) and the population and individual predicted data were in agreement with the observed data (Supplemental Figure 5). The parameter estimates returned by bootstrap analysis were similar to the parameter estimates of the final model, indicating the robustness of the model (Table 2).

### Clinical trial simulations

Our findings suggest that the concentration-QT relationship changes over time during a study occasion, while in a typical clinical trial this relationship is characterized by a single, linear, slope estimate. The predicted effect of dosing time on levofloxacin-induced QT prolongation is illustrated by clinical trial simulations (Table 3). We found that dosing time affects the linear concentration-QT relationship, the proportion of trials in which a significant drug effect was detected, and the proportion of trials in which the upper two-sided 90% confidence bound of ΔQTc at Cmax exceeded 10ms.

## DISCUSSION

In this study, we explored the implicit assumption that drug-induced QTc prolongation is not influenced by dosing time. Our results show that the relationship between the concentration of levofloxacin and the extent of QTc prolongation systematically varies over the course of the day. Using the developed PK-PD model to simulate clinical trials in which a therapeutic and a supratherapeutic dose of levofloxacin are administered, we show that dosing time would consequently influence the probability that a significant drug effect is detected. Likewise, the probability that the upper 90% confidence limit of the ΔQTc exceeds 10ms would depend on dosing time. Hence, if the developed model from this study on levofloxacin also applies to other drugs, dosing time influences the probability to detect drug-induced QT prolongation.

Our pharmacokinetic-pharmacodynamic model predicts that the largest drug effect occurs at 16:15, when the QTc interval increases by 1.7 ms per mg/L of levofloxacin, while the drug effect is virtually absent at 7:00. In a typical clinical trial, a linear slope is calculated to determine the concentration-QT relationship. Our model suggests that this slope estimate depends on the range of slope estimates that is present over time across the study period, but is most heavily influenced by the slope around the C¬max. For a clinical trial starting in the morning at 6:00 or 10:00, our simulations predict that the estimated concentration-QT relationship is -0.04 ms per mg/L and 0.71 ms per mg/L, respectively. This range of drug effects encompasses the value of 0.36 ms per mg/L that was found in a previous study that investigated the relationship between levofloxacin concentration and QTc interval in healthy subjects that was presumably started in the morning (Taubel et al., 2010).

Variation in pharmacodynamics can only be correctly analyzed if the variation in pharmacokinetics is properly accounted for. The concentration-time profiles of levofloxacin used in this study were derived from a pharmacokinetic model in which 24-hour variation in the pharmacokinetic parameters was implemented (Kervezee et al., 2016). Because this pharmacokinetic model was built using the same dataset as the current study, we used the individual post-hoc parameter estimates from this model as input for our PK-PD model. Therefore, the variation in the relationship between levofloxacin concentration and the QT interval can be attributed to variation in the sensitivity to levofloxacin, rather than to an artifact introduced by incorrect description of its pharmacokinetics.

Various physiological mechanisms may underlie the 24-hour variation in the extent of levofloxacin-induced QTc prolongation. We investigated whether variation in potassium levels may provide an explanation for our findings. Potassium levels showed 24-hour variation with higher levels during the day and lowest levels during the night, which is in line with previously published potassium profiles (Moore Ede et al., 1975; Schmidt et al., 2015; Sennels et al., 2012). However, we found that the variation in potassium cannot account for the 24-hour variation in the concentration-QT parameter. Another explanation may be 24-hour variation in the expression of ion channels in cardiomyocytes, which has been reported in experimental animal models (Jeyaraj et al., 2012; Schroder et al., 2013, 2015; Yamashita et al., 2003). It remains to be elucidated if rhythmic expression of cardiac ion channels affects the QT-prolonging potential of a drug and to what extent this applies to humans.

By showing that the sensitivity to the QTc prolonging effects of a drug varies systematically over the day and night, our study calls into question the implicit - but untested - assumption that the relationship between a drug and the QTc interval is independent of the time of day. This assumption is the basis of most clinical research on drug-induced QTc prolongation. For example, the current ICH E14 guidelines require the conduct of a thorough QT (TQT) study for every new drug under development (ICH, 2005). In a TQT study, dosing typically occurs at the same clock time in every occasion in order to perform time-matched baseline subtraction. This approach assumes a constant concentration-effect relationship over time, while our findings indicate that this relationship varies considerably over the course of the

24-hour period. Using clinical trial simulations, we show that the extent of drug induced QT prolongation may thus depend on the time of day that it is investigated. This finding is relevant in the current debate on the utility of the TQT study, in which it has been proposed that data from early phase clinical trials, combined with integrated PK-PD analysis, is a more informative approach to evaluate the QT prolonging potential of new drugs (Chain et al., 2011; Darpo et al., 2015; France and Della Pasqua, 2015; Rohatagi et al., 2009). By showing that potentially clinically relevant effects on the QT interval cannot be detected within the strict design of a TQT study, which is commonly limited to dosing in the morning, our study offers a strong argument in favor of assessing these effects by a more sophisticated design in which the dosing time is taken into account.

As this study was exploratory in nature, several limitations need to be considered. Firstly, the study population was relatively small and homogenous, consisting of healthy males between the age of 21 and 48 years, and factors such as food intake and sleep/wake rhythms were strictly standardized (Kervezee et al., 2016). Hence, to what extent our findings can be extrapolated to other populations and to real-life conditions remains to be investigated (Chain et al., 2013). Secondly, the use of continuous ECG recordings or triplicate recordings may have resulted in a richer dataset. Nevertheless, the high precision of the parameter estimates and the results of the bootstrap analysis suggest that the parameters could be precisely estimated with our dataset. Lastly, we did not include a placebo arm in our study, because the aim of the study was to investigate the effect of time of day on drug-induced QTc prolongation, and, as such, the subjects served as their own controls. Notwithstanding, we obtained sufficient pre-dose data in order to build a baseline model with precise parameter estimates that are comparable to previously published baseline models (Chain et al., 2011). Additionally, applying previously published baseline models to our data set results in a similar shape of the 24-hour variation in the concentration-QT relationship, further reducing the likelihood that our baseline model is misspecified.

An important question is to what extent our results are applicable to other drugs with QTc-prolonging potential. As the mechanism by which levofloxacin prolongs the QTc interval, namely blockade of the hERG channel is shared by most other QTc-prolonging drugs (Kannankeril et al., 2010), it is unlikely that the observed time-of-day dependency is a drug-specific property. Nevertheless, future research is warranted to extend our findings to other drugs that prolong the QTc interval. In this light, it will be useful to retrospectively and prospectively assess the effect of dosing time on the extent of drug-induced QTc prolongation in clinical studies with multiple daily dosing.

In conclusion, the tacit assumption that a drug's effect on the QTc interval is constant over the course of the day should not be taken for granted, as we show that the probability of detecting a significant drug effect depends on the time that a clinical trial is carried out, at least within the constraints of our study design. Future research into the relevance of our findings for other types of drugs as well as for other (patient) populations is crucial from both a regulatory as well as clinical perspective.

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## CONFLICT OF INTEREST

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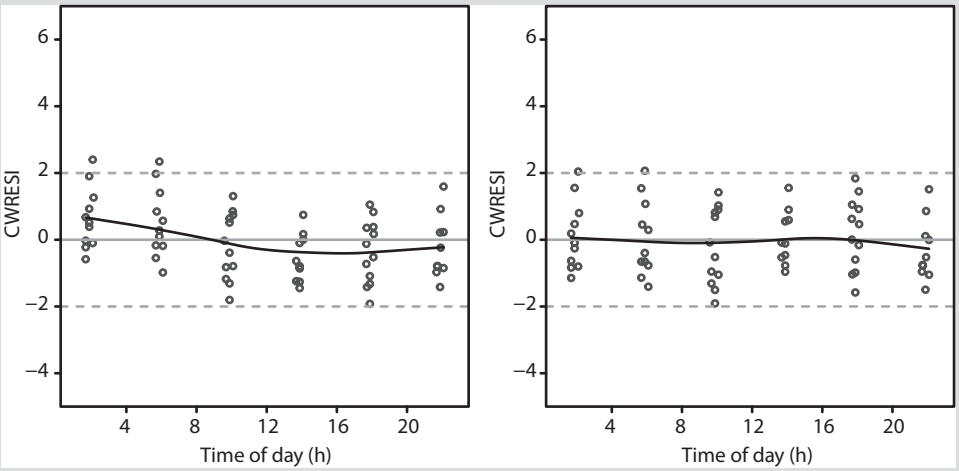
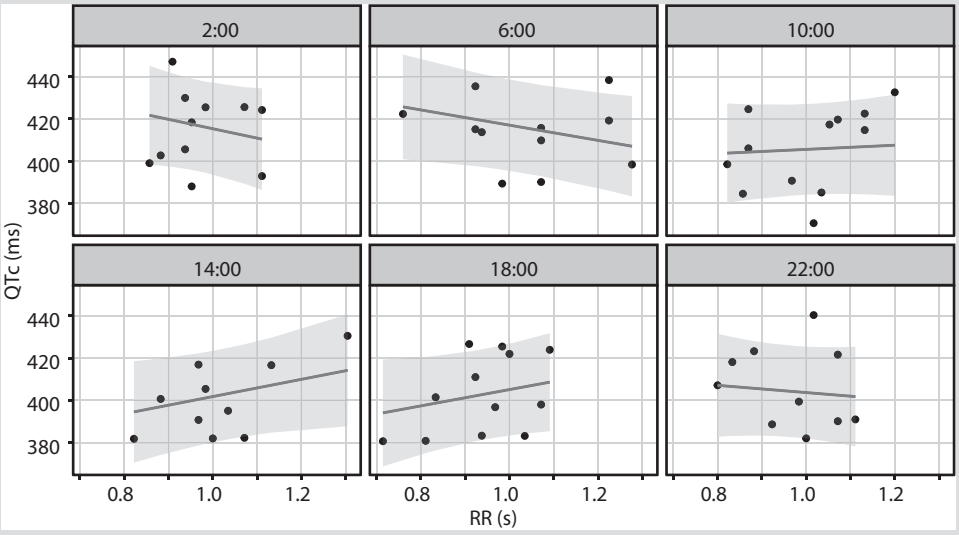
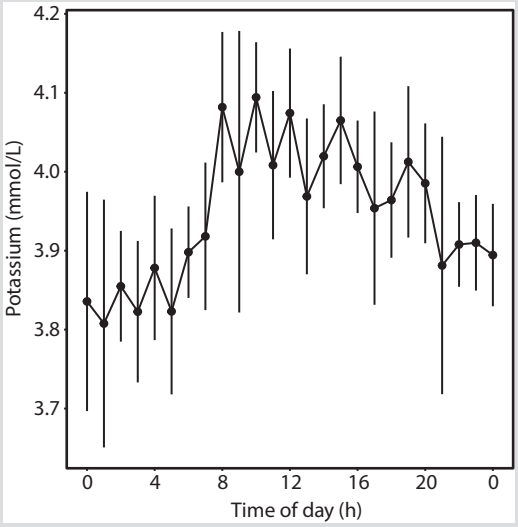


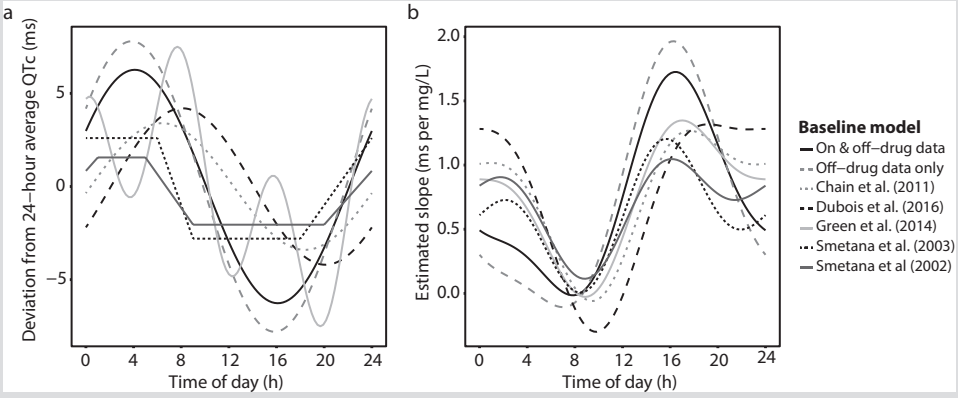

**Supplementary Figure 1** Conditional weighted residuals with interaction (CWRESI) of baseline model without **(left)** and with **(right)** correction for 24-hour rhythm in the QT interval.



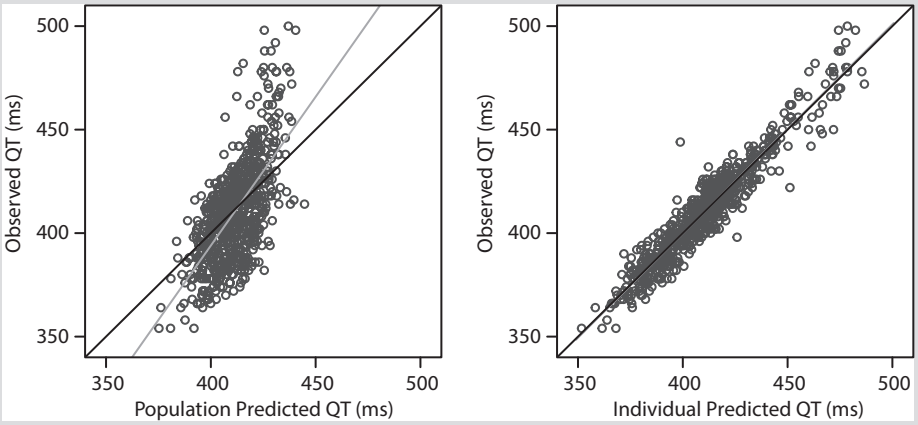

**Supplementary Figure 2** The relationship between the RR interval and the QTc interval in the baseline recordings split by time of day. The QT interval was corrected by the study-specific α obtained from the baseline model (α=0.216). A linear mixed effect with QTc as the dependent variable, RR as the fixed effect, dosing time as a categorical variable, interaction between RR and dosing time and subject as a random effect shows that the interaction between RR and dosing time (p=0.1617) and the effect of RR on QTc (p=0.5706) are not significant. Solid lines: predicted slopes of the model. Shaded areas: 95% prediction intervals.




**Supplementary Figure 3** Mean concentrations of potassium in plasma. Sampling times were rounded to the nearest hour. Error bars represent 95% confidence intervals.




**Supplementary Figure 4 (a)** 24-hour variation in baseline QT interval estimated with the full (on- and off-drug) data set (dark red line), with the pre-dose (off-drug) data set only (light red line) and reported in various publications (blue, green and orange lines). **(b)** Estimated 24-hour variation in concentration-QT relationship using the baseline models shown in panel a.




**Supplementary Figure 5** Goodness of fit plots of the final model. Observed versus population **(left)** and individual **(right)** predicted QT intervals.

**Supplemental Table 1** Parameter estimates of the baseline QT model

| Parameter | Estimate (RSE) |
|---|---|
| $QT_0$ (ms) | 407 (1.1%) |
| α | 0.216 (18%) |
| A (ms) | 7.8 (28%) |
| φ (h from midnight) | 3.84 (22%) |
| IIV $QT_0$ (%) | 3.5% (16%) |
| IOV $QT_0$ (%) | 2.3% (13%) |
| Proportional residual error (%) | 1.2% (45.2%) |

$QT_0$: intercept of QT-RR relationship; α correction term for RR interval, A: amplitude of the 24-hour variation in QT; φ: acrophase (time of peak) of the 24-hour variation in QT; IIV: interindividual variability; IOV: interoccasion variability; RSE: relative standard error.

**Supplemenatary Code 1** Model code of the final model used in NONMEM v7.3

```
$PROBLEM
$DATA 4_NONMEMDataFiles/CHDR1227_NMdataset_QT_LFX_v11.0.csv IGNORE=I;

$INPUT ID OCC TALD DV HR AMT MDV CMT EVID ADMINTIME TIME TSHPEAK TSHPEAK2 RR QTCF QTCR BMI
HGT WGT LBM AGE CLI VI MESORI AMPI PHASEI OBSCONC TINTERVAL

$SUB ADVAN6 TOL=5
$MODEL
COMP(DEPOT)
COMP(CENTRAL)
COMP(TRANSIT)

$PK
;Interocc var
OC1=0
IF(ADMINTIME.EQ.2)OC1=1
OC2=0
IF(ADMINTIME.EQ.6)OC2=1
OC3=0
IF(ADMINTIME.EQ.10)OC3=1
OC4=0
IF(ADMINTIME.EQ.14)OC4=1
OC5=0
IF(ADMINTIME.EQ.18)OC5=1
OC6=0
IF(ADMINTIME.EQ.22)OC6=1

BOV = ETA(10)*OC1+ETA(11)*OC2+ETA(12)*OC3+ETA(13)*OC4+ETA(14)*OC5+ETA(15)*OC6

; Baseline model
TVBSL = THETA(1)
BSL = TVBSL*EXP(ETA(1)+BOV)
ALPH = THETA(2)*EXP(ETA(2))
AMP24 = THETA(3)*EXP(ETA(3))
PHASE24 = THETA(4)*EXP(ETA(4))
CIRC = AMP24*COS(6.283185*(TIME-PHASE24)/24)

; Drug effect model
SLPMESOR = THETA(5)*EXP(ETA(5))
SLPAMP = THETA(6)*EXP(ETA(6))
SLPPHASE = THETA(7)*EXP(ETA(7))

SLPAMP12 = THETA(8)*EXP(ETA(8))
SLPPHASE12 = THETA(9)*EXP(ETA(9))

SLOPE = SLPMESOR+SLPAMP*COS(6.283185*(TIME-SLPPHASE)/24)+SLPAMP12*COS(6.283185*(TIME-
SLPPHASE12)/12)

;PK model
KA=MESORI+AMPI*COS(6.283185*(TIME-PHASEI)/24)
CL=CLI
V=VI
KTR=KA

K13 = KA
```

```
K32 = KTR
K20 = CL/V

$DES
DADT(1) = -K13*A(1)
DADT(2) = K32*A(3)-K20*A(2)
DADT(3) = K13*A(1)-K32*A(3)

$ERROR
CPPR = A(2)/V
IPRED=BSL*RR**ALPH+CIRC+SLOPE*CPPR
Y=IPRED*(1+EPS(1))

$THETA
407                     ; BSL
0.216                   ; ALPHA
7.8                     ; AMP24
3.84                    ; PHASE24
0.1                     ; SLPMESOR
(0,1,10)                ; SLPAMP24
(0.1,12,23.9)           ; SLPPHASE24
(0,1,10)                ; SLPAMP12
(0.1,12,23.9)           ; SLPPHASE12

$OMEGA
0.1                     ; BSL
0 FIX                   ; ALPH
0 FIX                   ; AMP24
0 FIX                   ; PHASE24
0 FIX                   ; SLOPE MESOR
0 FIX                   ; SLPAMP
0 FIX                   ; SLPPHASE
0 FIX                   ; SLPAMP12
0 FIX                   ; SLPPHASE12
$OMEGA BLOCK(1) 0.1                     ; IOV on BSL
$OMEGA BLOCK(1) SAME                    ; IOV on BSL
$OMEGA BLOCK(1) SAME                    ; IOV on BSL
$OMEGA BLOCK(1) SAME                    ; IOV on BSL
$OMEGA BLOCK(1) SAME                    ; IOV on BSL
$OMEGA BLOCK(1) SAME                    ; IOV on BSL

$SIGMA
0.05

$EST PRINT=5 MAX=9999 METHOD=1 INTERACTION POSTHOC NOABORT

$COV COMP PRINT=E

$TABLE ID OCC ADMINTIME TIME TALD IPRED PRED CWRESI RES WRES CWRES MDV SLOPE SLPMESOR SLPAMP
SLPPHASE SLPAMP12 SLPPHASE12 RR DV BSL ALPH AMP24 PHASE24 CPPR ETA1 BOV CLI VI KA MESORI AMPI
PHASEI OBSCONC NOPRINT ONEHEADER FILE=sdtabRun1101b_FINAL
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