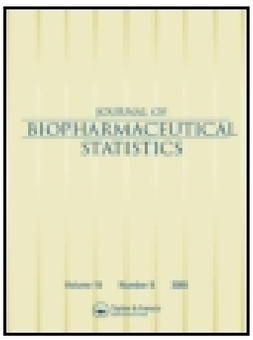

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Checking distributional assumptions for pharmacokinetic summary statistics based on simulations with compartmental models

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ABSTRACT

Bioequivalence (BE) studies are an essential part of the evaluation of generic drugs. The most common in vivo BE study design is the two-period two-treatment crossover design. *AUC* (area under the concentration–time curve) and *Cmax* (maximum concentration) are obtained from the observed concentration–time profiles for each subject from each treatment under each sequence. In the BE evaluation of pharmacokinetic crossover studies, the normality of the univariate response variable, e.g. log(*AUC*)[1] or log(*Cmax*), is often assumed in the literature without much evidence. Therefore, we investigate the distributional assumption of the normality of response variables, log(*AUC*) and log(*Cmax*), by simulating concentration–time profiles from two-stage pharmacokinetic models (commonly used in pharmacokinetic research) for a wide range of pharmacokinetic parameters and measurement error structures. Our simulations show that, under reasonable distributional assumptions on the pharmacokinetic parameters, log(*AUC*) has heavy tails and log(*Cmax*) is skewed. Sensitivity analyses are conducted to investigate how the distribution of the standardized log(*AUC*) (or the standardized log(*Cmax*)) for a large number of simulated subjects deviates from normality if distributions of errors in the pharmacokinetic model for plasma concentrations deviate from normality and if the plasma concentration can be described by different compartmental models.

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1. Introduction

In a typical pharmacokinetic bioequivalence (BE) study with a single dose administration, one of the drug products is a reference formulation and the other a test formulation. Each subject is administered both formulations in a randomized two-period crossover design (Jones and Kenward, 2003). A concentration–time profile is determined for each subject given each formulation. Each single concentration–time profile can be modeled by the pharmacokinetic compartmental model (Gibaldi and Perrier, 1982). There are many software programs available for estimating the pharmacokinetic parameters such as the absorption rate and the apparent volume of distribution (Beal et al., 2009). Then *AUC* (area under the concentration–time curve), *Cmax* (maximum concentration), and *Tmax* (time to reach *Cmax*), which are the pharmacokinetic metrics, can be obtained from the fitted pharmacokinetic model. However, the *AUC, Cmax*, and *Tmax* are obtained from the nonparametric method (Food and Drug Administration, 2001) for BE assessment. Obviously, *Tmax* is not a continuous random variable since *Tmax* has the fixed number of mass points due to a fixed available

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sampling times and further is not evaluated by the equivalence analysis. Because of this common practice, we in this article discuss the distribution of AUC and $Cmax$ obtained by the nonparametric method by simulating that distribution when the fundamental data generating mechanism is the (one-compartment) pharmacokinetics (PK) model.

In practice, the univariate response variables, such as log(AUC) and log($Cmax$), are often assumed to follow a normal distribution without much empirical evidence. In one published investigation of goodness of fit (Lacey et al., 1997), the number of subjects varied only from 29 to 69 and so the power of the Shapiro–Wilk test to detect departures from lognormal or normal distributions was not large.

In this article, we investigate the normality assumption of log(AUC) or log($Cmax$) using pharmacokinetic compartmental models typically used to describe concentration profiles over time under a set of special assumptions for variance and covariance structures. Specifically, we propose to generate data using the simplest (one and two compartment) pharmacokinetic models, to clarify which distributions for log(AUC) or log($Cmax$) are most plausible. Although there are many software packages (e.g., NONMEM, Beal et al., 2009) and many programs (e.g., SAS/IML program, Russek-Cohen et al., 2005) available for pharmacokinetic simulations, we write our own Statistical Analysis System (SAS) program to simulate the plasma concentration profiles for streamlining the derived response variables and incorporating the desired variance–covariance structures for errors and pharmacokinetic parameters. We systematically investigate the assumption of the normality of response variables (log(AUC) and log($Cmax$)) by simulating a large number of concentration–time profiles from the two-stage pharmacokinetic models for a wide range of variability, correlations, and measurement error structures for the pharmacokinetic parameters. In Stage 1, we simulate the mean plasma concentration–time profile of each subject from the one-compartment pharmacokinetic model using one particular drug's pharmacokinetic parameters (absorption rate, elimination rate, bioavailability, apparent volume of distribution, etc.) whose values follow the log-normal distribution from subject to subject (as suggested by Sheiner and Beal, 1981, and Davidian and David, 1995). In Stage 2, the plasma concentration–time profile of each subject is the result of the mean plasma concentration–time profile of each subject multiplied by the log-normally distributed residual errors. Then the distributions of log (AUC) and log($Cmax$) from simulated data are examined in three ways. We will compare the histogram of the standardized response variable (log(AUC) and log($Cmax$)) for 100,000 simulated subjects with the standard normal density curve and compare its percentiles with the standard normal percentile for several combinations of the variability, correlations, and measurement error structures of pharmacokinetic parameters, in order to highlight the most severe departures from normality. From practical experience, correlation coefficients among pharmacokinetic parameters ranging from 0.1 to 0.5 seem reasonable. A coefficient of variation (CV) of 0.2 is considered small for measurement errors and 0.4 is excessively large (cf. the discussion of a highly variable drug with CV>0.3 for within-subject variations of AUC or $Cmax$, by Blume and Midha, 1993; Blume et al., 1995). We model measurement errors within each subject as an Ornstein–Uhlenbeck process since they form a continuous-time process and it is reasonable to treat the measurement errors as being stationary over time. In practice, measurement errors are most commonly assumed to be independent for each subject in pharmacokinetic modeling (see Chapter 3, Beal et al., 2009). Third, we compare the rejection rates of the Shapiro–Wilk normality test for the log(AUC) and log($Cmax$) variables at 0.05 significance level for small (40-subject) studies for the above cases. In a very large sample size study, the sampling distribution will be very close to the true distribution for the response variable and we can determine what kinds of departure from normality occur. From the rejection rate of Shapiro–Wilk normality test for the response variable based on the small sample studies, we obtain the proportion of trials that could reject the null hypothesis of normality. If the rejection rate is much larger than the nominal significance level, then it is evident that the summary metric is not normally distributed, although that does not specify the nature of the departure. In addition, normality testing for a small sample (e.g., 40 subjects) has very low power to reject the null

hypothesis of normality. Hence the rejection rate – which may be all that one would see in biopharmaceutical practice – is not a sensitive description of true non-normality. We also conduct sensitivity analyses to investigate how the sampling distribution of the standardized log(AUC) (or the standardized log($Cmax$)) for a large number of simulated subjects deviates from normality if e_{ij} is distributed as t (a heavy tail distribution) or the mixture of two normal variables (two subgroups responding differently) or if the concentration–time profiles follow a two-compartment pharmacokinetic model with normal residual errors e_{ij}.

In Section 2, the two-stage one-compartment pharmacokinetic models are described in detail. In Section 3, we present the simulation scheme for the concentration profiles from a one-compartment pharmacokinetic model with first-order absorption and first-order elimination for many subjects to whom are administered a single 1 mg oral dose of Ropinirole for treatment of Parkinson's disease. The simulation is motivated by a real example (Kaye and Nicholls, 2000), and the estimated means for parameters from that reference are used as their true means in the simulation. Subsequently, the sampling distributions of log(AUC) and log($Cmax$) are obtained. In Section 4, we examine the departure from normality of the histogram of the standardized response variable (log(AUC) and log ($Cmax$)) for 100,000 subjects simulated from one of several combinations of the variances and correlations of pharmacokinetic parameters' vector and measurement error structures. In Section 5.1, sensitivity analyses illustrate how the distribution of the standardized log(AUC) (or the standardized log($Cmax$)) for a large number of simulated subjects deviates from normality if e_{ij} is distributed as t with 5 to 20 degrees of freedom. In Section 5.2, sensitivity analyses study the validity of the normality assumptions of log(AUC) and log($Cmax$) if there is a subgroup with a slower absorption process. In Section 5.3, sensitivity analyses study the effect of different pharmacokinetic compartment models on the validity of the normality assumptions of log(AUC) and log($Cmax$) under different combinations of the pharmacokinetic parameters and the measurement errors. In Section 6, the distributions of log(AUC) and log($Cmax$) are examined for a real case with 39 subjects.

2. Pharmacokinetic models and assumed distribution

Assume that a typical person takes one tablet with dose D orally and the plasma concentration–time curve obtained after oral administration of one tablet can be described by a one-compartment model with first-order absorption and elimination.

Let X be the true amount of drug in the body at time t after oral administration of one tablet with dose D. Let X_a be the true amount of drug at the absorption site at time t after oral administration of one tablet with dose D. For a drug that enters a body by a first-order absorption process, and is eliminated by a first-order process, and distributes in the body according to a one-compartment model, the change in the amount of drug follows the following differential equations (Gibaldi and Perrier, 1982):

$$\frac{dX}{dt} = ka \cdot X_a - ke \cdot X. \tag{1}$$

$$\frac{dX_a}{dt} = -ka \cdot X_a. \tag{2}$$

where ka is the first-order absorption rate constant and ke is the first-order elimination rate constant for the drug. Solving differential Equations (1) and (2), we obtain the relationship between the amount of drug and time:

$$X = \frac{F \cdot D \cdot ka}{(ka - ke)}\left(e^{-ka \cdot t} - e^{-ke \cdot t}\right).$$

[1]All logarithms in this article are taken to base e.

Assuming the apparent volume of distribution of a typical person is V_a and bioavailability fraction is F, we obtain the relationship between the true concentration of drug (μ_{Ct}) and time (t):

$$\mu_{Ct} = \frac{F \cdot D \cdot ka}{V_a \cdot (ka - ke)} \left(e^{-ka \cdot t} - e^{-ke \cdot t}\right).$$

Let C_{ij} denote the jth measurement of plasma concentration, j=1,…, n_i, for the ith subject, i=1,…, m, taken at time t_{ij} after dosing so that a total of $N = \sum_{i=1}^{m} n_i$ plasma concentrations are obtained. Assume that the relationship between the mean of C_{ij} and t_{ij} for a given subject i is a nonlinear function $f(t_{ij}, \boldsymbol{\beta}_i)$, where $\boldsymbol{\beta}_i$ is a $(p \times 1)$ vector of pharmacokinetic parameters for the ith subject which can vary from subject to subject and t_{ij} is a nonrandom design constant. We further assume that the form of f is common to all subjects, while $\boldsymbol{\beta}_i$ differs for each subject i. This may be written as $E(c_{ij}|\boldsymbol{\beta}_i) = \mu_{c_{ij}} = f(t_{ij}, \boldsymbol{\beta}_i)$, where $f(t_{ij}, \boldsymbol{\beta}_i)$ is often assumed to be a nonlinear function of t_{ij} and $\boldsymbol{\beta}_i$ in the form of a summation of exponential functions. It is common to represent the body as a system of compartments and to assume that the rates of transfer between compartments follow first-order or linear kinetics when we characterize the concentration of a drug in the human body (Gibaldi and Perrier, 1982). For example:

$$f\left(t_{ij}, \boldsymbol{\beta}_i\right) = \frac{F_i \cdot D \cdot ka_i}{V_{a,i}(ka_i - ke_i)} \left(e^{-ka_i \cdot t_{ij}} - e^{-ke_i \cdot t_{ij}}\right). \tag{3}$$

which is derived from the one-compartment linear pharmacokinetic model for plasma concentration after a single oral dose, D, where $\boldsymbol{\beta}_i = (ka_i, ke_i, F_i/(1 - F_i), V_{a,i})'$ and $ka_i > ke_i, 0 \leq F_i \leq 1$.

Now we can define the following two-stage models:

Stage 1 (between subject variability)

Variation among different subjects is accounted for through the subject-specific regression parameters ($\boldsymbol{\beta}_i$). Parameters may differ due to unexplained variation from the natural biological or physical variability among subjects or the run-to-run variation in assay procedures.

In general, subjects in pharmacokinetic BE studies are chosen from a relatively homogeneous population of healthy volunteers. Thus, variation among pharmacokinetic parameters across subjects is often attributable mainly to random variation among subjects rather than to differences in individual demographic and physiological characteristics that would be more pronounced in a heterogeneous patient population, e.g., body weight, genetics, and disease status.

In BE studies, it is appropriate to assume that inter-subject variation is due to unexplained noise:

$$\log\left(\boldsymbol{\beta}_i\right) = \log(\boldsymbol{\gamma}) + b_i. \tag{4}$$

In Equation (4), $\log\left(\boldsymbol{\beta}_i\right)$ is the vector of logarithms of the components of the vector $\boldsymbol{\beta}_i$, $\boldsymbol{\gamma}$ is a positive vector of population pharmacokinetic parameters, $\log(\boldsymbol{\gamma})$is the vector of logarithms of the components of the vector $\boldsymbol{\gamma}$, and the error vector $\mathbf{b_i}$ is the normal random component of inter-subject variation, which might be taken to have mean vector zero and covariance matrix Σ. In practice, the pharmacologists often assume $\log\left(\boldsymbol{\beta}_i\right)$ to be distributed as a normal random variable (Sheiner and Beal, 1981; Davidian and David, 1995). This assumption is based on physiological and biological qualitative properties such as positive skewness of $\boldsymbol{\beta}_i$ (Sheiner and Beal, 1981). On the contrary, there is not much empirical evidence about this assumption. However, one could anticipate instances where the population would actually be bimodal, where a small percentage of the population would have a slower absorption process. This two-subpopulation case corresponds to a two-component mixture of the multivariate-normal $\log\left(\boldsymbol{\beta}_i\right)$ distribution where two components differ only by $\log(ka)$.

Stage 2 (Within subject variability)

Assume that for subject i, the jth concentration follows the following model:

$$y_{ij} = \log(C_{ij}) = g(t_{ij}, \boldsymbol{\beta}_i) + e_{ij}. \tag{5}$$

where e_{ij} is a normal random measurement error with $E(e_{ij}) = 0$ and $Var(e_{ij}) = \sigma^2$, and $g(t_{ij}, \boldsymbol{\beta}_i) = \log\big(f(t_{ij}, \boldsymbol{\beta}_i) - \sigma^2/2\big)$. Let $\mathbf{y_i} = [y_{i1}, ..., y_{in_i}]'$ be the vector of log-transformed concentrations of the ith subject and $\mathbf{e_i} = [e_{i1}, ..., e_{in_i}]'$ the errors of the i th subject. Let $g_i(\boldsymbol{\beta}_i)$ denote he vector of functions of the ith subject:

$$g_i\ (\boldsymbol{\beta}_i) = [\, g(t_{i1}, \boldsymbol{\beta}_i) \quad g(t_{i2}, \boldsymbol{\beta}_i) \quad \ldots \quad g(t_{in_i}, \boldsymbol{\beta}_i)\,]'. \tag{6}$$

So we can summarize the data for the ith subject as $\mathbf{y_i} = \mathbf{g_i}(\boldsymbol{\beta}_i) + \mathbf{e_i}$, where we assume $E(\mathbf{e_i}) = 0$, $Var(\mathbf{e_i}) = \mathbf{R_i}$, and $\mathbf{R_i}$ is the variance–covariance matrix of log-transformed data within the i th subject.

Let $e_{ij} = X_i\big(t_{ij}\big)$, where $\{X_i(t), t \geq 0\}$ is Ornstein–Uhlenbeck process (Uhlenbeck and Ornstein, 1930) defined by the following stochastic differential equation:

$dX_i(t) = -\xi X_i(t)dt + \sigma dW(t)$, $X_i(0)\tilde{N}(0, \sigma^2)$, and $t \geq 0$. Here ξ and σ are unknown parameters with $\xi > 0$ and $\sigma > 0$. $W(t)$ is the standard unit Wiener process (Karlin and Taylor, 1975). The solution to the proceeding differential equation is the Ito stochastic integral $X_i(t) = \exp(-\xi t)X_i(0) + \sigma \int_0^t \exp(-\xi(t-s))dW_s$.

Then we have $e_{i1} = u_{i1}$, $e_{i,j+1} = e^{-(t_{j+1}-t_j)\xi} \cdot e_{ij} + u_{i,j+1}$, $j = 1, 2, .., m$, where $u_{i1}, ..., u_{im}$ are independent distributed normal variables with $E(u_{ij}) = 0$, $Var(u_{i1}) = \sigma^2$, and $Var(u_{ij}) = \sigma^2\big(1 - \exp\big(-2\big(t_{j+1} - t_j\big)\xi\big)\big)$. So $Cov(e_{ij}, e_{ik}) = \sigma^2 \exp\big(-2\big(t_k - t_j\big)\xi\big), k>j$. The variance of log-transformed concentration is computed by $\sigma^2 = \log(1+CV^2)$, where CV denotes the coefficient variation from untransformed concentration data.

We assume that $\mathbf{b_i}$ is independent of all e_{ij}. The assumption of normality of $\mathbf{b_i}$ and e_{ij} in Equations (3) and (4) cannot be justified on a physiological or pharmacological basis and hence some sensitivity analyses of bioavailability parameters ($\log(AUC)$ and $\log(Cmax)$) to these distributions are essential.

3. Simulation scheme

Motivated by a real example, we will simulate the concentration profiles from the model defined by Equations (3)–(5), a one-compartment pharmacokinetic model with first-order absorption and first-order elimination, for many subjects to whom are administered a single 1 mg oral dose of Ropinirole for the treatment of Parkinson's disease assuming the estimate means are the true value. This drug is a novel non-ergoline dopamine D2 receptor agonist, whose clinical pharmacokinetics is summarized (Kaye and Nicholls, 2000). We obtain the estimated means of untransformed pharmacokinetic parameters (*ka, ke, F*, and *V*) from the above reference. Here, *ka* is the absorption rate, hr^{-1}; *ke* the elimination rate, hr^{-1}; *F*, the bioavailability fraction, $0 \leq F \leq 1$; and *V*, the apparent volume of distribution, liters (L). In the reference, *F* is reported to be approximately 50%; *V* at steady state is approximately 7.2 L/kg after oral administration; *Tmax* ranges approximately from 0.5 to 4 hours after dosing, and the elimination half-life is approximately 6 hours after dosing. The average of *ke* is about 0.12 hr^{-1} is obtained from $ke = \frac{0.693}{t_{1/2e}}$, the well-known approximate relationship with elimination half-life $t_{1/2e}$ (Gibaldi and Perrier, 1982). The average of *ka* is 1.5 hr^{-1} obtained by $T\max = \frac{2.3026}{ka-ke}\log\big(\frac{ka}{ke}\big)$, an approximate relationship (Gibaldi and Perrier, 1982), among *Tmax*, the absorption rate *ka*, and *ke* when we assume *Tmax* to be 4.21 hours. The patients with Parkinson's disease usually have body weights in the range 65–75 kg (Kaye and Nicholls, 2000). The average weight of a patient is assumed 70 kg, and then the average of *V* is 525 L.

The following detailed steps describe how to simulate the plasma concentration profiles:

(1) The coefficients of variation for person-level untransformed pharmacokinetic parameters are assumed after considering estimated values (Kaye and Nicholls, 2000) as follows:

$$\boldsymbol{\eta} = E\left[\, ka, \quad ke, \quad \tfrac{F}{1-F}, \quad V_a \,\right]' = \left[\, 1.5, \quad 0.12, \quad 1, \quad 525 \,\right]' \text{ and } \mathbf{cv} = \left[\, cv\_ka, \quad cv\_ke, \quad cv\_\tfrac{F}{1-F}, \quad cv\_V_a \,\right]'.$$

(2) Assume that the log-transformed vector of pharmacokinetic parameters for the *i*th subject follow a multivariate normal distribution and write this as the component-wise log of vector entries: $\log\left[\, ka_i, \quad ke_i, \quad \left(\frac{F}{1-F}\right)_i, \quad V_{a,i} \,\right]' \sim N(\boldsymbol{\lambda}, \boldsymbol{\Lambda})$. For a log-normal (λ,σ^2) variable, the CV squared cv_{pk}^2 (=variance divided by the square of mean) is $e^{\sigma^2} - 1$ and therefore $\sigma = \sqrt{1 + cv_{pk}^2}$. The correlation matrix of these log-transformed pharmacokinetic parameters is denoted as $\boldsymbol{\Phi}$, a matrix of 1's on the diagonal with all off-diagonal entries assumed equal to a number ρ_0. We further assume each parameter on this scale is equally correlated. Here we need to convert the marginal mean $(\eta[j], \forall \mathrm{j} \in \{1,2,3,4\})$ and CV $(cv_{pk}[j], \forall \mathrm{j} \in \{1,2,3,4\})$ for each untransformed pharmacokinetic parameter obtained from the reference into the marginal mean $(\lambda[j], \forall j \in \{1,2,3,4\})$ of the log-transformed pharmacokinetic parameter and the variance matrix $(\boldsymbol{\Lambda})$ of log-transformed pharmacokinetic parameters by the following formulas:

$$\lambda[j] = \log(\eta[j]) - 0.5 * \log(1 + (cv_{pk}[j])^2), \forall \mathrm{j} \in \{1,2,3,4\}, \mathbf{M} = (diag\left\{ \sqrt{\log(1 + (cv_{pk}[j]^2))} \right\})_{4*4}, \text{and } \boldsymbol{\Lambda} = \mathbf{M} \cdot \boldsymbol{\Phi} \cdot \mathbf{M}$$

.

(3) Independently generate m subjects' random vectors of log-transformed pharmacokinetic parameters, $\log\left(ka_i, ke_i, F_i/(1-F_i), V_{a,i}\right)'$, from the distribution in Step 2. Then convert this to $\left(ka_i, ke_i, F_i, V_{a,i}\right)'$. Here i=1, 2,…, m.

(4) For a given individual i, simulate concentration profile at time points $\mathbf{t} = (0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 28, 32, 36)'$, measured in hours. Let n_i be the number of sampling times. The choice of the sampling schedule is seen in these kinds of studies and follows the general rule: more frequent samplings just after dosing (e.g., sampling every 15 minutes for the first few samples and sampling every half hour for the next few samples) and less frequent samplings later (e.g., sampling every 2 or 3 hours after half-life). This flexible sampling schedule allows more information for the rapidly changing period prior to the half-life.
Let t_{ij} be the j th sampling time point after dosing to Subject i and C_{ij} be the concentration at t_{ij}, j=1,2,…,n_i. Here $\log(C_{ij}) \sim \text{normal}\left(g(t_{ij}, \boldsymbol{\beta}_i), \sigma_{ij}^2\right)$, $f(t_{ij}, \boldsymbol{\beta}_\mathbf{i}) = \frac{F_i \cdot D \cdot ka_i}{V_{a,i} \cdot (ka_i - ke_i)} \left(e^{-ka_i \cdot t_{ij}} - e^{-ke_i \cdot t_{ij}}\right)$, and $g(t_{ij}, \boldsymbol{\beta}_i) = \log\left(f(t_{ij}, \boldsymbol{\beta}_i) - \sigma^2/2\right)$.

(5) Obtain AUC_i by the trapezoidal-rule integration $\sum_{j=1}^{n_i - 1} 0.5\left(C_{ij} + C_{i,j+1}\right)\left(t_{i,j+1} - t_{ij}\right)$ for Subject i since analytical integration from this complicated nonlinear and stochastic model is not tractable, and the AUC is calculated this way in practice (Food and Drug Administration, 2001).

(6) Obtain $Cmax_i = \underset{j}{Max}\left(C_{ij}\right)$ from all observed values for Subject i as is done in practice (Food and Drug Administration, 2001).

(7) Obtain the Shapiro–Wilk W test for the goodness of fit of log-normal distribution of AUC_i and $Cmax_i$, i = 1,2,…,m. Small sample sizes such as m = 40 will be investigated and large sample size of 400,000 will also be investigated for the true distribution arising from random-effects pharmacokinetic models.

(8) Repeat Steps 2 to 7 for *Snum* times when m=40. Here *Snum* is 10,000.

(9) Calculate rejection rate of goodness of fit of log-normal at 0.05 significance level by $\frac{\sum_{l=1}^{Snum} I(pvalue_l \leq 0.05)}{Snum}$. The 0.05 significance level is chosen based on the usual type I error rate of 5% used by the regulatory and industrial statisticians for each small BE study (m = 40).

4. Distributions of log(*AUC*) and log(*Cmax*)

4.1. *An example of simulated plasma concentration–time profiles*

We begin with illustrative simulated plasma concentration profiles generated from one particular set of parameters in a one-compartment model with CV=0.2, ξ =log(2), $\rho_0 = 0.5, cv_{pk} = (0.3, 0.3, 0.3, 0.3)'$, and $\left(k_{a,i}, k_{e,i}, F_i/(1-F_i), V_i\right)'$= (1.5,0.12,1,525)'. Figure 1 shows concentration–time profiles for a sample of 20 subjects simulated under model (3)–(5).

Throughout this article, the log(AUC) and log($Cmax$) are each standardized through centering at their sample means and scaling by their sample standard deviations. The density curve of the standardized log(AUC) (or log($Cmax$)) is estimated by the normal kernel density estimation method with bandwidth which is 0.9 times the minimum of the standard deviation and the interquartile range divided by 1.34 times the sample size to the negative one-fifth power (Sheather and Jones, 1991).

4.2. *Examination of distributions of response variables for 8 scenarios*

In this section, we simulate the plasma concentration–time profiles for eight cases defined by combinations of parameters (ρ_0, cv_{pk}, ξ, CV), shown in Table 1. Clearly, it can be seen that ρ_0 covers a practical wide range from 0.1 to 0.5, cv_{pk} from 0.1 to 0.3, ξ from 0 to –log(0.5), and CV from 0.2 to 0.4. We compare the distributions of the standardized log(AUC) and log($Cmax$) for 100,000 simulated subjects to the standard normal distribution. We also obtain the rejection rate of the normality testing for 2500 trials, each of which has 40 subjects. Note that the normality testing for a small sample (e.g., 40 subjects) has very low power to reject the null hypothesis of normality.

From Table 1, it can be seen that:

(1) the mean of the standardized log(AUC) differs from 0 by less than 0.1 for all cases; (2) its 75th percentile is about 0.15 larger than the 75th standard normal quantile for Cases 1 to 4 and about 0.15 smaller for Cases 5 to 8, and (3) its 25th percentile is 0.07 smaller than the 25th standard normal quantile for Cases 1 to 4 and about 0.08 larger for Cases 5 to 8. The sample percentiles of the

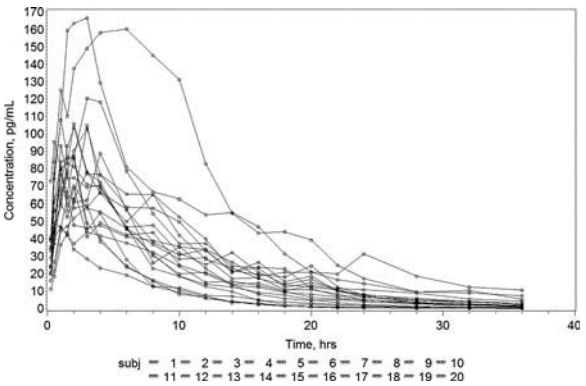

Figure 1. An example of concentration–time profiles from 20 subjects simulated from one-compartment model with CV = 0.2, ξ = log(2), $\rho_0 = 0.5, cv_{pk} = (0.3, 0.3, 0.3, 0.3)'$and $\left(k_{a,i}, k_{e,i}, F_i/(1-F_i), V_i\right)'$= (1.5,0.12,1,525)'.

Table 1. Comparison of the standard normal quantiles and sample quantiles of the standardized log(*AUC*) and log(*Cmax*) of simulated 100,000 subjects from models (1)–(5) where PK parameters with $\eta = E\left[\, ka, \quad ke, \quad \frac{F}{1-F}, \quad V \,\right]' = [\,1.5, \quad 0.12, \quad 1, \quad 525\,]'$, $cv_{pk} = 0.2$ or 0.3, and $\mathbf{e}_i = [e_{i1}, \ldots, e_{in_i}]'$.

					Quantiles for $N(0,1)$: (25th, 50th, 75th) = (−0.674,0,0.674)					
	$\log \eta\tilde{N}(\lambda, \wedge)$		Residual error e_{ij}		Quantiles of the standardized log(*AUC*)			Quantiles of the standardized log(*Cmax*)		
Case	ρ_0	cv_{pk}	ξ	CV	25th	50th	75th	25th	50th	75th
1	0.5	0.3	0	0.4	−0.749	0.022	0.780	−0.413	0.152	0.737
2	0.5	0.3	log(2)	0.2	−0.699	0.097	0.856	−0.910	−0.244	0.486
3	0.1	0.3	0	0.4	−0.766	0.009	0.773	−0.490	0.148	0.804
4	0.1	0.3	log(2)	0.2	−0.715	0.075	0.852	−0.973	−0.253	0.539
5	0.5	0.2	0	0.4	−0.590	−0.052	0.478	−0.337	0.136	0.625
6	0.5	0.2	log(2)	0.2	−0.551	0.017	0.576	−0.865	−0.294	0.382
7	0.1	0.2	0	0.4	−0.597	−0.050	0.483	−0.378	0.134	0.673
8	0.1	0.2	log(2)	0.2	−0.560	0.008	0.574	−0.894	−0.294	0.413

standardized log(*AUC*) for four cases with $\rho_0 = 0.5$are the almost same as those for four cases with $\rho_0 = 0.1$.

Figure 2 shows that the histogram (300 grouping-intervals) of the standardized log(*AUC*) has heavy tails compared to the density of $N(0,1)$ for four cases with $\rho_0 = 0.5$. Figure 2 also shows that the spread of the distribution is much narrower for cv_{pk} = 0.2 than that for cv_{pk} = 0.3.

The rejection rates of Shapiro–Wilk normality test of log(*AUC*) at 0.05 significance level are about 5% for Cases 3, 5, 6, 7, and 8. The rejection rates of Shapiro–Wilk normality test of log(*AUC*) at 0.05 significance level are about 6.5% for Cases 1, 2, and 6.

From Table 1, it can be seen that (1) the mean of the standardized log(*Cmax*) is less than 0 by 0.15 for Cases 1, 3, 5, and 7; (2) its 75th percentile is about 0.1 larger than the 75th standard normal quantile for Cases 1 and 3, the same for Cases 5 and 7, and about 0.3 smaller for Cases 2, 4, 6, and 8; and (3) its 25th percentile is 0.2 to 0.3 larger than the 25th standard normal quantile for Cases 1, 3, 5, and 7 and about 0.2 smaller for Cases 2, 4, 6, and 8. The sample percentiles of the standardized log (*Cmax*) for 4 cases with $\rho_0 = 0.5$are the almost same as those for 4 cases with $\rho_0 = 0.1$.

Figure 3 shows that the histogram (300 grouping-intervals) of the standardized log(*Cmax*) is skewed to the right of $N(0,1)$ if $\xi = 0$ or to the left if $\xi = -\log(0.5)$ for 4 cases with $\rho_0 = 0.5$. Figure 3 also shows that the spread of the distribution of the standardized log(*Cmax*) is much narrower when cv_{pk}=0.2 than that for cv_{pk}=0.3.

All rejection rates of Shapiro–Wilk normality test of log(*Cmax*) at 0.05 significance level for Cases 1, 3, 5, 6, 7 and 8 are less than 7.5% and for Cases 2 and 4 are greater than 23%.

Examination of distributions of 8 cases above with large samples shows some minor deviations of log(*AUC*) from normality. The 25th and 75th percentiles of log(*AUC*) are slightly different from the 25th and 75th percentiles for the standard normal distribution, respectively. The 50th percentile of log(*AUC*) is close to 0. It also shows that log(*Cmax*) distributes skewed to the right or the left. The 25th, 50th, and 75th percentiles of log(*Cmax*) are all different from the 25th, 50th, and 75th percentiles for the standard normal distribution, respectively. But the rejection rate of the Shapiro–Wilk normality test of log(*Cmax*) is slightly higher than 5% percent, but less than 7.5% for all cases. Clearly the Shapiro–Wilk normality test of log(*AUC*) with small sample sizes for all cases has very low power to reject the null hypothesis of normality.

5. Sensitivity analyses

Since simulations in Section 4 are based on one-compartment pharmacokinetic models with normal measurement errors, we explore in our next simulations whether log(*AUC*) and log(*Cmax*) approximately follow normal distributions if data come from the one-compartment pharmacokinetic models with symmetric measurement errors distributed as *t* and with a bimodal population corresponding

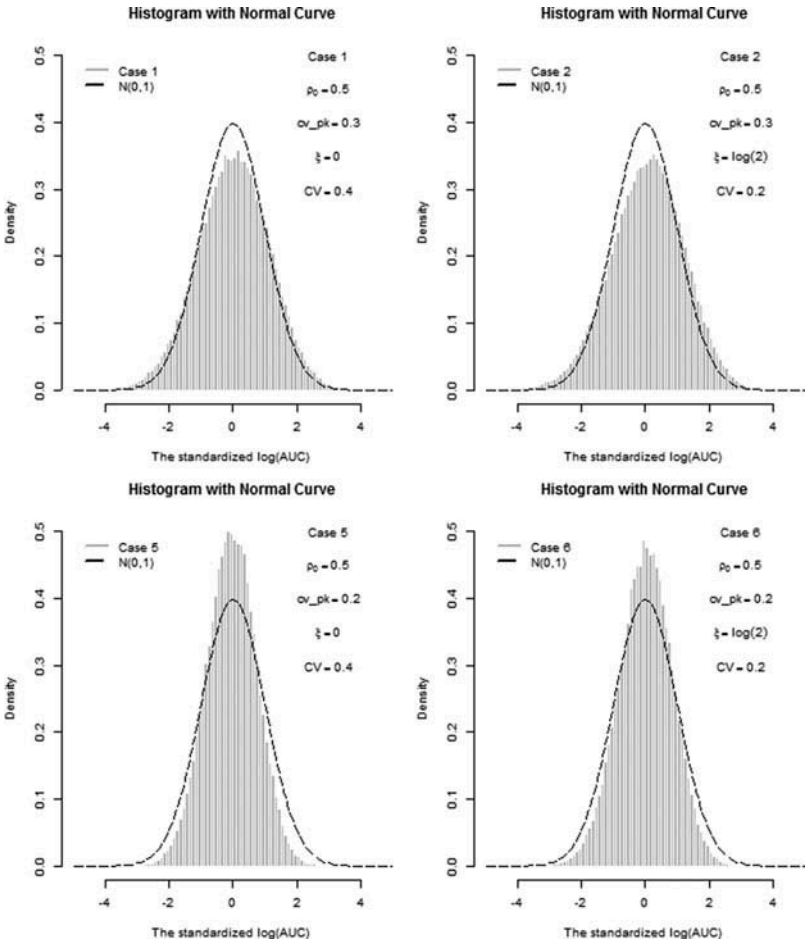

Figure 2. Histograms of the standardized log(*AUC*) for cases in Table 1 compared to *N*(0,1).

to one subgroup having a slower absorption process or if data come from two-compartment pharmacokinetic models with normal measurement errors.

5.1. *T-distributed measurement errors*

We investigate how the distribution of the standardized log(AUC) (or the standardized log($Cmax$)) for large samples of simulated subjects deviates from normality if e_{ij} is distributed as a $t(\nu)$ with $\nu = 5, 10, 15,$ or 20. We compare the histogram of the empirical standardized log(AUC) (or the standardized log($Cmax$)) with the standard normal density curve.

The cases summarized in Table 2 are now defined in terms of (ξ,ν, CV). For Cases 1 to 8 in Table 2, we assume that $e_{i1}, \ldots, e_{im}$ are independent and identically distributed t-variables for each i by letting $\xi = 0$ and we also vary the ν values from 5 to 20 by 5 and CV values from 0.2 to 0.4 by 0.2 but fix the pharmacokinetic parameters' CVs and correlation at $cv_{pk} = (0.3, 0.3, 0.3, 0.3)'$ and $\rho_0 = 0.5$, respectively. For Cases 9 to 12, $e_{i1}, \ldots, e_{im}$ are the correlated t-variables for each i by letting $\xi = -\log(0.5)$.

Figure 4 shows that the histogram of the standardized log(AUC) for 100,000 simulated subjects is very close to the standard normal density curve for all cases except Cases 1 and 9 (ν = 5 and CV = 0.4) in which the histograms are slightly skewed to the right of the standard normal density curve. The values of ν and CV don't affect the sample distribution of standardized log(AUC) much. Table 2 shows that the 25th, 50th, and

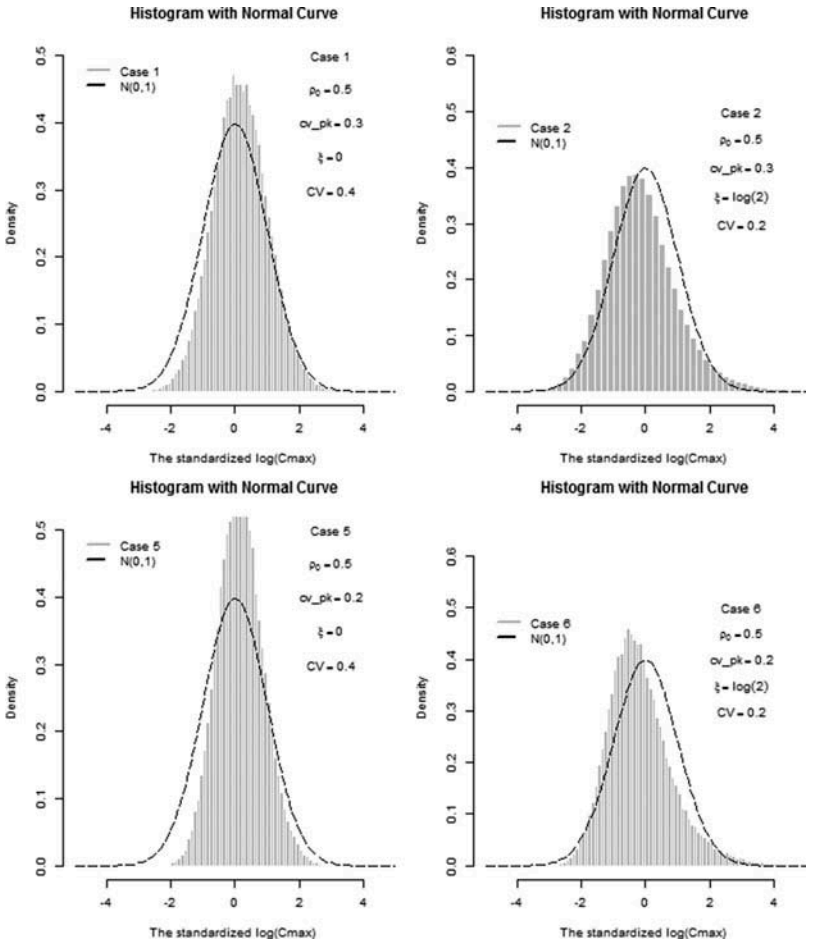

Figure 3. Histograms of the standardized log(*Cmax*) for cases in Table 1 compared to *N*(0,1).

75th percentiles of log(*AUC*) are all very close to the 25th, 50th, and 75th percentiles for the standard normal distribution, respectively. The rejection rate of Shapiro–Wilk normality test at 0.05 significance level for log (*AUC*) is about 5% if $\nu \geq 10$ and greater than 5% if ν=5.

Figure 5 shows that the histogram of the standardized log(*Cmax*) is skewed to the right compared to the standard normal density when $\nu \geq 5$ and CV = 0.4 and the histogram of the standardized log(*Cmax*) has a sharper peak and skew to the left compared to the standard normal density when $\nu \geq 5$ and CV = 0.2. The sample distribution of standardized log(*Cmax*) is affected by values of ν since its sample density curve is more skewed when ν = 5 than that when ν = 20. The 25th, 50th, and 75th percentiles of log(*Cmax*) are all different from the 25th, 50th, and 75th percentiles for the standard normal distribution, respectively. The rejection rate of Shapiro–Wilk normality test at 0.05 significance level for log(*Cmax*) is about 5% when $\nu \geq 10$ except Case 3 (8.1%) and above 10% when ν=5.

In conclusion, the distribution of log(*AUC*) seems approximately normal if the independent and identical measurement errors are assumed to be $t(10)$, i.e. symmetric with moderately heavy tails. The distribution of log(*Cmax*) is skewed even if the independent and identical measurement errors are assumed to follow t distribution.

Table 2. Comparison of the standard normal quantiles and sample quantiles of the standardized log(*AUC*) and log(*Cmax*) of simulated 100,000 subjects from Models 1 to 5 where PK parameters with $\rho_0 = 0.5$ **and** $\eta = E\left[ka, ke, \frac{F}{1-F}, V\right]' = [1.5, 0.12, 1, 525]'$ and $\mathbf{e_i} = [e_{i1}, \ldots, e_{in_i}]'$ **with** $cv_{pk} = (0.3, 0.3, 0.3, 0.3)'$.

				Quantiles for $N(0,1)$: (25th, 50th, 75th) = (−0.674,0,0.674)					
	Error $e_{ij}\sim t(v)$			Standardized log(*AUC*)			Standardized log(*Cmax*)		
Case	ξ	v	CV	25th	50th	75th	25th	50th	75th
1	0	5	0.4	−0.626	0.081	0.774	−0.366	0.353	1.160
2		5	0.2	−0.651	0.025	0.677	−0.768	−0.226	0.343
3		10	0.4	−0.669	0.020	0.694	−0.460	0.197	0.892
4		10	0.2	−0.668	−0.001	0.656	−0.815	−0.298	0.232
5		15	0.4	−0.675	0.013	0.681	−0.491	0.153	0.823
6		15	0.2	−0.672	0.001	0.653	−0.829	−0.317	0.211
7		20	0.4	−0.691	0.003	0.669	−0.506	0.133	0.793
8		20	0.2	−0.670	−0.005	0.648	−0.833	−0.323	0.191
9	log(2)	5	0.4	−0.645	0.070	0.763	−0.514	0.123	0.837
10		5	0.2	−0.622	0.040	0.682	−0.698	−0.171	0.435
11		10	0.4	−0.669	0.015	0.676	−0.565	0.020	0.666
12		10	0.2	−0.630	0.018	0.648	−0.716	−0.212	0.352

5.2. *Bimodal population: One subgroup with a slower absorption process*

We assume in this subsection that the population of subjects consists of two subgroups. One subgroup has a slower absorption process. To illustrate how the distribution of the standardized log(*AUC*) (or the standardized log(*Cmax*)) for large samples of simulated subjects deviates from normality if there is a subgroup with a slower absorption rate, we will compare the histogram of the standardized log(*AUC*) (or the standardized log(*Cmax*)) with the standard normal density curve. In this subsection we simulate the pharmacokinetic plasma concentration-time profiles from two subpopulations: 70% of population has the mean ka = 1.5 hr^{-1} and the rest of population has the mean ka = 0.2 hr^{-1} while we still assume that $e_{i1}, \ldots, e_{im}$ are independent and identically distributed normal variables for each i by letting $\xi = 0$, $CV = 0.2$, and the pharmacokinetic parameters' cv values and correlation at $cv_{pk} = (0.2, 0.2, 0.2, 0.2)'$ and $\rho_0 = 0.3$.

The left graph in Figure 6 shows that the histogram of the standardized log(*AUC*) for 100,000 simulated subjects is very close to the standard normal density curve. The right graph in Figure 6 shows the histogram of the standardized log(*Cmax*) for 100,000 simulated subjects to be bimodal.

5.3. *Two-compartment pharmacokinetic models with normal measurement errors*

To contrast the results of previous simulations with those of analogous simulations from a two-compartment pharmacokinetic model, we would ideally consider the two-compartment model for the same drug Ropinirole for which we simulated the one-compartment model in Section 4. However, the pharmacokinetic parameters for the two-compartment model of Ropinirole are not available in the literature. Hence, we have to switch to a different drug, Digoxin, whose pharmacokinetic parameters for the two-compartment pharmacokinetic model were published (Kramer et al., 1974). The cardiac glycoside Digoxin (Kramer et al., 1974) has a low therapeutic index and serious side effects.

In a two-compartment model, Compartment 1 represents the central compartment, compartment 2 the "tissue" or peripheral compartment, V_k the apparent volume of distribution of the kth compartment, and k_{jk} the first-order rate constant for transfer of drug from the jth to the kth compartment (k = 0 represents an elimination process). The equation (Kramer et al., 1974) describing the time course of drug concentration in the central compartment of this model for the ith subject at time t_{ij} after an intravenous bolus injection (dose D) is:

$$E(C_{ij}|\boldsymbol{\beta}_i) = \mu_{C_{ij}} = f^*(t_{ij}, \boldsymbol{\beta}_i) = \frac{D}{V_{a,i1} \cdot (\lambda_i - \gamma_i)} \left((k_{21i} - \gamma_i) e^{-\gamma_i \cdot t_{ij}} - (k_{21i} - \lambda_i) e^{-\lambda_i \cdot t_{ij}} \right), \tag{7}$$

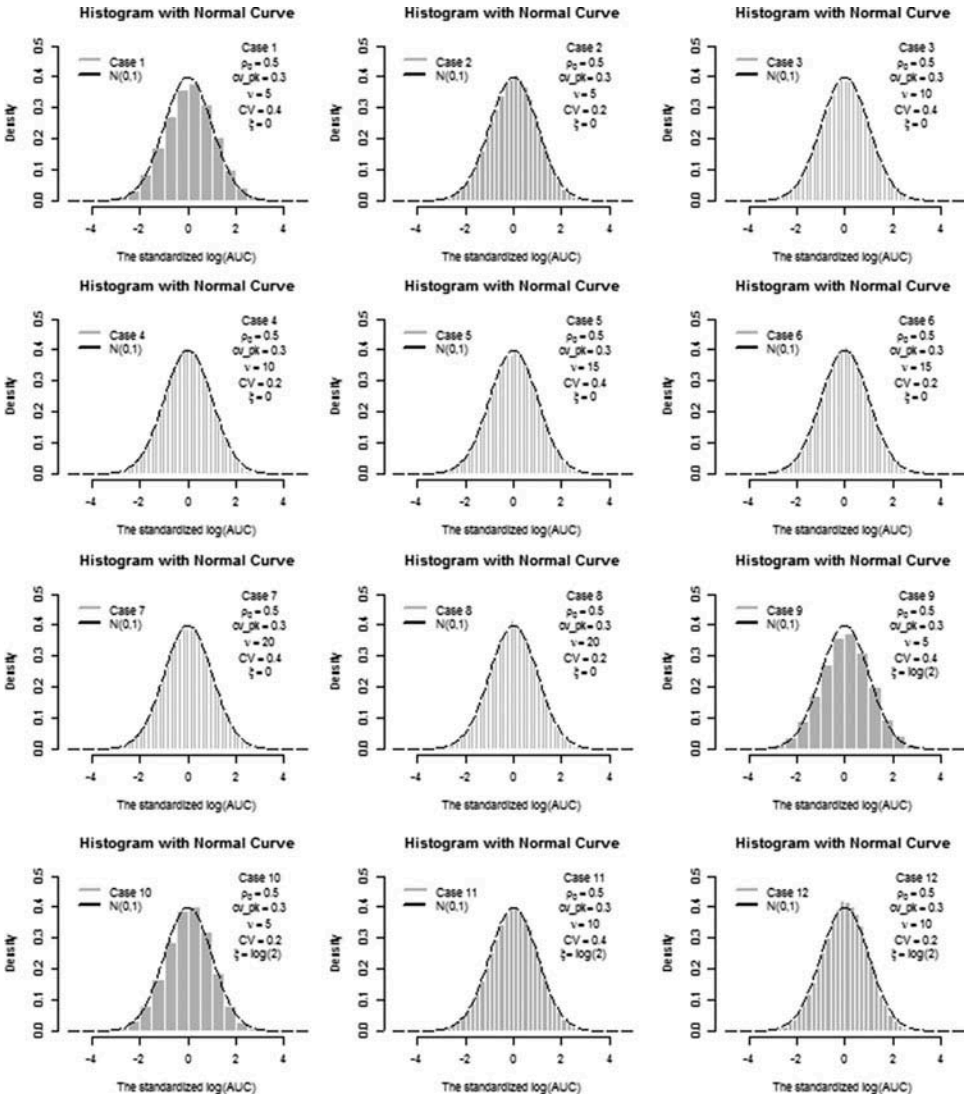

Figure 4. Histograms of the standardized log(*AUC*) for cases in Table 2 compared to *N*(0,1).

where $\lambda_i = 0.5\left[(k_{12i}+k_{21i}+k_{10i}) + \sqrt{(k_{12i}+k_{21i}+k_{10i})^2 - 4k_{21i}k_{10i}}\right]$, $\gamma_i = 0.5[(k_{12i}+k_{21i}+k_{10i}) - \sqrt{(k_{12i}+k_{21i}+k_{10i})^2 - 4k_{21i}k_{10i}}]$, and $\boldsymbol{\beta_i}=(k_{12i}, k_{21i}, k_{10i}, V_{i1})'$.

Based on Equations (3)–(5), we simulate the concentration profiles in the central compartment of the two-compartment pharmacokinetic model (6) for an average person assumed to weigh 70 kg, who administers 1 mg Digoxin by rapid bolus injection.

The following steps provide details of how to simulate the plasma concentration profiles:

(1) Obtain the means of pharmacokinetic parameters $(k_{12}, k_{21}, k_{10}, V_{a,1})'$ from (Kramer et al., 1974). The apparent volume of distribution (V_1) is 53.69 L (for an average person weighed 70 kg); k_{12} is 0.76 hr^{-1}, k_{21} is 0.12 hr^{-1}, and k_{10} is 0.29 hr^{-1}.

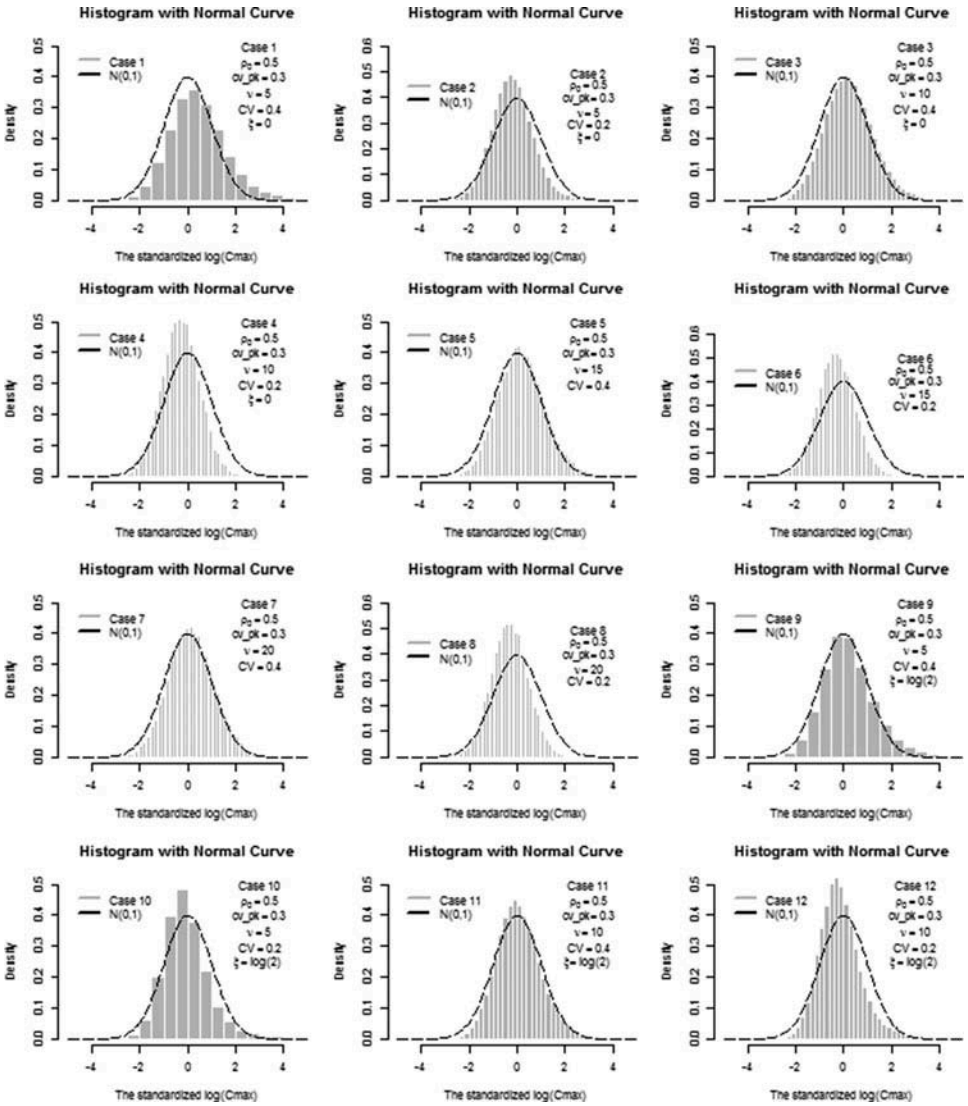

Figure 5. Histograms of the standardized log(*Cmax*) for cases in Table 2 compared to *N*(0,1).

(2) Several sets of coefficients of variation of pharmacokinetic parameters are assumed after considering estimated values (Kramer et al., 1974):

$$Let\ \ \boldsymbol{\eta} = E\begin{pmatrix} k_{12} \\ k_{21} \\ k_{10} \\ V_{a,1} \end{pmatrix} = \begin{pmatrix} 0.76 \\ 0.12 \\ 0.29 \\ 53.69 \end{pmatrix}, \text{and } \mathbf{cv} = \begin{pmatrix} cv\_k_{12} \\ cv\_k_{21} \\ cv\_k_{10} \\ cv\_V_a \end{pmatrix}.$$

(3) Assume that the vector of pharmacokinetic parameters and transformed parameters for the *i*th subject follow log-normal distribution, denoted as $\begin{pmatrix} k_{12,i} & k_{21,i} & k_{10,i} & V_{a,1,i} \end{pmatrix}' \sim \log-\text{normal}(\boldsymbol{\lambda}, \boldsymbol{\Lambda})$. The correlation matrix of these log-transformed pharmacokinetic parameters is assumed to be $\boldsymbol{\Phi}$ without any reference. Here we need to convert the marginal mean $(\eta[j], j \in \{1,2,3,4\})$ and CV $(\text{cv}[j], j \in \{1,2,3,4\})$ for each untransformed pharmacokinetic parameter obtained from the

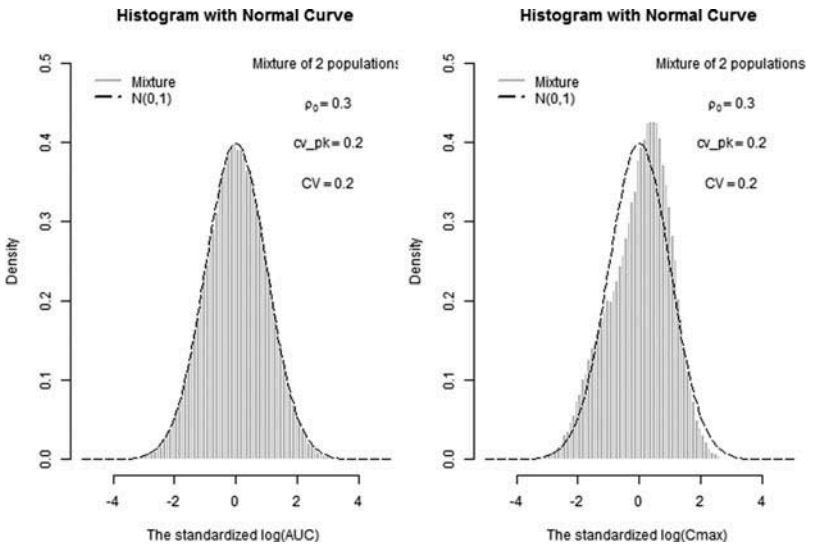

Figure 6. Comparison of standard normal density and sample histogram of the standardized log(*AUC*) and log(*Cmax*) from 100,000 subjects simulated from one-compartment model (1) with $CV = 0.2$, $\xi = 0$, and $\rho_0 = 0.3, cv_{pk} = (0.2, 0.2, 0.2, 0.2)'$for a bimodal population: 70% of population with the mean vector $\left(k_{a,i}, k_{e,i}, F_i/(1-F_i), V_i\right)' = (1.5, 0.12, 1, 525)'$ and 30% of population with the mean vector $\left(k_{a,i}, k_{e,i}, F_i/(1-F_i), V_i\right)' = (0.2, 0.12, 1, 525)'$.

reference into the marginal mean ($\lambda[j], j \in \{1,2,3,4\}$) of each log-transformed pharmacokinetic parameter and variance matrix ($\mathbf{\Lambda}$) of log-transformed pharmacokinetic parameters by the following formulas: $\lambda[j] = \log(\eta[j]) - 0.5 * \log(1 + (cv_{pk}[j])^2), j \in \{1,2,3,4\}$, and $\mathbf{M} = (diag\left\{\sqrt{\log(1 + (cv_{pk}[j]^2))}\right\})_{4*4}$.

$$Let\ \mathbf{\Phi} = \begin{pmatrix} 1 & \rho_0 & \rho_0 & \rho_0 \\ \rho_0 & 1 & \rho_0 & \rho_0 \\ \rho_0 & \rho_0 & 1 & \rho_0 \\ \rho_0 & \rho_0 & \rho_0 & 1 \end{pmatrix}, \text{then } \mathbf{\Lambda} = \mathbf{M} \cdot \mathbf{\Phi} \cdot \mathbf{M}.$$

(4) Generate random m subjects' vector of pharmacokinetic parameters and transformed parameter, $\left(k_{12,i}, k_{21,i}, k_{10,i}, V_{a1,i}\right)'$, from distribution in Step 2. Here i=1,2,. . .,m.
(5) For a given individual i, simulate concentration profile (C_{ij}) at time points, hr, $\mathbf{t} = (1/30, 1/15, 1/10, 2/15, 1/6, 7/30, 3/10, 11/30, 1/2, 3/4, 1, 2, 3, 4, 6, 8, 16, 24, 48, 72)'$ and n_i is the number of sampling time. The choice of sampling schedule follows (Kramer et al., 1974).
Let t_{ij} be the jth sampling time point after dosing to Subject i and C_{ij} be the concentration at t_{ij}, j=1,2,. . .,n_i.
(6) Repeat Steps 5 to 9 in Section 3, we can obtain the rejection rate of goodness of fit of log-normal for *AUC*, or *Cmax* at 0.05 significance level

To illustrate how the distribution of the standardized log(*AUC*) (or the standardized log(*Cmax*)) deviates from normality if the data is described by a two-compartment model, we examine the sampling distribution of the standardized log(*AUC*) (or the standardized log(*Cmax*)) under different combinations of pharmacokinetic parameters' variation and measurement errors. Since there are more pharmacokinetic parameters that vary from subject to subject in the two-compartment model than those in the one-compartment model, it is even more restrictive than before to assume that

their across-subject joint distribution is multivariate lognormal. From Table 3, we can easily see that the 25th, 50th, and 75th quantiles for log(AUC) (or log($Cmax$)) simulated from the two-compartment model are correspondingly similar to the 25th, 50th, and 75th quantiles for log(AUC) (or log ($Cmax$)) simulated from the one-compartment model. Hence the sampling distributions of the standardized log(AUC) for all cases (not shown here) are similar to the cases in Figure 2 of Section 4.2. The sampling distributions of the standardized log($Cmax$) for all cases (not shown here) are similar to the cases in Figure 3 of Section 4.2. It seems that the sampling distributions of the standardized response variables for the studied cases here and in Section 4.2 are not affected by the particular choice of compartment model, but by the distributions of the pharmacokinetic parameters and distribution of the measurement errors.

6. One real case

We would like to examine the sampling distributions of AUC and $Cmax$ using a real dataset from a pharmacokinetic study of an orally administered agent. This was a single-dose, randomized, open-label, two-period, two-sequence, two-treatment, and crossover, comparative bioavailability study of the generic product to the innovative product. The products were studied using a crossover design with 40 normal, healthy volunteers being administered a single oral dose under fasting conditions. There were 39 subjects in the study because one subject withdrew from the study. Plasma concentration sampling times are pre-dose and at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 24.0, and 36.0 hours post dose.

The null hypothesis of normality of log(AUC) (or log($Cmax$)) is not rejected at 0.05 significance level because P-values from Shapiro–Wilk normality test of log(AUC) and log($Cmax$), respectively, are 0.7103 and 0.0981, larger than 0.05. The histograms of log(AUC) and log($Cmax$) are not presented here due to small number of subjects.

7. Discussion and conclusions

Strictly speaking from statistical theory, AUC_i is not a log-normal variable even under the assumption that the plasma concentration (C_{ij}) at each time point (t_{ij}) is a log-normal random variable since $AUC = \sum_{j=1}^{n_i-1} 0.5\left(C_{ij} + C_{i,j+1}\right)\left(t_{i,j+1} - t_{ij}\right)$, which is a weighted sum of dependent log-normal random variables. Examination of the sampling distributions of the standardized log(AUC) for many cases in Section 4 and in Section 5.3 with large samples shows that the sampling distribution of the standardized log(AUC) sometimes has heavy tails compared to the normal distribution regardless of the choice of compartmental models. But normality assumption of log(AUC) seems reasonable if the independent and identical measurement errors are assumed to be t with more than 10 degrees of freedom, moderately heavy tails.

Since $Cmax_i = \max_j\left(C_{ij}\right)$ from dependent observed values for subject i, the distribution of $Cmax_i$ is affected by the sampling schedule. So $Cmax_i$ should not be log-normal variable even if $C_{ij}$is a log-

Table 3. Quantiles of log(AUC) and log($Cmax$) at 0.05 significance level (10,000 simulations) for 40 subjects with cv_{pk} = 0.2 or 0.3, residual errors $\mathbf{e_i} = [e_{i1}, \ldots, e_{in_i}]'$, ξ= 0, and CV = 0.4.

			Quantiles for $N(0,1)$: (25th, 50th, 75th) = (−0.674,0,0.674)					
	$\log \eta \tilde{N}(\lambda, \wedge)$		the standardized log(AUC)			the standardized log($Cmax$)		
Case	ρ_0	cv_{pk}	25th	50th	75th	25th	50th	75th
1	0.5	0.3	−0.792	0.044	0.875	−0.446	0.317	1.094
3	0.1	0.3	−0.728	0.011	0.741	−0.419	0.317	1.061
5	0.5	0.2	−0.616	−0.030	0.549	−0.356	0.248	0.878
7	0.1	0.2	−0.572	−0.048	0.475	−0.350	0.240	0.853

normal random variable. Examination of the sampling distributions of the standardized log(*Cmax*) for many cases in Section 4 and in Section 5.3 with large samples shows that the distribution of log (*Cmax*) is obviously skewed to the right or the left regardless of the choice of compartmental models. The sampling distribution of log(*Cmax*) is skewed even if the independent and identical measurement errors are assumed to be *t*. Especially, the normality assumptions of log(*Cmax*) is severely violated if there is a subpopulation with a slower absorption process. However, the rejection rate of the Shapiro–Wilk normality test when simulating many small studies does not provide the significant evidence for these cases.

In conclusion, the sampling distribution of log(*AUC)* with large samples seems not to deviate very far from normality for a limited number of cases studied in this article, but often has heavy tails. The sampling distribution of log(*Cmax*) is skewed either to the left or to the right and is not robust to many perturbations studied in this article. Examination of the sampling distributions of log(*AUC)* (or log(*Cmax*)) for a large number of simulated subjects helps to identify the nature of non-normality of log(*AUC)* (or log(*Cmax*)). On the contrast, the rejection rate of Shapiro–Wilk normality test which is not sensitive for many small samples (e.g. 40 subjects) cannot provide such insight. Hence it is necessary to examine the sampling distribution of the response variable for more large sample size simulations with more extensive variation of pharmacokinetic parameters and distributions so that the nature of the distribution for log(*AUC*) (or log(*Cmax*)) can be further evaluated. We must point out the limitation of our investigation since it is based on the simulations generated from the pharmacokinetic compartmental models and a lognormal measurement error structure with specific assumed values, while the real concentration–time profile may not fit the pharmacokinetic compartmental model and measurement error structure may be very different from lognormal. It would be reasonable to conclude that the non-normality situation might probably be worse than what our article found because of the relatively few deviations from lognormal parameters and normal measurement-error distributions and stationary correlations that we had tried.

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