Statistics
in Medicine

Research Article

Received 20 December 2012, Accepted 27 August 2013 Published online 16 September 2013 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/sim.5978

New approach to assess bioequivalence parameters using generalized gamma mixed-effect model (model-based asymptotic bioequivalence test)‡

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In the pharmacokinetic (PK) study under a 2x2 crossover design that involves both the test and reference drugs, we propose a mixed-effects model for the drug concentration-time profiles obtained from subjects who receive different drugs at different periods. In the proposed model, the drug concentrations repeatedly measured from the same subject at different time points are distributed according to a multivariate generalized gamma distribution, and the drug concentration-time profiles are described by a compartmental PK model with between-subject and within-subject variations. We then suggest a bioequivalence test based on the estimated bioavailability parameters in the proposed mixed-effects model. The results of a Monte Carlo study further show that the proposed model-based bioequivalence test is not only better on maintaining its level but also more powerful for detecting the bioequivalence of the two drugs than the conventional bioequivalence test based on a non-compartmental analysis or the one based on a mixed-effects model with a normal error variable. The application of the proposed model and test is finally illustrated by using data sets in two PK studies.

Keywords: bioequivalence test; crossover design; mixed-effects model; multivariate generalized gamma distribution

1. Introduction

To investigate whether a test drug is bioequivalent to a reference drug, a pharmacokinetic (PK) study is often implemented under a 2x2 crossover design. In the PK study, some healthy volunteers or subjects are recruited and administered with the drugs under investigation, and the drug concentrations in blood or plasma are then repeatedly measured after the subject was administered with the drug. Note that, under the 2x2 crossover design, the subjects in one sequence receive the reference (R) drug and then the test (T) drug in two different periods between with a washout time, while the subjects in the other sequence take the drugs in reverse order in the two periods [1]. To test against the bioequivalence of the two drugs, both the Food and Drug Administration of the United States (US FDA) [2] and the European Medicines Evaluation Agency (EMEA) [3] recommended the area under the drug concentration-time curve (AUC) and the maximum drug concentration (C_{max}) as the bioavailability parameters. Moreover, the test drug is claimed to be bioequivalent to the reference one if the ratio of the bioavailability parameters associated with different drugs is between the two boundary values 0.8 ($= 4/5$) and 1.25 ($= 5/4$).

The bioequivalence test currently employed in industry is constructed based on the estimated bioavailability parameter that is, in fact, the geometric mean of the estimated bioavailability parameters each from individual drug concentration-time profile [4]. However, when the prior knowledge of the kinetic of the drug is available, a reasonable compartmental PK model [5] can be employed in a statistical model to describe the evolution of the drug concentration over time and hence provides more information

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about the bioavailability of the drug under investigation. To do so, Lindsey *et al.* [6] considered a fixed-effects statistical PK model for the concentration-time profiles observed from a group of subjects, where the one-compartment PK model with first-order absorption and elimination serves as the mean curve. In addition, Lindsey *et al.* [6] suggested some particular distributions for the nonnegative and right-skewed drug concentration. Chen and Huang [7] further proposed a fixed-effects statistical PK model for the drug concentrations in a 2x2 crossover design that are distributed according to the generalized gamma distribution [8, 9] because it includes many well-known righted-skewed distributions, for example, gamma, Weibull, and lognormal distributions. However, both the statistical PK models suggested in Chen and Huang [7] and Lindsey *et al.* [6] did not consider the correlation between the drug concentrations that are measured from the same subject either in different periods or at different time points in any period.

Due to the diversity of the patient population, Sheiner and Ludden [10] proposed a nonlinear mixed-effects model, referred to as the NLMEM, for the drug concentration-time profiles in a PK study. Note that the NLMEM is, in fact, an additive model that describes directly the drug concentrations with a normal error variable. Davidian and Giltinan [11, 12] then discussed some methods for estimating the parameters in the NLMEM, which is either based on an approximate likelihood function or the time-consuming Monte Carlo expectation maximization (EM) algorithm [13, 14]. Several authors, for example, Pentikis *et al.* [15], Hu *et al.* [16], Fradette *et al.* [17], Panhard *et al.* [18], and Panhard *et al.* [19], further considered the bioequivalence test with respect to *AUC* based on the NLMEM in a 2x2 crossover design where only the between-subject variation (BSV) is under study. On the other hand, Dubois *et al.* [20] considered an NLMEM for the drug concentration-time profiles in a 2x2 crossover design, which includes both the BSV and within-subject variability (WSV). Moreover, to speed up the computation, Dubois *et al.* [20] suggested one estimate the parameters based on the stochastic approximation EM (SAEM) algorithm [21]. Although the NLMEM is getting popular in the PK study for a handy software *Monolix* [22], the NLMEM may not be appropriate for the drug concentrations that are usually nonnegative and right-skewed distributed.

To provide an alternative mixed-effects model in a PK study under a 2x2 crossover design, we propose, in this paper, a multiplicative model for the right-skewed drug concentrations. In the proposed mixed-effects model, a multivariate generalized gamma (MGG) distribution is developed for the joint distribution of the repeatedly measured drug concentrations, and the BSV and WSV are both considered in a one-compartment PK model as discussed in Dubois *et al.* [20]. In fact, in the MGG distribution, the marginal distribution of the drug concentrations observed at each time point is a generalized gamma distribution, and the correlations between drug concentrations measured at two different time points from the same subject may depend on the time lag. We then employ the SAEM algorithm to estimate the parameters in the proposed mixed-effects model, hereafter, referred to as multivariate generalized gamma mixed-effects model (MGGMEM), and finally suggest an MGGMEM-based bioequivalence test.

In Section 2, we introduce the MGGMEM for the drug concentration-time profiles in a PK study under a 2x2 crossover design and discuss the estimation of the parameters in the proposed model. In Section 3, we consider a bioequivalence test based on the ratio of two estimated *AUC*s from the fitted MGGMEM. In Section 4, we report the results of a simulation study designed for investigating the performances of the MGGMEM-based bioequivalence test relative to other competitive tests. We then demonstrate the use of the proposed model and test by illustrating two real data sets in Section 5. Finally, in Section 6, we give some conclusions and discussions on the application of the proposed model.

2. A multivariate generalized gamma mixed-effects model

2.1. The proposed mixed-effects model

Let $Y_{ijk\ell}$ be the drug concentration measured from the jth subject in the ith sequence during period k at time t_ℓ, $i, k = 1, 2$, $j = 1, \cdots, n_i$, $\ell = 1, \cdots, m$. For an orally administered drug, the mean drug concentrations at time t is usually described by a one-compartment PK model with first-order absorption and elimination after a lag time as given by

$$\mu(t;\boldsymbol{\eta}) = \frac{dk_a}{Vk_a - CL}\left[\exp\left(-\frac{CL}{V}(t - Tlag)^+\right) - \exp(-k_a(t - Tlag)^+)\right], \tag{1}$$

where $\boldsymbol{\eta} = (\log k_a, \log CL, \log V, \log Tlag)'$ is the vector of PK parameters, d is the dose level applied, k_a and CL are the absorption and clearance rates, respectively, V is the volume parameter, $Tlag$ is the lag time, and $a^+ = \max(0, a)$. Note that, under model (1), we have

$$\log AUC = \log(d/CL),$$

and

$$\log C_{\max} = \log\left(\frac{d}{V}\right) + \frac{CL\log k_a - \log(CL/V)}{Vk_a - CL}.$$

To take into account of the variability of the subjects under a 2x2 crossover design, we consider two multivariate normal random vectors, $\boldsymbol{b}_{ij} = \left(b_{ij}^a, b_{ij}^{CL}, b_{ij}^V, b_{ij}^{Tlag}\right)'$ and $\boldsymbol{w}_{ijk} = \left(w_{ijk}^a, w_{ijk}^{CL}, w_{ijk}^V, w_{ijk}^{Tlag}\right)'$, corresponding to BSV and WSV, respectively, where both the random vectors have the same mean vector $\mathbf{0}$ but different covariance matrices Ω and Ψ, respectively. Let $I_A = 1$ if A is true, and 0, otherwise. Then, the random vector for each subject is $\boldsymbol{\eta}_{ijk} = (\log k_{aijk}, \log CL_{ijk}, \log V_{ijk}, \log Tlag_{ijk})'$, $i, k = 1, 2$, $j = 1, \cdots, n_i$, with

$$\begin{aligned}
\log k_{aijk} &= \mu_0^a + \delta^a I_{\{i\neq k\}} + \pi_k^a + \xi_i^a + b_{ij}^a + w_{ijk}^a,\\
\log CL_{ijk} &= \mu_0^{CL} + \delta^{CL} I_{\{i\neq k\}} + \pi_k^{CL} + \xi_i^{CL} + b_{ij}^{CL} + w_{ijk}^{CL},\\
\log V_{ijk} &= \mu_0^V + \delta^V I_{\{i\neq k\}} + \pi_k^V + \xi_i^V + b_{ij}^V + w_{ijk}^V,\\
\log Tlag_{ijk} &= \mu_0^{Tlag} + \delta^{Tlag} I_{\{i\neq k\}} + \pi_k^{Tlag} + \xi_i^{Tlag} + b_{ij}^{Tlag} + w_{ijk}^{Tlag},
\end{aligned} \tag{2}$$

where $\boldsymbol{\delta} = (\delta^a, \delta^{CL}, \delta^V, \delta^{Tlag})'$, $\boldsymbol{\pi}_k = \left(\pi_k^a, \pi_k^{CL}, \pi_k^V, \pi_k^{Tlag}\right)'$, and $\boldsymbol{\xi}_i = \left(\xi_i^a, \xi_i^{CL}, \xi_i^V, \xi_i^{Tlag}\right)'$ are the vectors of constants corresponding to the drug, period and sequence effects, respectively, with $\boldsymbol{\pi}_1 = \boldsymbol{\xi}_1 = \mathbf{0}$. Let $\boldsymbol{\theta}_1 = \left(\boldsymbol{\mu}_0', \boldsymbol{\delta}', \boldsymbol{\pi}_2', \boldsymbol{\xi}_2'\right)'$ and $\boldsymbol{\theta}_2 = (vec(\Omega)', vec(\Psi)')'$, corresponding to the fixed and random effects, respectively, where $\boldsymbol{\mu}_0 = \left(\mu_0^a, \mu_0^{CL}, \mu_0^V, \mu_0^{Tlag}\right)'$, and $vec(\mathrm{A})$ is the vectorization of matrix A. Then, the random vector $\boldsymbol{\eta}_{ij} = \left(\boldsymbol{\eta}_{ij1}', \boldsymbol{\eta}_{ij2}'\right)'$ is distributed according to a multivariate normal distribution with mean vector

$$E(\boldsymbol{\eta}_{ij}) = \left(\boldsymbol{\mu}_0' + \boldsymbol{\delta}' I_{\{i\neq 1\}} + \boldsymbol{\xi}_i', \boldsymbol{\mu}_0' + \boldsymbol{\delta}' I_{\{i\neq 2\}} + \boldsymbol{\pi}_2' + \boldsymbol{\xi}_i'\right)'$$

and covariance matrix

$$\mathrm{Cov}(\boldsymbol{\eta}_{ij}) = \begin{pmatrix} \Omega + \Psi & \Omega \\ \Omega & \Omega + \Psi \end{pmatrix}.$$

Note that, under model (2), the expected $\log AUC$ for subject in sequence i at period k is $\log d - \left(\mu_0^{CL} + \delta^{CL} I_{\{i\neq k\}} + \pi_k^{CL} + \xi_i^{CL}\right)$, $i, k = 1, 2$. If $\pi_2^{CL} = \xi_2^{CL} = 0$, then the expected $\log AUC$ becomes $\log d - \mu_0^{CL}$ for reference drug and $\log d - \left(\mu_0^{CL} + \delta^{CL}\right)$ for test drug.

To link the drug concentration and sampling time under a 2x2 crossover design, we consider the mixed-effects model as follows:

$$\log Y_{ijk\ell} = \log \mu(t_\ell, \boldsymbol{\eta}_{ijk}) + \log \varepsilon_{ijk\ell}, \tag{3}$$

where the $\boldsymbol{\varepsilon}_{ijk} = (\varepsilon_{ijk1}, \ldots, \varepsilon_{ijkm})'$ is a random vector of measurement errors that is independent of the $\boldsymbol{b}_{ij}$ and $\boldsymbol{w}_{ijk}$ in (2). Note that the NLMEM considered in Dubois *et al.* [20] is an additive model with $Y_{ijk\ell} = \mu(t_\ell, \boldsymbol{\eta}_{ijk}) + \varepsilon_{ijk\ell}$, where the $\varepsilon_{ijk\ell}$ are identically and independently distributed normal variables with zero mean and a time-dependent variance. In this paper, however, we assume that the marginal distribution of the $\varepsilon_{ijk\ell}$, $\ell = 1, \ldots, m$, in model (3) is a generalized gamma distribution, denoted by GG_ℓ, $\ell = 1, \cdots, m$, with mean 1, scale parameter σ, and shape parameter λ. Hence, the related location parameter is given by

$$\beta = g(\sigma, \lambda) = \log\Gamma(\lambda^{-2}) - 2\sigma(\log\lambda)/\lambda - \log\Gamma(\lambda^{-2} + \sigma/\lambda).$$

Note that the PDF of the generalized gamma distribution is given by

$$f(\varepsilon) = \begin{cases} \dfrac{|\lambda|[\lambda^{-2}(e^{-\beta}\varepsilon)^{\lambda/\sigma}]^{\lambda^{-2}} \exp[-\lambda^{-2}(e^{-\beta}\varepsilon)^{\lambda/\sigma}]}{\sigma\varepsilon\Gamma(\lambda^{-2})} & , \lambda \neq 0 \\ \dfrac{\exp[-(\log\varepsilon-\beta)^2/(2\sigma^2)]}{\sqrt{2\pi}\sigma\varepsilon} & , \lambda = 0 \end{cases},$$

and the associated cumulative distribution function (CDF) is obtained as

$$F(\varepsilon) = \begin{cases} \Gamma[\lambda^{-2}(e^{-\beta}\varepsilon)^{\lambda/\sigma}; \lambda^{-2}] & , \lambda > 0 \\ 1 - \Gamma[\lambda^{-2}(e^{-\beta}\varepsilon)^{\lambda/\sigma}; \lambda^{-2}] & , \lambda < 0 \\ \Phi[(\log\varepsilon-\beta)/\sigma] & , \lambda = 0 \end{cases},$$

where $\Gamma(s;\gamma) = \int_0^s u^{\gamma-1}e^{-u}du/\Gamma(\gamma)$, and $\Phi(\cdot)$ is the distribution function of a standard normal random variable.

Next, we consider the joint distribution of $\boldsymbol{\varepsilon}_{ijk}$, $i,k = 1,2$, $j = 1,\cdots,n_i$, as a multivariate generalized gamma distribution. We employ the Gaussian copula [23, 24] to link the marginal CDFs, $F_1,\cdots,F_m$, of the error variables $\varepsilon_{ijk1},\ldots,\varepsilon_{ijkm}$. In other words, we assume that the random vector of normal scores, $\boldsymbol{q} = (\Phi^{-1}(F_1),\cdots,\Phi^{-1}(F_m))'$, is distributed according to a multivariate normal distribution with mean vector $\mathbf{0}$ and correlation matrix $\mathbf{R} = (r_{\ell\ell'})$. Because the correlation between the drug concentrations measured at different time points may depend on the time lag, we further assume

$$r_{\ell\ell'} = r\,(|t_\ell - t_{\ell'}|) \text{ for } \ell,\ell' = 1,\ldots,m,$$

where $r(0) = 1$ and $r(u) = \rho^u$ with $u > 0$ and $0 \leqslant \rho < 1$. Then, the PDF of the Gaussian copula is

$$c((\Phi^{-1}(F_1),\cdots,\Phi^{-1}(F_m));\rho) = |\boldsymbol{R}|^{-1/2}\exp\left\{\frac{1}{2}\boldsymbol{q}'(I_m - \boldsymbol{R}^{-1})\boldsymbol{q}\right\},$$

which depends on the parameter vector $\boldsymbol{\theta}_3 = (\rho,\sigma,\lambda)'$.

Note that due to a different kinetic of the oral drug under study, we may have, for instance, a zero-order one-compartment PK model as given by

$$\mu(t;\boldsymbol{\eta}) = \frac{d}{T_a CL}\left[1 - \exp\left(-\frac{CL}{V}(t-(t-T_a)^+)\right)\right]\exp\left(-\frac{CL}{V}(t-T_a)^+\right), \tag{4}$$

where $\boldsymbol{\eta} = (\log T_a, \log CL, \log V)'$ is the vector of PK parameters, d is the dose level applied, T_a is the absorption duration, CL is the clearance rate, and V is the volume parameter. In fact, we can also construct a mixed-effects model such as models (2) and (3) based on the model in (4). Note that, under model (4), we have

$$\log AUC = \log(d/CL),$$

again, and

$$\log C_{\max} = \log\frac{d}{T_a CL} + \log\left[1 - \exp\left(-\frac{CL}{V}(T_a)\right)\right].$$

2.2. *Estimation of the parameters in the mixed-effects model*

In fact, under the 2x2 crossover design, the observed data set is $\boldsymbol{y} = \{\boldsymbol{y}_{ij}, i = 1,2, j = 1,\cdots,n_i\}$, where $\boldsymbol{y}_{ij} = \{y_{ijk\ell}, k = 1,2, \ell = 1,\cdots,m\}$, and the unobserved data set is $\boldsymbol{\eta} = \{\boldsymbol{\eta}_{ij}, i = 1,2, j = 1,\cdots,n_i\}$. Let $f_\ell(y_{ijk\ell}|\boldsymbol{\eta}_{ijk})$ be the conditional PDF of $\mu(t_\ell,\boldsymbol{\eta}_{ijk})\varepsilon_{ijk\ell}$ given $\boldsymbol{\eta}_{ijk}$ and $F_\ell(y_{ijk\ell}|\boldsymbol{\eta}_{ijk})$ the associated conditional CDF, where both the conditional PDF and CDF depend on σ and λ. Then, the likelihood function of $\Theta = \left(\boldsymbol{\theta}_1',\boldsymbol{\theta}_2',\boldsymbol{\theta}_3'\right)'$ given $(\boldsymbol{y}_{ij},\boldsymbol{\eta}_{ij})$ is

$$L(\Theta|\boldsymbol{y}_{ij},\boldsymbol{\eta}_{ij}) = \varphi(\boldsymbol{\eta}_{ij};\boldsymbol{\theta}_1,\boldsymbol{\theta}_2)d(\boldsymbol{y}_{ij};\boldsymbol{\eta}_{ij},\boldsymbol{\theta}_3),$$

where $\varphi(\boldsymbol{\eta}_{ij};\boldsymbol{\theta}_1,\boldsymbol{\theta}_2)$ is the joint PDF of $\boldsymbol{\eta}_{ij}$, and $d(\boldsymbol{y}_{ij};\boldsymbol{\eta}_{ij},\boldsymbol{\theta}_3)$ is the conditional joint PDF of $\boldsymbol{y}_{ij}$ given $\boldsymbol{\eta}_{ij}$ as given by

$$d(\boldsymbol{y}_{ij};\boldsymbol{\eta}_{ij},\boldsymbol{\theta}_3)=\prod_{k=1}^{2}\left\{c(\Phi^{-1}(F_1(y_{ijk1}|\boldsymbol{\eta}_{ijk})),\ldots,\Phi^{-1}(F_m(y_{ijkm}|\boldsymbol{\eta}_{ijk}));\rho)\prod_{\ell=1}^{m}f_\ell(y_{ijk\ell}|\boldsymbol{\eta}_{ijk})\right\}.$$

Hence, the log-likelihood function of Θ given the observed data set $\boldsymbol{y}$ is

$$l(\Theta|\boldsymbol{y})=\sum_{i=1}^{2}\sum_{j=1}^{n_i}\log\left(\int L(\Theta|\boldsymbol{y}_{ij},\boldsymbol{\eta}_{ij})d\,\boldsymbol{\eta}_{ij}\right).$$

Note that the setting under study involves incomplete data. Therefore, the EM algorithm [25] is usually employed to find the maximum likelihood estimate (MLE) of Θ. Let $l_c(\Theta|\boldsymbol{y},\boldsymbol{\eta})=\sum_{i=1}^{2}\sum_{j=1}^{n_i}\log L(\Theta|\boldsymbol{y}_{ij},\boldsymbol{\eta}_{ij})$ be the log-likelihood of Θ given $(\boldsymbol{y},\boldsymbol{\eta})$ and set $Q(\Theta|\Theta^*)=E(l_c(\Theta|\boldsymbol{y},\boldsymbol{\eta})|\boldsymbol{y},\ \Theta^*)$. Then, in the rth iteration of the EM algorithm, $Q_r(\Theta)=Q\left(\Theta|\hat{\Theta}_{r-1}\right)$ is evaluated at the E step and then maximized to obtain an updated value $\hat{\Theta}_r$ at the M step. Because there is no analytic form for $Q_r(\Theta)$, we further employ the SAEM algorithm [21] to evaluate $Q_r(\Theta)$ in which the E step is divided into a simulation step and a stochastic approximation step. At the simulation step, we generate $\left\{\boldsymbol{\eta}_{ij}^{(s)},s=1,\cdots,S\right\}$ from the conditional distribution

$$p\left(\boldsymbol{\eta}_{ij}|\boldsymbol{y}_{ij},\hat{\Theta}_{r-1}\right)=\frac{L\left(\hat{\Theta}_{r-1}|\boldsymbol{y}_{ij},\boldsymbol{\eta}_{ij}\right)}{\int L\left(\hat{\Theta}_{r-1}|\boldsymbol{y}_{ij},\boldsymbol{\eta}_{ij}\right)d\,\boldsymbol{\eta}_{ij}}$$

by using the Metropolis-Hasting algorithm [26]. At the approximation step, we then update $Q_r(\Theta)$ according to

$$Q_r(\Theta)=(1-\delta_r)Q_{r-1}(\Theta)+v_r\left\{\frac{1}{S}\sum_{s=1}^{S}\left[\sum_{i=1}^{2}\sum_{j=1}^{n_i}\log L\left(\Theta|\boldsymbol{y}_{ij},\boldsymbol{\eta}_{ij}^{(s)}\right)\right]\right\}$$

by introducing $(v_r)_{r\geq 0}$, a sequence of positive numbers decreasing to 0. Finally, we find $\hat{\Theta}_r$ at the M step such that $Q_r\left(\hat{\Theta}_r\right)=\max_{\Theta}Q_r(\Theta)$. The SAEM algorithm has been shown in Kuhn and Lavielle [26] to be more efficient than the Monte Carlo EM algorithm for computing the MLE of Θ, and the estimates given by SAEM algorithm converge toward the MLE. To further estimate the standard error of the estimated parameter in the MGGMEM, we estimate the Fisher information matrix by combining the stochastic approximation approach and the missing information principle in Louis [27] as suggested in Kuhn and Lavielle [26].

3. Model-based bioequivalence test

We consider testing against the average bioequivalence between the test and reference drugs with respect to $\Delta_A=\log AUC_T-\log AUC_R=-\delta^{CL}$ based on the MGGMEM in (3). In other words, we construct a test for the null hypothesis

$$H_0:\{\Delta_A\leq\log(0.8)=-0.2231\text{ or }\Delta_A\geq\log(1.25)=0.2231\}$$

versus the alternative hypothesis

$$H_A:\{-0.2231<\Delta_A<0.2231\}$$

based on the estimator $\hat{\Delta}_A=-\hat{\delta}^{CL}$.

Note that the null hypothesis H_0 for the bioequivalence test can expressed as a union of the two one-sided hypotheses; namely,

$$H_{01}:\{\Delta_A\leq-0.2231\}\text{ and }H_{02}:\{\Delta_A\geq 02231\}.$$

The alternative hypothesis is then an intersection of the two hypotheses as follows:

$$H_{A1}:\{\Delta_A > -0.2231\} \text{ and } H_{A2}:\{\Delta_A < 02231\}.$$

Let

$$W_1 = \left(\hat{\Delta}_A + 0.2231\right)/se\left(\hat{\Delta}_A\right) \text{ and } W_2 = \left(\hat{\Delta}_A - 0.2231\right)/se\left(\hat{\Delta}_A\right),$$

where $se\left(\hat{\Delta}_A\right) = se\left(\hat{\delta}^{CL}\right)$ is the estimated standard error of $\hat{\Delta}_A$. Therefore, under the significance level α, a Wald-type test rejects the H_{01} if $W_1 \geqslant z_\alpha$, while H_{02} is rejected when $W_2 \leqslant -z_\alpha$, where z_α is the upper αth percentile of a standard normal distribution. An intersection-union test then concludes, under the significance level α, that two drugs are bioequivalent if the two one-sided hypotheses are both rejected [28, 29]. Moreover, suppose that the observed values of W_1 and W_2 are w_1 and w_2, respectively. The theory of intersection-union tests implies that The p-value of the two one-sided test is then given by $\max\{1-\Phi(w_1), \Phi(w_2)\}$ [29].

In fact, we can construct an approximate $(1-2\alpha)\times 100\%$ confidence interval for Δ_A; namely, $\left(\hat{\Delta}_A - z_\alpha se\left(\hat{\Delta}_A\right), \hat{\Delta}_A + z_\alpha se\left(\hat{\Delta}_A\right)\right)$. If the $(1-2\alpha)\times 100\%$ confidence interval is within (-0.2231, 0.2231), then the bioequivalence of the two drugs is concluded under the significance level α [29].

Note that the standard bioequivalence test recommended by the US FDA [2] and the EMEA [3] is based on lognormally distributed individual AUC estimated from each drug concentration-time profile, but without assuming any PK model for the profile. Let AUC_{Rij} and AUC_{Tij} be the estimated individual AUC for subject j in sequence i receiving reference and test drugs, respectively, $j = 1, \cdots, n_i$, $i = 1, 2$. Set

$$D = \left(\sum_{i=1}^{2}\sum_{j=1}^{n_i} \log AUC_{Tij} - \log AUC_{Rij}\right) \Big/ (n_1 + n_2)$$

and

$$\text{var}(D) = \frac{(n_1+n_2)\sum_{i=1}^{2}\sum_{j=1}^{n_i}\left[\log AUC_{Tij} - \log AUC_{Rij} - \left(\overline{\log AUC_{Ti\cdot}} - \overline{\log AUC_{Ri\cdot}}\right)\right]^2}{4n_1n_2(n_1+n_2-2)}.$$

Then $T_1 = (D + 0.2231)/\sqrt{\text{var}(D)}$ and $T_2 = (D - 0.2231)/\sqrt{\text{var}(D)}$ are the test statistics for H_{01} and H_{02}, respectively. Under the significance level α, H_{01} is rejected when $T_1 \geqslant t_\alpha(n_1+n_2-2)$, and H_{02} is rejected when $T_2 \leqslant -t_\alpha(n_1+n_2-2)$, where $t_\alpha(\text{DOF})$ is the upper αth percentile of a Student's t-distribution with DOF. Because the standard bioequivalence test is free of any PK model, the test is, hereafter, referred to as a non-compartmental analysis (NCA)-based test.

4. A simulation study

4.1. Design of the simulation study

We conducted a Monte Carlo study to investigate the level and power performances of the proposed MGGMEM-based test, denoted by MGG, relative to the NCA-based test, denoted by NCA, and NLMEM-based test, denoted by Norm, for the bioequivalence of two drugs with respect to AUC. In the simulation study, we consider the situation where the two drugs are administered to $n = 16$ or 24 volunteers in a 2x2 crossover design, and the drug concentrations are measured at (i) 0.5, 2, 4, 6, 10, 14 h ($m = 6$) or (ii) 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 14 h ($m = 14$) after the drug administration.

In the simulation study, for simplicity, we consider the one-compartment PK model (1) without time lag ($Tlag = 0$), period effect or sequence effect for the drug concentrations. Taking a reference from the data analysis in Section 5, we generate data with $\left(\mu_0^a, \mu_0^{CL}, \mu_0^V\right)' = (0.37, 1.43, 2.77)'$,

$$\Omega = \begin{pmatrix} 0.09 & 0 & 0 \\ 0 & 0.09 & 0.027 \\ 0 & 0.027 & 0.01 \end{pmatrix} \text{ and } \Psi = \begin{pmatrix} 0.25 & 0.015 & 0 \\ 0.015 & 0.01 & 0.003 \\ 0 & 0.003 & 0.0025 \end{pmatrix}.$$

The marginal generalized gamma distribution for the errors variables has mean 1 and shape parameter $\lambda = 0.60$, but the standard deviation can be 0.15 or 0.30. To study the level performances of the bioequivalence tests, we consider $\delta^{CL} = -0.2231$ or 0.2231. We also take $\delta^{a} = 0$ and $\delta^{V} = \delta^{CL}$ for a bioequivalence with respect to $C_{\max}$. To study the power performance, we then consider $\delta^{a} = 0$ and $-\delta^{CL} = -\delta^{V} = \log(0.9), \log(1.0), \log(1.1)$ or $\log(1.2)$.

To further study the effect of BSV or WSV on the level and power performances of the bioequivalence tests, we also conduct a simulation study where two different levels of BSV and WSV as suggested in Dubois *et al.* [20] are considered. In this simulation study, we only take into account $n = 16$, $m = 14$, and the standard deviation of the error variables as 0.3. In addition, two different BSV and WSV under study are

$$\Omega_L = \begin{pmatrix} 0.04 & 0 & 0 \\ 0 & 0.04 & 0 \\ 0 & 0 & 0.01 \end{pmatrix} \text{ and } \Psi_L = \begin{pmatrix} 0.01 & 0 & 0 \\ 0 & 0.01 & 0 \\ 0 & 0 & 0.0025 \end{pmatrix}$$

for low level variation, and

$$\Omega_H = \begin{pmatrix} 0.25 & 0 & 0 \\ 0 & 0.25 & 0 \\ 0 & 0 & 0.25 \end{pmatrix} \text{ and } \Psi_H = \begin{pmatrix} 0.0225 & 0 & 0 \\ 0 & 0.0225 & 0 \\ 0 & 0 & 0.0225 \end{pmatrix}$$

for high level variation.

In each of the settings under study, 500 replicates were used to obtain the level and power estimates under the significance level $\alpha = 0.05$. Therefore, the approximate standard deviation of the level estimate is 0.010 $\left(\approx \sqrt{0.05 \times 0.95/500}\right)$, and the maximum standard deviation of the power estimate is about 0.022 $\left(\approx \sqrt{0.5 \times 0.5/500}\right)$. The estimated levels and powers are then presented in Tables I, II, and III, respectively.

4.2. *Results of the simulation study*

The results in Table I show that both the MGGMEM-based and NCA-based tests are generally superior to the NLMEM-based test on maintaining the significance level. The type I error rate of the NCA-based test is well under control when $m = 14$, but it tends to be inflated when $m = 6$, especially, when $\Delta_A = \log(1.25)$ with $n = 16$. Moreover, the type I error rate of the NLMEM-based test is far beyond

Table I. Estimated level for drug concentrations measured from n individuals at m time points under $\alpha = 0.05$.

			(i) $m = 6$						(ii) $m = 14$					
			sd(ε) = 0.15			sd(ε) = 0.30			sd(ε) = 0.15			sd(ε) = 0.30		
n	Δ_A	ρ	NCA	MGG	Norm	NCA	MGG	Norm	NCA	MGG	Norm	NCA	MGG	Norm
16	log(0.8)	0	6.4	6.4	15.6	4.2	6.8	21.2	5.2	4.8	11.4	6.2	6.2	16.2
		0.2	6.2	5.2	15.0	4.2	6.8	24.2	6.4	4.2	10.6	5.6	4.6	12.4
		0.5	5.8	6.2	13.8	4.4	4.6	22.6	5.6	4.8	9.6	5.4	5.6	9.8
		0.8	4.8	7.0	12.2	4.6	6.0	16.2	5.6	4.4	7.8	5.6	4.2	6.8
	log(1.25)	0	7.4	6.6	16.4	7.0	4.8	23.8	5.6	5.4	11.8	5.6	6.2	17.6
		0.2	7.4	6.6	17.3	6.8	5.8	26.2	6.0	6.4	11.4	6.0	4.2	14.6
		0.5	7.4	4.2	17.4	7.2	6.8	27.4	5.8	4.4	8.8	4.4	4.0	8.8
		0.8	7.4	6.7	13.8	7.2	5.4	16.0	5.0	4.8	8.2	5.0	3.8	7.0
24	log(0.8)	0	5.2	4.6	13.6	6.0	4.8	18.2	6.2	3.6	8.4	5.4	5.2	11.6
		0.2	5.0	4.6	15.6	5.6	6.6	18.2	5.4	4.4	7.4	5.2	5.6	10.2
		0.5	5.4	5.8	11.6	4.8	6.0	19.0	5.2	5.2	6.2	4.8	6.4	7.2
		0.8	5.8	5.0	9.0	5.8	6.7	12.6	5.6	5.8	6.0	4.8	4.8	5.2
	log(1.25)	0	4.2	4.2	12.6	3.6	5.6	21.6	4.6	4.6	9.8	5.0	6.2	15.4
		0.2	4.2	4.6	12.2	3.4	6.6	21.2	4.8	4.4	8.0	5.6	6.0	14.6
		0.5	3.8	5.0	11.8	3.4	6.4	15.1	4.8	4.8	8.2	5.8	4.0	10.4
		0.8	3.6	5.8	9.2	3.0	3.8	9.8	5.8	5.8	6.8	6.0	3.6	7.6

Table II. Estimated power for drug concentrations measured from n individuals at m time points under $\alpha = 0.05$.

			(i) $m = 6$						(ii) $m = 14$					
			sd(ε) = 0.15			sd(ε) = 0.30			sd(ε) = 0.15			sd(ε) = 0.30		
n	Δ_A	ρ	NCA	MGG	Norm	NCA	MGG	Norm	NCA	MGG	Norm	NCA	MGG	Norm
16	log(0.9)	0	83.4	88.0	90.2	57.2	75.6	78.4	91.8	92.8	93.2	80.0	86.0	85.8
		0.2	82.8	84.0	90.6	55.0	80.6	77.6	88.0	90.8	91.2	67.4	82.6	77.2
		0.5	79.0	83.2	88.0	46.2	75.4	63.0	82.4	87.8	81.6	51.2	78.2	57.4
		0.8	67.6	80.8	75.6	30.0	69.4	52.6	68.4	83.0	72.4	31.2	67.4	38.4
	log(1.0)	0	100	97.6	99.8	91.2	92.2	96.0	100	99.8	99.6	99.8	99.0	99.2
		0.2	100	97.0	99.0	89.8	92.2	95.2	99.8	99.2	99.8	98.0	98.2	98.8
		0.5	100	98.0	99.6	79.2	91.0	75.6	99.8	99.0	97.6	87.0	93.6	86.0
		0.8	97.8	95.4	99.0	52.8	83.8	74.0	98.4	95.4	97.8	58.6	86.6	66.6
	log(1.1)	0	88.8	86.6	93.0	63.6	83.4	82.7	95.0	95.4	96.0	85.2	90.6	90.6
		0.2	87.8	89.2	93.8	62.2	80.8	82.8	92.2	94.0	94.2	76.6	88.2	82.6
		0.5	83.6	87.2	89.6	53.4	81.2	60.8	87.2	91.2	88.0	57.2	84.2	64.8
		0.8	74.4	84.4	81.4	32.8	64.8	58.8	77.6	84.0	80.4	35.4	72.4	43.4
	log(1.2)	0	25.8	52.2	44.6	18.0	52.0	46.2	29.6	51.6	44.4	22.4	42.6	42.6
		0.2	25.6	53.6	45.6	17.8	55.6	44.8	27.4	53.0	35.2	18.6	50.8	37.0
		0.5	24.0	54.8	44.0	15.4	46.0	45.0	23.0	52.4	31.0	14.6	51.2	23.6
		0.8	20.0	51.4	33.0	10.8	38.6	25.2	19.6	56.2	23.8	10.8	45.4	16.0
24	log(0.9)	0	95.0	94.9	96.8	77.8	89.6	87.4	99.4	98.0	98.0	93.6	93.6	92.8
		0.2	94.8	94.2	96.4	75.2	88.2	86.0	97.0	96.0	97.6	82.8	91.6	88.2
		0.5	93.4	91.9	96.0	64.0	84.6	84.8	94.0	93.8	93.6	67.6	85.6	74.6
		0.8	85.8	90.5	90.8	46.2	78.9	62.0	85.0	92.6	86.4	46.4	80.6	53.6
	log(1.0)	0	100	99.8	100	99.4	98.8	97.4	100	100	99.8	100	99.4	99.8
		0.2	100	99.7	99.4	99.0	98.2	97.4	100	99.8	100	99.8	99.4	99.2
		0.5	100	99.1	99.8	96.8	98.1	96.8	100	99.2	99.8	97.8	98.8	97.6
		0.8	100	99.1	99.8	78.6	92.7	92.0	100	99.6	99.7	82.8	93.8	85.0
	log(1.1)	0	98.2	95.9	97.4	79.0	91.7	89.2	100	99.2	99.6	96.0	96.2	96.6
		0.2	98.2	96.0	97.4	78.0	89.4	85.6	99.0	97.8	98.6	88.8	92.4	92.2
		0.5	96.0	94.6	96.8	70.4	85.8	84.8	97.0	95.2	97.2	75.6	87.6	81.4
		0.8	87.4	91.9	91.2	49.6	80.8	64.8	89.8	94.6	90.4	56.6	83.4	62.0
	log(1.2)	0	33.8	53.6	50.8	19.2	54.4	47.0	40.6	49.2	47.6	31.0	46.0	46.6
		0.2	33.2	52.2	50.4	19.0	49.8	49.2	36.6	51.0	42.0	23.4	49.6	38.4
		0.5	31.4	54.0	46.2	16.2	53.2	46.0	30.6	55.8	38.0	17.6	51.8	24.6
		0.8	23.0	53.6	31.8	13.2	50.4	24.8	24.6	57.2	27.8	14.4	53.6	17.6

Table III. Estimated level and power of bioequivalence tests for different between-subject and within-subject variability with n=16 and m=14 under $\alpha = 0.05$.

		Δ_A					
Situation	Test	log(0.8)	log(0.9)	log(1.0)	log(1.1)	log(1.2)	log(1.25)
(Ω_L, Ψ_L)	NCA	3.4	31.6	60.0	43.0	17.2	4.0
	MGG	4.2	87.0	97.6	91.0	63.0	6.6
	Norm	8.4	44.6	72.4	53.0	29.2	11.4
(Ω_H, Ψ_L)	NCA	3.4	31.4	57.6	45.6	18.6	4.2
	MGG	3.8	71.0	84.2	72.4	52.8	4.4
	Norm	14.0	49.0	70.0	45.2	28.0	13.8
(Ω_L, Ψ_H)	NCA	3.6	23.0	51.0	36.0	14.0	4.0
	MGG	4.6	73.0	85.0	77.0	56.6	6.0
	Norm	7.8	36.0	67.0	40.0	25.4	12.0
(Ω_H, Ψ_H)	NCA	3.4	23.8	51.0	39.0	16.0	4.0
	MGG	5.2	70.4	72.4	66.0	51.2	5.6
	Norm	14.6	36.0	60.0	19.8	24.4	16.4

the significance level under all the situations under study, in particular, when the number of subjects is small, blood samples are taken less frequently or the error variable suffers a large variation.

The results in Table II indicate that the power of each test is higher for more frequently sampling of blood from the subjects. This is not surprising because, for more available data, the *AUC* associated with each test under study can be more accurately estimated from the drug concentration-time profiles. Note that the type I error rate of the NLMEM-based test is beyond the significance level, and hence, its power is overestimated. However, the power of the MGGMEM-based test is even higher than that of the NLMEM-based test, especially, when the highly correlated drug concentrations are subject to a large variation. Finally, under the mixed-effects model considered in the simulation study, the individual *AUC* may not be lognormally distributed and is possibly more variable, especially when the individual drug concentration-time profile is subject to a larger variation or a stronger correlation. Therefore, the MGGMEM-based test is more powerful than the NCA-based test when the standard deviation of the error variables is larger, or the correlation associated with the drug concentrations is stronger. Note that the individual *AUC* is estimated based on each drug concentration-time profile, and hence, the estimated *AUC* would be less accurate if the blood samples are less frequently taken from the subjects under study. Therefore, in this case, the MGGMEM-based test is superior to the NCA-based test on the power performance.

The results in Table III show that, again, both the MGGMEM-based and NCA-based tests are superior to the NLMEM-based test on maintaining the significance level when the random effects are subject to different BSV or WSV in the PK compartmental model. The power of the NLMEM-based test is even lower than that of the MGGMEM-based test, though the type I error rate of the NLMEM-based test is far beyond the specified significance level. In fact, the power performance of the NCA-based test is robust to the BSV or WSV because the test does not depend on the PK compartmental model. Note that the power of MGGMEM-based test tends to be lower with higher BSV or WSV. Moreover, the effect of BSV is more profound than that of WSV on the power performance of the MGGMEM-based test.

5. Data analysis

5.1. Data for benzbromarone drug

We illustrate the use of MGGMEM for testing against the bioequivalence between two brands of benzbromarone tablets in a 2x2 crossover design, where the test drug, Euricon, and the reference drug, Urinorm, are manufactured by two different pharmaceutical companies, respectively. Note that the benzbromarone, a well-known uricosuric agent, reduces serum uric acid concentrations probably by blocking tubular reabsorption. In the 2x2 crossover study, 16 healthy adult volunteers were randomly allocated to two sequences. In sequence 1, eight volunteers were orally administered with one tablet of 50 mg of Urinorm and then, after 1 week, one tablet of 50 mg of Euricon. On the other hand, the other eight volunteers in sequence 2 receive the two drugs in reverse order in the two periods. The blood samples were taken, and the benzbromarone concentration was measured 14 times at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 14 h after the drug administration. The drug concentrations-time profiles are displayed in Figure 1.

For the analysis based on MGGMEM, we employ model (3) to describe the drug concentrations-time profiles. The parameters in model (3) were estimated by using the SAEM with

$$v_r = \begin{cases} 1, & 1 \leqslant r \leqslant 300 \\ \dfrac{1}{r-300}, & 301 \leqslant r \leqslant 500 \end{cases}.$$

Note that, according to a data analysis, the MGGMEM gives smaller values of the estimated -2log-likelihood and BIC [30] than those produced by the NLMEM. Therefore, the MGGMEM is better than the NLMEM for fitting into the data set under investigation. In fact, the MGGMEM estimates the correlation measure ρ as 0.824 with a standard error 0.022, which indicates the repeatedly measured drug concentrations are significantly correlated. For the fixed effects in the MGGMEM, the mean of $\log k_a$ and that of *Tlag* can be regarded as zero. Moreover, there is no period or sequence effect on *CL* or V. However, different drugs may result into different means of *CL* and V. The random effects in the MGGMEM further suggest that the BSV exit only for *CL* and V, while the WSV appear in all the four PK parameter-variables. Finally, the MGGMEM gives $\hat{\Delta}_A = -0.045$ (or $AUC_T/AUC_R = 0.956$) with $se\left(\hat{\Delta}_A\right) = 0.007$.

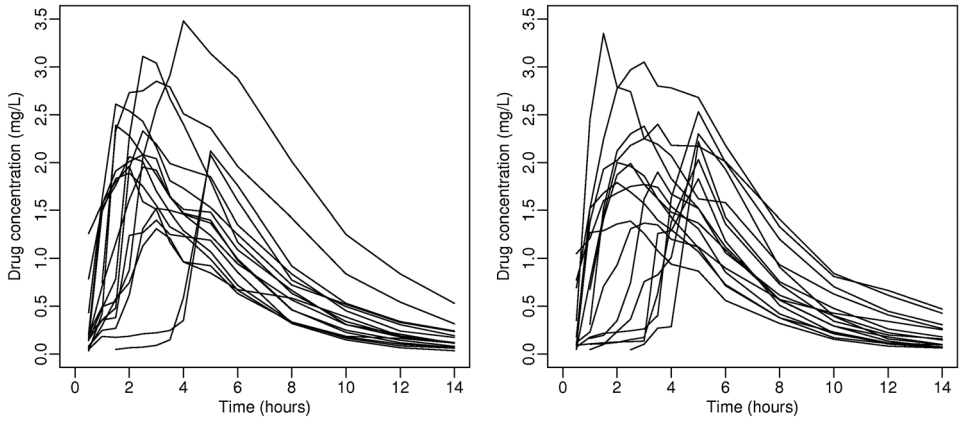

Figure 1. The benzbromarone concentration-time profiles for Urinorm (left) and Euricon (right).

Table IV. Bioequivalence test for two data sets.

	NCA	NLMEM	MGGMEM
	benzbromarone data		
$\hat{\Delta}_A$	-0.030	-0.032	-0.045
se $\left(\hat{\Delta}_A\right)$	0.051	0.027	0.007
p-value	0.001	7.24×10^{-13}	$< 1.0 \times 10^{-20}$
90% CI for Δ_A	(-0.119, 0.060)	(-0.076, 0.012)	(-0.057, -0.033)
	ciprofloxacin data		
$\hat{\Delta}_A$	0.141	0.153	0.142
se $\left(\hat{\Delta}_A\right)$	0.048	0.054	0.040
p-value	0.062	0.097	0.022
90% CI for Δ_A	(0.052, 0.230)	(0.066, 0.240)	(0.076, 0.208)

The summary statistics for the bioequivalence tests based on NCA, NLMEM, and MGGMEM, respectively, are presented in Table IV. The p-values indicate that the Euricon and Urinorm are bioequivalent based on the NCA-based, NLMEM-based or MGGMEM-based test. Nevertheless, the MGGMEM-based test gives the smallest p-value and hence provides with the most significant evidence for concluding the bioequivalence of the two drugs.

5.2. *Data for ciprofloxacin drug*

A comparative PK study of two ciprofloxacin conventional formulations was carried out on ten healthy male volunteers in a 2x2 crossover design [31]. In sequence 1, five volunteers were orally administered with one tablet of 500 mg of Cipro M.E. and then, after 1 week, one tablet of 500 mg of Cipro Teva. The other five volunteers in sequence 2 received the two drugs in reverse order in two periods. The blood samples were taken and the ciprofloxacin concentrations were measured $m = 10$ times at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 from the antecubital vein. The 10 ciprofloxacin concentration-time profiles are displayed in Figure 2.

For the analysis based on MGGMEM or NLMEM, we consider the mean concentrations follow the one-compartment model with zero-order absorption as in (4). In the SAEM algorithm, we employed, again, the sequence of $\{v_r\}_{r\geq 0}$ used for analyzing the benzbromarone concentrations. Note that the values of the estimated -2log-likehood and BIC given by MGGMEM are smaller than those produced by the NLMEM. Therefore, the MGGMEM is, again, better than the NLMEM for describing the ciprofloxacin concentrations under study. A data analysis under the MGGMEM estimates the correlation measure ρ as 0.355 with a standard error 0.089. Moreover, the drug, period, or sequence effect does not exit on CL, T_a or V. However, the random effect of WSV appears for T_a and V, whereas the random effect

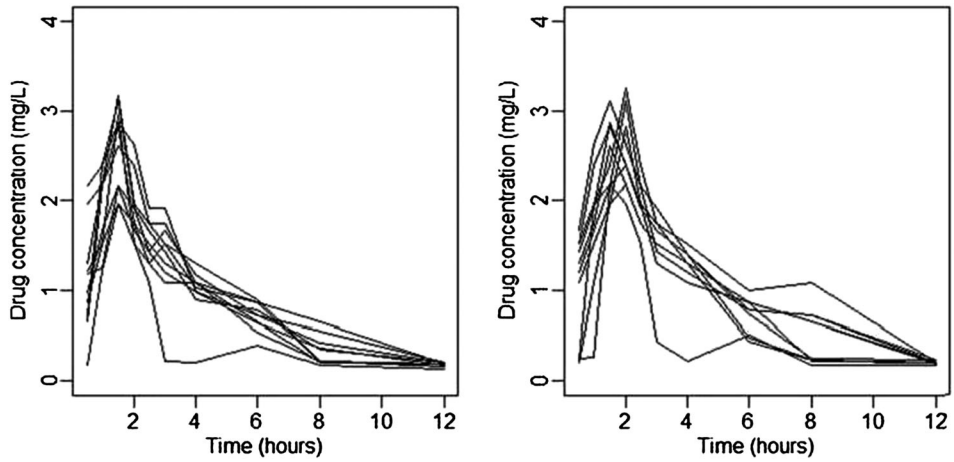

Figure 2. The ciprofloxacin concentration-time profiles for Cipro M.E. (left) and Cipro Teva (right).

of BSV is not significant. Finally, the MGGMEM gives $\hat{\Delta}_A = 0.142$ (or $AUC_T/AUC_R = 1.153$) with $se\left(\hat{\Delta}_A\right) = 0.040$.

The summary statistics for the bioequivalence tests based on NCA, NLMEM, and MGGMEM, respectively, are also presented in Table IV. The associated p-values show that, under the significance level 0.05, both the NCA-based and NLMEM-based tests do not conclude the bioequivalence between the Cipro M.E and Cipro Teva. However, the proposed MGGMEM-based bioequivalence test provides a significant evidence for the bioequivalence of the two drugs.

To investigate the power performances of the three bioequivalence tests particularly for the data set, we conducted a simulation study based on 500 data sets each generated from the estimated MGGMEM. We obtain the power estimates as 0.228, 0.452, and 0.612 for the NCA-based, NLMEM-based, and MGGMEM-based bioequivalence tests, respectively. Therefore, we expect that the MGGMEM-based bioequivalence test is the most powerful one among the three for the data from the current estimated MGGMEM. In fact, the results of the data analysis are in a good agreement with the simulation result.

6. Discussions and conclusions

In this paper, we discuss the use of a mixed-effect model to describe the drug concentration-time profiles, which are distributed according to a multivariate generalized gamma distribution, obtained in PK study under a 2x2 crossover design. Note that, in the PK study, the repeatedly measured drug concentrations are usually right-skewed and correlated. Moreover, the subjects involved in the study or the potential drug consumers may be of great variety. Therefore, the proposed mixed-effects model, MGGMEM, would be more appropriate for the drug concentration-time profiles in a 2x2 crossover design. Based on the proposed model, MGGMEM, we then suggest an approximate two one-sided test for testing against the bioequivalence of the two drugs. The simulation results presented in Section 4 show that the MGGMEM-based bioequivalence test maintains well its significance level and has a better power performance than the non-compartmental test conventionally used by the drug company as suggested by US FDA [2] or EMEA [3] especially when the estimated individual AUC fails the assumption of lognormal distribution. Therefore, using the MGGMEM-based bioequivalence test not only protects the drug consumers from taking the non-qualified generic drug but also helps the drug company get an approval of the test drug as a generic drug. In this way, the potential patients would then have a better chance to benefit from the generic drug in terms of economic and health care.

Note that the SAEM algorithm gives a sequence of parameter estimates that converge almost surely to the values that maximize the log-likelihood [26]. In the estimation of the parameters in the MGGMEM, however, we need to input some initial values when applying the SAEM algorithm. According to our computating experience from the data analysis and simulation study, the fixed-effects model [7] is recommended for finding the scale and shape parameters of the marginal generalized gamma distribution. These estimated parameters can then be of great help to provide with reasonable initial values so that the necessary computing time will be shorten.

Acknowledgement

This study is supported by the National Science Council of Taiwan (NSC97-2118-M-008-003-MY2 and NSC101-2118-M-008-004-MY2).

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