Journal of Biopharmaceutical Statistics

ISSN: 1054-3406 (Print) 1520-5711 (Online) Journal homepage: www.tandfonline.com/journals/lbps20

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To cite this article: Meiyu Shen & Stella G. Machado (2017) Bioequivalence evaluation of sparse sampling pharmacokinetics data using bootstrap resampling method, Journal of Biopharmaceutical Statistics, 27:2, 257-264, DOI: 10.1080/10543406.2016.1265543

To link to this article: https://doi.org/10.1080/10543406.2016.1265543

Published online: 11 Jan 2017.

JOURNAL OF BIOPHARMACEUTICAL STATISTICS
2017, VOL. 27, NO. 2, 257–264
http://dx.doi.org/10.1080/10543406.2016.1265543

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Bioequivalence evaluation of sparse sampling pharmacokinetics data using bootstrap resampling method

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ABSTRACT

Bioequivalence studies are an essential part of the evaluation of generic drugs. The most common in vivo bioequivalence study design is the two-period two-treatment crossover design. The observed drug concentration–time profile for each subject from each treatment under each sequence can be obtained. *AUC* (the area under the concentration–time curve) and C_{max} (the maximum concentration) are obtained from the observed drug concentration–time profiles for each subject from each treatment under each sequence. However, such a drug concentration–time profile for each subject from each treatment under each sequence cannot possibly be available during the development of generic ophthalmic products since there is only one-time point measured drug concentration of aqueous humor for each eye. Instead, many subjects will be assigned to each of several prespecified sampling times. Then, the mean concentration at each sampling time can be obtained by the simple average of these subjects' observed concentration. One profile of the mean concentration vs. time can be obtained for one product (either the test or the reference product). One *AUC* value for one product can be calculated from the mean concentration–time profile using trapezoidal rules. This article develops a novel nonparametric method for obtaining the 90% confidence interval for the ratio of AUC_T and AUC_R (or $C_{T,max}/C_{R,max}$) in crossover studies by bootstrapping subjects at each time point with replacement or bootstrapping subjects at all sampling time points with replacement. Here *T* represents the test product, and *R* represents the reference product. It also develops a novel nonparametric method for estimating the standard errors (SEs) of AUC_h and $C_{h,max}$ in parallel studies by bootstrapping subjects treated by the *h*th product at each time point with replacement or bootstrapping subjects treated by the *h*th product at all sampling time points with replacement, $h = T, R$. Then, 90% confidence intervals for AUC_T/AUC_R and $C_{T,max}/C_{R,max}$ are obtained from the nonparametric bootstrap resampling samples and are used for the evaluation of bioequivalence study for one-time sparse sampling data.

ARTICLE HISTORY
Received 7 July 2016
Accepted 16 October 2016

KEY WORDS
Bioequivalence; bootstrap; ophthalmic solution; sparse data

1. Introduction

Bioequivalence studies are an essential part of the evaluation of generic drugs. The most common in vivo bioequivalence study design is the two-period two-treatment crossover design. The observed drug concentration–time profile for each subject from each treatment under each sequence can be obtained. *AUC* (the area under the concentration–time curve) and C_{max} (the maximum

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This article reflects the views of the authors and should not be construed to be those of the US Food and Drug Administration. This work was done by the authors during the time when Dr. Machado was a former employee at Center for Drug Evaluation and Research, U.S. Food and Drug Administration prior to her retirement in 2013.

concentration) are obtained from the observed drug concentration–time profiles for each subject from each treatment under each sequence. However, such a drug concentration–time profile for each subject from each treatment under each sequence cannot possibly be available during the development of generic ophthalmic products since there is only one-time point measured drug concentration of aqueous humor for each eye. Instead, many subjects will be assigned to each of several prespecified sampling times. Then, the mean concentration at each sampling time can be obtained by the simple average of these subjects' observed concentration. One profile of the mean concentration vs. time can be obtained for one product (either the test or the reference product). One AUC value for one product can be calculated from the mean concentration–time profile using trapezoidal rules. The following provides the detail how to calculate one AUC value for each product.

Let C_{hij} be the hth product concentration of the jth subject at the ith sampling time point, $h = T, R$, $I = 1,..,k_h$, and $j = 1,\ldots,n_{hi}$. Here k_h is the number of sampling time points for the hth product and n_{hi} is the number of subjects in the hth product at the ith sampling time point. Let $\bar{C}_{hi}$ be the mean concentration of the hth product at the ith sampling time point. Thus, $\bar{C}_{hi} = \sum_{j=n_{hi}} C_{hij}/n_{hi}$. The mean profile of drug concentration–time of the hth product is reconstructed by $(t_i, \bar{C}_{hi})$, $i = 1,.., k_h$. Here t_i is the time at the ith sampling time point. From this mean profile, AUC of the hth product is calculated by:

$$AUC_{h,0\to t_i} = t_1 * \bar{C}_{h1}/2 + \sum_{i=1}^{k_h-1} (\bar{C}_{hi} + \bar{C}_{h,i+1}) \cdot (t_{i+1} - t_i)/2 \tag{1}$$

$C_{h,max}$ is also obtained from the mean profile by:

$$C_{h,\max} = \max_{i\in(1,..,k_h)} (\bar{C}_{hi}). \tag{2}$$

Generally speaking, parallel studies and crossover studies are two popular study designs used for pharmacokinetic (PK) bioequivalence evaluation in the development of ophthalmic products.

In parallel studies, each subject contributes one eye with cataract. For a fixed sampling time point, the test and reference products are randomly assigned to each subject with an equal probability. One aqueous humor concentration per subject is obtained since only one eye per subject receives either the test product or the reference product and each subject's aqueous humor from the dosed eye is extracted only once at a prespecified sampling time point. We can calculate the sample mean of drug concentrations over many subjects for each of two products at each time point. In other words, we can derive one drug concentration–time profile for each of two products. From the mean drug concentration–time profile, one value of AUC_h (or $C_{h,max}$) can be obtained for the hth product, $h = T, R$. Obviously, AUC_T is independent of AUC_R. At each sampling time point, the observed concentration for each subject is assumed to be a random normal variable. Since multiple subjects contribute one value per subject at a given sampling time point, the sample mean and sample variance can be obtained. Then, the variability of AUC_h can be obtained for the hth product, $h = T, R$.

In the crossover studies, each subject with bilateral cataracts is randomly assigned one of two treatments (the test and reference products) to one of two eyes (the left and right eyes). If the test product is randomly assigned to the left eye, then the reference product is assigned to the right eye and vice versa. A single sample of aqueous humor is collected from each eye at the same assigned sampling time. Hence, a pair of aqueous humor concentrations per subject is obtained at a prespecified sampling time point since each subject contributes one concentration value per eye (if one eye receives the test product, the other eye receives the reference product, and vice versa.). Assuming one eye of each subject contributes one replicated measurement at the assigned sampling time point for one product, we can calculate the sample mean of drug concentrations over many subjects for each of two products at each time point. In other words, we can derive one drug

concentration–time profile for each of two products. From the mean drug concentration–time profile, one value of AUC_h (or $C_{h,max}$) can be obtained for each product. Note that AUC_T is not independent of AUC_R since each subject contributes a pair of concentrations at one sampling time point.

Bailer (1988) used Equation (1) for estimating the AUC of the mean drug concentration–time profile. Since AUC in Equation (1) is a linear combination of the sample means at various sampling time points, then Bailer estimated the variance of estimator for AUC as a linear combination of sample variances at various sampling time points since the mean concentration at one sampling time point is independent of the mean concentration at another sampling time point. Takemoto et al. (2006) expanded Bailer's algorithm to PK metrics, e.g., mean residence time (MRT_h), total clearance (CL_h), and volume of distribution at steady state ($V_{ss,h}$) for calculating the estimated mean and estimated variance of these parameters and compared the mean and standard deviation by Bailer's algorithm and by the bootstrap method for one-point sampling data. The procedures based on the bootstrap method for one-point sampling data of the hth product in (Takemoto et al., 2006) are as follows:

(1) Construction of time course of blood level or tissue concentrations, which consist of three or four points at each time, from data obtained by one-point sampling in animal experiments (one point at each time is collected from one animal).
(2) Selection of one point from three or four points at each time, permitting replacement using random number, and construction of pseudo-profile (Mager and Göller, 1995).
(3) Calculation of PK metrics (e.g., AUC_h, MRT_h, CL_h, $V_{ss,h}$) from time course obtained in Step 2.
(4) Construction of the histograms and calculation of moment characteristics (e.g., mean, SD) of AUC_h, MRT_h, CL_h, and $V_{ss,h}$, respectively, with resampling of bootstrap number (B) times.
(5) Assessment of histograms, including normal distribution and log-normal distribution.
(6) Comparison of AUC_h, MRT_h, CL_h, and $V_{ss,h}$, respectively, between two animal groups depending on the type of statistical distribution.

Bailer's method (1988) can be used for statistical evaluation of AUC_h for the hth product in parallel bioequivalence studies. The pseudo-profile from bootstrap method (Takemoto et al., 2006) may not resemble the mean profile due to subject to subject variability. It is difficult to determine an appropriate $C_{h,max}$ from the pseudo-profile of the hth product from the bootstrap method (Takemoto et al., 2006). In addition, either method (Bailer, 1988; Takemoto et al., 2006) is not suitable to the crossover design for the PK bioequivalence study used in the development of ophthalmic products since the concentrations of two products come from the same subject. Furthermore, none of available method discusses how to obtain the standard deviation for $C_{h,max}$, $h = T, R$.

This article develops a novel nonparametric method for obtaining the 90% confidence interval for the ratio of AUC_T and AUC_R (or $C_{T,max}/C_{R,max}$) in crossover studies by bootstrapping subjects at each time point with replacement or bootstrapping subjects at all sampling time points with replacement. Here T represents the test product and R represents the reference product. It also develops a novel nonparametric method for estimating the SEs of AUC_h and $C_{h,max}$ in parallel studies by bootstrapping subjects treated by the hth product at each time point with replacement or bootstrapping subjects treated by the hth product at all sampling time points with replacement, $h = T, R$. In Section 2, we describe the two one-sided tests for univariate bioequivalence testing. In Section 3, we describe the proposed bootstrap method for obtaining the variability of AUC and the variability of C_{max} for parallel studies and crossover studies. In Section 4, we present a real case study (Shen, 2008).

2. Two one-sided tests for univariate bioequivalence testing

We denote by $\mu_T$and μ_R the mean of the statistic used (AUC or C_{max}), respectively, for the test product and reference product investigated.

In order to conclude the bioequivalence of the test product and the reference product, we should reject the null hypothesis in the following hypothesis tests (U.S. Food and Drug Administration, 2001):

$$\begin{array}{l} H_0 : \mu_T/\mu_R \leq \theta_1 \text{ or } \mu_T/\mu_R \geq \theta_2 \\ H_a : \theta_1 < \mu_T/\mu_R < \theta_2 \end{array}. \tag{3}$$

Here θ_1 and θ_2 are prespecified constants, also called equivalence margins and $\theta_1 < \theta_2$. Equivalence margins $\theta_1 = 0.8$ and $\theta_2 = 1.25$ are recommended in (U.S. Food and Drug Administration, 1992), the FDA Guidance for Industry.

The null hypothesis, H_0, states that μ_T and μ_R are not equivalent. The alternative hypothesis, H_a, representing equivalence is the intersection of the two one-sided parameter regions, $\{\theta_1 < \mu_T/\mu_R\}$ and $\{\mu_T/\mu_R < \theta_2\}$.

3. Statistical methods

In crossover studies, log(AUC) and log(C_{max}) can be derived for each subject under each treatment in each sequence. In parallel studies, log(AUC) and log(C_{max}) can be derived for each subject under each treatment. For these rich sampling designs, the 1992 guidance recommended logarithmic transformation of PK data (AUC and C_{max}). 90% confidence interval for the mean difference in log(AUC) between the test and the reference can be obtained from log-transformed data. Thus, 90% confidence interval for μ_T/μ_R can be antilog transformation of 90% confidence interval calculated from log(AUC) data.

In a situation such as one-time point measured drug concentration of aqueous humor for each eye during development of generic ophthalmic products, the distribution of AUC (C_{max}) is unknown since there is one value obtained from the mean profile of each product. However, Bailer (1988) assumed that the concentrations at each time point follow a normal distribution in order to calculate the variability of AUC from the variability of mean concentration at each time point and further to calculate 90% confidence interval for the ratio of true AUC of the test vs. true AUC of the reference . Bailer's assumption (1988) is different from the normality assumption for log(AUC).

To obtain the 90% confidence interval for $\mu_T/\mu_R(AUC)$ in crossover studies without assuming the normality of observed concentrations at each time point, we develop the following novel nonparametric bootstrap method for estimating the SEs of AUC_h of the hth product, $h = T, R$. The correlation of two observations from the same subject is taken care of by bootstrapping subjects who has two treatments:

(1) Bootstrapping n_i subjects treated at the ith time point to select n_i, $i = 1,.. k$, with replacement or bootstrapping $\sum_{i=1}^{k} n_i$ subjects at all k sampling time points to select $\sum_{i=1}^{k} n_i$ with replacement repeatedly 10^5 times.
(2) For the jth bootstrap replicate for the hth product, we compute $AUC_{h,j}$ by Equation (1) and obtain $C_{h,max,j}$ by Equation (2), $h = T, R$.
(3) For the jth bootstrap replicate, we compute $AUC_{T,j}/AUC_{R,j}$ and $C_{T,max,j}/C_{R,max,j}$.
(4) The 5th percentile and 95th percentile of AUC_T /AUC_R from all bootstrap replicates comprise the 90% confidence interval for $\mu_T/\mu_R(AUC)$.

Similarly, we can obtain the 90% confidence interval for $\mu_T/\mu_R(C_{max})$ by the 5th percentile and 95th percentile of $C_{T,max}/C_{R,max}$ from all bootstrap replicates from the above steps 1 to 3.

To obtain the 90% confidence interval for $\mu_T/\mu_R(AUC)$ in parallel studies without assuming the normality of observed concentrations at each time point, we develop the following novel nonparametric bootstrap method for estimating the SEs of AUC_h of the hth product, $h = T, R$. For each treatment, we bootstrap subjects. Hence, two treatment groups in a bootstrap replicate are still independent:

(1) Bootstrapping n_{hi} subjects treated by the hth product at the ith time point to select n_{hi}, i =1,.. k_h, with replacement or bootstrapping $\sum_{i=1}^{k_h} n_{hi}$subjects at all sampling time points to select $\sum_{i=1}^{k_h} n_{hi}$with replacement repeatedly 10^5 times.
(2) For the jth bootstrap replicate for the hth product, we compute $AUC_{h,\,j}$ by Equation (1) and obtain $C_{h,max,j}$ by Equation (2), $h = T, R$.
(3) For the jth bootstrap replicate, we compute $AUC_{T,j}/AUC_{R,j}$ and $C_{T,max,j}/C_{R,max,j}$.
(4) The 5th percentile and 95th percentile of AUC_T/AUC_R from all bootstrap replicates comprise the 90% confidence interval for $\mu_T/\mu_R(AUC)$.

Similarly, we can obtain the 90% confidence interval for $\mu_T/\mu_R(C_{max})$ by the 5th percentile and 95th percentile of $C_{T,max}/C_{R,max}$ from all bootstrap replicates from the above steps 1 to 3.

4. A real case example

On February 13, 2009, Tobradex AF Suspension (Tobramycin 0.3%/dexamethasone 0.05% ophthalmic suspension) manufactured by Alcon Inc. was approved to be bioequivalent to the reference product Tobradex® ophthalmic suspension by the Center of Drug Evaluation and Research at Food and Drug Administration (CDER, 2009). The statistical evaluation of a double-masked, parallel group, randomized, single-dose bioequivalence study of Tobradex AF suspension and TOBRADEX ophthalmic suspension (Shen, 2008) provided the scientific evidence for this approval.

In this study, concentrations of dexamethasone in the aqueous humor of cataract surgery patients following a single topical ocular dose of the Tob 0.3%/Dex 0.05% formulation or TOBRADEX were measured. Aqueous humor samples were obtained using a sparse sampling scheme, whereby the time of sample collection will either be 0.5 h (±5 min), 1 h (±5 min), 2 h (±10 min), 3 h (±10 min), or 5 h (±20 min.) following a single preoperative dose of test article on the day of surgery.

Nine hundred eighty-seven male and female patients 18 years of age and older, of any race, who required cataract surgery, were enrolled to be able to collect PK data for at least 75 patients for each of the five post-dose time points per treatment. The per protocol (PP) population included all patients who received study medication, satisfied pre-randomization protocol inclusion/exclusion criteria that were relevant to the assessment of PK parameters, and had an aqueous humor sample collected within the protocol defined window for their assigned time and for whom adequate PK data were collected and available.

Starting here, we denote Tobradex AF Suspension by the Test product (A) and Tobradex® ophthalmic suspension by the reference product (B). The concentration data of PP population treated by A and B are displayed in Figure 1.

In the statistical analysis, BLQ (below the limit of quantitation) is replaced with one-half the limit of quantitation. To obtain the 90% confidence interval for AUC_{0-5}:

(1) First, bootstrap all 886 PP patients to select 886 with replacement repeatedly 5000 times.
(2) Second, for each bootstrap sample, compute AUC_{0-5} for the test product and the reference product, separately.

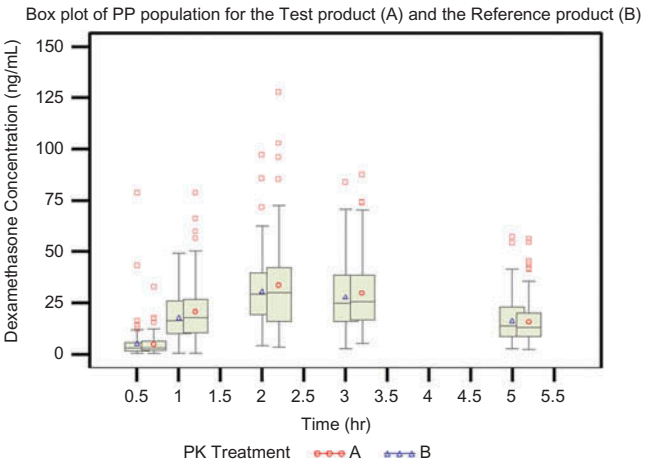

Figure 1. Boxplot of concentration vs. time for PP population: 0.2 is added to the variable time of the test product such that two boxplots can be displayed side by side.

(3) Third, for each bootstrap sample, compute the ratio of AUC_{0-5} for the test product over AUC_{0-5} for the reference product. The 5th percentile and 95th percentile of the ratio of AUC_{0-5} for the test over AUC_{0-5} for reference product comprise the 90% confidence interval.
(4) Fourth, 90% confidence interval for ratio of AUC_{0-5} for the test product and the reference product is obtained as just described in the third step. The results are listed in Table 1.

Similarly, 90% confidence intervals for ratio of AUC_{0-3}, AUC_{0-2}, and AUC_{0-1} for the test product and the reference product are obtained, respectively.

A refinement to the simple bootstrap method is to bootstrap the data using stratification by time point. This method is illustrated for PP with AUC_{0-5}. To estimate 90% confidence interval for PP for AUC_{0-3}, AUC_{0-2}, and AUC_{0-1}, we followed the same steps:

(1) First, bootstrap all PP patients at 0.5 h (say $n1$) to select $n1$ with replacement repeatedly 5000 times. We repeated this for other time points. Then, we combined these bootstrap samples at all time points.
(2) Second, for each bootstrap sample, compute AUC_{0-5} for the test product and the reference product, separately.
(3) Third, for each bootstrap sample, compute the ratio of AUC_{0-5} for the test product over AUC_{0-5} for the reference product. The 5th percentile and 95th percentile of the ratio of AUC_{0-5} for the test over AUC_{0-5} for reference product comprise the 90% confidence interval.
(4) Fourth, 90% confidence intervals for ratio of AUC_{0-5} for the test product and the reference product are obtained as just described in the third step. The results are listed in Table 2.

Table 1. The 90% confidence intervals for ratio of AUC_{0-5}, AUC_{0-3}, AUC_{0-2}, and AUC_{0-1} for the test product vs. the reference product using unstratified bootstrap method.

	Population	5% percentile	AUC_T/AUC_R	95% percentile
AUC_{0-5}	PP	0.983	1.069	1.159
AUC_{0-3}	PP	0.997	1.095	1.197
AUC_{0-2}	PP	0.995	1.110	1.235
AUC_{0-1}	PP	0.906	1.079	1.268

Table 2. The 90% confidence intervals for ratio of AUC_{0-5}, AUC_{0-3}, AUC_{0-2}, and AUC_{0-1} for the test product vs. the reference product using stratified bootstrap method.

	Population	5% percentile	AUC_T/AUC_R	95% percentile
AUC_{0-5}	PP	0.981	1.065	1.153
AUC_{0-3}	PP	1.000	1.095	1.194
AUC_{0-2}	PP	1.007	1.116	1.230
AUC_{0-1}	PP	0.905	1.090	1.282

Table 3. The 90% confidence intervals for ratio of AUC_{0-5}, AUC_{0-3}, AUC_{0-2}, and AUC_{0-1} for the test product vs. the reference product using Fieller's method.

Parameter	Population	5% percentile	AUC_T/AUC_R	95% percentile
AUC_{0-5}	PP	0.983	1.067	1.158
AUC_{0-3}	PP	0.995	1.092	1.197
AUC_{0-2}	PP	0.993	1.106	1.230
AUC_{0-1}	PP	0.901	1.069	1.274

Using the stratified bootstrap method, we got almost the same results as when we used the unstratified bootstrap method.

In order to check how well bootstrap methods work, we obtained the 90% confidence intervals for the ratio of AUC_{0-5}, AUC_{0-3}, AUC_{0-2}, and AUC_{0-1} for the test product vs. the reference product with the Fieller's method.

The method for estimating 90% confidence interval was illustrated with AUC_{0-5}. To estimate 90% confidence interval for AUC_{0-3}, AUC_{0-2}, and AUC_{0-1}, we repeated the following steps:

(1) First, compute AUC_{0-5} using formula (1) for the test and reference products.
(2) Second, compute the SE for each AUC_{0-5}.
(3) Third, use the Fieller's method to compute the 90% confidence interval for the ratio of the (AUC_{0-5}) of the test vs. (AUC_{0-5}) of the reference.

Clearly, 90% confidence interval obtained with a bootstrap method is similar to that obtained with the Fieller's method. The results are presented in Table 3.

In summary, the results support equivalence of the two products for AUC_{0-5}, AUC_{0-3}, and AUC_{0-2}, but not for AUC_{0-1}. Note that AUC_{0-1} does not represent the average exposure due to the short time interval in which the elimination has not finished yet.

5. Discussion and conclusion

The bootstrap method has been successfully applied to obtain the 90% confidence interval for AUC_T/AUC_R for sparse concentrations data in parallel studies as illustrated in Section 4. It can be applied to obtain the 90% confidence interval for AUC_T/AUC_R for sparse concentrations data in crossover studies. It can be also applied to obtain the 90% confidence interval for $C_{T,max}/C_{R,max}$ for sparse concentrations data in parallel studies and crossover studies. However, existing methods such as Bailer's method can be applied only to obtain the 90% confidence interval for AUC_T/AUC_R for sparse concentrations data in parallel studies. Furthermore, after two new drug applications were approved based on the bootstrap method in the review (Shen, 2008), a bootstrap method was recommended for evaluation of the bioequivalence study of dexamethasone and tobramycin with PK endpoints using a design as single-dose, parallel design, in vivo in aqueous humor and for evaluation of in vitro bioequivalence study of dexamethasone and tobramycin using a design as in vitro microbial kill rate study (U.S. Food and Drug Administration, 2013).

Acknowledgments

The authors would like to thank the anonymous reviewers for their comments. The first author would like to thank her mentor and supervisor, Dr. Yi Tsong at the Food and Drug Administration, for his longtime support and encouragement.

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