Assessment of Bioequivalence in Pharmacokinetic Parameters Using Mixed-Design ANOVA

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Abstract

The objective of this study was to assess the bioequivalence of the test and reference formulations in a controlled clinical setting, using a mixed-design analysis of variance (ANOVA) approach. The study focused on key pharmacokinetic parameters, including CMAX, AUCT, and AUCINF. The data were analyzed to determine the geometric mean ratios and their corresponding 90% confidence intervals. The primary outcome showed that the 90% confidence intervals for CMAX, AUCT, and AUCINF were (0.8753, 1.1967), (0.8771, 1.0843), and (0.8815, 1.0269), respectively. These intervals indicate that the test and reference formulations have no significant difference in the rate and extent of absorption, falling within the conventional bioequivalence range of 0.80-1.25. The study findings suggest that the test formulation is bioequivalent to the reference formulation, meeting the regulatory criteria for bioequivalence.

Introduction

Bioequivalence involves assessing the similarity between two pharmaceutical products, often a generic drug and its brand-name counterpart, in their bioavailability and pharmacokinetic characteristics. The U.S. Food and Drug Administration (FDA) defines bioequivalence as “the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study” (Definition from 21 CFR § 320.1). For two products to be considered bioequivalent, they must demonstrate equivalence in the rate and extent of availability of the active pharmaceutical ingredient (API) at the site of action. Bioequivalence is crucial as it ensures that a generic drug performs in a manner similar to its brand-name counterpart in terms of efficacy and safety. Regulatory bodies such as the FDA mandate bioequivalence studies for approving generic medications.

In the conduct of bioequivalence studies, small groups of healthy subjects typically receive treatment with both the brand-name drug and its generic form in a crossover design. The amount of drug in the bloodstream and the time it takes for the drug to be absorbed are measured. The main parameters measured are the maximum concentration of the drug in the bloodstream (Cmax) and the total exposure to the drug over time (AUC). Bioequivalence is generally established if the key pharmacokinetic parameters of the generic drug lie within an accepted range (usually 80-125%) compared to the brand-name drug.

Study Methods

Data Description and Manipulation

This report demonstrates the progress of a bioequivalence study using example data.

	Drug 1			Drug 2		
	CMAX	AUCT	AUCINF	CMAX	AUCT	AUCINF
Summary Statistics						
Min	20.7	305.87	402.05	21.3	252.65	398.53
Max	349	3401	3495.9	445	3069.13	3186.74
Arithmetic Mean	107.98	1213.95	1423.94	114.57	1207.10	1361.57
Standard Deviations	69.05	713.69	732.01	83.56	700.55	682.82
Coefficient of Variations	63.94924	58.79	51.41	72.94	58.04	50.15
Geometric Means	90.46482	1047.64	1265.03	92.21	1021.68	1205.28

Table 1. Summary statistics (min, max, arithmetic means, standard deviations, coefficient of variations, geometric means) grouped by the drug type

This bioequivalence study employs a crossover design, as indicated by the presence of multiple sequences and periods. In such studies, subjects receive different treatments in different periods, allowing for a direct comparison of the pharmacokinetics of the test drug (Drug 1) and the reference drug (Drug 2) within the same individual. The dataset includes four sequences, each representing a different order of receiving the test and reference drugs. The number of periods, four in this case, reflects the crossover nature, where each subject receives both treatments at different times. There are two treatment levels in this study, corresponding to the test formulation (Drug 1) and the reference formulation (Drug 2). In crossover designs, a washout period between treatments is crucial to ensure that the effect of the first treatment does not carry over into the second treatment period. This washout period needs to be sufficiently long to allow the first drug to be eliminated from the body before the second drug is administered.

The dataset under examination exhibits instances of missing data, specifically in the measurement categories of CMAX, AUCT, and AUCINF. Handling missing data is a critical aspect of statistical analysis and can be approached through various methodologies. Given that the dataset presents a relatively minor proportion of missing data, our strategy will involve substituting the missing values with the average values of the corresponding measurements for each subject. This method, known as mean imputation, will help maintain the integrity of the dataset while ensuring that the analysis is not skewed by the absence of these data points.

Statistical Methods

The objective of this study is to establish bioequivalence between the test formulation (Drug = 1) and the reference formulation (Drug = 2). To achieve this objective, three variables including CMAX, AUCT and AUCINF will be analyzed to determine if their geometric mean ratios between Drug 1 and Drug 2 are within an acceptable range.

The hypotheses in the bioequivalence testing are

- Null Hypothesis (H_0): The means of the key pharmacokinetic variables (such as CMAX, AUCT, AUCINF) are significantly different between the test and reference formulations.
- Alternative Hypothesis (H_1): There is no significant difference in the means of these variables between the two formulations.

When evaluating the bioequivalence between Drug 1 and Drug 2, a critical step involves examining the 90% confidence intervals of the geometric mean ratios for CMAX, AUCT, and AUCINF. If these confidence intervals fall within an acceptable range (usually 80-125%), it provides a basis for

rejecting the null hypothesis. The null hypothesis in this context typically posits that there is a significant difference in bioavailability between the two drugs. Therefore, if the confidence intervals of the geometric mean ratios are indeed within the acceptable limits, it indicates a lack of significant difference in the pharmacokinetic parameters of the drugs. Consequently, this allows for the assertion of bioequivalence between Drug 1 and Drug 2.

In this report, mixed-design analysis of variance (ANOVA) model will be applied. This statistical method is particularly suited for testing differences across two or more independent groups, while also accommodating repeated measures on the same participants. The mixed-design ANOVA integrates both fixed and random effects, making it an excellent choice for handling data with complex structures, such as bioequivalence studies. In this report, the fixed effects include drug type, period, and sequence. The random effect include subject.

Key pharmacokinetic measures like the area under the curve (AUC) and peak concentration (Cmax) are analyzed to assess bioequivalence. The mixed-design ANOVA can decompose the variability in these measures into components attributable to treatment effects, subject effects, period effects, and other sources. The relevant formulas for selected mixed-design ANOVA can be written as

$$Y_{ijkl} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \pi_k + (\pi\tau)_{ik} + \epsilon_{ijkl}$$

where Y_{ijkl} is the log scaled observed value for the lth observation in the ith treatment, jth period, and kth sequence. μ is the overall mean. τ_i is the fixed effect of the ith treatment. β_j is the fixed effect of the jth period. $(\tau\beta)_{ij}$ is the interaction between treatment and period. π_k is the random effect of the kth subject (within sequence). $(\pi\tau)_{ik}$ is the interaction between subject and treatment. ϵ_{ijkl} is the random error term.

To successfully conduct an ANOVA (Analysis of Variance) test, certain assumptions must be met by the dataset, including Normality, Homogeneity of Variance, and Sphericity of the covariance matrix. These criteria are critical for ensuring the reliability of the ANOVA results.

Result

Variable	CMAX				AUCT				AUCINF			
Random effect												
Subject(Sequence)	0.1863				0.2213				0.1913			
Residual	0.2182				0.1004				0.0521			
Fixed effect												
Source	numDF	denDF	F-value	p-value	numDF	denDF	F value	p-value	numDF	denDF	F-value	p-value
Drug	1	71	0.0416	0.8389	1	71	0.157	0.6934	1	71	1.124	0.2927
Sequence	3	71	0.4143	0.7433	3	71	0.153	0.9274	3	71	0.339	0.7973

Period	3	21	1.2092	0.3308	3	21	1.378	0.2769	4	21	1.375	0.2779
Geometric mean ratio												
Point estimate			1.0235				0.9752				0.9514	
90% CI			0.8753-1.1967				0.8771-1.0843				0.8815-1.0269	

* CI: confidence interval.
* numDF: Numerator Df
* denDF: Denominator Df

Table 2. Summary of the mixed-design analysis of variance (ANOVA) model on CMAX, AUCT and AUCINF

The mixed-design ANOVA model was applied to assess the bioequivalence of the pharmacokinetic parameters: CMAX, AUCT, and AUCINF. We focus on the 90% confidence intervals for the geometric mean ratios of these parameters, as they are critical for determining bioequivalence.

- **CMAX** (Peak Concentration):
  The 90% confidence interval for the geometric mean ratio of CMAX was found to be between 0.8753 and 1.1967. This interval falls within the accepted bioequivalence range.
- **AUCT** (Area Under the Curve - Time):
  The 90% confidence interval for the geometric mean ratio of AUCT was found to be between 0.8771 and 1.0843. This interval also falls within the accepted bioequivalence range.
- **AUCINF** (Area Under the Curve - Infinity):
  The 90% confidence interval for the geometric mean ratio of AUCINF was found to be between 0.8815 and 1.0269, which remains within the accepted bounds for bioequivalence.

Discussion and Conclusions

The outcomes derived from the mixed-design ANOVA model indicate that the pharmacokinetic attributes of the two drugs under examination demonstrate bioequivalence. Specifically, the 90% confidence intervals for the geometric mean ratios of key parameters like CMAX, AUCT, and AUCINF align with the established criteria for bioequivalence. This alignment suggests that the test drug and the reference drug are statistically analogous in several respects: the rate of absorption (CMAX), the extent of absorption (AUCINF), and the total drug exposure over time (AUCT). Therefore, based on these pharmacokinetic measures, the test and reference formulations can be considered bioequivalent within the accepted regulatory criteria.

However, there are some potential limitations in a study employing a mixed-design ANOVA for bioequivalence assessment. For example, sample size of this study is not enough, it may increase the risk of Type II errors. Also, factors such as environmental conditions and lifestyle factors such as diet or smoking can introduce variability that affects the pharmacokinetic parameters.

Reference

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2. Park, G., Kim, H., Bae, K.S. (2020). Bioequivalence data analysis. *Transl Clin Pharmacol*. 2020 Dec;28(4):175-180. https://doi.org/10.12793/tcp.2020.28.e20
3. Chow S. C. (2014). Bioavailability and Bioequivalence in Drug Development. *Wiley interdisciplinary reviews. Computational statistics*, *6*(4), 304–312. https://doi.org/10.1002/wics.1310

Appendix

The R code used in bioequivalence study is

```
require(sasLM)
require(nlme)
setwd("C:/Users/Leon/Desktop/PHST")
BEdata = read.csv("./DTA01.csv", as.is=TRUE)
colnames(BEdata)[1]="SUBJ"
BEdata = af(BEdata, c("SUBJ", "SEQ", "PERIOD", "DRUG", "REPL"))
min(BEdata[BEdata$DRUG==1,"CMAX"])
max(BEdata[BEdata$DRUG==1,"CMAX"])
mean(BEdata[BEdata$DRUG==1,"CMAX"])
sd(BEdata[BEdata$DRUG==1,"CMAX"])
sd(BEdata[BEdata$DRUG==1,"CMAX"]) / mean(BEdata[BEdata$DRUG==1,"CMAX"]) * 100
exp(mean(log(BEdata[BEdata$DRUG==1,"CMAX"])))
min(BEdata[BEdata$DRUG==1,"AUCT"])
max(BEdata[BEdata$DRUG==1,"AUCT"])
mean(BEdata[BEdata$DRUG==1,"AUCT"])
sd(BEdata[BEdata$DRUG==1,"AUCT"])
sd(BEdata[BEdata$DRUG==1,"AUCT"]) / mean(BEdata[BEdata$DRUG==1,"AUCT"]) * 100
exp(mean(log(BEdata[BEdata$DRUG==1,"AUCT"])))
min(BEdata[BEdata$DRUG==1,"AUCINF"])
max(BEdata[BEdata$DRUG==1,"AUCINF"])
mean(BEdata[BEdata$DRUG==1,"AUCINF"])
sd(BEdata[BEdata$DRUG==1,"AUCINF"])
sd(BEdata[BEdata$DRUG==1,"AUCINF"]) / mean(BEdata[BEdata$DRUG==1,"AUCINF"]) *
    100
exp(mean(log(BEdata[BEdata$DRUG==1,"AUCINF"])))
min(BEdata[BEdata$DRUG==2,"CMAX"])
```

```r
max(BEdata[BEdata$DRUG==2,"CMAX"])
mean(BEdata[BEdata$DRUG==2,"CMAX"])
sd(BEdata[BEdata$DRUG==2,"CMAX"])
sd(BEdata[BEdata$DRUG==2,"CMAX"]) / mean(BEdata[BEdata$DRUG==2,"CMAX"]) * 100
exp(mean(log(BEdata[BEdata$DRUG==2,"CMAX"])))
min(BEdata[BEdata$DRUG==2,"AUCT"])
max(BEdata[BEdata$DRUG==2,"AUCT"])
mean(BEdata[BEdata$DRUG==2,"AUCT"])
sd(BEdata[BEdata$DRUG==2,"AUCT"])
sd(BEdata[BEdata$DRUG==2,"AUCT"]) / mean(BEdata[BEdata$DRUG==2,"AUCT"]) * 100
exp(mean(log(BEdata[BEdata$DRUG==2,"AUCT"])))
min(BEdata[BEdata$DRUG==2,"AUCINF"])
max(BEdata[BEdata$DRUG==2,"AUCINF"])
mean(BEdata[BEdata$DRUG==2,"AUCINF"])
sd(BEdata[BEdata$DRUG==2,"AUCINF"])
sd(BEdata[BEdata$DRUG==2,"AUCINF"]) / mean(BEdata[BEdata$DRUG==2,"AUCINF"]) *
    100
exp(mean(log(BEdata[BEdata$DRUG==2,"AUCINF"])))
Result1 = lme(log(CMAX) ~ DRUG + PERIOD + SEQ, random=~1|SUBJ,
data=BEdata)
summary(Result1)
anova(Result1)
VarCorr(Result1)
ci = intervals(Result1, 0.90) # 90% CI of log scale difference
exp(ci$fixed["DRUG2",])
Result2 = lme(log(AUCT) ~ DRUG + PERIOD + SEQ , random=~1|SUBJ,
data=BEdata)
summary(Result2)
anova(Result2)
VarCorr(Result2)
ci = intervals(Result2, 0.90) # 90% CI of log scale difference
exp(ci$fixed["DRUG2",])
Result3 = lme(log(AUCINF) ~ DRUG + PERIOD + SEQ , random=~1|SUBJ,
data=BEdata)
summary(Result3)
anova(Result3)
VarCorr(Result3)
ci = intervals(Result3, 0.90) # 90% CI of log scale difference
exp(ci$fixed["DRUG2",])
```