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OUTLIER DETECTION IN AVERAGE BIOEQUIVALENCE STUDY USING METHOD OF BOOTSTRAP

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Abstract

A **generic drug** is defined as "a drug product that is comparable to brand/reference drug product in dosage form, strength, route of administration, quality and performance characteristics, and intended use." The average bioequivalence criterion stipulates that two drugs are to be considered bioequivalent when the 90% confidence interval, for the ratio of Geometric means (Test/Reference) is between 80% and 125%. The result is expressed as average bioequivalence (ABE), as it compares average values for both test and reference drug bio availabilities. The bioavailability of a drug is defined as the rate and extent to which the active drug ingredient from a drug product is absorbed and becomes available at the site of drug action. A bioequivalence study data which contains a statistical outlier, may affect the inference of the study. In this paper, the Bootstrap method is applied for detection of an outlier subject or unusual subject in a two way crossover design in bioequivalence study as it is the most acceptable design in BE studies which separates intra subject variability from inter subject variability. Five data sets are simulated, for a two way crossover design, assuming Bivariate Log Normal distribution for pharmacokinetic parameter Cmax. For each data set, statistical outlier is identified using method based on statistical theory of Bootstrapping. In addition, statistical inference using above method is compared with inferences drawn from more commonly used methods of outlier detection such as i) Maximum Normalized Residual Method (MNR) ii) Estimates Distance Method (ED) iii) Lund's Method. It is observed that inferences drawn from the existing methods of Outlier detection viz. Estimates Distance, Lund's Method and MNR (Maximum Normalized residual) method are not consistent. Lund's method is less likely to detect "true" outlier whereas Estimates distance method is more likely to indicate a subject as an outlier in the absence of "true" outlier. The method based on bootstrapping is more robust in identifying the "true" outlier.

Keywords: Bioequivalence, Confidence interval, Two Way crossover design, Outlier detection methods, Bootstrapping

Area of research: Statistics

1. INTRODUCTION

A **generic drug** (generic drugs, generics) is a drug defined as "a drug product that is comparable to brand/reference listed drug product in dosage form, strength, route of administration, quality and performance characteristics, and intended use.

Bioavailability is a measurement of the extent of a therapeutically active medicine that reaches the systemic circulation and is therefore available at the site of action.

The average bioequivalence criterion stipulates that two drugs are to be considered bioequivalent when the 90% confidence interval, considering the average bioavailability of the test drug (T) and the reference drug (R) and the T/R ratio, is between 80% and 125%, for data converted to the logarithmic scale.

The result is expressed as average bioequivalence (ABE), as it compares average values for both test and reference drug bio availabilities. The two drugs are bioequivalent if the 90% Confidence Interval for the ratio of geometric means of test preparation to standard preparation (T/R ratio) lies in 80% to 125%.

Outlier data in Bioequivalence (BE) studies are defined as subject data for one or more BA measures that are discordant with corresponding data for that subject and/or for the rest of the subjects in a study. Because BE studies are usually carried out as crossover studies, the most important type of subject outlier is the within-subject outlier, where one subject or a few subjects differ notably from the rest of the subjects with respect to a within-subject T-R comparison.

In this paper a new statistical method for detection of an outlier subject or unusual subject in a bioequivalence study is developed using bootstrap technique. This research paper exhibits the use of this new method of outlier detection to the simulated data for pharmacokinetic parameter for a Two way cross over design in BE studies. Two ways crossover design is the most acceptable design in BE studies which separate intra subject variability from interring subject variability.

2. METHODOLOGY

The concept of Bootstrapping is used to detect statistical outlier.
The method is applied to simulated data (for the pharmacokinetic parameter Cmax) of varying sample sizes (Sample sizes 18, 28, 38, 50 and 64, based on different values of Intra subject coefficient of variation) for a two way crossover design. The data are simulated using bivariate log normal distribution.

Resamples are drawn from the simulated data of sample size n.
a) 100 samples of 'n' subjects are drawn with replacement from the original sample excluding i^{th} subject. (i=1,2…….. n).
b) 100 samples of 'n' subjects are drawn with replacement from the original sample including i^{th} subject. (i=1,2…….. n).
The objective is to test the hypothesis
H_0: i^{th} subject is not an outlier.
H_1: Not H_0.

The test statistic to test this hypothesis is based on two quantities, P_{1i} and P_{2i}, where P_{1i} is calculated as the proportion of times bioequivalence is established excluding i^{th} subject from the analysis data and P_{2i} is calculated as the proportion of times bioequivalence is established including i^{th} subject in the analysis data.

90% confidence interval for the ratio of geometric means is calculated using statistical analysis of the design of experiment viz, Two Way crossover design. The method can also be applied to any design of

Bioequivalence Clinical Trial like Parallel Design, Three Way Crossover Design, and Replicated Crossover Design.

In order to calculate P_{1i} and P_{2i}, the statistical analysis of the design of BE under consideration, is performed for every sample generated (using bootstrap).

An outlier is detected using the test statistic Zi (i=1,2,….n) where Zi is calculated as follows –

$$Z_i = \frac{P_{1i} - P_{2i}}{\sqrt{\frac{P_{1i} * (1 - P_{1i}) + P_{2i} * (1 - P_{2i})}{100}}} \qquad if \;\; P_{1i} \neq P_{2i}$$

$$Z_i = 0 \qquad if \;\; P_{1i} = P_{2i}$$

Z_i follows standard normal distribution.

Decision Criterion: Reject H_0 if $|Z_i| > 1.96$ (α=5%)

3. NUMERICAL ANALYSIS

3.1 Application of Bootstrap Method:

According to the 80/125 rule for assessment of average bioequivalence, the ratio of true averages (μ_T / μ_R) must be within (80%, 125%), with 90% assurance to claim Bioequivalence.

A typical approach is to construct a 90% confidence interval for μ_T/μ_R and compare it with (80%, 125%). If this constructed confidence interval is within (80%, 125%), then average bioequivalence is concluded. For every resample the entire analysis is performed and the criterion for establishing bioequivalence is compared with 90% confidence interval for μ_T/μ_R in order to estimate P_{1i} and P_{2i}.

After resampling two quantities P_{1i} and P_{2i} are defined such that P_{1i} will be proportion of bootstrap samples in which bioequivalence is achieved P_{2i} will be proportion of bootstrap samples in which bioequivalence is not achieved when i^{th} subject is excluded from the sample.

The test statistic for outlier detection is defined as
Zi (i=1, 2,……...18) where Zi is calculated as follows –

$$Z_i = \frac{P_{1i} - P_{2i}}{\sqrt{\frac{P_{1i} * (1 - P_{1i}) + P_{2i} * (1 - P_{2i})}{100}}} \qquad if \;\; P_{1i} \neq P_{2i}$$

$$Z_i = 0 \qquad if \;\; P_{1i} = P_{2i}$$

Following results are obtained for the above data set for two way crossover design with sample size 18.

Table 1: Calculations the test statistic using Bootstrap Method Two way Crossover Design **(Sample Size 18)**

Subject	P_{1i}	P_{2i}	Zi	Subject	P_{1i}	P_{2i}	Zi
1	0.54	0.39	2.151	10	0.55	0.33	3.2138
2	0.18	0.39	-3.3823	11	0.37	0.30	1.0516
3	0.53	0.34	2.7612	12	0.37	0.30	1.0516
4	0.41	0.46	-0.7141	**13**	**0.67**	**0.36**	**4.6135**
5	0.27	0.39	-1.8194	14	0.51	0.41	1.4260
6	0.28	0.35	-1.0686	15	0.28	0.41	-1.9521
7	0.33	0.38	-0.7399	16	0.31	0.41	-1.4812
8	0.35	0.43	-1.1637	17	0.34	0.38	-0.5898
9	0.33	0.32	0.1510	18	0.31	0.33	-0.3032

The decision criterion is if $Max\ Z_i > |1.96|$ then the corresponding subject is identified as a statistical outlier.

In the above case, **Max Z_i = 4.6135** and it corresponds to subject number 13.
Since this value is greater than 1.96, the subject number 13 is an outlier.

3.2 Application of Lund's Method, MNR Method and ED Method: Two way Crossover design (Simulated data with Sample Size 18)

Maximum Normalized Residual method, MNR, is the simplest method based on Residuals (Residuals are not based on any statistical Model). Estimates Distance method, is based on the Distance between the Parameter Estimates of the likelihood function, i) When all subjects are included and ii) ith Subject is excluded. This is computed for each subject. Very High distance indicates presence of outlier. Lund's Method uses Model based Residuals. Model is similar to Regression Model. Residuals are nothing but Unexplained Portions. The higher the value of the Residuals indicates presence of Outlier. Following table displays the results of application of these methods to simulated data of size 18 for Two way Crossover Design.

Table 2: Calculations the test statistic based on Lund's Method, MNR Method and ED Method

Subject	**Lund's method (Studentized residuals)**	**Z(MNR)**	**Estimates Distance**	**Subject**	**Lund's method (Studentized residuals)**	**Z(MNR)**	**Estimates Distance**
1	1.3974	0.8343	1.6126	10	1.0780	1.3558	2.4484
2	1.7242	1.1235	1.3455	11	0.6270	0.9568	1.3093
3	0.9023	0.3963	0.6480	12	0.1618	0.5452	4.3203
4	0.1562	0.2638	1.7272	**13**	**2.4684**	**2.5860**	**30.3361**
5	1.5556	1.7784	0.7746	14	0.2426	0.6167	1.1178
6	0.6088	0.1367	1.0397	15	0.7318	0.2455	1.1395
7	0.3728	0.7318	1.0326	16	0.1146	0.3006	0.8856
8	0.2534	0.6262	8.2885	17	0.4532	0.0010	0.9524
9	0.1794	0.5607	8.7419	18	0.8502	0.3503	0.6813

4. Discussion

It is observed that for the simulated data with sample size 18, the maximum value test statistic under Lund's method, Maximum Normalized Residual method (MNR) and Estimates Distance corresponds to Subject number 13 though, Subject Number 13 is not identified as an outlier by each of these methods. For Lund's Method, the test statistic, calculated Maximum Studentized Residual is **2.4684 (Subject No. 13)** whereas the Table Value from Prescott Lund's table is obtained as 2.8627. Hence according to Lund's method, there is no outlier as Maximum studentized residual is less than the Prescott Lund's table Value. For MNR Method, the test Statistic is Maximum Normalized Residual which is equal to 2.5860 (Subject No. 13) whereas, the corresponding Critical value is 2.6516. Hence, Subject number "13'with Maximum normalized Residual is not an outlier.

But according to ED method, the value of test Statistic is Maximum Estimates Distance which is 30.3361 (Subject No. 13). Also, the Critical value based on Chi-square distribution with 3 degrees of freedom is 7.8147. Since the value of test statistic is greater than the critical value, the subject number "13" is identified as an outlier.

Comparison among these four methods has clearly indicated that the subject number "13" is identified as an outlier using Method of Bootstrap and Estimates Distance method. Both Lund's Method and MNR method also resulted in the maximum value of the test statistic corresponding to the same subject (Subject No. 13) but failing to identify that subject as an outlier on the basis of the corresponding critical values. It is also observed that the 90% Confidence interval for this data is (74.87, 98.55) (Subject Number 13, included) indicating that Average Bioequivalence is not established. After excluding the subject number 13 from the analysis data, 90% Confidence interval is (81.12, 100.24). This indicates that Average bioequivalence is established after removing subject number 13 from the analysis. It is observed that Subject Number 13 has the impact on the study outcome. This indicates that Subject number 13 is a "True outlier'.

The above method of bootstrap for outlier detection is applied to the simulated data with sample sizes 28, 38, 50 and 64 and the results are also compared with the existing methods of outlier detection viz. MNR, ED and Lund's method.

For data with sample size 28, comparison among these three methods has clearly indicated that the subject number "11" is identified as an outlier using Estimates Distance method whereas both Lund's Method and MNR method do not identify any outlier. Method of bootstrap has also not detected any outlier for this data.

For other simulated data sets with sample sizes 38, 50 and 64 Estimates Distance method has identified an outlier which may not be a true outlier. In general, Estimates Distance method is more likely to detect an outlier even in absence of true outlier.

It is observed that existing methods of Outlier detection viz. Estimates Distance, Lund's Method and MNR (Maximum Normalized residual) method are not consistent. Lund's method is less likely to detect "true" outlier whereas Estimates distance method is more likely to indicate a subject as an outlier in the absence of "true" outlier.

The new method of outlier detection based on bootstrapping is more robust in identifying the "true" outlier.

This method of bootstrap can be applied to data of any sample size and any design in Bioequivalence studies.

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