A SAS PROCEDURE FOR OUTLIER DETECTION IN BIOAVAILABILITY/BIOEQUIVALENCE STUDIES

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ABSTRACT

In the pharmaceutical industry, a crossover experiment is often conducted to assess bioequivalence between a test formulation and a reference formulation of a drug product. Two formulations are considered bioequivalent if the ratio of means (test/reference) of the pharmacokinetic parameters of interest such as area under the blood concentration-time curve (AUC) and maximum blood concentration (Cmax) is within some reasonable limits (say, (80%, 120%)) with 90% assurance. A bioavailability analysis that includes possible outlying values may affect the decision on bioequivalence. Chow and Tse (1990) proposed two test procedures, the likelihood distance (LD) and the estimates distance (ED) for detection of an outlying subject. In this paper, a SAS procedure for the LD and ED test procedures is developed using the SAS Macro language. An example is presented to demonstrate the use of the SAS procedure.

1. INTRODUCTION

In the pharmaceutical industry, a crossover experiment is often conducted with some healthy volunteers to assess bioequivalence between a test formulation and a reference formulation of a drug product in terms of the rate and extent of absorption of the drug product. Rate and extent of absorption usually measured by the pharmacokinetic parameters: area under the blood or plasma concentration-time curve (AUC), maximum blood or plasma concentration (Cmax), and time to maximum concentration (Tmax). Two formulations are considered to be bioequivalent if the ratio of means (test/reference) for the pharmacokinetic parameters of interest is within some reasonable limits (say, (80%, 120%)) with 90% assurance. Along this line, several approaches have been proposed. For example, Westlake (1976), Anderson and Hauck (1983), Schuirmann (1987), and Chow and Shao (1990). However, a typical approach is to construct a 90% confidence interval for the ratio of means and compare it with the (80%, 120%) limits. If the confidence interval is within the limits, then the bioequivalence between the two formulations is claimed.

In practice, studies of bioequivalence commonly encounter the problem that the data set contains some outlying or extreme observations. These outlying observations may occur either as unexpected observations in the blood or plasma concentration-time curve or as the unusually subject who has extremely high or low bioavailability with respect to the reference formulation. In general, the unexpected observations in the plasma concentration time curve will have little effect on the comparison of bioavailability. However, a bioavailability analysis that includes possible outlying subjects may affect the decision on bioequivalence. To detect an outlying subject, Chow and Tse (1990) proposed two test procedures, likelihood distance (LD) and estimates distance (ED) under the assumption that there are no period effects and formulation effects. The main purpose of this paper is to develop a SAS procedure for LD and ED test procedures using the SAS Macro language.

In the next section, we describe the procedures for detection of an outlying subject. In Section 3, a SAS procedure for detection of outlying subjects is presented. An example concerning a three-way crossover experiment for the assessment of bioequivalence between two test formulations and a reference formulation of a drug product is used to illustrate the use of the developed SAS procedure.

2. TEST PROCEDURES FOR DETECTION OF AN OUTLYING SUBJECT

Consider the following crossover model

\[ Y_{ijt} = \mu + S_i + F_j + \epsilon_{ijt} \]

\[ j, t = 1, \ldots, n; i = 1, \ldots, k \]

where \( \mu \) is the overall mean, \( F_j \) is the fixed effect of the jth formulation with \( F_j F_j = 0 \); \( P_t \) is the fixed effect of the tth period. With \( S_i S_i = 0 \); \( S_i \) is the random effect of the ith subject; \( \epsilon_{ijt} \) is the error term; and \( Y_{ijt} \) is the response variable on the ith subject in the jth period under the jth formulation. In the above model, it is assumed that \( (S_i) \) and \( (\epsilon_{ijt}) \) are independently and normally distributed with means 0 and variances \( \sigma^2 \) and \( \sigma_e^2 \), respectively.

In the following, we describe two procedures for detection of an outlying
subject. For illustration, we focus on
the following reduced model with the
assumption that there are no period and
formulation effects.

\[ Y_{ij} = \mu + S_i + \epsilon_{ij} \]

\( j=1, \ldots, n; i=1, \ldots, k \)

In this case, the parameters of interest
are \( \mu, \sigma^2_\epsilon \), and \( \sigma^2_\epsilon \).

Let \( \hat{\theta} = (\hat{\theta}_1, \hat{\theta}_2, \hat{\theta}_3) \) \ WHERE \( \hat{\theta}_1 = \mu, \hat{\theta}_2 = \sigma^2_\epsilon, \) \ and \( \hat{\theta}_3 = \sigma^2_\epsilon \).

Then, the log-likelihood function is
given by

\[ L(\theta) = -\frac{n}{2} \log 2\pi - \frac{k}{2} \log(\sigma^2_\epsilon n^{-1}) \]

\[ -\frac{1}{2\sigma^2_\epsilon} \sum_{i=1}^{k} \sum_{j=1}^{n} (Y_{ij} - \hat{\mu})^2 \]

\[ -\left(\frac{n}{2}\right) \left(\frac{1}{\sigma^2_\epsilon} - \frac{1}{\hat{\sigma}^2_\epsilon}\right) \sum_{i=1}^{k} (Y_{i.} - \hat{\mu})^2. \]

The maximum likelihood estimator (MLE) \( \hat{\theta} \)
of \( \theta \) are given below

\( \hat{\theta}_1 = \bar{Y} \)

\( \hat{\theta}_2 = n \bar{Y} \)

\( \hat{\theta}_3 = \frac{(k-1)m_2}{k} \)

where \( m_1 = \frac{1}{k(n-1)} \sum_{i=1}^{k} \sum_{j=1}^{n} (Y_{ij} - \bar{Y})^2 \)

and \( m_2 = \frac{n}{k-1} \sum_{i=1}^{k} (Y_{i.} - \bar{Y})^2. \)

Note that if \( \hat{\theta}_2 < \theta \) (i.e., \( (k-1)m_2 < \theta \)), then the maximum likelihood
estimators of \( \theta \) modify to

\( \hat{\theta}_2 = \frac{1}{nk} \sum_{i=1}^{k} \sum_{j=1}^{n} (Y_{ij} - \bar{Y})^2. \)

This obtains by maximizing \( L(\theta) \) under the
condition that \( \hat{\theta}_2 < \theta \). The LD test
procedure is then given by

\[ LD_1(\hat{\theta}) = 2 \left[ L(\hat{\theta}) - L(\hat{\theta}(i)) \right], \]

WHERE \( \hat{\theta}(i) \) denotes the MLE of \( \theta \) with
deletion of the \( i \)th subject. As \( k \) tends to infinity, it can be verified that \( LD_1(\hat{\theta}) \) is asymptotically
chi-square distributed with three
degrees of freedom. Thus we consider
the \( i \)th subject as an outlying subject if

\[ LD_1(\hat{\theta}) > x^2_3(\alpha), \]

where \( x^2_3(\alpha) \) is the upper a percentile
point of \( x^2_3. \)

Similar idea can also be applied to the
estimates. Thus, we have

\[ ED_1(\hat{\theta}) = k^2(\hat{\theta}(i) - \hat{\theta})^2, \]

where \( \hat{\theta} \) is the estimate of

\[ \hat{\theta}(i) = \left[ \frac{\hat{\theta}_1}{n}, \frac{2\hat{\theta}_2}{n-1}, 0 \right] \]

\[ \Sigma = \left[ \begin{array}{ccc} \hat{\theta}_1 & 0 & 0 \\ 0 & \frac{2\hat{\theta}_2}{n-1} & 0 \\ 0 & 0 & \frac{2\hat{\theta}_3}{n} \end{array} \right] \]

Chow and Tse (1990) showed that \( ED_1(\hat{\theta}) \)
is distributed as chi-square with three
degrees of freedom as \( k \) tends to infinity. Thus we consider the \( i \)th
subject as an outlying subject if

\[ ED_1(\hat{\theta}) > x^2_3(\alpha). \]

3. SAS PROCEDURE

In this section, a SAS procedure
which computes the LD and ED statistics
using SAS Macro language is developed
for detection of an outlying subject
under the reduced model (i.e., it is
assumed that there are no period and
formulation effects). In order to
successfully run this SAS program, the
data matrix must be arranged in such a
way that row and column of the matrix
represent formulation and subject,
respectively. In addition, it is
required to specify the number of
subjects and that of formulations,
namely, \( k \) and \( n \) of the above model in
the SAS program. The main task of
this program is to calculate parameters
\( \hat{\theta}_1, \hat{\theta}_2, \) and \( \hat{\theta}_3 \) as well as test
statistics LD (likelihood distance) and
ED (estimates distance) based on
different data sets, i.e., SET0,
SET1, ..., SETk. In this case, SET0
contains \( k \) subjects, and SETi is the
data set with the \( i \)th subject deleted
from SET0, where \( i=1, 2, ..., k \). In
other words, with proper adjustments
such as number of subjects of the data
set, it is required to repeat the same
computations \( k+1 \) times for a data set of
k subjects. Because of the iterative
nature of this process, the SAS Macro
language is adapted for doing the job.

Upon completion of finding parameter
estimates \( \hat{\theta}_1, \hat{\theta}_2, \) and \( \hat{\theta}_3 \), along with
checking the condition imposed on \( \theta \)’s
and making adjustment if necessary,
the SAS program proceeds with
calculating test statistics LD and ED as
well as associated p-values for each
subject on the data set. Subsequently,
on the basis of test results, we may
determine whether or not a subject is an
outlier in a bioequivalence study.

The following is the SAS procedure
which can be used for outlier detection in bioequivalence study. This SAS program written using the Macro Language with name- and statement-style invocations. This SAS program is run on an IBM 3090 under VM/SP CMS operation system.

CMS FIX IN DISK STUDY DATA A;
OPTIONS MPRINT IMPLMAC;
%LET K=12;
%LET N=3;
%LET L=%EVAL(4*K-1);
DATA RAWDATA;
INFILE INI;
INPUT SUBJ1 - SUBJ&K;
DATA FIRST(DROP=SUBJ1-SUBJ&K I);
SET;
ARRAY SUBJ (*) NUMERIC ;
DO I=1 TO DIM(SUBJ);
SUBJECT=I;
OUTPUT;
END;
PROC SORT;
BY SUBJECT;
PROC MEANS NOPRINT MEAN;
VAR AUC;
BY SUBJECT;
OUTPUT OUT=SECOND MEAN=MEANAUC;
dataArray=
DATA SELECT;
SET RAWDATA;
%IF &SUBJNO NE 0 %THEN %DO;
DROP SUBJ&SUBJNO;
%END;
DATA GO(KEEP=AUC);
SET;
ARRAY SUBJ (*) NUMERIC ;
DO I=1 TO DIM(SUBJ);
AUC=SUBJ(I);
OUTPUT;
END;
PROC MEANS NOPRINT MEAN;
VAR AUC;
BY SUBJECT;
OUTPUT OUT=GO CCXCSS;
PROC MEANS NOPRINT MEAN;
VAR XBAR CSS;
OUTPUT OUT=MDATA CSS=CSSXBAR CSSCSS
SUM=SUMXBAR SUMCSS MEAN=MXBAR MCSS;
DATA GOGO;
SET;
%IF &SUBJNO=0 %THEN %DO;
THETA1=XBAR;
THETA2=CSS/(4*K*(N-1));
THETA3=(N/(&K-1))*CSSXBAR;
%END;
%ELSE %DO;
THETA1=XBAR;
THETA2=CSS/((4*K-1)*(N-1));
THETA3=(N/(&K-1))*CSSXBAR;
%END;
DATA GOGO;
IF _N_=1 THEN SET GOGO(KEEP=THETA1);
SET FIRST(KEEP=SUBJECT AUC MEANAUC);
QA1=(AUC-THETA1)**2;
QA2=(MEANAUC-THETA1)**2;
PROC MEANS NOPRINT SUM;
VAR QA1 QA2;
OUTPUT OUT=GOGOGO SUM=SUMQA1 SUMQA2;
DATA &DATA(KEEP=THETA1-THETA3 LKHD);
MERGE GO GOGO GOGOGO;
QA2=SUMQA2/(&N);
IF THETA3 LT THETA2 THEN DO;
THETA2=CSS/(&N*(&K));
THETA3=CSS/(&N*(&K-1));
END;
IF THETA3 LT THETA2 THEN DO;
THETA2=CSS/(&N*(&K-1));
THETA3=CSS/(&N*(&K-1));
END;
QC=SUMQA1/(2*THETA2);
QD=-(&N/2)*(1/THETA3-1/THETA2)*QA2;
LKHD=QA+QB+QC+QD;
%MEND STUDY;
%MEND LOOP;
%MEND DSN;
STUDY 0 &K SETO;
LOOP &K;
DATA FINAL (KEEP=THETA1-THETA3 LKHD)
MERGE GO GOGOGO;
QA2=SUMQA2/(&N);
IF THETA3 LT THETA2 THEN DO;
THETA2=CSS/(&N*(&K));
THETA3=CSS/(&N*(&K-1));
END;
IF THETA3 LT THETA2 THEN DO;
THETA2=CSS/(&N*(&K-1));
THETA3=CSS/(&N*(&K-1));
END;
QC=SUMQA1/(2*THETA2);
QD=-(&N/2)*(1/THETA3-1/THETA2)*QA2;
LKHD=QA+QB+QC+QD;
%MEND STUDY;
%MEND LOOP;
%MEND DSN;
STUDY 0 &K SETO;
LOOP &K;
DATA FINAL (KEEP=THETA1-THETA3 LKHD)
MERGE GO GOGOGO;
QA2=SUMQA2/(&N);
IF THETA3 LT THETA2 THEN DO;
THETA2=CSS/(&N*(&K));
THETA3=CSS/(&N*(&K-1));
END;
IF THETA3 LT THETA2 THEN DO;
THETA2=CSS/(&N*(&K-1));
THETA3=CSS/(&N*(&K-1));
END;
QC=SUMQA1/(2*THETA2);
QD=-(&N/2)*(1/THETA3-1/THETA2)*QA2;
LKHD=QA+QB+QC+QD;
%MEND STUDY;
%MEND LOOP;
%MEND DSN;
STUDY 0 &K SETO;
LOOP &K;
A 3x3 crossover experiment was conducted to compare the bioavailability of three formulations of a drug (one reference formulation and two test formulations). During each dosing period, each of 12 normal subjects was administered one of the three formulations. Each dose period was preceded by a 10 hour fast and then followed by a 4 day drug washout period. Blood samples were obtained just prior to dosing and at several predetermined time points after drug administration. The AUC's and individual subject ratios (with respect to reference) for each formulation is given in Table 1.

<table>
<thead>
<tr>
<th>subject</th>
<th>R</th>
<th>A</th>
<th>B</th>
<th>A/R</th>
<th>B/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>863.8</td>
<td>944.2</td>
<td>558.0</td>
<td>1.09</td>
<td>0.65</td>
</tr>
<tr>
<td>2</td>
<td>672.0</td>
<td>675.8</td>
<td>619.7</td>
<td>1.01</td>
<td>0.92</td>
</tr>
<tr>
<td>3</td>
<td>500.0</td>
<td>524.6</td>
<td>507.3</td>
<td>1.05</td>
<td>1.01</td>
</tr>
<tr>
<td>4</td>
<td>1662.0</td>
<td>1006.3</td>
<td>442.4</td>
<td>0.63</td>
<td>0.27</td>
</tr>
<tr>
<td>5</td>
<td>344.5</td>
<td>254.6</td>
<td>304.2</td>
<td>0.74</td>
<td>0.88</td>
</tr>
<tr>
<td>6</td>
<td>435.0</td>
<td>381.9</td>
<td>316.8</td>
<td>0.88</td>
<td>0.73</td>
</tr>
<tr>
<td>7</td>
<td>925.6</td>
<td>653.2</td>
<td>690.0</td>
<td>0.71</td>
<td>0.75</td>
</tr>
<tr>
<td>8</td>
<td>772.0</td>
<td>612.6</td>
<td>728.2</td>
<td>0.79</td>
<td>0.94</td>
</tr>
<tr>
<td>9</td>
<td>763.5</td>
<td>529.2</td>
<td>701.5</td>
<td>0.69</td>
<td>0.92</td>
</tr>
<tr>
<td>10</td>
<td>221.9</td>
<td>177.9</td>
<td>216.1</td>
<td>0.80</td>
<td>0.97</td>
</tr>
<tr>
<td>11</td>
<td>735.2</td>
<td>794.9</td>
<td>534.5</td>
<td>1.17</td>
<td>0.73</td>
</tr>
<tr>
<td>12</td>
<td>1087.0</td>
<td>976.1</td>
<td>813.6</td>
<td>0.90</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Table 2 gives the confidence intervals for the ratios of means of the three formulations based on a pair of one-sided five percent tests, namely, associated 90 percent confidence intervals.

Table 2. Confidence interval for ratio of means

<table>
<thead>
<tr>
<th>Ratio with subject 4</th>
<th>Without subject 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/R</td>
<td>67.1 - 100.8</td>
</tr>
<tr>
<td>B/R</td>
<td>54.8 - 88.6</td>
</tr>
<tr>
<td>B/A</td>
<td>65.3 - 105.6</td>
</tr>
</tbody>
</table>
According to FDA plus/minus 20 percent criterion, namely, the confidence interval for the ratio of means of the test formulation and the reference formulation is totally within 80 and 120 percent, we may conclude that, with inclusion of subject 4, the formulation A and the reference formulation R are not bioequivalent. However, they are considered to be bioequivalent with subject 4 deleted from the analysis based on the same FDA criterion. Thus, in this case, we can use proposed LD and ED tests to determine whether or not subject 4 is an outlier.

In this example, there are 12 subjects and 3 different formulations involved in the study. That is, we need to specify $k=12$ and $n=3$ in the proposed SAS procedure. The SAS program produces the following results:

### Deleted Subject

<table>
<thead>
<tr>
<th>Subject</th>
<th>Theta1 Estimate</th>
<th>Theta2 Estimate</th>
<th>Theta3 Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>623.4</td>
<td>41779.0</td>
<td>181196.2</td>
</tr>
<tr>
<td>2</td>
<td>635.5</td>
<td>45414.3</td>
<td>182131.3</td>
</tr>
<tr>
<td>3</td>
<td>648.7</td>
<td>45489.2</td>
<td>182571.5</td>
</tr>
<tr>
<td>4</td>
<td>600.8</td>
<td>11636.7</td>
<td>166995.1</td>
</tr>
<tr>
<td>5</td>
<td>667.7</td>
<td>45319.3</td>
<td>154637.8</td>
</tr>
<tr>
<td>6</td>
<td>666.7</td>
<td>45196.2</td>
<td>160535.6</td>
</tr>
<tr>
<td>7</td>
<td>682.6</td>
<td>45679.2</td>
<td>182699.9</td>
</tr>
<tr>
<td>8</td>
<td>637.1</td>
<td>44887.1</td>
<td>186995.4</td>
</tr>
<tr>
<td>9</td>
<td>634.7</td>
<td>44636.8</td>
<td>189008.9</td>
</tr>
<tr>
<td>10</td>
<td>685.4</td>
<td>45051.7</td>
<td>152764.6</td>
</tr>
<tr>
<td>11</td>
<td>632.5</td>
<td>43812.0</td>
<td>157602.1</td>
</tr>
<tr>
<td>12</td>
<td>607.9</td>
<td>43784.7</td>
<td>157437.9</td>
</tr>
</tbody>
</table>

### Likelihood Distance

<table>
<thead>
<tr>
<th>PR &gt; CHI Estimates Distance</th>
<th>PR &gt; CHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>0.0969</td>
</tr>
<tr>
<td>0.15</td>
<td>0.9682</td>
</tr>
<tr>
<td>0.16</td>
<td>0.9727</td>
</tr>
<tr>
<td>0.30</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.37</td>
<td>0.9459</td>
</tr>
<tr>
<td>0.20</td>
<td>0.9781</td>
</tr>
<tr>
<td>0.07</td>
<td>0.9950</td>
</tr>
<tr>
<td>0.13</td>
<td>0.9909</td>
</tr>
<tr>
<td>0.08</td>
<td>0.9938</td>
</tr>
<tr>
<td>0.94</td>
<td>0.8121</td>
</tr>
<tr>
<td>0.87</td>
<td>0.9049</td>
</tr>
<tr>
<td>0.28</td>
<td>0.9646</td>
</tr>
</tbody>
</table>

In the printout, the row indicated by deleted subject 1 gives the MLE estimates of parameters as well as the calculated test statistics and associated p-values with the exclusion of subject 1 from computations. In this case, with subject 4 deleted, the parameter estimates are quite different from other MLE's, which indicates subject 4 can be highly influential. Furthermore, the p-values of both tests are very close to zeros. Thus, at one percent level of significance, both likelihood distance and estimates distance test procedures conclude that subject 4 is an outlier under the proposed reduced model.

### References


