BIOEQ4: BIOEQUIVALENCE MACRO TO CREATE BOTH TABLE AND SAS-DATA-SET ACCORDING TO THE FDA BIOEQUIVALENCE GUIDELINES ISSUED IN 1992

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Introduction:

In the pharmaceutical industry bioequivalence analysis is required to prove that a given drug (generic or other) formulation has a similar distribution in the body as the original used in research. Bioequivalence analysis can be done by the company filing a new drug application in order to replace the trial (or reference) formulation with a marketed (or test) formulation, or by a generic company trying to market a generic formulation (test) of the patented (reference) drug. The Food and Drug Administration (FDA) has developed strict guidelines to ensure equivalent distribution of drugs in humans after administration.

Bioequivalence in humans is usually demonstrated using pharmacokinetic (PK) parameters which look at how drugs are distributed into and eliminated from the body. The PK parameters, $C_{max}$, AUC, and $t_{max}$, are estimated by looking at blood, plasma, or serum drug concentrations within a person over time. Bioequivalence analysis can focus on the population, the individual or the average distribution of drug in various subjects. This paper focuses on average bioequivalence and the subsequently required analysis.

Companies must prove average bioequivalence using the FDA guidelines before a New Drug Application (NDA) approvals can be made. The FDA guidelines have evolved over time in order to stay abreast of current statistical and pharmacokinetic methodologies yet provide tangible rules to guide companies in demonstrating bioequivalence. Older guidelines focus on arithmetic means, $\bar{x} = \frac{1}{n} \sum x_i$, while the newer 1992 guidelines focus on the geometric mean, $\bar{X} = \left( \prod x_i \right)^{1/n}$. The BIOEQ4 macro looks at the geometric mean of the ratios of an individual’s test and reference PK parameters as well as the ratio of the arithmetic means. Various statistical inferences and tests are then calculated based on these two forms.

Statistical Background:

Bioequivalence is usually demonstrated by implementing a basic $2 \times 2$ crossover where one group of subjects receives formulation A followed by washout time and then receives formulation B. The second group of subjects receives the formulations in reverse order. Occasionally a more intricate design than a $2 \times 2$ crossover is implemented and requires a different specification of the statistical model. The BIOEQ4 macro is capable of managing any design comparing two formulations or treatments and can be easily altered to handle studies that attempt to prove bioequivalence among several formulations.

The FDA requires companies use an ESTIMATE statement, which takes into consideration dependencies when estimating a standard error, to find the correct estimates of the mean difference and corresponding standard errors of the PK parameters. Since the ESTIMATE statement is the only way in PROC GLM (in SAS version 6.09) to include covariances when estimating the error of difference in least square means and there is no easy way to output and handle the results from an ESTIMATE statement, automating a bioequivalence analysis is virtually impossible.

A key component involved in the automation of bioequivalence analysis is the consideration of the covariance structure. By using the LSMEANS statement and options to output the variance-covariance matrix and using the following equation:

$$\text{Var}(\bar{X}_T - \bar{X}_R) = \text{Var}(\bar{X}_T) + \text{Var}(\bar{X}_R) - 2 \times \text{Cov}(\bar{X}_T, \bar{X}_R)$$

one can find the variance of the difference in means which corresponds to the results of the FDA-required ESTIMATE statement. The following estimate statement will calculate the LS mean difference (test - reference) where the formulations are ordered as reference first and test second in CLASS page of PROC GLM and also when the data is sorted using PROC SORT.

```
ESTIMATE 'TEST - REFERENCE' TRT -1 1;
```

Formats may affect this ordering which is crucial to the correct results when BIOEQ4 is used.

The variance/covariance matrix can be estimated by using the following equation:

$$\text{Variance Covariance} = (X'X)^{-1} \sigma^2 = \begin{bmatrix}
\text{Var}(\bar{X}_R) & \text{Cov}(\bar{X}_R, \bar{X}_T) \\
\text{Cov}(\bar{X}_T, \bar{X}_R) & \text{Var}(\bar{X}_T)
\end{bmatrix}$$

where $X$ is the design matrix and $\sigma^2$ is the estimate of the true error variability. A cross section of the data created by the LSMEANS statement has the following structure:
Sample part of data set output by LSMEANS statement within PROC GLM.

The variables COV1 and COV2 correspond to the 2 by 2 variance-covariance matrix

\[
\begin{bmatrix}
0.00216504 & 0.00000173 \\
0.00000173 & 0.00216504
\end{bmatrix}
\]

By applying the data to the equation:

\[\text{Var}(\bar{X}_T - \bar{X}_R) = \text{Var}(\bar{X}_T) + \text{Var}(\bar{X}_R) - 2 \cdot \text{Cov}(\bar{X}_T, \bar{X}_R)\]

the estimate of the Variance of the difference in LS means can be estimated as follows:

\[\text{Var}(\bar{X}_T - \bar{X}_R) = 0.00216504 + 0.00000173 - 2 \cdot 0.00000173\]

Involved in the analysis are the estimates based on the ratio (test/reference) of geometric means, the ratio of the arithmetic means and the difference of the arithmetic means. The difference of the means can be found simply by using the LSMEANS statement to find the formulation means and then calculating the difference. The confidence intervals can then be calculated accordingly using the above estimates, as follows:

\[\bar{X}_R - \bar{X}_T \pm t_{\alpha/2} \cdot \sqrt{\text{Var}(\bar{X}_R - \bar{X}_T)}\]

The geometric mean of the ratios can be found by using the log-transformed data in the GLM procedure to calculate an arithmetic mean (or average) from the LSMEANS statement and subsequently exponentiating the result. The following equation may clarify the process. A geometric mean of the ratios of test to reference could be expressed as:

\[
\left( \prod_{i=1}^{n} \frac{x_{n_i}}{x_{R_i}} \right)^{1/n} = \exp \left( \ln \left( \prod_{i=1}^{n} \frac{x_{n_i}}{x_{R_i}} \right) \right)^{1/n}
\]

\[= \exp \left( \frac{1}{n} \ln \left( \prod_{i=1}^{n} \frac{x_{n_i}}{x_{R_i}} \right) \right)
\]

\[= \exp \left( \frac{1}{n} \sum_{i=1}^{n} \ln \frac{x_{n_i}}{x_{R_i}} \right)
\]

\[= \exp \left( \frac{1}{n} \sum_{i=1}^{n} (\ln X_{n_i} - \ln X_{R_i}) \right)
\]

\[= \exp \left( \frac{1}{n} \sum_{i=1}^{n} \ln X_{n_i} - \frac{1}{n} \sum_{i=1}^{n} \ln X_{R_i} \right)
\]

where \(\bar{X}\) is the mean using log-transformed data,

\(X_{n_i}\) is the value of the test measurement of the \(i^{th}\) subject, and

\(X_{R_i}\) is the value of the reference measurement of the \(i^{th}\) subject.

Likewise one can also find the estimate of the standard error and confidence limits of the ratios simply by exponentiating the corresponding standard error and confidence limits of the difference in means obtained using log-transformed data:

\[
\exp \left( \bar{X}_R - \bar{X}_T \pm t_{\alpha/2} \cdot \sqrt{\text{Var}(\bar{X}_R - \bar{X}_T)} \right)
\]

Power associated with the F-test testing for differences in formulation is calculated assuming that one is either interested in a difference (test-reference) no larger than \(\log(1.2)\) for the log-transformed data or 0.2 x reference mean for untransformed data. The noncentrality parameter can be calculated as follows:

\[
NC = \frac{\delta^2}{\text{Var}(\bar{X}_R - \bar{X}_T)}
\]

where

\[
\delta = \log(1.2)\] for log-transformed data,

\[= 0.2 \times \text{reference mean} \] for non-transformed data,

\[
\text{Var}(\bar{X}_R - \bar{X}_T) = \text{variance of the difference of the means}.
\]

A situation where the power reaches 80% is often referred to as the ±80/20 rule since bioequivalence is declared if the test mean falls within 20% of the reference mean with 80% power.

The power calculation may be slightly different from those of other programs since it includes covariances.
that may possibly exit in the estimate of the treatment mean difference standard error. Many noncentrality parameter estimates are simplified by using either of the following equations:

\[ NC = \left( \frac{\delta}{\delta \cdot \sqrt{2/n}} \right)^2 = \frac{\delta^2 \cdot n}{\delta^2 \cdot 2} \]

or

\[ NC = \left( \frac{\delta}{\delta \cdot \sqrt{1/n_R + 1/n_T}} \right)^2 = \frac{\delta^2}{\delta^2 \cdot (1/n_R + 1/n_T)} \]

which do not include covariance structures in the estimate of the difference of the LS means. Thus, the power estimate may be slightly different than in a simple software package, though it is a more accurate estimate.

Macro:

The macro is set up in several sections: data preparation, statistical analysis, bioequivalence analysis, and table creation. Various amenities such as by-variable processing, printing/no-printing, and table titling and footnoting are concurrently programmed within the sections. The macro can easily be altered to suit other situations if there is adequate understanding of the GLM procedure and its output.

The data can be entered into SAS and be structured so that each subject’s PK parameters for one formulation and all by-processing variables be located within one record, as shown below:

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>DOSE</th>
<th>FORMULA</th>
<th>SEQ</th>
<th>DAY</th>
<th>TREAT</th>
<th>CMAX</th>
<th>TMAX</th>
<th>AUCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 A</td>
<td>STR('MONT')</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10 B</td>
<td>STR('WWPI')</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sample data set before invoking BIOEQ4

DOSE corresponds to a by-variable for processing while the values of CMAX, TMAX and AUCT are PK parameters that will be on the left hand side of the model equation in the GLM procedure. FORMULA is the variable that defines the bioequivalence comparators. The variables SEQUENCE, PERIOD and SUBJECT are common statistical effects used in a crossover Analysis of Variance (ANOVA).

BIOEQ4 can be invoked by using the following code:

```sas
%BIOEQ4(
    DRUGXPPP, %* input data set;
    BIOEQ, %* output data set;
    PK1 PK2 PK3, %* PK variables;
    3, %* number of PK vars;
    DOSE, %* by-variable;
    %STR('MONT'), %* Reference formulation;
    %STR('WWPI'), %* Test formulation;
    SUB(SEQ) SEQ DAY TREAT, %* stat model;
    SUB SEQ DAY TREAT, %* Class variables;
    TREAT, %* Formulation var;
    TITLE1, TITLE2, TITLE3, %* Formulation var;
    %STR("CMAX" "TMAX" "AUCT"), %*Labels;
    FOOTNOTE1, FOOTNOTE2, PRINT, %* Print results and output;
    )
```

and changing the above information to correspond to the desired set of data.

BIOEQ4 will rename the variables (to VAR1, VAR2, VAR3, etc) to simplify naming conventions throughout BIOEQ4, calculate the log-transformed data and then proceed to use the GLM procedure to analyze both log-transformed and untransformed data according to the specified model.

By using the OUTSTAT option in the GLM procedure, the degrees of freedom associated with the \( \hat{\sigma}^2 \) (estimate of the error) can be obtained. Below is a sample of the data produced by this option. DOSE refers to one of the by-processing variables, LVAR1 shows that the analysis was performed on the log-transformed data of the first variable entered into the macro, and _SOURCE_ refers to the effect entered into the model statement.
Industry Applications

Sample of the data set created with the OUTSTAT option within PROC GLM:

An LSMEANS statement with COV and OUT options is included in the GLM procedure to create a SAS-data-set containing the LS means as well as the variance-covariance matrix.

<table>
<thead>
<tr>
<th>S</th>
<th>O</th>
<th>N</th>
<th>U</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>A</td>
<td>R</td>
<td>Y</td>
<td>P</td>
</tr>
<tr>
<td>O</td>
<td>M</td>
<td>C</td>
<td>P</td>
<td>R</td>
</tr>
<tr>
<td>B</td>
<td>S</td>
<td>E</td>
<td>E</td>
<td>D</td>
</tr>
<tr>
<td>S</td>
<td>E</td>
<td>-</td>
<td>-</td>
<td>F</td>
</tr>
</tbody>
</table>

1 25 LVAR1 DAY SS3 2 0.48 3.59 0.05
2 25 LVAR1 ERROR ERROR 24 1.39 0.19
3 25 LVAR1 SEQ SS3 2 0.17 0.46 0.63
4 25 LVAR1 SUB (SEQ) SS3 23 8.54 6.38 0.01
5 25 LVAR1 TREAT SS3 1 0.16 1.52 0.23

Sample output from LSMEAN statement with option COV

DOSE is again the by-processing variable, _NAME_ gives the names of the variables that correspond to those entered into the macro (VAR1 = CMAX, VAR2 = TMAX, etc) as well as their log-transformed counterparts (LVAR1, LVAR2, etc), FORM denotes formulation or treatment that defines the bioequivalence comparator, LSMEAN contains the LS mean, and COV1 and COV2 respectively correspond to columns one and two of the variance-covariance matrix associated with the LS mean estimates.

The Power associated with finding a difference in the means can be calculated by using the PROBF function with the error degrees of freedom (df) found in the data set created by using the OUTSTAT option in the GLM procedure statement, the noncentrality parameter and F-statistic as follows:

\[
\text{Power} = 1 - \text{Probf(Probfinv(.90,1,\text{df},0),1,\text{df},\text{nc})}
\]

where

\[
\text{NC} = \frac{\delta^2}{\text{Var}(\bar{X}_r - \bar{X}_g)}
\]

\[
\delta = \log(1.2) \text{ for log-transformed data,}
\]

\[
= 0.2 \times \text{reference mean for non-transformed data.}
\]

One and two dimensional arrays are heavily used throughout the statistical and bioequivalence sections to refer to the statistics and variables for each of the PK parameters. When a two dimensional array is used, it helps distinguish transformed and non-transformed data and their corresponding statistics.

The remainder of BIOEQ4 contains program code in the form of a DATA _NULL_ to develop a simple table containing only bioequivalence statistics. The table is created assuming a line size of 132 and page size of 60 (usually landscape). The code can be easily modified to create whatever table is desired since it is a typical DATA _NULL_ data statement. Following the SAS program code is a more realistic copy of the table in readable format.

One data set is provided containing the necessary information for those users who would like to customize their tables structure and format.

Printed results can be obtained from the macro to verify various steps of the data processing. These results can be used to check the end results of the macro at various points in the analysis, to assure the macro user that she/he is using BIOEQ4 correctly, and to verify the results against previously used data processes.

BIOEQ4 can easily be developed to handle statistical designs that have more than two formulations or treatments present. The issue of main concern is expanding the code to find the correct variance-covariance structures out of a matrix (n x n where n is the number of formulations or treatments) for each specific comparison to the reference.
Sample table created by BIOEQ4:

<table>
<thead>
<tr>
<th>Sample table created by BIOEQ4:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

The above figure is a bitmap file showing the general structure and organization of the actual table BIOEQ4 creates. The full table assumes SAS fonts and is created with a Data_Null_.

BIOEQ4 provides most required statistics from the FDA’s 1992 guidelines to as well as some older statistics that were used to test average bioequivalence. It does not provide outlier analysis, nonparametric analysis or other analysis that may have been referenced in the 1992 guidelines.

Conclusion:

Average bioequivalence analysis programs exist in which one must hand enter the data to be used and have a fairly simple experimental design; however, if there is a wealth of data to be used in the analysis or if a more complex design than a 2 by 2 crossover design is implemented, this process can be cumbersome and difficult, if not impossible.

This bioequivalence macro allows the data to be entered into SAS in whatever manner desirable and then allows one to specify the statistical model used in the PROC GLM statement and returns the bioequivalence estimates, in both data set and table format, according to the FDA’s specifications. Thus regardless of the complexity of the statistical model or the enormity of the data, this macro enables one to expedite the results of the bioequivalence trial and meet new drug application (NDA) deadlines in a more timely manner.

All data and references to drug and protocol names have been changed in this paper to protect the confidentiality policies of the Wyeth-Ayerst Research. Furthermore, in order to fit many of the data examples into a two column format of this paper, numbers may have been truncated and changed in the word processing software and may not necessarily reflect the true nature of the data (numeric formats may be different and variance covariance matrices may not be symmetric, nonsensical statistical results, etc.).

This code is continually evolving to reflect the changes and adaptations of Phase I bioequivalence analysis for various governmental agencies. In no way does the BIOEQ4 macro claim to perform all required analysis for any agency at any one point in time since requirements may change. Furthermore, recognition should be made to Stephanie Giel for her contributions in converting the guidelines to SAS code, without which this macro would have been extremely difficult to create.

Endnotes:

BIOEQUIVALENCE PARAMETER ESTIMATES WHERE DOSE IS 17.5 MG

<table>
<thead>
<tr>
<th>Power Based on Log-Transformed Data (%)</th>
<th>Cmax</th>
<th>Tmax</th>
<th>AUCT</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>79</td>
<td>76</td>
<td>96</td>
<td>97</td>
</tr>
</tbody>
</table>

Ratio of Least Squares Geometric Means *(%) 

<table>
<thead>
<tr>
<th>90% Confidence Limits Around the Ratio of the Geometric Means *(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>109</td>
</tr>
<tr>
<td>93 - 121</td>
</tr>
<tr>
<td>90 - 123</td>
</tr>
<tr>
<td>92 - 111</td>
</tr>
<tr>
<td>92 - 110</td>
</tr>
</tbody>
</table>

Ratio of Least Square Arithmetic Means 

<table>
<thead>
<tr>
<th>Two One-Sided Tests:</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(R&lt;0.8)</td>
</tr>
<tr>
<td>P(R&gt;1.2)</td>
</tr>
</tbody>
</table>

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.001</td>
<td>0.04</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Based on the Mean Square Error and LS Means from the Log-Transformed ANOVA

This is where the footnote goes

Sample BIOEQ4 table 2.2.4.5.6.7