

Use Of Macros In Generating Statistical Comparisons For Bioequivalence Reports

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Introduction

With health care costs rising at steady rates, consumers are seeking to save money wherever they can. One way of cutting health costs is to choose generic equivalent drugs over brand-name drugs. Generics offer substantial savings over brand-name drugs in both over-the-counter and prescription drugs. But, are these generics really equivalent to the brand-name drugs they seek to replace?

For a generic drug to be marketed, the company manufacturing it must submit an application for approval to the Food and Drug Administration (FDA). This application is supported by clinical trials which seek to show statistically that the generic is, within set limits, equivalent to the brand-name drug. A basic trial is set up as a two-way crossover. The test formulation (generic) and the reference formulation (brand-name) are both given to the same two groups of subjects. One group will get the test form first, followed by a washout period, then the reference form. The other group will get the formulations in the opposite order. In this way, each subject serves as his own control because the subject got both study formulations. This helps reduce the number of subjects needed to achieve an acceptable level of statistical power for the tests.

The subjects have blood samples taken immediately before dosing and at specified intervals after dosing. These samples are analyzed quantitatively for the concentration of the drug in the sample. When the concentrations are plotted versus time, a profile appears. This plot of the time course of the drug through the body is the pharmacokinetic profile of the drug. The profile can be analyzed for certain pharmacokinetic parameters such as the maximum concentration (C_{MAX}), time of C_{MAX} (T_{MAX}), the trapezoidal area under the concentration-time curve (AUC), and the half-life of elimination (HALF-LIFE).

The natural logarithms of the C_{MAX} and AUC are also of interest. These parameters for the test formulation are compared to those of the reference formulation to see if the pharmacokinetics of the test form are close enough to those of the reference form for it to be called equivalent. The theory here is that if the pharmacokinetic profile is the same, the effect profile will be the same.

The FDA requires certain statistical tests to be done in order to show the equivalence of the pharmacokinetics, known as bioequivalence. The test formulation must be within $\pm 20\%$ of the reference formulation to be considered bioequivalent. The primary basis of comparison is a 90% confidence interval based on calculations of two one-sided tests (1). This interval must be within 80% to 120% of the reference value for untransformed parameters and 80% to 125% for log-transformed parameters for bioequivalence to be concluded. Secondary tests include calculation of the *a posteriori*, or post-study, power and the p-value of the test of the hypothesis that the two formulations mean values are equal ($H_0: \mu_t = \mu_r$). Harris includes a 95% confidence interval calculation and a calculation of the mean ratio of the log-transformed parameters which are required for Australian (2) and Canadian (3) submissions.

Harris Laboratories has developed a set of SAS procedure and DATA step code and macros to facilitate the calculation of these tests and the generation of report summary tables, listings, and figures. Restrictions of this code are that only pairwise comparisons are made (one test treatment versus one reference treatment). The output files are created as 144 column width, centered with left justified headings and centered titles and footnotes. The figures are generated as PostScript files.

The SAS code to perform these calculations and generate the tables and figures was developed from examples in literature (4) and evolved over several years with tests being added or removed or refined. The problem was that all the calculations were being done by a batch job with the data edited into the program itself for each comparison. This led to transcription errors and made the program unfriendly. There were a very limited number of people who could make use of the program. Also, the program could not be easily modified to keep up with new developments in database use at Harris. So, the macro-izing of the program was undertaken. Now, the program is fairly transparent to the end user and does not require knowledge of SAS to run. Also, the program can now be dovetailed into the database system at Harris much more easily.

Example Study

The data used in the example are from a two-way crossover study in 26 subjects. This single dose study has the following pharmacokinetic parameters:

CMAX	= maximum concentration
TMAX	= time of CMAX
AUC	= Area under the curve to the last measurable concentration
AUC-INF	= AUC extrapolated to infinite time
KEL	= elimination rate constant
HALF-LIFE	= elimination half-life
LCMAX	= $\ln(\text{CMAX})$
LAUC	= $\ln(\text{AUC})$
LAUC-INF	= $\ln(\text{AUC-INF})$

The pharmacokinetic parameters along with the subject number (VOLN), treatment identifier (TREAT), study period (PHASE), and treatment sequence (SEQ) are included in the dataset HERE.TEST which is first sorted by TREAT VOLN into WORK.TOTAL. The macros called later are looking for the WORK.TOTAL dataset.

Statistical Calculations

The statistical calculations are based on an analysis of variance (ANOVA) using the treatment sequence, subject within sequence, treatment, and period effects in the model. Least square means for each treatment are calculated for the parameters and are used in the statistical calculations.

The equation for the confidence intervals is:

$$\text{Eq. 1. } \text{limit} = (\text{lsmean}_t - \text{lsmean}_r) \pm (t * \text{SE}) \quad (1),$$

where limit is the lower or upper limit of the interval, lsmeant is the test least-square mean, lsmeanr is the reference least-square mean, t is the t value at either 0.05 (for 90%) or 0.025 (for 95%). SE is calculated by:

$$\text{Eq. 1.1 } \text{SE} = \text{RMSE} * \sqrt{0.5 * (1/n1 + 1/n2)},$$

where RMSE is the square root of the mean square of the error term from the ANOVA and n1 and n2 are the number of subjects in each treatment sequence group. For a balanced two-way crossover study, $n1 = n2 = N/2$, where N is the number of subjects in the study. The limit is converted to a percentage of lsmeanr for easier comparison to the 80%-120% or 80%-125% limits.

Power is calculated by the following:

$$\text{Eq. 2.1 } T = [(\text{lsmean}_t * 0.2)/(\text{SE})] - t_{0.025}, \text{ then}$$

$$\text{Eq. 2.2 } \text{power} = \text{probt}(T, \text{df}) \quad (3),$$

where df is the degrees of freedom of the error term from the ANOVA. The power is expressed as a percentage. For example, a power of 0.8 is expressed as 80%. There is a slight modification of Eq. 2.1 for the log-transformed parameters:

$$\text{Eq. 2.3 } T = [0.2/(\text{SE})] - t_{0.025}.$$

The mean ratio of the log-transformed parameters is calculated by:

$$\text{Eq. 3.1 } \text{Mean ratio} = 100 * \exp(\text{lsmean}_t - \text{lsmean}_r) \quad (4).$$

Macro Variables

The GLOBAL macro variables used by all of the macros are:

CLIENT	=	client name for page headers
PROTOCOL	=	protocol number for page headers
HARRIS	=	Harris Labs. project number for page headers
CM	=	output file prefix

Macro variables that are specific for the comparison of interest are:

CCM	=	compound of interest
T1	=	test treatment id
T2	=	reference treatment id
TRT1	=	test treatment name
TRT2	=	reference treatment name
N1	=	number of subjects on first treatment sequence (N/2)
N2	=	number of subjects on second treatment sequence (N/2)
FIGURE	=	first figure number
PLIST	=	ordered list of parameters to be plotted
TABLE	=	summary table number

Program Flow

A flowchart of the SAS program is included in Attachment A. The macro code is inserted into the program by using the %INCLUDE statement. This allows the macros to be updated without having to make changes to a number of programs.

Macro SETCNTR

This macro is called to include the code which sets the CNTR variable in the datasets and to create and modify the PARAM variable. Underscores in the parameter names are replaced by hyphens for better presentation. SETCNTR is not included on the flowchart in Attachment A.

Macro PTITLE(CCM,T1,T2,TRT1,TRT2)

```
.  
.   
.   
TITLE "&CLIENT.";   
TITLE2 "&PROTOCOL.";   
TITLE3 "&HARRIS.";   
TITLE5 "&CCM. - Treatment &t1. versus Treatment &t2.";   
FOOTNOTE "Test Treatment: TREAT &t1. = &trt1.";   
FOOTNOTE2 "Reference Treatment: TREAT &t2. = &trt2.";   
%MEND PTITLE;
```

The PTITLE macro takes CLIENT, PROTOCOL, HARRIS, CCM, T1, T2, TRT1, and TRT2 and creates the TITLE and FOOTNOTE statements used by the procedures generating text output. Since the CENTER options is active, but the page headers or blurbs are to be left-justified, the macro variables CLIENT, PROTOCOL, and HARRIS are padded with blanks to 144 characters in length.

Code Section 1

The first section of code used to generate the outputs consists of PRINT, MEANS, and GLM procedures. The data is sorted out to the TOTAL dataset by TREAT and then printed, and averaged by TREAT. The GLM procedure is used to calculate the ANOVA because general linear models are less affected by unbalanced data. Also, GLM calculates least-square means and has output options available to send statistical information to work datasets. These output options are the OUTSTAT option in the PROC GLM statement and the OUT= option in the LSMEANS statement. A CONTRAST is used to test the hypothesis of equality instead of the more desirable ESTIMATE statement because with the OUTSTAT option, the CONTRASTs are written to the output dataset while ESTIMATE data are not. This way the p-value of the hypothesis test is output to the OUTSTAT dataset for use later. Other values output to OUTSTAT are the sums of squares and degrees of freedom for each effect tested and the error term. The _TYPE_ of sum of square and the _SOURCE_ are listed for each observation.

Code Section 2

Code Section 2 includes OUTSTAT2 DATA step. OUTSTAT2 assigns a counter variable to each parameter using the SETCNTR macro and selects the error term data from the OUTSTAT dataset (_TYPE_="ERROR"). Since the RMSE is not on the OUTSTAT dataset, it is calculated by taking the square root of the sum of squares divided by the degrees of freedom.

Macro PROBY(T1,T2)

DATA PROB&T1.&T2.; SET OUTSTAT;

IF _TYPE_="CONTRAST" AND _SOURCE_="TREAT &T1. VS TREAT &T2.";

.

.

.

PROB&T1.&T2.=PROB;

OUTPUT;

RUN;

.

.

.

%MEND PROBY;

The macro PROBY creates work datasets containing the equality hypothesis p-values for each comparison being investigated. In the example, the two treatments involved are A and B. These values are assigned to the macro variables T1 and T2. The dataset PROBAB is created from OUTSTAT by selecting the CONTRAST data with _SOURCE_="TREAT A VS TREAT B" and assigning the value in PROB to PROBAB. The CNTR variable is also created. The dataset is then sorted by CNTR. The use of a macro here facilitates multiple pairwise comparisons. For example, in a three-way crossover study, comparisons of A to B, A to C, and B to C are commonly needed. Then, three CONTRAST statements are used and three datasets, PROBAB, PROBAC, and PROBBC are created by three calls to macro PROBY.

Macro LSMEANY(T)

DATA LSM&T.; SET LSMEANS;

IF TREAT="&T.";

.

.

.

LSMEAN&T.=LSMEAN;

RUN;

.

.

.

%MEND LSMEANY;

The macro LSMEANY selects out the least-square mean information for each treatment. The LSMEAN statement in the GLM procedure calculates the lsmeans for each parameter by the variable TREAT. The OUT= option outputs the lsmeans to the LSMEANS dataset. The treatment to be selected is passed to the macro variable T and is used to select the proper value of TREAT. Again, the CNTR value is set and the PARAM value created. For the two-way crossover, two calls are made to macro LSMEANY, one for Treatment A and one for Treatment B. The two datasets, LSMEANA and LSMEANB, are sorted by CNTR.

Macro LCOMB(T1,T2,N1,N2)

```
.
.
.
PROC PRINTTO NEW PRINT="&CCM.&T1.&T2.TEST.LIS"; RUN;
DATA LSM&T1.&T2.; MERGE OUTSTAT2 LSM&T1. LSM&T2.; BY CNTR;
DATA PARM; SET LSM&T1.&T2.;
REFERENC=LSMEAN&T2.;
TESTT=LSMEAN&T1.;
PVALUE=PROB&T1.&T2.;

SE=RMSE*SQRT(0.5*(1/&N1. + 1/&N2.));
.
.
.
RUN;
.
.
.
%MEND LCOMB;
```

LCOMB is the workhorse macro of the set. This set of instructions merges together data for the two treatments and does the calculations of power, confidence intervals, and mean ratios. The output for each set of calculations is routed to a file by PROC PRINTTO for inclusion in the statistical appendix of the report. LCOMB is passed values for T1 and T2, the treatment IDs for the treatments being compared, and for N1 and N2, the number of subjects in each treatment sequence for a 2-way crossover, or N/2 each for a more than 2-way crossover.

Data LSMAB merges the PROBAB, LSMEANA, LSMEANB, and OUTSTAT2 datasets which have been sorted by CNTR.

Data PARM begins calculation of the statistics. The least-square means, degrees of freedom, RMSE, and p-values are brought in from LSMAB. The SE is calculated. The variables T_90 and T_95 represent the $t_{0.05}$ and $t_{0.025}$ parameters, respectively. The variable LOGPARAM is set to 'YES' if the first character of PARAM is an "L" indicating a log-transformed parameter.

Data POWER calculates the power for each comparison. The variables ACT_DIFF and ACT_PCT are the actual difference between the least-square means and actual difference as a percent of the reference least-square mean. The POWER is calculated and then converted to a percentage. If the reference mean is zero, all calculated values are set to missing.

Data steps ONESIDE and ONESIDE2 calculate the 90% and 95% confidence intervals, respectively. Variables LL and LL2 are the lower limits and UL and UL2 are the and upper limits. In ONESIDE2, the mean ratio of the log-transformed parameters is also calculated.

The data sets POWER, ONESIDE, and ONESIDE2 are sorted by CNTR and printed out to the output file which is included in an appendix in the report.

```
Macro LGRAPH(CCM,T1,T2,TRT1,TRT2,FIGURE,PLIST)
TITLE1 F=SIMPLEX J=L H=.3 CM LS=2 CM " &CLIENT."
      J=L      " &PROTOCOL."
      J=L      " &HARRIS.";
.
.
.
FILENAME GRAPH "&CCM.&T1.&T2.TEST.GSF";
.
.
.
PROC GPLOT GOUT=GRAPH&T1.&T2.;
TITLE2 F=SWISS LS=0.5 CM H=0.4 CM "Figure &FIGURE.";
.
.
.
TITLE6 H=.4 CM F=SWISS "&CCM. - Treatment &T1. versus Treatment &T2.";
.
.
.
%MEND LGRAPH;
```

The LGRAPH macro generates graphical representations of the 90% and 95% confidence intervals for each parameter. Since the graphical titles can include a justification (J= option) and must be set up differently to maintain the correct margins, LGRAPH resets the TITLES and FOOTNOTES. The macro variables passed to LGRAPH are T1, T2, TRT1, TRT2, CCM, FIGURE, and PLIST. FIGURE is the figure number for the first of the two plots. It is used to calculate FIGURE2 which is the second figure number. PLIST is the list of parameters being plotted and is used in an AXIS statement to set up the order of plot. The GOPTIONS statement set up the options to generate PostScript plots.

The first data step GRAPH selects data from ONESIDE, the 90% confidence intervals. The lower and upper limits are output as separate observations with the same PARAM value. The vertical axis is free scale, that is, the axis limits are not set in the AXIS statement. So, if all LL and UL values are between 80 and 120, blank values of 70 and 130 are output to the dataset to force the axis to include those values. Otherwise the axis is limited by the minimum value of LL and the maximum value UL.

When plotting, the AXIS1 statement orders the parameters horizontally as they appear in PLIST. The PLIST macro variable includes the parameter names within single quotes. Reference lines are plotted at 80, 100, 120, and 125 on the vertical axis to indicate critical levels of the interval. The symbol used to plot the interval is I=HILOB, which creates a box between the minimum and maximum values for each parameter. The page header is set up as left-justified with leading blanks to get the correct amount of margin, while the figure titles are centered. The footnotes are also left-justified.

The second data step GRAPH performs the same functions as before, but with the ONESIDE2 data for the 95% confidence intervals. The GPLOT is also the same except using FIGURE2 as the figure number. Both figures are GREPLAYed to produce the correct margins.

```

Macro STATRPT(T1,T2,TRT1,TRT2,CCM,TABLE)
.
.
.
PROC PRINTTO NEW PRINT="&CCM-STATS-TABLE.LIS"; RUN;
.
.
.
TITLE5 "Table &TABLE.";
TITLE7 "Bioequivalence With Respect to &CCM.";
TITLE9 "Treatment &T1. versus Treatment &T2.";
PROC REPORT....;
.
.
.
COMPUTE AFTER;
.
.
.
LINE @17 "Treatment &T1. = &TRT1. - test";
LINE @17 "Treatment &T2. = &TRT2. - reference";
.
.
.
endcomp;

run;
quit;
%MEND STATRPT;

```

Macro PTITLE is rerun to set up the first three titles for STATRPT. The TITLE5 and the FOOTNOTES are reset in STATRPT.

STATRPT produces a summary table of the statistical comparisons. This table appears in the main part of the report and is referred to in the discussion and conclusions. The variables passed are T1, T2, TRT1, TRT2, CCM, and the table number, TABLE.

First, the sets POWER, ONESIDE, and ONESIDE2 are sorted by CNTR. This is probably unnecessary as the datasets were previously sorted, but has been left in just in case. The three sets are then merged by CNTR.

The footnotes are cleared and the options set to produce a 144 column, 48 line output with no page numbers, no date, and centered. Old output files are deleted to insure that the file printed for the report is the latest iteration.

The output is routed by PROC PRINTTO and the merged file resorted if necessary. The table header and titles are set up by PTITLE. Again, in this macro, the variables CLIENT, PROTOCOL, and HARRIS are padded to 144 characters to force them to be left-justified.

PROC REPORT is used to generate the table. The COLUMN statement orders the variables to be printed as PARAM, TESTT, REFERENC, ACT_PCT, PVALUE, Z, POWER, LL, ZZ, UL, LL2, ZZ2, UL2, and RATIO. Z is a computed variable that is set to "*" if PVALUE is less than 0.05. This indicates a

statistically significant PVALUE. ZZ and ZZ2 are also computed, but are simply hyphens (" - ") to go between the lower and upper confidence interval limits. Because the dataset has been sorted out by CNTR, no GROUP or ORDER variables are defined. The table footnotes are set up in the LINE statements of the COMPUTE AFTER block.

Summation

Prior to the development of these macros, the procedure for doing a comparison of pharmacokinetic data was to run the GLM procedure and print the output. The RMSE, degrees of freedom, N, p-values, and LSMEANS would then be transcribed manually into the comparison program. The TITLES and FOOTNOTES would be edited and the program run. This amount of manual entry led to a larger than acceptable number of transcription errors and took a relatively large amount of time. Use of the output options in the GLM procedure and macros to select data from the output datasets and calculate the statistical measures have greatly decreased the incidence of transcription error and shortened the time needed to accomplish the task. There has not been a great improvement in CPU efficiency. The integration of the SAS macros and code with the newly developed database at Harris should further increase accuracy and time savings.

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