Application of component-wise rank method to bivariate bioequivalence case

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One of the most common analyses in pharmaceutical research is bioequivalence test of two drugs. The FDA has endorsed the usage of Schuirmann's two one-sided hypotheses for the analyses in such studies. Generally, however, several measures on the drugs are taken simultaneously such that the data are multivariate. Nandakumar and McKean generalized Schuirmann's procedure to this multivariate setting. For bivariate data, the results can be summarized in a graphical display. These procedures are least-squares-type procedures and hence, are quite sensitive to mild outliers. To counter this sensitivity, Nandakumar and McKean also developed a simple highly efficient and robust analogue to their multivariate least-squares procedure. The robust results can also be displayed graphically, overlaid with the least-squares graphical results. In this paper, a SAS algorithm is presented, which implements these least-squares and robust multivariate tests for bioequivalence, including their graphical summaries.

Keywords: Component-wise ranks, Average bioequivalence, Hodges–Lehmann's estimator, Confidence ellipse, Robust statistics

Introduction

Bioequivalence studies are performed to demonstrate that different formulations or regimens of drug products are similar to each other in terms of their therapeutic benefit and non-therapeutic side effects. Pharmaceutical equivalents for the drugs are identified in the industry with AUC (area under the concentration versus time curve), Cmax (maximum drug absorbed) and Tmax (time to reach Cmax). Although the data are multivariate, usually, Schuirmann's[1] univariate two one-sided hypotheses test is employed to test this equivalence setup. Usually the measurements are ln-transformed ($\log_e$) because:

1. the ratio, rather than the difference between average parameter data from the two formulations, is of interest;
2. a multiplicative model is postulated for pharmacokinetic measures AUC and Cmax.

There are some issues surrounding the usage of lognormal assumption. Schuirmann's *t*-test fails in the presence of mild outliers.[2,3] Secondly, many bioequivalence studies have very small sample sizes. The problem is also compounded as the normality assumption of the parameters of interest has been challenged by Ghosh and Gonen.[4]

The other paradigm of interest is the usage of multivariate procedures on AUC and Cmax in bioequivalence studies. Even though many multivariate procedures are suggested in the past,[5] they have not been actively implemented by the industry. The FDA in its guidance has previously identified the need of multiple endpoints to support bioequivalence (ex: nasal spray studies). This motivates the necessity of setting up the study as multivariate response with correlated data as we discuss later.

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Component-wise Rank Method

In this section, brief discussion in the development of location and spread of the bivariate problem is presented. This can be easily generalized to a higher level multivariate set-up. The multivariate method (as shown later) proposed by Nandakumar and McKean[2] depends on a vector of location estimators and its variance covariance matrix. The traditional procedure is based on component-wise means. The robust Wilcoxon procedure is based on component-wise Hodges–Lehmann estimator. The Hodges–Lehmann estimator is highly efficient relative to the sample means and its efficiency is 95.5% at the normal distribution. A program to estimate the location[6] using Wilcoxon's signed rank statistic is shown below.

```
%macro pairs(data=, out=, var=, whr=);
proc sort data=&data.;
      by    &var.;
run;

proc sql noprint;
create table data_2 as select a.&var. as
x, b.&var. as y, a.i, b.j
      from
            (select *, monotonic() as i
                  from &data.) a,
            (select *, monotonic() as j
                  from &data.) b
```


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```
      order by x, y;

create table data_3 as select *
      from data_2
      %if "&whr."="leq" %then %do;
            where i <= j
      %end;
      %if "&whr."="less" %then %do;
            where i < j
      %end;
      order by x, y;

create table data_4 as select (x+y)/2 as
loc
      from data_3;
quit;
proc means data=data_4 noprint;
      var loc;
      output out=loc median=median;
run;

%global loc&var.;
proc sql noprint;
select median into:loc&var.
      from loc;
quit;

data loc;
set loc;
      var="HL&var.";
run;
%mend pairs;

%pairs( Data  =  Name of the input
dataset,
Out    =    Name of the output dataset,
Var    =    Variable whose Hodges-
Lehmann estimate is needed,
Whr    =    is a where statement that can
be one of
1.    %str(leq)
2.    %str(less));
```

It is beneficial to use the 'leq' option over the 'less' option in the above pairs macro as it includes 'n' more observations for analysis. Consider a dummy data created below to execute the above code. This dummy data are used throughout the paper to illustrate the programming steps.

```
data dummy;
      do k=1 to 10;
            x=normal(1);
            y=normal(10);
            output;
      end;
run;
%pairs( Data     =  dummy,
        Out      =  Datax,
        Var      =  X,
        Whr      =  %str(leq));
%pairs( Data     =  dummy,
        Out      =  Datay,
        Var      =  Y,
        Whr      =  %str(leq));
%put &locx.;     0.3407105493
%put &locy.;     -0.355420706
```

From the above program, the Hodges–Lehmann's estimates of location for the variables X and Y are 0.34071 and −0.35542, respectively. The least-squares estimates of location are the sample means which are 0.25530 and −0.35598, respectively.

Consider a bivariate random sample $(X_{1,1},X_{2,1})$, …, $(X_{1,n},X_{2,n})$ with the joint probability density function (pdf) $f(x_1,x_2)$ and marginal pdfs $f_1(x_1)$ and $f_2(x_2)$. Assume that f_1 and f_2 are symmetric about 0. Hettmansperger and McKean[7] in their book lay out the details of the component-wise rank, a robust analogue to the least-squares variance covariance matrix. The component-wise rank method uses the vector of Wilcoxon signed-rank statistics on each component. The covariance matrix can easily be generalized to any $m \times m$ multivariate setup. A consistent estimator of the bivariate covariance is

$$\sum_{HL} = \begin{bmatrix} \tau_A^2 & \tau_A\tau_B\delta \\ \tau_A\tau_B\delta & \tau_B^2 \end{bmatrix}$$

where

$$\tau_i = \frac{1}{(12)^{1/2}\int f_i^2(t)\mathrm{d}t} \text{ and}$$

$$\delta = \frac{1}{n}\sum_{i=1}^{n} \frac{R_{it}R_{jt}}{(n+1)(n+1)} \mathrm{sgn}(X_{it})\mathrm{sgn}(X_{jt})$$

where R_{it} is the rank of $|X_{it}|$ among $|X_{i1}|$, …, $|X_{in}|$ for i=1, 2. The only assumption made for this set-up is that the underlying distribution is symmetric. From the below SAS program, ties are handled by taking averages of the tied ranks. For Wilcoxon-based estimators, correction for ties is not required. Note that the estimates are based on differences of the residuals, so the estimate is invariant to the location estimator. The below SAS code computes the robust covariance matrix using the component-wise rank method. The quantile (qt is usually 0.90 or 0.95) used in the estimation of τ (tau)[8] is for the empirical distribution of the absolute differences of the residuals (see p. 204 of Ref. 7).

```
%macro tau(data=, var=, qt=);
proc sql noprint;
create table data2 as select
abs(a.&var.-b.&var.) as d&var.
      from &data. a, &data. b
      order by d&var.;
quit;

proc univariate data=data2 noprint;
      var d&var.;
      output out=pt pctlpre=p_
      pctlpts=&qt.;
run;

%global &var.;
proc sql noprint;
select p_&qt./sqrt(&n.) into:tn
      from pt;

create table data3 as select a.*,
b.p_&qt.,
```

```
      case when d&var. le &tn. then 1
            else 0
      end as gtn
      from data2 a, pt b;

select sum(gtn)/(&n.**2) into:gtn
      from data3;

select distinct (1/((sqrt(3)/
&tn.)*&gtn.*sqrt((&n.-2)/&n.)))**2
into:&var.
      from data3;
quit;
%mend tau;
%global n;
proc sql noprint;
select max(monotonic()) into:n
      from dummy;
quit;
```

```
%pairs (data=dummy, out=datax, var=x,
whr=%str(leq));
proc append base=_loc_ data=loc;
run;

%pairs (data=dummy, out=datay, var=y,
whr=%str(leq));
proc append base=_loc_ data=loc;
run;

proc transpose data=_loc_ out=_loc_;
var median;
id var;
run;

Proc sql;
create table resid as select a.x-b.HLx as
residx, abs(a.x-b.HLx) as aresidx, a.y-
b.HLy as residy, abs(a.y-b.HLy) as
aresidy
from dummy a, _loc_ b;
quit;

%tau (data=resid, var=residx, qt=90);
%tau (data=resid, var=residy, qt=90);

data resid (drop=i);
set resid;
      array num{*} residx aresidx residy
      aresidy;
      do i=1 to dim(num);
            num{i}=round(num{i},
            .0000000001);
      end;
run;

proc rank data=resid out=a_resid
ties=mean;
     var aresidx aresidy;
     ranks aresx aresy;
run;

proc sql noprint;
select 3*sqrt(&residx.)*sqrt(&residy.)
*sum(rankx*ranky*sign(residx)*
sign(residy))/
(&n.-1) into:covfrom
      (select residx, aresidx, aresx/
(&n.+1) as rankx, residy,
aresidy, aresy/(&n.+1) as ranky
            from a_resid);
quit;

%put &residx.;    2.327785
%put &residy.;    0.276367
%put &cov.;       -0.25962
```

For the least-squares analyses, a similar summary of location (means) and covariance matrix is obtained using the below SAS code.

```
proc corr data=dummy cov;
var x y;
run;
```

Utilizing the dummy data from above, the robust covariance structure using component-wise rank method is

$$\sum_{\mathrm{HL}} = \begin{bmatrix} 2.327785 & -0.25962 \\ -0.25962 & 0.276367 \end{bmatrix}$$

The ordinary least-squares estimate of covariance structure for the same data is

$$\sum_{\mathrm{LS}} = \begin{bmatrix} 1.321717066 & -0.176266298 \\ -0.176266298 & 0.214504740 \end{bmatrix}$$

These location and spread estimators obtained for the least-squares and robust procedures are implemented in the context of bivariate average bioequivalence analyses.

Bivariate Average Bioequivalence Hypothesis

The FDA[9] directs testing the equivalence between two treatments using Schuirmann's two one-sided hypotheses. The limits 0.8 and 1.25 are mandated by the FDA. The multivariate hypotheses take the form

$$\mathrm{Ho}: |\Delta\mu_{\mathrm{AUC}}| > \ln(1{\cdot}25) \text{ or } |\Delta\mu_{\mathrm{Cmax}}| > \ln(1{\cdot}25)$$

$$\mathrm{Ha}: |\Delta\mu_{\mathrm{AUC}}| \leq \ln(1{\cdot}25) \text{ and } |\Delta\mu_{\mathrm{Cmax}}| \leq \ln(1{\cdot}25)$$

where $\Delta\mu_{\mathrm{AUC}}$ is the mean difference between the two drugs for ln (AUC) and $\Delta\mu_{\mathrm{Cmax}}$ is the mean difference between the two drugs for ln (Cmax). The above null hypothesis states that the difference in location parameter of ln (AUC) or the difference in location parameter of ln (Cmax) for the two drugs are less than –ln (1.25) or greater than ln (1.25). Rejecting the null hypothesis suggests that the overall test on the difference in parameters for the two drugs are bound between –ln (1.25) and ln (1.25) and concludes average bioequivalence.

Model and Assumptions

Consider the 2×2 crossover design for multivariate average bioequivalence endorsed by FDA. In a two-sequence, two-period crossover design, the multivariate responses AUC and Cmax are measured for each of two treatments. Assume that out of n subjects,

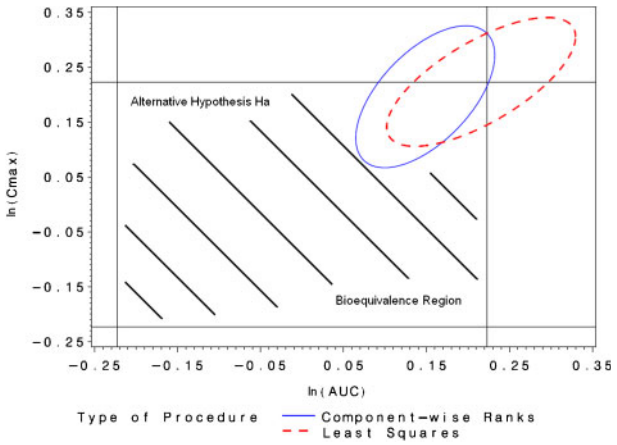

Figure 1 95% Confidence ellipse plotting the difference in test and reference drug's AUC and Cmax along with the region of alternative hypothesis

n_1 subjects are randomly assigned to Sequence 1, while the remaining n_2 subjects are assigned to Sequence 2. As discussed previously, the subjects' responses AUC and Cmax are of interest, but these procedures are easily generalized to more than two responses.

$$Y_{ijk} = \pi_i + \mu_k + s_{j(i)} + e_{ijk}$$

The response Y_{ijk} is the ln-transformed AUC or ln-transformed Cmax for treatment k and subject j within sequence i, $s_{j(i)}$ is the random effect and e_{ijk} is the random error. Assume the random subject effect $s_{j(i)}$ to be independently and identically distributed as N (0, Φ1) and the random error e_{ijk}, also independently and identically distributed as N (0, Φo). For the rank-based method, the only assumptions required are that the below differences have finite Fisher information and symmetry. Since the responses are ln-transformed, the symmetry assumption is credible. Random effects $s_{j(i)}$ and e_{ijk} are mutually independent. The difference between the two drug responses eliminates the random subject effect[10] as shown.

$$Y_{1j1} - Y_{1j2} = \mu_1 - \mu_2 + e_{1j1} - e_{1j2}$$

$$Y_{2j1} - Y_{2j2} = \mu_1 - \mu_2 + e_{2j1} - e_{2j2}$$

Based upon the hypothesis stated above, the square bound on either axis by the coordinates (ln (0.80), ln (1.25)) forms the region of alternative hypothesis. A 95% confidence ellipse[11] is plotted for the difference in the two treatment's AUC on the X-axis and Cmax on Y-axis. The least-squares ellipse is constructed with the ordinary mean differences as estimates of location and least-squares covariance structure for spread. The above differences are assumed to be continuous, symmetric, and have finite Fisher information. A robust confidence ellipse is constructed with the Hodges–Lehmann's location estimate of differences as estimates of location and component-wise rank method for robust spread. Several standardized programs and procedures are available to produce these confidence ellipses (ex:[12] from the SAS community, the SGPLOT procedure). If the ellipse falls completely inside the region of alternative hypothesis, the null hypothesis is rejected and average bioequivalence is concluded.

Table 1 The 2 × 2 cross-over example

	Period			No. of subjects
Sequence	1	Wash-out	2	*N*
1	Test	No carryover	Reference	*n*/2
2	Reference	No carryover	Test	*n*/2

Example of Bivariate Bioequivalence

Consider an example (GEN #28) of the bioequivalence problem sourced from the FDA website.[13] In this small sample (n=20 subjects) crossover study, subjects are initially randomized into one of two treatment groups: Test or Reference drugs as shown in Table 1. There is wash-out period to prevent any carryover effect between the two treatments. Subjects are randomly assigned to one of two sequences. The snippet of the data is shown in Table 2.

The traditional bioequivalence procedure analyses the data as two univariate hypotheses. The SAS program to test the univariate bioequivalence hypothesis for AUC is shown below.

```
proc mixed data=dose_equivalence;
class subj seq trt;
```

Table 2 Snippet of the data

Subj	Sex	Age	Wt	Seq	Per	TRT	Cmax	AUCr
1	M	42	197	1	1	A	123.0	1766.200
2	M	48	153	2	2	A	323.0	2580.175
4	F	58	281	2	2	A	385.0	4707.000
6	F	27	145	2	2	A	127.0	1954.400
7	F	38	140	2	2	A	162.0	1138.650
…								
16	F	30	206	2	1	B	146.0	1881.250
17	F	…	…	1	2	B	199.0	2067.925
18	F	44	160	1	2	B	147.0	1885.650
19	F	48	123	2	1	B	131.0	1319.725
20	M	33	160	2	1	B	144.0	1536.275
21	M	41	250	1	2	B	163.0	1968.675

Table 3 Summary of the univariate bioequivalence analyses

Variable	Mean difference	Standard error	Lower CI	Upper CI
AUC	0.2159	0.08150	0.07496	0.3568
Cmax	0.2231	0.08398	0.07784	0.3683

```
        model logAUC=seq subj(seq)
        trt;
        random subj(seq);
        lsmeans trt/pdiff cl
        alpha=0.1;
run;
```

Similarly, the univariate results are derived for ln (Cmax). The results of the univariate analyses are summarized in Table 3.

The 90% confidence intervals are not completely captured within $(-\ln 1.25, +\ln 1.25)$ leading to failure of rejecting the null hypotheses. Hence, the bioequivalence of the two drugs cannot be concluded.

The least-squares location of the treatment differences for ln (AUC) and ln (Cmax) are (0.2159, 0.2231) and the covariance matrix is

$$\sum_{LS} = \begin{bmatrix} 0.132841 & 0.095938 \\ 0.095938 & 0.141056 \end{bmatrix}$$

(using the proc corr procedure). Similarly, the Hodges–Lehmann's location of the treatment differences for ln (AUC) and ln (Cmax) are (0.148492, 0.196017) and the robust covariance matrix is

$$\sum_{HL} = \begin{bmatrix} 0.070494 & 0.063633 \\ 0.063633 & 0.167822 \end{bmatrix}$$

A 95% confidence ellipse is plotted with difference between test and reference drug's ln-transformed AUC as the x-axis and ln-transformed Cmax as the y-axis in Fig. 1. Two confidence ellipses are plotted (a least-squares ellipse and a robust ellipse) along with the square region of alternative hypothesis.

Since both the ellipses fall partly outside the region of alternative hypothesis, the null hypotheses cannot be rejected. Hence, bioequivalence between the test and reference drugs cannot be concluded. It is also seen that the least-squares ellipse (identified with the broken curve) is larger than the robust ellipse (identified by the solid curve), suggesting that the least-squares ellipse may be affected by mild outliers. A strong positive correlation between AUC and Cmax is also seen by the shape of the ellipses. The robust properties of the ellipse using component-wise rank method are studied in detail by Nandakumar and McKean.[2]

Conclusion

Nandakumar and McKean[2] developed a multivariate analysis for bioequivalence studies. Taking advantage of the multivariate structure of the data is generally more powerful than the standard univariate analysis. Besides the least-squares multivariate analysis, Nandakumar and McKean also proposed a Wilcoxon-type multivariate analysis which is robust in the presence of outliers and is analogous to the least-squares multivariate procedure. In this paper, SAS algorithms are developed for both the least-squares and the Wilcoxon multivariate analyses. The SAS graphics algorithm portrays the results of these analyses in the elliptical form. These algorithms are for the bivariate case, in practice, usually for the bioequivalence variables AUC and Cmax. However, they can easily be generalized to higher dimensions enabling users to use convenient SAS code to run these powerful bioequivalence procedures.

Besides the sequence effect, sometimes a period effect is also included in the bioequivalence model.[9] This can be handled by traditional and robust multivariate regression. In a balanced crossover study, regression of the residuals can be used and the location estimate, i.e. period effect, can be estimated by simple means for least-squares and Hodges–Lehmann for robust analyses. This work is under current investigation.

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