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Multivariate Bioequivalence

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ABSTRACT

Conventional measure of concluding bioequivalence is based on comparison of the AUC and C_{max} of the plasma drug concentration between the test and reference drug. However, regulators sometimes ask that similarity of the whole profile be shown because the areas can be equivalent without the entire profiles over time being the same. This can be done by comparing concentrations at each time point one at a time. But it does not take care of dependence in the concentrations over the time for each subject. Hence multivariate statistical methods are required.

Two procedures are proposed for testing bioequivalence and properties of these are examined using simulation in SAS 9.2 ®. A macro has been developed which involves sampling from multivariate normal distribution under various scenario of the null and alternative hypothesis. The results from simulation have been validated using R.

INTRODUCTION

Bioequivalence is defined as the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study. Bioequivalence of test formulation with reference formulation is concluded based on plasma, serum, or blood concentration-time profile.

A typical bioequivalence (PK) study is conducted using a two-treatment crossover study design. Alternately, a four-period, replicate design crossover study is sometimes used as well. Single doses of the test and reference drug products are administered and blood or plasma levels of the drug are measured over time. Pharmacokinetic parameters characterizing rate and extent of drug absorption rate are evaluated statistically. The PK parameters of interest are the resulting area under the plasma concentration-time curve (AUC), calculated to the last measured concentration ($AUC_{(0-t)}$) and extrapolated to infinity ($AUC_{(0-\infty)}$) for extent of absorption and the maximum or peak drug concentrations (C_{max}) for rate of absorption.

Conventional statistical methodology for checking bioequivalence is the two one-sided tests (TOST). First of the two one-sided tests determines whether bioavailability of a generic product (test), when substituted for a brand-name product (reference) is significantly less. Second of the two one-sided tests determines whether bioavailability of a generic product (test), when substituted for a brand-name product is significantly higher. If bioavailability of test formulation is within 80% to 125% of that of reference formulation then the two are considered bioequivalent.

Equivalence of the summary measures, in general, need not guarantee equivalence of the entire profile. For some drugs it may be reasonable to assess bioequivalence with respect to one or another summary measure. However for some other drug classes this may not be enough. Instead, equality of the whole profile may be necessary. This can be checked by comparing concentrations at each time point individually. One difficulty with this approach is that controlling error rate for an individual test does not ensure control on overall type I error, particularly when the observations (over time) are dependent. Hence multivariate approach is more appropriate.

In fact even in conventional bioequivalence studies, wherein multiple summary parameters are compared between test and reference products (AUC, C_{max}, T_{max}) simultaneously, a multivariate approach is technically necessary. In practice, however, this was rarely adopted. Perhaps the first paper to discuss this issue is due to Wang et al (1999).

This multivariate approach can be used for comparison of entire profiles. In one more situation entire profile comparison may be convenient. There are drugs for which computation of traditional summary measures is difficult. This may be because the profile has an unusual shape (very slow decline towards the end, non-monotone tail etc.). Comparison of full profile by passes these difficulties.

Present paper will discuss profile comparison between test and reference formulations to conclude bioequivalence using methods based on multivariate approach. The use of these methods has also been explored for testing equivalence.

METHODOLOGY

A 2x2 cross over experiment is considered. Let $\underline{X}_{iT}$ and $\underline{X}_{iR}$ be the p vector of natural log transformed concentrations for subject i on test and reference drug respectively, where p is the number of time points at which concentration is measured.

Then $\underline{D}_i = \underline{X}_{iT} - \underline{X}_{iR}$ is the vector of difference in profiles.

We assume that $\underline{D}_i$ follows a multivariate normal distribution with $(\underline{\mu}, \Sigma)$.

Bioequivalence of test with reference can be concluded if $\left|\underline{\mu_j}\right| < \delta_j$ for j = 1, , p. where δ_j is the bioequivalence boundary for j^{th} coordinate.

If the AUC, C_{max} and T_{max} are the parameters of interest then each bioequivalence boundary may be different and with different units. On the other hand for profile comparison the variable measured at each time point is the same. We will further assume that in fact the equivalence boundary specified is also the same. Hence in our case bioequivalence criteria will be

$$\left|\underline{\mu_j}\right| < \delta \quad \forall \text{ j = 1, , p.}$$

Under the above setup, we propose two methods for testing bioequivalence:-

Non-bioequivalence - H_0: $\mu_j \geq \delta$ or $\mu_j \leq -\delta$ for some j vs. bioequivalence - H_1: $-\delta < \mu_j < \delta$ for all j

Method a) Intuitive approach-

Declare bioequivalence if $\max_{j=1\ldots p} \left|\overline{D_j}\right| < c$ where $\overline{D_j}$ is the average at the j^{th} time point over n subjects.

Here 'c' is so chosen as to ensure $P[\max_{j=1,2\ldots p} \left|\overline{D_j}\right| < c] \leq \alpha$ if $\underline{\mu} = \delta E_{1\times p}$ under null hypothesis of non-bioequivalence.

Method b) Quadratic form-

Declare bioequivalence if $n\overline{D}' S^{-1}\overline{D} < d$; where S is the estimated variance covariance matrix.

Here'd' is so chosen that $P[n\overline{D}' S^{-1}\overline{D} < d] \leq \alpha$ if $\underline{\mu} = \delta E_{1\times p}$ under null hypothesis of non-bioequivalence.

A simulation approach is adopted to assess the properties of above procedures. Both procedures discussed above are based on the assumption of multivariate normal distribution. Hence sampling from multivariate normal distribution is essential. Samples have been generated from univariate standard normal distribution and converted into multivariate normal sample using the result of Cholesky decomposition.

Result which is used for sampling from multivariate normal distribution is given below:

If $\underline{X} \sim MVN_p(\mu, \Sigma)$ then $\underline{X} = \mu + L\underline{Y}$ where $\underline{Y} \sim N(0,1)$ is p-vector and $LL' = \Sigma$, L is the lower triangular matrix of Σ obtained by using Cholesky decomposition.

The multivariate samples have been used to get the empirical distribution of the above tests under null distribution. The appropriate cutoffs are then used to estimate the power for the various values of mean vector under the alternative hypothesis. SAS 9.2 is used and a macro is developed for the entire methodology. The results obtained using SAS have been validated using R.

IMPLEMENTATION USING SAS

The simulation methodology involves sampling from multivariate normal distribution with specified mean and variance covariance matrix. 'PROC FCMP' is used for sampling from multivariate normal distribution. The SAS Function Compiler (FCMP) Procedure allows users to create, test, and store SAS functions and subroutines for use by other SAS procedures. PROC FCMP is a powerful tool when complex computations are at hand as it supports SAS functions, FCMP functions and CALL routines. PROC FCMP simplifies computations by wrapping common computations into PROC FCMP "functions/CALL routines". The macro written for the multivariate normal sampling explores the use of some SAS functions and call routines. Use of the procedure FCMP made easy the task of simulation from multivariate normal distribution using functions RAND, ARRAY, DYNAMIC_ARRAY, WRITE_ARRAY and call routines such as CHOL, MULT, ADDMATRIX. Following is the sample SAS code which is a part of the macro and used for sampling from multivariate normal.

FUNCTIONING OF THE SAS MACRO

Following is a SAS code illustration for generating multivariate sample based on the proc FCMP. Suppose we are required to generate a sample of size 10 each with 5 variables from multivariate normal distribution with mean μ and Σ. Sampling is carried out using the mean vector with each co-ordinate equal to 2.5 and estimated variance co-variance of the data which is stored as a SAS dataset 'COV.sas7bdat' in the WORK library. Following is the SAS code which can be used to generate a sample from multivariate normal distribution with above specifications:

```
proc fcmp;
   array a2[5, 5]/ nosymbols;
   array a1[5, 5]/ nosymbols;
   array b1[5, 10]/ nosymbols;
   array b2[5, 10]/ nosymbols;
   array one[10, 5]/ nosymbols;

 do i = 1 to 5;
      do j = 1 to 10;
            b1[i, j] = RAND('NORMAL',0,1);

      end;
   end;

   rc1 = read_array("COV", a1);
   call chol(a1, a2);
   call mult(a2, b1, b2);
   call dynamic_array(b1, 10, 5);
   call transpose(b2, b1);
   call addmatrix(2.5, b1, one);
   rc2= write_array("RESULT", one);
quit;
```

Defining Arrays required for Matrix Calculations

Sampling from Univariate Standard Normal Distribution (SAS Function 'RAND')

CALL routines and Functions of PROC FCMP

Above mentioned SAS functions and CALL routines are used together to get random samples from multivariate normal distribution with specified mean and variance covariance matrix. The SAS dataset 'RESULT.sas7bdat' in WORK library stores a simulated sample.

Below is the macro call:

```
%simulation(rep = ,
            dscov = ,
            cots1 =,
            cots2 =,
            ns = ,
            nt = ,
            hypval=,
            path =
            );
```

Following is the description of above the macro parameters and their role in computations:

1. rep (Required): Represents the number of simulations required.
2. dscov (Required): SAS dataset name of the estimate of the Variance Co-variance matrix.
3. cots1 (Optional): Critical value for estimating power under Method (a).
4. cots2 (Optional): Critical value for estimating power under Method (b).
5. ns (Required): Number subjects/ Size of each simulated sample.
6. nt (Required): Number of time points/ Dimension of multivariate variable.
7. hypval (Required):Co-ordinate of the mean vector. Note: This macro is designed to simulate samples with each coordinate of the mean vector equal to the hypothesized value.
8. path(Required): Path where the outputs need to be stored.

VALIDATION USING R

The R function "mvnorm" from the package MASS has been used to generate samples from multivariate normal distribution. The test statistic values and the power behavior observed from the samples simulated in R are similar to the results obtained in SAS.

SIMULATION STUDY

The power is estimated for different values of mean vector. The values considered are $0E_{1\times p}$, $\pm\frac{\delta}{3}E_{1\times p}$, $\pm\frac{\delta}{2}E_{1\times p}$, $\pm\frac{2\delta}{3}E_{1\times p}$, $\pm\delta E_{1\times p}$, and $\pm 2\delta E_{1\times p}$. Here δ is the specified equivalence boundary. The last two cases belong to the null hypothesis space.

RESULTS OF SIMULATION

Simulations have been carried out for different mean vectors. Power of the above two test statistic is calculated and discussed in the following sections.

PLASMA CONCENTRATION TIME PROFILE

This section presents the data of plasma time concentration profile and the results of simulations for checking the bioequivalence of test drug based on the entire profile. The test and reference drugs are given to subjects in a two period cross-over fashion. The results are based on drug plasma concentration measured over 24 time points for 54 subjects. The bioequivalence boundary of ln(1.25) has been used.

Figure 1: Individual Difference Profile

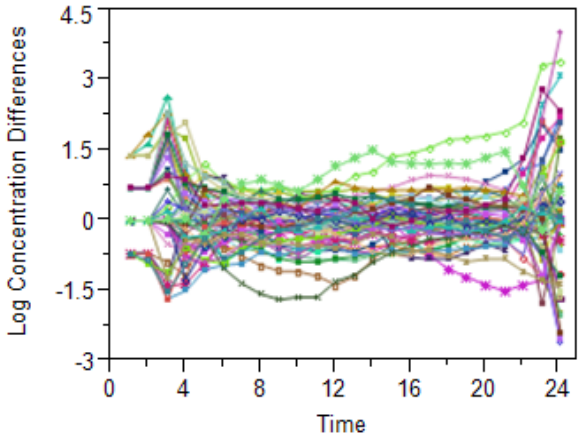


Figure 2: Mean Difference Profile

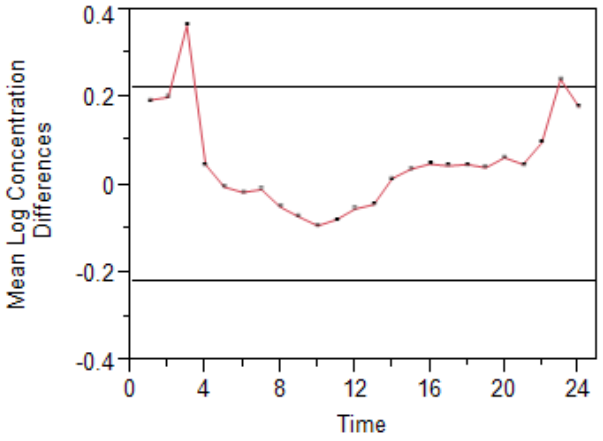


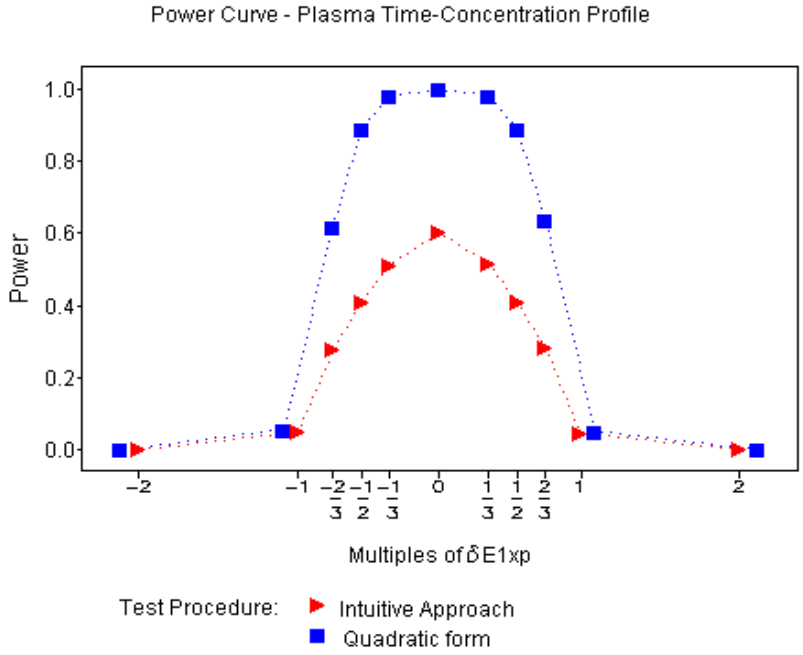
Figure 1 represents time profile of differences ($\ln(C_T)$- $\ln(C_R)$). Figure 2 is mean difference profile.

Table 1: Empirical Power for Two Methods
(Based on 5000 Simulations)

	Multiples of $\delta E_{1\times p}$										
Test Procedure	–2	–1	$-\frac{2}{3}$	$-\frac{1}{2}$	$-\frac{1}{3}$	0	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{2}{3}$	1	2
Intuitive Approach	0.000	0.049	0.277	0.409	0.513	0.601	0.516	0.407	0.282	0.046	0.000
Quadratic form	0.000	0.057	0.619	0.889	0.981	0.998	0.982	0.887	0.637	0.052	0.000

Note: $\delta = \ln(1.25)$ is used as bioequivalence limit for concentration data.

Figure 3: Empirical Power Curve: Testing Bioequivalence
(Based on 5000 simulations)



Both power curves are highest at origin and decline progressively as we move away from origin. At equivalence limit, empirical power is close to nominal α (0.05). This is consistent with expectation. Power should be low under the null hypothesis and high under the alternative. Origin represents the case of identical average profiles. Hence the power is expected to be maximum here. Clearly power curve for test based on quadratic form is above that for test based on intuitive approach. Power for quadratic form based test increases sharply as soon as we move away from the null hypothesis. In view of the difference in power curves, use of quadratic form test is recommended. It would be of interest to examine whether above conclusions persist for other situations also. We have examined two more data sets described in the next section.

QTC AND HEART RATE TIME PROFILE

We have explored power function of the proposed methods for QTc and heart rate data. For the parameters QTc and heart rate the suggested equivalence boundaries are of 5 ms and 5 bpm respectively. Each of these data sets is from a 2X2 cross over design. Each one consists of 18 time points and 28 subjects.

Figure 4: Mean Difference Profile for QTc

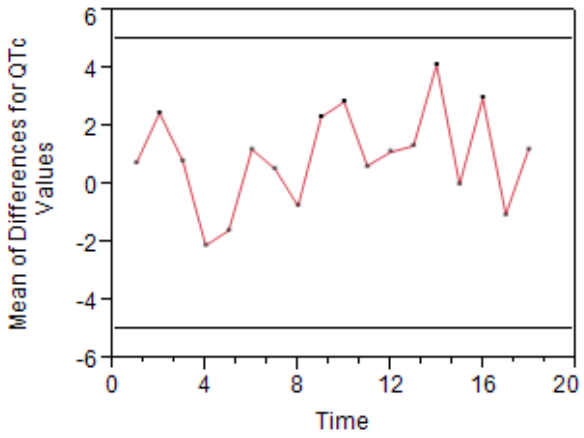
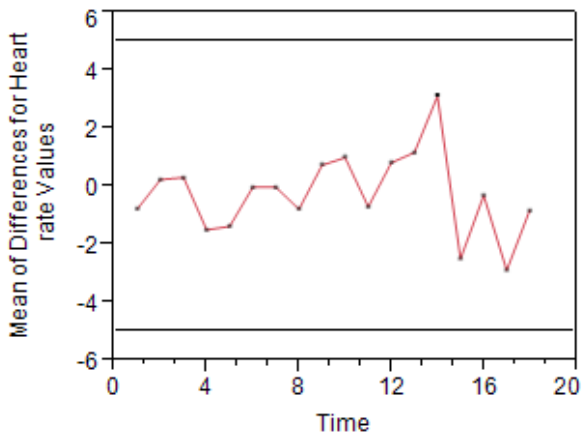


Figure 5: Mean Difference Profile for Heart Rate

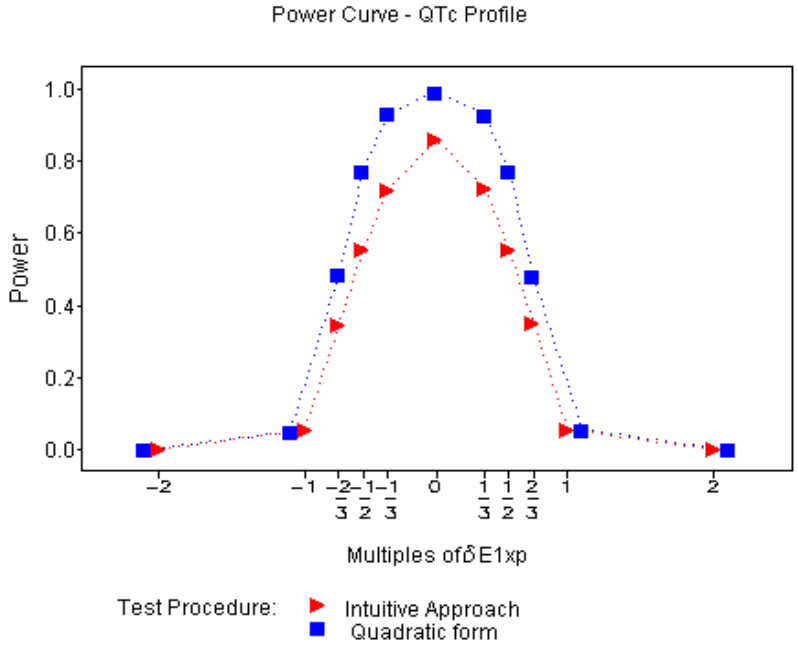


Figures 4 and 5 represent the mean of differences between test and reference drug for QTc and Heart rate data.

Table 2: Empirical Power for Two Methods for QTc Profile
(Based on 5000 Simulations)

	Multiples of $\delta E_{1\times p}$										
Test Procedure	–2	–1	$-\frac{2}{3}$	$-\frac{1}{2}$	$-\frac{1}{3}$	0	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{2}{3}$	1	2
QTc ($\delta = 5ms$)											
Intuitive Approach	0.000	0.055	0.346	0.554	0.719	0.859	0.726	0.555	0.352	0.057	0.000
Quadratic form	0.000	0.051	0.485	0.772	0.931	0.992	0.929	0.770	0.480	0.056	0.000

Figure 6: Empirical Power Curve: Testing Equivalence using QTc Profile

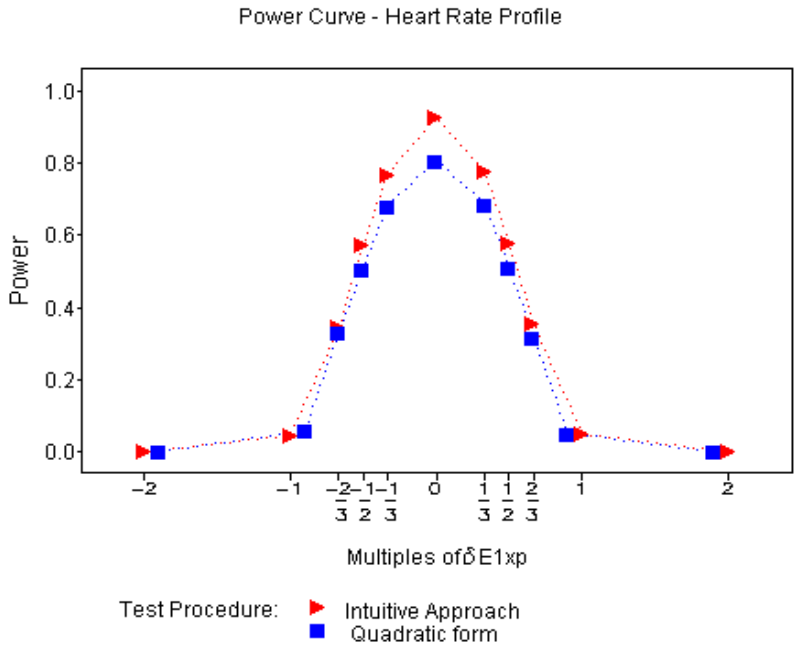


Monotone decreasing behavior (as we move away from the origin on either side) is observed here too. In this case also test based on quadratic form performs better. The difference between two curves is smaller than in the previous case.

Table 3: Empirical Power for Two Test Procedures for Heart Rate Profile
(Based on 5000 Simulations)

	Multiples of $\delta E_{1\times p}$										
Test Procedure	–2	–1	$-\frac{2}{3}$	$-\frac{1}{2}$	$-\frac{1}{3}$	0	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{2}{3}$	1	2
Heart Rate ($\delta = 5bpm$)											
Intuitive Approach	0.000	0.045	0.345	0.574	0.768	0.928	0.776	0.578	0.357	0.049	0.000
Quadratic form	0.000	0.058	0.331	0.508	0.680	0.808	0.687	0.512	0.318	0.052	0.000

Figure 7: Empirical Power Curve: Testing Equivalence using Heart Rate Profile



In case of heart rate data as well, monotone decreasing behavior is observed as we move away from origin. Surprisingly, in this case performance of intuitive test is slightly better.

CONCLUSION

The test based on quadratic form seems to have an edge over intuitive test. This work has demonstrated that it is possible to compare full profiles in PK/ PD studies without getting involved in intractable multivariate mathematics even though the dimension increase from 3 (Wang et al paper) to as high as 24 (our data sets)

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