Pak. J. Statist.
2016 Vol. 32(5), 371-391

INDIVIDUAL BIOEQUIVALENCE WITH MULTIVARIATE APPROACH

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ABSTRACT

This research proposes a multivariate generalization of the criterion used for testing individual bioequivalence. Although various approaches have been reported in literature for multivariate equivalence, majority of these contemplate simultaneous comparisons of means in each dimension for multivariate equivalence region. Unlike previous approaches, our proposed criterion combines not only the comparison of means and variances but also the within-subject correlations into a single aggregate criterion. The exact distribution of proposed criterion is not traceable, therefore, bootstrap method as recommended by FDA is proposed. The performance of our multivariate individual bioequivalence criterion has been evaluated through a simulation study. The power and size of the study using multivariate individual bioequivalence criterion are determined by varying sample sizes, mean differences, within-subject variances as well as within-subject correlations between pharmacokinetic parameters.

KEYWORDS

Individual bioequivalence studies, Multivariate equivalence, Crossover design, Bootstrapping.

1. INTRODUCTION

Generic drug products, the ones manufactured by generic drug companies or innovator companies themselves, have gained remarkable popularity during the past few years. Successful FDA approval can only be achieved by different generic drugs through conducting bioavailability and bioequivalence studies (Shao, Chow & Wang, 2000). Bioavailability refers to the rate and extent to which active ingredient / moiety of a drug is absorbed and becomes available at its site of action whereas two drug products are considered bioequivalent provided they exhibit similar bioavailability indicating their therapeutic equivalency (Chow & Liu, 2009). Therefore, bioequivalence studies are substantially important as the compliance of a new drug application is both tedious and expensive to acquire.

As average bioequivalence (ABE) has few limitations the focus of the bioequivalence testing in recent years has shifted from ABE to population and individual bioequivalence (PBE and IBE) studies. Chen (1997) mentioned that, ABE focuses only on the comparison of the mean distances between formulations and neglects the distribution of the pharmacokinetic (PK) parameters (bioavailability metric). In addition, ABE neither considers the within-subject variability of the formulation nor subject-by-formulation

interaction. Thus the safe and interchangeable use of a generic drug remains questionable even after approval by the method of average bioequivalence.

Drug interchangeability is described as drug prescribability or switchability. For the first time prescription, the practitioner's available options to choose between a reference and a test formulation is defined as the drug's prescribability. On the other hand, drug switchability refers that a patient who is already on an established regimen using one of the two drug formulations, can easily switch to the other formulation. Switchability can also be described as is the exchangeability of two formulations within a subject maintaining the same or gaining the better efficacy and safety. Prescribability requires the population bioequivalence (PBE) whereas switchability requires individual bioequivalence (IBE) between both test and reference formulations. The individual bioequivalence approach evaluates within-subject variability for test and reference formulations, as well as subject-by-formulation interaction, which cannot be neglected while considering the drug switchability (Chen, 1997; D. & Chinchilli, 1994; FDA, 1999; Hauck & Anderson, 1992).

The inadequacy of ABE to assure drug switchability between formulations was initially demonstrated by Anderson and Hauck (1990) which strengthen the need of individual bioequivalence studies. When a drug is marketed, its safety and efficacy in an individual patient may be of concern if the generic manufacturers intend to change the formulation or of their drug. This scenario relates to the drug switchability and an IBE assessment is required for the approval of new formulations (FDA, 1999, 2001).

Generally, bioequivalence studies are conducted to demonstrate the similar efficacies of a brand drug and its generic copy, for which obtained responses called pharmacokinetic (PK) parameters are derived from serum concentration-time profile. The most frequently used PK parameter in bioequivalence analysis is area under the curve (AUC), while another important PK parameter being C_{max}, because both drugs are required to show similar specific plasma concentration levels in order to exhibit same therapeutic effect. In other words, C_{max} is closely correlated to the AUC. Other additional PK parameters of considerable importance in bioequivalence analysis are time to reach maximum concentration (T_{max}) and ratio C_{max} / AUC. Since most of the PK parameters employed in the bioequivalence analysis are derived from blood concentration-time, therefore, ignoring the correlation between them is unjustifiable. Extensive research material is available in literature for the univariate bioequivalence analysis (ABE, PBE and IBE) where the analysis of bioequivalence data is carried out considering one PK parameter at a time. Contrary to this, only scarce material is found for the multivariate situations for average bioequivalence where more than one PK parameters are considered simultaneously (Berger & Hsu, 1996; E. B. Brown, Iyer, & Wang, 1997; L.D. Brown, Casella, & Gene Hwang, 1995; Munk & Pfluger, 1999; Tamhane & Logan, 2004; Wang, Hwang, & Dasgupta, 1999). Although, three criteria have also been developed for multivariate population bioequivalence (Bassam, 2009; Chervoneva, Hyslop, & Hauck, 2007; Dragalin, Fedorov, Patterson, & Jones, 2003), the criterion for multivariate individual bioequivalence considering more than one pharmacokinetic parameter at a time is still lacking.

However, literature reveals an approach for multivariate equivalence which concurrently compares the means in each dimension (L. D. Brown et al., 1995; Munk & Pfluger, 1999; Wang et al., 1999).

In this study we have adopted another approach by considering aggregated equivalence criterion. For this purpose, not only the comparison of means and within-subject variances for the test and reference formulations are incorporated but the within-subject correlations are also included. Hence, this research can be regarded as a first attempt proposing a multivariate individual bioequivalence criterion with inclusion of within-subject correlations among PK parameters.

In univariate cases, the criterion and statistical method for the assessment of IBE are initially proposed by Anderson and Hauck (1990). Afterwards a general approach for the assessment of IBE was proposed by Sheiner (1992) and R. Schall and Luus (1993). The approach of R. Schall and Luus (1993) depends on the comparison of the expected discrepancy between bioavailabilities Y_{T} and Y_{R} of the test and reference formulations, with the expected discrepancy between two bioavailabilities Y_{R} and $Y_{R'}$ from the reference formulation. Thus a comparison of the reference formulation to itself makes the basis for the comparison of the test with the reference formulation. In assessment of individual bioequivalence, the discrepancies between Y_{T} and Y_{R}, and Y_{R} and $Y_{R'}$ are computed for within-subject differences of bioavailabilities (Robert Schall, 1995). The individual bioequivalence criterion is defined as

$$\frac{E(Y_T - Y_R)^2 - E(Y_R - Y_{R'})^2}{E(Y_R - Y_{R'})^2/2} < \theta \tag{1.1}$$

For IBE, $Y_T - Y_R$ and $Y_R - Y_{R'}$ represent within subject differences, where Y_T, Y_R and $Y_{R'}$ are bioavailabilities following the administration of the one test and two reference formulations, these bioavailabilities are believed to be from the same individual and must be considered as dependent and θ represents predetermined bound for IBE criterion.

Assuming $Y_T \sim N\ (\mu_T, \sigma_T^2\ )$ and $Y_R \sim N\ (\mu_R, \sigma_R^2\ )$ are dependent and after taking the expectation, the above criterion can be written in terms of population parameters as.

$$\frac{(\mu_T - \mu_R)^2 + \sigma_D^2 + (\sigma_{WT}^2 - \sigma_{WR}^2\ )}{\sigma_{WR}^2} < \theta \tag{1.2}$$

Or linearized as

$$(\mu_T - \mu_R) + \sigma_D^2 + \sigma_{WT}^2 - \sigma_{WR}^2 - \sigma_{WR}^2\theta_{IBE} < 0 \tag{1.3}$$

For the assessment of IBE Hyslop et al. (2000) assumed normally distributed (logarithmic transformed) pharmacokinetic (PK) parameters and derived a parametric confidence interval for the above linearized criterion.

Here in this research we have introduced the multivariate generalization of criterion (1.1) based on comparison of true means, within subject variances and within-subject correlations for test and reference formulations.

2. METHODS

Crossover design is often used in bioequivalence studies which is defined as the design where each study subject is administrated an either of the two formulations (T=test and R=Reference) of a drug, then after washout period study subjects are switched over to the other formulation. Therefore, in a standard 2X2 crossover design, each study subject is allocated either RT or TR sequence in two periods at random. A crossover design where either number of periods or number of sequences are greater than number of formulations to be compared is known as higher order or replicated crossover design (Chow & Liu, 2009). For assessment of individual bioequivalence the higher order crossover designs are recommend by FDA (1999). Hence in this research we consider a higher order crossover design, e.g., an s-sequence and 4-period crossover design and let n_i represents the number of subjects in the i[th] sequence. Reference and test drugs are denoted by R and T respectively. Let $\mathbf{y_{ijkl}} = (y_{ijkl1}, y_{ijkl2}, y_{ijkl3} \dots . y_{ijklp})'$ be the log transformed p-variate vector of responses for k[th] formulation of the j[th] subject in the i[th] sequence; $i = 1,2, \dots \mathrm{s}, \mathrm{j} = 1,2, .. n_i,$ $\mathrm{k} = \mathrm{T}, \mathrm{R}, \mathrm{l} = 1,2$. Here p represents the number of pharmacokinetics parameters, i.e., responses obtained on each subject in the study, e.g., $p = 2$ if data are taken on AUC and C_{max}.

The multivariate version of the statistical model that was used in the FDA (2001) document is given as

$$\mathbf{Y}_{ijkl} = \boldsymbol{\mu}_k + \boldsymbol{\gamma}_{ikl} + \boldsymbol{\eta}_{ijk} + \boldsymbol{\epsilon}_{ijkl} \tag{2.1}$$

In the above model $\boldsymbol{\mu}_T$ and $\boldsymbol{\mu}_R$ represent population mean responses under test and reference formulations respectively, $\boldsymbol{\eta}_{ijk}$ is the random subject effect corresponding to k[th] treatment of j[th] subject in i[th] sequence, $\boldsymbol{\gamma}_{ikl}$ is the fixed effect corresponding to l[th] application of k[th] treatment in i[th] sequence, satisfying estimability condition $\sum_{i=1}^{s}\sum_{l=1}^{2}\boldsymbol{\gamma}_{ikl} = 0$ and $\boldsymbol{\epsilon}_{ijkl}$ are random within subject errors. It is assumed that $\boldsymbol{\epsilon}_{ijkl}{}'s$ are normally and independently distributed random vectors with mean 0 and covariance matrix Σ_{WK} i.e., $\boldsymbol{\epsilon}_{ijkl} \sim N(0, \Sigma_{WK})$, k=T, R. it is also assumed that $(\boldsymbol{\eta}'_{ijT}, \boldsymbol{\eta}'_{ijR})'$ follows 2p-variate normal distribution with mean 0 and variance-covariance matrix Σ_B (the between subject covariance matrix).

$$\Sigma_B = \begin{bmatrix} \Sigma_{BT} & \Sigma_{BTR} \\ \Sigma_{BRT} & \Sigma_{BR} \end{bmatrix}$$

Here Σ_{BT} and Σ_{BR} are p x p between subject variance-covariance matrices under test and reference formulations respectively and Σ_{BRT} is between subject variance-covariance matrix of test and reference formulations.

For multivariate individual bioequivalence $\mathbf{Y}_T - \mathbf{Y}_R$ and $\mathbf{Y}_R - \mathbf{Y}_{R'}$ represent within subject differences, where $\mathbf{Y}_T, \mathbf{Y}_R$ and $\mathbf{Y}_{R'}$ are considered to be from the same individual as used in univariate individual bioequivalence in FDA (2001) and thus $\mathbf{Y}_T, \mathbf{Y}_R$ and $\mathbf{Y}_{R'}$ must be treated as dependent as mentioned by Hauschke et al. (2007) in univariate case. In multivariate bioequivalence the variance of difference between $\mathbf{Y}_T$ and $\mathbf{Y}_R$ is

$$Var\,(\mathbf{Y}_T - \mathbf{Y}_R) = Var\left(\boldsymbol{\eta}_{ijT} - \boldsymbol{\eta}_{ijR}\right) + Var\left(\boldsymbol{\epsilon}_{ijTl} - \boldsymbol{\epsilon}_{ijRl}\right)$$

where $Var\left(\boldsymbol{\eta}_{ijT} - \boldsymbol{\eta}_{ijR}\right) = \Sigma_D$ is subject by formulation interaction matrix and defined as

$$\Sigma_D = \Sigma_{BT} - \Sigma_{BTR} - \Sigma_{BRT} + \Sigma_{BR}$$

We propose following criterion for the multivariate individual bioequivalence

$$C_I = \{E\ (\boldsymbol{Y}_T - \boldsymbol{Y}_R)'\Sigma_{WR}^{-1}(\boldsymbol{Y}_T - \boldsymbol{Y}_R)\} - \{E\ (\boldsymbol{Y}_R - \mathbf{Y}_{R'})'\Sigma_{WR}^{-1}(\boldsymbol{Y}_R - \mathbf{Y}_{R'})\} \tag{2.2}$$

We simplify our proposed criterion (2.2) by using following suppositions

$$\mathbf{Z} = \Sigma_{WR}^{-1/2}(\boldsymbol{Y}_T - \boldsymbol{Y}_R)\ and\ \mathbf{K} = \Sigma_{WR}^{-1/2}(\boldsymbol{Y}_R - \mathbf{Y}_{R'})$$

Now equation 2.2 can be written as

$$C_I = E\ \{\mathbf{Z}'\mathbf{Z}\} - E\ \{\mathbf{K}'\mathbf{K}\} \tag{2.3}$$

Here we consider $E\ \{\mathbf{Z}'\mathbf{Z}\}$

$$E\ \{\mathbf{Z}'\mathbf{Z}\} = E\ \left\{(z_1 z_2)\begin{pmatrix} z_1 \\ z_2 \end{pmatrix}\right\} = E\ \left\{\sum_{v=1}^{2} z_v^2\right\} = \sum_{v=1}^{2}\{\sigma_{zv}^2 + \{E(z_v)\}^2\}$$

$$E\{\mathbf{Z}'\mathbf{Z}\} = tr(\Sigma_{\mathbf{Z}}) + E\ \{\mathbf{Z}'\}\ E\{\mathbf{Z}\}$$

Similarly,

$$E\{\mathbf{K}'\mathbf{K}\} = tr(\Sigma_{\mathbf{K}}) + E\{\mathbf{K}'\}\ E\{\mathbf{K}\}$$

Now equation 2.3 becomes

$$C_I = tr(\Sigma_{\mathbf{Z}}) + E\{\mathbf{Z}'\}E\{\mathbf{Z}\} - tr(\Sigma_{\mathbf{K}}) - E\{\mathbf{K}'\}E\{\mathbf{K}\} \tag{2.4}$$

Now we consider $E\{\mathbf{Z}\}$and $E\{\mathbf{Z}'\}$

$$E\{\mathbf{Z}\} = E\{\Sigma_{WR}^{-1/2}(\boldsymbol{Y}_T - \boldsymbol{Y}_R)\} = \Sigma_{WR}^{-1/2}E\{(\boldsymbol{Y}_T - \boldsymbol{Y}_R)\} = \Sigma_{WR}^{-1/2}(\boldsymbol{\mu}_T - \boldsymbol{\mu}_R)$$

$$\mathrm{E}\{\mathbf{Z}'\} = (\boldsymbol{\mu}_T - \boldsymbol{\mu}_R)'\Sigma_{WR}^{-1/2}$$

Now we solve $E\{\mathbf{K}\}$ and $E\{\mathbf{K}'\}$

$$E\{\mathbf{K}\} = \Sigma_{WR}^{-\frac{1}{2}}\left(\boldsymbol{\mu}_R - \boldsymbol{\mu}_{R'}\right) = \Sigma_{WR}^{-\frac{1}{2}}(\boldsymbol{\mu}_R - \boldsymbol{\mu}_R) = 0$$

Similarly, $E\{\mathbf{K}'\} = 0$

Now we consider covariance matrices of **Z** and **K**

$$\Sigma_{\mathbf{Z}} = \Sigma_{WR}^{-1/2}\ Cov\ (\boldsymbol{Y}_T - \boldsymbol{Y}_R)\Sigma_{WR}^{-1/2}$$

$$\Sigma_{\mathbf{Z}} = \Sigma_{WR}^{-1/2}\ (\Sigma_{BT} + \Sigma_{WT} + \Sigma_{BR} + \Sigma_{WR} - \Sigma_{BTR} - \Sigma_{BRT})\Sigma_{WR}^{-1/2}$$

$$\Sigma_{\mathbf{Z}} = \Sigma_{WR}^{-1/2}\Sigma_{WT}\Sigma_{WR}^{-1/2} + \mathbf{I} + \Sigma_{WR}^{-1/2}\Sigma_D\Sigma_{WR}^{-1/2}$$

and

$$\Sigma_{\mathbf{K}} = \Sigma_{WR}^{-1/2}\ Cov\ (\boldsymbol{Y}_R - \mathbf{Y}_{R'})\Sigma_{WR}^{-1/2}$$

$$\Sigma_{\mathbf{K}} = \Sigma_{WR}^{-1/2}\left(\Sigma_{BR} + \Sigma_{WR} + \Sigma_{BR'} + \Sigma_{WR'} - \Sigma_{BR'R} - \Sigma_{BRR'}\right)\Sigma_{WR}^{-\frac{1}{2}}$$

$$= \Sigma_{WR}^{-1/2}(2\Sigma_{WR})\Sigma_{WR}^{-1/2} = 2\ \mathbf{I}$$

After putting values of $\Sigma_{\mathbf{Z}}, E\{\mathbf{Z}'\}, E\{\mathbf{Z}\}, \Sigma_{\mathbf{K}}, E\{\mathbf{K}'\}$ and $E\{\mathbf{K}\}$ in equation 2.4 the multivariate IBE criterion becomes

$$C_I = tr\left(\Sigma_{WR}^{-\frac{1}{2}}\Sigma_{WT}\Sigma_{WR}^{-\frac{1}{2}} + \mathbf{I} + \Sigma_{WR}^{-\frac{1}{2}}\Sigma_D\Sigma_{WR}^{-\frac{1}{2}}\right) + (\boldsymbol{\mu}_T - \boldsymbol{\mu}_R)'\Sigma_{WR}^{-1}(\boldsymbol{\mu}_T - \boldsymbol{\mu}_R) - tr(2\,\mathbf{I})$$

$$C_I = tr\left(\Sigma_{WT}\Sigma_{WR}^{-1}\right) + tr\left(\Sigma_D\Sigma_{WR}^{-1}\right) + (\boldsymbol{\mu}_T - \boldsymbol{\mu}_R)'\Sigma_{WR}^{-1}(\boldsymbol{\mu}_T - \boldsymbol{\mu}_R) - P \qquad (2.5)$$

If $p = 1$ then above multivariate individual bioequivalence criterion in equation 2.5 reduces to univariate individual bioequivalence criterion defined in equation 1.2.

We further simplify $\Sigma_{\mathbf{Z}}$ as

$$\Sigma_{\mathbf{Z}} = \Sigma_{WR}^{-1/2}\left(\frac{1}{2}\Sigma_{WT} + \frac{1}{2}\Sigma_{WT} + \frac{1}{2}\Sigma_{WR} + \frac{1}{2}\Sigma_{WR} + \Sigma_D\right)\Sigma_{WR}^{-1/2}$$

Here if we define

$$\mathbf{I}_{ij} = \frac{\left(\mathbf{Y_{ijT1}} + \mathbf{Y_{ijT2}}\right)}{2} - \frac{\left(\mathbf{Y_{ijR1}} + \mathbf{Y_{ijR2}}\right)}{2}$$

With $\mathbf{I} = \mathbf{I}_{1j} + \mathbf{I}_{2j}$ then it can be easily proved that $\Sigma_{\mathrm{I}} = \Sigma_D + \frac{1}{2}(\Sigma_{WT} + \Sigma_{WR})$

$$\Sigma_{\mathbf{Z}} = \Sigma_{WR}^{-1/2}\left(\frac{1}{2}\Sigma_{WT} + \frac{1}{2}\Sigma_{WR} + \Sigma_{\mathbf{I}}\right)\Sigma_{WR}^{-1/2}$$

Now resulting multivariate individual bioequivalence criteria (2.4) becomes

$$C_I = tr\left(\frac{1}{2}\Sigma_{WR}^{-1/2}\Sigma_{WT}\Sigma_{WR}^{-1/2} + \frac{1}{2}\Sigma_{WR}^{-1/2}\Sigma_{WR}\Sigma_{WR}^{-1/2} + \Sigma_{WR}^{-1/2}\Sigma_{\mathbf{I}}\Sigma_{WR}^{-1/2}\right) + \left(\boldsymbol{\mu}_T - \boldsymbol{\mu}_R\right)'\Sigma_{WR}^{-1}\left(\boldsymbol{\mu}_T - \boldsymbol{\mu}_R\right) - tr(2\,\mathbf{I})$$

Now using the property of trace

$$C_I = \frac{1}{2}\,tr\left(\Sigma_{WT}\Sigma_{WR}^{-1}\right) + \frac{1}{2}tr(\mathbf{I}) + tr\left(\Sigma_{W\mathbf{I}}\Sigma_{WR}^{-1}\right) + (\boldsymbol{\mu}_T - \boldsymbol{\mu}_R)'\Sigma_{WR}^{-1}(\boldsymbol{\mu}_T - \boldsymbol{\mu}_R)tr(2\,\mathbf{I})$$

$$C_I = \frac{1}{2}\,tr\left(\Sigma_{WT}\Sigma_{WR}^{-1}\right) + tr\left(\Sigma_{W\mathbf{I}}\Sigma_{WR}^{-1}\right) + (\boldsymbol{\mu}_T - \boldsymbol{\mu}_R)'\Sigma_{WR}^{-1}(\boldsymbol{\mu}_T - \boldsymbol{\mu}_R) - \frac{3}{2}P \qquad (2.6)$$

It is simple to show that if $p = 1$ then our proposed multivariate individual bioequivalence criterion (MIBE) in equation 2.6 reduces to the linearized version of univariate individual bioequivalence criterion as defined in equation 1.3. Furthermore, our developed criteria (2.5) and (2.6) account not only for within-subject variances but also the within-subject correlations among the PK parameters.

Hypothesis for proposed multivariate individual bioequivalence criterion are

$H_0: C_I \geq \boldsymbol{\theta}$ vs $H_a: C_I < \boldsymbol{\theta}$

where C_{I} is the p-variate individual bioequivalence criterion and $\boldsymbol{\theta}$ is the predefined constant as the upper value for the acceptance region. Since the exact distribution of test statistics C_I is not tractable, therefore numerical methods such as the bootstrap, as recommended by FDA, has to be employed to determine the confidence interval for the C_I. If the corresponding one-sided upper $(1-\alpha)$ confidence bound of C_{I} is less than $\boldsymbol{\theta}$ then multivariate individual bioequivalence will be concluded.

The estimated multivariate individual bioequivalence criterion could be obtained as

$$\hat{C}_I = \frac{1}{2}\, tr\left(\hat{\Sigma}_{WT}\hat{\Sigma}_{WR}^{-1}\right) + tr\left(\hat{\Sigma}_{W\mathbf{I}}\hat{\Sigma}_{WR}^{-1}\right) + (\hat{\boldsymbol{\mu}}_T - \hat{\boldsymbol{\mu}}_R)'\hat{\Sigma}_{WR}^{-1}(\hat{\boldsymbol{\mu}}_T - \hat{\boldsymbol{\mu}}_R) - \frac{3}{2}P$$

The difference of mean vectors under test and reference formulations can be estimated as

$$\hat{\boldsymbol{\delta}} = \hat{\boldsymbol{\mu}}_T - \hat{\boldsymbol{\mu}}_R = \bar{\mathbf{Y}}_{\boldsymbol{T}} - \bar{\mathbf{Y}}_{\boldsymbol{R}} = \frac{\mathbf{1}}{\mathbf{2}}\sum_{\boldsymbol{i=1}}^{\boldsymbol{s}}\frac{\mathbf{1}}{\boldsymbol{n_i}}\sum_{\boldsymbol{j=1}}^{\boldsymbol{n_i}}\mathbf{I}_{ij}$$

Now here in multivariate situation we consider following re-parameterization likewise FDA (2001) proposed in univariate case, here for each subject j in sequence i, $\mathbf{T_{1j}}$ and $\mathbf{R_{1j}}$ denote difference vectors between the bioavailabilities vectors under the test and reference formulations for the first and second administration of the respective formulation

$$\mathbf{T_{1j}} = \mathbf{Y_{1jT1}} - \mathbf{Y_{1jT2}} \qquad \mathbf{T_{2j}} = \mathbf{Y_{2jT1}} - \mathbf{Y_{2jT2}}$$

$$\mathbf{R_{1j}} = \mathbf{Y_{1jR1}} - \mathbf{Y_{1jR2}} \qquad \mathbf{R_{2j}} = \mathbf{Y_{2jR1}} - \mathbf{Y_{2jR2}}$$

With $\frac{1}{2}Cov\left(\mathbf{T_{ij}}\right) = \Sigma_{WT}$ $\quad \frac{1}{2}Cov\left(\mathbf{R_{ij}}\right) = \Sigma_{WR}$ $\quad \mathrm{Cov}\left(\hat{\boldsymbol{\delta}}\right) = \frac{1}{4}\sum_{i=1}^{s}\frac{1}{\boldsymbol{n_i}}\mathrm{Cov}(\mathbf{I}_{ij})$

The estimators of Σ_{WT}, Σ_{WR} and Σ_I are

$$\hat{\Sigma}_{WT} = \begin{bmatrix} \hat{\sigma}_{WT1}^2 & \hat{\sigma}_{WT12} \\ \hat{\sigma}_{WT21} & \hat{\sigma}_{WT2}^2 \end{bmatrix} \quad \hat{\Sigma}_{WR} = \begin{bmatrix} \hat{\sigma}_{WR1}^2 & \hat{\sigma}_{WR12} \\ \hat{\sigma}_{WR21} & \hat{\sigma}_{WR2}^2 \end{bmatrix} \quad \hat{\Sigma}_{\mathrm{I}} = \begin{bmatrix} \hat{\sigma}_{\boldsymbol{I1}}^{\mathbf{2}} & \hat{\sigma}_{\boldsymbol{I12}} \\ \hat{\sigma}_{\boldsymbol{I12}} & \hat{\sigma}_{\boldsymbol{I2}}^{\mathbf{2}} \end{bmatrix}$$

Here

$$\hat{\sigma}_{WTv}^2 = \frac{1}{2}\frac{\sum_{i=1}^{s}\sum_{j=1}^{n_i}\left(T_{ijv} - \bar{T}_{i.v}\right)^2}{n-2} \qquad \hat{\sigma}_{WRv}^2 = \frac{1}{2}\frac{\sum_{i=1}^{s}\sum_{j=1}^{n_i}\left(R_{ijv} - \bar{R}_{i.v}\right)^2}{n-2}$$

$$\hat{\sigma}_{Iv}^2 = \frac{1}{n-2}\frac{\sum_{i=1}^{s}\sum_{j=1}^{n_i}\left(I_{ijv} - \bar{I}_{i.v}\right)^2}{n-2}$$

where, $\mathrm{T_{ijv}}$ and $\mathrm{R_{ijv}}$ are defined for univariate bioavailability under test and reference formulations respectively. So $\hat{\sigma}_{WK1}^2$ and $\hat{\sigma}_{WK2}^2$ represent within-subject variances for $\mathrm{k^{th}}$ formulation (K=T, R) of first and second PK parameters usually, AUC and $\mathrm{C_{max}}$ respectively. Moreover, $\sigma_{WK12} = \rho_{WK}\sigma_{WK1}\sigma_{WK2}$ here ρ_{WK} is the within-subject correlation between PK parameters for $\mathrm{k^{th}}$ formulation.

2.1 The$100(1-\alpha)th$ confidence bound of C_{I}

Since, the exact distribution of MIBE C_{I} is not tractable, therefore, bootstrap method (Efron & Tibshirani, 1993) as recommended by the FDA, is proposed. The steps of bootstrap methods are

1. Obtain the estimates of population parameters $\boldsymbol{\mu}_T$, $\boldsymbol{\mu}_R, \Sigma_{WT}, \Sigma_{WR}$ and $\Sigma_{\mathbf{I}}$ and calculate MIBE criterion (2.6).
2. Let $\mathrm{Y}_{ijv} = \left(y_{ijT1v}, y_{ijR1v}, y_{ijT2v}, y_{ijR2v}\right)'$ and $\mathrm{Y}_{iv} = \left(Y_{i1v}, Y_{i2v}, \dots\dots, Y_{in_iv}\right)'$ for each PK parameter v and fixed sequence i, draw a simple random sample $\mathrm{Y}_{iv}^{*b} = \left(Y_{i1v}^{*b}, Y_{i2v}^{*b}, \dots\dots Y_{in_iv}^{*b}\right)'$ with replacement from Y_{iv}. That is the bootstrap samples are obtained using subjects as sampling units. As per FDA recommendation repeat this process for b= 1,2,…..B=2000 to obtained B bootstrap samples $\mathrm{Y}_{iv}^{*1}, \dots \mathrm{Y}_{iv}^{*B}, i = 1,2$ for each PK parameter $v = 1,2$
3. For each b=1, 2,….,B, compute $\hat{\boldsymbol{\mu}}_T$,$\hat{\boldsymbol{\mu}}_R$, $\widehat{\Sigma}_{WT}$, $\widehat{\Sigma}_{WR}$ and $\widehat{\Sigma}_{\mathbf{I}}$ by using the similar method as employed in step 1 but with the data set $(\mathrm{Y}_{1v}, \mathrm{Y}_{2v})$ replace by the bootstrap data set $(\mathrm{Y}_{1v}^{*b}, \mathrm{Y}_{2v}^{*b})$. Calculate the bootstrap estimate of multivariate individual bioequivalence criterion $\hat{C}_I^b$ for each bootstrap sample.
4. Calculate $\widehat{C}_I^b\ (1-\alpha)$, the $100(1-\alpha)^{\text{th}}$ percentile of C_I^b, b= 1, 2...B.
5. MIBE will be concluded if $\hat{C}_I^b\ (1-\alpha) < \boldsymbol{\theta}$

2.2 Identifying Upper Limit θ for MIBE

Hauschke et al. (2007) explained the calculation for the value of θ for univariate individual bioequivalence using limits of BE acceptance determined by FDA. Here, these limits are extended to the multivariate situation and calculation of $\boldsymbol{\theta}$ for multivariate individual bioequivalence is carried out by setting the natural logarithm of 1.25 as maximum difference between the means of the test and reference PK parameters; the maximum difference between the test and reference within-subject variances as 0.02, and the lowest within-subject variances as 0.04 and a maximum value of 0.03 for the subject-by-formulation interaction. There are no corresponding guidelines for including the correlation among PK parameters such. Therefore, various combinations of within-subject correlations have been used and to account for these correlations the value of $\boldsymbol{\theta}$ could be calculated for a range of values of ρ_{WR}, ρ_{WR} and ρ_D. When test and reference within-subject correlations are 0 then $\boldsymbol{\theta}$ reduces to p-multiples of univariate θ i.e. p x θ = p x 2.484, where p is the number of PK parameters.

Figure 1 depicts variation in the value of $\boldsymbol{\theta}$ in the range of within-subject correlations of test and reference PK parameters when interaction correlation (ρ_D) = 0. The horizontal reference line crosses the y-axis at 4.989 which is the value of $\boldsymbol{\theta}$ when within-subject reference and test correlations are 0 i.e. ($\rho_{WR} = \rho_{WT} = 0$) this value is noted as $\boldsymbol{\theta}_0$. When within-subject reference correlation is 0 the value of $\boldsymbol{\theta}$ is constant ($\boldsymbol{\theta}$ = 4.989) regardless of within-subject test correlation (ρ_{WT}). This figure further illustrates that when within-subject reference correlation (ρ_{WR}) is less than or equal to 0.35 the value of $\boldsymbol{\theta}$ is always less than $\boldsymbol{\theta}_0$. For $\rho_{WR} > 0.35$, the value of $\boldsymbol{\theta}$ is smaller or greater than $\boldsymbol{\theta}_0$ depending on the values of ρ_{WR} and ρ_{WT}.

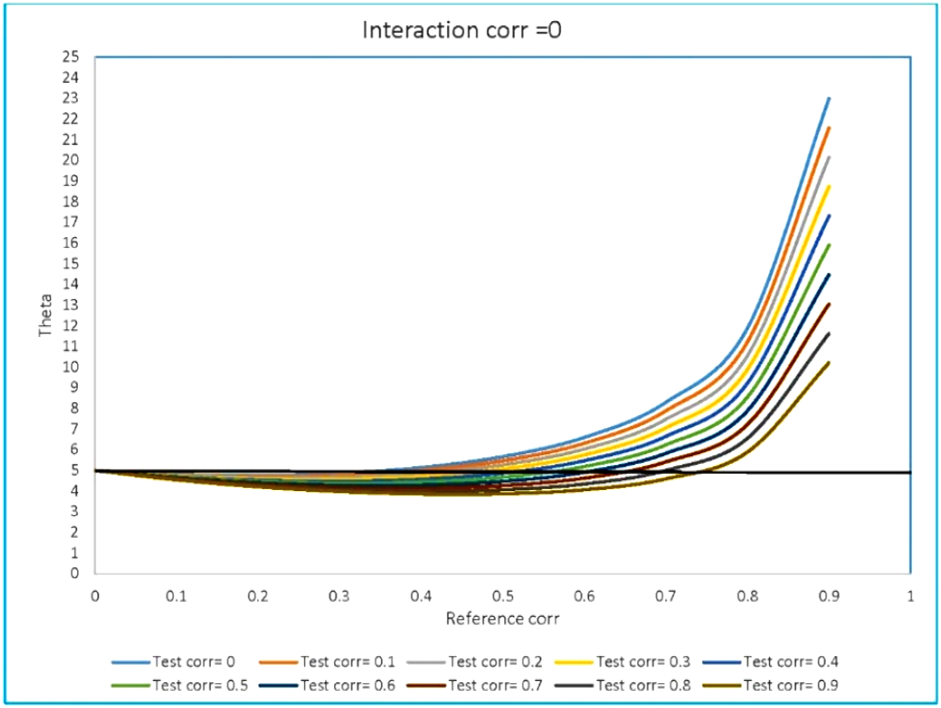

Figure 1: Effect of ρ_{WR} and ρ_{WT} on θ

2.3 Asymmetry of the Test

In Table 1 the upper bounds of multivariate IBE region $\boldsymbol{\theta}$ were calculated according to within-subject test and reference correlations when interaction correlation was assumed as 0. The calculated values of $\boldsymbol{\theta}$ were compared with $\boldsymbol{\theta}_0$, which was calculated in the absence of within-subject correlations. The difference between $\boldsymbol{\theta}$ and $\boldsymbol{\theta}_0$ represents the difference between BE regions when accounting and ignoring the correlations. The '+' shows the situation where BE region is larger under within-subject correlations, while '–' shows situation where BE region is larger under absence of within-subject correlations and equal sign shows that both BE regions are equal. The diagonal of the table 1 shows the conditions where within-subject reference and test correlations are equal. It is obvious that the table is asymmetric, because entries below and above the diagonal are not the mirror image. Therefore, BE regions are not equal for the same combinations of within-subject correlations depending on which drug is considered the reference. Similar results were also observed when interaction correlations were assumed as 0.5 and 0.9 (tables were not presented).

Table 1
Effect of the Within-Subject Test and Reference Correlations when $\rho_D = 0$ on MIBE (C_I) as a Function of $\boldsymbol{\theta}_0$

ρ_{WR} \ ρ_{WT}	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**
0	=	=	=	=	=	=	=	=	=	=
0.1	-	-	-	-	-	-	-	-	-	-
0.2	-	-	-	-	-	-	-	-	-	-
0.3	-	-	-	-	-	-	-	-	-	-
0.4	+	+	-	-	-	-	-	-	-	-
0.5	+	+	+	+	-	-	-	-	-	-
0.6	+	+	+	+	+	-	-	-	-	-
0.7	+	+	+	+	+	+	+	+	+	-
0.8	+	+	+	+	+	+	+	+	+	+
0.9	+	+	+	+	+	+	+	+	+	+

$= : \boldsymbol{\theta} = \boldsymbol{\theta}_0$, $+ : \boldsymbol{\theta} > \boldsymbol{\theta}_0$, $- : \boldsymbol{\theta} < \boldsymbol{\theta}_0$

3. SIMULATION STUDY

A simulation study was carried out to evaluate the power and size of the test of our proposed multivariate individual bioequivalence criterion. In simulations, following steps were taken for each of the combinations of difference in means, difference in within-subject variances, within-subject correlation of reference and test PK parameters and samples sizes. Each simulation assumed two-sequence, four-period replicated crossover design with equal number of subjects in each sequence ($n=n_1=n_2$), hence ($N= n_1 + n_2$) was the total number of subjects in each replicate. All simulations were performed in SAS 9.2.

It is important to evaluate the performance of the test under a variety of sample sizes therefore, we considered sample sizes $n= n_1= n_2 =15$, 30 and 60. We selected standard deviations for subject-by-formulation interaction as 0.1 and 0.01. Different values for the difference between means were selected as -log (1.25), -0.5log (1.25), 0, 0.5log (1.25) and log (1.25) as selected by Bassam (2009) for assessment of multivariate population bioequivalence. We chose values of differences for within-subject variances under test and reference formulations as 0.00, 0.01, 0.02, 0.03, and 0.04. A wide range of within-subject correlations among test and reference PK parameters and interaction correlations were selected. The correlations between 0 and 0.8 in increments of 0.2 were used to evaluate the effect of the correlations on the power and the size of the proposed test.

1. Generate 300 replicates (samples) of size N from bivariate normal distribution under two-sequence, four-period replicated crossover design with reference means μ_{R1} and μ_{R2} and test means μ_{T1} and μ_{T2} and within-subject variances σ^2_{WT1}, σ^2_{WT2} and σ^2_{WR1}, σ^2_{WR2} and subject-by-formulation interactions $\sigma^2_{D1}, \sigma^2_{D2}$ and within-subject correlations ρ_{WT}, ρ_{WR} and ρ_D that define configuration.
2. Estimate the mean vectors and within-subject variance covariance matrix for each replication.

3. According to the FDA draft guidelines (FDA, 1997) at least 2000 replications are recommended for bootstrap sampling, therefore, we bootstrap each replicate 2000 times using bootstrap method. Calculate estimated MIBE criterion $\hat{C}_I^b$ for each bootstrap sample. Here subscript *b* shows bootstrap.
4. For each simulation configuration calculate 95th percentile for 2000 calculated bootstrap estimates of $\hat{C}_I^b$. Let this percentile be denoted as $\hat{C}_{I(0.95)}$ which is the upper limit of one-sided confidence interval of C_I.
5. For each replication, reject the null hypothesis if the upper limit of one-sided 95% confidence interval, $\hat{C}_{I(0.95)}$ is less than predefined $\boldsymbol{\theta}$.
6. Calculate the size and power of the test by calculating the percentage of times the null hypothesis was rejected among 300 replicates, if the null hypothesis was true or was not true respectively.

3.1 Evaluation of Size and Power of the Test

In the example of bioequivalence, power of the test could be explained as probability of determining two drug as bioequivalent when they are actually bioequivalent.

Tables 2 to 6 compare two tests, the first ignores the correlation and the second one accounts for correlation in $\boldsymbol{\theta}$**.** Tables 2 and 3 compare the power of the proposed MIBE test as the function of the sample size, the within-subject correlations and the difference in means. Table 2 exhibits power of the test where subject-by-formulation interaction is assumed as 0.1. As expected power is highest when there is no difference in true means of the test and reference formulations and sample is large. The power drops gradually as the difference between means increases in either direction. Furthermore, power increases as within-subject correlations increases. Accounting for the correlation in $\boldsymbol{\theta}$ results in a test with relatively less power than ignoring the correlation. Table 3 shows the results for the power of the test when value of subject-by-formulation interaction is assumed as 0.01. Here again highest power is observed when true mean difference is 0. Comparison of tables 2 and 3 shows that low value of subject-by-formulation interaction produces relatively higher power.

Tables 4 and 5 compare the power of the proposed MIBE test as the function of the sample size, the within-subject correlations and the difference in within-subject variances. In Table 4 we present the results of power of the test where subject-by-formulation interaction is assumed as 0.1. Highest power is observed when there is no difference in within-subject variances with larger sample size. The power drops gradually as difference in within-subject variances increases. Likewise, tables 2 and 3 here again power increases as within-subject correlations increases. Similar pattern is observed in table 5 where subject-by-formulation interaction is assumed as 0.01. Comparison of tables 4 and 5 illustrate that low value of subject-by-formulation interaction produces relatively high value of power of the test.

In bioequivalence setting the size of the test could be assessed as the probability of determining two drugs as bioequivalent when they are actually not bioequivalent. Here we used maximum allowable value of the difference between means as 0.2231 and $\sigma_{WT1} = \sigma_{WR1} = 0.25,\ \sigma_{WT2} = \sigma_{WR2} = 0.25$ and therefore, to assess the size of the test chose other parameter values so that $\boldsymbol{\theta} = 4.989$. These values define the boundaries of

the MIBE acceptance region. The estimated sizes of the proposed test are summarized in table 6. In most of the cases our proposed test maintains expected 5 percent type I error and this probability of Type I error is too conservative in most of the cases. However, in only few cases these probabilities exceed from 0.05 when sample size is large and within-subject references correlation is greater than equal to 0.6.

Table 2
Power of the Test as the Function of Correlation, Difference in Means and the Sample Size when Subject-by-Formulation is 0.1

$\sigma_{D1} = \sigma_{D2} = 0.1$							
$\rho_{WR} = \rho_{WT} = \rho_D$	$\boldsymbol{\mu_T}$ - $\boldsymbol{\mu_R}$	**Ignoring Correlation in $\boldsymbol{\theta}$** $P(C_{\mathrm{I}} < \boldsymbol{\theta}_0)$			**Accounting for Correlation in $\boldsymbol{\theta}$** $P\,(C_{\mathrm{I}} < \boldsymbol{\theta})$		
		n=15	**n=30**	**n=60**	**n=15**	**n=30**	**n=60**
0	-0.2231	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	-0.1115	0.0909	0.4646	0.6768	0.0909	0.4646	0.6768
	0	0.4545	0.9293	0.9899	0.4545	0.9293	0.9899
	0.1115	0.1414	0.3535	0.6869	0.1414	0.3535	0.6869
	0.2231	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.2	-0.2231	0.0000	0.0000	0.0202	0.0000	0.0000	0.0101
	-0.1115	0.1818	0.6465	0.8182	0.1111	0.5657	0.7576
	0	0.5859	0.9798	1.0000	0.4949	0.9394	0.9899
	0.1115	0.2222	0.5556	0.8889	0.1616	0.4444	0.7879
	0.2231	0.0000	0.0101	0.0101	0.0000	0.0000	0.0000
0.4	-0.2231	0.0000	0.0101	0.0707	0.0000	0.0000	0.0202
	-0.1115	0.3030	0.8080	0.9293	0.2323	0.6667	0.8283
	0	0.7172	0.9899	1.0000	0.5758	0.9798	1.0000
	0.1115	0.2929	0.6869	0.9495	0.1717	0.5455	0.8687
	0.2231	0.0000	0.0303	0.0505	0.0000	0.0101	0.0101
0.6	-0.2231	0.0101	0.0808	0.1616	0.0000	0.0101	0.0707
	-0.1115	0.3434	0.8586	0.9798	0.2424	0.7475	0.8990
	0	0.7879	0.9899	1.0000	0.6061	0.9798	1.0000
	0.1115	0.4040	0.8182	0.9899	0.2626	0.6263	0.9192
	0.2231	0.0101	0.0505	0.1010	0.0000	0.0202	0.0404
0.8	-0.2231	0.0101	0.1616	0.3333	0.0000	0.0202	0.1212
	-0.1115	0.4848	0.9394	0.9899	0.2727	0.8081	0.9394
	0	0.8586	1.0000	1.0000	0.6364	0.9798	1.0000
	0.1115	0.4444	0.8788	0.9898	0.2828	0.6667	0.9495
	0.2231	0.0202	0.1010	0.2626	0.0000	0.0505	0.0707

- $\sigma^2_{WT1} - \sigma^2_{WR1} = 0, \sigma^2_{WT2} - \sigma^2_{WR2} = 0$
- Power of the test: the probability of rejecting the null hypothesis of "not bioequivalent" within the population BE region.

Table 3
Power of the Test as the Function of Correlation, Difference in Means and the Sample Size when Subject-by-Formulation is 0.01

$\sigma_{D1} = \sigma_{D2} = 0.01$							
$\rho_{WR} = \rho_{WT} = \rho_D$	μ_T - μ_R	**Ignoring Correlation in θ** $P(C_I < \theta_0)$			**Accounting for Correlation in θ** $P\ (C_I < \theta)$		
		n=15	**n=30**	**n=60**	**n=15**	**n=30**	**n=60**
0	-0.2231	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	-0.1115	0.1313	0.5859	0.7879	0.1313	0.5859	0.7879
	0	0.5455	0.9697	1.0000	0.5455	0.9697	1.0000
	0.1115	0.1717	0.4848	0.8182	0.1717	0.4848	0.8182
	0.2231	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.2	-0.2231	0.0000	0.0101	0.0303	0.0000	0.0000	0.0101
	-0.1115	0.2323	0.7273	0.8990	0.1818	0.6566	0.8283
	0	0.7172	0.9899	1.0000	0.6263	0.9798	1.0000
	0.1115	0.2929	0.6364	0.9293	0.2323	0.5556	0.8485
	0.2231	0.0000	0.0101	0.0101	0.0000	0.0101	0.0101
0.4	-0.2231	0.0101	0.0202	0.1010	0.0000	0.0101	0.0404
	-0.1115	0.3232	0.8384	0.9697	0.2323	0.7172	0.8586
	0	0.7778	1.0000	1.0000	0.6465	0.9798	1.0000
	0.1115	0.3636	0.7475	0.9697	0.2828	0.5960	0.9192
	0.2231	0.0000	0.0404	0.0505	0.0000	0.0101	0.0101
0.6	-0.2231	0.0101	0.0909	0.2121	0.0000	0.0101	0.0808
	-0.1115	0.4141	0.9091	0.9798	0.2727	0.8081	0.9394
	0	0.8687	1.0000	1.0000	0.6566	0.9798	1.0000
	0.1115	0.4444	0.8586	0.9899	0.2626	0.6667	0.9495
	0.2231	0.0101	0.0606	0.1111	0.0000	0.0404	0.0505
0.8	-0.2231	0.0101	0.1919	0.3636	0.0101	0.0606	0.1212
	-0.1115	0.5051	0.9394	0.9899	0.3030	0.8283	0.9394
	0	0.9091	1.0000	1.0000	0.6566	0.9798	1.0000
	0.1115	0.5253	0.8999	0.9899	0.3232	0.6869	0.9495
	0.2231	0.0202	0.1212	0.3030	0.0000	0.0505	0.0707

- $\sigma^2_{WT1} - \sigma^2_{WR1} = 0, \sigma^2_{WT2} - \sigma^2_{WR2} = 0$
- Power of the test: the probability of rejecting the null hypothesis of "not bioequivalent" within the population BE region.

Table 4
Power of the Test as the Function of Correlation, Difference in within Subject Variances and the Sample Size when Subject-by-Formulation is 0.1

$\sigma_{D1} = \sigma_{D2} = 0.1$							
$\rho_{WR} = \rho_{WT} = \rho_D$	$\sigma^2_{WT1} - \sigma^2_{WR1} = \sigma^2_{WT2} - \sigma^2_{WR2}$	**Ignoring Correlation in $\boldsymbol{\theta}$** $P(C_{\mathrm{I}} < \boldsymbol{\theta}_0)$			**Accounting for Correlation in $\boldsymbol{\theta}$** $P\,(C_{\mathrm{I}} < \boldsymbol{\theta})$		
		n=15	**n=30**	**n=60**	**n=15**	**n=30**	**n=60**
0	0	0.4545	0.9292	0.9899	0.4545	0.9292	0.9899
	0.01	0.1616	0.5758	0.8484	0.1616	0.5758	0.8484
	0.02	0.0606	0.2222	0.5556	0.0606	0.2222	0.5556
	0.03	0.0000	0.0303	0.1212	0.0000	0.0303	0.1212
	0.04	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.2	0	0.5859	0.9798	1.0000	0.4949	0.9394	0.9899
	0.01	0.3131	0.7879	0.9495	0.2121	0.6970	0.8889
	0.02	0.0909	0.3737	0.7374	0.0808	0.3030	0.6768
	0.03	0.0404	0.1313	0.2929	0.0101	0.0606	0.1919
	0.04	0.0000	0.0000	0.0606	0.0000	0.0000	0.0000
0.4	0	0.7172	0.9899	1.0000	0.5758	0.9798	1.0000
	0.01	0.4040	0.9091	0.9798	0.3030	0.7879	0.9596
	0.02	0.1212	0.5253	0.8586	0.0909	0.3535	0.6970
	0.03	0.0808	0.2323	0.5758	0.0303	0.0909	0.2828
	0.04	0.0202	0.0404	0.1212	0.0000	0.0000	0.0505
0.6	0	0.7879	0.9899	1.0000	0.6060	0.9798	1.0000
	0.01	0.4848	0.9596	0.9899	0.3131	0.8283	0.9596
	0.02	0.2323	0.7374	0.9394	0.1212	0.4242	0.7778
	0.03	0.1010	0.3535	0.6970	0.0303	0.1212	0.3434
	0.04	0.0202	0.1111	0.2626	0.0000	0.0101	0.0707
0.8	0	0.8586	1.0000	1.0000	0.6364	0.9798	1.0000
	0.01	0.5455	0.9798	1.0000	0.3535	0.8485	0.9596
	0.02	0.3030	0.8182	0.9596	0.1515	0.4646	0.7879
	0.03	0.1414	0.4141	0.7576	0.0505	0.1515	0.4040
	0.04	0.0505	0.1313	0.3737	0.0101	0.0101	0.0808

- $\mu_{T1} - \mu_{R1} = 0, \mu_{T2} - \mu_{R2} = 0$
- Power of the test: the probability of rejecting the null hypothesis of "not bioequivalent" within the population BE region.

Table 5
Power of the Test as the Function of Correlation, Difference in Within Subject Variances and the Sample Size when Subject-by-Formulation is 0.01

$\sigma_{D1} = \sigma_{D2} = 0.01$							
$\rho_{WR} = \rho_{WT} = \rho_D$	$\sigma^2_{WT1} - \sigma^2_{WR1} = \sigma^2_{WT2} - \sigma^2_{WR2}$	**Ignoring Correlation in $\boldsymbol{\theta}$** $P(C_{\mathrm{I}} < \boldsymbol{\theta}_0)$			**Accounting for Correlation in $\boldsymbol{\theta}$** $P\,(C_{\mathrm{I}} < \boldsymbol{\theta})$		
		n=15	**n=30**	**n=60**	**n=15**	**n=30**	**n=60**
0	0	0.5455	0.9697	1.0000	0.5455	0.9697	1.0000
	0.01	0.2929	0.7879	0.9495	0.2929	0.7879	0.9495
	0.02	0.0808	0.3535	0.7071	0.0808	0.3535	0.7071
	0.03	0.0404	0.1313	0.2525	0.0404	0.1313	0.2525
	0.04	0.0000	0.0000	0.0202	0.0000	0.0000	0.0202
0.2	0	0.7172	0.9899	1.0000	0.6263	0.9798	1.0000
	0.01	0.3939	0.8990	0.9798	0.3232	0.8182	0.9596
	0.02	0.1313	0.4949	0.7980	0.1111	0.3939	0.7576
	0.03	0.0505	0.2020	0.4646	0.0303	0.1515	0.3131
	0.04	0.0000	0.0303	0.0909	0.0000	0.0101	0.0606
0.4	0	0.7778	1.0000	1.0000	0.6465	0.9798	1.0000
	0.01	0.4444	0.9394	0.9899	0.3535	0.8384	0.9596
	0.02	0.2020	0.6667	0.8990	0.1111	0.4141	0.7778
	0.03	0.0808	0.2929	0.6566	0.0404	0.1616	0.3737
	0.04	0.0202	0.0909	0.2222	0.0000	0.0101	0.0808
0.6	0	0.8687	1.0000	1.0000	0.6566	0.9798	1.0000
	0.01	0.5354	0.9697	1.0000	0.3737	0.8889	0.9697
	0.02	0.2828	0.7980	0.9495	0.1313	0.5051	0.8182
	0.03	0.1010	0.3939	0.7374	0.0404	0.2020	0.4747
	0.04	0.0404	0.1111	0.3232	0.0101	0.0202	0.0909
0.8	0	0.9090	1.0000	1.0000	0.6566	0.9798	1.0000
	0.01	0.5859	0.9798	1.0000	0.3737	0.8889	0.9899
	0.02	0.3333	0.8889	0.9596	0.1515	0.6464	0.8384
	0.03	0.2828	0.4646	0.7879	0.1313	0.1717	0.5253
	0.04	0.0303	0.1717	0.4848	0.0101	0.0202	0.1010

- μ_{T1} - $\mu_{R1} = 0, \mu_{T2}$ - $\mu_{R2} = 0$
- Power of the test: the probability of rejecting the null hypothesis of "not bioequivalent" within the population BE region.

Table 6
Estimated size of the test

ρ_{WR}	ρ_D	ρ_{WT}	**Ignoring Correlation in θ** $\mathbf{P(C_I < \theta_0)}$			**Accounting for Correlation in θ** $P(C_I < \theta)$		
			n=15	**n=30**	**n=60**	**n=15**	**n=30**	**n=60**
0	0	0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	0.2	0.2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	0.4	0.4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	0.6	0.6	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	0.8	0.8	0.0000	0.0000	0.0101	0.0000	0.0000	0.0101
0.2	0	0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	0.2	0.2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	0.4	0.4	0.0000	0.0000	0.0101	0.0000	0.0000	0.0000
	0.6	0.6	0.0000	0.0000	0.0101	0.0000	0.0000	0.0000
	0.8	0.8	0.0000	0.0000	0.0202	0.0000	0.0000	0.0101
0.4	0	0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	0.2	0.2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	0.4	0.4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	0.6	0.6	0.0000	0.0000	0.0101	0.0000	0.0000	0.0000
	0.8	0.8	0.0000	0.0000	0.0404	0.0000	0.0000	0.0000
0.6	0	0	0.0000	0.0000	0.0000	0.0000	0.0202	0.0606
	0.2	0.2	0.0000	0.0000	0.0101	0.0000	0.0000	0.0303
	0.4	0.4	0.0000	0.0000	0.0303	0.0000	0.0000	0.0303
	0.6	0.6	0.0000	0.0000	0.0505	0.0000	0.0000	0.0101
	0.8	0.8	0.0101	0.0303	0.0606	0.0000	0.0000	0.0000
0.8	0	0	0.0101	0.0303	0.1717	0.0909	0.1717	0.6060
	0.2	0.2	0.0101	0.0404	0.1818	0.0303	0.1010	0.4040
	0.4	0.4	0.0101	0.0505	0.1717	0.0101	0.0505	0.1717
	0.6	0.6	0.0101	0.0505	0.2121	0.0101	0.0202	0.0606
	0.8	0.8	0.0101	0.0909	0.2020	0.0000	0.0101	0.0303

- Probability of rejecting the null hypothesis of "not bioequivalent" at the boundary of the BE region

4. NUMERICAL EXAMPLE

We considered an example mentioned in dataset 17a on US FDA http://www.fda.gov/Drugs/ScienceResearch/ucm301277.htm. In this dataset 37 subjects were used for BE evaluation of antihypertensive patch. They employed replicated crossover design of 2-sequence and 4-period with 18 and 19 subjects in sequence TRRT and RTTR respectively. The researchers at FDA revealed that the data were considered to have a large subject by formulation interaction (>0.15), this leads to the assessment of individual

bioequivalence. We constructed bivariate dataset, consisting of two PK parameters, AUC and C_{max} and used logarithmic transformation as recommended by FDA (2001). We used this two-dimensional, log-transformed data set for the assessment of multivariate individual bioequivalence using the proposed criterion (2.6). Using the above mentioned model the estimated matrices are obtained using SAS 9.2.

$$\hat{\boldsymbol{\mu}}_T - \hat{\boldsymbol{\mu}}_R = \begin{pmatrix} \hat{\mu}_{TAUC} \\ \hat{\mu}_{TCmax} \end{pmatrix} - \begin{pmatrix} \hat{\mu}_{RAUC} \\ \hat{\mu}_{RCmax} \end{pmatrix} = \begin{pmatrix} -0.0434 \\ -0.10881 \end{pmatrix}$$

$$\hat{\Sigma}_{WT} = \begin{bmatrix} 0.0956 & 0.11591 \\ 0.11591 & 0.16675 \end{bmatrix}$$

$$\hat{\Sigma}_{WR} = \begin{bmatrix} 0.06559 & 0.07827 \\ 0.07827 & 0.122788 \end{bmatrix}$$

$$\hat{\Sigma}_{D} = \begin{bmatrix} 0.05197 & 0.04862 \\ 0.04862 & 0.05725 \end{bmatrix}$$

As expected the log transformed AUC and C_{max} were found to be highly correlated in reference group ($\rho_{WR} = 0.872$) and test group ($\rho_{WR} = 0.917$) whereas $\rho_D = 0.894$. The proposed multivariate individual bioequivalence criterion $C_{\mathbf{I}}$was calculated using equation 2.6. The univariate criterion for IBE for each parameter AUC and C_{max} were also calculated using equation 1.3. The bootstrap method was used to determine the upper limit of 95% confidence interval for MIBE criterion and two univariate IBE criteria. The multivariate individual bioequivalence rule $\boldsymbol{\theta}$ which defines the upper boundary were calculated in two ways 1) when within-subject correlations between two PK parameters were ignored ($\boldsymbol{\theta_0} = 4.989$) and 2) when accounting those correlations ($\boldsymbol{\theta} = 3.227$). The parametric bootstrap was set to generate 2000 samples and multivariate IBE criterion was calculated for each generated sample. Then determined the upper limit of 95% confidence interval of MIBE criterion.

Table 7
Results of Univariate and Multivariate IBE

Test	Upper Limit of 95% C.I	Theta
Univariate IBE (AUC)	3.00379	2.494
Univariate IBE (Cmax)	2.51310	2.494
Multivariate IBE	4.64091	4.989 (ignoring correlation)
Multivariate IBE		3.227 (accounting for correlation)

Form Table 7 it is obvious that univariate tests rejected individual bioequivalence in this study as both upper limits of 95% C.I for AUC and C_{max} were greater than predefined value of 2.494. In multivariate situation data showed individual bioequivalence when correlations were ignored in $\boldsymbol{\theta}$**.** However, data did not confirm MIBE when within-subject correlations were considered in the calculation of $\boldsymbol{\theta}$ because calculated upper limit of 95% C.I of MIBE criterion was greater than 3.227. So these results show that for this study two formulations are not bioequivalent using either univariate or multivariate individual bioequivalence testing with account of correlation for $\boldsymbol{\theta}$. Effect of within-subject correlations can be observed in this study because when correlations were ignored for $\boldsymbol{\theta}$ the multivariate result was not in line when correlations were considered.

5. DISCUSSION

When a medicinal substitute of a marketed drug is synthesized its safety and efficacy are debatable and this situation is related to the drugs switchability. Individual bioequivalence takes into account within-subject and subject-by-formulation variances, thus being a relevant criterion to handle changes in treatment when a reference is substitute by a generic drug. Individual bioequivalence bears the advantage of permitting rather precise evaluation of bioequivalence for drugs with high pharmacokinetic variability.

Our proposed multivariate bioequivalence test will be applicable to all in vivo bioequivalence studies with higher order crossover design, intended to claim switchability between test and reference drug formulations.

The multivariate bioequivalence method will be practically applicable in situations where aggregation make sense for a specific application. For example, the usual pharmaceutical equivalence problems lie in in-vivo bioequivalence, where comparisons between two formulations are made on area under the curve (AUC) and maximum concentration (C_{max}). A generic drug sponsor is required to show equivalence to reference product regarding both of these measures. To make an attempt for considering of a multivariate bioequivalence approach is justified, because AUC and C_{max} both are derived from same serum concentration-time curve, hence likely to be combined into a single criterion.

As mentioned above important PK parameters are derived from serum concentration-time curve therefore, in bioequivalence assessment using multiple tests for each of four PK parameters, the assumption of independence among parameters is not justifiable. Clearly, correlations among PK parameters should be incorporated in multivariate tests of bioequivalence. Our proposed criterion for multivariate IBE that is equivalent to univariate IBE criterion approved by FDA not only incorporates comparisons of means and within-subject variances but also includes the within-subject correlations among PK parameters. Furthermore, our simulations result show that the proposed multivariate individual bioequivalence test performs well for higher correlation among PK parameters.

In univariate situation θ for individual bioequivalence is defined according to limits of BE acceptance defined by FDA. But there is no regulatory decision regarding θ which shows that whether it should be greater in multivariate situations or not. Therefore, we extended the limits previously defined for univariate case to the multivariate situations. Since there is no corresponding guideline regarding correlation in $\boldsymbol{\theta}$, we used various combinations of within-subject correlations between test and reference PK parameters for which $\boldsymbol{\theta}$ could be calculated. Our proposed test maintains high power at no or minimum differences of true means and within-subject variances with larger sample size. And this power continues to increase as within-subject correlations increases. Furthermore, this test maintains at least 80% power even at low sample size when within-subject correlations are high ($\geq$ 0.8) under no difference of true means and within-subject variances. Our simulation results compare power and size of the test when accounting and ignoring the correlations in $\boldsymbol{\theta}$**.** It is evident that accounting correlations for $\boldsymbol{\theta}$ slightly decrease the power (but still reasonably high ($\geq$ 80%) too at minimum differences and

large sample size) because calculated value of $\boldsymbol{\theta}$ become low in such cases. Our proposed test is too conservative if within-subject correlations are less than 0.6. There are only few cases where accounting the correlation for $\boldsymbol{\theta}$ produced relatively higher probability of type I error because parameter settings provide large value of $\boldsymbol{\theta}$ in such cases.

Our numerical example revealed the importance of accounting for correlations in defining the acceptable multivariate individual bioequivalence region because accounting and ignoring correlations in $\boldsymbol{\theta}$ did not provide same multivariate IBE conclusions.

Comprehensive SAS programs were written for simulations performed in this research study which are available from authors through email.

6. LIMITATIONS AND RECOMMENDATIONS

Our proposed criterion bears some special aspects, first it is not symmetric with respect to reference and test formulations. This asymmetry is not restricted only to the multivariate situations, but can also be displayed in univariate tests. In multivariate case, it is due to scaling of the criterion by within-subject variance covariance matrix. In case of evaluation of pharmaceutical generic products, it is reasonable where the reference is the current product, however, it is not devoid of limitation in some areas. Symmetrical criteria in multivariate and univariate situations can be found in Czado and Munk (1998) and Dragalin et al. (2003). Second, our proposed criterion due to its aggregated nature, is the sum of two entities. Therefore, the upper limit of this multivariate IBE criterion is achievable either by increasing both entities simultaneously or by increasing one of them while keeping the other one as minimum as possible. Due to this impact the multivariate bioequivalence could be achieved even if it is not bioequivalent at one of univariate tests when other parameters are close to zero. All aggregate methods proposed hitherto have such limitation hence our proposed criterion have also. This limitation makes the basis of FDA requirement for presenting univariate average and population bioequivalence in order to show univariate individual bioequivalence. Future research is recommended to find methods or measures that have hierarchical nature of average, population and individual bioequivalence because multivariate bioequivalence may be needed to be shown after demonstrating univariate average and population bioequivalence.

The performance of our proposed test was assessed through simulations which showed power and size of the test at different specific combinations of sample sizes, mean differences, within-subject variances, and within-subject correlations. One can also understand the properties and assess the performance of the proposed test at various combinations of parameters different from the ones reported in this research. Furthermore, our simulation study was limited to equal sample size in each sequence that might be not realistic in many bioequivalence studies. Hence, the effects of unequal sample size can be determined in future studies.

ACKNOWLEDGEMENT

The authors acknowledge Dr. Najia Mansoor, Department of Pharmacology University of Karachi for her valuable assistance that greatly improved manuscript formatting.

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