

# Study of regulatory requirements for the conduct of bioequivalence studies in US, Europe, Canada, India, ASEAN and SADC countries: Impact on generic drug substitution

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## ABSTRACT

In the last one decade, due to expiry of patented products as well as their exclusivity period, a drastic decline of branded pharmaceutical products and up streaming of generic drug market has been observed in developed as well as developing nations. This up rise in generic drug market is expected to rise in future till the arrival of new brand in market. This prevailing conditions could result in proliferation of generic drug manufacturing companies. The fact that generics do not undergo thorough extensive trials like innovator drugs, fuels further fears regarding their inferiority. Moreover, due to the hard competition amongst various companies to market their generics, the frequency of fraud and corruption have embarked doubts in consumers mind to reality. In order to blow away the doubts and re-establishing the credibility of generics in market, bioequivalence (BE) guidelines with stricter regulation should be the demand. The present study highlights the relevant regulatory guidelines for the conduct of bioequivalence studies in US, Europe, Canada, India, South Africa and South East Asian Nations. A comparative study of the differences in study design and specifications have also been addressed.

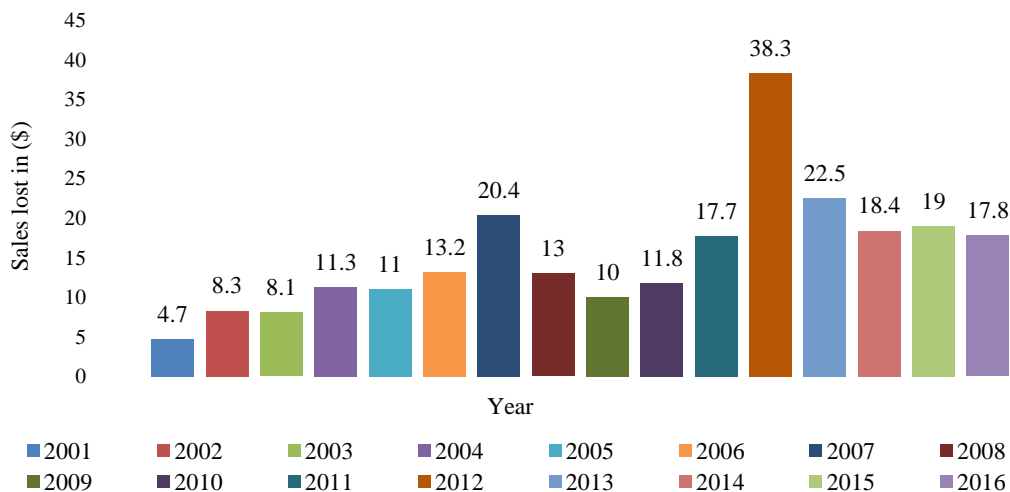
## INTRODUCTION

In last five years a depletion in sale and distribution of innovator drugs was observed and hence the present era in the pharmaceutical industry can be considered as the era of - "Innovator Drought". Between the years 2009 and 2013, pharmaceutical industries have faced the sharpest revenue decline in the history and any respite in the near future is unlikely. According to prediction, this economically depressing scenario is likely to continue till 2020 end. Another factor that contributes to this delicate situation is emergence of "Patent Cliff" or "Pharmageddon", which means loss of patent rights including their exclusivity. In a study it is reported that, 18 out of 20 blockbuster drugs have lost their patent protection which has led to "Brand Erosion", and again this is likely to continue till the end of 2020 (Pharmaceutical Online, 2012; Accenture Life Sciences, 2012). The depletion of patent protected innovator

drugs from market adds further pressure on the global generic drug industry. The global loss that industries faced due to expiry of all patented products in the last 12 years is shown in Fig.1. However, due to the prevailing condition of "Patent Cliff", generic industry is undoubtedly anticipating lavish gains especially in the pharma-emerging nations as their strength lies in patient numbers. This condition could result in mushrooming of generic drug manufacturing companies, but getting the product in market which can be substituted in place of the branded drug is a task with many hurdles. The perception of physicians, the consumers or the health care providers towards the generics play a major role in their acceptance. There have been number of cases, where the acceptance of generics has become questionable by the consumers (Meredith, 2003). This perception becomes more fixed as the severity of medical condition of the patient increases. It was found in a study that at least 20% to 30% of the consumers are in dilemma that generic products are less safe and effective than the branded drugs (Ganther and Kreling, 2000). This perception was found directly dependent upon the severity of the medical condition of the patient. In a study, it was observed that 14.2% of patients with cough opined that generic drugs were riskier than their branded counterparts.

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**Fig 1:** Sales lost due to patent expiry globally between 2001-2016 (<http://www.bioassociate.com/wp-content/uploads/2012/05/Bioassociate-The-significance-and-apparent-repercussions-of-the-2009-2015-pharmaceutical-patent-cliff.pdf>).

Further, this ratio increased to 53.8% in case of patients suffering from cardiac ailments (Ganther and Kreling, 2000). Substitution of generic drugs in place of branded drugs becomes even more questionable in case of occurrence of incidences that hold such consumer perception true, for example:

1. Generics of digoxin and some other cardiac agents have shown to be associated with anecdotal bioequivalence problems (Meredith, 2003).

2. The generic formulation of propranolol hydrochloride was reported to have a 40% higher incidence of adverse events than its branded counterpart (Sanderson and Lewis, 1986).

3. There has been found a difference in the absorption pattern of oral procainamide hydrochloride in healthy volunteers in comparison to individuals with acute myocardial infarction, questioning the validity of extrapolating bioequivalence (BE) in normal population to patient population (Meredith, 1996; Henderson and Eshan, 2001).

4. The composition of generic drugs differs in terms of excipients or inert substances from branded drugs. However, the use of lactose or gluten containing ingredients have significant effects on gut motility and absorption in lactose intolerant patients. Therefore, switch ability from branded to generics in such patients is more concerned (Reiffel, 1997; Dighe, 1999).

Likewise, in a study conducted by Elkoshi *et al.*, two omeprazole sodium formulations were found to be bioinequivalent due to the difference in their enteric coating (Elkoshi *et al.*, 2002). Although according to FDA, single dose studies are more sensitive to prove the bioequivalency but apparently, the inert substances used in generics can alter the distribution, metabolism and elimination at the steady state. This difference in the generic formulations of omeprazole sodium was found after the multiple dose studies that questions the validity of the studies conducted to prove the switchability (Besag, 2000).

5. It is just not about the inactive ingredients used, the compliance of generics also depends upon the appearance of the

drugs, multiple generics of single drug, inconsistent substitution to the patients by the pharmacy or, the physician. These differences have shown to cause confusion amongst patients especially geriatric patients causing anxiety (Besag, 2000; Gerbino and Joseph, 1993).

6. The use of generic drugs in pediatric patients has its own concerns. Generics are often tested on adult volunteers and when the same generics are administered to the children, there has been observed changes in the absorption, distribution, metabolism and excretion (ADME). For instance, omeprazole when orally administered in children have shown significant difference in plasma levels, area under curve (AUC), plasma half-life and concentration maxima ( $C_{max}$ ) than in adults. This is due to the difference in the metabolic rate in children as compared to adults (Andersson *et al.*, 2000; Israel and Hassel, 1998).

These kind of issues tend to reduce faith of health care providers as well as patients in generic drugs (Meredith, 1996). The fact that generics do not undergo thorough extensive trials like innovator drugs fuels further fears regarding their inferiority. Moreover, due to the hard competition amongst various companies to market their generics, the frequency of fraud and corruption have led to embarking the doubt in consumers mind to reality (Meredith, 1996; Dighe, 1999). Another hurdle in generic drug substitution and need for BE studies are the factors that affect bioavailability of drugs. Various factors that affect the bioavailability of drugs are shown in Fig 2.

Thus, establishing the generic market in pharma emerging countries can be a challenge for global pharma players. In order to blow away the doubts and re-establish the credibility of generics in market, bioequivalence (BE) guidelines with stricter regulation should be implemented. In case of pharma emerging markets, not only carrying out the bioequivalence trials for generics is important, but understanding the geographical variations of these countries has its own significance for carrying out trials for successful ANDA approvals.

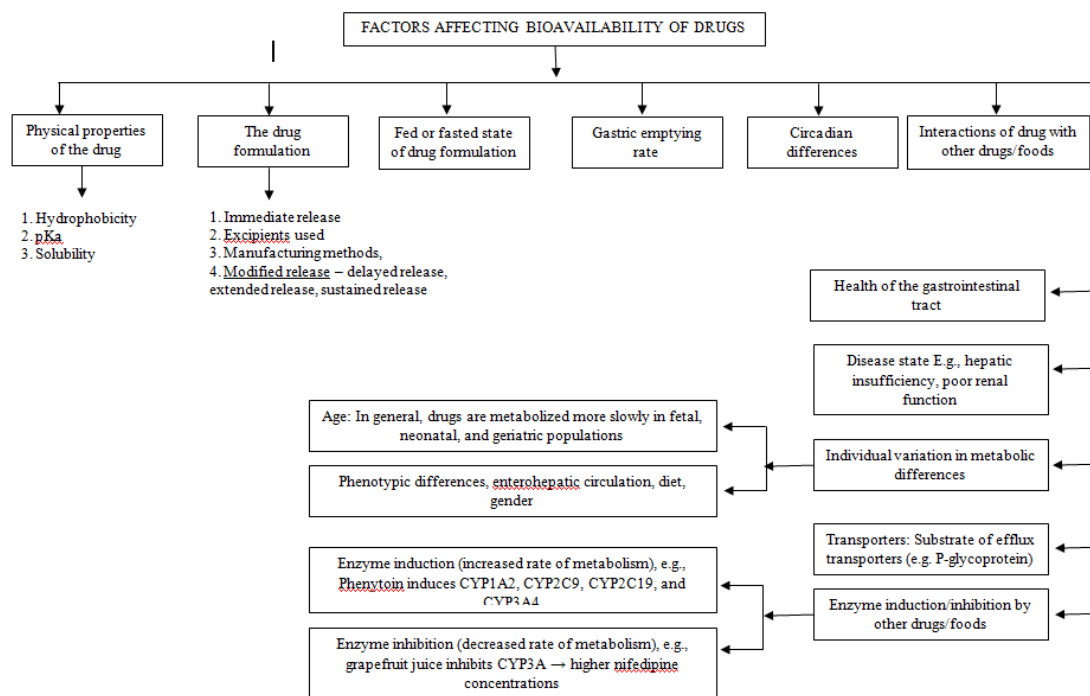


Fig. 2: Factors affecting bioavailability of drugs.

While dealing with the issue of geographical variation, establishing the local market can prove to be beneficial, local production can help the organization to shorten their supply chain, avoid any currency fluctuations and understand the specific market's requirements and needs. Furthermore, it can also help in meeting the urgent needs in the country.

Brand erosion will lead to generics flood in the pharma emerging countries. This will foster tough competition among generic drug companies (Pharmaceutical Online; 2012 Accenture Life Sciences, 2012) for getting early ANDA approvals in these countries, and thus resulting in an atmosphere conducive for the bioequivalence trials. In such a scenario, it becomes imperative to give careful consideration to the guidelines for conducting bioequivalence trials. The guidelines should be harmonized and well implemented. As it is clear that there is an off shore movement of BE studies to the Pharma emerging countries, the harmonization in the guidelines is the foremost requirement for required results due to:

1. In ideal circumstances, the generics tested in Pharma emerging countries should have access to global market. Hence, one harmonized guideline for conducting bioequivalence trials that is acceptable globally and ensures entry of generics in global market is the requirement of the day.

2. The geographic variation in Pharma emerging countries is one big challenge. The implications of variability in the staple food, climatic conditions and body mass index should be taken into account while framing the guidelines. This article addresses the comparative regulatory requirements of the developed and developing nations for the conduct of BE studies. Moreover, this article also highlights and recommends some of the

key aspects that are untouched or, yet to be resolved by the regulatory authorities during the conduct of BE studies.

### Significance of BE studies

“Bioequivalence studies are intended to look at the in vivo execution of a test pharmaceutical item (multi-source) contrasted with a reference pharmaceutical item. A typical outline for a bioequivalence study includes organization of the test and reference items on two events to volunteer subjects, with every organization isolated by a washout period. The washout period is decided to guarantee that medication given in one treatment is altogether dispensed with before organization of the following treatment. Only before organization, and for a suitable period a short time later, blood and/or pee tests are gathered and examined for the convergence of the medication substance and/or one or more metabolites. The ascent and fall of these fixations after some time in every subject in the study give an appraisal of how the medication substance is discharged from the test and reference items and retained into the body. To permit correlations between the two items, these blood (to incorporate plasma or serum) and/or pee focus time bends are utilized to compute certain bioequivalence measurements of hobby” (Ananthula, 2014; Atkinson *et al.* 2015; Genel *et al.* 2015; Mendes *et al.* 2015; Rita and Akhilesh, 2015; Tamayo *et al.* 2014). The major significance of BE studies can be understood by its use to define early and late clinical trials. Moreover, it helps the generic version of branded products, through ANDA approval, to reach the patients in a much easier way and in a cost effective manner. A number of products for which BE studies have been carried out in recent years is listed in Table 1.

**Table 1:** List of drugs for which BE studies have been recently reported.

Drug	Formulation tested	Reference formulation	Strength of test formulation	Study Design	References
<b>Artesunate and Mefloquine</b>	Fixed formulation (Immediate release tablets)	Non-Fixed treatment comprised of artesunate administered as 50 mg Arsumax <sup>®</sup> tablets Guilin Pharmaceutical and mefloquine administered as 250 mg tablets manufactured by Roche	100 mg artesunate and 200 mg mefloquine manufactured by Far Manguinhos	Randomised, cross-over design with a 90 day washout period between administrations of the study treatments	Oliario <i>et al.</i> 2010
<b>Ofloxacin (Oquin)</b>	Immediate release tablets	Ofloxacin immediate release tablets (Zanocin)	200 mg	Open labeled, two periods, single dose study	Shakya <i>et al.</i> 2010
<b>Alprazolam</b>	Sublingual tablets	Alprazolam immediate tablet (1mg)	1 mg	Randomized, open label, two-way crossover, single dose study	Damle <i>et al.</i> 2013
<b>Metformin and Canagliflozin</b>	Fixed formulation (Immediate release tablets)	Equivalent doses of single-component IR tablets (Reference)	2 canagliflozin/metformin IR FDC tablets (test) at 50 mg/500 mg, 50 mg/850 mg, 50 mg/1,000 mg, 150 mg/500 mg, 150 mg/850 mg, or 150 mg/1,000 mg	Randomized, open-label, single-center, single dose, 2-treatment, 2-period cross over trials consisting of 3 phases: a screening phase of approximately 3 weeks (day-22 through day-2); a treatment phase up to 20 days (including a washout period of 10 to 15 days between day 1 of each of treatment period), and a follow-up phase 7-10 days after day 4 of period 2 or at early withdrawal.	Devineni <i>et al.</i> 2014
<b>Erlotinib Hydrochloride</b>	Immediate release tablet	Erlotinib Hydrochloride tablets (Tarceva, OSI Pharmaceuticals, USA)	150 mg	A single center, randomized, single dose, laboratory-blinded, 2-period, 2 sequence, crossover design bioequivalence study	Jawhari <i>et al.</i> , 2014
<b>Desvenlafaxine succinate</b>	Extended release tablet (Tecnoquímicas S.A., Colombia laboratory)	Pristiq <sup>®</sup> -Desvenlafaxine 50 mg extended release tablets (Wyeth pharmaceuticals)	50 mg	An open label, two periods, two previously randomized sequences, crossover design	Vargas <i>et al.</i> , 2014
<b>Nicotine</b>	Lozenges	NIQUITIN <sup>®</sup> 2 mg Lozenge (Reference) of Glaxosmithkline consumer healthcare, uk	2 mg	An open label, randomized, two-treatment, two sequence, two-period, cross-over, single-dose comparative oral bioavailability study	Garg <i>et al.</i> , 2015
<b>Escitalopram oxalate</b>	Tablets [Laboratorios Tecnoquímicas S.A. (Jamundí – Colombia)]	Lexapro <sup>®</sup> (Escitalopram) made by H. Lundbeck A/S (Valby – Denmark)	20 mg	A crossover, 2 x 2, single-dose, two treatments, two periods, two sequences design was used, with a washout period of one week	Muñoz <i>et al.</i> , 2015
<b>Bosentan</b>	Tablets [Laboratorios Tecnoquímicas S.A. (Jamundí – Colombia)]	Tracleer <sup>®</sup> (Actelion Pharmaceuticals)	125 mg	An open label, four periods, two randomized sequences, crossover, with single pre- and fed 125 mg dose study was performed	Vargas <i>et al.</i> , 2015
<b>Losartan potassium/Amlodipine besylate</b>	Fixed Dose Combination Tablets (Losanet AM, Pharmaline, Lebanon)	Concomitant Administration of Single Components of Losartan and Amlodipine Tablets (Cozaar 100 mg, Merck Sharp & Dohme Ltd, UK and Norvasc 10 mg, Pfizer, Canada)	100 mg Losartan and 10 mg amlodipine	An open label, randomized, two-treatment, two sequence, two-period, cross-over, single-dose comparative oral bioavailability study	Bustami <i>et al.</i> , 2015
<b>Rosuvastatin</b>	Tablets (Losanet AM, Pharmaline, Lebanon)	Crestor <sup>®</sup> tablets made by Laboratorios AstraZeneca	40 mg	An open-label, two period and two sequences previously randomized, crossover study	Vargas <i>et al.</i> , 2015
<b>Enoxaparin</b>	IV bolus Enox <sup>®</sup> (Medis Laboratory, Tunisia)	Lovenox <sup>®</sup> (Sanofi US, Bridgewater, New Jersey)	Bolus dose	Using a table-generated randomization schedule	Boubaker <i>et al.</i> , 2015
<b>Zopiclone</b>	Zopiclone MK <sup>®</sup> and Zopicloteq TG <sup>®</sup> [Laboratorios Tecnoquímicas S.A. (Jamundí – Colombia)]	Imovane <sup>®</sup> [Sanofi-Aventis Farmacéutica Ltda (Brasil)]	7.5 mg	A single dose, randomized, crossover, with two periods, two sequences and a washout period of one week study	Ruiz <i>et al.</i> , 2015

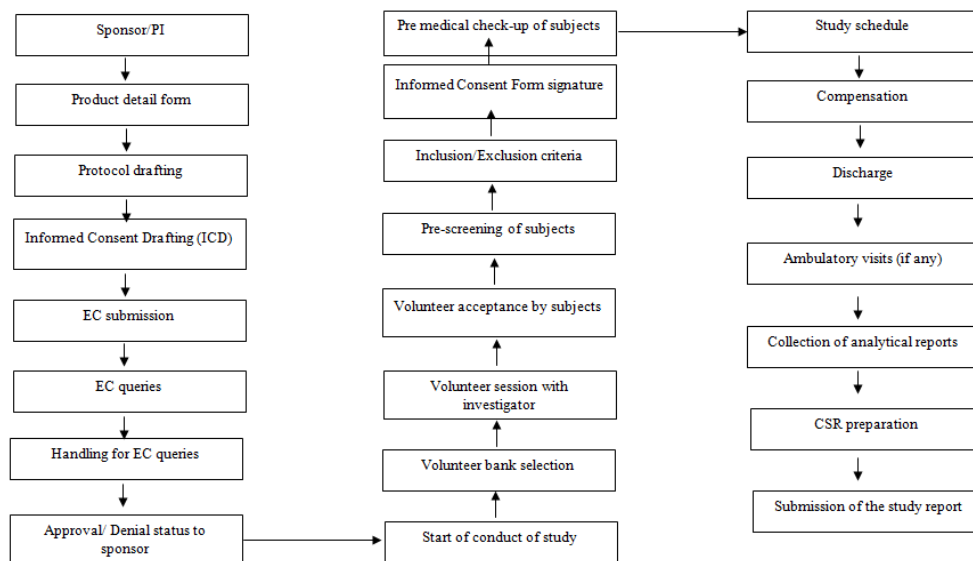


Fig. 3: Protocol for conduct of BE studies.

### Design and conduct of bioequivalence studies

The study protocol for conduct of BE studies is shown in Fig.3.

#### General study design

Single dose, non-replicate cross over designs are recommended for BE studies of immediate release and modified release dosage forms. As per United States Food and Drug Administration (USFDA) usually a single-dose, two-period, two-treatment, two-sequence cross study designs are recommended for fed BE studies where the test and reference formulations are compared following a test meal (FDA, 2003; Shaik *et al.*, 2011). European Medicines Agency (EMA), recommends a randomized, two-period, two-sequence single dose crossover design when two formulations are compared (EMA, 2010). Health Canada (HC) recommends the use of two-period cross-over design in which the subject is given the test and reference formulations (HC, 2012). As per Central Drugs Standard Control Organization (CDSCO), if two formulations are compared, a two-period and two-sequence cross over design is the design of choice (CDSCO, 2005). Similarly South African Development Community (SADC), considers a balance two-period, two-sequence crossover design as a standard design for the comparison of two formulations (SADC, 2007). Association of South East Nations (ASEAN) also recommends a two-period, two-sequence cross over design when two formulations have to be compared (ASEAN, 2004).

#### Long half-life drugs/highly variable drugs

According to USFDA, for a BE determination of an oral product of a drug with long half-life, a non-replicate, single dose, cross-over study can be conducted, provided an adequate washout period is used. If the crossover study is problematic, a BE study with a parallel design can be used [FDA, 2003]. The guidelines of EMA state that parallel study designs can be considered for substances with very long half-life and replicate designs for

substances with highly variable pharmacokinetic characteristics (EMA, 2010). HC also recommends the use of parallel designs while studying the drugs with very long elimination half-lives or some depot formulations (HC, 2012). Recommendations for using the parallel design for very long half-life substances or the replicate design for substances with highly variable disposition has been provided by CDSCO (CDSCO, 2005). As per SADC, well-established parallel designs for very long half-life substances could be considered and for long half-life drugs (>24 hours) the study should cover a minimum of 72 hours unless 80 % is recovered before 72 hours (SADC, 2007; ASEAN, 2004). ASEAN also recommends the use of parallel design for very long half-life substances and replicate designs for substances with highly variable disposition (ASEAN, 2004).

#### Blinding

There is as such no information provided by USFDA, EMA, CDSCO, ASEAN and SADC regarding blinding during the study (FDA, 2003; ASEAN, 2004; CDSCO, 2005; SADC, 2007; EMA, 2010).

HC recommends that to avoid biasness, comparative bioavailability studies should be conducted in such a manner that the subjects should not be aware of which product (test or reference) is being administered. Furthermore, the person responsible for recording adverse reactions and those conducting the bioanalysis of samples should not be aware of the treatment sequence (HC, 2012).

#### Number of subjects

USFDA recommends that the total number of subjects in the study should be adequate to prove the bioequivalence of the two formulations unequivocally. A minimum of 12 subjects should be involved in the BE study (FDA, 2003). According to EMA, the number of subjects to be included in the study should be based on an appropriate sample size calculation. The number of evaluable

subjects in a BE study should not be less than 12 (EMA, 2010). According to HC, the number of subjects to be used in a comparative bioavailability study should be estimated by considering the objectives of the study, the study design, the drug products being compared and the conditions under which the study is carried out.

The standard, the expected mean difference between the test and reference formulations and the anticipated intra-subject variance for the parameters stated in the standard, as well as the power, determine the number of subjects. All calculations are to be based on maintaining the overall Type I error rate at 5%. The minimum number of subjects in the study should be 12, but a larger number is usually required (HC, 2012). CDSCO recommendations state the number of subjects in a study should be statistically significant and this is determined by the following considerations:

- i. The error variance associated with the primary characteristic to be studied as estimated from a pilot experiment, from previous studies or from published data.
- ii. The significance level desired (p value): usually 0.05.
- iii. The expected deviation from the reference product should be compatible with bioequivalence.
- iv. The required (discriminatory) power, normally  $\geq 80\%$  to detect the maximum allowable difference (usually  $\pm 20\%$ ) in primary characteristics to be studied.

However, the minimum number of subjects should not be less than 16 unless justified for ethical reasons (CDSCO, 2005). As per SADC recommendations number of subjects need to be justified on the basis of providing at least 80% power of meeting the acceptance criteria. The minimum number of subjects should not be less than 12. If 12 subjects are unable to provide 80% power, more subjects should be included. However, in case of modified release oral dosage forms, a minimum of 20 subjects are required (SADC, 2007). ASEAN guidelines criteria is same as that of the CDSCO, for the sample size determination. However, the minimum number of subjects to be recruited in a study according to them should not be smaller than 12 unless justified (ASEAN, 2004).

#### ***Gender of the subject***

USFDA recommends if the drug product is intended for use in both sexes, then similar proportions of males and females should be included in the study. Furthermore, it recommends the inclusion of subjects of 60 years of age or more in case the drug product is to be used predominantly in the elderly (FDA, 2003). According to EMA recommendations, the subjects can belong to either sex (EMA, 2010). HC recommends comparative bioavailability studies to be conducted in normal, healthy male and/or female volunteers in order to minimize variability (HC, 2012). As per guidelines of CDSCO, the subjects of either sex may be used in the study, but the choice of gender should be governed by usage and safety criteria (CDSCO, 2005). Likewise, according to SADC and ASEAN, the subjects from either sex can be included in the study (SADC, 2007; ASEAN, 2004).

#### ***Female subjects***

There is no specific guidance provided with regard to the inclusion of female subjects in BE studies in USFDA (FDA, 2003). As per EMA, the risk to women of childbearing potential should be considered while conducting the BE studies (EMA, 2010). HC recommends the investigators to ensure that female volunteers are not pregnant, lactating or likely to become pregnant during the study, furthermore, the confirmation regarding pregnancy should be obtained by urine or serum tests prior to drug administration in each period (HC, 2012). CDSCO recommends that risks to women of childbearing potential should be considered on an individual basis. Women should be required to give assurance that they are not pregnant, nor likely to become pregnant until after the study and this should be confirmed by the pregnancy test immediately prior to the first and last dose of the study. Furthermore, women taking the contraceptive drugs should normally not be included in the study (CDSCO, 2005). According to SADC, the risk to women of childbearing potential should be considered on an individual basis, and the same holds true for the ASEAN guidance (SADC, 2007; ASEAN, 2004).

#### ***Replacement of subjects on withdrawal or dropouts***

There is as such no provisions provided by USFDA regarding the replacement of subjects on withdrawal or dropouts (FDA, 2003). According to EMA, the data from all the treated subjects should be considered for statistical analysis. The protocols that include 'spare subjects' that could be treated as replacement for 'excluded subjects' from the study for the purpose of data analysis are not acceptable. All the subjects should be included in the analysis, even if the number of the subjects are more than the minimum requirement and there are no drop-outs (EMA, 2010). HC recommends on assigning a fixed number of subjects, in addition to the number estimated by the sample size calculation. This strategy allows for possible drop outs and inclusion of evaluable data provided by all the subjects for both test and reference products in a cross-over study or for one treatment in parallel study for statistical analysis. Moreover, HC recommends identification and consideration of the outliers as a part of the protocol. No more than 5% of the subjects should be considered to be outliers, unless there are 20 or fewer subjects, in which case only 1 subject could be removed. The observations should be identified by a simple outlier test and its procedure should identify observations which are very different from all others collected. The parameters of evaluations are usually AUC and  $C_{max}$ , but in some instances other parameters might be required. There are no recommendations regarding the retesting of subjects identified as outliers (HC, 2012). According to them, it is acceptable to replace a subject withdrawn/ dropout from the study once it has begun, provided that the substitute follows the same protocol originally intended for the withdrawn subject and he/she is tested under similar environmental and other controlled conditions (CDSCO, 2005). ASEAN and SADC do not provide any specification regarding the withdrawals or dropouts, similar to USFDA (SADC, 2007; ASEAN, 2004).

### Age

As per USFDA and EMA, subjects to be recruited for the *in vivo* BE studies should be of 18 years of age or older and capable of giving the informed consent (FDA, 2003; EMA, 2010). The HC recommends the subjects to be between the age of legal majority and the age of onset of age-associated changes in organic function. This description typically coincides with an age range of 18-55 years (both inclusive) (HC, 2012). According to CDSCO, the studies should normally be performed on healthy adult volunteers with an aim to minimize variability and permit detection of differences between the study drugs (CDSCO, 2005). According to ASEAN and SADC, the subjects to be taken in the study should be between 18-55 years old capable of giving informed consent (SADC, 2007; ASEAN, 2004).

### Body mass index (BMI)

USFDA does not make any recommendations regarding BMI (FDA, 2003). As per HC, the subjects recruited preferably should have a BMI within 18.5 and 30 kg/m<sup>2</sup> (HC, 2012). EMA recommends on recruiting the subjects with BMI between 18.5 and 30 kg/m<sup>2</sup> (EMA, 2010). No specifications have been provided by CDSCO regarding the BMI of subjects (CDSCO, 2005). ASEAN guidelines recommend the BMI of Asian subjects to be 18-25 kg/m<sup>2</sup> (ASEAN, 2004).

In accordance with SADC, the subjects should have body mass within the normal range according to the accepted normal values for the BMI or within 15% of the ideal body mass, or any other recognized reference (SADC, 2007).

### Strength of the dosage form

According to USFDA, for the drug product with different strengths, an *in vivo* BE demonstration of one or more lower strengths can be waived based on dissolution tests and *in vivo* study on the highest strength. However, in few cases conducting the study on a strength that is not the highest strength may be appropriate for reasons of safety, provided that the following conditions are met: (a) Linear elimination kinetics has been shown over the therapeutic dose range. (b) The higher strengths of the test and reference products are proportionally similar to their corresponding lower strength. (c) Comparative dissolution testing on the higher strength of the test and reference products is submitted and found to be appropriate (FDA, 2003). According to HC, the comparative bioavailability studies for all the strengths may not be required for products in which the proportions of excipients and the dissolution characteristics are similar (HC, 2012).

According to EMA, if several strengths of a test product are applied for, it may be sufficient to establish the bioequivalence at only one or two strengths, depending upon the proportionality in composition between the different strengths and other product related issues. However, the strength to be evaluated depends upon the linearity in pharmacokinetics of the active substances. For products with the linear kinetics, it is sufficient to establish the bioequivalence with only one strength i.e. the highest strength.

Furthermore, for drugs with a less proportional increase in AUC with increasing dose over the therapeutic dose range (Non-linear Pharmacokinetics), bioequivalence should in most cases be established both at the highest strength and at the lowest strength (or strength in the linear range) i.e. in this situation two bioequivalence studies are needed (EMA, 2010). On contrary, CDSCO does not provide any recommendation regarding the use of higher or lower strengths while carrying out the BE studies (CDSCO, 2005). As per ASEAN, one unit of the highest marketed strength or a clinical usual dose should generally be given. A higher dose which does not exceed the maximal dose of the dosage regime or labeled dose range might be employed if there are any analytical difficulties (ASEAN, 2004). However, no recommendations are provided by SADC on the strength related issues for the conduct of BE studies (SADC, 2007).

### Single/Multiple dose

USFDA recommends the use of single-dose pharmacokinetic studies for both immediate and modified release drug products to demonstrate BE as they are generally more sensitive in assessing release of the drug substance from the drug product into the systemic circulation. However, in cases where the multiple-dose study is important, appropriate dosage administration and sampling should be carried out to document the attainment of the steady state (FDA, 2003). According to HC, to carry out the comparative bioavailability studies, the use of same dose of each product should be preferred as a single dosage form units (HC, 2012).

As per EMA, the conduct of a multiple dose study in patients is acceptable if a single dose study cannot be conducted in healthy volunteers due to tolerability reasons and in cases where the single dose study is not feasible in patients. However, the multiple dose study is less sensitive in detecting differences in C<sub>max</sub>, this will only be acceptable if the applicant can adequately justify that the sensitivity of the analytical method cannot be improved and when it becomes difficult to rely on the measurement of parent compound after the single dose administration (EMA, 2010). According to CDSCO, single dose studies are generally recommended. However, there are some situations where the steady study design is required such as:

- (a) Drugs with dose and time dependent pharmacokinetics.
- (b) Some modified release products.
- (c) When there is a problem of sensitivity in plasma concentration measurements after the single dose administration.
- (d) If the intra-individual variability is reduced at the steady state (CDSCO, 2005).

According to ASEAN, the single dose studies are usually recommended, however, in the situations where the steady state studies are required, are same as provided by CDSCO (ASEAN, 2004). SADC recommends single dose studies, but steady-state studies advocated when required (SADC, 2007).

### **Genetic Phenotyping**

USFDA does not provide any information on the genetic phenotyping (FDA, 2003). HC also does not provide any guidance related to the genetic phenotyping (HC, 2012). EMA recommends considering the phenotype and/ or genotype of subjects for safety or pharmacokinetic reasons (EMA, 2010). As per CDSCO, the phenotyping and/ or genotyping of subjects should be considered for exploratory bioavailability studies and all studies using parallel group design. It may also be considered in case of cross-over study designs for safety or pharmacokinetic reasons. Furthermore, if a drug is known to show altered pharmacokinetic profile due to major genetic polymorphism, studies could be performed in panels of subjects of known phenotype or genotype for the polymorphism in question (CDSCO, 2005). ASEAN also recommends on considering the phenotyping and/or genotyping of subjects for the exploratory bioavailability studies and all studies using parallel group. However, it can also be done for cross-over study designs for safety or pharmacokinetic reasons (ASEAN, 2004). Recommendations of SADC are on similar line as CDSCO and ASEAN (SADC, 2007).

### **Endogenous substances**

No recommendations have been provided by USFDA and HC on endogenous substances (FDA, 2003; HC, 2012). According to EMA, the endogenous substances study should be demonstrated, either in the pilot study or as a part of the pivotal bioequivalence study using different doses of the reference formulation, in order to ensure that the dose used for the bioequivalence comparison is sensitive to detect potential differences between formulations. Furthermore, in BE studies with endogenous substances, it cannot be directly assessed whether carry-over has occurred, and thus extra care should be taken to ensure that the washout period is of adequate duration (EMA, 2010). There has been no information provided by CDSCO, ASEAN and SADC regarding the endogenous substances (CDSCO, 2005; SADC, 2007; ASEAN, 2004).

### **Parent drug/Metabolite**

USFDA recommends the measurement of the parent drug released from the dosage form, rather than the metabolite because the concentration time-profile of the parent drug is more sensitive to changes in formulation performance than the metabolite, which is more reflective of the metabolite formation, distribution and elimination. However, there are few exceptions where the measurement of metabolite becomes important, for instance, the measurement of a metabolite may be preferred when the parent drug levels are too low to allow reliable analytical measurement in blood, plasma or serum for an adequate length of time or if the active metabolite may be formed as a result of gut wall or other presystemic metabolism. Therefore, if the metabolite contributes meaningfully to safety and/or efficacy, the measurement of metabolite and the parent drug is recommended (FDA, 2003). The other guidelines (HC, CDSCO, EMA, ASEAN and SADC) also suggest the use of parent drug data to estimate BE. However, their

opinions and justifications for the use of metabolites as a primary data are different. According to HC, the determination of bioequivalence should be based on data for the parent drug. The metabolite should be taken into consideration only if the parent drug is not detectable due to rapid biotransformation. However, the study should be designed for primary and major metabolite and appropriate scientific justification for waiver of the measurement of parent drug as well as use of metabolite data should be provided (HC, 2012). EMA also recommends the measurement of parent compound for BE evaluation as  $C_{max}$  of a parent compound is usually more sensitive to differences between formulations with respect to the rate of absorption than  $C_{max}$  of a metabolite. Also for inactive prodrugs, the demonstration of BE for parent compound is recommended instead of the metabolite. However, some prodrugs may have low plasma concentrations and be quickly eliminated resulting in difficulties in demonstration of BE of the parent compound, therefore, in this case the measurement of the main active metabolite is recommended without the measurement of the parent compound. Furthermore, the HC doesn't encourage the use of a metabolite as surrogate for an active parent compound (EMA, 2010). CDSCO, ASEAN and SADC recommends on measuring the active drug substance as the main evaluation criteria for BE, however, in some cases where the concentrations of the drug (s) may be too low to be accurately measured in the biological matrix or in case of the unstable drugs or drugs with the short half-lives or pro-drugs, measurement of the active main metabolite is considered for the evaluation purpose (CDSCO, 2005; SADC, 2007; ASEAN, 2004).

### **Posture and Physical Activity**

USFDA and EMA do not provide any guidance on the posture or physical activity (FDA, 2003; EMA, 2010). However, HC strongly recommends that the subjects should not be allowed to recline until at least two hours after drug ingestion. Physical activity and posture should be standardized as much as possible to limit effects on gastrointestinal blood flow and motility, and the same pattern should be maintained for each study period (HC, 2012). CDSCO recommends standardization of study environment, involving the post-dosing postures (CDSCO, 2005). As per ASEAN and SADC, the posture and physical activity may need to be standardized as the bioavailability of an active moiety from a dosage form could be dependent upon the gastrointestinal transit times and regional blood flow (SADC, 2007; ASEAN, 2004).

### **Emesis/Vomiting**

USFDA recommends that the data from subjects who experience emesis during the course of BE study for immediate-release products be deleted from statistical analysis if vomiting occurs at or before 2 times the median  $T_{max}$ . In case of modified-release products, data from subjects who experience emesis any time during the labeled dosing interval should be deleted (FDA, 2003). As per HC, the subjects who vomit should be evaluated for continued participation in the study based on the potential impact



of vomiting on the integrity of study results and the evaluation should take place as soon as possible after the episode (s) of vomiting and before initiation of analysis of the study samples (HC, 2012). According to EMA, events such as vomiting and diarrhea are the reasons to exclude the subjects from the study as these may render the plasma concentration-time profile unreliable (EMA, 2010). There has been no recommendations provided on this regard by CDSCO, ASEAN and SADC (CDSCO, 2005; SADC, 2007; ASEAN, 2004).

#### ***Fasting conditions***

USFDA recommends an overnight fasting of the subjects, a minimum of at least 10 hours, before the commencement of the study. Furthermore, no food should be allowed for at least 4 hours post-dose (FDA, 2003). According to HC, the subject should normally fast for 8 hours before drug administration, that means no food or solids are to be consumed, although alcohol-free, xanthine-free and flavonoid-free clear fluids are permissible the night prior to the study, and 4 hours after the drug administration, a standard meal may be taken (HC, 2012). EMA recommends on fasting for at least 8 hours prior to administration of the drug product, unless otherwise justified, and no food should be allowed for at least 4 hours post-dose (EMA, 2010). According to CDSCO guidelines, a single dose study should be conducted on overnight fasted subjects with a minimum fasting period of 10 hours and post dose fasting of 4 hours. In case of multiple dose studies, where an evening dose is also scheduled, 2 hours of fasting before and after the dose is considered acceptable (CDSCO, 2005). As per ASEAN guideline, the subjects should be kept on fast at least during the night prior to administration of the products (ASEAN, 2004). SADC recommends on standardizing and supervising the fasting prior to the dosing (SADC, 2007).

#### ***Food specification for fed studies***

USFDA, EMA and HC recommends on consumption of meal 30 minutes prior to the drug administration (following an overnight fast of at least 10 hours). The test meal should comprise of high-fat (approximately 50% of total caloric content of the meal) and high-calorie (approximately 800 to 1000 calories) meal. The meal should derive approximately 150, 250 and 500-600 calories from proteins, carbohydrates and fats respectively (FDA, 2003; EMA, 2010; HC, 2012). CDSCO recommends the consumption of a high-fat breakfast before dosing. Such a breakfast must be designed to provide 950-1000 Kcals. At least 50% of these calories must come from fat, 15-20% from proteins and the rest from carbohydrates. Furthermore, the vast ethnic and cultural variations of the Indian subcontinent preclude the recommendations on consumption of any single standard high-fat breakfast 15 minutes before dosing. A high-fat (approximately 50% of total caloric content of the meal) and high-calorie (approximately 800 to 1000 calories) meal should derive approximately 150, 250 and 500-600 kilocalories from proteins, carbohydrates and fats respectively (CDSCO, 2005). As per

ASEAN and SADC, all meals taken after the treatment should be standardized with respect to the composition and time of administration during the sampling period (SADC, 2007; ASEAN, 2004).

#### ***Fluid intake***

According to USFDA, the test or reference products can be administered with about 8 ounces (240 milliliters) of water under fasting conditions, unless the study is a food-effect BA and BE study. The subjects are allowed water as desired except for 1 hour before and after the drug administration (FDA, 2003). HC recommends on taking the dose with water of standard volume (150 to 250 milliliters) at a standard temperature. Furthermore, water may be permitted up to one hour before drug administration and one hour after drug administration xanthine and flavonoid-free fluids are permitted one hour after the drug administration (HC, 2012). According to EMA, the test and reference products should be administered with a standardized volume of fluid (at least 150 ml). It is recommended that water is allowed as desired except for one hour before and one hour after the drug administration (EMA, 2010). CDSCO recommends on standardization of the fluid intake in all studies (CDSCO, 2005). According to ASEAN guidelines, the fluid intake may profoundly influence gastric passage for oral administration forms, therefore, the volume of fluid should be constant (at least 150 ml) (ASEAN, 2004). As per SADC, the volume of fluid administered at the time of dosing should be constant (e.g. 200 ml) as it may influence the gastric transit of orally administered dosage forms (SADC, 2007).

#### ***Sampling***

USFDA recommends that 12-18 samples, including the pre dose sample, should be collected per subject per dose. Sampling can be continued for at least three or more terminal half-lives of the drug. It recommends withdrawal of the samples at appropriate times to describe the absorption, distribution and elimination phases of the drug (FDA, 2003). According to HC, the collection of minimum of 12 samples per subject per dose is recommended. The duration of sampling should be sufficient to account for at least 80% of the known AUC to infinity. Furthermore, the period should usually be of at least three times the terminal half-life of the drug. (HC, 2012). As per EMA, at least three to four samples are needed during the terminal log-linear phase for estimating the terminal rate constant accurately. The sampling schedule should be planned to avoid  $C_{max}$  being the first point of a concentration time curve, it should also cover the plasma concentration time curve long enough to provide a reliable estimate of the extent of exposure which is achieved if  $AUC_{(0-t)}$  covers at least 80% of  $AUC_{(0-\infty)}$ . Furthermore, a sampling period longer than 72 hours is not considered necessary for any immediate release formulation irrespective of the half-life of the drug (EMA, 2010). CDSCO recommends on extending the blood sampling to at least three-elimination half-lives in case of immediate release products. Sampling should be continued for a sufficient period, which should ensure that the area extrapolated

from the time of the last measured concentration to infinite time is only a small percentage (normally less than 20%) of the total AUC. Furthermore, there should be at least three sampling points during the absorption phase, three to four at the projected  $T_{max}$ , and four points during the elimination phase (CDSCO, 2005). According to ASEAN, the sampling schedule should be planned to provide an adequate estimation of  $C_{max}$  and to cover the plasma concentration time curve long enough to provide a reliable estimate of the extent of the absorption, and this is generally achieved if the AUC derived from the measurement is at least 80% of the AUC extrapolated to infinity (ASEAN, 2004). As per SADC, for most drugs 12 to 18 samples including a pre-dose sample should be collected per subject per dose. The sampling period should be approximately of three terminal half-lives of the drug. Furthermore, at least three to four samples above LOQ should be obtained during the terminal log-linear phase to estimate  $K_{el}$  by linear regression analysis (SADC, 2007).

#### Wash-out period

According to USFDA, an adequate washout period (e.g. more than 5 half-lives of the moieties to be measured) should separate the treatment (FDA, 2003). According to HC, the interval between the study days should be long enough to permit elimination of essentially all of the previous dose from the body. The minimum time between treatments should be the same for all subjects and to account for variability in elimination rate between subjects, normally should be not less than 10 times the mean terminal half-life of the drug. (Should not exceed three to four weeks) (HC, 2012). EMA recommends that in steady-state studies, washout period of the previous treatment can overlap with the build-up of the second treatment, provide the build-up period is sufficiently long (at least 5 times the terminal half-life) (EMA, 2010). CDSCO doesn't provide any recommendation on the washout period (CDSCO, 2005). According to ASEAN, the subsequent treatments should be separated by periods long enough to eliminate the previous dose before the commencement of next period. In steady-state studies washout of the previous treatment last dose can overlap with the build-up of the second treatment, provided the build-up period is sufficiently long (at least three times the terminal half-life) (ASEAN, 2004). As per SADC, to avoid the carry-over effects, treatments should be separated by adequate wash-out periods (SADC, 2007).

#### Statistical Parameters

USFDA recommends on providing information regarding  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ ,  $C_{max}$ ,  $T_{max}$ ,  $\lambda_z$  and  $t_{1/2}$ . If the steady state studies are employed,  $C_{min}$ ,  $C_{av}$ , degree of fluctuation and swing are employed (FDA, 2003). According to HC, the parameters to be measured are  $AUC_T$ ,  $AUC_1$ ,  $AUC_T/AUC_1$ ,  $C_{max}$ ,  $T_{max}$ ,  $\lambda$ ,  $t_{1/2}$ . For the multiple dose studies, the parameters to be measured are  $C_{min}$ , pre-dose concentrations determined immediately before a dose at steady state ( $C_{pd}$ ) and area under concentration versus time curve, over the dosing interval ( $AUC_{tau}$ ) (HC, 2012). According to EMA, for a single dose study, parameters to be evaluated are  $AUC_{(0-t)}$ ,

$AUC_{(0-\infty)}$ , residual area,  $C_{max}$  and  $T_{max}$ . In studies with sampling period of 72 hours,  $AUC_{(0-72h)}$  is to be measured. For immediate release formulations at steady state,  $AUC_{(0-\tau)}$ ,  $C_{max,ss}$  and  $T_{max,ss}$  should be determined (EMA, 2010). According to CDSCO, the parameters to be evaluated after the single-dose studies are  $AUC_{0-\tau}$ ,  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ ,  $C_{max}$  and  $K_{el}$ . For the steady-state studies, the parameters to be evaluated are  $AUC_{0-\tau (ss)}$ ,  $C_{max}$ ,  $C_{min}$ ,  $C_{pd}$  and degree of fluctuation (CDSCO, 2005). According to ASEAN, the parameters to be estimated are  $AUC_t$ ,  $AUC_{\infty}$ ,  $C_{max}$ ,  $t_{max}$ ,  $Ae_t$ ,  $Ae_{\infty}$  as appropriate. For studies in steady state,  $AUC_t$ ,  $C_{max}$ ,  $C_{min}$  and fluctuation should be provided (ASEAN, 2004). As per SADC, the parameters to be analyzed using ANOVA are  $AUC_t$ ,  $AUC_{\infty}$  and  $C_{max}$ . The analysis technique for  $t_{max}$  should be non-parametric and should be applied to untransformed data (SADC, 2007).

#### Acceptance Criteria

USFDA recommends that the traditional BE limit should be 80-125% for non-narrow therapeutic range drugs. However, for narrow therapeutic range drugs, the guideline recommends on additional testing and/or controls to ensure the quality of drug products and it is designed to provide increased assurance of interchangeability for drug products (FDA, 2003). As per HC, at 90% confidence limits, the range of  $AUC_T$  is 75.41%-103.74% and the range for  $C_{max}$  is 61.94%-107.06% (HC, 2012). According to EMA, for the parameters  $AUC_{(0-t)}$ ,  $C_{max}$  and  $AUC_{(0-72h)}$ , 90% confidence interval for the ratio of the test and reference products should be contained within the acceptance interval of 80-125%. In specific cases of products with a narrow therapeutic index, the acceptance interval for AUC should be limited to 90.00-111.11%. Furthermore, the acceptance range recommended for highly variable drugs is 69.84%-143.19% (highly variable drug products are those drugs whose intra-subject variability for a parameter is larger than 30% (EMA, 2010). As per CDSCO, the confidence interval for the ratio of geometric means of AUC (for both  $AUC_{0-\tau}$  and  $AUC_{0-t}$ ) and  $C_{max}$  determined using log-transformed data should generally be within the range of 80 to 125%, when the products are compared after single dose administration in both the fasting and fed state (CDSCO, 2005). According to ASEAN guidelines, the 90% confidence interval (AUC ratio and  $C_{max}$ ) for the measure of relative bioavailability should lie within an acceptance interval if 0.80-1.25. However, in certain cases a wider interval may be acceptable. The interval should be defined e.g. 0.75- 1.33 with the justification addressing in particular any safety or efficacy concerns for patients switched between formulations (ASEAN, 2004).

According to SADC, for single dose studies the 90% confidence interval for test/reference ratio should lie within the acceptance interval of 80 to 125% (AUC ratio) and for  $C_{max}$  the 90% confidence interval for the test/reference ratio should lie within an acceptance interval of 75-133% using the log-transformed data, except for narrow therapeutic range API's when an acceptance interval of 80-125% will apply. The acceptance window for steady state is similar to that of the single-dose studies (SADC, 2007). The comparison of guidelines recommended by

US, Europe, Canada, India (CDSCO), South Africa and South East Asian Nations for the conduct of bioequivalence studies is shown in Table 2.

### General Concerns Over Bioequivalence Study Design

Although with time, the bioequivalence regulations have made stricter, yet there is ample scope of improvement in present bioequivalence study designs. Areas where amendments are desired include: general study design, blinding, gender of subject, female subjects, body mass index, and replacement of subjects on withdrawal or, dropouts, genetic phenotyping, endogenous substances, emesis / vomiting and washout period, respectively. These are addressed in the subsequent sections.

### General Study Design

The guidelines for bioequivalence studies follow the same principle in general, FDA, in additions, also addresses the issues related to food intake in BE studies. The presence of food not only affect the tablet disintegration, drug dissolution and drug transit time through gastrointestinal tract, but also affects the metabolic transformation of drug in the gastrointestinal wall and the liver.

The outcome of single meal, single dose study conducted by Melander shows the variable effect of food on the oral bioavailability of the drugs, while the food intake enhanced the oral bioavailability of certain drugs like propranolol, metoprolol, hydralazine, hydrocholothiazide, spironolactone, nitrofurantoin, erythromycin, dicumarol, phenytoin and carbamazepine, its presence delayed the oral bioavailability of isoniazid, rifampicin, tetracycline, penicillin and ampicillin. No effect of food on oral bioavailability was observed in case of metronidazole, oxazepam, melperone, propylthiouracil and sulphasomidine (Melander, 1978). Further, the study showed that repeated intake of protein-rich diet enhanced, while the carbohydrate- rich diet declined, the rate of oxidation of antipyrine and theophylline (Melander, 1978). Another study that relates the presence of food with the efficacy of drugs was conducted by Mahesh *et al.* The authors reported that oral sulfonyl ureas were more effective when taken 30 min prior to the meals. When taken with meals, food interfered with absorption (Otoom *et al.*, 2001). Since food has significant effect on the oral bioavailability of the drugs, it is important that guidelines should mention whether the study should be conducted in the fasting state or the fed state.

**Table 2:** Comparison of bioequivalence guidelines of US, Europe, Canada, India (CDSCO), South Africa and South East Asian Nations (ASEAN, 2004; CDSCO, 2005; EMA, 2010; FDA, 2003; HC, 2012; SADC, 2007).

S.No.	Criteria	FDA	EMA	HC	CDSCO	SADC	ASEAN
1.	General	Single dose, non-replicate cross-over study for immediate release and modified release dosage forms and a single-dose, two-period, two-treatment, two-sequence cross study designs for fed BE studies.	Single dose, randomized, 2-Period, 2-Sequence cross over design.	Single dose, 2-Period cross over design.	Single dose, randomized, 2-Period, 2-treatment, cross-over study design.	Single dose, Balanced two period, two-sequence crossover design.	Single dose, two period, two sequence crossover design.
2.	Long half-life drugs/highly variable drugs	Non replicate single dose crossover with adequate washout period /parallel study design.	Parallel design for long half-life drug and replicate for highly variable drugs.	Parallel design and/or Alternate design when the uncertainty in the intra-subject variance is large and the collection of data should be done in stages based on the observed intra-subject variance from first stag using two strategies such as- (i) Group sequential designs (ii) Adaptive designs.	Parallel design for long half-life drugs and replicate designs for drugs with variable disposition.	Parallel design (the study should cover a minimum of 72 hours unless 80% drug is recovered before 72 hours).	Parallel design for long half-life drugs and replicate designs for drugs with highly variable disposition.
3.	Blinding	Not specified.	Not specified.	Double blind study where subjects, person recording adverse drug reactions and person conducting bioanalysis should not be aware of samples/ products as well as about the treatment sequence.	Not specified.	Not specified.	Not specified.
4.	Number of subjects	Healthy Volunteers, minimum number of volunteers to be taken in the study should be 12.	Healthy Volunteers, Minimum number of volunteers should not be less than 12 unless justified.	Healthy volunteers, not less than 12, larger number preferable for better statistical evaluation and ethical reasons.	Healthy Volunteers, Not less than 16 unless justified for ethical reasons.	Minimum number should not be less than 12. If 12 subjects do not provide 80% power, more subjects should be included.	Minimum number of subjects should not be smaller than 12 unless justified.

5.	Gender of subject	Male/female; If drug product is intended for use in both sexes, attempt should be made to include similar proportions of females and males in the study.	Male and/or female.	Male and/or female.	Male/female; the choice of gender should be consistent with usage and safety criteria. If drug product is intended for use in both sexes, attempt should be made to include similar proportions of females and males in the study.	Male and/or female.	Male and/or female.
6.	Female Subjects	Not specified.	Risk to women of childbearing potential should be considered.	Investigators should ensure that female volunteers participating in the study are not pregnant, lactating or likely to become pregnant during the study.	Women taking contraceptive drugs should normally not be included in the studies. Women are required to give assurance that they are not pregnant, nor likely to become pregnant until after the study and this should be confirmed by the pregnancy test immediately prior to the first and last dose of the study. Furthermore, women taking the contraceptive drugs should normally not be included in the study.	Risk to women of childbearing potential should be considered on individual basis.	Risk to women of childbearing potential should be considered on individual basis.
7.	Replacement of subjects on withdrawal or dropout	Not specified.	The data from all treated subjects should be included in the study. There is no such thing as 'spare' subjects in the study.	A fixed number of subjects, in addition to the number estimated by the sample size calculation should be recruited which allows for possible drop-outs. Reasons for withdrawal of subjects administered with at least one dose of drug should be reported, and the subject's plasma concentration data should be provided.	Acceptable to replace a subject withdrawn/drop-out from the study once the study has begun provided the substitute follows the same protocol originally intended for the withdrawn subject and the subject is tested under similar controlled conditions.	Not specified.	Not specified.
8.	Age criteria	18 years or older.	18 years or older.	18-55 years (both inclusive).	Healthy adult volunteers.	18-55 years.	18-55 years.
9.	BMI	Not specified.	18.5-30 kg/m <sup>2</sup> .	18.5-30 kg/m <sup>2</sup> .	Not specified.	Should be within the normal range according to accepted normal values for the BMI or within 15% of the ideal body mass, or any other recognised reference.	18-25 kg/m <sup>2</sup> .
10.	Strength of the dosage form	In most of the cases, the highest strength.	For drugs with linear pharmacokinetics, use of highest strength is preferred. For drugs with non-linear pharmacokinetics, the establishment of BE studies both at the highest and at the lower strength is required.	For drug products with similar proportions of excipients and the dissolution characteristics, it is not necessary to carry out the BE studies for all the strengths.	Not specified.	Not specified.	Use of one unit of highest marketed strength or a clinical usual dose is recommended.

11.	Single/ Multiple dose	Single dose studies are preferred for both immediate and modified release drug products. Multiple dose studies are conducted only wherever required.	Multiple dose studies are acceptable only in cases where it not possible to carry out single dose studies.	Single dose studies are preferred.	Single dose studies are preferred except for some special situations, where the conduct of steady state studies are acceptable.	Single dose studies are preferred except for some situations where steady state studies are motivated.	Single dose studies are preferred except for some special situations, where the conduct of steady state studies are acceptable.
12.	Genetic Phenotyping g	Not specified.	Consideration on phenotyping and/or genotyping of subjects should be given for safety or pharmacokinetic reasons.	Not specified.	Phenotyping and/or genotyping should be considered for parallel study designs and can be considered for cross-over designs as well as for safety or pharmacokinetic reasons.	Phenotyping and/or genotyping should be considered for parallel study designs and can be considered for cross-over designs as well for safety or pharmacokinetic reasons.	Phenotyping and/or genotyping should be considered for parallel study designs and it can also be considered for cross-over designs as well for safety or pharmacokinetic reasons.
13.	Endogenous substances	Not specified.	It should be demonstrated either in pilot study or as a part of the pivotal bioequivalence study.	Not specified.	Not specified.	Not specified.	Not specified.
14.	Parent drug/ Metabolite	Parent drug analysis is considered in most of the cases except for certain cases when the parent drug levels are too low to allow reliable analytical measurement in blood, plasma or serum or if the metabolite is formed as a result of gut wall or other presystemic metabolism, where the main active metabolite is considered.	Parent compound is the main moiety to be measured for the BE evaluation, however in case of prodrugs when the concentration of the parent drug is too low, main metabolite is measured without measuring the parent compound.	Parent compound to be measured except for cases when the parent drug is not detectable, then their active detectable metabolite could be considered.	Parent compound is the main moiety to be measured, exception is made of certain cases where the concentrations of drug is too low to accurately measure in the biological matrix or in case of the unstable drug or drugs with short half-life or the prodrugs, where the metabolite measurement with the parent drug is done.	Parent compound is the main moiety to be measured, exception is made of certain cases where the concentrations of drug is too low to accurately measure in the biological matrix or in case of the unstable drug or drugs with short half-life or the prodrugs, where the metabolite measurement with the parent drug is done.	Parent compound is the main moiety to be measured, exception is made of certain cases where the concentrations of drug is too low to accurately measure in the biological matrix or in case of the unstable drug or drugs with short half-life or the prodrugs, where the metabolite measurement with the parent drug is done.
15.	Posture/ Physical activity	Not specified.	Not specified.	Subjects should not be allowed to recline until at least two hours after drug ingestion of drug.	Standardization of post-dosing postured is recommended.	Posture and physical activity may need to be standardized on a case by case basis.	Posture and physical activity may need to be standardized on a case by case basis.
16.	Emesis/ vomiting	If the vomiting occurs at or before 2 times the median Tmax as in case of immediate-release products, the data of that subject should not be included in the statistical analysis. Furthermore, in case of modified release drug products, the subjects should be excluded from the study if they experience emesis.	Subjects experiencing the vomiting should be excluded from the study.	The evaluation of subjects for continued participation in the study should be done after the episodes of vomiting and before the analysis of the study samples.	Not specified.	Not specified.	Not specified.

17.	Fasting prior to study	10 h before and 4 h after drug administration.	8 h before and 4 h after the administration of product, unless otherwise justified.	8 h before and 4 h after the drug administration.	Single dose: At least 10 h Overnight and 4 h after dosing. Multiple dose: 2 h before and after dose.	Fasting prior to dosing should be standardized and supervised.	Subjects should preferably fast overnight prior to dose administration.
18.	Food Specification for "fed Studies"	A high-fat (approximately 50 percent of total caloric content of the meal) and high-calorie (approximately 800 to 1000 calories) meal is recommended as a test meal for "Food-effect BA" and fed BE studies. This test meal should derive approximately 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively.	High fat (approx. 50% of total caloric content of the meal) and high calorie (approx. 800-1000 kcal) meal.	High fat, high calorie meal. Meal should derive approximately 150, 250, and 500-600 kcal from protein, carbohydrates and fat respectively.	Requires consumption of a high-fat breakfast approx. 15 min. before dosing (950-1000KCalories) {50% of Calories should be derived from fats, 15-20% of Calories from proteins and Rest Carbohydrates }.	All meals taken after dosing should be standardised with regards to composition and time of administration and in accordance with any specific requirements for each study.	All the meals taken after the treatment should be standardized with regards to composition and time of administration during the sampling period.
19.	Fluid (water) intake	Drug should be administered with 8 ounces (240 ml) of water under fasting conditions. The subjects should not be allowed to consume water 1 h before and after the drug administration.	Drug should be administered with standard volume of fluid, at least 150 ml. Subjects are not recommended to consume water 1 h before and after the drug administration.	Drug recommends on administrating the drug with standard volume of water (150-250 ml). Water is not permitted 1 h before and after the drug administration.	Drug should be administered with standard quantity of fluid.	The volume of fluid to be administered at the time of dosing should be constant (e.g. 200 ml).	The volume of fluid to be administered during the drug administration should be constant (at least 150 ml).
20.	Sampling	12-18 samples including the pre-dose sample per dose per subject should be collected. Sampling should be distributed once three or more terminal half-lives of the drug.	Measurements should be taken to avoid $C_{max}$ being the first point of concentration time cure. At least 2-4 samples needed during the terminal log-linear phase.	Minimum 12 samples per subject per dose needed. The period should usually be of at least three times the terminal half-life of the drug.	At least 3 sampling points during the absorption phase, 3-4 at projected $T_{max}$ and 4 points during the elimination phase.	At least 12-18 samples including the pre-dose sample per subject per dose to be collected.	Enough sampling times to provide an adequate estimation of $C_{max}$ and to cover the plasma concentration time curve to provide reliable estimate of the extent of absorption.
21.	Wash-out period	More than 5 half-lives of the moieties to be measured.	For Steady State; at least 5 times the terminal half-life.	Not less than 10 times the mean terminal half-life of the drug.	Not specified.	Adequate wash-out period should be there to avoid the carry-over effects.	Subsequent treatments should be separated by adequate wash periods. In steady state studies, wash out period should be kept at least three times the terminal life.
22.	Parameters	$AUC_{0-t}$ , $AUC_{0-\infty}$ , $C_{max}$ , $T_{max}$ , $t_{1/2}$ Steady State: $C_{min}$ , $C_{av}$ , degree of fluctuation and swing.	$AUC_{0-t}$ , $AUC_{0-\infty}$ , $t_{max}$ , $C_{max}$ , residual area Steady State: $AUC_{0-\tau}$ , $C_{max, ss}$ , $T_{max, ss}$ .	$AUC_T$ , $AUC_1$ , $AUC_T/AUC_1$ , $C_{max}$ , $t_{max}$ , $\lambda$ , $t_{1/2}$ Steady State: $C_{pd}$ , $AUC_{tau}$ .	$AUC_{0-t}$ , $AUC_{0-\infty}$ , $AUC_{0-\tau}$ , $C_{max}$ , $K_{el}$ Steady State: $AUC_{0-\tau(ss)}$ , $C_{max}$ , $C_{min}$ , $C_{pd}$ and deg. of fluctuation.	Parameters derived from measures of concentration, e.g. $AUC_t$ , $AUC_{\infty}$ and $C_{max}$ should be analysed using ANOVA. Analysis technique for $t_{max}$ should be non-parametric and should be applied to untransformed data.	$AUC_T$ , $AUC_{\infty}$ , $C_{max}$ , $T_{max}$ , $A_e$ , $A_{e\infty}$ Steady State: $AUC_1$ , $C_{max}$ , $C_{min}$ and fluctuation should be provided.

23.	Acceptance criteria	90% confidence interval between 80-125%. It recommends additional tests and/or controls to ensure the quality of drug products containing Narrow Therapeutic Range Drugs.	90% confidence interval between 80-125%. AUC should be tightened to 90-111.11% for narrow therapeutic range drugs and 69.84%-143.19% for highly variable drugs.	90% confidence interval between 75.41%-103.74% (AUC ratio) and $C_{max}$ is 61.94-107.06%.	90% confidence interval between 80-125%. No specifications on narrow therapeutic drugs.	a) Single dose studies- i) AUC ratio- 90% confidence interval for the test /reference ratio should lie within the acceptance interval of 80-125% ii) $C_{max}$ ratio- 90% confidence interval for the test/reference ratio should lie within an acceptance interval of 75-133% except for narrow therapeutic range API's where acceptance interval of 80-125% will apply.	For AUC and $C_{max}$ , 90% of the confidence interval for the measure of relative bioavailability should lie within acceptable interval of 0.80-1.25.
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**Table 3:** Effect of gender of subject on ADME.

Absorption	Distribution	Metabolism	Excretion
Absorption occurs at different sites throughout the gastrointestinal tract and the rate of absorption is influenced by gut transit times. However, the transit time was found to be shorter in men (44.8 h) than in women (91.7 h) which leads to the delayed absorption in females (Soldin <i>et al.</i> , 2011). On the basis of evaluation done by the FDA (Soldin <i>et al.</i> , 2011; Anderson, 2005) on 26 bioequivalence studies including 94 datasets. Out of these there was > 20% sex related difference in $C_{max}$ and AUC in 30% of the datasets. For instance, it was observed that polyethylene glycol enhanced the bioavailability of ranitidine in males but decreased in females. After a fat-rich meal, Cyclosporine A was found to have decreased bioavailability in females while increased bioavailability in males (Soldin <i>et al.</i> , 2011)	Distribution of the drug is affected by the weight of the subject, and generally males weigh more than females. However, the dose is not adjusted in accordance with the body weight which has led to 20-88% higher AUCs in females in comparison to males, as evaluated by FDA in bioequivalence studies (Anderson, 2005). Furthermore, the volume of distribution ( $V_d$ ) also varies in males and females. For instance, in case of lipid-soluble drugs, the $V_d$ is increased in females while in case of water-soluble drugs, females have smaller $V_d$ as compared to males (Soldin <i>et al.</i> , 2011). In case of drugs like ofloxacin and salbutamol, the $V_d$ values were greater in males than in females due to the difference in body mass index (Soldin <i>et al.</i> , 2011).	The family of enzymes involved in the metabolism are cytochrome P450 (CYP), urine diphosphate glucuronosyl transferase (UGT) and N-acetyl transferase (NAT) enzymes. In an <i>in vitro</i> studies, it was found that, there was a 2-fold higher CYP3A4 level of expression in liver samples obtained from a group of 46 females as compared to the samples obtained from 48 males (Anderson, 2005).	Drug elimination also varies with the gender. There has been observed the marked differences between the glomerular filtration, tubular secretion and tubular reabsorption between males and females. For instance, the oral clearance of torasemide was shown to have 30-40% higher mean $AUC_{24}$ and $C_{max}$ values in females than males (Soldin <i>et al.</i> , 2011). Sufentanil and Clozapine have shown a faster clearance in males after oral administration than females (Koren, 2010).

### Blinding

Blinding is an important parameter that ensures that there is no biasness in the study created by the sponsors. The biasness might arise due to eagerness off the sponsors to ensure launch of their product, thus secure the financial stakes. Only Health Canada highlights the importance of blinding during the conduct of the bioequivalence study. In the era of patent drought when there is a mushrooming of look-alike generic drug products, the physicians as well as sponsors can make a huge money out of these practices. The ultimate outcome of such practices result in serious compromises with safety of patient population. Hence, blinding should be one of the key parameters that should be the part of all the guidelines for BE studies.

### Gender of Subject

Every guideline specifies the intake of either sex in the bioequivalence study, but no provision has been provided by any of the guidelines regarding the ratio of males and females to be recruited in the study. It has been found that the difference in the gender results in difference in the absorption, distribution,

metabolism and excretion of the drug. The data supporting this statement is shown in Table 3.

Thus, guidelines should recommend the recruitment of equal number of male and female subjects in the study. Otherwise, there should be a provision that the investigator should justify the choice of subjects based on BA data submitted while filing for NDA approval.

### Replacement of subjects on withdrawal or dropouts

EMA recommends that all the enrolled subjects should be treated equally for the statistical evaluation. It prohibits the use of 'spare subjects' as a replacement for 'excluded subjects'. While HC and CDSCO, recommends on assigning the fixed number of subjects, in addition to the number estimated by sample size calculation for possible drop-outs during the study, there are no recommendations regarding the issue by USFDA, ASEAN and CDSCO. It is worthwhile to include 2-3 subjects since the commencement of the study. The data, thus acquired, would be more significant in case of dropouts as compared to the data where subject are included subsequent to subject withdrawal.

### Genetic Phenotyping

Genetic phenotyping is a very important criteria to be considered while carrying out the BE studies. The fact was highlighted in the study conducted by Kandasamy *et al.*, conducted open-label, two-way randomized crossover designs with two periods and two treatments BA/BE studies of fluoxetine and norfluoxetine to identify the poor metabolizer (PM) phenotypes. After the studies in 144 subjects, the pharmacokinetic parameters were found distinct between two phenotypes: 1. PM phenotypes showed higher exposure (approximately 2.3 fold increase in  $AUC_{0-\infty}$  and much slower elimination (almost 2 fold increase in elimination half-life) for fluoxetine as compared to Extensive metabolizer (EM) phenotypes. 2. PM phenotypes showed approximately 0.5 fold lower exposure of norfluoxetine as compared to EM counterparts (Kandasamy *et al.*, 2010). Therefore, it becomes necessary to consider this parameter while conducting the BE studies on subjects of pharma emerging countries.

### 4. CONCLUSION

The present article has highlighted the relevant facts related to down streaming of branded pharmaceutical products and emergence/up streaming of generic drug market both in developed and under developed countries. The provisions in shortcomings of current regulatory guidelines like USFDA, EMA, HC, CDSCO, ASEAN and SADC have been discussed in detail. Recommendations for improvement in current BE guidelines on certain aspects like general study design, blinding, gender of subject, replacement of subjects on withdrawal or dropouts, genetic phenotyping respectively have been made. Despite the efforts made to make BE regulatory requirements much stricter, the subject of generic substitution of branded products is still debatable. Most of the current issues pertinent to generic substitution include: Can just the BE studies justify consumer perception of risk? Does the difference in product appearance and packaging of branded and generic products influence the choice of consumers and physicians? In a report submitted by Ganther and Kreling, at least 20% to 30% of consumers believe that generic prescription drugs are less safe and effective than their branded equivalent (Ganther and Kreling, 2000). Moreover, it was also reported that patient perception of risk was dependent on the severity of the medical condition for which treatment was administered (Meredith, 2003). At times there are marked differences in appearance of generic and branded equivalents. In cases, where more than one generic drug is available, difference could be marked. Moreover, the colorants, excipients as well as size, shape and delivery formulation of generic product may differ considerably from the branded product. Such differences in appearance may cause anxiety and confusion in patients, especially in elderly and occasionally result in a patient inadvertently taking two formulations simultaneously (Besag, 2000). The problem is exacerbated by the existence of multiple generic formulations and inconsistent substitution, within a single

pharmacy and among different pharmacists that can lead to frequent switching between formulations and increased risk of confusion (Germino and Joseph, 1993). The concerns related to geriatric population during the generic drug substitution could not be ignored. Geriatric patients usually suffer from one or more chronic medical conditions and are often receiving several concomitant drugs. To such population individual pharmacokinetic variation is of particular importance. Additionally, the physiological changes associated with age may affect drug absorption, distribution, metabolism and excretion (Germino and Joseph, 1993). Therefore, generic substitution should be carefully monitored in geriatric population. However, many of these concerns remain theoretical and with present designed regulations, major problems have not been reported due to generic substitution. However, there is a reason to remain careful, the fact is reflected in the statement stated by Meredith in 2003 that "The process is far from perfect and is not without risk, so potentially serious clinically relevant problems could arise" (Meredith, 2003).

Nevertheless, BE criteria has been refined, the future will witness further improvement that will address the concern regarding safety and efficacy of patient population. But we must support that in the era of patent drought, current BE studies will be definitely able to re-establish the credibility of the generic drug. Further refinement of existing guidelines and introduction of legislation that enforce stricter monitoring of product quality and bioequivalence shall re-establish the credibility of generic drug market in the era of patent drought.

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