International Journal for Pharmaceutical Research Scholars (IJPRS)

V-4, I-1, 2015 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Bioequivalence Study in India and South-Africa Country

Shah DB*[1], Patel HN[2], Maheshwari DG[3]

[1]*Assistant Professor, Department of Quality Assurance, L. J. Institute of Pharmacy, Between Katariya Motor and Sanand- Sarkhej Circle, S. G. Highway, Ahmedabad, Gujarat 382210, India.*

[2] *Department of Quality Assurance, L. J. Institute of Pharmacy, Between Katariya Motor and Sanand- Sarkhej Circle, S. G. Highway, Ahmedabad, Gujarat 382210, India.*

[3]*Head of Department of Quality Assurance, L. J. Institute of Pharmacy, Between Katariya Motor and Sanand-Sarkhej Circle, S. G. Highway, Ahmedabad, Gujarat 382210, India.*

Manuscript No: IJPRS/V4/I1/00043, Received On: 19/03/2015, Accepted On: 27/03/2015

ABSTRACT

Bioequivalence (BE) study play a major role in the drug development phase for both new drug products and their generic equivalents, and the reason that attract considerable attention globally. The present study was aimed to study the requirements of bioequivalence for registration of pharmaceutical products in Asian & African Country. It is essential for pharmaceutical industry to study the guidelines of bioequivalence for respective country where industry would like to apply for ANDA and thus want to enter into the generic market. The study gives insight about requirements of bioequivalence with study parameters such as design of study, fasting or fed state studies, volunteers recruitment, study dose, sampling points, pharmacokinetic parameters, criteria for bioequivalence, GCP requirements etc. which are needed for pharmaceutical industry to carry out bioequivalence studies and to file ANDA. Test products for bioequivalence studies are usually manufactured by a sponsor or manufacturer while reference is provided by the government laboratories of respective countries. Sampling points varies with the respect to the regulatory guidelines of these countries. India obey Indian GCP guidelines and South-Africa follow MCC GCP guidelines. Criteria of bioequivalence, for India is 90% CI 80-125% for C_{max}, AUC_t, $AUC_{o\text{-}inf.}$ and South-Africa 90% CI for 75-133% C_{max}, AUC_t, 80-125% $AUC_{o\text{-}inf.}$

KEYWORDS

Bioequivalence, Bioavailability, ANDA, Pharmacokinetics, Pharmacodynamic

INTRODUCTION

In pharmacokinetics bioequivalence is a term used to assess the expected *in vivo* biological equivalence of two proprietary preparations of a drug. If two products are a foresaid to be bioequivalent it means that they would be likely to be, for all intents and purposes, the same. Two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent and their bioavailability (rate and extent of availability) after administration in the same molar dose are similar to such a degree that their effects, with respect to one efficacy and another safety, can be predictable to be essentially the same. Pharmaceutical equivalence entail the same amount of the same active substance(s), in same dosage form, for the same route of administration and gathering the same or comparable standards. Bioequivalence studies are required by regulations to ensure therapeutic equivalence between a pharmaceutically equivalent test product and a reference product. Different *in vivo* and in *vitro* methods are used to measure product

***Address for Correspondence:**
Mr. Darshil Shah
Assistant Professor, Department of Quality Assurance,
L. J. Institute of Pharmacy, Ahmedabad, Gujarat, India.
E-Mail Id: darshilshah89@yahoo.com

quality[1]. As per United States Food and Drug Administration (FDA) bioequivalence is the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study[2].

In vivo certification of equivalence is needed when there is a risk that probable differences in bioavailability may result in therapeutic nonequivalence. Some of examples are critical use medicines, narrow therapeutic range (efficacy/safety margins), steep dose-response curve, pharmacokinetics complicated by incomplete absorption window, nonlinear pharmacokinetics, pre-systemic elimination or high first pass metabolism (>70%), unfavorable physicochemical properties, e.g., low solubility, less stability, meta-stable modifications, poor permeability, documented evidence for bioavailability problems related to the drug or drugs of similar chemical structure or formulations, where high ratio of excipients to active ingredients exists, non-oral, non-parenteral pharmaceutical products designed to act systemically (such as transdermal patches, suppositories, testosterone gel and skin inserted contraceptives), Modified release pharmaceutical products designed to act systemically, fixed combination products with systemic action, where at least one of the API requires an *in vivo* study, non-solution pharmaceutical products, which are for non-systemic use (e.g. for oral, nasal, ocular, dermal, rectal or vaginal application) and are intended to act without systemic absorption. In those cases, the equivalence is established through comparative clinical or pharmacodynamic, dermato-pharmacokinetic studies and/or in vitro studies. In certain cases, the measurement of the concentration of the API may still be required for safety reasons, i.e. in order to check unintended systemic absorption, in each comparison, the new formulation or new method of manufacture shall be the test product and the prior formulation (or respective method of manufacture) shall be the reference product[3].

Requirements for Registration of Bioequivalence Study in India

Bioequivalence studies are required in India for the new drugs as per the requirement detailed in schedule Y of the Drug and Cosmetics Rules and its amendments. The study should be premeditated in such a way that the formulation effect can be distinguished from other effects. Typically, if two formulations have to be compared, a two-period, two sequence crossover design is the design of choice with the two phases of treatment separated by an adequate washout period which should ideally be equal to or more than five half life's of the moieties to be measured. Other study designs include the parallel design for very long half-life substances or the replicate design for substances with highly variable disposition. Single dose studies generally are sufficient. Standardization of the study require for environment, diet, fluid intake, post-dosing postures, exercise, sampling schedules etc. is important in all studies. Conformity to these standardizations should be stated in the protocol and reported at the end of the study, in order to restore confidence that all variability factors are involved, except that of the products being tested, have been minimized. Unless the study design requires, subjects should withdraw from smoking, alcohol, coffee, tea, xanthine containing foods and liquor and fruit juices during the study and at least 48 hours before its commencement. To establish bioequivalence, the calculated 90% confidence interval for AUC and C_{max} should fall within the bioequivalence range, usually 80-125%. This is equivalent to the rejection of two one sided t tests with the null hypothesis of non bioequivalence at 5% level of significance. The non parametric 90% confidence interval for T_{max} should lie within a clinically acceptable range[4].

Bioequivalence Study Requirements for Registration in South Africa

The study should be premeditated in such a manner that the formulation effect can be notable from another effects. If number of formulations

have to be compared is two, a balanced two periods, two sequence crossover designs is considered to be the design of choice. However, under certain conditions and provided the study design and the statistical analysis are scientifically sound, alternatively well recognized designs such as parallel designs for very long half-life substances, could be considered. In general, single dose studies will meet requirement, but there are situations in which steady state studies may be required in which case the steady state study design should be provoked. To avoid carry over effects, treatments should be separated by adequate washout periods5. For bioavailability studies, measurement of individual enantiomers may be important. For bioequivalence studies, this guidance recommends measurement of the racemate using an achiral assay. For individual enantiomer measurement in BE studies are recommended only when the enantiomers exhibit different pharmacokinetic and pharmacodynamic characteristics, primary efficacy and safety activity resides with the minor enantiomers and non-linear absorption is present (as expressed by a change in the enantiomer concentration ratio with change in the input rate of the drug/API) for at least one of the enantiomers. In such cases, bioequivalence factors are applied to the enantiomers separately[5,6].

General Regulatory Considerations for BA/BE studies

The general considerations for BA/BE studies are:

• Study design and protocol.

• Bioanalysis.

• Selection of appropriate analyte(s).

• BE metrics and data treatment.

• Statistical approaches and analysis.

Assessment of Bioequivalence

The assessment of BE of different drug products is based on the fundamental assumption that two products are equivalent when the rate and extent of absorption of the test/generic drug does not show a significant difference from the rate and extent of absorption of the reference/brand drug under similar experimental conditions as defined. In different regulatory authorities of BE studies are generally classified as:

1. Pharmacokinetic endpoint studies.

2. Pharmacodynamic endpoint studies.

3. Clinical endpoint studies.

4. In vitro endpoint studies.

The general descending order of preference of this study includes Pharmacokinetic, Pharmacodynamic, clinical, and in vitro studies.[7]

Pharmacokinetic Endpoint Studies

These studies are most widely preferred to assess BE for drug products, where drug level has determined in an easily accessible biological fluid (such as plasma, blood and urine) and drug level has correlated with the clinical effect. The statutory definition of BA and BE, stated in rate and extent of absorption of the active moiety or ingredient to the site of action, maintain the use of pharmacokinetic measures to indicate release of the drug substance from the drug product with absorption into the systemic circulation. Regulatory guidance recommends that measures of systemic exposure be used to reflect clinically important differences between test and reference products in BA and BE studies.7These measures include

i) Total exposure (AUC_{0-t} or $AUC_{0-\infty}$for single-dose studies and $AUC_{0-\tau}$ for steady-state studies),

ii) Peak exposure (Cmax), and

iii) Early exposure (partial AUC to peak time of the reference product for an immediate-release drug product).On systemic exposure measures will reflect comparable rate and extent of absorption, which, will achieve the underlying goal of assuring comparable therapeutic effects. Single dose studies to document BE were preferred because they are generally more sensitive in assessing *in vivo* release of the drug substance from the drug product when compared to multiple dose studies. The following are the circumstances that demand multiple- dose study/steady state pharmacokinetics:[8,9]

- Doses- or time-dependent pharmacokinetics.
- For which the fluctuation in plasma concentration for modified-release products over a dosage interval at steady state needs to be assessed.
- If problems of sensitivity inhibit sufficiently precise plasma concentration measurements after single-dose administration.
- If the intra-individual variabe, in the plasma concentration or disposition precludes the possibility of Demonstrating BE in a reasonably sized single-dose study and this variability is reduced at steady state.
- When a single-dose study is not be conducted in healthy volunteers due to tolerability reasons and a single-dose study is not feasible in patients.
- If the medicine has a long terminal elimination half-life and blood concentrations after a single dose cannot be followed for a sufficient time.
- For those medicines that produce their own metabolism or show large intra-individual variability.
- For those having combination products for which the ratio of plasma concentration of the individual substances is important.
- If the medicine is likely to be accumulate in the body.
- For enteric coated preparations in which the coating is innovative. Under normal circumstances, blood should be the biological fluid sampled to measure drug concentrations.
- Most drugs may be measured in serum or plasma; however, in some drugs, whole blood (eg, tacrolimus) may be more appropriate for the analysis.[10,11]

Pharmacodynamic Endpoint Studies[8,12]

Pharmacokinetic study measures systemic exposure but are generally inappropriate to document local delivery BA and BE.

Based on a pharmacodynamic study, providing an appropriate pharmacodynamic endpoint is available. Pharmacodynamic evaluation is measurement of the effect on a pathophysiological process, such as function of time, after administration of two different products to serve as a basis for BE assessment.

Regulatory authorities request justification from the applicant for the use of pharmacodynamic effects/parameters for the establishment of BE criteria. These studies generally become necessary under two conditions

1) If the drugs and/or metabolite(s) in plasma or urine cannot be analyzed quantitatively with sufficient accuracy and sensitivity;

2) If drug concentration measurement cannot be used as surrogate endpoints for the demonstration of efficacy and safety of the particular pharmaceutical product. The other important specifications for pharmacodynamic studies include i) a dose-response relationship should be demonstrated; ii) sufficient measurements should be taken to provide an appropriate pharmacodynamic response profile; iii) the complete dose-effect curve should remain below the maximum physiological response; iv) all pharmacodynamic measurements/methods should be validated for specificity, accuracy, and reproducibility. Examples of these pharmacodynamic studies include locally acting drug products and oral inhalation drug products, like metered dose inhaler and dry powder inhaler, and topically applied dermatological drug products, such as creams and ointments.[13,14]

Clinical Endpoint Studies or Comparative Clinical Trials

In the absence of pharmacokinetic and pharmacodynamic approaches, adequate and well-controlled clinical trials can be used to establish BA/BE. Several international regulatory authorities provide general information about the conduct of clinical studies to establish BE.

***In Vitro* Endpoint Studies**

More recently, a Biopharmaceutics Classification System (BCS) has categorized drug substances as having either high or low solubility and

permeability and drug products as exhibiting rapid dissolution.[15] According to this approach, drug substances may be classified into four primary groups:

1) Highly soluble and highly permeable; 2) highly permeable and poorly soluble; 3) highly soluble and poorly permeable; 4) poorly soluble and poorly permeable. Using this BCS approach, a highly permeable and highly soluble drug substance formulated into a rapidly dissolving drug product may need only in vitro dissolution studies to establish BE.20 In addition, in vitro approaches to document BE for *nonbioproblem* drugs approved before 1962 remain acceptable as per FDA regulations. Dissolution tests can also be used to reduce the number of *in vivo* studies in other circumstances, and to i) assess batch-to-batch quality and support batch release; ii) provide process control and quality assurance; and iii) assess the need for further BE studies relative to minor post-approval changes, Where they function as a signal of bioinequivalence.[16] The broad spectrum of BA/BE in vitro studies specifications were provided by each regulatory authority.

Criteria for Bioequivalence

To establish bioequivalence, the calculated 90% confidence interval for AUC and Cmax should fall within the bioequivalence range, usually 80-125%. This is equivalent to the rejection of two one sided t-tests with the null hypothesis of non-bioequivalence at 5% level of significance.

The non-parametric 90% confidence interval for Tmax should lie within a clinically acceptable range. Limits for permissible differences in bioavailability may be required for drugs that have narrow therapeutic index, serious dose-related toxicity and steep dose response curve.

India

In a progress to ensure standards of quality, efficacy and safety in medical products, India's Central Drug Standard Control Organization (CDSCO) has revised its guidelines on bioequivalence and bioavailability for pharmaceuticals. The revisions will become part of Schedule Y of the Drugs and Cosmetics Act and new drug applications will have to meet these necessities.

Table 1: Regulatory criteria on sample size for BA/BE studies

Regulatory authority	Minimum	Sample size specifications
India	Should not be,12	The number of subjects required is checked by a) The error variance associated with the primary characteristic to be studied as estimated from a pilot experiment, from previous studies or from published data; b) The significance level desired; c) The expected deviation from the reference product compatible with BE (delta, ie, percentage difference from 100%); and d) the required power
South Africa	Should not be,12 (general); 20 subjects (for modified release oral dosage forms)	The number of subjects should be justified on the basis of providing at least 80% power of meeting the acceptance criteria; Alternatively, the sample size can be calculated using appropriate power equations, which should be presented in the protocol

The revisions indicate how a relative study should be executed, the design requirements, study population, the characteristics that need to be studied, facts of the bioanalytical methodology required, and parameters for statistical evaluation of the results.

South-Africa

Medicines Control Council of South-Africa follows MCC GCP guidelines for bioequivalence focuses on the equivalence of release of the active pharmaceutical ingredient from the pharmaceutical product and its successive absorption into the systemic circulation. Comparative study using clinical or pharmacodynamic end points may also be used to demonstrate bioequivalence. The 90 % confidence interval for the test/reference ratio should lie within the acceptance interval of 0.80-1.25 (80-125%).

Table 2: Regulatory criteria on number of studies required for conducting BA/BE studies

Regulatory authority	Immediate-release formulations	Modified-release formulations
India	Generally a single-dose, nonreplicate, fasting study Food-effect studies are required 1) when it is recommended that the study drug should be taken with food (as would be in routine clinical practice); 2) when fasting state studies make assessment of Cmax and Tmax difficult If multiple-study design is important, appropriate dosage administered and sampling be carried out to document attainment of steady state	Should conduct fasting as well as food-effect studies If multiple-study design is important, appropriate dosage administered and sampling carried out to document attainment of steady state
South Africa	Should be done under fasting conditions unless food effects affect bioavailability of drug or reference product dosage recommended	Both fed and fasted studies are required If multiple-study design is important, it should be carried out as per regulatory specifications

Table: 3 Regulatory criteria for conducting fasting and fed BA/BE studies

Regulatory authority	Fasting requirements	Fed study requirement
India	Overnight fast (at least 10 h), with a subsequent fast of 4 h following dosing for multiple-dose fasting studies, when an evening dose must be given, 2 h before and after the dosing	950–1000 kcal of high-fat breakfast approximately 15 min before dosing (at least 50% of calories must come from fat, 15%–20% from proteins and rest from carbohydrates) The vast ethnic and cultural restrictions of the Indian subcontinent preclude the recommendation by a single standard high fat; in this case protocol should specify the appropriate and suitable diet
South Africa	Fasting prior to dosing and after dosing should be standardized.	Use of high-calorie and high-fat meals is recommended

Table: 4 Regulatory criteria on fluid intake, posture and physical activity for BA/BE studies

Regulatory authority	Fluid intake	Posture and physical activity
India	Standardization of fluid intake and physical activity is required and it should be stated in protocol	
South Africa	The volume of fluid administered at the time of dosing should be constant (eg, 200 mL); fluids taken after dosing should also be standardized	Should be standardized

Table 5: Regulatory criteria on sampling and washout period for conducting BA/BE studies

Regulatory authority	Sampling criteria	Washout criteria
India	**Blood sampling** Should be extended to at least 3 elimination half lives; at least 3 sampling points during absorption phase, 3–4 at the projected Tmax, and 4 points during elimination phase; sampling should be continued for a sufficient period to ensure that AUC_{0-t} to $AUC_{0-\infty}$ is only a small percentage (normally, 20%) of the total AUC. Truncated AUC is undesirable except in the presence of enterohepatic recycling **Urinary sampling** Collect urine samples for 7 or more half-lives Adequate and ideally it should be ≥5 half-lives of the moieties to be measured	Adequate and ideally it should be ≥5 half-lives of the moieties to be measured
South Africa	**Blood sampling** Sampling should be sufficient to account for at least 80% of the known $AUC_{0-\infty}$, Cmax; collecting at least 3–4 samples above the LOQ during the terminal log-linear phase; sampling period is approximately thee terminal half-lives of the drug; AUC truncated at 72 h is permitted for long half-life drugs; 12–18 samples should be collected per each subject per dose; at least 3–4 samples above LOQ should be obtained during the terminal log-linear phase **Urine sampling** Sufficient urine should be collected over an extended period and generally no less than 7 times the terminal elimination half-life; for a 24-h study, sampling times of 0–2, 2–4, 4–8, 8–12, and 12–24 h post dose are usually appropriate Adequate washout period	Adequate washout Period

Table 6: Regulatory acceptance criteria for bioequivalence for single-dose study & steady-state study

Regulatory Authority	90% Confidence Interval on Log Transformed Data		
	Single-Dose Study		
	C_{max}	AUC_{0-t}	$AUC_{0-\infty}$
India	80–125	80–125	80–125
South Africa	75–133	80–125	Not applicable

Regulatory authority	90% confidence interval on Log transformed data		
	Steady-state study		
	C_{max}	AUC_{0-t}	$AUC_{0-\infty}$
India	80–125	80–125	80–125
South Africa	75–133	75–133	80–125

Table 7: Comparative Assessment of study parameters between India, South-Africa

Sr. No.	Parameters	India	South-Africa
1.	Study design	Non-replicated, randomized, crossover studies	Non-replicated, randomized, crossover studies
2.	Fasting/Fed state studies	Fasting	Fasting
3.	Volunteers	>16 subjects	Min 80% Power of acceptance criteria
4.	Study dose Test Reference	Test product made by the manufacturer Any internationally available product or Already approved Indian product	Test product made by the manufacturer US/Europe or South African reference product
5.	Sampling points	3 sample points during absorption phase, 3-4 at projected Tmax, 4 samples during elimination phase.	12 to 18 samples per subject/dose
6.	Analytical method validation parameters	Selectivity, accuracy, precision, recovery. Lower limit of quantification (LLOQ) and calibration curve. Stability of analyte in spiked samples	Specificity, accuracy, precision, stability of analyte, limit of detection and quantification, response function, robustness and ruggedness
7.	Moieties to be measured in plasma	Active Drug / Metabolites if applicable	Active Drug / Metabolites if applicable
8.	Pharmacokinetic parameters	Cmax, Tmax, AUC0-t, AUC0-∞, t1/2, λz	Cmax, Tmax, AUC0-t, AUC0-∞, t1/2, λz
9.	Criteria for Bioequivalence	90% CI 80-125% for Cmax, AUCt, AUC0-inf	90% CI 80-125% for Cmax, AUCt, AUC0-inf
10.	GCP Requirements	Indian GCP Guidelines	MCC GCP Guidelines

CONCLUSION

During the last few years, there is a major progress in policies and procedures concerning the determination of bioavailability and bioequivalence. Presently, there is international harmonization of regulatory requirements for bioequivalence studies. Comparative assessment of study parameters between India and South-Africa revealed that India and South-Africa follow GCP guidelines of respective countries. Sampling points and number of samples as well as analytical method validation parameters are well defined for India. Moieties to be measured in plasma, pharmacokinetic parameters and criteria for bioequivalence are same for these two countries. However, the trend in the near future appears towards achieving the appropriate choice of clinically relevant bioequivalence ranges based on therapeutic ranges, rate of absorption metrics, designs to resolve the issue of intra and inter subject variability etc.

ACKNOWLEDGEMENTS

The authors are highly thankful to Dr. K. Pundarikakshudu, Director of L. J. Institute of Pharmacy, Ahmedabad, India for providing valuable guidance and all facilities to carry out the Research work.

REFERENCES

1. Roger L. Nation, Lloyd N. Sanso. (1994). Bioequivalence *requirements for generic products and Pharmacological Therapy*, 62, 41-55.
2. Tomasz G., Marczak M., Jaroszewski, J. J., Whitmire, M., (2012). Comparison of bioequivalence study regulatory requirements for human and veterinary drugs. *Regulatory Toxicology and Pharmacology*, 64, 233–242.
3. Strober, B. E., Armour K., Romiti, R., and Catherine S., et al. (2012). Biopharmaceuticals and bio-similar in psoriasis: what the dermatologist needs to know. *Journal of American Academy of Dermatology*, 66(2), 317-322.
4. Government of India, Ministry of Health and Family Welfare. (2005). Guidelines for bioavailability and bioequivalence studies. (pp 1-33). Central Drug Standards and Control Organization, New Delhi.
5. Zwarenstein, M., Schoeman J. H., Caesar V., Lombard, C. J., et al. (1998). Randomised controlled trial of self-supervised and directly observed treatment of tuberculosis. *The Lancet Journal*, 352, 1340-1343.
6. Narang, V. S., Lulla, A., Malhotra G., Shrinivas P., (2004). Bioequivalence evaluation of two marketed brands of stavudine 40 mg capsules in healthy human South African volunteers. *Pharmacological Research*, 50, 511–516.
7. Chen, M. L., Shah, V., Patnaik, R., Adams, W., Hussain, A., Conner, D., (2001). et al. Bioavailability and Bioequivalence: An FDA Regulatory Overview. *Pharmaceutical Research Journal*, 18, 1645–1650.
8. Guidelines for Bioavailability and Bioequivalence Studies. (2005). Central Drugs Standard Control Organization, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India, New Delhi. http://cdsco.nic.in/html/be%20guidelines%20draft%20ver10%20march%2016,%2005.pdf. Accessed on 2 January, 2015.
9. Asean Guidelines for the Conduct of Bioavailability and Bioequivalence Studies. (2014) http://www.suregmp.com/forum/file_down_ok.asp?Folder=PDS&DownFile=Bioavailability_Bioequivalence%20Studies.pdf. Accessed on 5 January, 2015.
10. European Medicines Agency (2009). London, 24 May 2007. Doc. Ref. EMEA/ CHMP/EWP/200943/2007. Committee for Medicinal Products for Human Use (CHMP). Recommendation on the Need for Revision of (CHMP).Note For Guidance on the Investigation of Bioavailability and Bioequivalence. CPMP/EWP/QWP/1401/98. http://www.ema.europa.eu/docs/en_GB/docu

ment_library/Scientific_guideline/09/WC500003009.pdf. Accessed on 8 January, 2015.

11. European Medicines Agency (2009). Pre-Authorisation Evaluation of Medicines for Human Use London, 24 July 2008. Doc. Ref. CPMP/EWP/QWP/1401/98 Rev. 1. Committee for Medicinal Products for Human Use (CHMP). Draft. Guideline on the Investigation of Bioequivalence. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/09/WC500003011.pdf. Accessed on 8 January, 2015.
12. Midha, K. K., McKay, G., Bioequivalence: its history, practice, and future.(2009) Applications. *American Association of Pharmaceutical Scientists Journal*, 11, 664–670.
13. Juniper, E. R., Cockcroft, D. W., Hargreave, F. E. (1991). *Histamine and methacholine inhalation tests: tidal breathing method* (Vol. I). Laboratory procedure and standardization. Astra Draco, Lund, Sweden.
14. Sterk, P. J., Fabbri, L. M., Quanjer, P. H., et al. Airway responsiveness. (1993) Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Official statement of the European Respiratory Society. *European Respiratory Journal*, 6(16), 53–83.
15. Amidon, G. L., Lennernas, H. P., Shah, V. P., Crison, J. R. (1995) A theoretical basis for a Biopharmaceutics drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharmaceutical Research Journal*, 12, 413–420.
16. Davit, B. M., Conner, D. P., Fabian-Fritsch, B. L. et al. (2008) Highly Variable Drugs: Observations from Bioequivalence Data Submitted to the FDA for New Generic Drug Applications. *American Association of Pharmaceutical Scientists Journal*, 10(1), 148–156.

