The *in vitro* and *in vivo* Pharmaceutical equivalence and stability studies of some antihypertensive drugs manufactured in Bangladesh in rat model

The dissertation submitted to the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Bangladesh in the partial fulfillment of the requirements for the degree of Doctor of Philosophy (PhD)

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DECLARATION

I, do, hereby declare that the thesis entitled **"The *in vitro* and *in vivo* Pharmaceutical equivalence and stability studies of some antihypertensive drugs manufactured in Bangladesh in rat model"** prepared for submission to the University of Dhaka, Dhaka-1000, Bangladesh for the degree of Doctor of Philosophy in Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, is the original research work of mine and have not been submitted anywhere for the award of any degree or diploma.

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SUPERVISOR'S CERTIFICATE

This is to certify that the thesis work entitled **"The *in vitro* and *in vivo* Pharmaceutical equivalence and stability studies of some antihypertensive drugs manufactured in Bangladesh in rat model"** submitted by Rehana Begum, Reg. No.: 102/ 2015- 2016 and Re-reg. No.: 74/2020-2021, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka for the partial fulfillment of the requirements for the degree of Doctor of Philosophy, is a record of original research work. It has carried out by her under our direct supervision as per the code of academic and research ethics of Faculty of Pharmacy, University of Dhaka.

The results and findings incorporated here have not been submitted to any other institution for the award of any degree. The style and format are in accordance with the practices of this university. The contents and results of the thesis are satisfactory. We recommend the work for the award of the degree of Doctor of Philosophy in Pharmaceutical Chemistry, University of Dhaka.

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Dedicated to

My Father

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&

My Mother

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ABBREVIATIONS

ACE	Angiotensin converting Enzyme
ARB	Angiotensin receptor Blocker
AUC	Area Under the Curve
BE	Bioequivalence
BP	British Pharmacopeia
cGMP	Current Good Manufacturing Practices
C_{max}	Peak Plasma Drug Concentration
f_1	Difference factor
f_2	Similarity Factor
FDA	Food and Drug Administration
FTIR	Fourier-Transform Infrared Spectroscopy
HPLC	High Performance Liquid Chromatography
NDA	New Drug Application
NSAID	Non-steroidal anti- inflammatory drug
PE	Pharmaceutical Equivalence
TE	Therapeutic Equivalence
t_{max}	Time for Peak Plasma Drug Concentration
USA	United States of America
USP	United States Pharmacopeia

ABSTRACT

Background

Bangladesh is a densely populated country. To meet the healthcare needs of this huge population, huge amounts of medicines are required. Again, the first objective of National Drug Policy 2005 was to ensure that common people of Bangladesh should have easy access to effective, safe and good quality drug products at affordable prices. As hypertension is a very common disorder in Bangladesh, many pharmaceutical companies are now producing antihypertensive drugs from each class. But most of the companies do not conduct bioequivalence studies and for clinical trial and bioequivalence studies, even now we depend on another country like Malaysia, India and. No data are available in regard to pharmaceutical equivalence and bioequivalence studies of antihypertensive drugs manufactured in Bangladesh. The present study is carried out to perform *in vitro* and *in vivo* pharmaceutical equivalence and stability studies in comparison with reference innovator brands of some antihypertensive drugs manufactured in Bangladesh to compare the quality, efficacy and safety of these drug products by taking reference innovator brands as standard brands. This study will also help the physicians to choice a suitable brand which is easily available, have standards of quality, efficacy and safety.

Methods

In vitro pharmaceutical equivalence of some antihypertensive drugs was determined by comparing general quality assessment parameters such as weight variation, hardness, % friability, disintegration time, dissolution time and the amount of active substance between test brands and their respective reference innovator brands. Times required for 50% dissolution ($T_{50\%}$) and 90% dissolution ($T_{90\%}$) were also compared between test brands and their respective reference innovator brands. Mean of % dissolution versus time graph and statistical difference factor (f_1) and similarity factor (f_2) were also compared using dissolution profiles of test brands and their respective reference innovator brands. *In vivo*

pharmaceutical equivalence of some antihypertensive drugs was done by plotting plasma concentration- time curves of test brands with their respective reference innovator brands after administration of drug in rat models. Stability testing was compared between test brands and their respective reference innovator brands under stress conditions in acidic and basic conditions at different temperatures (29^0C, 60^0C and 70^0C).

Results

Experimental three brands of tablet atenolol 50 mg were randomly designated as AA, AB, AC and reference innovator brand as ARI. Eight brands of tablet Carvedilol 6.25 mg were randomly designated as CA, CB, CC, CD, CE, CF, CG, CH and reference innovator brand as CRI. Ten brands of tablet Losartan potassium 50 mg were randomly designated as LA, LB, LC, LD, LE, LF, LG, LH, LI, LJ and reference innovator brand as LRI. Four brands of tablet ramipril 5 mg were randomly designated as RA, RB, RC, RD and reference innovator brand as RRI.

All test brands including their respective reference innovator brands passed the general quality assessment parameters such as weight variation, hardness, % friability, disintegration time, dissolution and % potency. Still significant variations were observed in disintegration time of test brands of tablet losartan potassium and tablet ramipril with their respective reference innovator brands. A correlation was observed between disintegration time and the rate of dissolution in this study.

All test brands including their respective innovator brands were found within % weight variation test acceptance limit. Test brands of atenolol showed weight variation percentage limit between - 2.39% and + 2.77%, whereas reference innovator brand showed weight variation percentage limit between - 1.84% and + 1.40%. Test brands of carvedilol showed weight variation percentage limit between - 2.72% and + 5.87%, whereas reference innovator brand showed weight variation percentage limit between - 1.08% and + 0.92%. Test brands of losartan potassium showed weight variation percentage limit between - 4.66% and + 4.08%, whereas reference innovator brand showed weight variation percentage limit between -2.60% and +2.14%. Test brands of ramipril showed weight

variation percentage limit between -2.00% and + 2.85%, whereas reference innovator brand showed weight variation percentage limit between -1.85% and +1.49%.

All test brands of tablet atenolol, tablet carvedilol, tablet losartan potassium and tablet ramipril including their respective reference innovator brands were found satisfactory for hardness testing. Test brands of atenolol showed lowest hardness value hardness between 4.55 kg and 6.13 kg, whereas reference innovator brand showed hardness 5.32 kg. Hardness of test brands of carvedilol were found between 3.88 kg and 7.68 kg, whereas 6.26 kg was found for reference innovator brand. Hardness of test brands of losartan potassium were found between 6.28 kg and 9.96 kg, whereas 6.89 kg was found for reference innovator brand. Test brands of ramipril showed hardness between 7.51 and 13.19, whereas reference innovator brand showed 7.16 kg

All test brands of tablet atenolol, tablet carvedilol, tablet losartan potassium and tablet ramipril including their respective reference innovator brands met the acceptance criteria for % friability test. They had % friability values less than 1%.

All test brands of tablet atenolol, tablet carvedilol, losartan potassium and tablet ramipril including their respective reference innovator brands met the acceptance criteria for disintegration time. No major variations were found in disintegration time of different test brands of atenolol. They were found to disintegrate between 0.43 and 1.36 minutes, whereas reference innovator brand disintegrated in 1.44 minutes. No momentous variations were found in disintegration time of test brands of carvedilol. They disintegrated between 0.39 and 5.33 minutes, whereas innovator brand disintegrated in 0.78 minutes. Test brands with higher disintegration time were CE, CH and CG.

Significant variations were found in disintegration time of test brands of losartan potassium. They were found to disintegrate between 6.52 and 15.22 minutes, whereas reference innovator brand disintegrated in 7.19 minutes. Test brands with higher disintegration time were LA, LC, LF, LH and LI, having values >10 minutes. Test brands of ramipril showed significant variations in disintegration time. All test brands of ramipril disintegrated between 0.71 and 10.90 minutes, whereas innovator brand disintegrated in

1.09 minutes. Test brands with higher disintegration time were RA, RB having values >5 minutes and RC >10 minutes.

All test brands of tablet atenolol, tablet carvedilol, tablet losartan potassium and tablet ramipril including their respective reference innovator brands met the acceptance limit for % of dissolution. Test brands of tablet atenolol including their respective reference innovator brand attained more than 90% of dissolution within 30 minutes. Test brands of tablet carvedilol including their respective reference innovator brand achieved more than 90% of dissolution except brand CH which got more than 80% within 30 minutes. Test brands of tablet losartan potassium LA, LC, LE, LF, LG, LH and reference innovator brand LRI did more than 90% of dissolution except brands LB, LD, LI and LJ which got more than 80% within 30 minutes. Test brands of tablet ramipril including their respective reference innovator brand attained about 100% of dissolution within 30 minutes.

All test brands of tablet atenolol, tablet carvedilol, tablet losartan potassium and tablet ramipril including their respective reference innovator brands met the acceptance limit for assay content. They had % potency between 99% and 103%.

The mean % of drug dissolved of tablets of different test brands were compared with that of their respective innovator brands graphically by plotting the mean % of drug dissolved against time. All test brands including reference innovator of tablet atenolol released more than 80% of drug within 10 minutes. Except test brands CB, CC, CG, CH; all other brands including reference innovator brand of tablet carvedilol released more than 80% of drug within 20 minutes. Reference innovator brand and brands LC, LE, LF, and LG of tablet losartan potassium released more than 80% of drug in 20 minutes. Test brands LA, LB, LD, LH, LI, and LJ released more than 80% of drug in 30 minutes. Except brand RC; all test brands and reference innovator brand of tablet ramipril 5mg released more than 80% of drug in 10 minutes.

The time required for 50% dissolution ($T_{50\%}$) and 90% dissolution ($T_{90\%}$) were determined. All test brands of tablet atenolol and also tablet ramipril including their reference innovator brands showed $T_{50\%}$ values less than 10 minutes and $T_{90\%}$ values less than 30 minutes. For tablet carvedilol; all test brands including reference innovator brand showed $T_{50\%}$ values

less than 10 minutes and $T_{90\%}$ values less than 30 minutes except test brand CH. Test brand CH had $T_{50\%}$ less than 10 minutes but T_{90}% greater than 30 minutes. For tablet losartan potassium; test brands LA, LB, LD, LH, LI, LJ showed $T_{50\%}$ values greater than 10 minutes whereas, other brands less than 10 minutes. Test brands LB, LD, LI showed $T_{90\%}$ values greater than 30 minutes whereas, other brands less than 30 minutes.

The mean percentage of drug dissolved of tablets of test brands and their respective reference innovator brands were used to calculate difference factor(f_1) and similarity factor (f_2) using the respective equations. All test brands of antihypertensive drugs showing f_1 values less than 15 are acceptable in comparison with reference innovator brands. For test brands of tablet carvedilol CB and CH; f_2 values were less than 50. For test brands of tablet losartan potassium LB, LD and LI; f_2 values were less than 50. For test brand of tablet ramipril RC; f_2 values were less than 50. Test brands with f_2 values less 50 may not be equivalent to their respective reference innovator brands.

In vitro dissolution profiles showed variations in availability of drug substances from test brands and reference innovators brands. All test brands of tablet atenolol; all test brands of tablet carvedilol except two brands CB & CH; all test brands of tablet losartan potassium except brands LB, LD & LI and all test brands of tablet ramipril except brand RC were observed to have $T_{50\%}$ values less than 10 minutes, $T_{90\%}$ values less than 30 minutes, f_1 values less than 15 and f_2 values more than 50. They appeared to have very good bioavailability. Test brands CB and RC showing f_2 values less than 50 but $T_{50\%}$ values less than 10 minutes, $T_{90\%}$ values less than 30 minutes and f_1 values less than 15 also seemed to have very good bioavailability. Test brands CH and LB, LD, LI having $T_{50\%}$ values greater than 10 minutes, $T_{90\%}$ values greater than 30 minutes and f_2 values less than 50 were not equivalent to reference innovator brands in availability of drug substances.

In vivo pharmaceutical equivalence study was done by plotting plasma concentration- time curves of test brands with their respective reference innovator brands after administration of drug products in rat models. The curves indicated that the t_{max} value for test brands and innovator brand of atenolol was 2.5 hrs and C_{max} values for brands AA, AB, AC, ARI were 0.123, 0.128, 0.113, 0.129 µg/mL respectively. The t_{max} value for test brands and innovator

brand of carvedilol was 1.5 hrs and C_{max} values for brands CA, CB, CC, CD, CE, CF, CG, CH, CRI were 0.106, 0.106, 0.102, 0.103, 0.099, 0.096, 0.096, 0.098, 0.090, 0.106 μg/mL, respectively. The t_{max} value for test brands and innovator brand of losartan potassium was 1.5 hrs and C_{max} values for brands LA, LB, LC, LD, LE, LF, LG, LH, LI, LJ, LRI were 0.122, 0.123, 0.126, 0.118, 0.122, 0.123, 0.118, 0.123, 0.117, 0.120, 0.124 μg/mL, respectively. The t_{max} value for test brands and innovator brand of ramipril was 2.5 hrs and C_{max} values for brands RA, RB, RC, RD, RRI were 0.047, 0.061, 0.058, 0.053, 0.063 μg/mL, respectively. Comparing *in vivo* C_{max} and t_{max} values of test brands with their respective innovators, all antihypertensive testing brands may be considered equivalent to their respective reference innovator brands.

Stability studies of test brands of tablet atenolol, tablet carvedilol, tablet losartan potassium and tablet ramipril including their respective innovator brands were done by stress degradation in acidic and basic conditions at different temperatures (29^0C, 60^0C and 70^0C). Data showed no significant degradation of test brands and also their respective reference innovator brands. So, all antihypertensive test brands also may be considered equivalent to respective reference innovator brands regarding stability.

In conclusion, this study indicated that except test brands CH, LB, LD and LI; all other test brands may be considered *in vitro* and *in vivo* pharmaceutically equivalent to their respective reference innovator brands and also equivalent in case of stability. These brands may be similar in quality, efficacy, safety and may be used interchangeably. But test brands CH, LB, LD and LI are not similar to their respective reference innovator brands and cannot be used interchangeably.

CHAPTER ONE

INTRODUCTION

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INTRODUCTION

1.1 INTRODUCTION

Now-a-days hypertension is a very common cardiovascular disorder in the world. The occurrence of hypertension increases with increase of age. In the United States of America, almost fifty percent of people among the ages of 60 to 69 years old have hypertension. The occurrence is more increased after the age of 70 years.[1] In low and middle income countries; the prevalence of hypertension is also very high.[2] Hypertension is a very common disorder in our country. In Bangladesh, the number of patients having hypertension increases day by day.[3] Uncontrolled increased arterial pressures are responsible for the alterations in the vascular and hypertrophy of the left ventricle of the heart. As a result, hypertension is the main reason for stroke. It is a key risk element for coronary artery diseases and its related problems, myocardial infarction and sudden cardiac death. It is a principal contributor to cardiac failure, renal failure and aortic dissection.[4]

Hypertension is usually treated with different types of antihypertensive drugs. These different hypertensive drugs decrease blood pressure by different mechanisms. Widely utilized antihypertensive drugs are diuretic antihypertensive agents, alpha1 adrenergic antagonists, beta adrenergic receptor antagonists, combined alpha 1 and beta adrenergic receptor antagonists, calcium channel antagonists, angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists.[4]

Pharmaceutical equivalence is the term where the drug products having the equal quantity of active ingredient in a same dosage form, meet all relevant standards of same strength, quality and efficacy.[5-6] Drug products are said to be therapeutically equivalent if they are pharmaceutical equivalent and bioequivalent. Therapeutically equivalent products can be interchangeable. Pharmaceutical equivalence can be determined by comparing the quantity of active ingredient and other quality parameters of the test product with the innovator product.[7]

Recently a dissolution profile is more highlighted by FDA than a single point dissolution test. A dissolution profile can represent a drug product more accurately than a single point dissolution test. In the area of pre as well as post changes of drug products for scale up, post approval changes with different strengths, a dissolution profile comparison helps to ensure product performance similarity and indicates bioequivalence.[8-9]

When the patent of an innovator drug product has expired, it is then open to all pharmaceutical manufacturing companies to produce their own brands. To obtain approval for a new drug product, the applicant company must endorse that their generic drug brand is bioequivalent and pharmaceutically equivalent compared to the innovator drug.[10] Bangladesh is a densely populated country. To meet the healthcare needs of this huge population, huge amounts of medicines are required. Again, the first objective of National Drug Policy 2005 was to ensure that common people of Bangladesh should have easy access to effective, safe and good quality drug products at affordable prices.[10-11] As hypertension is a very common disorder in Bangladesh, many pharmaceutical companies are now producing antihypertensive drugs from each class. But most of the companies do not conduct bioequivalence studies and for clinical trial and bioequivalence studies, even now the pharmaceutical companies depend on another country like Malaysia, India and most of the companies do not conduct bioequivalence studies.

No data are available in regard to pharmaceutical equivalence and bioequivalence studies of antihypertensive drugs manufactured in Bangladesh. Therefore, the present study is carried out to perform *in vitro* and *in vivo* pharmaceutical equivalence and stability studies in comparison with reference innovator brands of some antihypertensive drugs manufactured in Bangladesh to compare the quality, efficacy and safety of these drug products. This study will also help the physicians to choice a suitable brand which is easily available, have standards of quality, efficacy and safety.

The present study was carried out to determine various quality assessment parameters of test brands of some antihypertensive drugs manufactured in Bangladesh and compared these brands with their respective innovator brands. The dissolution profiles of test brands were compared with their respective innovators brands graphically and also statistically

using similarity factor (f_2) and difference factor (f_1). *In vivo* pharmaceutical equivalence of some antihypertensive drugs was done by plotting plasma concentration- time curves of test brands with their respective reference innovator brands after administration of drug in rat models. The stress degradation studies were done to compare stability of the test brands with respective their reference innovators brands.

A brief background regarding the present study is introduced below:

1.2 PHARMACEUTICAL EQUIVALENCE (PE)

Pharmaceutical equivalence is the term where drug products having the equal quantity of active ingredient in a same dosage form, meet all relevant standards of same strength, quality, purity and potency. Pharmaceutical equivalent drug products should supply equal quantity of active substance on the same dosing time. They must meet the same compendial or other relevant standards on potency, content uniformity, disintegration and dissolution rate.

Pharmaceutically equivalent drug products may not have similar excipients such as color, flavor. They may have different quantity of allowed impurities. They may have different characteristics such as shape, release mechanism, scoring, packaging and labeling.[12-13]

1.3 RELATIONSHIP BETWEEN PHARMACEUTICAL EQUIVALENCE (PE), BIOEQUIVALENCE (BE) AND THERAPEUTIC EQUIVALENCE (TE)

Pharmaceutical equivalence and therapeutic equivalence are not same. Therapeutically equivalent drug products should be pharmaceutically equivalent and should have same safety and efficacy profile after same dosage administered that means should be bioequivalent. The relationship between pharmaceutical equivalence, bioequivalence and therapeutic equivalence can be shown as follows: [12,14]

Pharmaceutical equivalence (PE) + Bioequivalence (BE) = Therapeutic Pharmaceutical

1.3.1 Bioequivalence (BE)

Bioequivalence is the absence of a significant difference in the rate and extent of absorption and availability of active drug substance in pharmaceutical equivalents or pharmaceutical alternatives at the site of action after administration of same molar dose under same conditions. Bioequivalence of a drug product is the assessment of its bioavailability at the site of action when manufactured by different manufacturers. All aims and purposes will be same if two drug products are bioequivalent.

The clinical effects and the safety profiles of the drug products are expected to be similar if the products are pharmaceutical equivalent and bioequivalent. The products may be interchangeable to each other.[12,13,15-16]

1.3.2 Therapeutic Equivalence (TE)

If the drug products are pharmaceutically equivalent and bioequivalent, they are said to be therapeutic equivalents. When they are administered to patients under specific conditions, they are assumed to have identical clinical efficacy and safety profile.

According to Food and Drug administration (FDA) therapeutic equivalents should have following characteristics:

A. approved as clinically effective and safe;
B. having equal quantity of active ingredient in an identical dosage form and meet applicable standards of strength, quality and purity;
C. they are bioequivalent;
D. they are properly labeled and
E. they comply with Current Good Manufacturing Practices (cGMP) during manufacturing.

Therapeutic equivalents may have different excipients such as colors, flavors, preservatives. They may have different characteristics such as shape, scoring configuration, packaging, release mechanism and storage conditions.[7,15]

1.4 *IN VITRO* DISSOLUTION TESTING

In vitro dissolution tests are usually employed to measure the rate and extent of drug dissolution. Drug dissolution is the release of active drug substance from a drug product in a specific medium under specific conditions.[12]

In case of solid dosage forms, *in vitro* dissolution tests are very essential tests for a number of reasons. Importance of dissolution testing is as follows:

A. In the primary stages of drug development, dissolution testing permit differentiation between formulations and correlations obtained from bioavailability data.
B. As an integral part of the whole quality assurance program, dissolution testing may monitor the manufacturing of the drug product. Dissolution testing ensures regulation of variables of materials and process which may affect drug release and quality of the product.
C. Steady dissolution testing can ensure bioequivalence of drug products from batch to batch.
D. To get approval from regulatory agency for marketing the drug product dissolution is required. Submitted New Drug Applications (NDAs) should contain *in vitro* data.[9,17]

The first step for dissolution of a tablet dosage form is the disintegration of the tablet. To release the drug substance from a tablet, tablet should disintegrate properly. Many formulation and manufacturing techniques can affect the disintegration of a tablet and thus, its dissolution. The factors affecting disintegration are as follows: [16,19]

i. Drug substance's particle size;
ii. Hygroscopicity and solubility of the formulation;
iii. Category and content of the disintegrant, binder and lubricant;
iv. Method of manufacturing, especially the compactness of the granulation and compression forces; and
v. Any kind of in process variables.

The United States Pharmacopeia (USP) holds seven apparatus design for dissolution testing of immediate release dosage forms, extended release dosage forms, enteric coated

dosage forms and transdermal dosage forms. USP Apparatus 1 and USP Apparatus 2 are generally employed for immediate release oral dosage forms.[17]

For the drug product and its formulation each dissolution testing method is specific. A variety of conditions such as apparatus, media pH should be considered to develop optimal dissolution limits.[20]

1.5 DISSOLUTION PROFILE COMPARISON

Dissolution profile comparisons are employed to compare the similarity of dissolution characteristics of two formulations or different strengths of the same formulation. The evaluation is done to decide whether *in vivo* bioavailability or bioequivalence studies are required. For the scale up and post approval changes of immediate release dosage form and modified release dosage form, dissolution profile comparisons are needed.

Dissolution profiles may be assumed similar by whole dissolution profile similarity or by similarity at every dissolution sampling time point. The Food and Drug Administration (FDA) has formulated a guideline on dissolution testing in 1997. This guideline describes three methods for the assessment of dissolution profile similarity. These methods are as follows:[12]

A. Model independent similarity factor method;
B. Model-independent multivariate confidence region method and
C. Model- dependent method.

1.5.1 Model Independent Similarity Factor (f_2) and Difference Factor (f_1) Method

The model independent method uses two statistical factors, difference factor (f_1) and similarity factor (f_2) to compare dissolution profiles.[21] The difference factor (f_1) is a measurement of the relative error between the dissolution profile curves. It calculates the percent deference between the two curves at each time point. Difference factor expressing equation is as follows:

$$f_1 = \{[\Sigma \mid R_t - T_t \mid] / \Sigma R_t\} \times 100$$

Where

R_t = the dissolution value of the reference product at time t and

T_t = the dissolution value of the test product at time t.

The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error. It calculates the percent similarity between the two dissolution profile curves. Similarity factor expressing equation is as follows:

$$f_2 = 50 \times \log \{[1 / (1 + (\Sigma (R_t - T_t)^2) / N)]^{1/2} \times 100\}$$

Where

N = the number of time points,

R_t = the dissolution value of the reference product at time t and

T_t = the dissolution value of the test product at time t.

Similarity factor and difference factor are determined by comparing the dissolution profiles of 6-12 units of each of the test and reference products. Similarity factor and difference factor are calculated using the mean dissolution values from both dissolution profiles at each time interval. Three or more dissolution time points are required for the measurements. The dissolution measurements of the test and reference products should be done under the same experimental conditions. One measurement should be done after 85% dissolution of each product. The dissolution time points for both dissolution profiles should be the same.[12]

Two dissolution profile curves are considered similar when f_1 values are close to 0 and f_2 values are close to 100. Generally f_1 values less than 15 (0-15) and f_2 values more than 50 (50-100) ensure sameness or equivalence of the two dissolution profiles and thus, of performance of the test and reference products.[12,18]

1.6 MEASUREMENT OF PLASMA- DRUG CONCENTRATION

Determination of drug concentrations in blood, plasma or serum after drug administration is the most straight and independent way to measure systemic drug bioavailability. The methods used to determine the plasma drug concentration- time profile are as follows:

A. Measurement of time for peak plasma drug concentration (t_{max})

The time for peak plasma drug concentration (t_{max}) is the time required for the drug concentration in plasma after administration to become maximum. At t_{max}, maximum level

of drug absorption in plasma occurs and the rate of drug absorption becomes equal to the rate of drug elimination. When comparing the drug products, value of t_{max} can be used as an approximate indication of drug absorption rate.[12]

B. Measurement of peak plasma drug concentration (*Cmax*)

Peak plasma drug concentration (Cmax) is maximum amount of drug obtained in plasma after oral administration. For many drug products, a relationship is observed between the pharmacodynamic drug effect and the plasma drug concentration. Value of Cmax indicates that the drug product is adequately systemically absorbed to provide a therapeutic effect. Cmax value is often used in bioequivalence studies for the rate of drug bioavailability as a substitute measure.[12]

C. Measurement of area under the plasma drug concentration- time curve (AUC)

The area under the plasma concentration-time curve (AUC) is the measurement of the extent of drug bioavailability. The total amount of active drug substance that reaches the systemic circulation is revealed by AUC value. The AUC value is the area under the plasma concentration-time curve from t=0 to t= ∞. It is equal to the quantity of unchanged drug that reaches the systemic circulation divided by the clearance of drug.[12]

1.7 STABILITY OF DRUG PRODUCTS

Drug stability is the capability of a drug product to maintain it physicochemical, therapeutic, biopharmaceutical and microbial properties within stated limits all over its shelf life. Stability studies for drug products are done to predict, evaluate and ensure its stability. Stability testing is designed as the length of time under specific experimental conditions and storage so that drug product will maintain its characteristics within specified limits.[22,23-24]

1.7.1 Potential Adverse Effects of Instability in Drug Products

Drug products may undergo degradation by a number of pathways due to instability and thus a wide range adverse effects may occur. The adverse effects due to instability in drug products may be described as follows:[16]

A. Decreased amount of active ingredient;

B. Increased amount of active ingredient;

C. Alteration of bioavailability of drug products;

D. Loss of content uniformity of drug products;

E. Decline of microbiological status of drug products;

F. Decline of pharmaceutical elegance and patient's acceptability of drug product;

G. Degradation species formation from drug product;

A. Decline of package integrity of drug product;

B. Decline of quality of label of drug product; and

J. Alteration of any functionally relevant feature of drug product.

1.7.2 Mode of Degradations of Drug Substance and Drug Products

Drug substances and drug products can undergo the following degradations.[25]

A. Chemical degradation

B. Physical degradation

C. Biological degradation

A. Chemical Degradation

Degradation of drug substances and drug products are mainly chemical degradation. Possible chemical degradation pathways are as follows:[26]

i. Hydrolysis

Moisture in most scenarios is present only in sparse amount in solid dosage forms. Drugs are often exposed to humidity in case of most parental dosage forms. As a result, hydrolysis is a very prevalent degradation reaction that can occur in drug substances. For example, drug products containing ester and amide functional groups undergo hydrolysis degradation reactions.

ii. Dehydration

Dehydration is another important chemical degradation reaction seen with drug substances. As for example, erythromycin may undergo acid catalyzed dehydration reaction

iii. Isomerization and racemization

Reversible reactions such as isomerization and racemization occur between optical isomers. Many drug substances undergo racemization and epimerization degradation reactions. As for example, pilocarpine undergoes base catalyzed epimerization.

iv. Decarboxylation and elimination

Drug substances containing a carboxylic acid group are occasionally prone to a reaction of decarboxylation degradation. As for example, 4-Aminosalicylic acid shows decarboxylation degradation.

v. Oxidation

Oxidation is a common chemical degradation reaction for drug substances and drug products. Drug substances and drug products are exposed to oxygen either at manufacturing sites or at storage sites. For example, ascorbic acid is very susceptible to oxidation degradation.

vi. Photodegradation

A large number of drug substances show photodegradation reaction. Reaction mechanisms for photodegradation are generally very complicated. For example, chloroquine undergoes photodegradation to produce numerous products via complex pathways.

vii. Drug-drug and drug-excipients interactions

Degradation reactions may occur between the drug substance and one or more excipients. In the same way two drug substances may react with each other. For example, catecholamines such as epinephrine undergoes degradation reaction with additive bisulfite.

B. Physical degradation

Drug substances and excipients may exist in various physical states. They can change from unstable or metastable physical state to a more stable state with time. Some physical changes of drug substances and excipients are as crystallization of amorphous states, transitions in crystalline states, growth of crystals, vapor-phase transfers and adsorption of moisture.[26]

C. Biological especially microbiological degradation

Microorganisms are mainly involved in biological degradation of drug substances and products. Rats, cockroaches, ants, and other non-microbiological factors may also affect biological stability of drug products.[16]

1.7.3 Factors Affecting Chemical Stability

Mainly two types of factors affect the chemical stability of drug substances. First one is molecular structure of the drug itself. Second one is environmental factors. Factors are as follows:[26]

A. Role of molecular structure

The drug degradation can be governed by the molecular structure of the drug substance itself. The substituents surrounding the reaction center can also determine its degradation activity.

B. Role of temperature

Temperature is a very important factor affecting chemical stability of the drug substance. The relationship between the temperature and degradation rate constant can be described by the Arrhenius equation:

$$k = Ae^{-E/RT}$$

Where

k = Reaction rate constant,

E = Activation energy,

A = Frequency of reaction,

T = Absolute temperature and

R = Ideal gas constant.

The reaction indicates that a small increase of reaction temperature will increase the magnitude of reaction rate constant markedly. Most degradation reactions go rapidly at elevated temperature than at decreased temperature.

C. Role of pH

After temperature, pH is the second most important factor which affects chemical stability of drug. Most chemical degradation reactions are catalyzed by hydronium and hydroxide

ions. Water itself is a precarious degradation reactant. Acids and bases, usually buffer species present in solution can affect the reaction rate. Again in case of ionizeable drug, the reactivity of the drug will also be pH-dependent.

D. Role of buffer

Buffer existing in the solution of the drug substance can affect the chemical stability of the drug substance. For example, phosphate and acetate buffer catalyze the hydrolysis of chloramphenicol.

E. Role of ionic strength

The presence of other ionic species such as salts like sodium chloride can affect the degradation rate of a drug substance. When ionic strength increases, the reaction rate between the opposite charged ions decreases and the rate between the same charged ions increases.

F. Role of dielectric constants

Degradation reaction rates between the ions and dipoles presenting in the solution can be affected by the dielectric constants of the solvents. For example, the hydrolysis of chloramphenicol in water and propylene glycol mixture increases with decreasing dielectric constant of the solvent.

G. Role of availability of oxygen

The availability of oxygen can affect the oxidation degradation rates of drug substances. The rates of the oxidation of drug substances can be affected by the availability of oxygen. Sometimes photodegradation involving photo-oxidative mechanisms depends on the availability of oxygen.

H. Role of light

The photodegradation rate of drugs can be affected by the number and the wavelength of incident light. The wavelength depended degradation reaction varies among drug substances. As for example, Photodegradation of nifedipine tablets was shown to be maximum at 420 nm.

I. Role of crystalline state and polymorphism

The crystalline state of solid dosage form can affect the chemical stability of the drug substance. Drugs having crystalline state shows slower reactivity due to lower ground-state free energy. Many drug substances show polymorphism. Each crystalline state of the polymorphs having different ground-state free-energy level has a different chemical reactivity.

J. Role of moisture and humidity on solid and semisolid drugs

Moisture can affect the degradation of heterogeneous drug systems such as solids and semisolids. For example, moisture and humidity can affect the degradation rates of ascorbic acid.

K. Role of excipients

Excipients can play a vital role in the chemical stability of drug substance. The influence of sugars in the degradation of ascorbic acid in aqueous solution is an examples of chemical degradation by excipients.

1.8 STABILITY TESTING METHODS

In pharmaceutical industries stability testing procedures are regularly done on drug substances and drug products. Stability testing procedures can be divided into the following four types depending upon the aim and steps followed:[27]

A. Real time stability testing procedure

Real time stability testing is generally done for longer duration of the test period. It is performed to permit significant product degradation under suggested storage conditions. The test period depends upon the stability of the product which should be long enough to indicate no sign of degradation and must permit to differentiate degradation from inter-assay variation.[27-28]

B. Accelerated stability testing procedure

Accelerated stability testing is generally performed at a very early stage to determine the rates of chemical and physical degradation reactions and their relationships with their storage conditions such as temperature, moisture, light and others. It is a short-term

stability study done under exaggerated storage conditions to increase the rate of chemical or physical degradation of a drug substance or drug product. So, accelerated stability testing is also called stressed testing.

Arrhenius equation can be used to project stability from the degradation reaction rates observed at high temperatures. When the activation energy is identified, the degradation rate at low temperatures may be projected from those obtained at exaggerated temperatures.[32, 33-34]

C. Retained sample stability testing procedure

Stability data are generally required for every marketed product. In retained sample stability testing stability samples which are retained storage for at least one batch a year are selected. The stability samples should be tested at predetermined intervals. This type of conventional stability testing on retained storage samples is also known as constant interval method. [27,35]

D. Thermal cycling stress testing procedure

Most heterogeneous systems such as ointments, creams, suspensions, emulsions, lotions, inhalation aerosols and suppositories may be adversely affected by various elevated temperatures during distribution and shipping. These types of drug products should be tested under cycling temperature conditions to reveal shipping and distribution conditions. The studies are usually done on packaged drug products during stress testing of the drug development stage.[25]

1.9 IMPORTANCE OF PROPER FORMULATION OF TABLETS

The design and manufacture of pharmaceutical tablets is a complex multi-stage process. Correct quantity of drug substance in the right form should be delivered at the appropriate time, at the proper rate and in the desired location with its protected chemical integrity during manufacturing process. Most drug substances do not have the required properties which give adequate flow from the hopper to the die cavity of tablet presses. Thus, they are subjected to pre-treatment either alone or in combination with suitable excipients to form free-flowing granules which are necessary for tableting.

Tablets are usually manufactured by wet granulation, dry granulation or direct compression method. These methods are consisted of a series of steps. The steps are weighing, milling, mixing, granulation, drying, compaction and often coating, packaging. Nevertheless of the method used, the processes of weighing, milling and mixing are the same. Later steps may differ.

The primary goals of tablet manufacturing process should include:

A. Formulated tablets should be strong enough to withstand mechanical shock faced during manufacturing, packing, shipping, dispensing and patient use.

B. Formulated tablets should be uniform in weight and in drug content.

C. Formulated tablets should be bioavailable in relative to indication requirements.

D. Formulated tablets should be chemically and physically steady for a specific period of time.

E. Formulated tablets should have sophisticated product identity which is free from any tablet flaws.[69-70]

1.10 PROPERTIES OF EXCIPIENTS IN THE MANUFACTURE OF TABLETS

Pharmaceutical drug products generally contain inactive, non-therapeutic substances other than the active drug substance. These substances are called excipients. They are added to a drug product for safeguarding product acceptability in terms of manufacturability, appearance and performance. In tablet formulation, excipients are usually used at different quantities with the active drug substance to produce tablets with standard quality. The type and quantities of each excipient used depend on the type of tablet manufactured and the type of process used.

Excipients used in tablet formulation may be classified into two groups:

i. Excipients which are used to impart satisfactory processing and compression characteristics to the formulation e.g. diluents, binders, glidants, and lubricants.

ii. Excipients that are used to give additional desirable physical characteristics to the compressed tablets e.g., disintegrants, surfactants, colouring agents, flavouring agents and sweetening agents.

A. Binders

Binders are polymeric, natural or synthetic materials which that give cohesive qualities to powdered materials used in tableting. Commonly used binders in tablet formulation are corn starch, starch, gelatin, acacia, sodium carboxymethyl cellulose and methyl cellulose.

They should ensure that tablets remain intact after compression and improve the free-flowing qualities of the powdered materials without impeding disintegration or dissolution.

B. Diluents

When the quantity of active ingredient of a tablet is very small, diluents are added to tablet formula. They are added to increase the size of the tablets to get a significant tablet weight which can be handled or compressed. Examples of bulking agents used in tablet formulation include lactose, mannitol, dicalcium phosphate, calcium sulfate, dry starch, cellulose, kaolin, anhydrous lactose etc.

The amount of diluents that uses in a tablet formulation is normally determined by the quantity of the drug, the nature and amount of other ingredients in the formulation.

Diluents should be chemically inert, non-hygroscopic, hydrophilic and must have good compression properties. The compatibility of diluents with the drug substance must be considered as it may interfere with the absorption of drug substances from the gastrointestinal tract.

C. Disintegrants

Disintegrants are added to a tablet formulation to overcome the cohesive strength imparted during compression. They help the breakdown of the tablet into granules for drug availability when they come in contact with water. The mechanisms by which disintegrants cause their functions are still not fully understood

Disintegrants generally used in the manufacture of tablets are corn and potato starches, bentonite, guar gum, methylcellulose, carboxymethyl cellulose, cation exchange resins, alginic acid, agar etc.

Disintegrants may be added intra granularly, extra granularly or both. The higher the concentration of disintegrants does not always the quicker the rate of disintegration. The concentration may have a direct relationship with the rate of disintegration to its maximum

label. After this level disintegration rate decreases with increase in concentration of disintegrants.

D. Lubricants

Lubricants decrease friction between the mixed powder and the die walls during compression and ejection of tablets. They also prevent the powder mix or granules from sticking to the processing zone of the tablet press especially the punches and die. The best lubricants are those which have low shear strength but strong cohesive tendencies perpendicular to the line of shear.

Lubricants can be classified based on their solubility characteristics into

i. Soluble lubricants e.g., Polyoxyethylene stearates, polyethylene glycol and lauryl sulphate salts.
ii. Insoluble lubricants e.g., Stearic acid, magnesium stearate etc.

During tablet manufacturing process inadequate lubrication causes the production of tablets with a pitted surface. On the other hand, excessive use of lubricants produces tablets with decreased rates of disintegration and dissolution. The appearance of the tablet is an important consumer requirement and thus, inadequate or excessive lubrication will lead to dismissal of the tablet batch.

E. Glidants

Glidants are fine powders which increase the movement of powders or granules within the hopper and into the die cavity prior to compression. Enhanced flow rates of powders or granules causes less weight variability of the tablets manufactured which results in more consistent dosing of the drug substance. Examples of glidants used in tablet manufacture include corn starch, talc, colloidal silicon dioxide, etc.

Glidants are naturally hydrophobic. Therefore, precaution should be taken to ensure that the concentration of glidants used in the formulation does not badly affect tablet disintegration and drug dissolution.

F. Adsorbents

Whenever there is need to include a liquid or semisolid drug substance or excipients e.g., flavouring agent within a tablet formulation, adsorbents are used. Adsorbents adsorb moisture which may attack tablets or cause cohesiveness of tablet powder or granules from these liquid or semi-solid components. Thus, they allow proper tablet compression. Examples of adsorbents used in the manufacture of tablets include magnesium oxide or carbonate, bentonite or kaolin etc.

G. Sweetening agents

Sweetening agents are added in tablets to impart sweetness to the product. Thus, they improve the acceptability of tablets. When the conventional tablet contains a bitter drug substance or if the tablet is a chewable tablet, then this excipient is particularly important. Sucrose is a standard sweetening agent. Artificial sweeteners have the advantage of not effecting blood sugars of diabetic or pre-diabetic patients. They are also considered non-cariogenic. Sweetening agent generally used in tablet manufacture are dextrates, dextrose, fructose, sucrose, mannitol, acesulfame potassium, aspartame, confectioner's sugar, saccharin, sorbitol, sucralose etc.

H. Flavouring agents

Flavouring agents are excipients which are used to impart a pleasant flavour and often odour to pharmaceutical formulations. They may be derived from natural sources e.g., fruit components or prepared artificially. Their particular use in pharmaceutical dosage forms is depended on the desired flavour, their solubility properties and their physico-chemical compatibility with the drug substance and other excipients used in the formulation.

During selection of the flavouring agent the age of the intended patient should be considered. Because certain age groups appear to prefer certain flavours. Children for example prefer sweet candy-like preparations with fruity flavours. On the contrary, adults seem to prefer less sweet preparations. Flavouring agents can degrade due to exposure to light, temperature, water, headspace oxygen, enzymes, contaminants and other product components. Therefore, they must be carefully selected and tested for stability.

I. Colouring agents

Colouring agents are generally used in tablet manufacture either for pleasing appearance or for uniquely identifying finished tablets. Colouring agents can be divided into water-soluble dyes and water-insoluble pigments. The adverse effects colouring agent in food substances arises suspicions over the safety of these agents in pharmaceutical formulations. Each country has its own list of approved colouring agents that may be used in pharmaceutical products. The colours must be uniformly distributed throughout the tablet. Examples of colourants used in the manufacture of tablets include titanium dioxide, iron oxides, aluminium lakes etc.

J. Surfactants

Surfactants are excipients which are added into tablet formulation to increase the wetting properties of hydrophobic tablets. Thus, the rate of tablet disintegration increases. They may also increase the aqueous solubility of poorly soluble drug substance in the gastrointestinal tract and in this way they increase the rate of dissolution of the drug substance. The surfactants should not interact with the drug substance which may affect the dissolution rate of the drug substance.

Examples of surfactants used in the manufacture of tablets include glyceryl monooleate, sodium lauryl sulphate, cetylpyridine chloride, etc.[12,17, 33, 71- 74]

1.11 FACTORS INFLUENCING DRUG ABSORPTION AND BIOAVAILABILITY

To achieve the desired therapeutic effect, the drug product must deliver the active drug substance at an optimum rate and quantity. The proper biopharmaceutical design can change the rate and extent of drug absorption. Bioavailability can be varied from rapid and complete absorption to slow and sustained absorption. A series of events may occur following administration of a tablet dosage form until its absorption into systemic circulation. The chain of events consists of four steps:

i. Disintegration of the drug product.
ii. Deaggregation and succeeding release of the drug.
iii. Dissolution of the drug in the aqueous fluids of the absorption site.

iv. Absorption or movement of the dissolved drug through the GI membrane into the systemic circulation and away from the absorption site.

The drug may also dissolve before disintegration or deaggregation of the dosage form and before or after reaching the absorption site. Without the drug goes into solution, drug cannot be absorbed into the systemic circulation.[71-74]

In a series of kinetic or rate processes, the rate at which the drug reaches the systemic circulation is determined by the slowest of the various steps involved in the sequence. Such a step is called as the rate-determining or rate-limiting step. The rate and extent of drug absorption from its dosage form can be influenced by a number of factors in all these steps. The various factors that influence drug absorption can be classified as

A. Physicochemical factors

1. Drug solubility and dissolution rate
2. Particle size and effective surface area
3. Polymorphism and amorphism
4. Pseudo polymorphism
5. Salt form of the drug
6. Lipophilicity of the drug
7. pKa of the drug and gastrointestinal pH
8. Drug stability
9. Stereo chemical nature of the drug

B. Pharmaceutical factors

1. Disintegration time
2. Manufacturing variables
3. Pharmaceutical excipients
4. Nature and type of dosage form
5. Product age and storage conditions

1. Disintegration time

Disintegration time is of specific importance in case of solid dosage forms like tablets and capsules. *In vitro* disintegration test is not at all a guarantee of drug's bioavailability.

Because if the disintegrated drug particles do not dissolve, absorption is not possible. If a solid dosage form does not obey the Disintegration time, it creates bioavailability problems. Because the subsequent process of dissolution will be much slower and absorption may be inadequate. Coated tablets, especially sugar coated tablets have long Disintegration time. Rapid disintegration is thus significant in the therapeutic success of a solid dosage form. Disintegration time of a tablet is directly related to the amount of binder present and the compression force of a tablet. A harder tablet with large amount of binder has a long Disintegration time.[71- 74]

2. Manufacturing variables

Drug dissolution is the single most significant factor in the absorption of drugs. This is especially true for the most widely used conventional solid dosage forms, tablets and capsules. The dosage form related factors that influence dissolution and thus absorption of a drug from such formulations are:

a. Excipients and
b. Manufacturing processes.

The influence of excipients such as binders, lubricants, disintegrants on drug dissolution will be discussed later.

Several manufacturing processes influence drug dissolution from solid dosage forms. Processes of such importance in the manufacture of tablets are:

a. Method of granulation, and
b. Compression force.

a. Method of Granulation

The wet granulation process is the most conventional technique in the manufacture of tablets. It was once thought to produce tablets that dissolve faster than those made by other granulation methods. The limitations of this method are

i. Formation of crystal bridge by the presence of liquid,
ii. The liquid may act as a medium for affecting chemical reactions such as hydrolysis and
iii. The drying step may harm the thermolabile drugs.

iv. Involvement of large number of steps each of which can influence drug dissolution method

v. Now the method of direct compression has been utilized to yield tablets that dissolve at a faster rate.

b. Compression Force

The compression force employed in tableting process can influence density, porosity, hardness, disintegration time and dissolution of tablets. Higher compression force increases the density and hardness of tablet. Thus, decreases the porosity and the penetrability of the solvent into the tablet. This causes in slowing of the dissolution rate of tablets. Conversely, higher compression forces cause deformation, crushing or fracture of drug particles into smaller ones. They convert a spherical granule into a disc shaped particle with a large increase in the effective surface area. This results in an increase in the dissolution rate of the tablet. In brief, the influence of compression force on the dissolution rate is difficult to predict. Thus, a thorough study on each formulation should be made to ensure better dissolution and bioavailability.

3. Pharmaceutical excipients

Excipients can influence absorption of drugs in spite of their inertness and utility in the dosage form. The more the number of excipients in a dosage form, the more complex it is. Then greater the potential for absorption and bioavailability problems. Commonly used excipients which can effect dosage forms are discussed below.[71-74]

a. Diluents

A diluent may be organic or inorganic. Among organic diluents, carbohydrates are very widely used. For example, starch, lactose, microcrystalline cellulose etc. These hydrophilic powders are very useful in promoting the dissolution of poorly water-soluble, hydrophobic drugs like spironolactone and triamterene. They form a coat onto the hydrophobic surface of drug particles and make them hydrophilic. Among the inorganic diluents, dicalcium phosphate is most common. Example of drug-diluent interaction resulting in poor bioavailability is that of tetracycline and dicalcium phosphate. The cause is formation of divalent calcium- tetracycline complex which is poorly soluble and thus, unabsorbable.

b. Binders

Like diluents, the hydrophilic binders show better dissolution profile with poorly wettable drugs like phenacetin by imparting hydrophilic properties to the granule surface. But, the proportion of strong binders in the tablet formulation is very complex. Large amounts of such binders increase hardness and decrease disintegration and dissolution rates of tablets. Non-aqueous binders like ethyl cellulose also hinder drug dissolution.

c. Disintegrants

These agents overcome the cohesive strength of tablet and break them up on contact with water. This is an important prerequisite to tablet dissolution. Almost all the disintegrants are hydrophilic in nature. A decrease in the amount of disintegrant can significantly lower bioavailability.

d. Lubricants

The commonly used lubricants are hydrophobic in nature as for examples several metallic stearates and waxes. They are known to inhibit wettability, penetration of water into tablet and their disintegration and dissolution. Because the disintegrant gets coated with the lubricant if blended simultaneously. This problem however can be prevented by adding the lubricant in the final stage. The best alternative is use of soluble lubricants like carbowaxes which promote drug dissolution.

e. Coatings

In general, the harmful effect of various coatings on drug dissolution from a tablet dosage form is in the following order:

Enteric coat > Sugar coat > Non-enteric film coat.

The dissolution profile of certain coating materials change on aging. As for example, shellac coated tablets after prolonged storage may dissolve more slowly in the intestine.

f. Suspending Agents

Popular suspending agents are hydrophilic which primarily stabilize the solid drug particles. They decrease their rate of settling through an increase in the viscosity of the medium. These agents and some sugars are also used as viscosity imparters to affect palatability and pourability of solution dosage forms. Such agents can influence drug

absorption. Surfactants are widely used in formulations as wetting agents, solubilisers and emulsifiers. Their influence on drug absorption is very complex. They may increase or decrease drug absorption either by interacting with the drug or the membrane or both.

g. Coloring agents

A very low concentration of water-soluble dye can have an inhibitory effect on dissolution rate of several crystalline drugs. The dye molecules get adsorbed onto the crystal faces and hinder drug dissolution. As for example, brilliant blue retards dissolution of sulphathiazole. Dyes have also been found to hinder micellar solubilisation effect of bile acids. This may impair the absorption of hydrophobic drugs like steroids. Cationic dyes are more reactive than the anionic ones due to their greater power for adsorption on primary particles.

4. Nature and type of dosage form

In addition to the proper selection of drug, clinical effectiveness often depends in large part on the proper selection of dosage form of that drug. Depending upon the nature and type of dosage form for a given drug, a 2 to 5 fold or possibly more difference could be observed in the oral bioavailability. This difference is due to the relative rate at which a particular dosage form releases the drug to the biological fluids and the membrane. The relative rate at which a drug from a dosage form is accessible to the body depends upon the complexity of dosage form. The more complex a dosage form, greater the number of rate-limiting steps and greater the potential for bioavailability problems.

As a general rule, the bioavailability of a drug from various dosage forms decreases in the following order:

Solutions > Emulsions > Suspensions > Capsules > Tablets > Coated Tablets > Enteric Coated Tablets > Sustained Release Products.

Therefore, absorption of a drug from solution is fastest with least possiblilty for bioavailability risks whereas absorption from a sustained release product is slowest with greatest bioavailability problems.[71- 74]

5. Product age and storage conditions

Due to aging and alterations in storage conditions a number of changes especially in the physicochemical properties of a drug in dosage form can occur. These changes can

adversely affect bioavailability. Disintegration and dissolution rates of are greatly affected due to aging and storage conditions in case of solid dosage forms especially tablets. These parameters of tablets increase in excipients that harden on storage e.g. acacia. The decrease is mainly due to softening or crumbling of the binder during storage e.g. carboxymethyl cellulose.

Changes that occur during the shelf-life of a dosage form are affected mainly by large variations in temperature and humidity. Studies conducted on prednisone tablets had shown that prednisone containing lactose as the filler, high temperature and high humidity resulted in harder tablets that disintegrated and dissolved slowly.

C. Patient related factors

Patient related factors are those factors related to the anatomical, physiological and pathological characteristics of the patient[12,17,33,72-77]

1. Age
2. Gastric emptying time
3. Intestinal transit time
4. Gastrointestinal pH
5. Disease states
6. Blood flow through the GIT
7. Gastrointestinal contents
    a. Other drugs
    b. Food
    c. Fluids
    d. Other normal GI contents
8. Pre-systemic metabolism by:
    a. Luminal enzymes
    b. Gut wall enzymes
    c. Bacterial enzymes
    d. Hepatic enzymes

1.12 STUDIED ANTIHYPERTENSIVE DRUGS

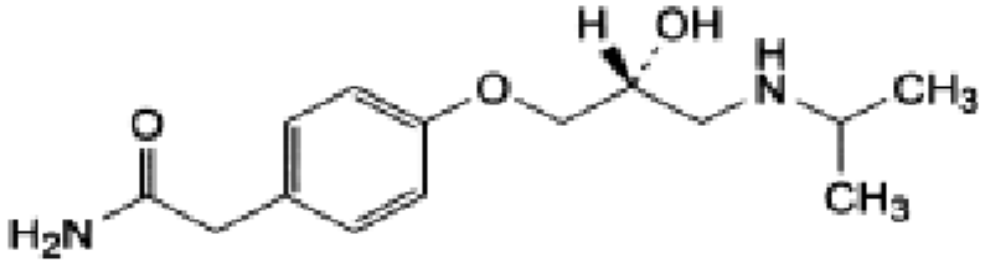
In the present research work, we have studied pharmaceutical equivalence and stability studies of some antihypertensive drugs manufactured in Bangladesh. The studied drugs are tablet atenolol 50 mg tablet, tablet carvedilol 6.25 mg, tablet losartan potassium 50 mg and tablet ramipril 5 mg. A brief note of these drugs is given below:

1.12.1 Atenolol

Atenolol is a cardio selective beta blocker. It has no intrinsic sympathomimetic activity and membrane stabilizing properties. Atenolol is used in the management of hypertension, angina pectoris, cardiac arrhythmias and myocardial infarction.

1. Physicochemical Parameters[37,78]

Molecular structure:



Nomenclature: 2-[4-[(2 *RS*)-2-hydroxy-3-[(1-methylethyl) amino] propoxy] phenyl]acetamide

Molecular formula: $C_{14}H_{22}N_2O_3$

Molecular weight: 266.3 g

Appearance: white or almost white powder

Melting point: 152 °C to 155 °C.

Solubility: Sparingly soluble in water, soluble in ethanol, slightly soluble in methylene chloride.

Loss on drying: Not more than 0.5 per cent.

Potency: Atenolol contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 2-[4-[(2 *RS*)-2-hydroxy-3-[(methylethyl) amino] propoxy] phenyl] acetamide, calculated with reference to the dried substance.

2. Pharmacokinetic Parameters

Only 50% of an oral dose of atenolol is absorbed from the gastrointestinal tract. Peak plasma concentrations achieve within 2 to 4 hours. Atenolol has low lipid solubility. It can cross the placenta. It can accumulate in breast milk where concentrations are higher than those in maternal plasma. Only small amounts cross the blood-brain barrier. Plasma protein binding is minimum. The plasma half-life is about 6 to7 hours. Atenolol undergoes little or no hepatic metabolism. It is excreted mainly in the urine. It is removed by haemodialysis.[79]

3. Pharmacological Parameters

Beta blockers are competitive antagonists of the effects of catecholamines at beta-adrenergic receptor sites. Atenolol is a beta blocker with a higher affinity for $beta_1$ than $beta_2$ receptors. It causes fewer non cardiovascular effects and is described as cardioselective or second generation beta blockers. $Beta_1$ blockade mainly affects the heart reducing heart rate, myocardial contractility, and rate of conduction of impulses through the conducting system. It also leads to suppression of adrenergic-induced renin release and lipolysis.[79]

4. Indications

Atenolol is used in the management of hypertension, angina pectoris, cardiac arrhythmias, and myocardial infarction. It may also be used for the prophylaxis of migraine.

5. Doses

   a. In hypertension atenolol i s given orally in a dose of 25 to 100 mg daily, as a single dose.
   b. The usual dose for angina pectoris is 50 to 100 mg daily orally, given as a single dose or in divided doses.
   c. For the treatment of cardiac arrhythmias, maintenance oral doses of 50 to 100 mg daily may be given.

d. Atenolol is also used in the management of acute myocardial infarction in a maintenance dose of 100 mg daily.
e. In the prophylaxis of migraine an oral dose of 50 to 200 mg daily has been used.

6. Side effects

Atenolol is generally well tolerated and most adverse effects are mild and transient. Reactions may be more severe after intravenous than oral doses. Among the most serious adverse effects are heart failure, heart block, and bronchospasm. Headache, depression, dizziness, hallucinations, confusion, amnesia, and sleep disturbances may occur. Fatigue is a common adverse-effect of beta blockers. Adverse gastrointestinal effects include nausea and vomiting, diarrhea, constipation, and abdominal cramping. Hypoglycemia, hyperglycemia, rashes, pruritus, exacerbation of psoriasis, excess sweating, and reversible alopecia may occur.[79]

7. Precautions

Atenolol should not be given to patients with

a. bronchospasm or asthma or to those with a history of obstructive airways disease.
b. metabolic acidosis, cardiogenic shock, severe peripheral arterial disease, sinus bradycardia, and second- or third-degree AV block.
c. uncontrolled heart failure.
d. increased sensitivity to allergens and also the severity of anaphylactic reactions.
e. in pregnancy shortly before delivery.

8. Drug interactions

Drugs which enhance the antihypertensive effects of atenolol are ACE inhibitors, calcium-channel blockers, clonidine, verapamil, sotalol, digoxin, adrenaline and general anesthetics. Drugs which decrease the antihypertensive effects of atenolol are aldesleukin and NSAIDs. In diabetic patients atenolol may reduce the response to insulin and oral hypoglycemic. Drugs which reduce absorption of atenolol are aluminium salts and bile-acid binding resins such as colestyramine. Metabolism may be increased by drugs such as

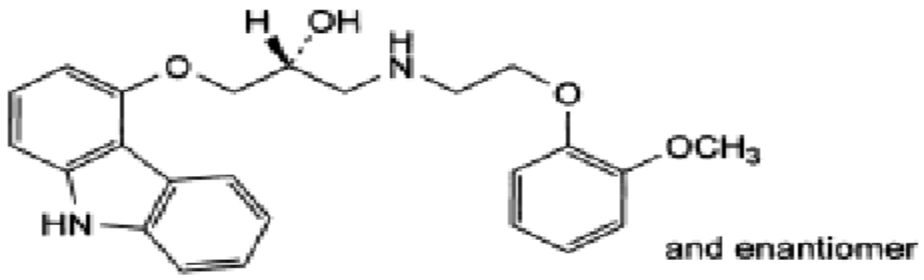
barbiturates and rifampicin and decreased with drugs such as cimetidine, erythromycin, fluvoxamine, and hydralazine. Cimetidine and hydralazine may decrease hepatic blood flow and thus decrease hepatic clearance.[79]

1.12.2 Carvedilol

Carvedilol is a Carvedilol is a non cardio selective beta blocker. It has vasodilating properties which are mainly responsible for its blocking activity at alpha I receptors. Calcium-channel blocking activity may contribute at higher doses. It also has antioxidant properties. Carvedilol has no intrinsic sympathomimetic activity and only weak membrane-stabilizing activity.

1. Physicochemical Parameters[37,78]

Molecular structure:



Nomenclature: (2*RS*)-1-(9*H*-Carbazol-4-yloxy)-3-[[2-(2methoxyphenoxy)ethyl]amino]propan-2-ol.

Molecular formula: C24H26N2O4

Molecular weight: 406.5 g

Appearance: White or almost white, crystalline powder.

Solubility: Practically insoluble in water, slightly soluble in alcohol, practically insoluble in dilute acids. It shows polymorphism.

Loss on drying: Not more than 0.5 per cent.

Potency: Carvedilol contains not less than 98.0 per cent and not more than102.0 per cent of C24H26N2O4, calculated on the dried basis.

Heavy metals: Maximum 10 ppm.[79]

2. Pharmacokinetic Parameters

Carvedilol is well absorbed from the gastrointestinal tract. However it has considerable first-pass metabolism in the liver. The absolute bioavailability is about 25%. Peak plasma concentrations occur within 1 to 2 hours after an oral dose. It has high lipid solubility. Carvedilol is more than 98% bound to plasma proteins. It is extensively metabolized in the liver by the cytochrome P450 isoenzymes CYP2D6 and CYP2C9. The metabolites are excreted mainly in the bile. Carvedilol has elimination half-life of about 6 to 10 hours. It may accumulate in breast milk as shown in animals.[79]

3. Pharmacological Parameters

Carvedilol is a third-generation β receptor blocker. It has a unique pharmacological profile. It blocks β_1, β_2, and α_1 receptors. It also has antioxidant and antiproliferative effects. It has membrane stabilizing activity but it has no intrinsic sympathomimetic activity. Carvedilol causes vasodilation. The additional properties such as antioxidant and antiproliferative effects may contribute to treat congestive heart failure.[80]

4. Indications

Carvedilol is used in the management of hypertension and angina pectoris. It is used as an adjunct to the standard therapy of symptomatic heart failure. It is also used after myocardial infarction in patients with left ventricular dysfunction to reduce mortality.

5. Doses

a. In hypertension carvedilol is given in an initial oral dose of 12.5 mg once daily, increased after two days to 25 mg once daily. A dose of 12.5 mg once daily may be adequate for elderly patients.
b. In angina pectoris an initial oral dose of 12.5 mg is given twice daily, increased after two days to 25 mg twice daily.
c. In heart failure, the initial oral dose is 3.125 mg twice daily. If tolerated, the dose should be increased gradually to the maximum dose tolerated. This should not

exceed 25 mg twice daily in patients with severe heart failure or 50 mg twice daily in patients with mild to moderate heart failure.

d. In patients with left ventricular dysfunction after myocardial infarction, the initial dose is 6.25 mg twice daily and then to a target dose of 25 mg twice daily.

6. Side effects

Bradycardia and hypotension; heart failure or heart block may be worsened in patients with cardiac disorders. Bronchospasm, shortness of breath, and dyspnea may be worsened in patients with a history of obstructive airways disease. Headache, depression, dizziness, hallucinations, confusion, amnesia, and sleep disturbances may occur. Fatigue is a common adverse-effect. Nausea and vomiting, diarrhea, constipation, and abdominal cramping may occur. Carvedilol can produce hypoglycemia, hyperglycemia.[79]

7. Precautions

a. Carvedilol should not be given to patients

b. With bronchospasm or asthma or to those with a history of obstructive airways disease.

c. With metabolic acidosis, cardiogenic shock, severe peripheral arterial disease, sinus bradycardia, and second or third degree AV block.

d. with uncontrolled heart failure

e. With increased sensitivity to allergens and also the severity of anaphylactic reactions.

f. In pregnancy shortly before delivery.

g. With liver function abnormalities

8. Drug interactions

Drugs which can enhance the antihypertensive effects of carvedilol are ACE inhibitors, calcium-channel blockers, clonidine, verapamil, sotalol, digoxin, adrenaline and general anesthetics. Drugs which can decrease the antihypertensive effects are aldesleukin and NSAIDs. In diabetic patients carvedilol can reduce the response to insulin and oral hypoglycemic. Drugs which can reduce absorption of carvedilol are aluminium salts and bile-acid binding resins such as colestyramine. Metabolism may be increased by drugs such

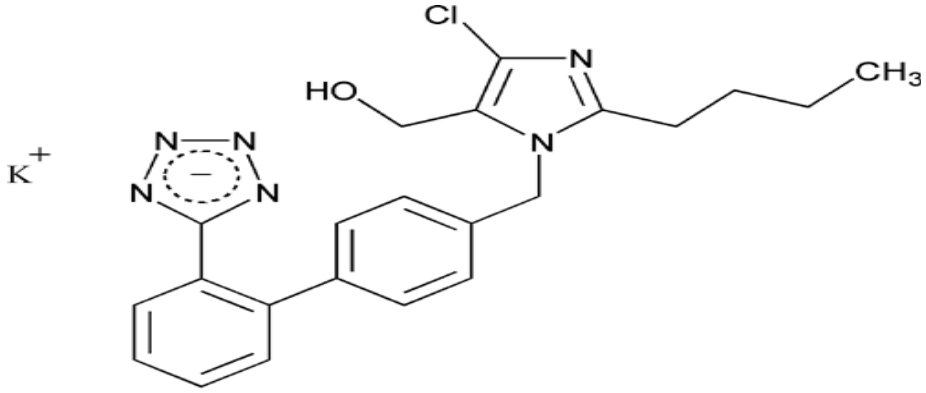
as barbiturates and rifampicin and decreased with drugs such as cimetidine, erythromycin, fluvoxamine, and hydralazine. Cimetidine and hydralazine may decrease hepatic blood flow and thus decrease hepatic clearance.[79]

1.12.3 Losartan Potassium

Losartan is an angiotensin II receptor antagonist with antihypertensive activity. These properties are mainly due to selective blockade of AT_1 receptors and the resulting reduced pressor response of angiotensin II. It is used in the management of hypertension and heart failure in patients who develop cough with ACE inhibitors. Losartan i s given orally as the potassium salt.

1. Physicochemical Parameters[37,78]

Molecular structure:



Nomenclature: 2-Butyl-4-chloro-1-[p-(o-lH-tetrazol-5-ylphenyl)benzyl] imidazole-5-methanol, monopotassium salt

Molecular formula: $C_{22}H_{22}ClKN_6O$

Molecular weight: 461.00 g

Appearance: White or almost white, crystalline powder.

Melting point: 263-265°C

Solubility: Freely soluble in water and in methanol, slightly soluble in acetonitrile.

Loss on drying: Not more than 0.5 per cent

Potency: Losartan potassium contains not less than 98.5 per cent and not more than 101.0 per cent of C22H22ClKN6O, calculated on the dried basis.[38]

2. Pharmacokinetic Parameters

After oral doses losartan is readily absorbed from the gastrointestinal tract. However, it undergoes considerable first-pass metabolism which results in a systemic bioavailability of about 33%. It is metabolized to an active metabolite and some inactive metabolites. Active metabolite E- 3174 (EXP- 3174 has greater pharmacological activity than losartan. Peak plasma concentrations of losartan and E3174 occur about hour and 3 to 4 hours respectively. Both losartan and E- 3174 are more than 98% bound to plasma proteins. Losartan is excreted as unchanged drug and metabolites in the urine and in the faeces via bile. The elimination half-lives of losartan and E - 3174 are about 1.5 to 2.5 hours and 3 to 9 hours respectively.[79]

3. Pharmacological Parameters

Losartan is an AT_1- Angiotensin II Receptor Blockers (ARBs). They are selective drugs. They bind to the AT_1 receptor of angiotensin II with high affinity than the AT_2 receptor. By antagonizing the effects of angiotensin II, they relax smooth muscle and thus promote vasodilation, increase renal salt and water excretion, reduce plasma volume, and decrease cellular hypertrophy.

4. Indications

Losartan is used in the management of hypertension and heart failure in patients who develop cough with ACE inhibitors. It is also used to reduce the risk of stroke in patients with left ventricular hypertrophy and in the treatment of diabetic nephropathy.

5. Doses

   a. In hypertension the usual dose of losartan potassium is 50 mg once daily. The dose may be increased, if necessary, to100 mg daily as a single dose or in two divided doses.
   b. Losartan potassium is used for heart failure in those aged 60 years and over. An initial dose of 12.5 mg is given once daily, and may be doubled at weekly intervals to a usual maintenance dose of 50 mg once daily.
   c. In diabetic nephropathy losartan potassium is given in an initial dose of 50 mg once daily, increased to l00 mg once daily depending on the blood pressure.

6. Side effects

Adverse effects of losartan are usually mild and transient. These include dizziness, headache and dose-related orthostatic hypotension. Impaired renal function, rash, urticaria, pruritus, angioedema, and raised liver enzyme values may occur. Hyperkalemia, myalgia, and arthralgia may. Other adverse effects include respiratory-tract disorders, back pain, gastrointestinal disturbances, fatigue, and neutropenia.

7. Precautions

Losartan should not be given to patients

a. in pregnancy
b. in severe hepatic impairment
c. in renal artery stenosis
d. with volume depletion
e. with renal impairment

8. Drug interactions

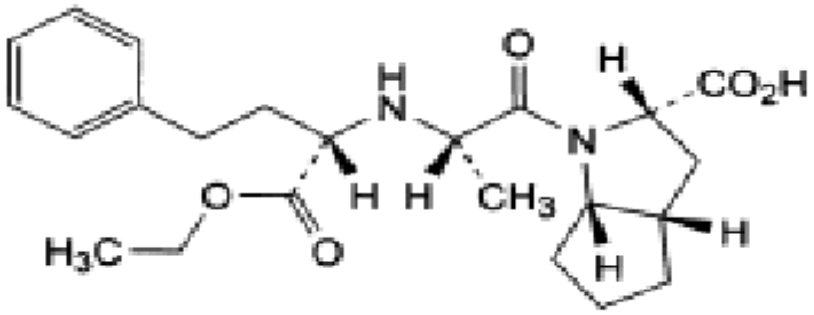
The antihypertensive effects of losartan may be increased by drugs lowering blood pressure. An additive hypokalemic effect may occur with potassium supplements and potassium-sparing diuretics. NSAIDs may increase the risk of renal impairment and may also weaken the hypotensive effect of losartan. The use of losartan with an ACE inhibitor may increase the risk of hyperkalemia, hypotension, and syncope. The use of losartan with the renin inhibitor, aliskiren should be escaped in renal impaired patients.[79]

1.12.4 Ramipril

Ramipril is an ACE inhibitor. It is used in the treatment of hypertension and heart failure. It is used after myocardial infarction in patients with clinical evidence of heart failure to progress survival. It is also used to reduce the risk of cardiovascular events in patients having certain risk factors. After oral dose ramipril is converted to its active form ramiprilat.

1. Physicochemical Parameters[37,78]

Molecular structure:



Nomenclature: (2S,3aS,6aS)-1-[(2S)-2-[[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl] octahydrocyclopenta[b]pyrrole-2-carboxylic acid.

Molecular formula: C23H32N2O5

Molecular weight: 416.5 g

Appearance: White or almost white crystalline powder.

Melting point: 105 °C to 112 °C.

Solubility: Sparingly soluble in water, freely soluble in methanol.

Loss on drying: Not more than 0.2 per cent

Potency: Ramipril contains not less than 98.0 per cent and not more than 102.0 per cent of C23H32N2O5, calculated on the dried basis.[38]

2. Pharmacokinetic Parameters

Ramipril acts as a prodrug of its active metabolite ramiprilat. About 50 to 60 % of ramipril is absorbed oral doses. Ramipril is metabolized in the liver to ramiprilat. Peak plasma concentrations of ramiprilat occur within 2 to 4 hours after an oral dose of ramipril. Ramiprilat is about 56 % bound to plasma proteins. Ramipril is excreted mainly in the urine as ramiprilat, other metabolites, and some unchanged drug. About 40% of an oral dose appears in the faeces. This may represent biliary excretion. The effective half-life for ramiprilat is 13 to 17 hours after multiple doses.[79]

3. Pharmacological Parameters

Ramipril is an Angiotensin Converting Enzyme (ACE) Inhibitor. They inhibit the conversion of the comparatively inactive angiotensin I to the active angiotensin II. Thus it weaken or abolish responses to angiotensin I but not to angiotensin II. So ramipril is highly

selective drug. It increases bradykinin levels and bradykinin stimulates prostaglandin biosynthesis. Blockade of bradykinin receptors lessens the acute blood pressure reduction.

4. Indications

Ramipril is used in the treatment of hypertension. It is also used in the management of heart failure. It is used after myocardial infarction to improve survival in patients with clinical evidence of heart failure. It is also used to reduce the risk of cardiovascular events in patients having certain risk factors. Ramipril is used in the treatment of diabetic and non-diabetic nephropathy.

5. Doses

a. In the treatment of hypertension an initial oral dose of 2.5 mg once daily is given. The usual maintenance dose is 2.5 to 5 mg daily as a single dose.
b. In the management of heart failure, ramipril is given in an initial dose of 1.25 mg once daily. The usual maximum dose is 10 mg daily.
c. After myocardial infarction, treatment with ramipril may be started in hospital 3 to I 0 days after the infarction at a usual initial dose of 2.5 mg twice daily. The usual maintenance dose is 2.5 to 5 mg twice daily.
d. In the treatment of diabetic and non-diabetic nephropathy, an initial oral dose of 1.25 mg once daily may be given. The maintenance dose is 5 mg once daily.
e. For the prophylaxis of cardiovascular events, ramipril is given in an initial dose of 2.5 mg once daily. The usual maintenance dose is 10 mg once daily.

6. Side effects

The most common adverse effects are hypotension, dizziness, fatigue, headache and nausea. Noticeable hypotension may occur at the start of therapy, particularly in patients with heart failure and sodium or volume-depletion. Other cardiovascular effects are tachycardia, palpitations, and chest pain. Worsening of renal function, proteinuria, and nephrotic syndrome may occur. Reversible acute renal failure also may occur. Hyperkalemia and hypernatremia may develop due to decreased aldosterone secretion. Persistent dry cough, angioedema, Skin rashes, photosensitivity and alopecia may occur.

Blood disorders such as neutropenia, agranulocytosis, thrombocytopenia and anemia may develop.

7. Precautions

Ramipril should not be given to patients

a. with aortic stenosis or outflow tract obstruction.
b. with renovascular disease or suspected renovascular disease.
c. with peripheral vascular diseases or generalized atherosclerosis.
d. with renal disease.
e. with a history of idiopathic or hereditary angioedema.
f. receiving treatment with diuretics or dialysis.
g. during pregnancy.

8. Drug interactions

Extreme hypotension may occur when ramipril is used with diuretics, other antihypertensive drugs and alcohol. An additive hypokalemic effect is probable in patients receiving potassium-sparing diuretics, potassium supplements and other drugs which can cause hyperkalemia such as cyclosporine or indomethacin. The adverse effects of ramipril on the kidneys may be increased by NSAIDs.[79]

1.13 THE IMPORTANCE OF THE PRESENT STUDY

Innovation of drug products is currently controlled by a patent system. The patent system protect the innovator of new medicines for a period of time. When the patent of an innovator drug product has expired, it is then open to all pharmaceutical manufacturing companies to produce their own brands. Due to differences in excipients and manufacturing processes, bioavailability and stability of these drugs may differ. Again, to obtain approval for a new drug product, the applicant company must endorse that their generic drug brand is bioequivalent and pharmaceutically equivalent compared to the innovator drug.[10]

Bangladesh now has become one of the cheapest sources of quality medicines in the world. So the generic pharmaceutical market of the world is now open for Bangladesh. She is capable of producing high-quality pharmaceutical products. Our pharmaceutical industry

now uses state-of-the-art manufacturing technology, very sophisticated QC equipment and highly skilled human resource. But for clinical trial and bioequivalence studies, even now we depend on another country like Malaysia, India and most of the companies do not conduct bioequivalence studies.[81]

Hypertension is a very common disorder in Bangladesh. Approximately 20% of adult and 40- 65% of elderly people suffer from hypertension in Bangladesh.[82-83] The number of patients having hypertension increases every year. Uncontrolled and elevated blood pressure causes many heart related diseases. Hypertension is the main reason for stroke. It is a key risk element for coronary artery diseases and myocardial infarction and sudden cardiac death.[4] Calcium-channel blocker (45%) and beta-blockers (40%) were the most commonly prescribed antihypertensive drugs in Bangladesh. Diuretics, ACE inhibitors, and angiotensin-receptor blockers were used in 30.8%, 25% and 24.2% cases, respectively.[84-85]

Bangladesh is a densely populated country. To meet the healthcare needs of this huge population, huge amounts of medicines are required. Again, the first objective of National Drug Policy 2005 was to ensure that common people of Bangladesh should have easy access to effective, safe and good quality drug products at affordable prices.[11] As hypertension is a very common disorder in Bangladesh, many pharmaceutical companies are now producing antihypertensive drugs from each class of antihypertensive drugs.

No data are available in regard to pharmaceutical equivalence and bioequivalence studies of antihypertensive drugs manufactured in Bangladesh. Therefore, the present study is carried out to perform *in vitro* and *in vivo* pharmaceutical equivalence and stability studies in comparison with their respective reference innovator brands of some antihypertensive drugs manufactured in Bangladesh to compare the quality, efficacy and safety of these drug products by taking reference innovator brands as standard brands. This study will also help the physicians to choice a suitable brand which is easily available, have standards of quality, efficacy and safety.

1.14 THE OBJECTIVES OF THE PRESENT STUDY

In vitro and *in vivo* pharmaceutical equivalence and stability studies of some antihypertensive drugs manufactured in Bangladesh were done by comparing these test brands with their respective reference innovator brands. Reference innovator brands are those drug products that contain an active substance or combination of substances that has not authorized before. They are invented first. In this study reference innovator brands were taken as standard brands to compare the quality, efficacy and safety of test brands. The objectives of the present study were given below:

A. To assess *in vitro* pharmaceutical equivalence by comparing the following parameters between test brands and their respective reference innovator brands.
    i. By comparing general quality assessment parameters such as % weight variation, hardness, % friability, disintegration time, dissolution time and the amount of active substance;
    ii. By comparing time required for 50 % dissolution and 90 % dissolution;
    iii. By comparing dissolution profiles using graphs and
    iv. By comparing dissolution profiles using statistical factors such as difference factor and similarity factor.
B. To assess *in vivo* pharmaceutical equivalence by comparing plasma drug concentration time curves of test brands with their respective reference innovator brands in rat models.
C. To assess the stability by comparing stability of test brands with their respective reference innovator brands under stress conditions.

CHAPTER TWO

MATERIALS AND METHODS

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2.1 MATERIALS

2.1.1 Drugs

A. Standard drugs:

Standard atenolol, losartan potassium and ramipril were kind gifts from Healthcare Pharmaceuticals Ltd., Gazipur, Bangladesh. Standard carvedilol was a kind gift from Incepta Pharmaceuticals Ltd., Savar, Bangladesh.

B. Experimental drugs:

Experimental drugs were some antihypertensive drugs manufactured in Bangladesh. These drugs are purchased as their availability in the local market of Dhaka city and they are labeled as follows:

i. Tablet atenolol 50 mg: Three brands of tablet atenolol 50 mg of different manufacturers were randomly designated as AA, AB, AC and reference innovator brand as ARI.

ii. Tablet carvedilol 6.25 mg: Eight brands of tablet Carvedilol 6.25 mg of different manufacturers were randomly designated as CA, CB, CC, CD, CE, CF, CG, CH and reference innovator brand as CRI.

iii. Tablet losartan potassium 50 mg: Ten brands of tablet Losartan potassium 50 mg of different manufacturers were randomly designated as LA, LB, LC, LD, LE, LF, LG, LH, LI, LJ and reference innovator brand as LRI.

iv. Tablet ramipril 5 mg: Four brands of tablet ramipril 5 mg of different manufacturers were randomly designated as RA, RB, RC, RD and reference innovator brand as RRI.

2.1.2 Chemicals and Reagents

Chemicals and reagents were of analytical grade and were purchased from local suppliers. Chemicals and reagent used in the present study were as follows:

i. Hydrochloric acid (37%, reagent grade, Merck, Germany)
ii. Orthophosphoric acid (85%, reagent grade, Merck, Germany)
iii. Sulfuric acid (98%, reagent grade, Merck, Germany)
iv. Acetic acid (99.8%, reagent grade, Merck, Germany)
v. Sodium acetate (Reagent grade, Merck, Germany)
vi. Sodium Hydroxide (Reagent grade, Merck, Germany)
vii. Methanol (Reagent grade, Merck, Germany)
viii. Acetonitrile (Reagent grade, Merck, Germany)
ix. Ammonium dihydrogen phosphate (Reagent grade, Merck, Germany)
x. Potassium dihydrogen phosphate (Reagent grade, Merck, Germany)
xi. Ethyl acetate (Reagent grade, Merck, Germany)
xii. Potassium bromide (Reagent grade, Merck, Germany)
xiii. Distilled water (Center for Advanced Research in Sciences, University of Dhaka)
xiv. Demineralized water (Center for Advanced Research in Sciences, University of Dhaka)

2.1.3 Apparatus

Apparatus used in the present study were as follows:

i. Volumetric flask and conical flask,
ii. Glass- stoppered test tube and normal test tube,
iii. Test tube holder,
iv. Funnel,
v. Graduated beaker and graduated cylinder,
vi. Graduated pipette and micropipette,
vii. Pipette filler,
viii. Wash bottle,
ix. Centrifuge tube,
x. Syringe and tips,
xi. Whatmanfilter paper No.1,
xii. Disc filter

xiii. Desiccators,

2.1.4 Equipment

Equipment used in the present study were as follows:

i. UV-Visible spectrophotometer (Model UV-800 Shimadzu, Japan)
ii. Hardness tester (Model HDT-300F, Logan Instrument Corp., USA)
iii. Friability tester (Model FIB-2S, Logan Instrument Corp., USA)
iv. Disintegration tester (Model DST-3, Logan Instrument Corp., USA)
v. Dissolution tester (Model UDT-804, Logan Instrument Corp., USA)
vi. Fourier-Transform Infrared Spectrometer (Model 8400- S, Shimadzu, Japan)
vii. High Performance Liquid Chromatography (UFLC Shimadzu, Japan,)
viii. Sonicator (Ultrasons Medi- II, P. Selecta, Spain)
ix. Analytical balance (Model AS-220.R2 , Radwag, Poland)
x. pH meter(P Selecta, Spain)
xi. Thermostatic water bath (Unitronic OR, P Selecta, Spain)
i. Centrifuge machine (Z36 HK, Hermle, Germany)
ii. Dryer
iii. Freeze

2.1.5 Animals

Ninety (90) rats weighing about 150 ± 25 g were used in this study as the experimental animals for the *in vivo* experiment. The rats were collected from Jahangirnagar University, Savar, Bangladesh.

2.1.6 Preparation of Stock Solutions [37-38]

i. Preparation of standard stock solution for dissolution testing of tablet atenolol 50 mg

100 mL stock solution of 50 µg/mL was prepared by dissolving 0.05 g of atenolol in 0.1N acetate buffer, pH4.6 and made the volume up to 100 mL with the same solvent. 10 mL of this solution was diluted with 0.1N acetate buffer, pH4.6 and finally made the volume up to 100 mL with the same solvent. The stock solution was then diluted to the desired strength by 0.1N acetate buffer, pH 4.6.

ii. **Preparation of standard stock solution for dissolution testing of tablet carvedilol 6.25 mg**

100 mL stock solution of 50 µg/mL was prepared by dissolving 0.05 g of carvedilol in 10 mL methanol and made up to 100 mL with hydrochloric acid adjusted to a pH of 1.45 ± 0.2. 10 mL of this solution was diluted with hydrochloric acid adjusted to a pH of 1.45 ± 0.2 and finally made the volume up to 100 mL with the same solvent. The standard stock solution was prepared on the day of analysis.

iii. Preparation of standard stock solution for dissolution testing of tablet losartan potassium 50 mg

100 mL stock solution of 50 µg/mL was prepared by dissolving 0.050 g of losartan potassium in distilled water and made up to 100 mL with the same solvent. Taken 10 mL from this, diluted with distilled water and finally made the volume up to 100 mL with the same solvent. The stock solution was diluted to the desired strength by distilled water.

iv. **Preparation of standard stock solution for dissolution testing of tablet ramipril 5 mg**

100 mL stock solution of 50 µg/mL was prepared by dissolving 0.050 g of ramipril in 0.1N hydrochloric acid and made up to 100 mL with the same solvent. 10 mL of this solution was diluted with 0.1N hydrochloric acid and finally made the volume up to 100 mL with the same solvent. The standard stock solution was prepared on the day of analysis.

v. Preparation of standard stock solution for assay of tablet atenolol 50 mg

100 mL stock solution of 20 µg/mL was prepared by dissolving 0.050 g of atenolol in methanol and made the volume up to 50 mL with the same solvent. 2 mL of this solution was diluted with the mobile phase and finally made the volume up to 100 mL with the same solvent. The stock solution was then diluted to the desired strength by the mobile phase.

vi. **Preparation of standard stock solution for assay of tablet carvedilol 6.25 mg**

100 mL stock solution of 10 µg/mL was prepared by dissolving 0.025 g of carvedilol in methanol and made the volume up to 50 mL with the same solvent. 2 mL of this solution was diluted with the mobile phase and finally made the volume up to 100 mL with the same solvent. The stock solution was then diluted to the desired strength by the mobile phase.

vii. **Preparation of standard stock solution for assay of losartan potassium 50 mg**

100 mL stock solution of 20 µg/mL was prepared by dissolving 0.050 g of losartan potassium in demineralized water and made the volume up to 50 mL with the same solvent. 2 mL of this solution was diluted with the mobile phase and finally made the volume up to 100 mL with the same solvent. The stock solution was then diluted to the desired strength by the mobile phase.

viii. **Preparation of standard stock solution for assay of ramipril 5 mg**

100 mL stock solution of 10 µg/mL was prepared by dissolving 0.025 g of ramipril in mobile phase and made the volume up to 50 mL with the same solvent. 2 mL of this solution was diluted with the mobile phase and finally made the volume up to 100 mL with the same solvent. The stock solution was then diluted to the desired strength by the mobile phase.

ix. **Preparation of sample solution for assay of tablet atenolol 50 mg**

Powder equivalent to 50 mg of atenolol was dissolved in methanol. First dilution was done methanol and filtered through Whatman filter paper No. 1. Then second dilution was done with the mobile phase to get a concentration of 20 µg/mL and further filtered through 0.45 nm membrane filter.

x. **Preparation of sample solution for assay of tablet carvedilol 6.25 mg**

Powder equivalent to 25 mg of carvedilol was dissolved in methanol. First dilution was done methanol and filtered through Whatman filter paper No. 1. Then second dilution was done with the mobile phase to get a concentration of 10 µg/mL and further filtered through 0.45 nm membrane filter.

xi. Preparation of sample solution for assay of losartan potassium 50 mg

Powder equivalent to 50 mg of losartan potassium was dissolved in water. First dilution was done with water and filtered through Whatman filter paper No. 1. Then second dilution was done with the same solvent to get a concentration of 20 µg/mL and further filtered through 0.45 nm membrane filter.

xii. Preparation of sample solution for assay of ramipril 5 mg

Powder equivalent to 25 mg of ramipril was dissolved in mobile phase and first dilution was done with mobile phase and filtered through Whatman filter paper No. 1. Then second dilution was done with same solvent to get a concentration of 10 µg/mL and further filtered through 0.45 nm membrane filter.

xiii. **Preparation of sample solution for stress degradation study**

20 tablets of losartan potassium was powdered. Powder equivalent to 50 mg of losartan potassium was dissolved in water and first dilution was done with water. The solution was filtered through Whatman filter paper No. 1. Then second dilution was done with same solvent to get a concentration of 0.5 mg/mL.

20 tablets of atenolol, 20 tablets of carvedilol and 20 tablets of ramipril were powdered separately. Powder equivalent to 50 mg of atenolol, 25 mg of carvedilol and 25 mg of ramipril were dissolved in methanol separately and first dilutions were done with methanol separately. The solutions were filtered through Whatman filter paper No. 1. Then second dilutions were done with the same solvent to get a concentration of 0.5 mg/mL separately.

2.1.7 Preparation of Dissolution Media [37-38]

i. 0.1N acetate buffer, pH 4.6 for tablet atenolol 50 mg

1000 mL of 0.1N acetate buffer, pH4.6 was prepared by mixing 449 mL of 0.1N sodium acetate with 551 mL of 0.1N acetic acid solution and adjusted with diluted acetic acid to a pH of 4.6.

ii. Hydrochloric acid adjusted to pH of 1.45 ± 0.2 for tablet carvedilol 6.25 mg

1000 mL of 0.1N HCl was prepared by taking 300 mL of distilled water in n a volumetric flask and 8.5 mL of concentrated hydrochloric acid was added to it. The

volume was made up to 1000 mL with distilled water. pH of this solution was adjusted to 1.45 ± 0.2 with diluted sodium hydroxide solution.

iii. **Distilled water for tablet losartan potassium 50mg**

iv. 0.1N HCl for tablet ramipril 5mg

1000 mL of 0.1N HCl was prepared by taking 300 mL of distilled water in a volumetric flask and 8.5 mL of concentrated hydrochloric acid was added to it. The volume was made up to 1000 mL with distilled water.

2.1.8 Preparation of Buffer Solutions [37-38]

i. Preparation of 0.1N acetate buffer,pH4.6

1000 mL of 0.1N acetate buffer, pH4.6 was prepared by mixing 449 mL of 0.1N sodium acetate with 551 mL of 0.1N acetic acid solution and adjusted with diluted acetic acid to a pH of 4.6. 1000 mL of 0.1N sodium acetate was prepared by dissolving 13.608 g of sodium acetate in distilled water and volume was made up to 1000 mL with the same solvent. 1000 mL of 0.1N acetic acid was prepared by mixing 5.8 mL of concentrated acetic acid with distilled water and volume was made up to 1000 mL with the same solvent.

ii. Preparation of ammonium dihydrogen phosphate buffer pH 3.0

1000 mL of 50 mM ammonium dihydrogen phosphate buffer pH 3.0 was prepared by dissolving 5.7515 g of ammonium dihydrogen phosphate in demineralized water and volume was made up to 1000 ml with same the solvent. pH was adjusted with diluted orthophosphoric acid.

iii. Preparation of potassium dihydrogen phosphate buffer pH 2.5

1000 mL of 50 mM potassium dihydrogen phosphate buffer pH 2.5 was prepared by dissolving 6.8045 g of potassium dihydrogen phosphate in demineralized water and volume was made up to 1000 ml with same the solvent. pH was adjusted with diluted orthophosphoric acid.

iv. Preparation of potassium dihydrogen phosphate buffer pH 3.0

1000 mL of 50 mM potassium dihydrogen phosphate buffer pH 3.0 was prepared by dissolving 6.8045 g of potassium dihydrogen phosphate in demineralized water and

volume was made up to 1000 ml with same the solvent. pH was adjusted with diluted orthophosphoric acid.

2.2 METHODS FOR COMPARISON OF GENERAL QUALITY ASSESSMENT PARAMETERS

2.2.1 Method for Comparison of Weight Variation

The weight variation test would be a satisfactory method for determining the drug content uniformity of tablets if the uniformity of drug distribution in the granulation or powder from which the tablets were made were perfect. According to USP the weight variation test is done by weighing twenty tablets individually, calculating the average weight and comparing the individual tablet weights to the average. The tablets meet the USP test if no more than 2 tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage limit. The weight variation tolerances for uncoated tablets having average weight 130 mg or less and 130 mg- 324 mg are 10% and 7.5%, respectively.

In this study, the weight variation tests were done for the tablets of test brands and their respective reference innovator brands. The weight variations of tablets of test brands were compared with that of their respective reference innovator brands.[33,38]

2.2.2 Method for Comparison of Hardness

Tablets should be sufficiently hard enough so that they can resist breaking during normal handling and yet soft enough so that they can disintegrate properly after swallowing. Tablet hardness could influence other quality parameters such as friability and disintegration. Tablet hardness test is referred to as non-compendia test. A force of about 4 kg is considered the minimum hardness requirement for a standard tablet.

In this study, the multifunctional hardness testers were used to determine the hardness of tablets of test brands and their respective reference innovator brands. Six tablets were randomly selected from each brand. The degree of forces required to break the tablets were measured in kilogram. The hardness of tablets of test brands was compared with that of their respective reference innovator brands.[17,33]

2.2.3 Method for Comparison of % Friability

A friabilator is used to determine a tablet's durability. The friabilator determines the tablet's friability by allowing it to roll and fall within a drum. The tablets are weighed before and after a specific number of rotations. Then any weight loss is determined. A tablet's resistance to loss of weight indicates its ability to withstand abrasion in handling, packaging and shipment. A maximum weight loss of not more than 1 % is generally considered acceptable for a standard tablet.

In this study, a friabilator is used to determine the % friability of tablets of test brands and their respective reference innovator brands. Ten tablets of each brand were weighed and subjected to abrasion in the friabilator. The % friability of tablets was determined from weight loss. The % friability of tablets of test brands was compared with that of their respective reference innovator brands.[17,33,38]

2.2.4 Method for Comparison of Disintegration Time

To be readily available to the body a tablet must be in solution. The first important step toward solution for most tablets is breakdown of the tablet into smaller particles or disintegration. A tablet disintegration tester is used to measure the time that it takes a tablet to disintegrate. A tablet disintegration tester consists of a basket and rack assembly containing six open ended transparent tubes which are held vertically upon a10-mesh stainless steel wire screen. Tablets are placed in each of the six tubes of the basket during testing. The basket is raised and lowered in the immersion fluid through the use of a mechanical device. Tablets must disintegrate within the times specified in the individual monograph.

In this study, a tablet disintegration tester is used to determine the disintegration time of tablets of test brands and their respective reference innovator brands. Six tablets of each brand were used for the test in water of an automatic disintegration tester at 37°C employing plastic discs. The disintegration time of tablets were recorded when no particles remained on the basket. The disintegration time of tablets of test brands were compared with that of their respective reference innovator brands. [17,33]

2.2.5 Method for Comparison of Dissolution

The rate of dissolution of may be directly related to the efficacy of the tablet product as well as to the bioavailability differences between formulations. The USP includes seven apparatus designs for drug release and dissolution testing of different dosage forms. USP Apparatus 1 and USP Apparatus 2 are mainly for evaluation of dissolution of tablet dosage forms. In the present study, USP Apparatus 2 is used. The apparatus consists of (*a*) a variable speed stirrer motor; (b) a stainless steel paddle on a stirrer shaft; (c) a 1000 mL vessel of glass material fitted with a cover having ports; (d) a water bath to maintain temperature of the dissolution medium. The test tolerance is expressed as a percentage of the labeled amount of drug dissolved in the time limit stated in the monograph.

In this study, a volume of the dissolution medium as stated in the individual monograph was placed in the vessel and allowed to reach to 37°C ± 0.5°C. Then the stirrer was rotated at the specified speed and at stated intervals, test brand samples and their respective reference innovator brand samples were withdrawn for chemical analysis of the percentage of drug dissolved. The percentage of drug dissolved of tablets of test brands were compared with that of their respective reference innovator brands.[17,33]

2.2.7 Method for Comparison of Assay Content Using High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) is a technique for the separation of components of mixtures by differential migration through a column containing a micro-particulate solid stationary phase. Solutes are detected in the mobile phase as they are eluted from the end of the column. The detector generates an electrical signal that can be amplified and presented in the form of a chromatogram of solute concentration as a function of time.

In this study, the chromatographic separation was reversed- phase separation (stationary phase less polar than mobile phase), eluting power decreases with increasing solvent polarity. Elution was done under isocratic condition (constant mobile phase composition). The mobile phase is a blend of methanol or acetonitrile with water or an aqueous buffer. Column wasC18 (250 x 4.6 mm) using octadecyl silica (C_{18} or ODS) as the stationary

phase. UV- visible absorbance detector was used which is based on the absorbance of UV or visible radiation in the range 190-800 nm by solute species containing chromophoric groups or structures. Quantification of solute was done from peak area measurements and calibration graphs using external standards. Integrated peak areas are directly proportional to the quantity of a solute injected when working in the linear range of the detector. Calibration is done with the external standards chromatographed separately from the samples.

Twenty tablets were weighed and pulverized by gentle grinding. Powder equivalent to the amount of solute to prepare standard solutions were used to make sample solutions. Percentage potencies of tablets of test brands and their respective reference innovator brands were made from their peak areas measurement and calibration graphs. The percentage potencies of tablets of test brands were compared with that of their respective reference innovator brands.[39,40-43]

A. Chromatographic conditions for assay of tablet atenolol 50 mg

Column was C18 (250 x 4.6 mm). The mobile phage was a mixture of water and methanol in the ratio of 70:30 v/v. Flow rate of 1.0 mL/min was maintained. The detection was carried out at the wavelength of 225 nm.

B. Chromatographic conditions for assay of tablet carvedilol 6.25 mg

Column was C18 (250 x 4.6 mm). The mobile phage was a mixture of 50 mM potassium dihydrogen phosphate buffer pH 2.5 and acetonitrile in the ratio of 80:20 v/v. Flow rate of 1.0 mL/min was maintained. The detection was carried out at the wavelength of 250 nm.

C. Chromatographic conditions for assay of tablet losartan potassium 50 mg

Column was C18 (250 x 4.6 mm). The mobile phage was a mixture of 50 mM ammonium dihydrogen phosphate buffer pH 3.0 and acetonitrile in the ratio of 65:35 v/v. Flow rate of 1.0 mL/min was maintained. The detection was carried out at the wavelength of 254 nm

D. Chromatographic conditions for assay of tablet ramipril 5 mg

Column was C18 (250 x 4.6 mm). The mobile phage was a mixture of 50 mM potassiumdihydrogen phosphate buffer pH 2.5 and acetonitrile in the ratio of 60:40 v/v.

Flow rate of 0.8 mL/min was maintained. The detection was carried out at the wavelength of 225 nm.

2.3 METHODS FOR COMPARISON OF DISSOLUTION PROFILE

2.3.1 UV-Visible Spectrophotometric Method for Comparison of Dissolution Profile Using Graphs

Every chemical substance absorbs, transmits or reflects light (electromagnetic radiation) over a certain range of wavelength. Spectrophotometry is a method to measure the intensity of light absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known substance (concentrations) can be determined by measuring the intensity of light detected. UV- visible spectrophotometer uses light over the ultraviolet range (185- 400 nm) and visible range (400- 700nm) of electromagnetic radiation spectrum.

In this study, twelve tablets were taken for each of tablets of test brands and their respective reference innovator brands. The dissolution measurements were done at different time points. The percentage of drug dissolved of tablets of test brands and their respective reference innovator brands at different time points were calculated from the absorbances of dissolution solutions and calibration graphs. The mean percentage of drug dissolved for twelve tablets of test brands and their respective reference innovator brands were calculated at different time points. The mean percentage of drug dissolved of tablets of test brands were compared with that of their respective reference innovator brands by plotting the mean percentage of drug dissolved against time.[39,44-46]

2.3.2 Comparison of Time Required for 50% Dissolution and 90% Dissolution

The values for $T_{50\%}$ and $T_{90\%}$ were determined as they are used as good guides for effective dissolution. The value for $T_{50\%}$ is the length of time required to 50% of the drug to go into solution. The value for $T_{90\%}$ is the length of time required to 90% of the drug to go into solution. The values for $T_{50\%}$ and $T_{90\%}$ of tablets of test brands were determined from dissolution profiles using UV- visible spectrophotometric method and compared with that of their respective reference innovator brands.[33]

2.3.3 Comparison of Dissolution Profile Using Difference Factor and Similarity Factor

A model independent mathematical approach was used to compare the dissolution profiles of tablets of test brands with their respective reference innovator brands. Two factors, difference factor (f_1) and similarity factor (f_2) were used to compare the curves of the dissolution profiles of test brands and reference innovator brands.

The difference factor (f_1) calculates the percent (%) difference between the two curves of the dissolution profiles at each time point and is a measurement of the relative error between the two curves. The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves of the dissolution profiles at each time point.

The following equations were used to calculate difference factor (f_1) and similarity factor (f_2):

$$f_1 = \{[\Sigma \mid R_t - T_t \mid] / \Sigma R_t\} \times 100$$

$$f_2 = 50 \times \log \{[1 / (1 + (\Sigma (R_t - T_t)^2) / N)]^{1/2} \times 100\}$$

Where N is the number of time points, R_t is the dissolution value of reference product at time 't' and T_t is the dissolution value for the test product at time 't'.

The mean percentage of drug dissolved for twelve tablets of test brands and their respective reference innovator brands were calculated at different time points. The mean percentage of drug dissolved of tablets of test brands and their respective reference innovator brands were used to calculate difference factor and similarity factor using the respective equations.[12, 47-51]

2.4 *IN VIVO* PHARMACEUTICAL EQUIVALENCE STUDY BY COMPARING PLASMA DRUG CONCENTRATION – TIME CURVES IN RAT MODELS

A. Experimental animals

The experiment was performed to compare plasma drug concentration- time curves of tablets of test brands with their respective reference innovator brands in rat models. The

UV- Visible Spectrophotometric method was used for determination of plasma- drug concentration after oral single administration of test and respective reference innovator brands of tablet atenolol, tablet carvedilol, tablet Losartan potassium and tablet ramipril in rats.

The animals used for in vivo experiments were adult healthy rats (150± 25 g). The animals were acclimatized for one week prior to the experiment. They were given normal diet. The animals were divided into 4 groups. Each group was again sub divided according to the test brands and respective reference innovator brands each having 3 (three) rats.

B. Oral administration of studied drug products in rats

Powdered tablets were dissolved in water for oral administration. Powder equivalent to the doses calculated based on the body weight of rats were administered orally. Atenolol (both test and reference) as1.25 mg/kg body weight, carvedilol (both test and reference) as 0.156 mg/kg body weight, losartan potassium (both test and reference) as1.25 mg/kg body weight and ramipril (both test and reference) 0.125 mg/kg body weight were administered separately to a rat of specific group by oral route.

C. Procedure for *in-vivo* experiment

Blood samples (0.5 mL) were withdrawn from cutting the tip of the tail into centrifuge tubes at 0, 1, 2, 2.5, 3, 4, 5 hours administration. The blood samples were centrifuged at 3000 rpm for 10 minutes. The plasma samples were separated into vials and kept in deep freeze until assayed. Samples are vortexed for 1 minute after adding 5 ml of hexane for tablet losartan, 5 mL of methanol for tablet atenolol, 5 mL of methanol for tablet carvedilol and 5 mL of ethyl acetate for tablet ramipril as extraction solvents. Then samples were centrifuged for 5 minutes at 3000 rpm. The supernatant samples were collected. In case of tablet ramipril supernatant was evaporated. Dry residue was dissolved with methanol. Absorbances were measured at 218 nm for tablet atenolol, at 285 nm for tablet carvedilol, at 201 nm for tablet losartan potassium and at 201 nm for tablet ramipril, respectively.

D. Preparation calibration curves

Stock solutions of 10 µg /mL were prepared for each drug separately. The stock solutions were used to prepare solutions of 0.5, 0.75, 1.0, 1.25, 1.5µg /mL, respectively for each

drug. Absorbances were taken of these solutions for each drug individually using a UV-Visible Spectrophotometer. Absorbances were plotted against concentrations for each drug individually to produce calibration curves.[52-59]

2.5 METHOD FOR STABILITY STUDIES UNDER STRESS CONDITIONS

Stress degradations were carried out by acidic and basic hydrolysis at different temperatures. Stress degradation patterns of text brands were compared with their respective reference innovator brands

A. Stress degradation procedure for acidic hydrolysis

10 mL of sample solutions were mixed with 10 mL of 0.1N HCL and 0.5N HCL solutions separately. These solutions were kept in a water bath at 60^{o}C and 70^{o}C for 1 hour separately and then were neutralized with equimolar strength and volume of sodium hydroxide before further dilution to get final concentration 5µg/mL for losartan potassium and 10µg/mL for atenolol, carvedilol and ramipril. Absorbances were taken using UV spectrophotometer.

B. Stress degradation procedure for basic hydrolysis

10 mL of sample solutions were mixed with 10 mL of 0.1N NaOH and 0.5N NaOH solutions separately. These solutions were kept in a water bath at 60^{o}C and 70^{o}C for 1 hour separately and were neutralized with equimolar strength and volume of hydrochloric acid before further dilutionto get final concentration 5µg/mL for losartan potassium and 10µg/mL for atenolol, carvedilol and ramipril. Absorbances were taken using UV spectrophotometer.[60-68]

CHAPTER THREE

RESULTS AND DISCUSSION

CHAPTER THREE

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3.1 COMPARISON OF GENERAL QUALITY ASSESSMENT PARAMETERS

For determination of pharmaceutical equivalence of some antihypertensive drugs manufactured in Bangladesh; general quality assessments parameters of tests brands were compared with their respective reference innovator brands. The performed quality assessment tests were weight variation, hardness, % friability, disintegration time, dissolution and assay content.

3.1.1 Comparison of Weight Variation

The weight variation tests were done for the tablets of antihypertensive testing brands and compared with that of their respective reference innovator brands. The tests were done according to USP weight variation test method. The results of % weight variation tests of all test and reference innovator brands were expressed as mean ± SD.

Test brands of atenolol showed average weight between 210 and 290 mg. The % weight variations were between - 2.39% and + 2.77%. Reference innovator brand showed average weight 220 mg and % weight variation between - 1.84% and + 1.40% (Table 3.1).

According to USP the weight variation tolerance for uncoated tablets having average weight 130 mg to 324 mg is 7.5%. The tablets meet the USP test if no more than 2 tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage limit.[33,38]

The weight variation tests showed no significant variation in results and all test brands of tablet atenolol including their reference innovator brand met the USP weight variation standard limit.

Table 3.1: Comparison of % weight variation of test brands of tablet atenolol 50 mg with their reference innovator brand.

Brand	Average weight	Range of % weight variation
AA	209.25 ± 2.92	-2.39 to 1.77
AB	216.98 ± 2.76	-1.65 to 2.77
AC	289.50 ± 2.23	-0.97 to1.63
ARI	219.34 ± 2.09	-1.84 to1.40

Average weight values are given as mean ± SD; n = 20.

Test brands of carvedilol showed average weight between 85 mg and 186 mg. The % weight variations were found between - 2.72% and + 5.87%. Reference innovator brand showed average weight 155 mg and % weight variation between - 1.08% and + 0.92% (Table 3.2).

The weight variation tests of tablet carvedilol showed no significant variation in results and all test brands including their reference innovator brand met the USP weight variation standard limit. The weight variation limits for tablets having average weight 130 mg or less and 130 mg- 324 mg are 10% and 7.5%, respectively. [33,38]

Table 3.2: Comparison of weight variation of test brands of tablet carvedilol 6.25 mg with their reference innovator brand.

Brand	Average weight	Range of % weight variation
CA	180.62 ± 1.48	-1.89 to 1.21
CB	185.79 ± 1.70	-2.63 to1.25
CC	137.12 ± 3.85	-2.72 to 5.87
CD	143.34 ± 1.38	-1.63 to1.79
CE	89.63 ± 0.91	-2.49 to1.86
CF	165.60 ± 1.55	-1.75 to1.75
CG	101.47 ± 0.84	-1.25 to1.70
CH	84.72 ± 0.97	-1.96 to1.81
CRI	155.27 ± 1.05	-1.08 to 0.92

Average weight values are given as mean ± SD; n = 20.

Test brands of losartan potassium showed average weight between 143 mg and 270 mg. The % weight variations were observed between - 4.66% and + 4.08%. Reference innovator brand showed average weight 154 mg and % weight variation between - 2.60% and + 2.14% (Table 3.3).

All test brands of tablet losartan potassium including their reference innovator brand met the USP weight variation standard limit. The weight variation limit for tablets having average weight 130 mg - 324 mg is 7.5%. [33,38]

Table 3.3: Comparison of weight variation of test brands of tablet losartan potassium 50 mg with their reference innovator brand.

Brand	Average weight	Range of % weight variation
LA	142.85 ± 1.80	-1.65 to 2.49
LB	194.17 ± 1.20	-1.42 to1.05
LC	193.41 ± 4.28	- 4.66 to 4.08
LD	194.26 ± 3.36	- 4.61 to 2.29
LE	155.95 ± 2.05	-2.08 to 2.73
LF	201.27 ± 2.83	-2.17 to 2.45
LG	265.48 ±2.38	-1.39 to1.36
LH	269.29 ± 3.03	-1.67 to 2.35
LI	199.59 ± 2.57	-1.90 to 0.92
LJ	169.76 ± 3.58	-2.45 to 3.97
LRI	153.81 ± 2.18	-2.60 to 2.14

Average weight values are given as mean ± SD; n = 20.

Test brands of ramipril had average weight between 95 mg and 220 mg. The % weight variations were found between - 2.00% and + 2.85%. Reference innovator brand showed average weight 102 mg and % weight variation between - 1.85% and + 1.49% (Table 3.4). No significant variations were found in weight variation test results and all test brands with reference innovator brand met weight variation limits.

Table 3.4: Comparison of weight variation of test brands of tablet ramipril 5 mg with their reference innovator brand

Brand	Average weight	Range o% weight variation
RA	221.17 ± 2.13	-1.48 to 1.82
RB	186.53 ± 1.86	-2.00 to 1.54
RC	95.39 ± 1.33	-1.87 to 2.85
RD	175.40 ± 1.35	-1.43 to1.37
RRI	101.64 ± 1.52	-1.85 to 1.49

Average weight values are given as mean ± SD; n = 20.

3.1.2 Comparison of Hardness

Tablet hardness may affect other quality parameters like friability and disintegration of a tablet. Hardness testing a non-compendia test. Usually a force of about 4 kg is minimum hardness requirement for a tablet dosage form.[17,33]

The results of tablet hardness of antihypertensive testing and reference innovator brands were expressed as mean ± SD and analyzed by Student's t- test. Difference between the means of test brands and innovator brands were considered statistically significant at $p < 0.05$.

Test brands of atenolol showed no significant variation in tablet hardness in comparison with their reference innovator brand. Innovator brand ARI and test brands AA, AC had hardness values >5 kg; AB had value <5 kg (Table 3.5). Test brands of tablet atenolol and their reference innovator brand are considered optimal for hardness test with variations within limit.

Table 3.5: Comparison of hardness of test brands of tablet atenolol 50 mg with their reference innovator brand.

Serial No.	Brand	Hardness (kg)
1	AA	6.13 ± 0.37*
2	AB	4.55 ± 0.15**
3	AC	5.06 ± 0.39*
4	ARI	5.32 ± 0.50

*p <0.05; **p <0.01; compared with the reference innovator brand, ARI; values are given as mean ± SD; n = 6.

Test brands of tablet carvedilol showed variation in tablet hardness in comparison with their reference innovator brand. Innovator brand CRI and test brands CB, CD, CF, CG had hardness values >5 kg; CA, CC, CE, CH had value <5 kg (Table 3.6). Test brands of tablet carvedilol and their reference innovator brand are found optimal for hardness with variations within limit.

Table 3.6: Comparison of hardness of test brands of tablet carvedilol 6.25 mg with their reference innovator brand.

Serial No.	Brand	Hardness (kg)
1	CA	$4.01 \pm 0.39^{***}$
2	CB	$7.68 \pm 1.00^{**}$
3	CC	$3.88 \pm 0.14^{**}$
4	CD	$5.82 \pm 0.46^{*}$
5	CE	$4.45 \pm 0.38^{***}$
6	CF	$6.02 \pm 0.13^{*}$
7	CG	$5.44 \pm 0.21^{***}$
8	CH	$4.18 \pm 0.16^{***}$
9	CRI	6.26 ± 0.18

*p <0.05; **p <0.01; ***p <0.001; compared with the reference innovator brand, CRI; values are given as mean ± SD; n = 6.

Test brands of tablet losartan potassium showed variation in tablet hardness in comparison with their reference innovator brand. Innovator brand LRI and test brands LB, LC had hardness values >6 kg; LF, LH had hardness values >7 kg; LA, LE, LG, LJ had value >8kg and LD, LI had values >9 kg (Table 3.7). Test brands of tablet losartan potassium and their reference innovator brand having hardness variations within limit are considered optimal for hardness.

Table 3.7: Comparison of hardness of test brands of tablet losartan potassium50 mg with reference their innovator brand.

Serial No.	Brand	Hardness (kg)
1	LA	$8.44 \pm 1.03^{**}$
2	LB	$6.28 \pm 0.28^{**}$
3	LC	$6.88 \pm 0.53^{*}$
4	LD	$9.4\ 8\pm 0.3^{***}$
5	LE	$8.96 \pm 0.23^{***}$
6	LF	$7.91 \pm 0.17^{***}$
7	LG	$8.64 \pm 0.11^{***}$
8	LH	$7.97 \pm 0.19^{***}$
9	LI	$9.9\ 6\pm 0.14^{***}$
10	LJ	$8.07 \pm 0.14^{***}$
11	LRI	6.89 ± 0.35

*p <0.05; **p <0.01; ***p <0.001; compared with the reference innovator brand, LRI; values are given as mean ± SD; n = 6.

Test brands of tablet ramipril showed variation in tablet hardness in comparison with their reference innovator brand. Innovator brand RRI and test brand RC showed hardness values >7 kg; RA, RB and RD had hardness values >10 kg (Table 3.8). Test brands of tablet ramipril and their reference innovator brand are considered optimal for hardness test.

Table 3.8: Comparison of hardness of test brands of tablet ramipril 5 mg with their reference innovator brand.

Serial No.	Brand	Hardness (kg)
1	RA	10.67 ± 0.63**
2	RB	13.19 ± 1.18**
3	RC	7.51 ± 0.29*
4	RD	12.19 ± 0.55**
5	RRI	7.16 ± 0.72

*p <0.05; **p <0.001; compared with the reference innovator brand, RRI; values are given as mean ± SD; n = 6.

3.1.3 Comparison of % friability

Friability is included in the United States Pharmacopoeia as a quality assessment test. According to USP the standard specification for % friability is not more than 1%. Tablets of antihypertensive test brands including their respective reference innovator brands met the acceptance criteria for % friability test (Tables 3.9- 3.12).[17,33,38]

Table 3.9: Comparison of % friability of test brands of tablet atenolol 50 mg with their reference innovator brand.

Serial No.	Brand	% friability
1	AA	0.097
2	AB	0.044
3	AC	0.038
4	ARI	0.052

Table 3.10: Comparison of % friability of test brands of tablet carvedilol 6.25 mg with their reference innovator brand.

Serial No.	Brand	% friability
1	CA	0.025
2	CB	0.062
3	CC	0.059
4	CD	0.024
5	CE	0.045
6	CF	0.033
7	CG	0.037
8	CH	0.066
9	CRI	0.028

Table 3.11: Comparison of % friability of test brands of tablet losartan potassium 50 mg with their reference innovator brand.

Serial No.	Brand	% friability
1	LA	0.042
2	LB	0.031
3	LC	0.093
4	LD	0.061
5	LE	0.032
6	LF	0.064
7	LG	0.030
8	LH	0.052
9	LI	0.040
10	LJ	0.044
11	LRI	0.059

Table 3.12: Comparison of % friability of test brands of tablet ramipril 5 mg with their reference innovator brand.

Serial No.	Brand	% friability
1	RA	0.042
2	RB	0.039
3	RC	0.073
4	RD	0.061
5	RRI	0.059

3.1.4 Comparison of disintegration time

A tablet should disintegrate properly to release drug substance from it. Disintegration could affect dissolution of a tablet and thus drug absorption. The British Pharmacopoeia specifies that uncoated tablets should disintegrate within 15 minutes and film coated tablets in 30 minutes.[17,33]

The results of disintegration time of tablet of all testing and reference innovator brands were expressed as mean ± SD and analyzed by Student's t- test. Difference between the means of test brands and reference innovator brands were considered statistically significant at $p < 0.05$.

No major variations were found in disintegration time of antihypertensive test brands of atenolol. They were found to disintegrate between 0.43 and 1.36 minutes, whereas reference innovator brand disintegrated in 1.44 minutes (Table 3.13). All test brands of tablet atenolol including their respective reference innovator brand met the acceptance criteria for disintegration time.

Table 3.13: Comparison of disintegration time of test brands of tablet atenolol 50 mg with their reference innovator brand.

Serial No.	Brand	Disintegration time (min)
1	AA	1.23 ± 0.02**
2	AB	0.43 ± 0.01**
3	AC	1.36 ± 0.01*
4	ARI	1.44 ± 0.01

*p < 0.05; **p < 0.001; compared with the reference innovator brand, ARI; values are given as mean ± SD; n = 6.

No momentous variations were found in disintegration time of test brands of carvedilol. They disintegrated between 0.39 and 5.33 minutes, whereas innovator brand disintegrated in 0.78 minutes (Table 3.14). Test brand with higher disintegration time was CH which was about 5 minutes. All test brands of tablet carvedilol including their respective reference innovator brand met the acceptance criteria for disintegration time.

Table 3.14: Comparison of disintegration time of test brands of tablet carvedilol 6.25 mg with their reference innovator brand.

Serial No.	Brand	Disintegration time (min)
1	CA	$0.39 \pm 0.03^{***}$
2	CB	$0.45 \pm 0.03^{***}$
3	CC	$0.40 \pm 0.02^{*}$
4	CD	$1.25 \pm 0.06^{*}$
5	CE	$2.12 \pm 0.06^{***}$
6	CF	$0.64 \pm 0.10^{*}$
7	CG	$2.45 \pm 0.03^{*}$
8	CH	$5.33 \pm 0.16^{***}$
9	CRI	0.78 ± 0.08

*p <0.05; **p <0.01; ***p <0.001; compared with the reference innovator brand, CRI; values are given as mean ± SD; n = 6.

Significant variations were found in disintegration time of test brands of losartan potassium. They were found to disintegrate between 6.52 to 15.22 minutes, whereas reference innovator brand disintegrated in 7.19 minutes (Table 15). Test brands with higher disintegration times were LA, LC, LF, LH and LI, having disintegration time values >10 minutes. In spite of variations in disintegration time, all test brands of tablet losartan potassium including their respective reference innovator brand met the acceptance limit for disintegration time.

Table 3.15: Comparison of disintegration time of test brands of tablet losartan potassium 50 mg with their reference innovator brand.

Serial No.	Brand	Disintegration time (min)
1	LA	$10.29 \pm 0.35^{**}$
2	LB	$6.88 \pm 0.3^{*}$
3	LC	$15.22 \pm 0.39^{**}$
4	LD	$8.15 \pm 0.34^{**}$
5	LE	$8.44 \pm 0.1^{**}$
6	LF	$12.34 \pm 0.09^{**}$
7	LG	$6.52 \pm 0.13^{*}$
8	LH	$11.45 \pm 0.07^{**}$
9	LI	$12.34 \pm 0.08^{**}$
10	LJ	$7.22 \pm 0.05^{*}$
11	LRI	7.19 ± 0.62

*p <0.05; **p <0.001; compared with the reference innovator brand, LRI; values are given as mean ± SD; n = 6.

Test brands of ramipril showed significant variations in disintegration time. All test brands of ramipril disintegrated between 0.71 and 10.90 minutes, whereas innovator brand disintegrated in1.09 minutes (Table 3.16). Test brands with higher disintegration time were RA, RB having values >5 minutes and RC >10 minutes. Despite variations in disintegration time, all test brands of tablet ramipril including their respective reference innovator brand met the acceptance limit for disintegration time.

Table 3.16: Comparison of disintegration time of test brands of tablet ramipril 5 mg with their reference innovator brand.

Serial No.	Brand	Disintegration time (min)
1	RA	5.64 ± 0.47*
2	RB	5.54 ± 1.08*
3	RC	10.90 ± 0.96*
4	RD	0.71 ± 0.16*
5	RRI	1.09 ± 0.13

*p <0.001; compared with the reference innovator brand, RRI; values are given as mean ± SD; n = 6.

3.1.5 Comparison of dissolution

Dissolution rate could directly affect the bioavailability and thus the efficacy of a tablet dosage form. According to USP not less than 80% of the labeled amount of tablet atenolol, not less than 80% of the labeled amount of tablet carvedilol, not less than 75% of the labeled amount of tablet losartan potassium and not less than 80% of the labeled amount of tablet ramipril should dissolved in 30 minutes separately. Antihypertensive test brands including their respective reference innovator brands met the acceptance limit for dissolution test (Tables 3.17– 3.20).[17,33]

Table 3.17: Comparison of % of dissolution of test brands of tablet atenolol 50 mg with their reference innovator brand.

Brand	% of dissolution in 30 minutes
ARI	97.26
AA	100.60
AB	100.55
AC	99.81

Table 3.18: Comparison of dissolution of test brands of tablet carvedilol 6.25 mg with their reference innovator brand.

Brand	% of dissolution in 30 minutes
CA	96.11
CB	91.81
CC	90.92
CD	97.17
CE	97.19
CF	96.70
CG	93.45
CH	88.90
CRI	97.04

Table 3.19: Comparison of dissolution of test brands of tablet losartan potassium 50 mg with their reference innovator brand.

Brand	% of dissolution in 30 minutes
LA	91.68
LB	85.47
LC	95.85
LD	84.58
LE	95.64
LF	95.34
LG	97.42
LH	92.48
LI	86.78
LJ	89.71
LRI	96.36

Table 3.20: Comparison of dissolution of test brands of tablet ramipril 5 mg with their reference innovator brand.

Brand	% of dissolution in 30 minutes
RA	100.15
RB	100.34
RC	99.84
RD	100.70
RRI	100.21

3.1.6 Comparison of % potency

Percentage potencies of tablets of test brands and their respective reference innovator brands were determined from their peak areas measurement and calibration graphs using High Performance Liquid Chromatography method.

According to USP tablets atenolol, carvedilol and ramipril should contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of active drug. Tablet losartan potassium should contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of active drug. [39,40-43]

Tablets of all test brands including their reference innovator brands met the acceptance criteria for assay content. Graphs for % potency are shown in Figures 3.1 – 3.4. Some chromatograms are shown in Figures 3.5 – 3.8.

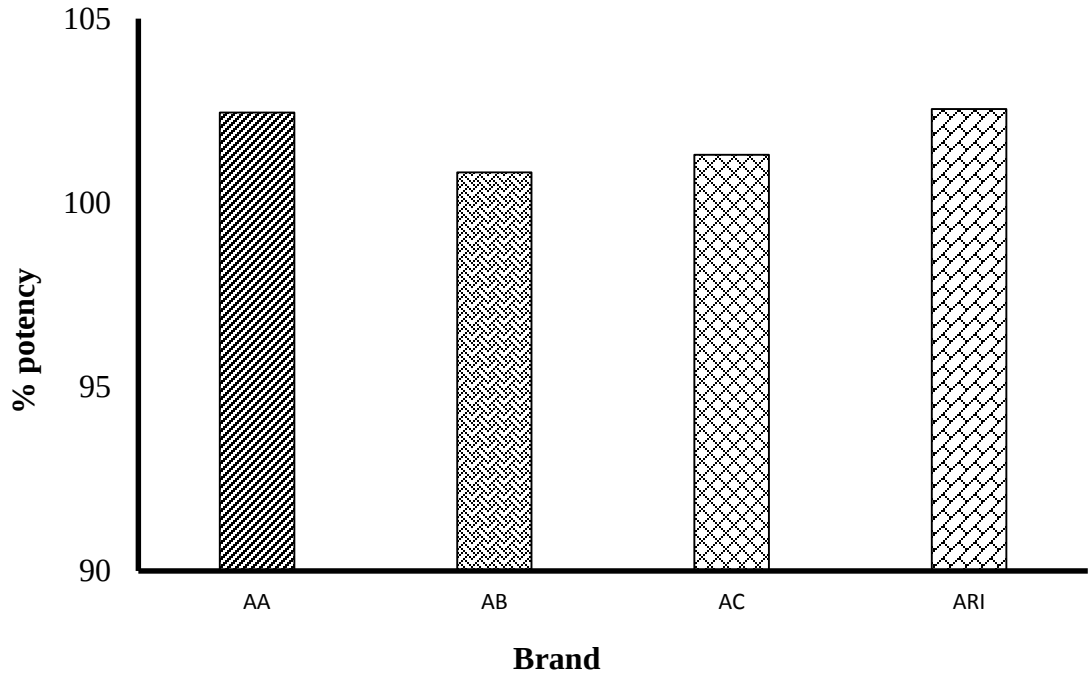


Figure 3.1: Comparison of % potency of test brands of tablet atenolol 50 mg with their reference innovator brand.

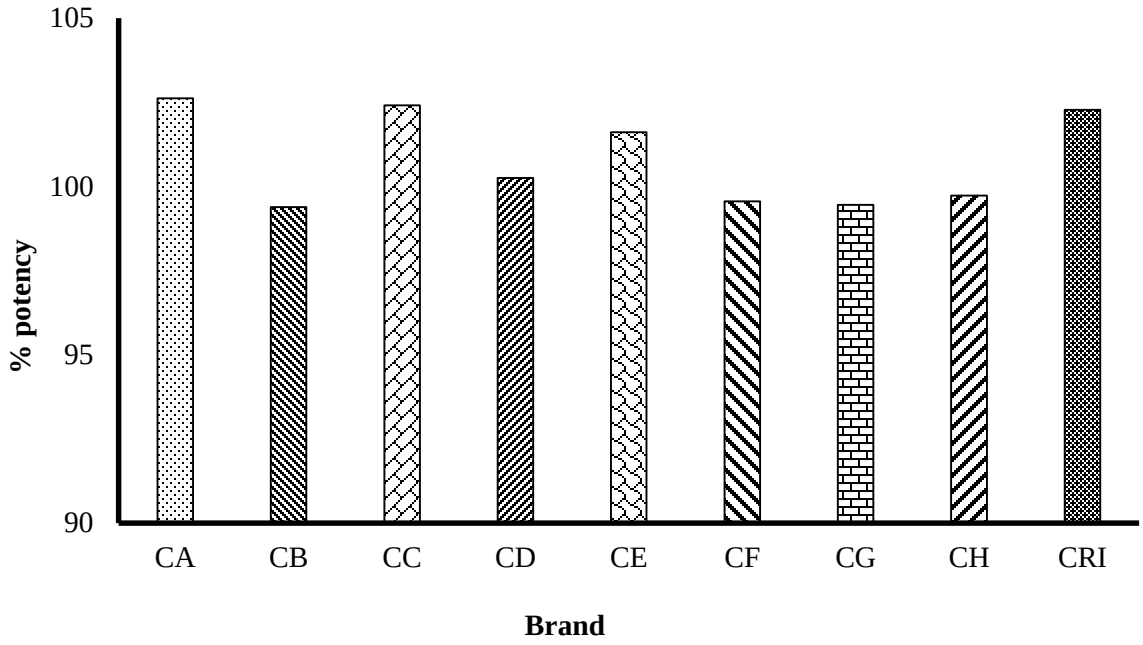


Figure 3.2: Comparison of % potency of test brands of tablet carvedilol 6.25 mg with their reference innovator brand.

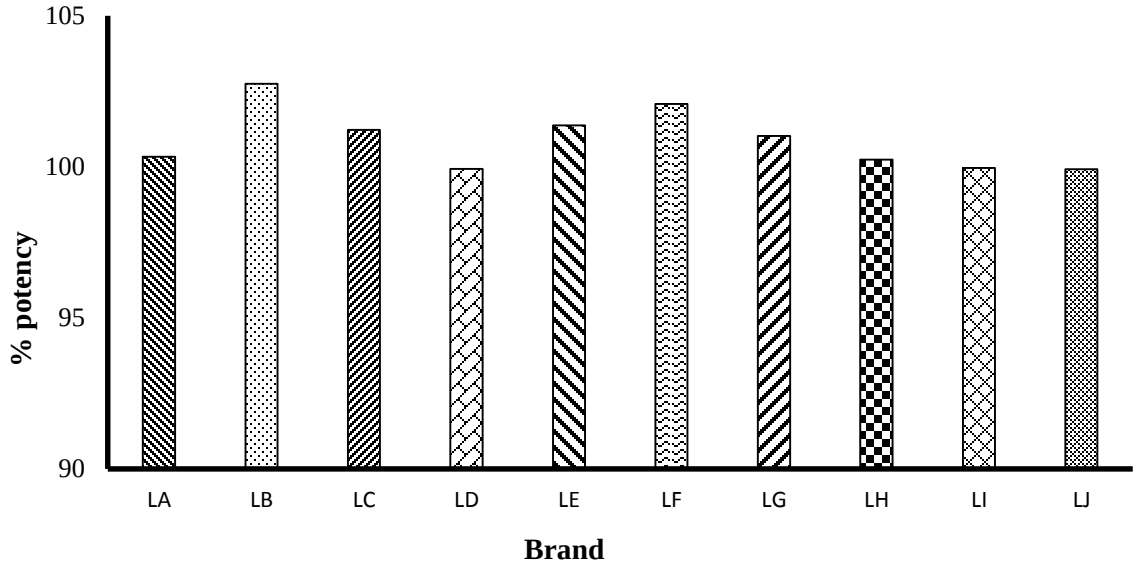


Figure 3.3: Comparison of % potency of test brands of tablet losartan potassium 50 mg with their reference innovator brand.

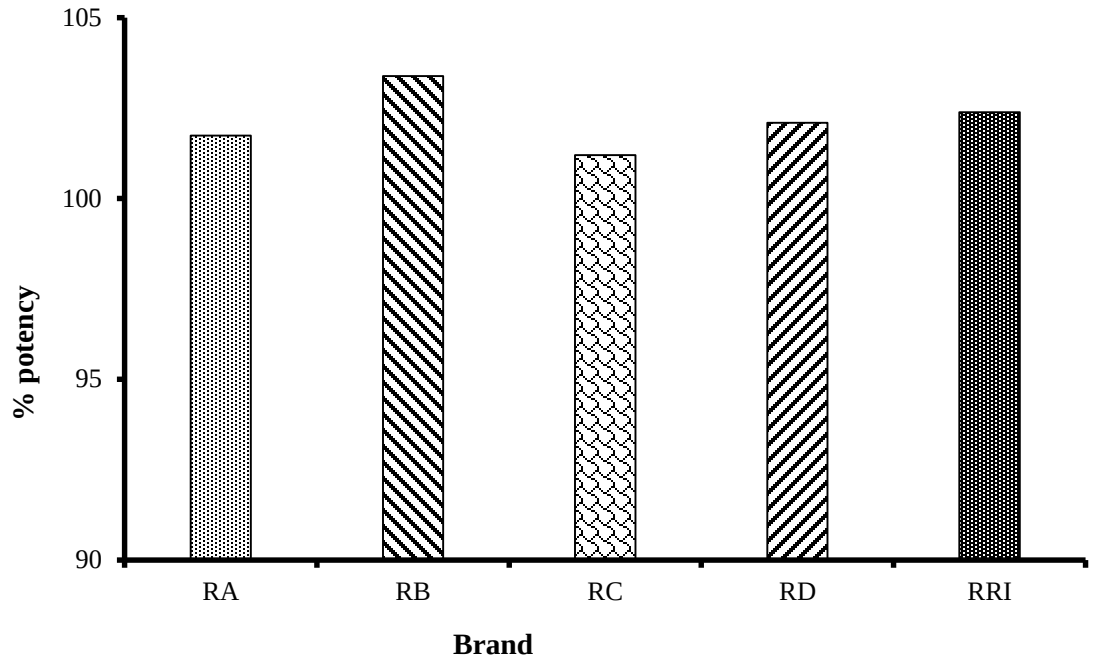
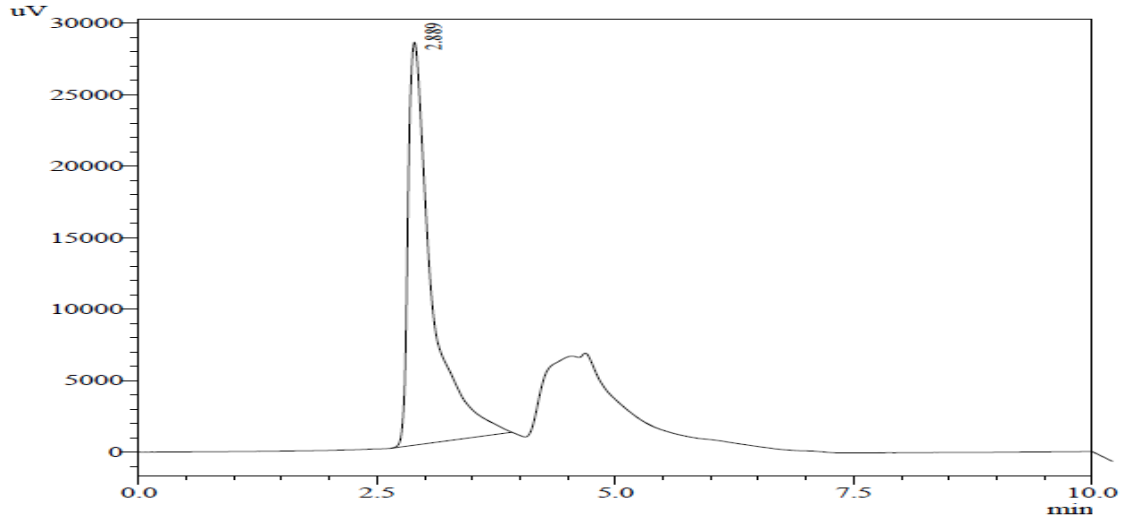
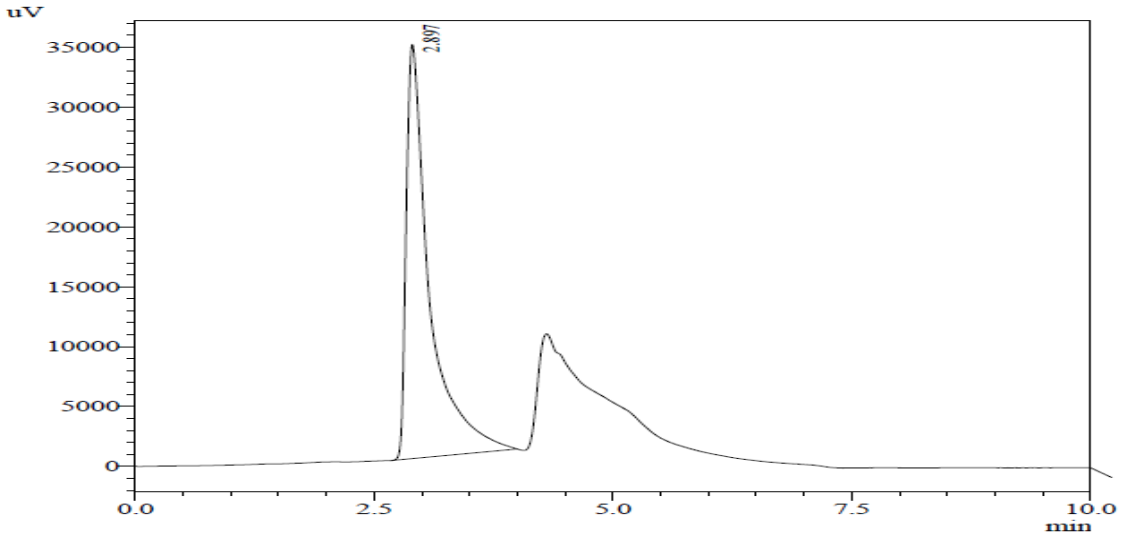


Figure 3.4: Comparison of % potency of test brands of tablet ramipril 5 mg with their reference innovator brand.

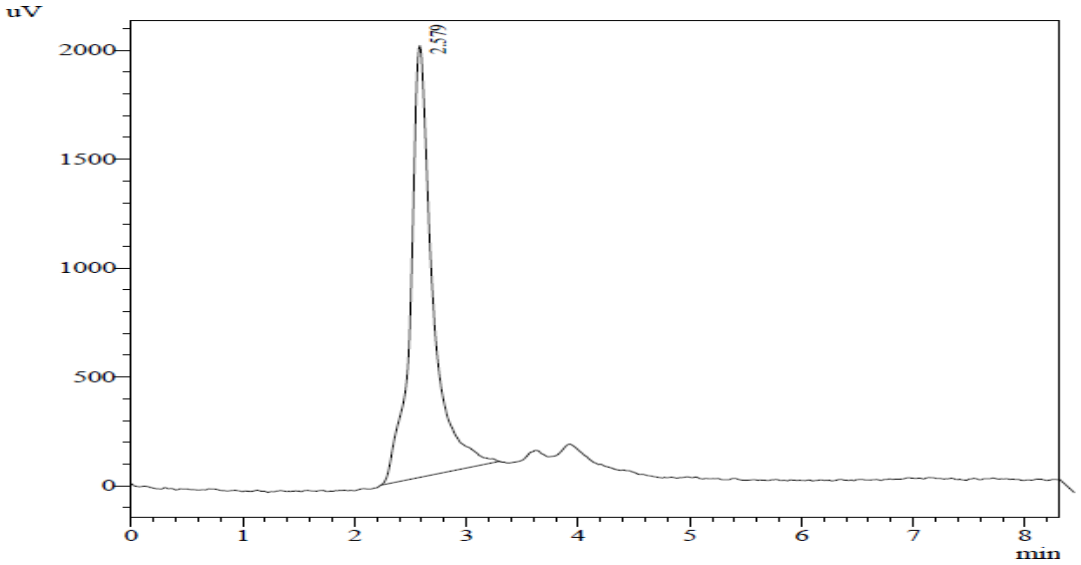


(A)

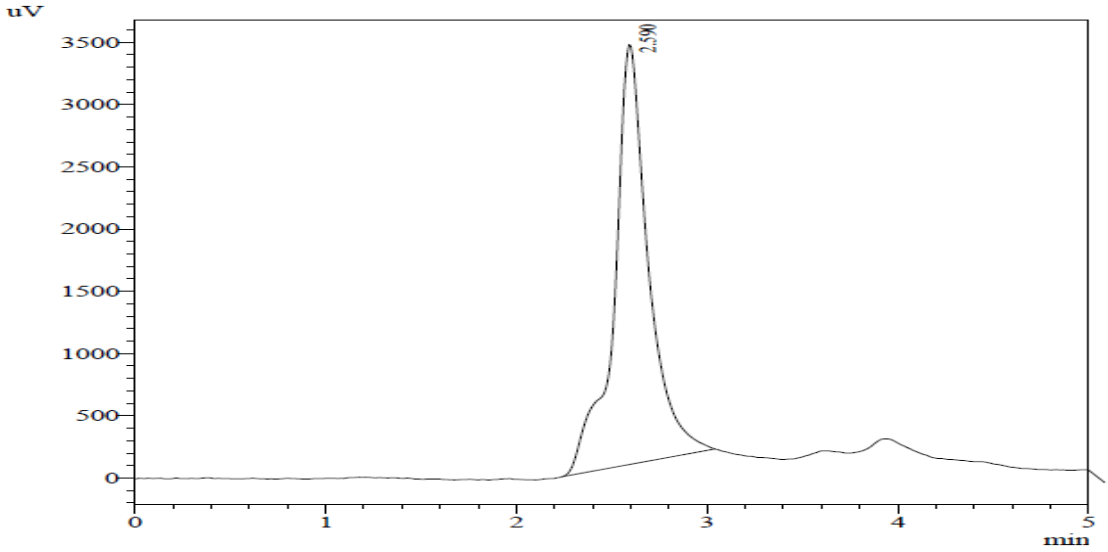


(B)

Figure 3.5: HPLC chromatograms of (A) test brand and (B) reference innovator brand of tablet atenolol 50 mg.

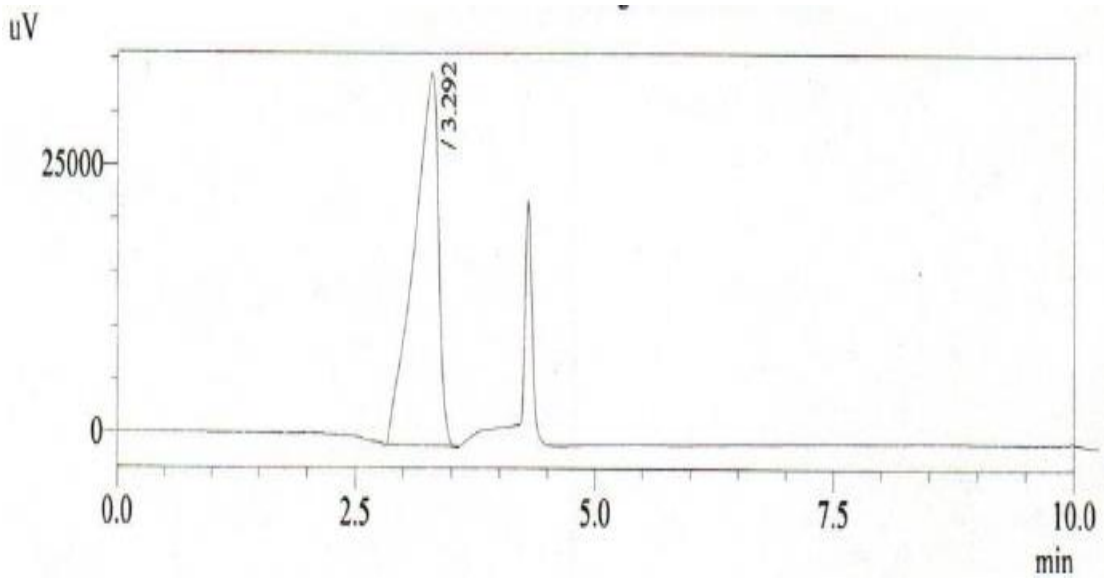


(A)

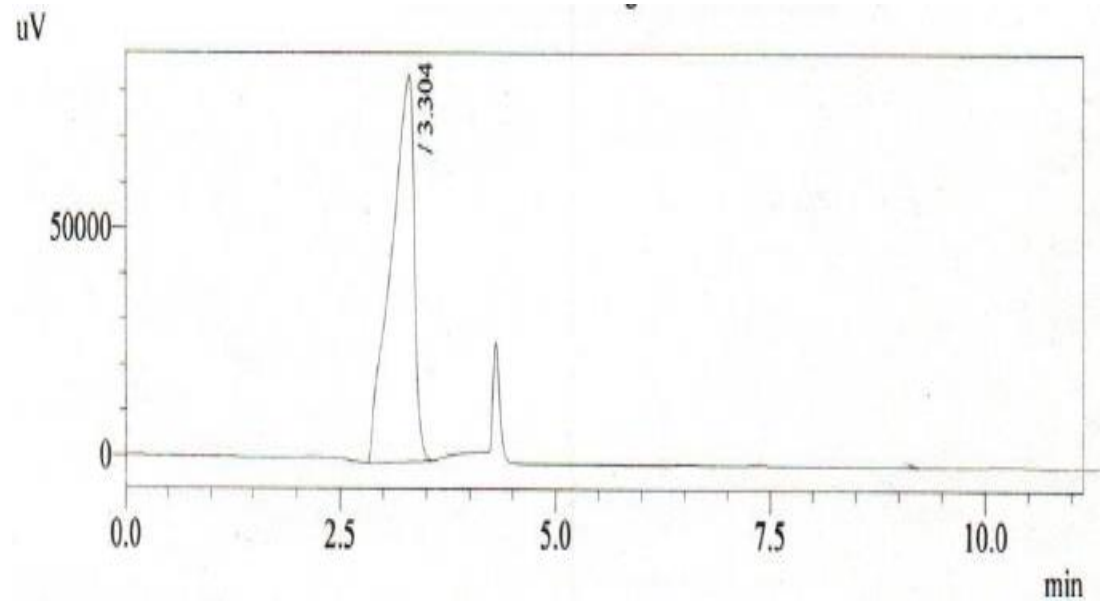


(B)

Figure 3.6: HPLC chromatograms of (A) test brand and (B) reference innovator brand of tablet carvedilol 6.25 mg.

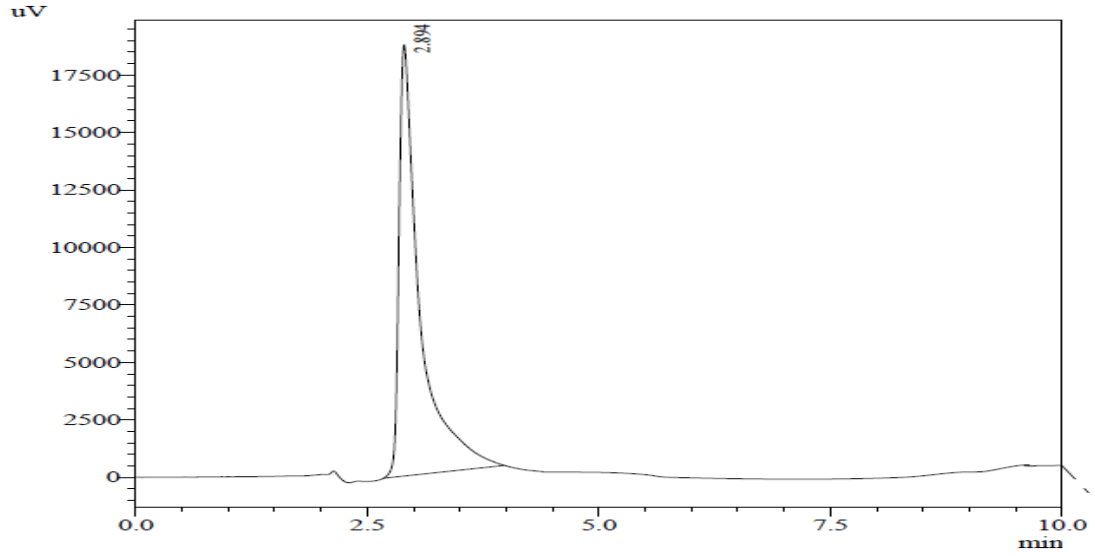


(A)

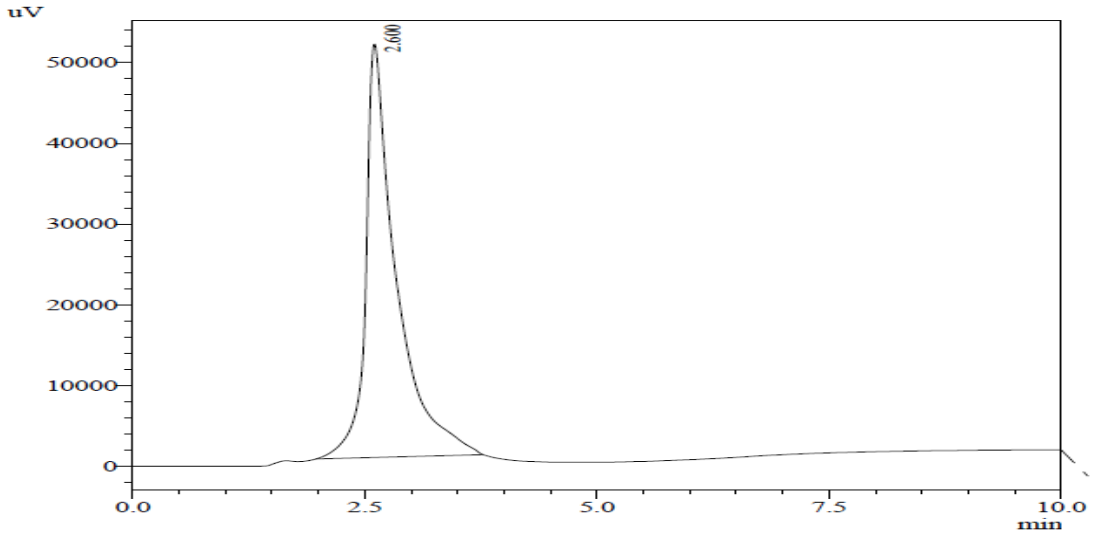


(B)

Figure 3.7: HPLC chromatograms of (A) test brand and (B) reference innovator brand of tablet losartan potassium 50 mg.



(A)



(B)

Figure 3.8: HPLC chromatograms of (A) test brand and (B) reference innovator brand of tablet ramipril 5 mg.

3.2 COMPARISON OF DISSOLUTION PROFILE

3.2.1 Comparison of Dissolution Profile Using Graphs

3.2.1.1 Determination of Mean of % Dissolution

The mean percentage of drug dissolved for twelve tablets of test brands and their respective reference innovator brands were calculated at different time points from the absorbances of dissolution solution and calibration graphs. Data for determination of the mean percentage of drug dissolved of tablets of test brands including reference innovator brands are shown in Tables 3.21 – 3.49. [39,44-46]

The results of the mean percentage of drug dissolved of tablet of all testing and reference innovator brands were expressed as mean ± SD and analyzed by Student's t- test.

Table 3.21: Data for determination of mean of % dissolution of test brand AA.

Tablet No.	% dissolution				
	5 min.	10 min.	15 min.	30 min.	45 min.
1	73.18	90.52	99.84	100.49	101.79
2	74.91	89.65	99.19	99.19	100.92
3	77.08	89.44	98.76	100.06	101.79
4	77.73	93.77	100.92	100.49	100.92
5	75.56	89.44	99.19	101.58	101.36
6	78.16	93.55	99.84	100.49	101.36
7	79.47	92.69	100.92	101.36	100.92
8	76.00	94.64	100.27	99.84	101.58
9	80.12	92.47	99.84	101.14	100.49
10	76.21	90.52	99.62	100.06	101.58
11	78.60	94.64	99.41	100.92	100.71
12	76.65	93.12	100.49	101.58	100.71
Mean	44.72 ± 2.92*	72.21 ± 2.66*	85.47± 1.21*	98.30 ± 1.91*	101.18 ± 0.45

*p <0.001; compared with the reference innovator brand, ARI; values are given as mean ± SD; n = 12.

Table 3.22: Data for determination of mean of % dissolution of test brand AB.

Tablet	**% dissolution**				
No.	**5 min.**	**10 min.**	**15 min.**	**30 min.**	**45 min.**
1	75.46	95.70	97.88	101.58	100.93
2	81.99	94.40	99.19	100.28	99.84
3	76.76	91.79	98.10	99.84	101.80
4	78.07	95.49	101.15	100.49	100.71
5	79.38	95.27	98.97	101.58	101.15
6	80.68	94.62	100.06	100.93	100.71
7	82.21	93.74	100.93	101.15	100.93
8	78.72	95.92	98.53	99.19	101.58
9	71.76	92.44	99.62	100.49	99.84
10	74.80	95.05	100.28	99.40	101.15
11	82.21	93.96	101.36	100.71	101.58
12	75.89	94.40	98.75	100.93	100.06
Mean	78.16 ± 3.33*	94.40±1.27*	99.57±1.19*	100.55±0.77*	100.86±0.67

*p <0.001; compared with the reference innovator brand, ARI; values are given as mean ± SD; n = 12.

Table 3.23: Data for determination of mean of % dissolution of test brand AC.

Tablet	**% dissolution**				
No.	**5 min.**	**10 min.**	**15 min.**	**30 min.**	**45 min.**
1	74.40	93.52	99.33	101.26	101.05
2	75.69	92.02	98.25	98.04	99.54
3	78.91	92.45	99.54	100.83	99.75
4	77.41	90.52	97.82	99.11	100.40
5	78.48	91.16	96.75	100.19	100.41
6	77.84	89.66	98.90	99.54	99.76
7	79.13	93.09	99.76	100.19	100.19
8	75.69	91.81	99.75	99.76	99.75
9	76.55	90.09	96.96	100.19	98.90
10	78.27	92.45	99.54	98.25	101.27
11	76.33	90.09	97.61	99.76	101.26
12	79.77	89.87	99.97	100.62	100.83
Mean	77.37±1.64**	91.39±1.34*	98.68±1.16**	99.81±0.97**	100.26±0.75

*p <0.05; **p <0.001; compared with the reference innovator brand, ARI; values are given as mean ± SD; n = 12.

Table 3.24: Data for determination of mean of % dissolution of reference innovator brand ARI.

Tablet No.	% dissolution				
	5 min.	**10 min.**	**15 min.**	**30 min.**	**45 min.**
1	68.93	88.96	93.74	98.53	100.49
2	71.97	86.12	95.27	99.19	97.88
3	69.58	87.87	93.31	97.66	101.58
4	72.19	84.82	94.40	98.75	100.28
5	70.23	86.56	92.00	97.01	99.62
6	72.19	84.60	93.96	95.05	100.93
7	68.27	86.78	94.62	96.57	102.24
8	73.72	88.08	91.13	98.32	101.80
9	71.76	85.25	92.66	95.92	99.62
10	70.23	88.52	95.27	98.53	100.93
11	67.62	85.47	92.00	95.27	101.80
12	72.41	85.91	95.70	96.36	100.28
Mean	70.76 ± 1.89	86.58 ± 1.48	93.67 ± 1.47	97.26 ± 1.43	100.62 ± 1.22

Values are given as mean ± SD; n = 12.

Table 3.25: Data for determination of mean of % dissolution of test brand CA.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	73.18	84.93	96.83	101.68
2	71.71	86.69	95.51	98.89
3	68.47	84.20	95.07	101.24
4	69.36	87.58	97.57	99.48
5	66.56	79.20	95.51	101.39
6	76.11	83.02	96.83	99.48
7	70.38	83.46	94.19	99.33
8	73.76	81.84	97.71	99.77
9	75.53	83.90	96.39	98.89
10	74.64	86.99	94.33	99.92
11	70.97	83.17	98.01	101.09
12	69.36	88.02	95.36	100.65
Mean	71.67 ± 3.00*	84.42 ± 2.59*	96.11 ± 1.30**	100.15 ± 1.01

*p <0.05; **p <0.001; compared with the reference innovator brand, CRI; values are given as mean ± SD; n = 12.

Table 3.26: Data for determination of mean of % dissolution of test brand CB.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	64.39	73.81	94.12	98.55
2	63.10	74.36	93.56	99.29
3	63.10	69.19	88.76	99.66
4	57.75	68.46	90.61	100.76
5	60.15	71.78	89.32	98.36
6	65.50	72.70	91.53	99.29
7	55.16	75.29	94.86	101.50
8	64.21	68.27	91.53	98.18
9	65.50	76.21	90.42	100.76
10	59.78	74.18	91.35	100.39
11	65.13	69.56	94.12	99.29
12	57.19	74.92	91.53	100.58
Mean	61.75 ± 3.60*	72.3 9± 2.85*	91.81 ± 1.96*	99.72 ± 1.07

*p <0.001; compared with the reference innovator brand, CRI; values are given as mean ± SD; n = 12.

Table 3.27: Data for determination of mean of % dissolution of test brand CC.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	65.17	81.47	92.82	100.51
2	70.12	80.03	93.34	99.73
3	60.99	76.51	89.82	100.51
4	62.17	77.95	88.12	98.95
5	69.47	78.86	93.73	100.77
6	60.60	84.47	93.08	99.86
7	66.34	77.03	91.77	100.64
8	66.47	79.77	90.21	101.17
9	70.38	78.08	87.86	98.69
10	66.34	76.25	89.69	99.47
11	65.69	82.90	87.99	101.30
12	61.38	82.90	92.56	100.77
Mean	65.43 ± 3.51*	79.69 ± 2.73*	90.92 ± 2.22*	100.20 ± 0.85*

*p <0.001; compared with the reference innovator brand, CRI; values are given as mean ± SD; n = 12.

Table 3.28: Data for determination of mean of % dissolution of test brand CD.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	70.69	87.90	95.14	99.65
2	73.01	86.67	98.97	100.88
3	75.61	83.67	95.69	99.24
4	74.38	86.54	96.51	101.29
5	71.78	82.30	97.33	99.52
6	69.73	85.31	98.42	100.88
7	74.92	83.94	97.74	101.84
8	68.64	86.26	93.64	99.79
9	71.37	82.98	98.42	101.43
10	71.92	87.22	96.78	100.88
11	73.42	82.44	98.42	100.61
12	74.65	88.18	98.97	101.16
Mean	72.51 ± 2.19**	85.28 ± 2.13**	97.17 ± 1.68*	100.60 ± 0.84

*p <0.05; **p <0.001; compared with the reference innovator brand, CRI; values are given as mean ± SD; n = 12.

Table 3.29: Data for determination of mean of % dissolution of test brand CE.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30min.**	**45 min.**
1	73.40	81.67	98.47	99.12
2	67.19	83.87	97.05	98.99
3	70.81	82.96	96.41	101.83
4	67.71	85.81	97.05	101.45
5	68.74	85.55	96.66	101.83
6	70.55	83.22	98.73	100.41
7	73.14	86.32	97.96	100.54
8	67.97	81.41	95.89	100.67
9	71.20	87.23	97.57	102.09
10	74.17	85.55	95.24	99.38
11	67.06	84.12	98.47	101.83
12	69.52	83.87	96.79	100.67
Mean	70.12 ± 2.50**	84.30 ± 1.83**	97.19 ± 1.09*	100.74 ± 1.11

*p <0.05; **p <0.001; compared with the reference innovator brand, CRI; values are given as mean ± SD; n = 12.

Table 3.30: Data for determination of mean of % dissolution of test brand CF.

Tablet	**% dissolution**			
No.	**10 min.**	**20 min.**	**30 min.**	**45 min.**
1	75.66	83.83	98.36	101.22
2	74.75	86.30	94.86	99.53
3	67.74	87.59	95.77	100.18
4	73.32	85.52	95.12	99.40
5	69.56	82.79	98.10	100.57
6	72.16	86.94	93.43	100.44
7	73.71	86.56	97.06	99.79
8	75.79	86.30	97.97	100.70
9	71.64	83.31	97.06	99.14
10	69.17	87.72	98.10	100.57
11	73.71	83.70	96.29	99.92
12	72.16	87.98	98.23	100.57
Mean	72.45 ± 2.57*	85.71 ± 1.8*5	96.70 ± 1.61*	100.17 ± 0.66

*p <0.001; compared with the reference innovator brand, CRI; values are given as mean ± SD; n = 12.

Table 3.31: Data for determination of mean of % dissolution of test brand CG.

Table	% dissolution			
t No.	**10 min.**	**20 min.**	**30 min.**	**45 min.**
1	69.00	76.18	92.87	99.41
2	70.92	74.90	94.02	100.57
3	64.25	77.72	95.56	99.67
4	68.23	80.29	91.07	101.21
5	71.69	76.57	94.54	99.67
6	74.77	75.03	93.38	100.18
7	66.17	81.57	92.36	100.18
8	72.21	76.18	95.05	99.54
9	65.53	79.39	92.61	100.83
10	67.33	78.88	94.79	101.34
11	68.10	84.01	91.97	100.18
12	67.33	80.93	93.13	100.44
Mean	68.79 ± 3.07*	78.47 ± 2.86*	93.45 ± 1.37*	100.27 ± 0.66*

*p <0.001; compared with the reference innovator brand, CRI; values are given as mean ± SD; n = 12.

Table 3.32: Data for determination of mean of % dissolution of test brand CH.

Tablet No.	**% dissolution**			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	56.63	71.34	89.49	99.63
2	61.49	69.06	87.06	98.49
3	58.06	73.06	90.06	99.06
4	57.49	74.34	89.77	97.91
5	55.77	67.06	86.34	99.91
6	58.49	69.63	91.49	100.34
7	64.77	77.20	86.34	98.49
8	55.63	73.49	93.06	100.06
9	65.34	69.63	86.49	98.34
10	55.34	73.34	85.49	97.49
11	60.49	75.77	93.63	101.34
12	55.77	70.91	87.63	98.63
Mean	58.77 ± 3.52*	72.07 ± 2.98*	88.90 ± 2.77*	99.14 ± 1.13

*p <0.001; compared with the reference innovator brand, CRI; values are given as mean ± SD; n = 12.

Table 3.33: Data for determination of mean of % dissolution of reference innovator brand CRI.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	80.34	89.46	98.57	100.18
2	76.32	85.97	97.64	99.92
3	74.64	85.43	96.29	101.26
4	76.59	88.12	94.28	100.45
5	72.43	86.11	97.64	101.26
6	75.92	92.14	96.16	99.78
7	72.16	85.30	95.36	101.39
8	74.84	90.93	98.17	99.51
9	77.26	89.59	95.22	100.45
10	76.59	86.91	98.71	99.11
11	73.23	89.32	99.65	100.59
12	74.04	84.50	96.83	101.26
Mean	75.36 ± 2.31	87.81 ± 2.46	97.04 ± 1.62	100.43 ± 0.76

Values are given as mean ± SD; n = 12.

Table 3.34: Data for determination of mean of % dissolution of test brand LA.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	50.95	67.07	88.76	99.36
2	45.62	72.64	90.62	97.75
3	40.66	73.57	88.64	98.18
4	50.02	73.39	92.36	100.60
5	41.65	63.41	90.87	99.67
6	46.36	62.60	94.96	100.17
7	50.95	72.33	89.07	98.43
8	53.49	71.40	93.29	99.17
9	53.24	73.26	93.04	98.80
10	54.11	74.26	89.07	99.79
11	49.21	70.23	97.69	99.17
12	55.04	79.40	91.86	98.93
Mean	49.28 ± 4.77*	71.13 ± 4.74*	91.68 ± 2.78*	99.17 ± 0.82

*p <0.001; compared with the reference innovator brand, LRI; values are given as mean ± SD; n = 12.

Table 3.35: Data for determination of mean of % dissolution of test brand LB

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	43.68	74.83	86.73	100.64
2	45.44	74.10	85.64	100.28
3	45.92	74.22	83.45	100.58
4	44.34	75.38	86.85	98.64
5	46.71	75.56	87.28	100.09
6	50.72	71.37	86.19	99.24
7	45.01	66.63	83.45	94.81
8	44.65	70.88	84.73	97.97
9	45.86	71.19	85.40	96.57
10	44.71	71.67	85.21	97.18
11	40.28	69.85	85.58	97.18
12	39.30	70.88	85.15	96.45
Mean	44.72 ± 2.92*	72.21 ± 2.66**	85.47 ± 1.21**	98.30 ± 1.91

*p <0.05; **p <0.001; compared with the reference innovator brand, LRI; values are given as mean ± SD; n = 12.

Table 3.36: Data for determination of mean of % dissolution of test brand LC.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	46.72	82.94	99.59	99.39
2	51.14	83.33	95.16	98.15
3	50.10	84.56	94.71	99.13
4	52.18	85.54	95.55	98.15
5	52.63	82.55	94.51	97.96
6	53.02	85.67	95.68	101.54
7	54.65	84.89	94.12	101.21
8	54.65	84.76	97.18	101.47
9	54.72	85.47	95.23	101.54
10	57.06	85.15	95.16	99.78
11	58.29	83.39	97.11	100.76
12	57.90	84.37	96.14	100.50
Mean	53.59±3.37*	84.38±1.08*	95.85±1.51**	99.96±1.39

*p <0.01; **p <0.001; compared with the reference innovator brand, LRI; values are given as mean ± SD; n = 12.

Table 3.37: Data for determination of mean of % dissolution of test brand LD.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	46.71	71.06	82.21	99.99
2	45.43	72.46	82.14	100.41
3	40.62	69.30	86.16	99.99
4	41.96	72.71	87.26	100.35
5	43.96	69.73	82.21	98.59
6	41.35	71.31	83.00	100.05
7	46.58	70.21	82.02	97.67
8	39.30	74.29	86.71	96.64
9	40.31	72.04	87.08	94.93
10	45.73	72.46	88.40	98.65
11	41.16	72.77	86.35	95.97
12	46.71	68.87	81.41	95.85
Mean	43.32 ± 2.81*	71.43 ± 1.64*	84.58 ± 2.60*	98.26 ± 1.99

*p <0.001; compared with the reference innovator brand, LRI; values are given as mean ± SD; n = 12.

Table 3.38: Data for determination of mean of % dissolution of test brand LE.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	47.59	83.06	99.75	99.09
2	52.71	84.31	94.16	101.19
3	49.43	83.46	94.56	98.83
4	58.95	85.16	97.06	99.16
5	53.76	85.69	95.87	97.98
6	55.08	84.97	94.36	99.95
7	54.62	84.44	96.33	101.33
8	57.57	86.54	95.22	99.42
9	58.36	85.30	94.89	100.93
10	53.83	84.05	95.22	99.16
11	57.77	82.47	96.07	100.34
12	56.52	86.35	94.16	101.19
Mean	54.68±3.52**	84.65±1.26*	95.64±1.59*	99.88±1.11

*p <0.01; **p <0.001; compared with the reference innovator brand, LRI; values are given as mean ± SD; n = 12.

Table 3.39: Data for determination of mean of % dissolution of test brand LF.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	50.26	85.21	94.07	98.89
2	56.88	82.83	98.44	97.86
3	48.85	83.02	94.59	98.51
4	54.31	82.83	94.39	100.43
5	52.57	81.87	94.65	97.86
6	56.69	85.14	95.04	101.52
7	56.30	82.32	94.01	99.60
8	55.01	83.92	95.61	100.63
9	53.86	84.05	94.84	99.66
10	52.25	81.35	95.94	100.82
11	55.46	84.44	95.74	100.11
12	49.10	82.25	96.71	100.05
Mean	53.46 ± 2.86**	83.27 ± 1.27*	95.34 ± 1.27*	99.66 ± 1.17

*p <0.05; **p <0.001; compared with the reference innovator brand, LRI; values are given as mean ± SD; n = 12.

Table 3.40: Data for determination of mean of % dissolution of test brand LG.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	54.42	86.43	97.43	99.99
2	58.88	85.94	98.04	99.75
3	54.90	85.88	98.71	99.81
4	53.25	86.55	96.76	100.18
5	56.74	83.87	96.57	99.57
6	58.33	86.25	97.31	99.51
7	51.42	85.76	97.12	98.53
8	55.76	84.17	98.90	99.20
9	56.07	85.58	96.57	99.14
10	55.27	85.09	95.29	98.10
11	56.92	87.35	97.98	99.09
12	56.62	85.82	98.41	100.48
Mean	55.71 ± 2.07*	85.72 ± 0.98*	97.42 ± 1.05*	99.46 ± 0.68

*p <0.01; compared with the reference innovator brand, LRI; values are given as mean ± SD; n = 12.

Table 3.41: Data for determination of mean of % dissolution of test brand LH.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	44.03	81.26	94.57	99.30
2	46.91	78.32	91.62	100.20
3	44.55	77.04	92.39	100.58
4	43.39	78.38	94.95	100.39
5	46.27	78.96	91.18	100.64
6	40.96	77.17	90.60	101.09
7	45.31	79.22	93.03	99.30
8	43.27	75.89	90.60	98.34
9	45.63	79.98	95.01	98.98
10	41.41	77.42	94.89	98.34
11	45.31	80.62	91.24	100.00
12	47.49	81.07	89.64	98.28
Mean	44.55 ± 2.03**	78.78 ± 1.72**	92.48 ± 1.96*	99.62 ± 1.00

*p <0.01; **p <0.001; compared with the reference innovator brand, LRI; values are given as mean ± SD; n = 12.

Table 3.42: Data for determination of mean of % dissolution of test brand LI.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	39.08	73.95	89.41	96.59
2	42.70	69.64	85.55	97.90
3	44.07	70.77	84.61	99.95
4	38.96	74.69	85.17	98.02
5	38.33	72.45	89.23	100.33
6	44.69	70.14	86.67	97.65
7	38.89	72.64	87.36	98.46
8	39.30	73.95	85.11	98.02
9	44.01	71.70	86.42	99.95
10	42.82	70.39	87.79	99.95
11	41.33	73.76	88.73	98.71
12	43.63	69.08	85.36	96.96
Mean	41.49 ± 2.43*	71.93 ± 1.91*	86.78 ± 1.70*	98.54 ± 1. 25

*p <0.001; compared with the reference innovator brand, LRI; values are given as mean ± SD; n = 12.

Table 3.43: Data for determination of mean of % dissolution of test brand LJ.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	40.38	79.01	89.14	101.03
2	48.56	76.48	88.17	99.86
3	45.77	75.44	92.65	100.83
4	42.58	72.78	92.13	98.62
5	47.39	76.22	89.14	101.09
6	49.53	74.92	88.17	98.62
7	47.91	77.06	90.38	99.21
8	48.17	73.43	88.75	100.38
9	46.29	77.45	89.73	98.04
10	47.58	74.60	86.22	99.79
11	45.90	75.83	91.29	99.99
12	46.03	73.23	90.70	100.44
Mean	46.34 ± 2.59*	75.54 ± 1.86*	89.71 ± 1.83*	99.82 ± 1.01

*p <0.001; compared with the reference innovator brand, LRI; values are given as mean ± SD; n = 12.

Table 3.44: Data for determination of mean of % dissolution of reference innovator brand LRI.

Tablet No.	% dissolution			
	10 min.	**20 min.**	**30 min.**	**45 min.**
1	55.94	88.19	98.48	97.16
2	60.93	88.01	98.48	96.78
3	61.06	81.38	96.15	99.11
4	60.36	81.32	93.75	99.62
5	61.69	89.65	93.56	99.93
6	59.60	90.40	98.73	97.16
7	57.33	86.24	98.92	99.30
8	53.67	86.81	93.81	99.30
9	62.38	86.93	94.00	95.52
10	55.19	78.29	93.94	100.50
11	62.82	83.34	99.30	100.63
12	56.26	84.91	97.22	98.55
Mean	58.94 ± 3.10	85.45 ± 3.70	96.36 ± 2.40	98.63 ± 1.62

Values are given as mean ± SD; n = 12.

Table 3.45: Data for determination of mean of % dissolution of test brand RA.

Tablet No.	% dissolution			
	5 min.	**10 min.**	**15 min.**	**30 min.**
1	67.89	82.05	92.83	101.97
2	67.16	83.88	94.57	98.95
3	72.19	87.17	95.48	100.32
4	68.53	88.63	94.04	99.32
5	73.19	84.06	90.09	100.78
6	74.20	82.51	93.01	99.68
7	66.89	85.52	90.27	100.51
8	71.27	86.62	90.18	99.23
9	72.19	84.79	92.10	99.77
10	68.53	88.36	93.65	101.42
11	75.38	86.99	91.19	99.23
12	73.74	85.34	92.83	100.60
Mean	70.93 ± 2.99*	85.49 ± 2.14**	92.52 ± 1.79*	100.15 ± 0.95

*p <0.01; **p <0.001; compared with the reference innovator brand, RRI; values are given as mean ± SD; n = 12.

Table 3.46: Data for determination of mean of % dissolution of test brand RB.

Tablet No.	% dissolution			
	5 min.	**10 min.**	**15 min.**	**30 min.**
1	62.99	86.89	91.05	99.65
2	66.43	83.36	88.70	101.46
3	61.90	79.28	85.26	100.46
4	65.34	83.45	90.51	101.55
5	60.27	82.36	92.86	99.20
6	64.07	81.64	90.87	100.74
7	64.80	84.89	92.68	99.74
8	68.60	83.26	91.41	101.28
9	64.25	81.45	89.06	99.83
10	65.88	82.63	93.40	99.56
11	62.26	81.27	93.95	101.64
12	65.43	83.81	88.88	99.02
Mean	64.35±2.26*	82.86±1.93*	90.72±2.46*	100.34±0.96

*p <0.001; compared with the reference innovator brand, RRI; values are given as mean ± SD; n = 12.

Table 3.47: Data for determination of mean of % dissolution of test brand RC.

Tablet No.	% dissolution			
	5 min.	**10 min.**	**15 min.**	**30 min.**
1	58.74	77.20	82.54	99.65
2	60.45	72.76	84.89	101.46
3	55.02	76.11	88.70	99.02
4	56.92	78.11	88.15	99.65
5	61.54	76.84	86.52	99.20
6	54.57	74.12	83.90	100.74
7	56.20	77.83	87.52	99.74
8	57.10	74.03	84.35	98.56
9	58.37	73.13	87.43	99.83
10	61.00	78.56	83.90	99.56
11	62.26	75.12	88.33	101.64
12	54.75	78.56	85.98	99.02
Mean	58.08±2.74*	76.03±2.13*	86.02±2.06*	99.84±0.96

*p <0.001; compared with the reference innovator brand, RRI; values are given as mean ± SD; n = 12.

Table 3.48: Data for determination of mean of % dissolution of test brand RD.

Tablet No.	% dissolution			
	5 min.	**10 min.**	**15 min.**	**30 min.**
1	72.17	88.31	92.28	99.95
2	75.61	89.63	93.07	101.45
3	68.91	92.45	94.66	100.92
4	71.20	90.60	95.54	101.98
5	72.35	87.07	91.66	100.65
6	77.37	85.93	93.07	99.68
7	71.46	86.81	92.63	100.74
8	69.88	87.43	94.74	100.04
9	74.73	88.04	93.51	101.45
10	69.61	90.42	95.01	99.24
11	70.93	91.57	94.57	101.62
12	72.79	90.78	95.45	100.65
Mean	72.25 ± 2.54*	89.09 ± 2.10*	93.85 ± 1.31*	100.70 ± 0.84

*p <0.05; compared with the reference innovator brand, RRI; values are given as mean ± SD; n = 12.

Table 3.49: Data for determination of mean of % dissolution of reference innovator brand RRI.

Tablet No.	% dissolution			
	5 min.	**10 min.**	**15 min.**	**30 min.**
1	76.14	89.77	95.38	99.03
2	77.12	92.44	94.31	100.19
3	68.57	89.50	96.00	99.30
4	74.27	91.19	93.78	100.64
5	76.50	89.50	92.98	101.53
6	70.88	91.73	94.31	101.79
7	76.67	93.42	95.83	98.59
8	73.02	90.48	92.44	101.08
9	75.25	94.31	96.09	99.66
10	71.15	89.41	93.33	100.37
11	74.09	90.12	96.18	100.28
12	77.12	89.68	93.06	100.10
Mean	74.23±2.81	90.96±1.67	94.47±1.37	100.21±0.97

Values are given as mean ± SD; n = 12.

3.2.1.2 Dissolution Profile Graphs

The mean percentage of drug dissolved of tablets of antihypertensive test brands were compared with that of their respective innovator brands graphically by plotting the mean percentage of drug dissolved against time. Graphs were shown in Figures 3.9 – 3.12. [39, 44-46]

All antihypertensive test brands including reference innovator of tablet atenolol released more than 80% of drug within 10 minutes. Except test brands CB, CC, CG, CH all other test brands including reference innovator brand of tablet carvedilol released more than 80% of drug within 20 minutes. Reference innovator brand and brands LC, LE, LF, and LG of tablet losartan potassium released more than 80% of drug in 20 minutes. Test brands LA, LB, LD, LH, LI, and LJ released more than 80% of drug in 30 minutes. Except brand RC all other test brands and reference innovator brand of tablet ramipril released more than 80% of drug in 10 minutes.

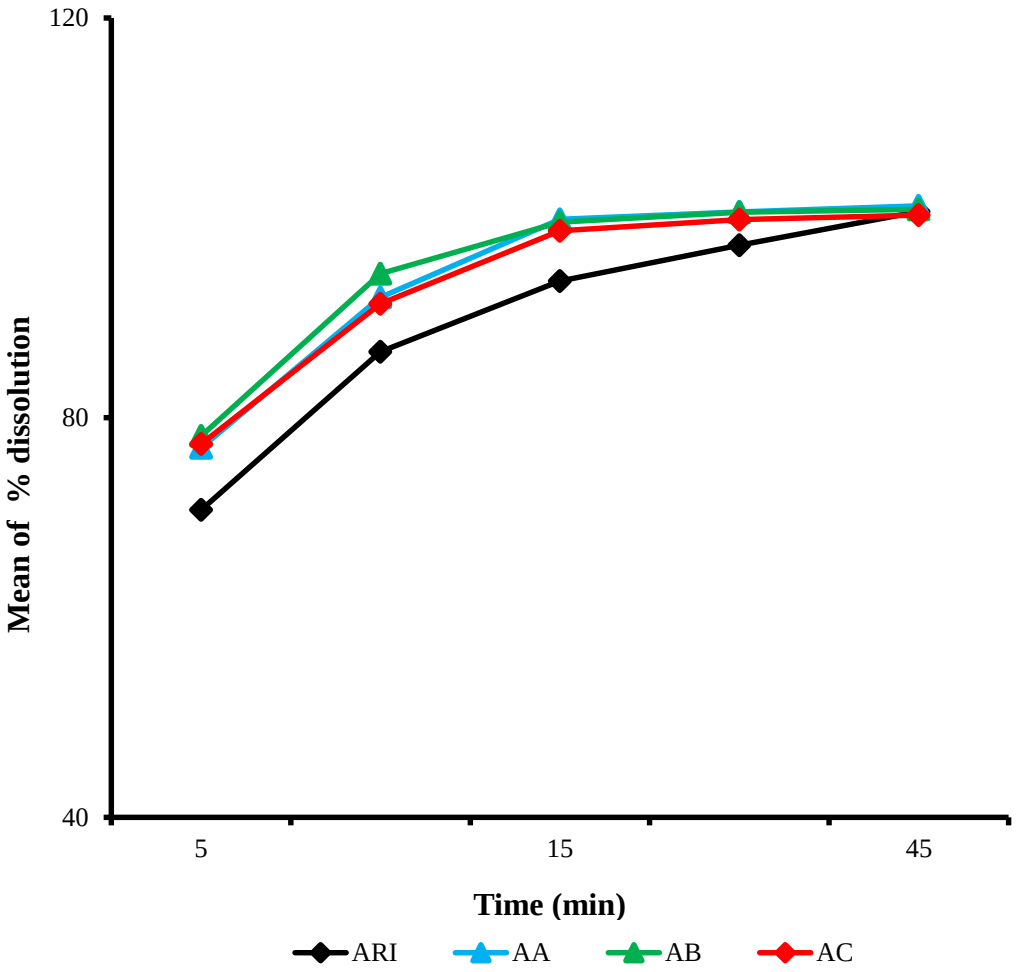


Figure 3.9: Comparison of dissolution profiles of test brands of tablet atenolol 50 mg with their reference innovator brand.

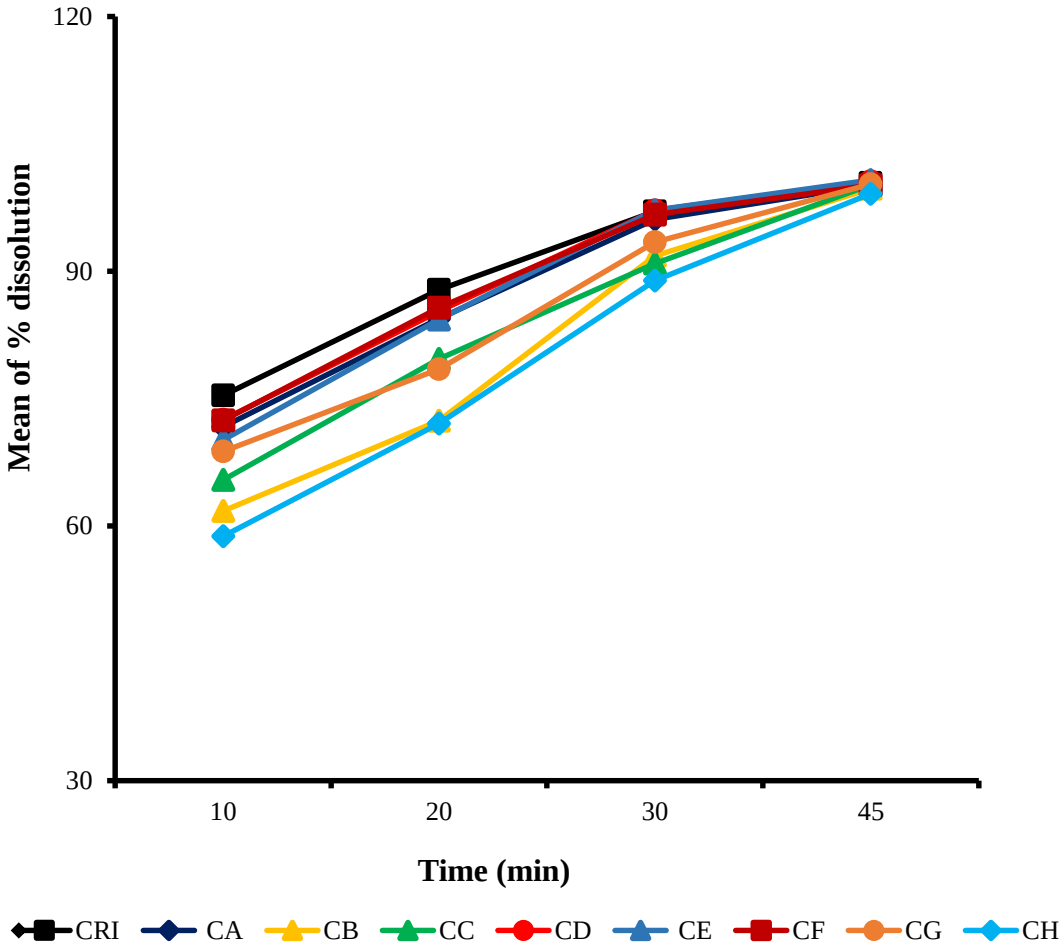


Figure 3.10: Comparison of dissolution profiles of test brands of tablet carvedilol with their reference innovator brand.

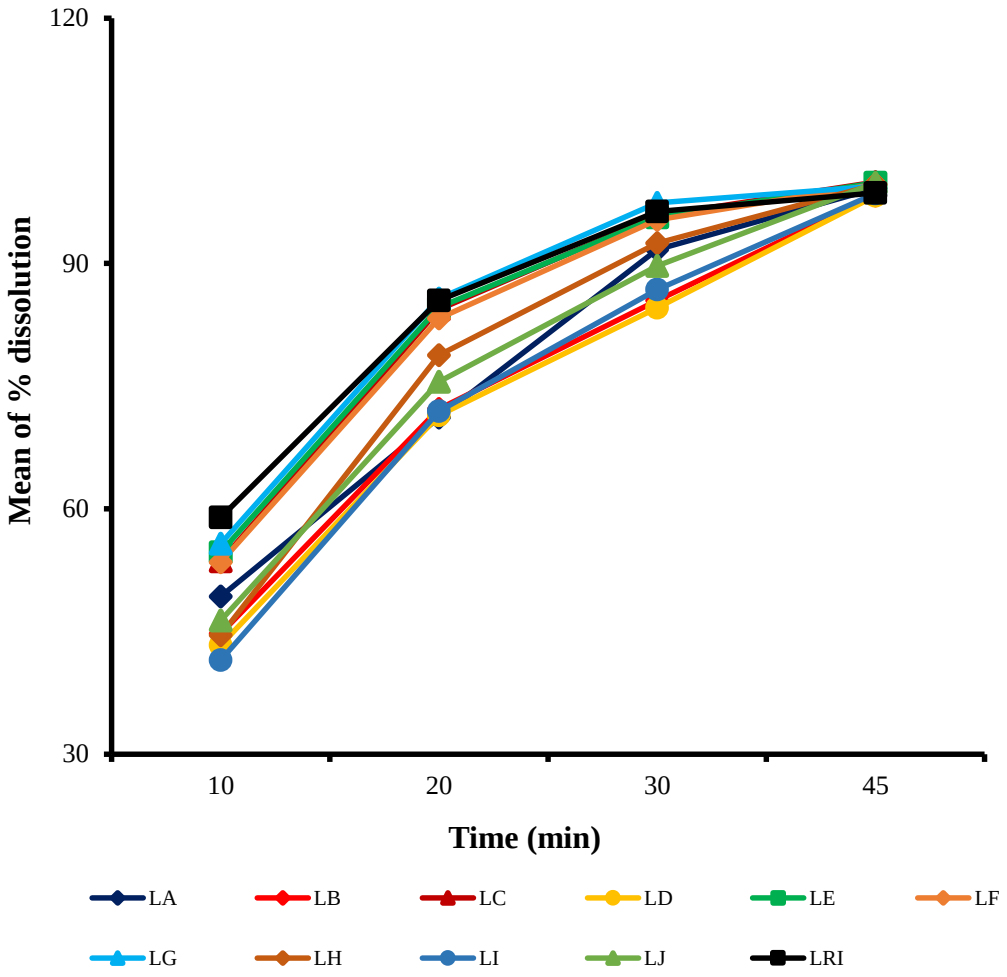


Figure 3.11: Comparison of dissolution profiles of test brands of tablet losartan potassium 50 mg with their reference innovator brand.

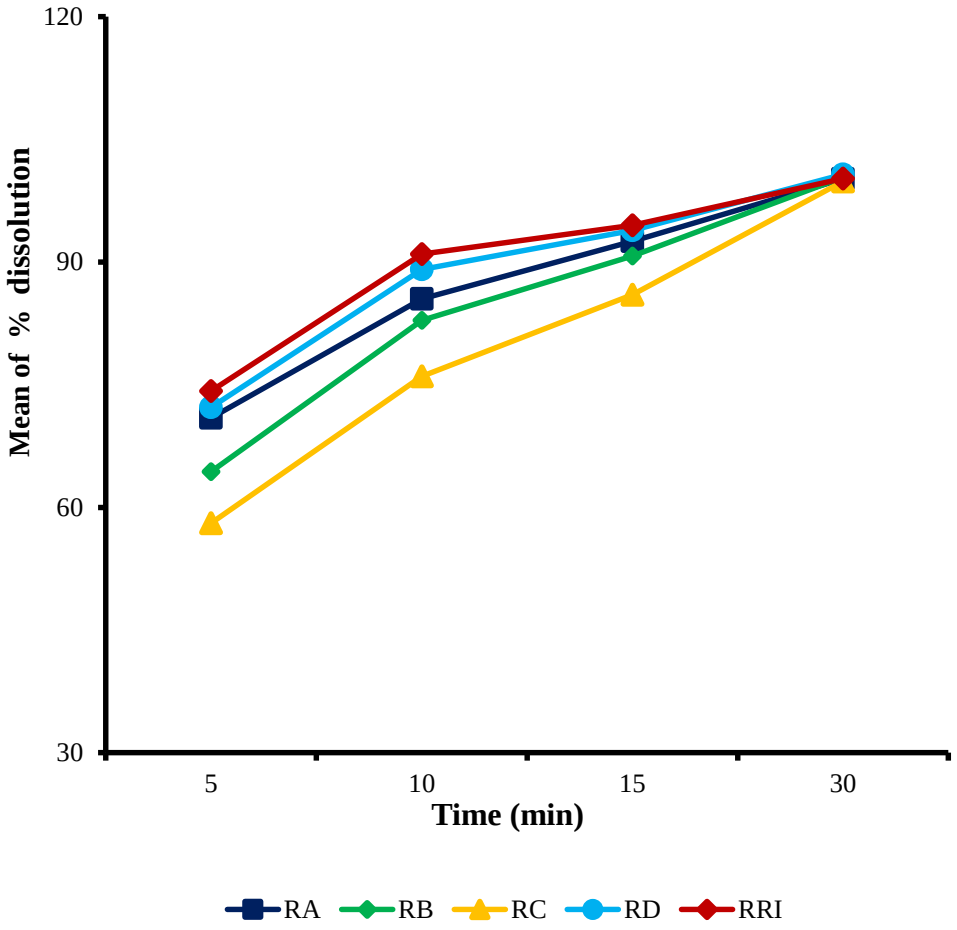


Figure 3.12: Comparison of dissolution profiles of test brands of tablet ramipril 5 mg with their reference innovator brand.

3.2.2 Comparison of Time Required for 50% Dissolution and 90% Dissolution

The time required for 50% dissolution ($T_{50\%}$) and 90% dissolution ($T_{90\%}$) were determined (Tables 3.50 – 3.53). All test brands of tablet atenolol and also tablet ramipril including their reference innovator brands showed $T_{50\%}$ values less than 10 minutes and $T_{90\%}$ values less than 30 minutes.[33]

For tablet carvedilol; all test brands including reference innovator brand showed $T_{50\%}$ values less than 10 minutes and $T_{90\%}$ values less than 30 minutes except test brand CH. Brand CH had $T_{50\%}$ less than 10 minutes but T_{90}% greater than 30 minutes. For tablet losartan potassium; reference innovator brand LRI and test brands LA, LB, LD, LH, LI, LJ showed $T_{50\%}$ values greater than 10 minutes whereas, other test brands had less than 10 minutes. Test brands LB, LD, LI showed $T_{90\%}$ values greater than 30 minutes whereas, other test brands including reference innovator brand LRI showed less than 30 minutes.

Table 3.50: Comparison of $T_{50\%}$ and $T_{90\%}$ values of test brands of tablet atenolol 50 mg with their reference innovator brand.

Brand	$T_{50\%}$ (min)	$T_{90\%}$ (min)
AA	<10	<30
AB	<10	<30
AC	<10	<30
ARI	<10	<30

Table 3.51: Comparison of $T_{50\%}$ and $T_{90\%}$ values of test brands of tablet carvedilol 6.25 mg with their reference innovator brand.

Brand	$T_{50\%}$ (min)	$T_{90\%}$ (min)
CA	<10	<30
CB	<10	<30
CC	<10	<30
CD	<10	<30
CE	<10	<30
CF	<10	<30
CG	<10	<30
CH	<10	>30
CRI	<10	<30

Table 3.52: Comparison $T_{50\%}$ and $T_{90\%}$ values of test brands of tablet losartan potassium 50 mg with their reference innovator brand.

Brand	$T_{50\%}$ (min)	$T_{90\%}$(min)
LA	>10	<30
LB	>10	>30
LC	<10	<30
LD	>10	>30
LE	<10	<30
LF	<10	<30
LG	<10	<30
LH	>10	<30
LI	>10	>30
LJ	>10	<30
LRI	>10	<30

Table 3.53: Comparison of $T_{50\%}$ and $T_{90\%}$ values of test brands of tablet ramipril 5 mg with their reference innovator brand.

Brand	$T_{50\%}$ (min)	$T_{90\%}$ (min)
RA	<10	<30
RB	<10	<30
RC	<10	<30
RD	<10	<30
RRI	<10	<30

3.2.3 Comparison of Dissolution Profile Using Difference Factor and Similarity Factor

The Model independent similarity factor method was used to compare dissolution profiles between test brands and their respective reference innovator brands statistically. The mean percentage of drug dissolved of tablets of test brands and their respective reference innovator brands were used to calculate difference factor (f_1) and similarity factor (f_2) using the respective equations. Difference factor (f_1) values up to 15 (0-15) and similarity factor (f_2) values greater than 50 (50-100) ensures sameness or equivalence of the test and the reference innovator brand.[12,47-51]

All test brands of atenolol having $f_1$values less than 15 and f_2 values more than 50 may be considered equivalent to reference innovator brand (Table 3.54). Except two test brands CB, CH; all other test brands of tablet carvedilol showing f_1 values less than 15 and f_2 values more than 50 seem to be equivalent to reference innovator brand (Table 3.55).

Except test brands LB, LD, LI; all other test brands of tablet losartan potassium having f_1 values less than 15 and f_2 values more than 50 may be equivalent to reference innovator brand (Table 3.56). Except brand RC, all other test brands of tablet ramipril showing f_1 values less than 15 and f_2 values more than 50 like to have very good bioavailability and may be equivalent to reference innovator brand (Table 3.57).[12,18]

Table 3.54: Data for difference factor (f_1) and similarity factor (f_2) values for test brands of tablet atenolol 50 mg.

Test brand	Difference factor (f_1)	similarity factor (f_2)
AA	4.84	65.21
AB	5.48	61.93
AC	4.14	67.08

Table 3.55: Data for difference factor (f_1) and similarity factor (f_2) values for test brands of tablet carvedilol 6.25 mg.

Test brand	Difference factor (f_1)	Similarity factor (f_2)
CA	2.30	78.08
CB	9.70	48.60
CC	6.77	57.18
CD	1.57	83.33
CE	2.55	73.98
CF	1.56	84.23
CG	5.45	60.84
CH	11.58	45.69

Table 3.56: Data for difference factor (f_1) and similarity factor (f_2) values for test brands of tablet losartan potassium 50 mg.

Test brand	Difference factor (f_1)	Similarity factor (f_2)
LA	8.60	52.27
LB	11.39	47.57
LC	2.44	60.35
LD	12.31	45.9
LE	2.58	80.18
LF	3.53	74.77
LG	1.59	84.78
LH	7.64	54.2
LI	11.97	45.91
LJ	8.95	52.88

Table 3.57: Data for difference factor (f_1) and similarity factor (f_2) values for test brands of tablet ramipril 5 mg.

Test brand	Difference factor (f_1)	Similarity factor (f_2)
RA	3.00	72.88
RB	6.08	58.58
RC	11.09	46.35
RD	1.38	88.00

3.3 *IN VIVO* PHARMACEUTICAL EQUIVALENCE STUDY BY COMPARING PLASMA DRUG CONCENTRATION – TIME CURVES IN RAT MODELS

When comparing the drug products, t_{max} value can be used as an approximate indication of drug absorption rate. Again, C_{max} value can be used in the bioequivalence studies for the rate of drug bioavailability. C_{max} and t_{max} values of test brands were compared with their respective reference innovator brands from plasma drug concentration- time curves after administration of drug in rat models to study the pharmaceutical equivalence *in vivo*.[86-100]

Plasma drug concentration- time curves of test brands with their respective reference innovator brands are given in Figures 3.13 – 3.16. The curves indicated that t_{max} value for test brands and innovator brand of tablet atenolol was 2.5 hrs and C_{max} values for brands AA, AB, AC, ARI were 0.123, 0.128, 0.113, 0.129 μg/mL respectively. t_{max} value for test brands and innovator brand of tablet carvedilol was 1.5 hrs and C_{max} values for brands CA, CB, CC, CD, CE, CF, CG, CH, CRI were 0.106, 0.106, 0.102, 0.103, 0.099, 0.096, 0.098, 0.090, 0.106 μg/mL, respectively. The t_{max} value for test brands and innovator brand of tablet losartan potassium was 1.5 hrs and C_{max} values for brands LA, LB, LC, LD, LE, LF, LG, LH, LI, LJ, LRI were 0.122, 0.123, 0.126, 0.118, 0.122, 0.123, 0.118, 0.123, 0.117, 0.120, 0.124 μg/mL, respectively. The t_{max} value for test brands and innovator brand of tablet ramipril was 2.5 hrs and C_{max} values for brands RA, RB, RC, RD, RRI were 0.047, 0.061, 0.058, 0.053, 0.063 μg/mL, respectively

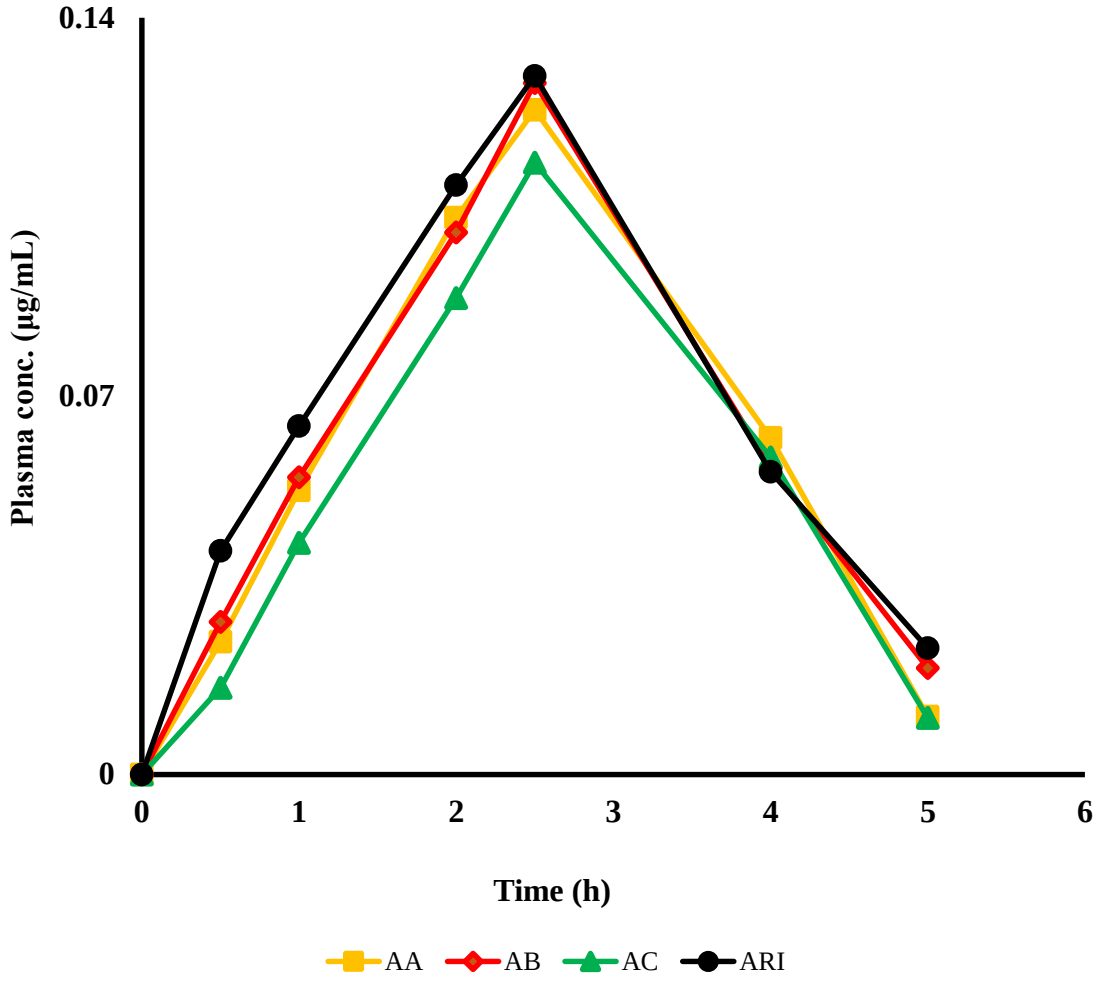


Figure 3.13: Comparison of plasma drug concentration- time curve of test brands of tablet atenolol 50 mg with their reference innovator brand.

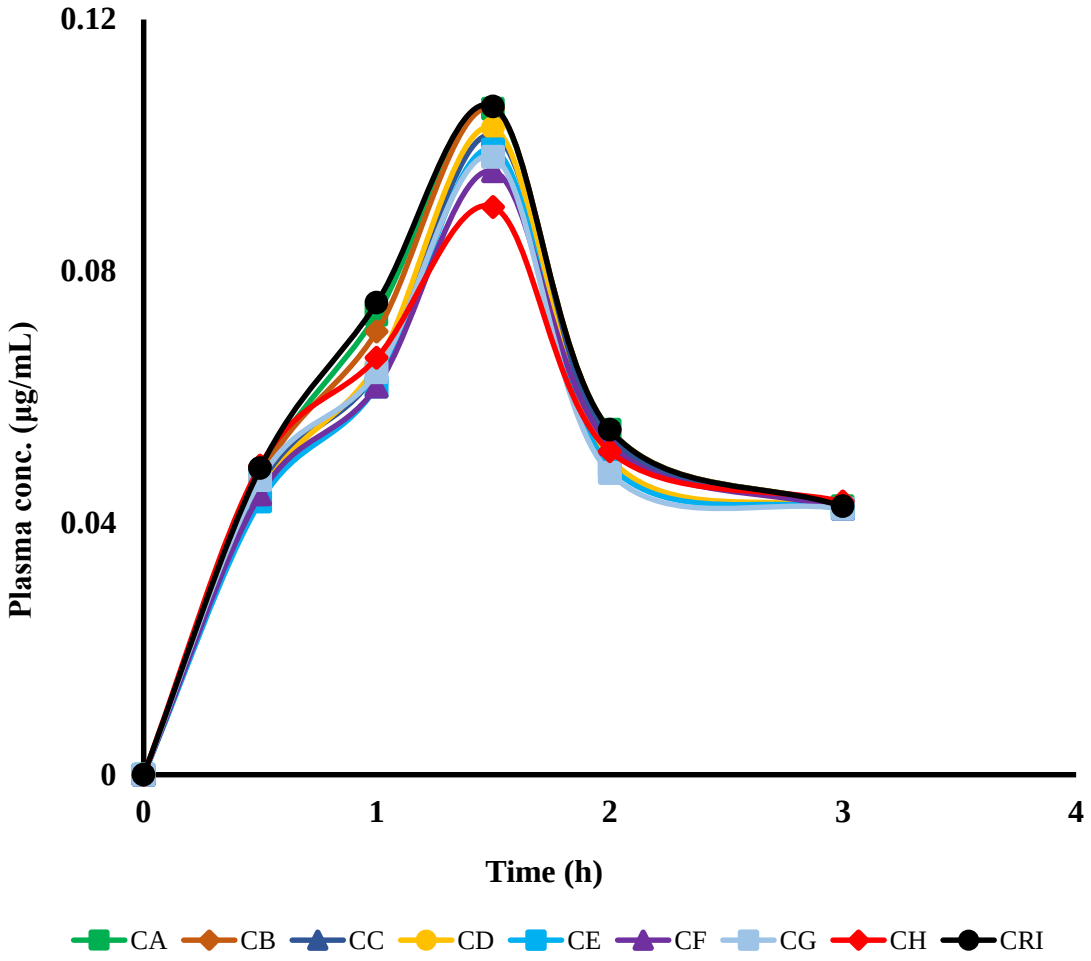


Figure 3.14: Comparison of plasma drug concentration- time curve of test brands of tablet carvedilol 6.25 mg with their reference innovator brand.

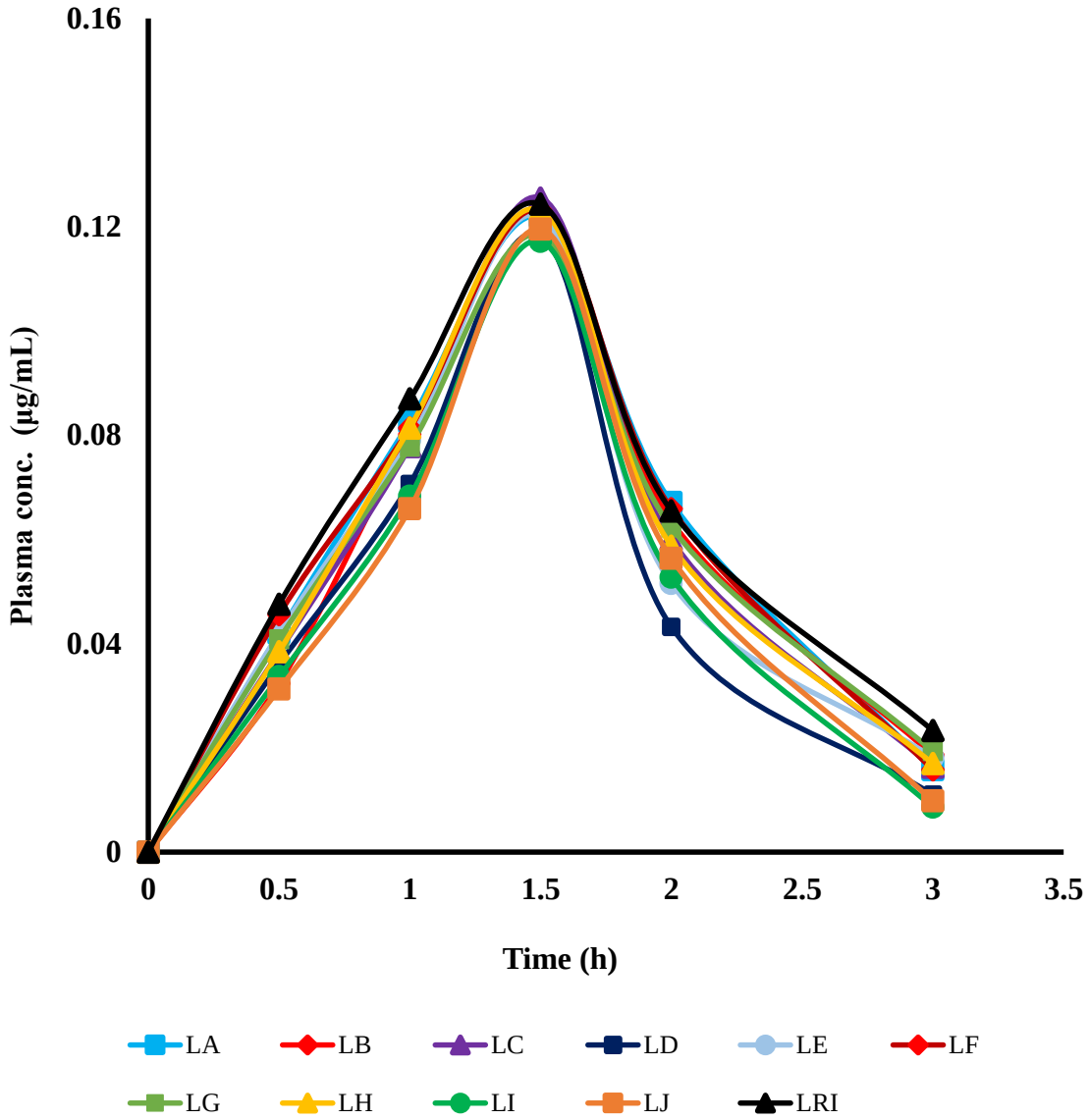


Figure 3.15: Comparison of plasma drug concentration- time curve of test brands of tablet losartan potassium with their reference innovator brand.

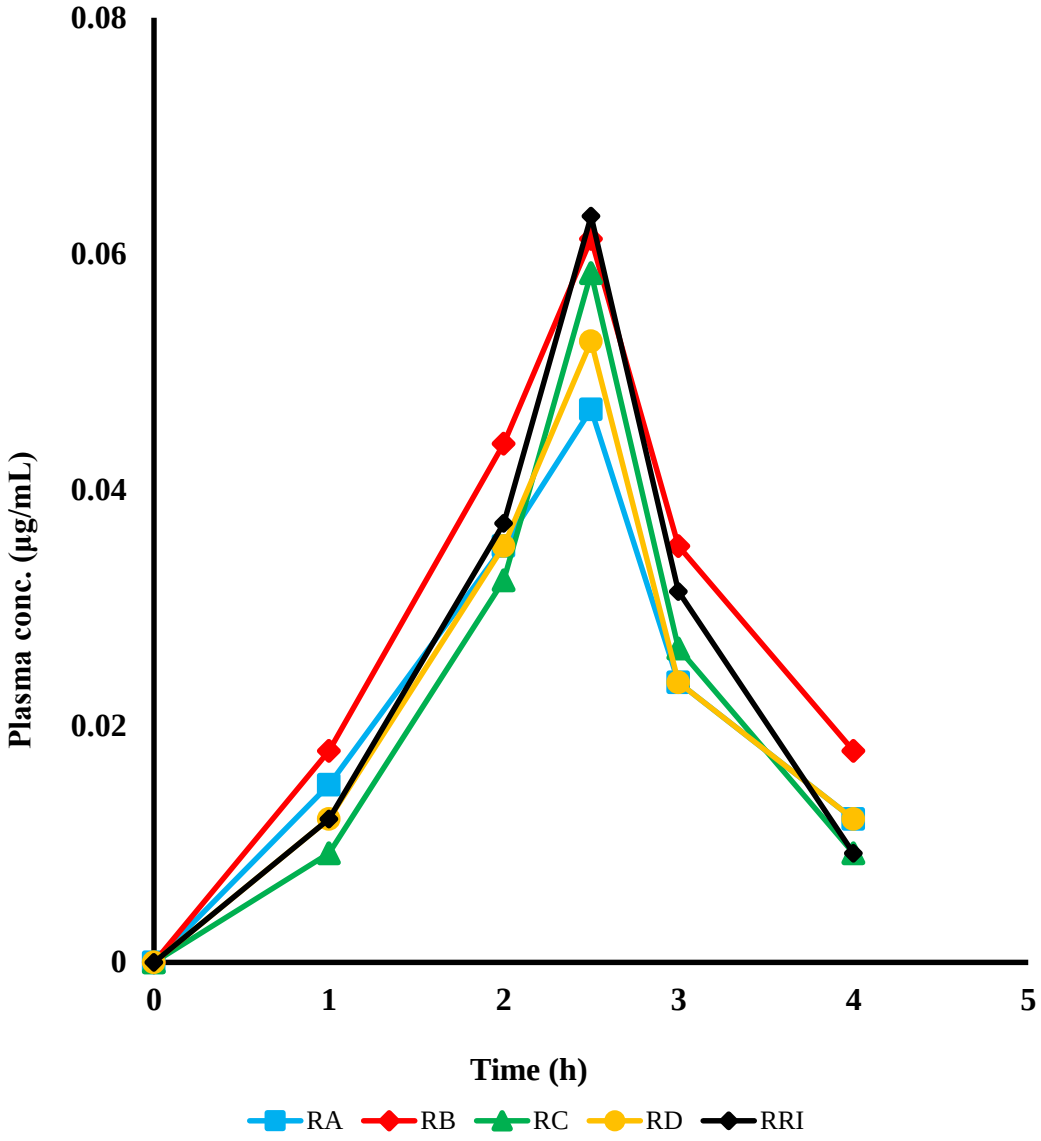


Figure 3.16: Comparison of plasma drug concentration– time curve of test brands of tablet ramipril with their reference innovator brand.

3.4 COMPARISON OF STABILITY UNDER STRESS CONDITIONS

Stability studies of test brands of tablet atenolol, tablet carvedilol, tablet losartan potassium and tablet ramipril including their respective innovator brands were done by stress degradation in acidic and basic conditions at different temperatures (29^0C, 60^0C and 70^0C). Figures 3.17– 3.24 showed no significant degradation of test brands and also their respective reference innovator brands. So, it can be assumed that all antihypertensive test brands and their respective reference innovator brands may be considered equivalent to respective reference innovator brands regarding stability. [101-103]

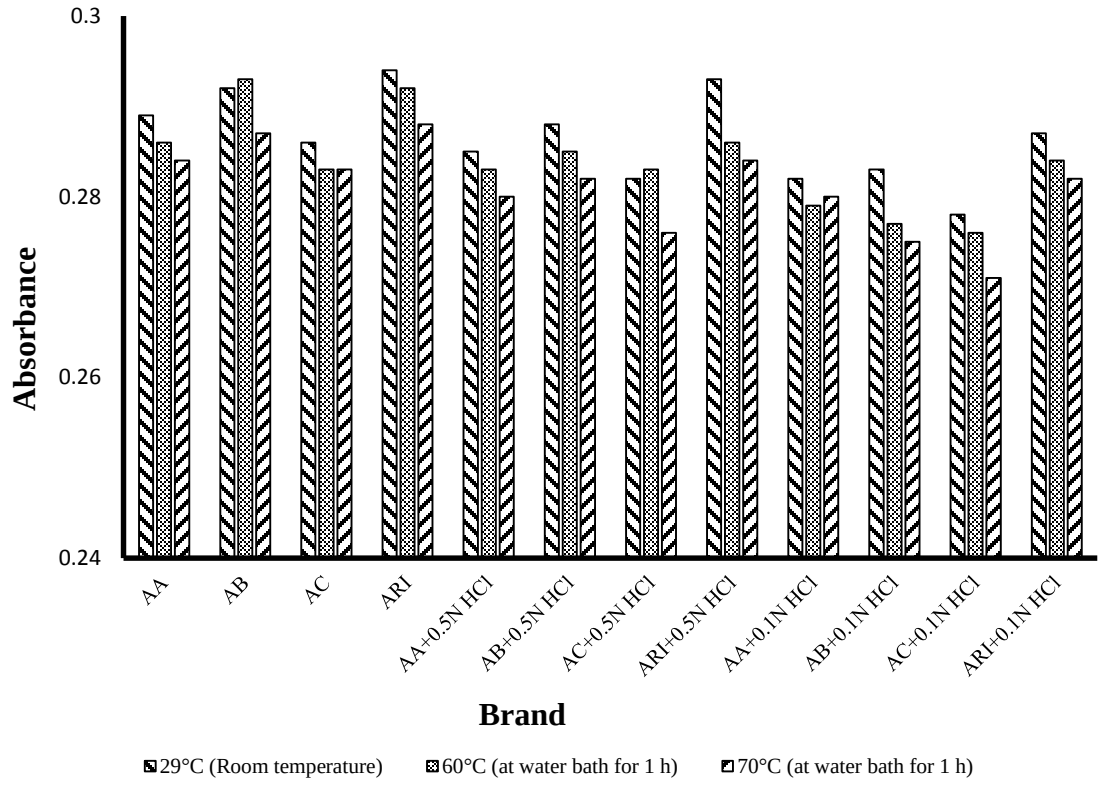


Figure 3.17: Comparison of stress degradation of test brands of tablet atenolol 50 mg with their reference innovator brand in acidic condition.

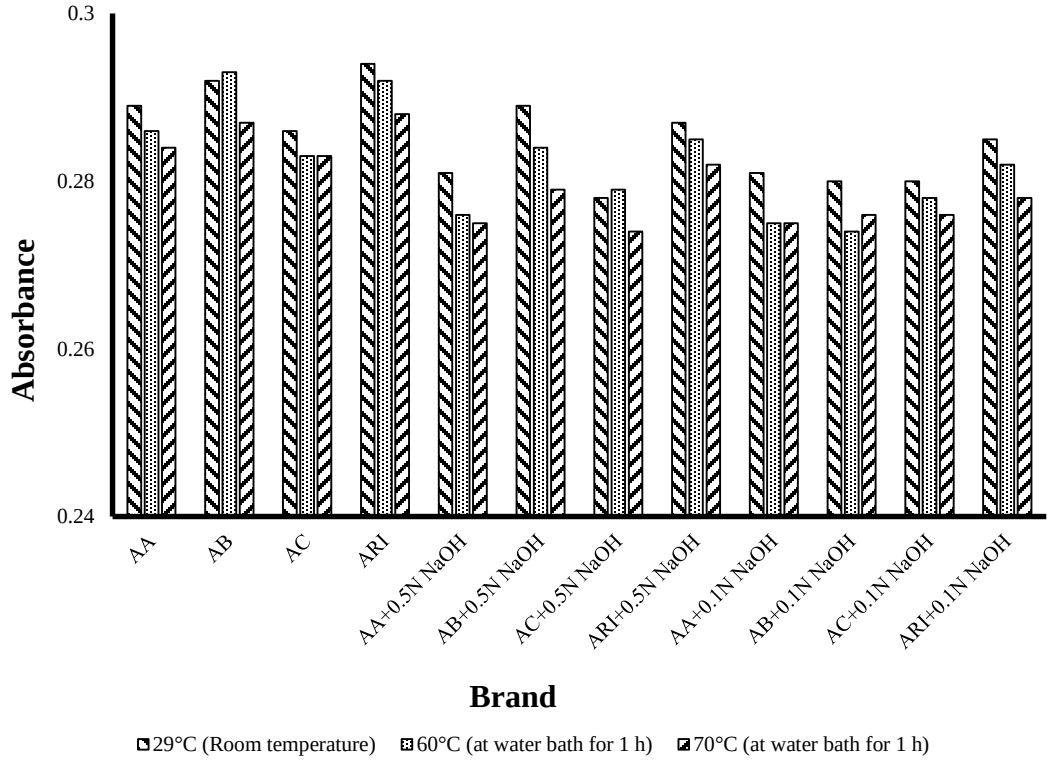


Figure 3.18: Comparison of stress degradation of test brands of tablet atenolol 50 mg with their reference innovator brand in basic condition.

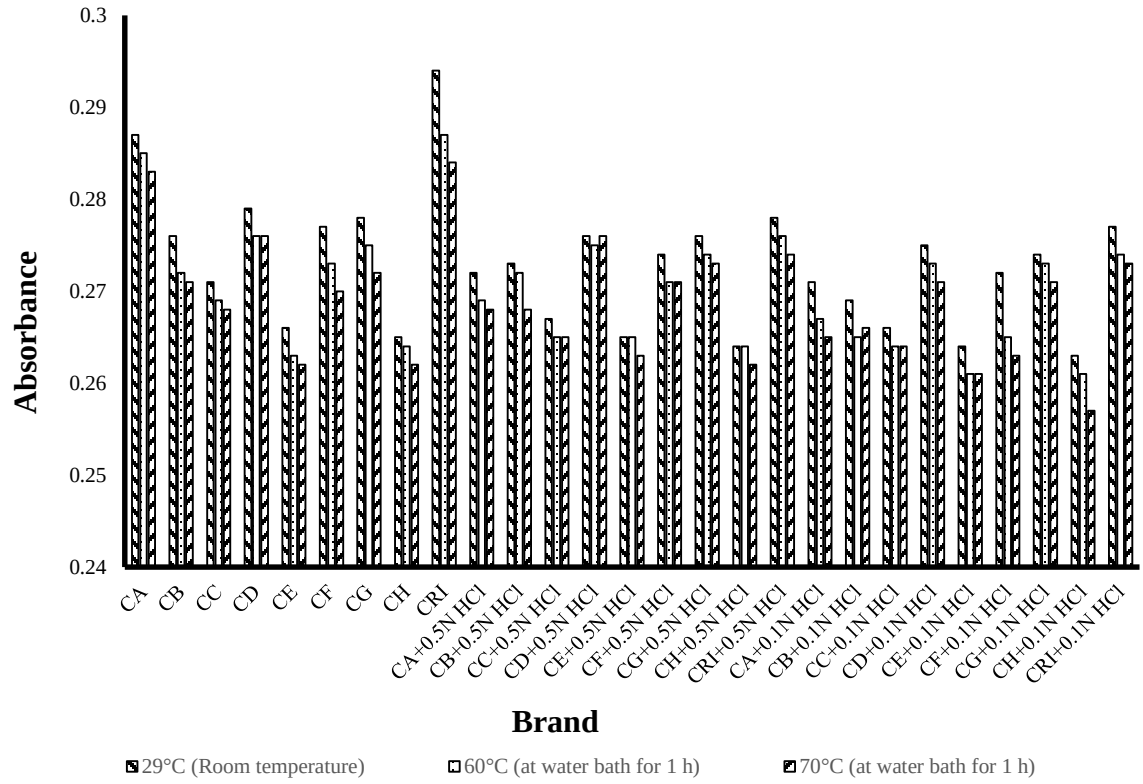


Figure 3.19: Comparison of stress degradation of test brands of tablet carvedilol 6.25 mg with their reference innovator brand in acidic condition.

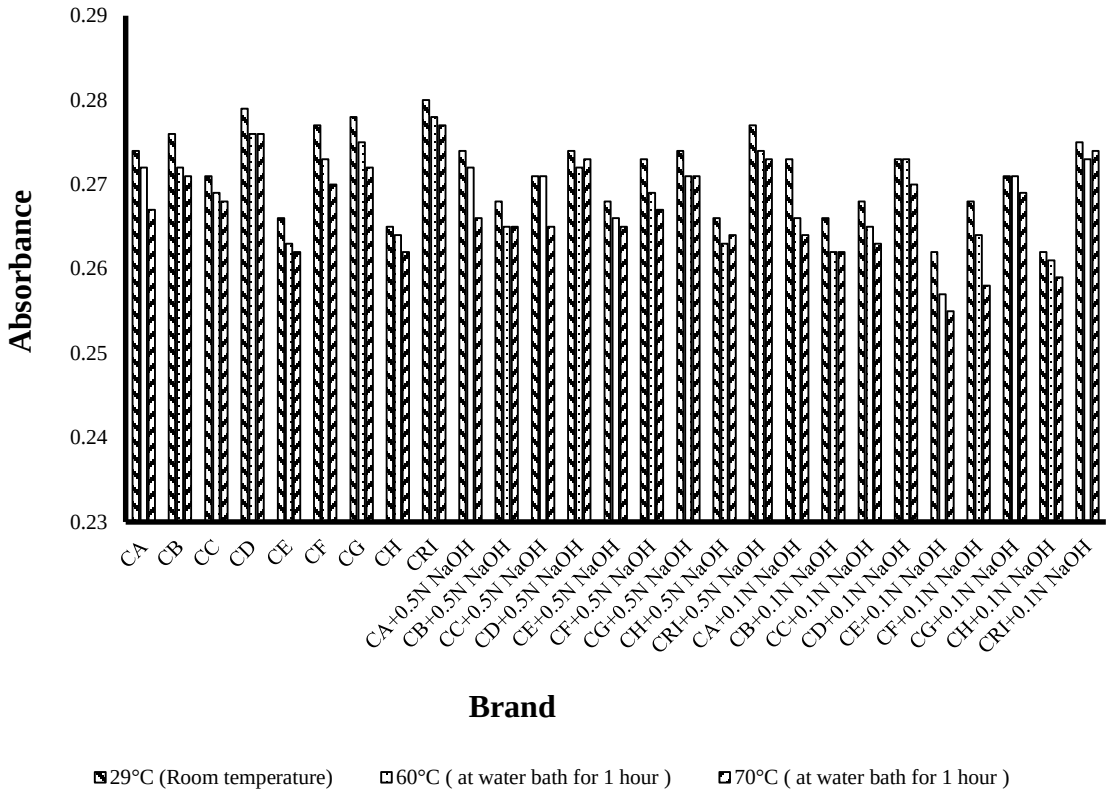


Figure 3.20: Comparison of stress degradation of test brands of tablet carvedilol 6.25 mg with their reference innovator brand in basic condition.

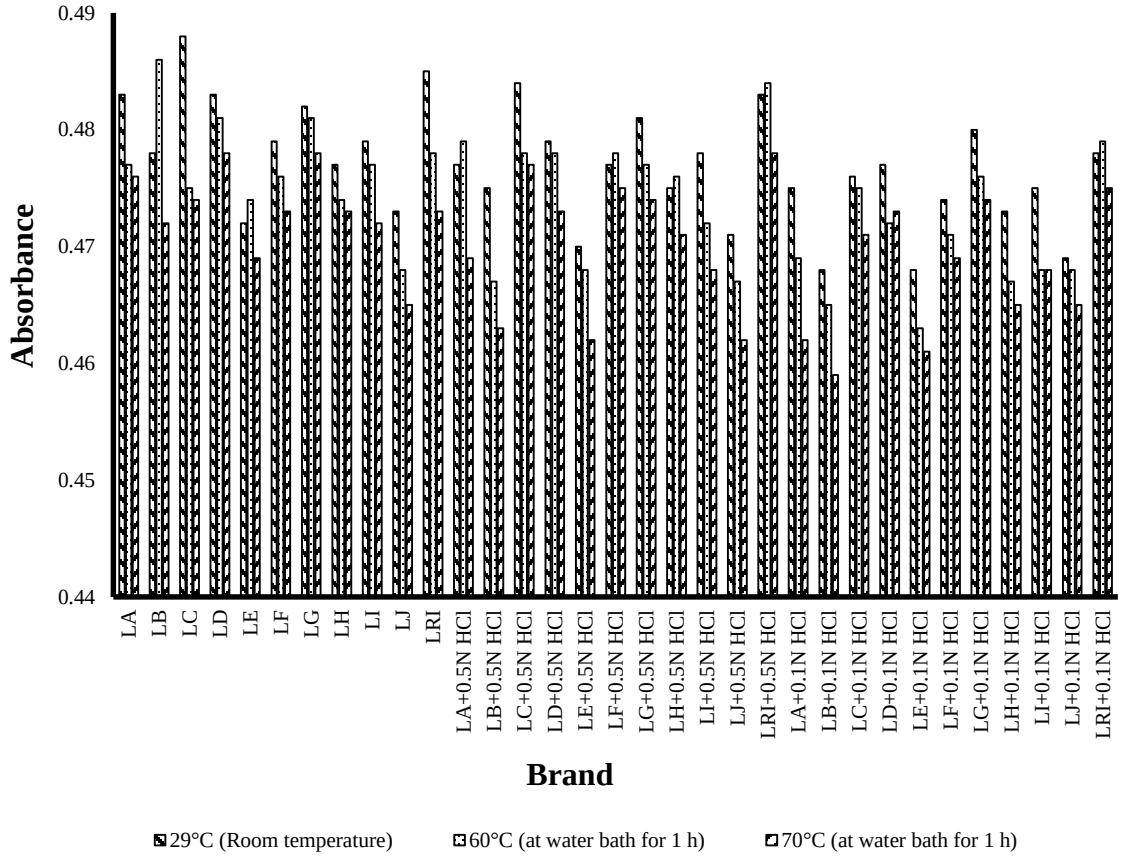


Figure 3.21: Comparison of stress degradation of test brands of tablet losartan potassium 50 mg with their reference innovator brand in acidic condition.

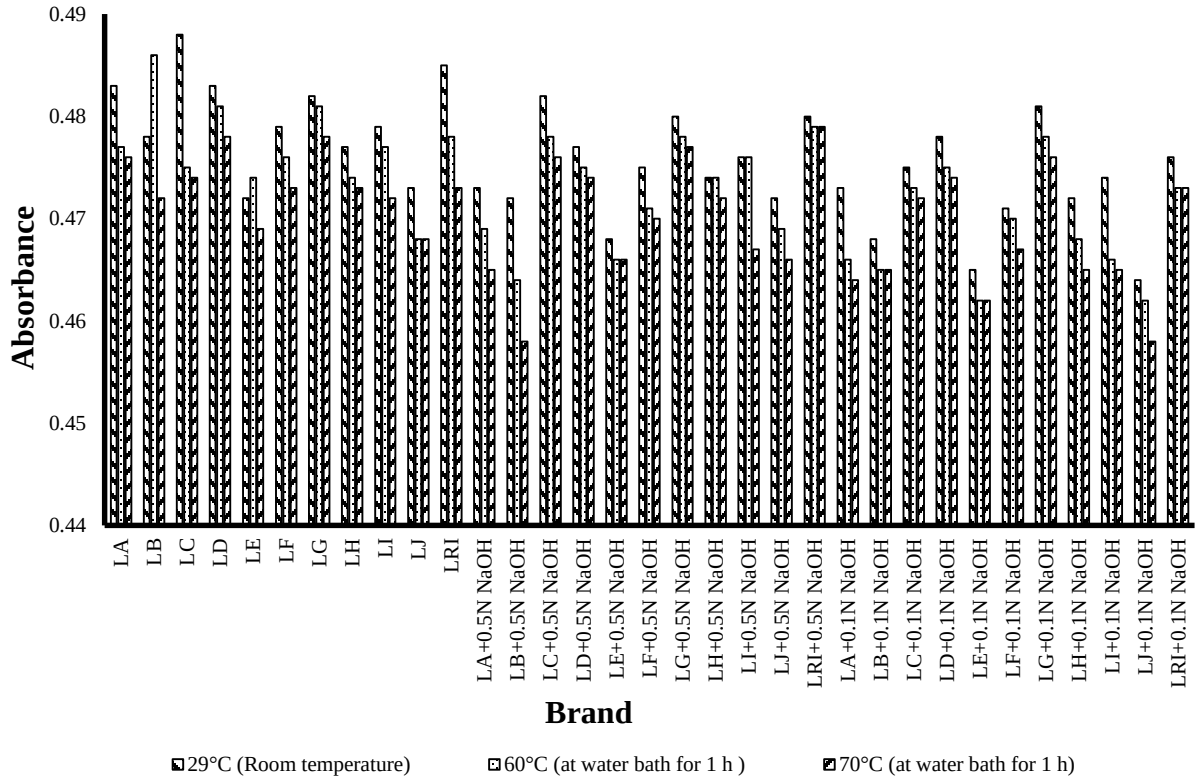


Figure 3.22: Comparison of stress degradation of test brands of tablet losartan potassium 50 mg with their reference innovator brand in basic condition.

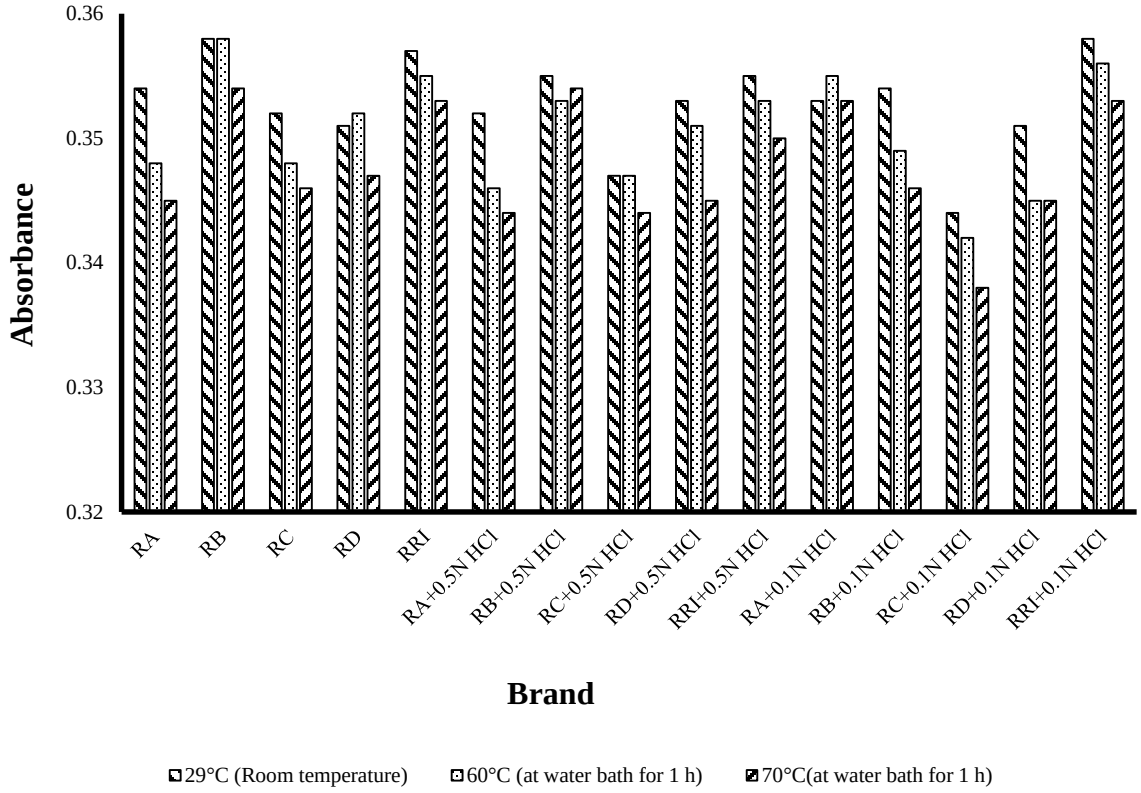


Figure 3.23: Comparison of stress degradation of test brands of tablet ramipril 5 mg with their reference innovator brand in acidic condition.

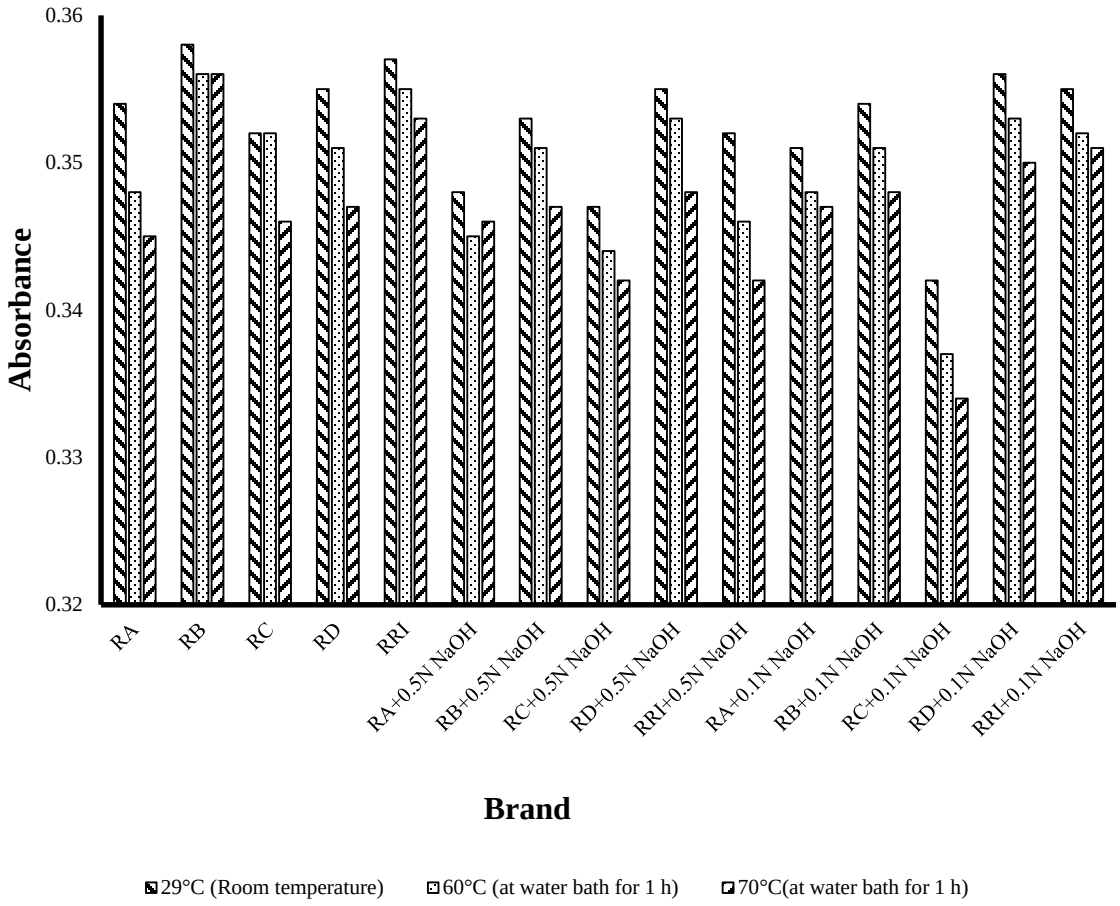


Figure 3.24: Comparison of stress degradation of test brands of tablet ramipril 5 mg with their reference innovator brand in basic condition.

CHAPTER FOUR

CONCLUSION AND RECOMMENDATIONS

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CONCLUSION AND RECOMMENDATIONS

4.1 CONCLUSION

The results obtained from this study revealed no significant difference between antihypertensive test brands and their respective reference innovator brands regarding general quality parameters such as weight variation, hardness, % friability, dissolution and % potency. In case of disintegration time of tablet losartan potassium and ramipril, significant variations were observed in test brands but they were within the specified limit. This study seemed to support a correlation between disintegration time and the rate of dissolution which was indicated in the previous study.[42] Except brand LB which showed low disintegration time but low dissolution rate and brand LC which showed high disintegration time but high dissolution rate.

In this study, parameters like $T_{50\%,}$ $T_{90\%,}$ difference factor (f_1) and similarity factor (f_2) derived from the dissolution profiles of test brands and their respective reference innovator brands were used as indicators for the availability of the drugs for absorption; thus, their equivalence. The i*n vitro* dissolution profiles showed variations in availability of drugs for absorption from the test brands and reference innovator brands.

All test brands of atenolol having $T_{50\%}$ values less than 10 minutes, $T_{90\%}$ values less than 30 minutes, $f_1$values less than 15 and f_2 values more than 50 seem to have very good bioavailability and hence, may be considered equivalent to their reference innovator brand. Except two test brands CB, CH; all other test brands of tablet carvedilol having $T_{50\%}$ values less than 10 minutes, $T_{90\%}$ values less than 30 minutes, f_1 values less than 15 and f_2 values more than 50 seem to have very good bioavailability. Test brand CB showed f_2 values less than 50 but $T_{50\%}$ values less than 10 minutes, $T_{90\%}$ values less than 30 minutes and f_1 values less than 15. Hence, it has also very good bioavailability. Brand CH showed $T_{50\%}$ values greater than 10 minutes, $T_{90\%}$ values greater than 30 minutes and f_2 values less than 50. It cannot be considered equivalent to reference innovator brand.

Except test brands LB, LD, LI; all other test brands of tablet losartan potassium having $T_{50\%}$ values less than 10 minutes, $T_{90\%}$ values less than 30 minutes, f_1 values less than 15 and f_2 values more than 50 appear to have very good bioavailability. Brands LB, LD and LI having $T_{50\%}$ values greater than 10 minutes, $T_{90\%}$ values greater than 30 minutes and f_2 values less than 50 cannot be considered equivalent to their reference innovator brand.

Except brand RC, all other test brands of tablet ramipril having $T_{50\%}$ values less than 10 minutes, $T_{90\%}$ values less than 30 minutes, f_1 values less than 15 and f_2 values more than 50 like to have very good bioavailability and may be equivalent to their reference innovator brand. Brand RC showed f_2 values less than 50 but $T_{50\%}$ values less than 10 minutes, $T_{90\%}$ values less than 30 minutes and f_1 values less than 15. Hence, it has also good bioavailability and may be equivalent to their reference innovator brand.

In vivo pharmaceutical equivalence study was done by plotting plasma concentration- time curves of test brands with their respective reference innovator brands after administration of drug in rat models. Comparing *in vivo* of C_{max} and t_{max} values of test brands with their respective reference innovators, all test brands may be considered equivalent to their respective reference innovator brands.

Stability studies by stress degradation in acidic and basic conditions at different temperatures (29^0C, 60^0C and 70^0C) revealed no significant degradation of test brands including their respective reference innovator brands. So, test brands also may be considered equivalent to reference innovator brands regarding stability.

In conclusion, this study indicated that except test brands CH, LB, LD and LI; all other test brands may be considered *in vitro* and *in vivo* pharmaceutically equivalent to their respective reference innovator brands and also similar in case of stability. These brands may be similar in quality, efficacy, safety and may be used interchangeably. But test brands CH, LB, LD and LI are not similar to their respective reference innovator brands and cannot be used interchangeably.

4.2 RECOMMENDATIONS

This study has emphasized that in vitro pharmaceutical equivalence and in vivo pharmaceutical equivalence studies in rat model of some antihypertensive drugs manufactured in Bangladesh do not indicate bioequivalency of these drug products in human body. One brand substituted with another brand on assumption of *in vitro* and *in vivo* pharmaceutical equivalence studies may always not be able to give the similar clinical effects. *In vivo* bioequivalence in human volunteers may be required for therapeutic equivalency of these antihypertensive drugs. Then these antihypertensive drugs will have identical clinical effects and safety profiles.

CHAPTER FIVE

REFERENCES

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REFERENCES

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APPENDICES

APPENDIX I

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THE *IN VITRO* PHARMACEUTICAL EQUIVALENCE STUDIES OF LOSARTAN TABLETS OF DIFFERENT MANUFACTURERS AVAILABLE IN BANGLADESH

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ABSTRACT

The study was aimed to assess the pharmaceutical equivalence of some losartan potassium tablets of different manufacturers marketed in Bangladesh using *in vitro* dissolution study. The dissolution was carried out using the apparatus II according to USP guidelines. Other general quality assessment tests like hardness, friability, disintegration time were also determined. All brands complied with the official specification for hardness, friability and disintegration time. The dissolution profiles showed inter brand and intra brand variability. All samples attained more than 85% dissolution within 30 minutes. The results were subjected to statistical analysis to compare the dissolution profiles. A model independent approach of similarity factor (f_2) was employed. The data indicated that only two brands may be used interchangeably.

Key words: Losartan potassium, pharmaceutical equivalence, *in vitro* dissolution.

INTRODUCTION

Blood pressure is the force of blood against the artery walls as it circulates through the body. Hypertension is the constant pumping of blood through blood vessels with excessive force. When blood pressure becomes persistently high then it is 140/90 mmHg or higher. Hypertension is an important public health challenge all over the world. An increasing trend in the prevalence of hypertension has been shown in the studies from India and Bangladesh.[1,2]

To treat hypertension the antihypertensive drugs are used. There are many classes of antihypertensive drugs available all over the world. The antihypertensive drugs lower blood pressure by different mechanisms. The most widely used antihypertensive drugs are the beta-adrenoceptor blockers, the centrally acting drugs, the ACE inhibitors and the angiotensin II receptor antagonists.[3]

Losartan potassium is one of the widely used antihypertensive drugs in Bangladesh. It is an angiotensin II receptor antagonist. It also reduces the combined risk of cardiovascular death, stroke and myocardial infarction in hypertensive patients with left ventricular hypertrophy and gives renal protection for type 2 diabetic patients with proteinuria.[4]

Pharmaceutical equivalence is the condition in which drug products; containing the identical quantity of active substance in an identical comparable dosage form, meet all applicable standards of identical strength, quality, purity and potency. The following criteria should be considered in the determination of pharmaceutical equivalence- (i) identical amount of active substance(s) (e.g. salt or ester), (ii) same dosage form or comparable dosage form (e.g. tablets versus capsules), (iii) same route of administration.

Pharmaceutical equivalence is not same to therapeutic equivalence which requires a product to be pharmaceutically equivalent and to have the same safety and efficacy profile after administration of the same dosage. Drug products are considered to be therapeutic equivalents only if they are pharmaceutically equivalents and if they have the same clinical effect and safety profile after administered to the patients.[5]

According to FDA, therapeutically equivalent drugs are those drug products that meet the following general criteria- (i) they are safe and effective; (ii) they are pharmaceutically equivalents in that they- (a) contain identical amounts of the same active drug ingredient in the same dosage form and route of administration, and (b) meet applicable standards of strength, quality, purity and identity; (iii) they are bioequivalent; (iv) they are adequately labeled and (v) they are manufactured in compliance with current Good Manufacturing Practice regulations.

Bioavailability refers to the rate and extent to which the active ingredient or therapeutic ingredient is absorbed from a drug product and becomes available at the site of drug action. Bioequivalence is the equivalent release of the same drug substance from two or more drug products or formulations. This gives to an equivalent rate and extent of absorption from these formulations. If a drug product containing chemically identical drug substance is delivered to the site of action at the same rate and extent as another drug product, then it is equivalent and can be substituted for that drug product. Methods used to define bioequivalence and bioequivalence studies include- (i) pharmacokinetic studies, (ii) pharmacodynamic studies, (iii) comparative clinical trials, and (iv) *in vitro* studies. The choice of study used depends on the site of action of the drug and the ability of the study design to compare drug delivered to that site by the two products.[6]

Despite the considerable use in Bangladesh, to the best of our knowledge, there are no reports on the pharmaceutical equivalence of the various losartan potassium tablets manufactured in Bangladesh. Therefore, in the present study, we set out to assess *in vitro* pharmaceutical equivalence of some losartan potassium tablets manufactured in Bangladesh. This study will help us to know the scenario of losartan potassium tablets manufactured in Bangladesh in respect of quality, safety and efficacy. The purpose of the study is to determine dissolution profiles of locally manufactured losartan potassium tablets and to compare those profiles statistically with drugs from innovator company (as reference) using similarity factor (f_2).

MATERIALS and METHODS

Drugs and chemicals: Standard losartan potassium was a kind gift from Healthcare Pharmaceuticals Ltd., Bangladesh. Three brands of losartan potassium (50 mg) were purchased from local drug store in Dhaka city. They were randomly designated as A, B and C. The local manufacturers are Incepta Pharmaceuticals Ltd., Opsonin Pharmaceuticals Ltd., and Orion Pharma Ltd. Tablet Cozaar 50 mg [Merck Sharp & Dohme, (New Zealand) Ltd.] was the innovator's product and it was designated as reference innovator (RI). Chemicals and all other reagents were of analytical grade and were purchased from local suppliers.

Preparation of stock solutions of losartan potassium: Hundred milliliter stock solution of 50 µg/mL was prepared by dissolving 0.05 g of losartan potassium in distilled water and made up to 100 mL with the same solvent. Ten milliliter of this solution was taken, diluted with distilled water and finally made up to 100 mL with the same solvent. The stock solution was diluted to the desired strength by distilled water.

Dissolution Medium: Distilled water.

Preparation of Calibration Curve: Serial diluted solutions of 4.0, 4.5, 5.0, 5.5, 6.0 µg/mL of losartan potassium were prepared from a stock solution (50 µg/mL) in distilled water. Absorbances were taken at 201 nm using a UV-Visible spectrophotometer (Model UV-800 Shimadzu, Japan). A plot of absorbance versus concentration of losartan potassium was made from which the regression equation was calculated.

Hardness test: The hardness was determined with an automatic tablet hardness tester (Model HDT–300F, Logan Instrument Corp.). Six tablets were randomly selected from each brand and the pressure at which each tablet crushed was recorded.

Friability test: Twenty tablets of each brand were weighed and subjected to abrasion by using a friability tester (Model FIB-2S Logan Instrument Corp.) at 25 rotation/minutes for 4 minutes. The tablets were then weighed and percentage friability was calculated.

Disintegration test: Six tablets of each brand were used for the test in distilled water at 37°C with an automatic disintegration tester (Model DST-3, Logan Instrument Corp.) employing plastic discs. The

disintegration time was taken as the time when no particles remained on the basket of the tester.

Dissolution test: The dissolution test was carried out using a dissolution tester (Model UDT-804, Logan Instrument Corp.) according to USP guidelines in 6 replicates for each brand.[7] The dissolution medium was 900 mL of distilled water which was maintained at 37.0±0.5°C. In all the experiments, 10 mL of dissolution sample was withdrawn at 10, 20, 30 and 45 minutes and replaced with equal volume of distilled water to maintain sink condition. Samples were filtered, diluted and the absorbance reading determined at 201 nm using spectrophotometer using distilled water as blank. The concentration was determined from the calibration curve of pure losartan potassium. The percent dissolutions were computed. The data were tailed and computed the means. The percent dissolutions of the samples and reference innovator were graphed against time. The values for $T_{50\%}$ and $T_{90\%}$ were determined as they are used as guides for dissolution.[8]

Analysis of similarity factor: The dissolution profiles were analyzed by a mathematical model, similarity factor (f_2). Mean dissolution values were employed to estimate the similarity factor (f_2). A factor value of 50 or greater (50-100) ensures sameness or equivalence of the two products. The following equations were used to calculate similarity factor (f_2).[9]

$$f_2 = 50 \times \log \left[\frac{100}{\sqrt{1 + \frac{\sum [Rt - Tt]^2}{n}}} \right]$$

Where n is the number of time points, Rt is the dissolution value of reference product at time 't' and T_t is the dissolution value for the test product at time.

Statistical analysis: The results were expressed as mean ± standard deviation (SD), where, n= 5.

RESULTS and DISCUSSION

Hardness is referred to as non-compendial test. It could influence other parameters such as friability and disintegration. A force of about 4kg is the minimum requirement of a satisfactory tablet.[10] The tablets of all brands were satisfactory for hardness. Tablets hardness was found to be within 6.28 to 8.44 kg (**Table 1**).

Friability test is included in the USP.[7] The standard specification for friability is 1%. Friability for all the brands was below 1% (**Table 1**). Disintegration times of all the brands were within the limit. The USP specifies that uncoated tablets should disintegrate within 15 minutes and film coated tablets in 30 minutes.[11] All losartan potassium tablets were film coated and disintegrated in <16 minutes (**Table 1**).

The calibration curve as shown in **Figure 1** has good correlation (r^2= 0.9961). The USP specifies that the amount of drug released (dissolution) should not be less than 80% of the labeled amount in 30 minutes. All brands complied with the specification. The dissolution mean values of the generic and reference innovator in water were shown in **Table 2**.The results of dissolution studies were presented in **Figure 2**. Both inter- and intra-brand variations in dissolution profiles were observed. Brand A released more than 90% drug within 30 minutes, brand B released less than 90% drug within 30 minutes and brand C released more than 95% drug within 30 minutes. From these data it was clear that although hardness, friability and disintegration time were almost similar within different brands but the brands differ in case of drug release. Similarity factor (f_2) has been adopted by FDA and the European Agency for the Evaluation of Medicinal Products to compare dissolution profiles.[12,13] Two dissolution profiles are considered similar and bioequivalent, if f_2 is between 50 and 100.[12] A $T_{90\%}$ of 30 minutes is satisfactory and is an excellent goal.[8] In this study parameters like $T_{50\%}$, $T_{90\%}$ and f_2 were derived from the dissolution profiles of the different brands. **Table 3** showed the f_2 values of different brands in respect of brand RI. For brand A and brand C, f_2 values were more than 50. So they are similar with brand RI and can be used interchangeably. For brand B, f_2 value was less than 50. So it is not similar with brand RI and can not be used interchangeably.

CONCLUSION

This study has emphasized that pharmaceutical equivalence does not indicate bioequivalence of drug product and one brand substituted with another brand on assumption of pharmaceutical equivalence may not give the desired onset of action and subsequent therapeutic effectiveness. However, *in vivo* test may be required for final comments regarding the quality of different brands of losartan tablet

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Table 1: Results of hardness, % friability and disintegration time of losartan tablet.

Formulation	Hardness ± SD	% Friability	Disintegration Time (minutes) ± SD
A	8.44 ± 1.03	0.04	10.29 ± 0.35
B	6.28 ± 0.28	0.03	6.88 ± 0.30
C	6.88 ± 0.53	0.34	15.22 ± 0.39
RI	6.89 ± 0.35	0.06	7.19 ± 0.62

Table 2: Mean percent dissolution of losartan tablet.

Time	RI ± SD	A ± SD	B ± SD	C ± SD
10	58.94 ± 3.37	49.28 ± 4.77	44.72 ± 2.92	53.59 ± 3.37
20	85.46 ± 3.86	71.13 ± 4.74	72.21 ± 2.66	84.39 ± 1.08
30	96.36 ± 2.91	91.68 ± 2.78	85.47 ± 1.21	95.85 ± 1.51
45	98.63 ± 1.73	99.18 ± 0.82	98.30 ± 1.91	99.97 ± 1.39

Table 3: $T_{50\%}$, $T_{90\%}$ and f_2 of three brands of losartan tablet.

Formulation	$T_{50\%}$ (minutes)	$T_{90\%}$(minutes)	Similarity factor (f_2)
A	>10	<30	52.26
B	>10	>30	47.58
C	<10	<30	76.21

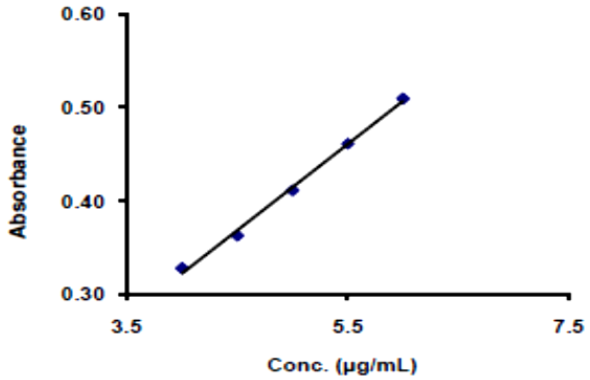

Figure 1: Calibration curve of losartan tablet for calculation of dissolution profiles.

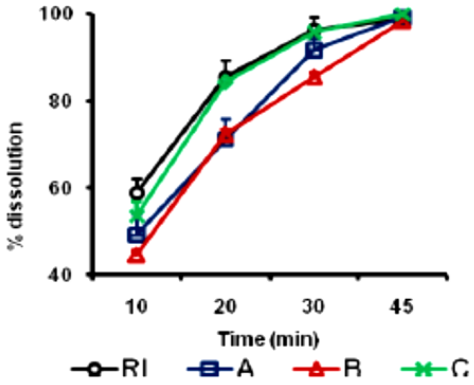

Figure 2: Comparison of dissolution profiles of different brands (A-C) of losartan tablet with innovator product (RI).

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In vitro Pharmaceutical Equivalence Study of Three Brands of Atenolol Tablets Available in Bangladesh

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ABSTRACT: The aim of the present work was to assess the pharmaceutical equivalence of three brands of atenolol (50 mg) tablets available in Bangladesh using *in vitro* dissolution study. The dissolution study was carried out using the paddle apparatus according to the guidelines of United States Pharmacopoeia (USP). The dissolution profiles of three locally manufactured atenolol tablets were determined and compared with the dissolution profile of atenolol tablet from innovator's company. All samples attained more than 85% dissolution within 10 minutes. Mean dissolution values were employed to estimate difference factor (f_1) and similarity factor (f_2). Difference factor (f_1) and similarity factor (f_2) were used to assess *in vitro* bio-equivalency among the three brands. Other general quality assessment parameters such as hardness, friability and disintegration time were also determined. All brands complied with the official specifications for hardness, friability and disintegration time. The study indicated that all brands can be prescribed interchangeably.

Key words: Atenolol, pharmaceutical equivalence, *in vitro*, dissolution.

INTRODUCTION

Hypertension is the most common cardiovascular disease in the world. The prevalence of hypertension increases with age. About 50% of people between the ages of 60 to 69 years old have hypertension, and the prevalence is further increased beyond age 70 in USA.[1] Hypertension is also common in our subcontinent. In India and Bangladesh, an increasing trend of hypertensive disease has been reported.[2] Elevated arterial pressure causes pathological changes in the vasculature and hypertrophy of the left ventricle. As a consequence, hypertension is the principal cause of stroke, a major risk factor for coronary artery disease and its attendant complications myocardial infarction and sudden cardiac death. Hypertension is a major contributor to cardiac failure, renal insufficiency and dissecting aneurysm of the aorta.[3]

The antihypertensive drugs are used to treat hypertension. There are different classes of antihypertensive drugs available all over the world. These drugs lower blood pressure by different mechanisms. The most widely used antihypertensive drugs are the β-adrenoceptor blockers, the centrally acting drugs, the ACE inhibitors and the angiotensin II receptor antagonists. Atenolol is a β-adrenoceptor blocker.[4] Atenolol is widely used as antihypertensive agent. It is widely used because of good patient acceptability and cardio-protective potentiality. This drug is also increasingly used for the treatment of angina pectoris, cardiac arrhythmias and myocardial infraction.[5]

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The bio-pharmaceutical characteristic of atenolol is described as sparingly soluble in water in British Pharmacopoeia (BP). On the basis of studied bio-pharmaceutical data, atenolol could be clearly classified into Bio-pharmaceutics Classification System (BCS) Class III.[6] BCS Class III drugs have high solubility and low permeability.[7]

Pharmaceutical equivalence is the condition in which drug products containing the identical quantity of active ingredient in an identical dosage form, meet all applicable standards of identical strength, quality, purity and potency. The following criteria should be considered in the determination of pharmaceutical equivalence - (i) identical amount of active ingredient(s); (ii) same dosage form or comparable dosage form (e.g. Tablets versus Capsules); (iii) same route of administration.[8]

Determination of pharmaceutical equivalence of tablets can be done by comparing the amount of active ingredient, dissolution time, hardness, friability and disintegration time of the test product against the reference product (innovator product).[9]

In recent years, FDA has placed more emphasis on a dissolution profile comparison in the area of post-approval changes and biowaivers. Under appropriate test conditions, a dissolution profile can characterize the product more precisely than a single point dissolution test. A dissolution profile comparison between pre-change and post-change products for scale-up and post approval change (SUPAC) related changes, or with different strengths helps assure similarity in product performance and signals bioinequivalence.[10]

Atenolol tablets are widely used in Bangladesh due to its effectiveness and affordable price. To the best of our knowledge, no reports are available on the pharmaceutical equivalence of various atenolol tablets manufactured in Bangladesh. The availability of numerous brands of atenolol tablets in drug market of Bangladesh makes physicians in a difficult situation to choice a suitable brand or to use of effective alternative brand.

Hence the present study was set out to assess the *in vitro* pharmaceutical equivalence of atenolol tablets manufactured in Bangladesh. The purpose of the study was to determine dissolution profiles of locally manufactured atenolol tablets and to compare those profiles graphically with drug from innovator's company (as reference standard). In addition to that the results were evaluated statistically using difference factor (f_1) and similarity factor (f_2). This study would provide a rationale for the interchangeability of the selected brands with the innovator brand.

MATERIALS AND METHODS

Drugs and chemicals. Standard atenolol was a kind gift from Healthcare Pharmaceuticals Ltd., Gazipur, Bangladesh. Three brands of atenolol (50 mg) tablets were purchased from the local market of Dhaka city. They were randomly designated as A, B and C. Tablet Tenormin 50 mg (AstraZeneca, the innovator company) was designated as reference innovator (RI). Chemicals and all other reagents were of analytical grade and were purchased from local suppliers.

Preparation of 0.1N acetate buffer, pH 4.6. 0.1N acetate buffer, pH 4.6 was prepared by mixing 44.9 parts (v/v) of 0.1N sodium acetate with 55.1 parts (v/v) of 0.1N acetic acid solution and adjusted with diluted acetic acid to a pH of 4.6.

Preparation of stock solution of atenolol. A stock solution (100 mL) of 50 µg/mL was prepared by dissolving 0.05 g of atenolol in 0.1N acetate buffer, pH 4.6 and made up to the mark volume with the same solvent. Then 10 mL from this was diluted with 0.1N acetate buffer at pH 4.6 and finally the volume was adjusted up to 100 mL with the same solvent. The resulting solution is called stock solution of 50 µg/mL. The stock solution was then diluted to the desired strength by 0.1N acetate buffer at pH 4.6.

Preparation of calibration curve. Serial diluted solutions of 5.0, 7.5, 10.0, 12.5, 15.0 µg/mL of atenolol were prepared from the stock solution (50 µg/mL) with 0.1N acetate buffer, pH 4.6. The absorbances were taken at 218 nm using a UV-Visible spectrophotometer (Model UV-800 Shimadzu, Japan). A plot of absorbance versus

concentration of atenolol was made from which the regression equation was calculated.[11]

Hardness test. The hardness was determined with an automatic tablet hardness tester (Model HDT-300F, Logan Instrument Corp., USA). Six atenolol tablets were randomly selected from each brand and the pressure at which each tablet crushed was recorded.

Friability test. Twenty atenolol tablets of each brand were weighed and subjected to abrasion by using a friability tester (Model FIB-2S, Logan Instrument Corp., USA) at 25 rev/min for 4 minutes. The tablets were then weighed and percentage friability was calculated.

Disintegration test. Six atenolol tablets of each brand were used for the test in distilled water with an automatic disintegration tester (Model DST-3, Logan Instrument Corp., USA) employing plastic discs. The disintegration time was taken as the time when no particles remained on the basket of the tester.

Dissolution test. The dissolution test was carried out using a dissolution tester (Model UDT-804, Logan Instrument Corp., USA) according to USP guidelines in 6 replicates for each brand.[12] The dissolution medium was 900 mL of 0.1N acetate buffer, pH 4.6 which was maintained at 37°±0.5°C. The dissolution tester was operated at 50 rpm. In all the experiments, 5 mL of dissolution sample was withdrawn at 5, 10, 15, 30 and 45 minutes and replaced with equal volume of 0.1N acetate buffer, pH 4.6 to maintain sink condition. Samples were filtered, diluted and the absorbences were taken at 218 nm using spectrophotometer where 0.1N acetate buffer, pH 4.6 used as blank. The concentrations of samples were determined from the calibration curve of pure atenolol. The percent dissolutions were computed. The data were tailored and computed the means.

Dissolution profile comparison using graph. The percent dissolutions of the samples and reference innovator were graphed versus time.

Determination of 50% and 90% dissolution. The time required for 50% dissolution ($T_{50\%}$) and 90% dissolution ($T_{90\%}$) were determined as they are used as good indicators for dissolution.[13]

Dissolution profile comparison using difference factor and similarity factor. A model independent mathematical approach was used to compare the dissolution profiles of the samples and the reference product using two factors, difference factor (f_1) and similarity factor (f_2). Mean dissolution values were employed to estimate difference factor (f_1) and similarity factor (f_2). The f_1 values up to 15 (0-15) and f_2 values greater than 50 (50-100) ensures sameness or equivalence of the test results and the reference product. The following equations were used to calculate difference factor (f_1) and similarity factor (f_2) for the studied tablets.[14]

$$f_1 = \{[\textstyle\sum_{t=1}^{n} |R_t - T_t|] \div [\sum_{t=1}^{n} R_t]\} \times 100$$

$$f_2 = 50 \times \log\left\{\left[1 + \left(\tfrac{1}{n}\right)\textstyle\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\}$$

Where, n is the number of time points, R_t is the dissolution value of reference product at time 't' and T_t is the dissolution value for the test product at time 't'.

Data analysis. The data were express as mean±standard deviation (SD).

RESULTS AND DISCUSSION

Hardness is referred to as non-compendial test. It may influence other quality parameters such as friability and disintegration. The crushing strength about 4 kp is the minimum requirement for a standard tablet.[15] Tablets of all brands were found to be satisfactory for hardness. Hardness was found to be within 4.55 to 6.13 kp for all brands. The results are shown in table 1.

Friability test is included in the United States Pharmacopoeia.[12] The standard specification for friability is not more than 1%. It was found to be less than 1% for each brand of tablets (Table 1).

Disintegration times of all the brands were within the limit. The British Pharmacopoeia specifies that uncoated tablets should disintegrate within 15 minutes and film coated tablets within 30 minutes.[16] All atenolol tablets were disintegrated in less than 2

minutes (Table 1). The calibration curve has good correlation ($r^2 > 0.9999$).

The United States Pharmacopoeia specifies that the amount of atenolol dissolved should not be less than 80% of the labeled amount in 30 minutes.[12] All brands complied with the specification. The dissolution mean values of the test products and reference innovator were shown in table 2.

The results of dissolution studies were presented graphically in figure 1. All the tested brands released more than 85% drugs within 10 minutes.

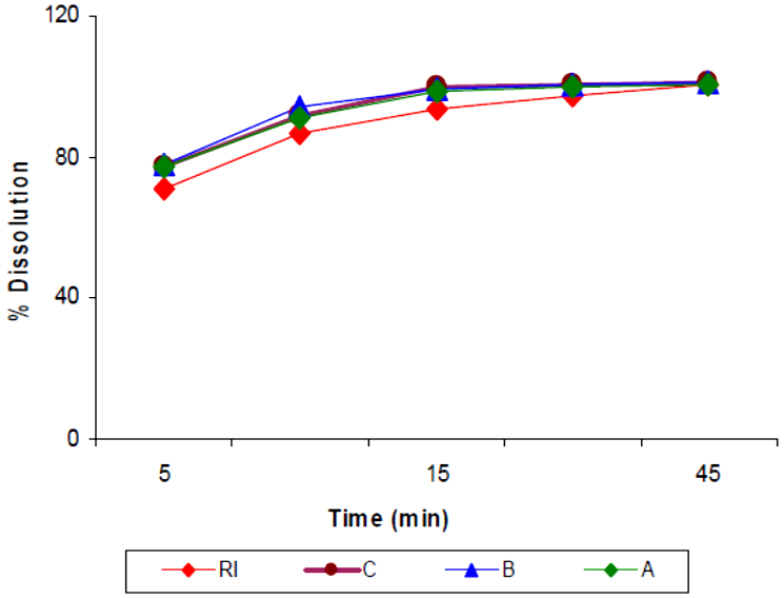

Figure 1. Dissolution profiles of different brands of atenolol tablets.

Table 1. Hardness, % friability and disintegration time of different brands of atenolol tablets.

Formulation	Hardness (kp)	% Friability	Disintegration time (minutes)
A	6.13 ± 0.37	0.097	1.23 ± 0.02
B	4.55 ± 0.15	0.044	0.43 ± 0.01
C	5.06 ± 0.39	0.038	1.36 ± 0.01
RI	5.32 ± 0.50	0.052	1.44 ± 0.02

Table 2. Mean percent dissolution of different brands of atenolol tablets.

	Brands of Tablets			
Time (minutes)	RI	A	B	C
5	70.76 ± 1.89	76.97 ± 2.91	78.16 ± 3.33	77.37 ± 1.64
10	86.58 ± 1.48	92.04 ± 2.66	94.40 ± 1.27	91.39 ± 1.34
15	93.67 ±1.47	99.86 ± 1.21	99.57 ± 1.19	98.68 ± 1.55
30	97.26 ± 1.43	100.60 ± 1.9	100.55 ± 0.77	99.81 ± 0.97
45	100.62 ± 1.22	101.17 ± 0.45	100.86 ± 0.67	100.26 ± 0.75

RI = Reference Innovator

Table 3. $T_{50\%}$, $T_{90\%}$, f_1 and f_2 values of different brands of atenolol tablets.

Formulation	$T_{50\%}$ (min)	$T_{90\%}$ (min)	Difference factor (f_1)	Similarity factor (f_2)
A	<5	<10	4.84	65.21
B	<5	<10	5.48	61.93
C	<5	<10	4.14	67.08

Similarity factor (f_2) has been adopted by the Food and Drug administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMEA) to compare dissolution profiles.[14,17] Two dissolution profiles are considered similar and bioequivalent, if f_1 is between 0 and 15 and f_2 is between 50 and 100.[14] In this study, parameters like f_1, f_2 and $T_{50\%}$, $T_{90\%}$ values were derived from the dissolution profiles of the different test brands of atenolol tablets. A $T_{90\%}$ of 30 minutes is satisfactory and is an excellent indicator of good dissolution.[13] Table 3 showed that brands A, B and C had $T_{50\%}$ values less than 5 minutes and $T_{90\%}$ values less than 10 minutes. Table 3 showed that f_1, f_2 values of different test brands in comparison of brand RI and it was observed that brands A, B and C had f_1 values less than 15 and f_2 values more than 50. They, therefore, were similar with brand RI and may be used interchangeably.

CONCLUSION

The results obtained from the *in vitro* pharmaceutical equivalence study of three brands of atenolol (50 mg) tablets showed that atenolol tablets of tested brands were equivalent to the brand of reference innovator. It can be inferred that these brands may have similar bioavailability and may be prescribed interchangeably.

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