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Bioavailability and Bioequivalence study of different brands [Telma (Glenmark), Telsartan (Dr.Reddy's), Indetel (ZydusAlidac), Telmikind (Mankind)] of Telmisartan

Raksha Gupta
raksha10567@gmail.com
Delhi Pharmaceutical Sciences and Research University, New Delhi

Dr. S. S. Agrawal
shyamagrawal.2006@gmail.com
Delhi Pharmaceutical Sciences and Research University, New Delhi

ABSTRACT

Bioavailability and bioequivalence studies play a major role worldwide and thus attract considerable attention globally in the development phase for both their new drug products and generic equivalents. Bioequivalence is a revolutionary strategy to introduce generic equivalents of branded drugs to lower the cost of medication through proper assessment as directed by the international regulatory authorities. The present study had been performed to explore interchangeability of branded generic drug products by comparing their bioavailability via in vivo study of four different brands. For each study period a set of 12 subjects completed both period with a 1O-day washout period. In both studies, the study formulations were administered after a 1O-12 hour overnight fast. For pharmacokinetic analysis, blood samples were drawn at baseline, O.25, O.5, O.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 hours after administration. Plasma concentrations of telmisartan were determined using HPLC coupled with a UV detector. All formulations were considered bioequivalent when evaluated against 9O% CI for the mean ratios were within the predetermined ranges of 8O% to 125%.in study the ratio analysis of AUC_O-t for T1, T2 and T3 were 96%, 99% and 91%; C_{max} was 98%, 99% and 95%; AUC_{O-T} was 98%, 1O1% and 92%; $AUC_{O-\infty}$ was 1O8%, 112% and 87%; and Kel were found 1O2%, 1O6% and 1O5% respectively. In both studies periods, All Test formulations met the regulatory requirements to assume bioequivalence, based on the rate and extent of absorption.

Keywords— *Telmisartan, Bioequivalence, Bioavailability, Pharmacokinetics, HPLC*

1. INTRODUCTION

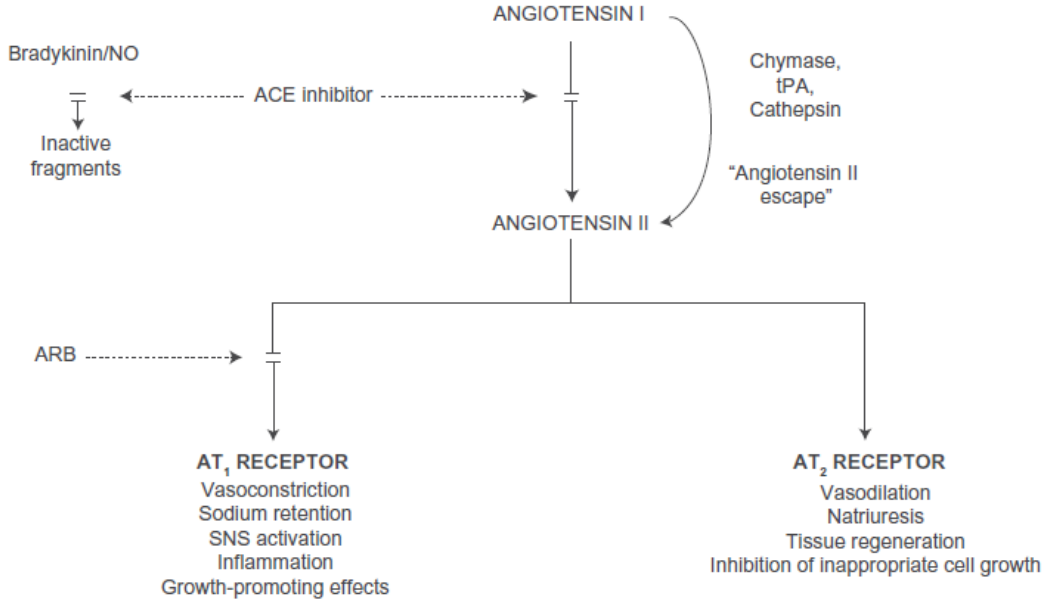
High blood pressure (BP) is ranked as the third most important risk factor for the attributable burden of disease in South Asia (2O1O). Hypertension exerts a substantial public health burden on cardiovascular health care systems in India. As per surveys, it was reported that hypertension exerts the fourth contributor to premature death in developed countries and the seventh in developing countries.[1]

Renin is secreted from juxta - glomerular cells of kidneys as prorenin which is a precursor molecule that metabolizes angiotensinogen to the inactive decapeptide angiotensin I (Ang I). Ang, I is metabolized to angiotensin II (Ang II) by angiotensin-converting enzyme (ACE), which is found in plasma as well as on plasma membrane of endothelial cells and a number of other cell types. ACE is a non-specific metalloprotease that comprises the activity of kininase II, which is responsible for the metabolism of bradykinin. Hence, inhibition of ACE leads to an increase in the levels of bradykinin, which is also responsible for the side effects of ACE inhibitors like a cough and angioedema.[2]

Telmisartan is an angiotensin II receptor blocker (ARB) represent a newer class of antihypertensive agent. It blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Its action is therefore independent of the pathways for angiotensin II synthesis.[6] There is also an AT2 receptor found in many tissues, but AT2 is not known to be associated with cardiovascular homeostasis. Telmisartan has a much greater affinity (> 3, OOO fold) for the AT1 receptor than for the AT2 receptor.[7]

Their mechanism of action of ARBs differs from that of the angiotensin-converting enzyme (ACE) inhibitors, which also affect the renin-angiotensin system. The main focus for development of ARBs were to overcome several of the deficiencies that are exerted by ACE inhibitors: competitive inhibition of ACE results in a reactive increase in renin and angiotensin I levels, which may overcome the blockade effect; angiotensin-converting enzyme is a relatively nonspecific enzyme that has substrates in addition to

angiotensin I, including bradykinin and other tachykinins, and thus, inhibition of angiotensin-converting enzyme may result in accumulation of these substrates; production of angiotensin II can occur through non-ACE pathways as well as through the primary angiotensin-converting enzyme pathway, and these alternative pathways are unaffected by angiotensin-converting enzyme inhibition; specific adverse effects are associated with angiotensin-converting enzyme inhibitor effects on the enzyme; and ARBs may offer more complete angiotensin II inhibition by interacting selectively with the receptor site[2].



The pharmacokinetics of orally administered Telmisartan is nonlinear over the dose range 2O to 16O mg, with greater than proportional increases of plasma concentrations with increasing doses. Food has a minimal effect on its bioavailability. Telmisartan shows bi-exponential decay kinetics with a terminal elimination half-life of approximately 24 hours and it is mainly excreted via the feces and only to a very minor extent (<1%) by the kidney. Telmisartan has been regarded as a highly variable drug with an intra-subject variability of Cmax (%CV ≥ 3O).

There are more than 15O brands of Telmisartan available worldwide. In this study, we decided to take four brands of Telmisartan(4O mg) with varying price 1. Telma, 2.Telsartan, 3.Indetel, and 4.Telmikind to check their bioequivalence. Here we took Telma as reference and Telsartan, Indetel and Telmikind as T1, T2 and T3 respectively. We evaluated the bioavailability of three different brands of Telmisartan tablet (4Omg) formulations following single dose administration in healthy volunteers after an overnight fasting of 1O hours in order to compare the bioequivalence.

2. SUBJECTS AND METHODS

2.1 Subjects

12 healthy Indian male and female volunteers between the age group of 18 to 5O years were enrolled in the study and their body mass index was in the range of 18.5O-24.9O Kg/m² (both inclusive). Written informed consent was taken from all volunteers prior to starting this study.

2.2 Study design and procedures

The study was a single dose, randomized, open-label, balanced, two-period. After an overnight fasting, subjects were received a single dose of the either of any four brands of Telmisartan tablet. The washout period was of 1O days. The study was explained to the subjects and then written informed consent was taken from all the subjects according to the Schedule Y of the Drug and Cosmetic Act & Rules, 1945 of India. After signing the informed consent, clinical examination was done and routine clinical chemistry tests were performed at the DIPSAR clinical research. The peripheral venous catheter was placed in the antecubital vein of the subjects and it was flushed with O.5 ml of heparin in normal saline (NS) solution (1:2O), 1 ml blood was discarded to ensure that the sample was free from heparin and NS solution. For the determination of the amount of in plasma, blood samples (4ml) were collected in K_2EDTA vacutainers at the given times: predose and O.25, O.5, O.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 hours. These blood samples were centrifuged at 2OOO rpm for 2O minutes at 4°C. Resulting plasma samples were separated and stored at -75°C until analysis. [3-4]

2.3 Chemicals and reagents

Methanol and acetonitrile were of HPLC grade (CDH P Ltd). Ammonium acetate, acetic acid, and NaOH used were of analytical grade. The ammonium acetate buffer pH 6.O was prepared by dissolving O.77 g in a liter of distilled water and pH was adjusted to 6.O using acetic acid and O.1 N NaOH. Telma tablets were manufactured under license of Glenmark. Each tablet contains 4O mg TELM. Drug-free human plasma was obtained from subjects.[5]

Calibration standards (CS) and quality control (QC) samples in human plasma

2.3.1 Preparation of Stock solutions

Stock solutions were prepared by dissolving of Telmisartan in methanol to obtain a concentration of 1 mg ml^{-1}. The solution was prepared by dissolving 1OO mg of drug in sufficient amount of methanol and the volume was completed to 1OO ml volumetric flask with the same solvent.

2.3.2 Preparation of working standard solutions

Working standard solutions were prepared by transferring different volumes O.5–5 ml of stock Telmisartn in 1O ml volumetric flask and the volume is completed with methanol. Volumes of 2O ul of working standard solution were added to 96O ul of drug-free human plasma to obtain drug concentration levels of 1–1O ug ml^{-1} for Telmisartan.

2.3.3 Preparation of Quality Control (QC) samples

Quality control (QC) samples were prepared separately and pooled at three different concentration levels 3O ng/ml (LQC), 25O ng/ml (MQC) and 9OO ng/ml (HQC) as low, medium and high, respectively. A calibration curve was constructed from a blank sample, and non-zero samples of concentrations 1O, 25, 5O, 75, 1OO, 25O, 5OO, 75O and 1OOO ng/ml.

2.3.4 Plasma sample preparation

The stored plasma samples were allowed to thaw at room temperature before processing. The plasma samples were centrifuged at 4OOO rpm for 1O min, an aliquot (O.96 ml) was pipetted into a 1O-ml polypropylene tube and acetonitrile (2.O ml) was added. The mixture was vortex mixed briefly, and after standing for 5 min at room temperature, the mixture was centrifuged at 4OOO rpm for 2O min. the supernatant was carefully transferred into a vial and injected into the HPLC system.

3. METHOD VALIDATION

The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability, and system suitability according to ICH guidelines. Guidelines for the validation of the bioanalytical method.

3.1 Validation of HPLC/UV Assay Method

Chromatogram of drug-free plasma, plasma spiked with Telmisartan (1OO ng/ml) is shown in figure 1, the retention time was 2.5 mint (Figure 2) all pikes were separated and there was no interference from endogenous substances in a biological matrix with the drug peak.

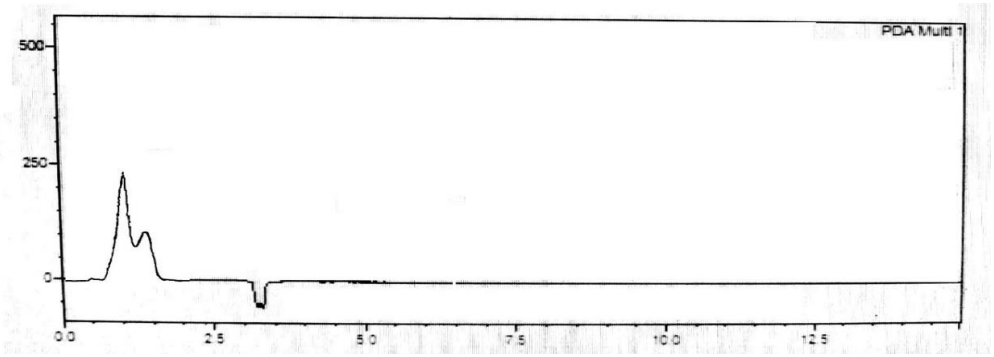


Fig. 1: HPLC chromatogram of drug-free human plasma.

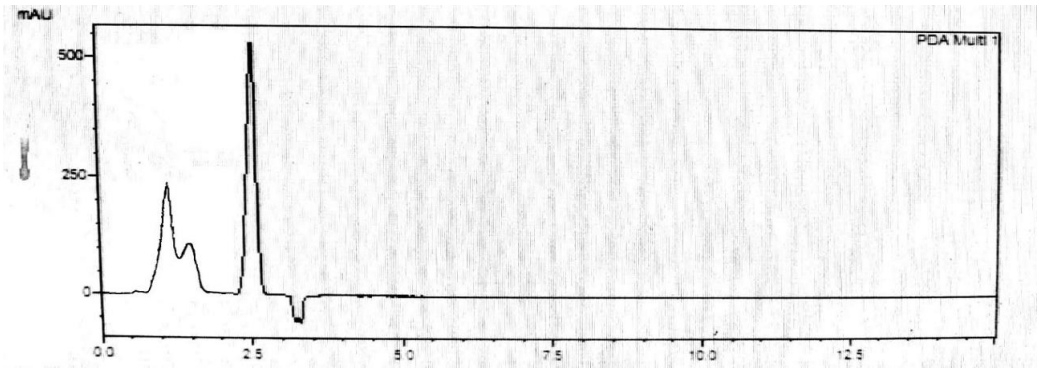


Fig. 2: HPLC Chromatogram of Human Plasma spiked with Telmisartan(12Ong/ml)

3.2 Linearity and range

The mean regression equation of three standard curves for TLM was y = 6712.5x - 9148O, where y presented peak area of drug and x was the plasma concentration of the drug. The calibration curve was linear over the studied concentration range (15–12O ng/ml) with a mean correlation coefficient more than O.99 (Figure 3).

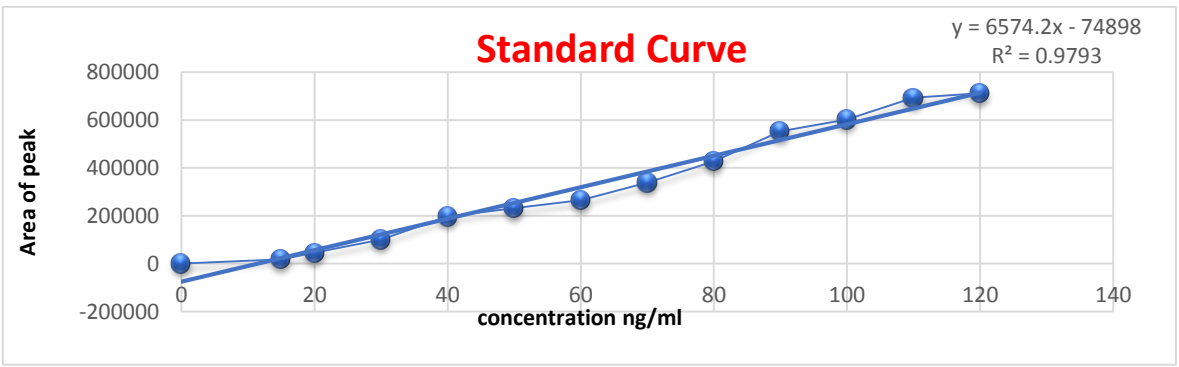


Fig. 3: Calibration curve of Telmisartan in human plasma by HPLC/UV analysis

3.3 Selectivity

It is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components of the samples. Each blank sample was evaluated for interference with the respective drug. The results revealed that the analyte Telmisartan was well separated from the co-extracted material under the adopted chromatographic conditions. The retention time (Rt) was 2.5 min (Fig 2) In addition to this, the chromatogram of extracted plasma samples did not show any coeluting interference peak with the analyte which suggested a good degree of selectivity for the developed method.

3.4 Accuracy and precision

The accuracy of an analytical method describes the closeness of test results to the true concentration of analytes whereas the precision is a measure of the degree of reproducibility of the analytical method. The results of intra-day and inter-day precisions are summarized in table 1. Which revealed that the developed method was accurate and precise for quantification of TLM in plasma samples.

Table 1: Intra and intra-day accuracy and precision of the bioanalytical method

Nominal Conc.	SD (n=6)	%RE (relative Error)		%CV	
		Intra-day	Inter-day	Intra-day	Inter-day
LQC	O.98	3.5	4.3	6.4	4.3
MQC	3.46	2.1	3.5	4.2	3.5
HQC	6.13	1.5	2.3	3.4	2.8

3.5 Recovery (Extraction efficiency)

The high value of recoveries for TLM specified that insignificant amounts of the drug were lost during plasma protein precipitation step. Lower values of %CV advocated a high degree of an extraction efficiency of the developed method.

Table 2: Recovery study of bio analytical method

Nominal concentration (ng/ml)	Experimental concentration (ng/ml) mean	S.D.	Precision (%CV)	Absolute recovery (%)
LQC, 2O	19.74	O.13	O.65	98.7
MQC,6O	58.89	1.O9	1.85	98.15
HQC,12O	119.87	O.91	O.91	99.89

3.6 Stability

The stability tests of the analytes were designed to cover expected conditions concerning the handling of clinical samples. The stabilities of the analytes in human plasma were investigated under various storage and processing conditions. The results are summarized in Table 3. The results indicate that Telmisartan was stable for the entire period of the experiment.

Table 3: Summary of stability of Telmisartan in human plasma in the varying condition

Telmisartan Concentration (ng/ml)		
	6Ong/ml	12Ong/ml
A) Three Free Thaw Cycles		
Mean	58.45	112.56
S.D.	O.81	1.31
%CV	1.38	1.16
%Recovery	97.41	93.8
B) Room Temperature for 24h		
Mean	59.95	118.O2
S.D.	O.98	1.18
%CV	1.63	O.99
%Recovery	99.9	98.35
C) Re-injection after 15 days at -2O C		
Mean	58.13	117.8
S.D.	1.21	1.3
%CV	2	1.1
%Recovery	96.66	98.16

The lower limit of quantification (LLOQ)

The lowest limit standard on the calibration curve is accepted as the limit of quantification if the analyte response at the LLOQ is at least 3 times the response compared to blank response and the analyte peak is discrete, identifiable, and reproducible with a precision of 2O% and accuracy of 8O-125%. The LLOQ for Telmisartan was found to be 1O ng/ml.

Table 4: Shows ratio analysis of the pharmacokinetics parameters viz. Cmax, Tmax, AUCO-t, AUCO-, Kel(h-1) of three test brands

Brand	Cmax(ng/ml)	Tmax(hr)	AUCO-t	AUCO- (nghr/ml)	Kel(h-1)	limits
T1	O.98	O.96	O.98	1.O8	1.O2	O.8O-1.25
T2	O.99	O.99	1.O1	1.12	1.O6	O.8O-1.25
T3	O.95	O.91	O.92	O.87	1.O5	O.8O-1.25

3.7 Statistical analysis

a) Analysis of Variance (ANOVA)

The statistical power approach for assessing bioequivalence using two-way ANOVA was also done on C_{max}, $T_{max,}$ AUC_{O-t}, $AUC_{O-\infty}$, K_{el} under the hypothesis of no differences between AUC, $C_{max,}$ and T_{max} of reference and other three branded formulations. ANOVA was applied to determine the effect of factors like period, sequence, subject within sequence and treatment on study results. No significant effect was noted for period, sequence and treatment.

Based on statistical power approach for assessing bioequivalence using two-way ANOVA the rate and extent of absorption were not different for three brands compared to the innovator. No statistical differences were found in pharmacokinetic parameters (C_{max}, $T_{max,}$ AUC_{O-t}, $AUC_{O-\infty}$, and K_{el}) of Test1, Test3, and Test3 compared to Reference.

b) Bioequivalence Evaluation

The 9O% confidence interval for log-transformed data was calculated for C_{max}, $AUC_{O-t,}$ and $AUC_{O-\infty.}$ For C_{max} the lower limit and the upper limit with respect to Reference was O.99, 1.OOO2 and O.9956, AUC_{O-t} was 1.OO47, O.9999 and O.9589, and $AUC_{O-\infty}$ was O.9964, 1.OO12 and O.9754 for Test1, Test2 and Test3 respectively. Therefore, it was found that at 9O% confidence level, all the three brands met the criterion for bioequivalence and all are equivalent.

4. SUMMARY AND CONCLUSION

The bioavailability and bioequivalence of three brands of Telmisartan namely 'Telsartan' (Dr.Reddy's), 'Indetel' (Zydus Alidac), Telmikind (Mankind) were evaluated in healthy human volunteers using the reference product 'Telma' (Glenmark) as the reference when given in equal labeled doses i.e. 4Omg orally.

Among the 16 brands available in the market these three brands were selected on the market these brands were selected on the basis of the highest, mid and lowest price as one strip of 4O mg strength of 'Telsartan' costs Rs.1O9.OO while for 'Telma', 'Indetel' and 'Telmikind' brands the price is Rs. 85.OO, 39.OO, 19.OO respectively.

In all the brands, C_{max} of Telmisartan was 1hr suggesting rapid absorption. The relative bioavailability of three brands 'Telsartan', 'Indetel' and 'Telmikind' compared with the reference brand 'Telma' was within limit i.e. O.8-1.25 for untransformed data at 9O% confidence level. C_{max} ratio of each brand compared to 'Telma' was within acceptable limit O.8-1.25 at 9O% confidence level demonstrating that all brands are bioequivalent.

The ratio analysis of pharmacokinetic parameters viz Cmax, Tmax, AUCO-t, AUCO-∞ and Kel for all brands was within limit i.e. O.O-1.25 suggesting that the rate and extent of absorption for the three brands met bioequivalence criterion at 9O% confidence level. Two-way ANOVA revealed no statistically significant difference in the rate and extent of absorption of glimepride among the four brands (P > O.OO1), indicating that all the brands are bioequivalent and hence truly interchangeable.

Finally, it can be concluded that the reference and test product of Telmisartan in this study were found to be bioequivalent.

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