BIOEQUIVALENCE EVALUATION OF TWO CLARITHROMYCIN TABLET FORMULATIONS: A RANDOMIZED, SINGLE-DOSE, OPEN-LABEL, TWO-TREATMENT PERIOD CROSS OVER STUDY IN HEALTHY MALE PAKISTANI VOLUNTEERS

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ABSTRACT

Objective: To conduct the bioequivalence study of commercially available two brands of clarithromycin tablets (500 mg) in healthy Pakistani male volunteers.

Methodology: The study was performed using a randomized, open labeled, two treatment periods and cross over study design. Healthy volunteers (n =12) were recruited following strict inclusion criteria. Their blood samples were collected at various time intervals over a period of 24 hrs after oral administration of test and reference formulations of clarithromycin tablets (500 mg). A validated reversed-phase high performance liquid chromatographic method with ultraviolet detection (RP-HPLC-UV) was used for the quantification of plasma concentrations of clarithromycin. Various pharmacokinetic parameters were determined using non-compartmental analysis approach.

Results: The C_{max}, t_{max}, AUC_{0-t} and half-life ($t^{1/2}$) values of clarithromycin from test formulation were 2.882 ±0.13 µg/mL, 1.75 ±0.45 hr, 11.33 ±0.68 µg.hr/mL and 2.84 ±0.17 hr, while for reference formulation these were 3.089 ±0.19 µg/mL, 1.83 ±0.39 hr, 12.87 ±1.09 µg.hr/mL and 3.10 ±0.28 hr, respectively. The 90% confidence interval values of C_{max}, t_{max}, AUC_{0-t} and half life for test and reference formulations of clarithromycin were 0.89-0.98, 0.80-1.14, 0.84-0.93 and 0.88-0.95, respectively, which satisfied the acceptance ranges of WHO and FDA bioequivalence guidelines i.e., 0.80-1.25. Similarly, in-vitro evaluation studies were also performed for both test and reference formulations of clarithromycin as specified in the official monographs in USP-30. Both formulations qualified all the in-vitro tests as per specifications in their respective monographs. The f1 and f2 values were also within the acceptance ranges for test and reference formulations.

Conclusion: The test and reference formulations of clarithromycin were bioequivalent.

Key Words: Bioequivalence, Clarithromycin, RP-HPLC-UV, Pharmacokinetics

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INTRODUCTION

Clarithromycin is a semi-synthetic broad-spectrum macrolide, having excellent antibacterial spectrum against a big number and range of strains of bacteria. Mechanism of its activity is mainly associated with its inhibitory action of protein synthesis. It can be efficiently and safely used in various infections such as streptococcal tonsillitis, oropharyngitis, acute otitis media, maxillary sinusitis, pertussis, pneumonia, mycoplasma pneumoniae, skin and skin structure infections, mycobacterium avium-intracellular infections, other non-tuberculous mycobacterial infections, helicobacter pylori and gastroduodenal infection[1]. Clarithromycin metabolism takes place in the liver by the cytochrome P-450 complex to at least 8 metabolites including a microbiologically-active metabolite i.e., 14-hydroxy clarithromycin[2,3]. It is mainly excreted through renal and hepatic routes of excretion; however, some dose adjustment may be required in some forms of renal impairment[4].

In Pakistan, significant variety and considerable bulk of pharmaceutical dosage forms are produced by na-

tive pharmaceutical industry. There is great difference among retail prices of respective brand of same generic drug and products of local companies, which are substantially more inexpensive than international contestants. Currently, more than 20 brands of clarithromycin tablet formulations are obtainable from our national market[5], but reported data of their bioequivalence with special reference to currently executed clinical trial followed by in-vivo quantitation by HPLC coupled with UV detection can rarely be found.

This research work was conducted, considering the assigned project by the Federal Ministry of Health (MOH), Pakistan, to relate the bioequivalence of local brand/manufactured dosage form of clarithromycin tablet versus reference standard. During this study, their dissolution profiles, comparative blood concentrations and other pharmacokinetic values were evaluated as per stated guidelines of U.S. Food and Drug Administration and the European Medicine Evaluation Agency[6,7].

METHODOLOGY

Clafax® tablets (500 mg), from local manufacturer Medicraft Pharmaceuticals Pvt. Ltd. (Peshawar, Pakistan), were used as test formulations, whereas Klaricid® tables (500 mg), from Abbott Laboratories Pvt. Ltd. (Karachi, Pakistan), as reference formulations.

Clarithromycin standard (purity 99.3%) and Diclofenac sodium reference standard (purity 98.9%) were used as raw materials. Solvents used for mobile preparation were of HPLC grade and included triethylamine, ammonium acetate, acetonitrile and methanol. Clarithromycin tablets were purchased from the local market.

The in-vitro release profiles of active ingredient (clarithromycin) from its tablet dosage form were studied using Dissolution Test Apparatus, Model DA-8D3+ of Instrumends® Pvt. Ltd., (Lahore, Pakistan).

Chromatography was performed using a RP-HPLC added with UV-Visible detector (Perkin Elmer Series-200; Norwalk, USA). The output of the detector was monitored with a chromatocorder12 (SIC System Instruments). A Discovery® HS-C_{18} column (250 x 4.6 mm, 5µm; Bellefonte PA, USA) was used for the separation of analytes.

The analysis of clarithromycin samples were performed at ambient conditions using isocratic mode of separation. Mixture of solvents and acetate buffer was used as mobile phase at ratio of acetate buffer (pH 6.0), methanol and acetonitrile (40:10:50 v/v). The lambda maximum was fixed at 205 nm in the detector. The flow rate of mobile phase for elution purpose was maintained at 1.5 mL/min.

Clarithromycin contents in both formulations and blood samples were assessed by reported chromatographic method of analysis with appropriate modifications and validation regarding following parameters.

The linearity of the method was determined by spiking standard mixtures of clarithromycin in the concentration range of 0.2 to 10 µg/mL and diclofenac sodium (at a fixed concentration of 0.5µg/mL) into the serum samples. Calibration curves for standard solutions (in the mobile phase) and spiked samples were calculated by square of regression values.

The precision of the method was determined in terms of repeatability and intermediate precision (intra-day and inter-days reproducibility). The injection repeatability was determined by analysing five replicate injections of the same diluted volunteer's serum sample.

Intra-day and inter-days reproducibility was assessed by spiking serum samples with 0.250 and 0.500 µg/mL of clarithromycin and analyzing these samples three times a day at 08:30 hr, 14:00 hr, and 19:30 hr. These serum samples were then placed under specified temperature conditions (8 °C) and evaluated for 3 successive days.

Accuracy of the results was assessed after spiking serum samples with known concentrations of clarithromycin (0.5 and 1.0 µg/mL) and analyzing 5 times.

The in-vitro drug release and in-vivo bioavailability studies of the test formulation in comparison with reference formulation were conducted. In-vitro release profile of both products of clarithromycin tablets (500 mg) was evaluated by adopting standard procedure as specified in USP-30, 2007; using dissolution test apparatus-II (Paddle Method). The dissolution medium was acetate buffer (pH 5.0, 900 mL) and rotation of the paddle was fixed at 50 rpm for the specified time. The samples were collected periodically and then analyzed.

The dissolution profiles of test and reference formulations were compared using a difference factor (f1) and similarity factor (f2). It is determined by the following equation [3]:

$$f1 = \left\{\left[\sum_{t=1}^{n}\left|R_j - T_j\right|\right] \Big/ \left[\sum_{t=1}^{n} R_t\right]\right\} * 100 \quad (1)$$

Where, n is the number of time points, Rt is the dissolution value of the reference formulation at time t, and Tt is the dissolution value of the test formulation at time t. Generally, f1 value varies from 0 to 15 [6].

Similarity factor (f2) is also an important parameter for comparing the dissolution profiles of test and reference products [7]. Mathematically f2 can be given as [8]:

$$f2 = 50log\left\{\left[1 + (1/m)\sum_{j=1}^{m} w_j\left|R_j - T_j\right|^2\right]^{-0.5} * 100\right\} \quad (2)$$

According to FDA and EMEA, two dissolution profiles are considered similar if f2 is between 50 and 100[8].

The in-vivo bioequivalence study was a randomized; open labeled, two treatment periods, single dose and cross over design with two weeks washout period in between. The protocol was approved by the local ethical committee of the Department of Pharmacy, University of Peshawar, Pakistan and was conducted according to the principles of the declaration of Helsinki and its amendments[12]. Informed consent was obtained from the selected healthy volunteers at the start of the study.

Healthy male Pakistani volunteers aged between 20-35 years were selected for this study and a written informed consent was obtained from each participant. Based on physical examination, medical history and biochemical evaluation of the laboratory tests (such as serum creatinine, alkaline phosphates, SGPT or SALT, total bilirubin, hemoglobin level, total blood count), only those volunteers were recruited who possessed good health.

Volunteers with history of serious illnesses (like hematological, G.I.T, hepatic, renal or cardiovascular abnormalities) or having drug allergy and any abnormal biochemical tests conducted before trials were not included. Smokers, alcohol users and those taking medicines chronically were also not allowed to participate in this study.

Fourteen volunteers were enrolled in this study but two were excluded due to abnormal liver function tests. The volunteers were randomly divided into two equal groups (A and B). During first treatment period, after an overnight fasting, each volunteer of group A was given a dose of 500 mg of clarithromycin of test formulation and each volunteer of group B was given a dose of 500 mg of clarithromycin of reference formulation (Klaricid®) with 240 ml of water. During second treatment period, after providing a two week drug free washout, reverse of plan was adapted for drug administration as presented in table 1. Volunteers remained in normal seated position for the initial 1 hr and the fasting was extended for further 3 hrs. After 3 and 8 hrs of the drug administration they were provided with standardized lunch and dinner. They were not allowed to take any acidic and xanthine containing drinks. They were also informed to avoid smoking and physical exercise.

Blood samples were collected using disposable syringes via catheter from basilica vein of cubital fossa of the volunteers at various time intervals i.e., 0.5, 1, 2, 3, 4, 6, 8, 12 and 16 hrs. These were then transferred to Pyrex glass test tubes and allowed to coagulate in dark, centrifuged at 4300 rpm for 10 min. The serum portions were collected and stored at -20 ˚C until analysis. These samples were analyzed within two weeks after collection.

Extraction of clarithromycin from blood samples was performed by dichloromethane. The extraction by dichloromethane was performed by adding 3 mL of dichloromethane (as 1 mL x 3) to serum samples, vortexed for 30 seconds and centrifuged at 4300 rpm for 5 min. The organic layer was collected and evaporated under stream of nitrogen. Remaining residues after organic layer evaporation were re-constituted in mobile phase (100 µL) and injected into RP-HPLC system for analysis.

The pharmacokinetics parameters of clarithromycin were calculated using "non-compartmental model" approach. The C_{max} and t_{max} were measured from peak serum concentrations of clarithromycin, while AUC_{0-t} and half-life values were determined by using the statistical package PK Solutions 2.0 (SummitPK, Montrose, Colorado).

The bioequivalence between two formulations was assessed by applying "ANOVA" test for logarithmically transformed parameters like C_{max}, t_{max}, AUC_{0-t} and half-life. Then the 90 % CI of the test/reference formulations were determined. Based upon US FDA and EMEA guidelines they were considered to be bioequivalent if the 90 % CI of the test/reference formulations were within the acceptance ranges of 0.80-1.25[6,7].

RESULTS

The release profiles were evaluated according to USP-30 official monograph, and were considered passed i.e., >80% releases were seen with in 30 min from each test and reference formulations. The results are summarized in table 2 and presented in figure 1.

The HPLC-UV method developed for the quantification of clarithromycin showed good linearity in the concentration range 0.2 to 10 µg/mL. The correlation co-efficient for clarithromycin in both standard solutions and spiked serum samples were 0.999 and 0.994, respectively, whereas the corresponding regression equations were y =0.0058x-0.0012 and y =0.0055x–0.0007, respectively. The lower limits of detection and quantification for clarithromycin were 0.018 µg/mL and 0.6 µg/mL, respectively. The average % recoveries of clarithromycin were 94% and 88% at the 2 nominal concentration levels (Table 3). Results of the precession data (intra-day and inter-days reproducibility) reveal complete harmony among the repeated analyses and intra-day and inter-days studies, as shown in table 4.

The in-vivo data represented that the serum concentration of clarithromycin after administration of test formulation at 0.50 hr was 0.322 ± 0.0316 µg/mL, while in case of reference formulation it was 0.208 ± 0.0358µg/mL. The maximum concentrations (C_{max}) of clarithromycin for test and reference formulations were observed 2.88 ± 0.1303 µg/mL and 3.023 ± 0.19 µg/mL, respectively, whereas the t_{max} values (i.e., time to reach maximum concentrations) for both test and reference formulation were 1.75 ±0.45 hr and 1.83 ±0.39

hr, respectively. Based upon non-compartmental model calculations the half-lives of clarithromycin from test and reference formulations were 2.84 ±0.17 hr and 3.10 ±0.28 hr, respectively (Figure 2, Table 5).

The 90% confidence interval values of C_{max}, t_{max}, AUC_{0-t} and half-life for test and reference formulations of clarithromycin were 0.89-0.98, 0.80-1.14, 0.84-0.93 and 0.88-0.95, respectively (Table 5 and 6).

Table 1: Study design for the bioequivalence study of two clarithromycin tablet formulations

Clinical Trial (Treatment period)	Group N=No. of Volunteers	Clarithromycin tablets administered (single dose)
First	A N=6	Test formulation of Clarithromycin 500 mg tablet
	B N=6	Reference formulation of Clarithromycin 500 mg tablet
Two Weeks Wash-Out period		
Second	A N=6	Reference formulation of Clarithromycin 500 mg tablet
	B N=6	Test formulation of Clarithromycin 500 mg tablet

Table 2: Comparison of dissolution release profiles of clarithromycin from test and reference formulations through difference factor (f1) and similarity factor (f2)

Time (min.)	Mean % Release of Test Formulation	Mean % Release of Reference Formulation	Difference Factor (f1)	Similarity Factor (f2)
0 min.	0	0	6.4	87.76
10 min.	71.4	69.8		
20 min.	84	83.6		
30 min.	93.88	92		

Table 3: Accuracy, linearity, recovery and sensitivity of the HPLC-UV method developed for determination of clarithromycin in human serum

Parameters	Clarithromycin
Accuracy (mean ± SD; RSD; % recovery) Spiked Concentration	
0.5 µg/mL	0.47 ± 0.047; 10.071; 94.0
1.0 µg/mL	0.882 ± 0.0946; 10.726; 88.2
Linearity Standard mixture	
Slope	0.0058
Intercept	0.0012
Correlation coefficient	0.999
Spiked serum samples	
Slope	0.0055
Intercept	0.0007
Correlation coefficient	0.994
Sensitivity	
LOD (µg/mL)	0.018
LOQ (µg/mL)	0.06

Table 4: Intra-day and inter-days precision data of the HPLC-UV method developed for determination of clarithromycin

Known concentration added (µg/mL)	Intra-day			Inter-days		
	Time	Found values (mean ± SD)	% RSD	Days	Found values (mean ± SD)	% RSD
0.250	8:30 Hr	0.24 ± 0.030	12.5	DAY 1	0.25 ± 0.017	6.80
	14:00 Hr	0.22 ± 0.016	7.27	DAY 2	0.22 ± 0.024	10.91
	19:30 Hr	0.23 ± 0.022	9.56	DAY 3	0.20 ± 0.020	10.00
0.500	8:30 Hr	0.49 ± 0.039	7.96	DAY 1	0.47 ± 0.045	9.57
	14:00 Hr	0.46 ± 0.040	8.70	DAY 2	0.45 ± 0.039	8.67
	19:30 Hr	0.45 ± 0.062	13.78	DAY 3	0.41 ± 0.032	7.80

Table 5: Statistical comparison of various pharmacokinetic parameters (logarithmically transformed) of clarithromycin after administration of 500 mg single oral dose of test and reference formulations in healthy volunteers

PK Parameter	Test (T) Formulation (mean± SD)	Reference (R) Formulation (mean± SD)	T / R Point Estimate	Lower Limits of CI	Upper Limits of CI	p-value
C_{max}	2.882 ± 0.13	3.089 ± 0.19	0.933	0.89	0.98	> 0.05
t_{max}	1.75 ± 0.45	1.83 ± 0.39	0.956	0.80	1.14	> 0.05
AUC_{0-t}	11.33 ± 0.68	12.87 ± 1.09	0.880	0.84	0.93	< 0.05
$t_{½}$	2.84 ± 0.17	3.10 ± 0.28	0.916	0.88	0.95	< 0.05
MRT	4.10 ± 0.24	4.48 ± 0.41	0.915	0.87	0.95	< 0.05

Table 6: Analysis of variance (one-way) of pharmacokinetic parameters of reference vs. test formulations of clarithromycin tablets (500 mg)

C_{max}	nlog. Transformed Data					Non Transformed Data				
Source	**DF**	**SS**	**MS**	**F**	**P**	**DF**	**SS**	**MS**	**F**	**P**
Sequence	1	0.0284	0.0284	9.96	0.0052	1	0.2588	0.2588	9.59	0.005
Error	22	0.06278	0.0028	...	...	22	0.593	0.027	...	...
Total	23	0.09119	...	...	...	23	0.8524	...	...	...
AUC_{0-t}	nlog. Transformed Data					Non Transformed Data				
Source	**DF**	**SS**	**MS**	**F**	**P**	**DF**	**SS**	**MS**	**F**	**P**
Sequence	1	0.09461	0.09461	17.00	0.0004	1	14.153	14.153	17.3	0.000
Error	22	0.12241	0.00556	...	...	22	17.994	0.818	...	...
Total	23	0.21702	...	...	...	23	32.14	...	...	...

Figure 1: Mean in-vitro release profiles of clarithromycin from test and reference formulations using USP-method (dissolution test apparatus-II)

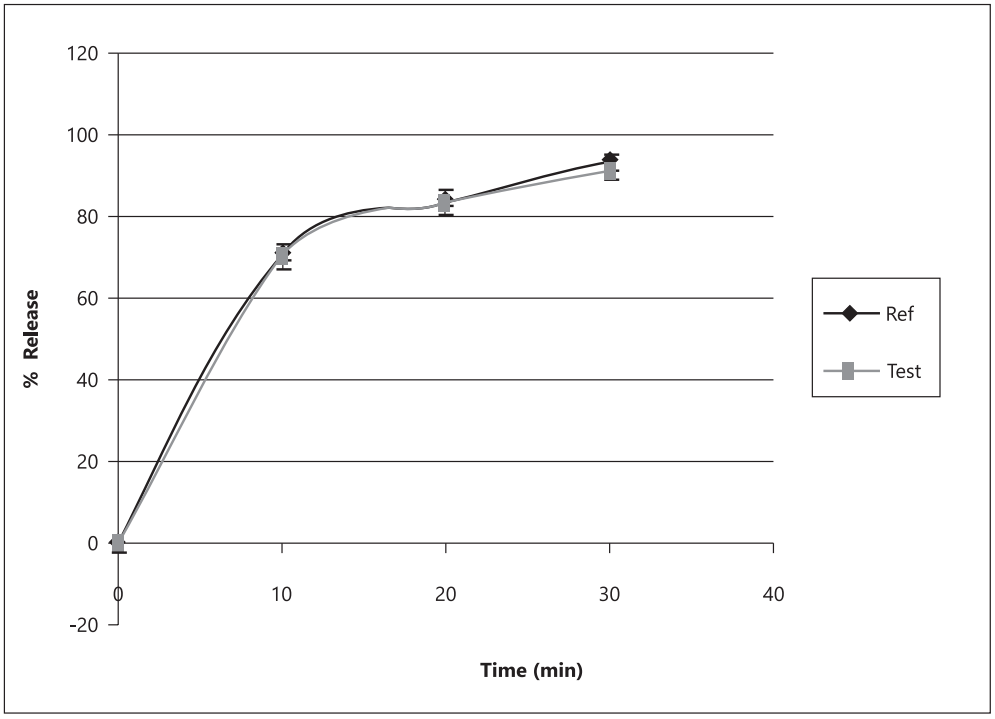
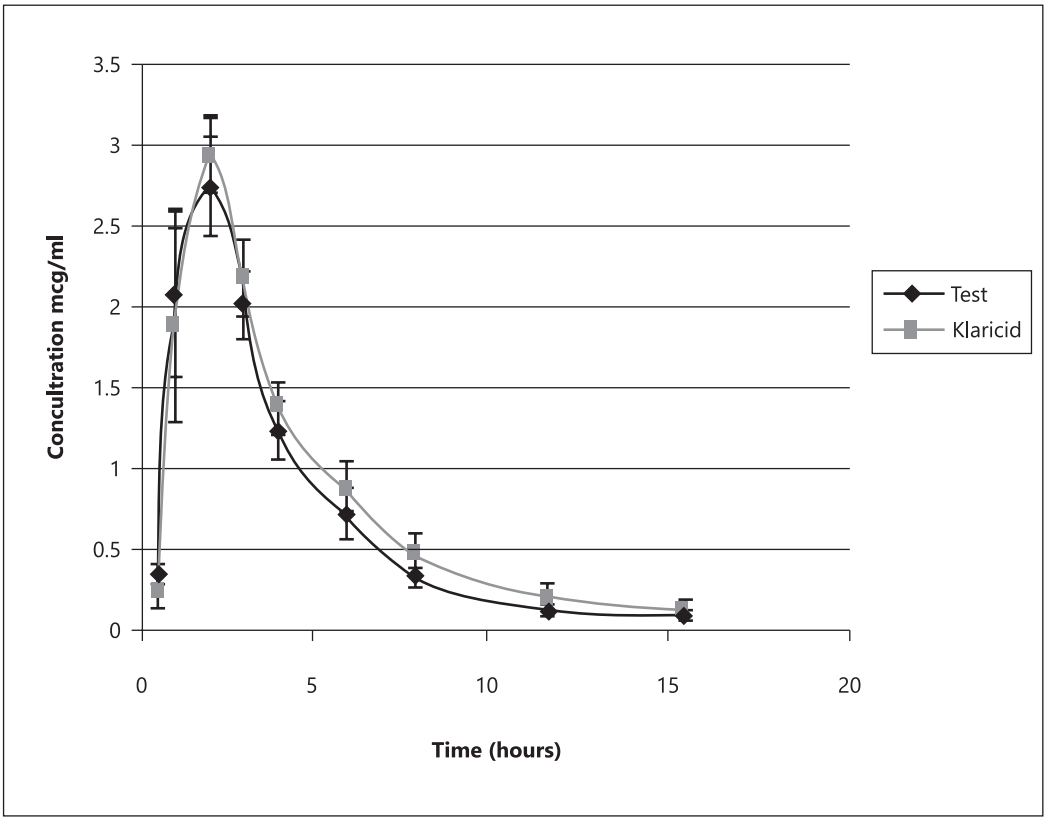


Figure 2: Serum-drug concentration profiles of test formulation, Medicraft Pharmaceutical Pvt. Ltd. and reference formulation (Klaricid®, Abbott; Lab. Pvt. Ltd.) of clarithromycin (500 mg) after single dose oral administration to healthy male volunteers (n=12)



DISCUSSION

The study was designed to evaluate the bioequivalence characteristics of commercially available brands of clarithromycin, Clafax® tablets 500 mg (Medicraft Pharmaceuticals Pvt. Ltd. Pakistan), that is manufactured and marketed in Khyber Pukhtunkhwa, against the reference formulation, Klaricid® tablets 500 mg (Abbott Laboratories Pvt. Ltd. Pakistan).

The HPLC method for analysis of clarithromycin in serum sample was adopted from the reported method with modifications. Validation was performed considering variable parameters. Various mobile phases with variable compositions and parameters were tested and finally mobile phase of ammonium acetate buffer (pH=6), methanol and acetonitrile with 40:10:50 ratios, at wavelength of 205 nm and flow rate of 1.5 mL/min were selected based upon its appropriate sensitivity, selectivity, accuracy and reproducibility. This method has several advantages over the previously reported methods of Bahrami et al, 2005, Ramakrishna et al, 2005, Barrett et al, 2005[9-11]. Sample preparation is relatively more simple, rapid and inexpensive and the chromatographic conditions and systems i.e. detector, HPLC column and internal standards (IS) used are easily available. The use of internal standard is useful to compensate the loss of the analyte in the samples containing unknown concentrations. Besides this, the low limits of quantification obtained with a UV detector allowed avoiding using more expensive detectors in the form of flourimetric and electrochemical detectors.

Comparison between dissolution profiles were achieved using a difference factor f1 and a similarity factor f2. In case of clarithromycin formulations, f1 was 6.4, which lie well within the acceptable range of pre-determined 0-15. Therefore, these dissolution profiles of test and reference formulations were concluded to be similar[9]. While dissolution profiles comparison of clarithromycin's test and reference formulations gave f2 value of 87.76 that was again within the acceptance range of FDA and EMEA for similarity declaration i.e., 50-100 therefore we considered that dissolution profiles of test and reference formulations were similar[12,13].

The study showed that, concentration of clarithromycin in serum after administration of test formulation at 0.50 h was 0.322 ±0.0316 µg/ml, while in case of reference formulation it was 0.208 ±0.0358 and absorption increased that concentration with passage of time. The maximum concentrations (C_{max}) of clarithromycin, for test and reference formulations were observed at 1.75 ±0.45 hours and 1.83 ±0.39 hours, respectively. Those quick onsets of maximum concentrations reflected the rapid absorption behavior through gastric lumen into the circulatory system, where the concentration of test formulation was 2.88 ±0.1303 µg/ml and reference formulation had serum concentration of 3.023 ±0.19 µg/ml. Low levels of maximum concentration in serum is mainly because of its comparatively less bioavailability i.e. 50-55 % reported by Sunil et al[13]. Based upon non-compartmental model calculations the half lives of clarithomycin from test and reference formulations were 2.84 ±0.17 h and 3.10 ±0.28 Hours. These calculated values were comparable to the corresponding parameters obtained by single oral dose of 500 mg clarithromycin reported by Sung et al, 2001[1].

These data of maximum concentration of clarithromycin from test and reference formulations were comparable to the reported values of this parameter by Lohitnavy et al, 2003 and Sung et al, 2001[1,14,15] i.e. 3.018 $\mu g ml^{-1}$ and 2.64 $\mu g ml^{-1}$, respectively. Further calculation and evaluation showed that the difference of peak serum concentration of the clarithromycin from test and reference formulations was not significant ($p>0.05$). The mean ± SD data for t_{max} values of clarithromycin following oral administration of test formulation was 1.75± 0.45 h and reference formulation had given the value of 1.83 ±0.39 h. These values were also well comparable to the reported data by Lohitnavy et al, 2003, i.e. 1.8 h and 2 h while, Sung et al, 2001, reported values of 1 h that was also seen in some of the volunteers in this study. The difference of time for peak serum concentration of the clarithromycin from test and reference formulations was not significant ($p>0.05$). The elimination half life for clarithromycin in case of test formulation and reference formulation were 2.84 ±0.17 hours and 3.10 ±0.28 hours, respectively. These values of half life of clarithromycin from test and reference formulations were comparable to the reported data by Sung et al, 2001, i.e. 3.31 h.

The difference in half lives of test and reference formulation was significant ($p<0.05$). The mean ±SD data regarding AUC values of clarithromycin after single oral administration of test and reference formulations were 11.33 ±0.68 µg ml^{-1}·h and 12.87 ±1.09 $\mu g ml^{-1}$·h, respectively. These values were also comparable to the data of AUC i.e. 12.13 $\mu g ml^{-1}$·h, reported by Sung et al, 2001. The difference of AUC of the clarithromycin from test and reference formulations was significant ($p<0.05$).

Statistical calculations of natural logarithmically transformed data of both formulations of clarithromycin regarding the above mentioned pharmacokinetic parameters were well with-in range of US FDA and WHO ranges for bioequivalence i.e. 0.80–1.25. Therefore, test and reference formulations of clarithromycin were concluded to be bioequivalent. Both formulations were well tolerated by all the volunteers participated in this study. No unexpected incidents were reported during the conductance of this trial that influenced the study outcomes. All volunteers continued participation till the end of this study and were discharged in good health.

CONCLUSION

In-vitro evaluation of both formulations including their dissolution profiles indicated statistically non-significant differences among these formulations. Pharmacokinetic behaviors of test and reference formulations were almost overlapping. Statistical evaluation of various pharmacokinetic parameters showed that 90 % confidence interval values of C_{max}, t_{max}, AUC_{0-t} and half-lives of test and reference formulations of clarithromycin were well within the acceptance ranges of WHO and US FDA for bioequivalence 0.80-1.25. Tolerability evaluation of both test and reference formulations indicated that both formulations were well tolerated. Therefore, it was concluded that test and reference formulations of clarithromycin were bioequivalent and can be used interchangeably without any dose adjustments and monitoring.

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CONTRIBUTORS

SU conceived the idea, designed the study, sample analysis and manuscript drafting. O helped samples collection, treatment and preparation. IK helped biological samples collection, treatment and preparation. AK did HPLC analysis of samples. YS did statistical data interpretation. ZN did manuscript review. ZI was principal investigator/supervisor. All authors contributed significantly to the submitted manuscript.