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Research Article

TWO-WAY CROSSOVER BIOEQUIVALENCE STUDY OF ROSUVASTATIN TABLETS 5 MG IN HEALTHY, ADULT, ASIAN-INDIAN MALE VOLUNTEERS UNDER FASTING CONDITION

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ABSTRACT

Objective: To compare the bioavailability and characterize the pharmacokinetic profile of the test and reference formulations of rosuvastatin tablets 5 mg in healthy, adult, Asian-Indian male volunteers under fasting condition.

Methods: An open label, randomized, two-treatment, two-period, two-sequence, single dose, two-way crossover study was conducted in forty healthy adult volunteers. All the subjects were randomly allocated to receive either of the treatment arms separated by a washout period of 9 days. In each period 20 blood samples including 0.00 to till 48.0 h post dose were collected and were analyzed by a validated LC-MS/MS method. Pharmacokinetic parameters C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, K_{el} and $T_{1/2}$ of rosuvastatin were characterized using non-compartmental analysis, and 90% confidence interval were estimated for ln-transformed parameters C_{max} and AUC_{0-t}.

Results: Pharmacokinetic and statistical analysis was estimated in 37 completed subjects. The estimated mean (±SD) values of C_{max} (ng/mL) for test and reference were 6.348 (±2.656) and 6.204 (±2.825), respectively; and the mean (±SD) values of AUC_{0-t} (ng.h/mL) for test and reference were 55.084 (±23.745) and 54.041 (±23.949), respectively.

Conclusions: The 90% CI for C_{max} (93.40-116.4) and AUC_{0-t} (93.61-112.10) were within the regulatory acceptance limit 80.00-125.00%, hence bioequivalence was concluded between test (rosuvastatin tablets 5 mg) and reference (Crestor® tablets 5 mg) formulations. The reported adverse events i.e. high SGPT and diarrhea in 2 subjects were followed up until resolution, however both the formulations appeared to be well tolerated.

Keywords: Bioavailability, Bioequivalence, Pharmacokinetic, Rosuvastatin.

INTRODUCTION

Rosuvastatin (CAS: 287714-41-4) is a synthetic lipid lowering drug acts by competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase, which is a rate limiting enzyme involves in the conversion of HMG-CoA to mevalonate, a precursor of cholesterol. It is used in conjunction with the diet and regular exercises to treat patients with hypertriglyceridemia and other cardiovascular diseases [1-5]. Following oral administration of rosuvastatin under fasting conditions the peak plasma levels of rosuvastatin occur at 3 to 5 h hours and the elimination half life is around 16-19 h [6, 7].

Strengths available for rosuvastatin in Australia are 5, 10, 20 and 40 mg once daily oral administration, and usual maximum dose is 20 mg once daily. It is well known that the highest available strength of rosuvastatin 40 mg has contraindications among Asian patients. In comparison with the Caucasian population, a 2-fold elevated median exposure (in terms of rate and extent of absorption) was observed in Asians [8]. A pharmacokinetic study conducted with 40 mg strength compared the plasma levels of white and Asian subjects residing in the same environment reported the higher plasma concentrations in Chinese (~2.3 fold), Malay subjects (~2 fold) and Asian-Indian (~1.6 fold) study population than the white subjects [9]. Due to complications with increased plasma concentrations of rosuvastatin, therapy should be initiated with 5 mg once daily in Asian population [7, 8].

This present study was aimed to estimate the pharmacokinetics of rosuvastatin tablets 5 mg strength and to assess the bioequivalence of test and reference formulations. Crestor® (rosuvastatin) tablets 5 mg of AstraZeneca Pty Limited; Australia is a branded formulation of rosuvastatin tablets 5 mg available in Australia [10]. Dr. Reddy's Laboratories Limited, Hyderabad, India developed a generic version of test formulation rosuvastatin tablets 5 mg subjected to assess the bioequivalence for marketing in to the Australia. The bioequivalence of the two treatments is concluded based on the lack of any differences in rate (C_{max}) and extent (AUC) of absorption, this present study was conducted in consideration of all the applicable TGA regulatory requirements to conclude bioequivalence of test and reference formulations, and able to address the proportional summary results of rosuvastatin tablets 5 mg strength in healthy, adult, Asian-Indian male volunteers under fasting condition [11].

MATERIALS AND METHODS

Study subjects and ethical considerations

Non alcoholic, non-smoking, healthy, male volunteers between 18 to 45 years of age, having a body mass index (BMI) between 18.5-27.0 kg/m^2 of western India and belonging to Asian-Indian race were enrolled as the study population. Homogenous subject population was selected with the aim to minimize variability and permit detection of differences between pharmaceutical products. No female volunteers were participated. All the subjects (n=40) were screened for 28 days prior to the dosing in period-I. Health status of the volunteers was elicited by general clinical examination, measurement of blood pressure, heart rate, oral temperature, respiratory rate, 12-Lead ECG, clinical laboratory evaluations including alkaline phosphatase and creatine phosphokinase (CPK), glomerular rate (GFR), Chest X-Ray (posterior-anterior view), immunological tests for HIV (Human Immunodeficiency Virus), HBsAg (Hepatitis B Surface Antigen) and HCV (Hepatitis C Virus) by general clinical examination and different laboratory tests. Informed consent presentations were carried out in local language on the day of check-in for period-I, and all the subjects voluntarily provided written informed consent to participate in the study.

This was a single dose, open-label, two-treatment, two-period, two-sequence, two-way crossover, bioequivalence study in healthy adult volunteers under fasting conditions. This study was performed at Lambda Therapeutic Research Limited, Ahmedabad, India. Based up on the prior approval taken from Aditya Independent Ethics Committee study was conducted in accordance with the basic principles of ICH-GCP E6 (International Conference on Harmonization-Guideline for Good Clinical Practices) and the principles enunciated in the Declaration of Helsinki (revised version of Seoul, Korea, 2008).

Drug administration and study restrictions

Dosing was done as per the randomization schedule generated by SAS® software version 9.1.3 (SAS Institute Inc., USA). After an overnight fast of at least 10 h, a single oral dose (5 mg) of either test or reference formulation was administered under open-label conditions with 240 mL of drinking water at ambient temperature. Each subject was exposed to both the treatments in a crossover manner, so that every subject acts as his own control. Compliance to the dose administration was assessed by conducting a thorough examination of the oral cavity by trained study personnel. Washout period of 9 days was maintained between the successive dosing days.

Volunteers were instructed to refrain from drinking water from 1 h prior to dosing till 1 h after dose except during administration of the drug. Prior to and thereafter water was allowed at all other times. Subjects were restricted to consuming any alcoholic products, xanthine containing beverages (like chocolate, tea, coffee, cola drinks) and tobacco containing products from 24 h prior to dosing till the last sample collection in both the study periods. They were also instructed to avoid using any medicine for at least 30 days prior to first study drug administration and until study completion. Urine scan for drugs of abuse and breath test for alcohol was carried out prior to check in of period-I. Subjects were instructed to remain in sitting posture or ambulatory position for the first 3 h after dose administration. Standard meal was served at appropriate interval during the study periods.

Blood sample collection

In each period, a total of 20 blood samples (5 mL each) were collected through an indwelling intravenous cannula (Venflon) at pre-dose sample (0 h) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 8.0, 10.0, 12.0, 16.0, 24.0, 36.0 and 48.0 h after dosing. Blood samples were transferred into pre-labeled sample collection tubes containing K_2EDTA as anticoagulant kept in a wet ice bath. The samples were centrifuged at 3000 rcf for 5 minutes below 10°C (and above 0°C) to separate plasma. The resultant samples were stored at -65 ± 10°C until analysis.

Analytical method

Plasma samples from the subjects completed both the clinical phases of the study were analyzed. In addition to this, subject withdrawn due to adverse events, and withdrawn on protocol non-compliance were also analyzed for safety reasons. The plasma concentrations of rosuvastatin in the subject samples were determined by validated LC-MS/MS method using rosuvastatin d6 as the internal standard. The analyte and internal standard were extracted from the plasma using liquid-liquid extraction method. The calibration curve was established by using an 8-point calibration curve and 4 quality control concentrations (high, medium, lower medium and low) were used during assay of samples. The linearity of the assay method ranged from 0.05-25.127 ng/mL for rosuvastatin. The range of precision and accuracy of the back-calculated concentrations of the calibration curve standard points were 1.1-5.9% and 99.0-102.0%, respectively.

Safety and tolerability evaluation

Safety was assessed from the screening period to end of the study. It was assessed through clinical examinations, vital signs assessment, 12-lead Electrocardiogram (ECG), X-ray (postero-anterior view) recording, clinical laboratory parameters (e.g. biochemistry, hematology, immunology and urine analysis), subjective symptomatology and monitoring of adverse events.

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters (C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, K_{el} and $T_{½}$) for rosuvastatin were calculated from the plasma concentration versus time profile by non-compartmental analysis using WinNonlin professional software version 5.3 (Pharsight Corporation, USA). The pharmacokinetic parameters including, maximum measured plasma concentration (C_{max}) was determined as the peak concentration for each volunteer for each formulation, and T_{max} was the time corresponding to C_{max}. Area under the plasma concentration time curve from time zero to the last quantifiable concentration (AUC_{0-t}), and from time zero to infinity ($AUC_{0-\infty}$) were estimated by numerical integration using the linear trapezoidal method. Terminal phase elimination rate constant (K_{el}) was estimated via linear regression of time versus log concentration, this was calculated by linear least squares regression analysis using at least last three or more non-zero plasma concentration values. The adjusted *r*-square value 0.80 was considered as cutoff point for the estimation of K_{el}. Elimination half-life ($T_{½}$) was calculated using the formula $0.693/K_{el}$.

Statistical comparison of the ln-transformed pharmacokinetic parameters of the two formulations was carried out using SAS® version 9.1.3 (SAS Institute Inc., USA). Analysis of variance was carried out by employing PROC GLM of SAS® Version 9.3 (SAS Institute Inc., USA) for ln-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for Rosuvastatin. ANOVA model included sequence, formulation, period and subject (sequence) as fixed effects. An F-test was performed to determine the statistical significance of the effects involved in the model at a significance level of 5% (alpha=0.05). Two one-sided tests for bioequivalence (90% confidence interval (CI) for the ln-transformed least squares mean ratios) and power analyses were estimated. Bioequivalence for ln-transformed pharmacokinetic parameters C_{max} and AUC_{0-t} were concluded if the 90% confidence interval for the test-to-reference ratio was within the 80.00-125.00% limit.

Based on pilot study data, a sample size of 40 subjects under fasting condition was estimated to be sufficient to meet the 80.00 to 125.00% bioequivalence range at 5% level of significance with a statistical power of at least 80%. This also takes into account the variations around the estimated intra-subject CV as well as the possibility of observing drop-outs [12].

RESULTS

Subjects

Forty healthy, adult, male volunteers were enrolled in the study, however only 37 completed both the clinical phases. 2 volunteers were withdrawn from the study on the medical ground, and 1 volunteer did not turn up for period-II dosing. Demographic profiles of the volunteers included in the pharmacokinetic and statistical calculations (n=37) are summarized in Table 1.

Table 1: Demographics of volunteers included in pharmacokinetic and statistical analysis.

Demographic Variable (Unit)	Pharmacokinetic population (n=37)
Age (y)	29.7 ± 6.42
Height (cm)	167.53 ± 6.124
Body Weight (kg)	62.535 ± 8.7399
BMI (kg/m^2)	22.262 ± 2.6557

Data are presented as mean ± standard deviation

BMI, Body mass index

Safety results

The test and reference formulations of rosuvastatin tablets were well tolerated by all the study volunteers, and there were no deaths and no serious adverse events reported during the conduct of the study. Conversely, two adverse events (AEs) high SGPT and diarrhoea were reported by two volunteers during the conduct of the study. The AEs of high SGPT and diarrhoea were reported after administration of the reference and test formulations, respectively. Both the volunteers were withdrawn from the study on medical grounds and were followed up for their AEs until resolution. The causality assessment was judged as unlikely related for diarrhoea and possibly related for high SGPT.

Table 2: Pharmacokinetic parameters of rosuvastatin following single dose administration of test and reference formulations of rosuvastatin tablets 5 mg under fasting condition (n=37).

Parameter (unit)	Test Product	Reference Product
C_{max} (ng/mL)	6.348 ± 2.656	6.204 ± 2.825
AUC_{0-t} (ng.h/mL)	55.084 ± 23.745	54.041 ± 23.949
$AUC_{0-\infty}$ (ng.h/mL)	57.276 ± 24.006	56.468 ± 25.080
T_{max} (h)*	4.500 (2.000-5.000)	4.500 (1.500-6.000)
K_{el} (1/h)	0.064 ± 0.020	0.066 ± 0.025
$T_{1/2}$ (h)	11.883±3.454	11.819 ± 4.164

Data expressed as mean ± standard deviation

* Values are given as median (min-max).

Test: Rosuvastatin tablets 5mg of Dr. Reddy's Laboratories Limited, India

Reference: Crestor® tablets 5 mg of AstraZeneca Pty Limited; Australia

Pharmacokinetics and bioequivalence results

Volunteers completed both the treatment periods were included in pharmacokinetic and statistical analysis. Pharmacokinetic parameters of test and reference formulations were computed for rosuvastatin using actual time of sample collection. Descriptive statistics (mean±SD) for all the pharmacokinetic parameters were calculated and are summarized in Table 2.

The mean (±SD) plasma concentrations (C_{max}) for test 6.348 (±2.656) ng/mL and reference 6.204 (±2.825) ng/mL formulations and 4.500 (1.500-6.000) h, respectively. In terms of extent of were observed at median (min-max) T_{max} of 4.500 (2.000-5.000) h absorption (AUC_{0-t}) the mean (±SD) values observed as follows, for test 55.084 (±23.745) ng.h/mL and reference 54.041 (±23.949) ng.h/mL formulations; and for $AUC_{0-\infty}$, for test 57.276 (±24.006) ng.h/mL and reference 56.468 (±25.080) ng.h/mL formulations are comparable with each other. The mean (±SD) elimination half life ($T_{1/2}$) calculated for test and reference formulations are 11.883 (±3.454) h and 11.819 (±4.164) h, respectively.

The linear and semi logarithmic plots of mean plasma concentration versus time for rosuvastatin presented in Fig. 1 and Fig. 2, respectively, indicting that the curves are closely similar, and are almost superimposed on each other for test and reference formulations.

The geometric least square mean (LSM) ratio and 90% confidence interval (CI) for the primary pharmacokinetic parameters [C_{max} and AUC_{0-t}] of rosuvastatin under fasting condition was presented in Table 3.

Table 3: Summary statistics of rosuvastatin

Parameter (Unit)	Geometric Least Square Means		(T/R) Ratio (%)	90 % CI limits		
	Test	Reference			ISCV (%)	Power (%)
C_{max} (ng/mL)	5.864	5.623	104.3	93.40 – 116.41	28.5	95.4
AUC_{0-t} (ng.h/mL)	50.490	49.288	102.4	93.61 – 112.10	23.2	99.1

The results demonstrates that the ratios and corresponding 90% CI of the relative C_{max} and AUC_{0-t} geometric LSM of the test to reference formulations were within the regulatory bioequivalence criteria of 80.00% to 125.00% under fasting conditions.

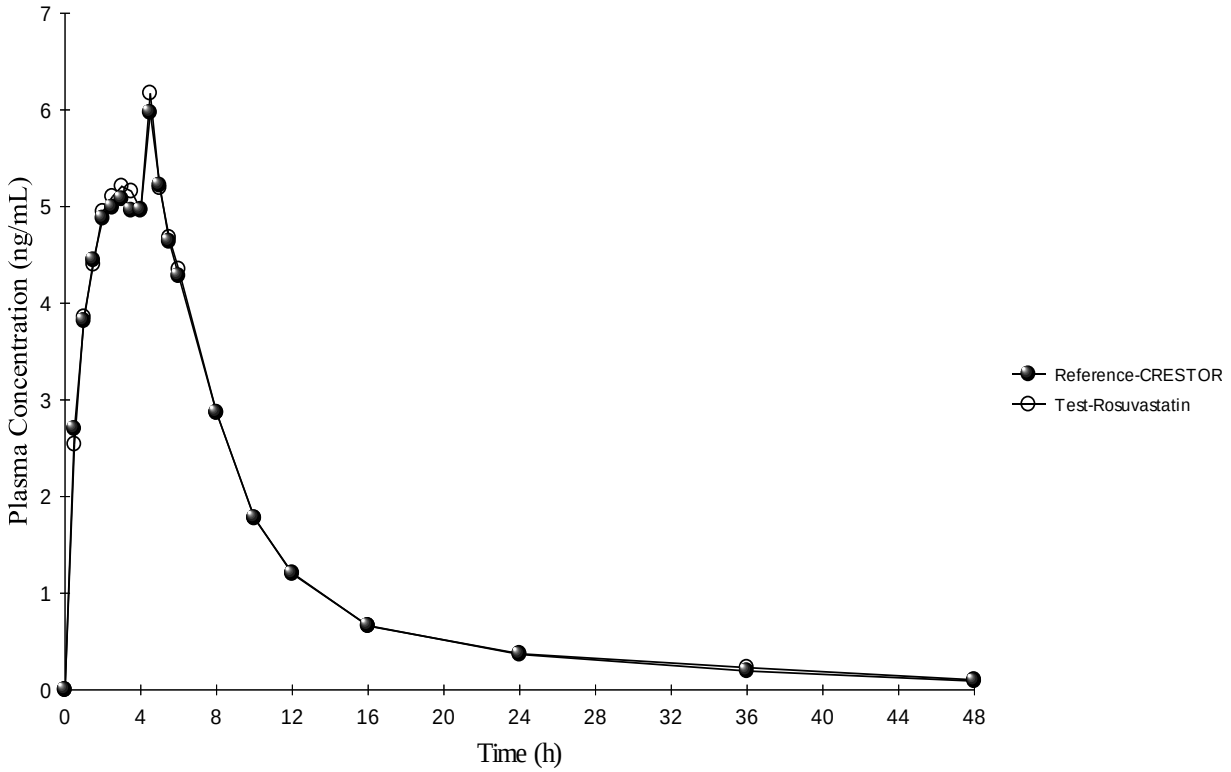

Fig. 1: Linear plasma concentration-time profile of rosuvastatin following single dose administration of test and reference formulations of rosuvastatin tablets 5 mg under fasting conditions (n=37).

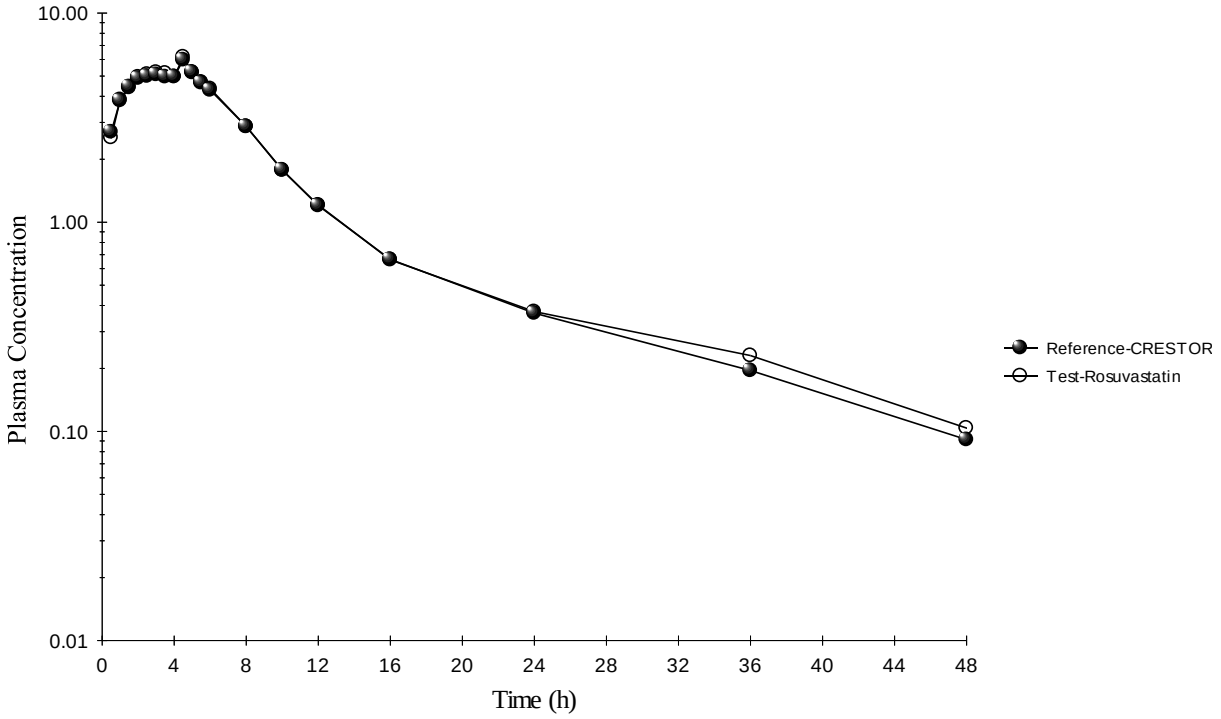

Fig. 2: Semi logarithmic linear plasma concentration-time profile of rosuvastatin following single dose administration of test and reference formulations of rosuvastatin tablets 5 mg under fasting conditions (n=37).

DISCUSSION

The use of generic preparation of a therapeutically well-established active drug principle has to be justified by the appropriate bioequivalence study, because the proof of bioequivalence of the test and reference formulations assures the equal therapeutic efficacy [13]. Hence to estimate bioequivalence between test and reference formulations, the generic test formulation rosuvastatin tablets 5 mg has been developed by Dr.Reddy's Laboratories Limited, Hyderabad, India, and subjected to compare with the reference formulation Crestor® rosuvastatin tablets 5 mg of AstraZeneca Pty Ltd., Australia for generic submission.

Rosuvastatin calcium tablets are indicated for hyperlipidemia as an adjunct therapy to diet to treat elevated cholesterol levels. It is available in 5, 10, 20 and 40 mg strengths and shows linear pharmacokinetics throughout all the strengths. However the highest recommended strength of the rosuvastatin 40 mg in the global market has contraindications in Asian patients [7, 8]. This present study was aimed to assess the pharmacokinetics of rosuvastatin 5 mg tablets in Asian-Indian population, residents of western India (Ahmedabad city). Normal, healthy, adult, male volunteers were chosen as study population with the aim to minimize variability and to permit detection of differences between test and reference formulations.

Based on the reported half life (3 to 5 h) of the rosuvastatin and other potential advantages with the cross over study design, this study was planned to in a cross over manner, in which each volunteer (except the withdrawn subjects) received both the treatments during the two periods. Hence all the subjects serve as their own control and no separate group of subjects was required to act as the control group. A washout period of 9 days was kept in between the two dosing days of the study periods. Blood samples were collected at pre dose (0.00 h) to up to 48.0 h after drug administration. This sampling was planned in order to provide a reliable estimate of the extent of absorption [13].

All the pharmacokinetic parameters were computed using non-compartmental analysis, and ANOVA was applied on the ln-transformed values. In comparison of pharmacokinetics of test and reference formulations a similar rate (C_{max}) and extent (AUC_{0-t}) of absorption was observed. This similarity was also observed with respect to elimination pharmacokinetic parameters ($AUC_{0-\infty}$, K_{el} and $T_{1/2}$). As such there is limited published literature available on pharmacokinetics of rosuvastatin tablets 5 mg strength on comparison of different ethnic groups especially Asian-Indian population, plasma levels calculated in this present study are lower than the values reported in a study conducted with 5 mg strength in Chinese population of the mean (standard deviation) values of C_{max} 10.22 (8.05) ng/mL and AUC_{0-t} 73.67 (48.78) ng.h/mL [14]. The mean (±SD) values for C_{max} and AUC of this study are 2-fold higher than the reported values of study conducted on rosuvastatin tablets 5 mg by Teva Pharmaceutical Industries Limited [15]. ANOVA applied on ln-transformed values for C_{max} and AUC_{0-t} parameters for rosuvastatin for the difference between all the effects namely formulation, sequence and period were found to be statistically insignificant ($p>0.05$) indicative of absence of significant differences between the test and reference formulations.

Rosuvastatin can be administered with and without food, however the scope of this study was limited to test under fasting condition only, and the study was conducted in accordance with the applicable study conditions as per the Australian regulatory requirement. The inclusion of only male subjects can be considered as another limitation of the present study, however it does not impact on the study outcome due to the crossover design.

CONCLUSIONS

In conclusion, both the formulations were well tolerated after single oral dose administration. Bioequivalence with respect to rosuvastatin was concluded based on the pre-specified regulatory acceptance criteria. The statistical results i.e. 90% CI of ln-transformed data for both the primary pharmacokinetic parameters (C_{max} and AUC_{0-t}) were within the acceptance limit of 80.00-125.00%, indicating the bioequivalence of the test and reference formulations, and the two formulations are interchangeable.

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