International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

In-vitro and In-vivo Bioequivalence Assessment of Azithromycin Tablet Formulations at Fasting and Fed Conditions

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Abstract: ***This study aimed to determine the bioequivalence of Azithromycin tablets 500 mg manufactured by ACI HealthCare Limited and ZITHROMAX® (azithromycin) tablets 500 mg from Pfizer Labs Division of Pfizer Inc. It was a randomized, double-blind crossover trial conducted at Raptim Research Pvt. Ltd., India, involving healthy adult male subjects in both fasting and fed conditions. Key pharmacokinetic parameters, including Cmax and AUC0-t, were evaluated. The 90% confidence intervals for the ratio of geometric least square means based on Ln-transformed primary PK parameters were as follows: 1) Cmax (fasting: 91.33-104.86, fed: 89.64 – 109.64) 2) AUC0-t (fasting: 93.13-102.76, fed: 90.97-104.79) 3) AUC0-inf (fasting: 93.37-102.67, fed: 90.70-105.00). These intervals were found within the acceptable bioequivalence limits of 80.00% to 125.00% for Azithromycin. The study observed a maximum intra-subject coefficient of variation (ISCV) of 27.42% for Cmax. Based on this ISCV and a ratio estimate (Test/Reference) of 93-107%, a sample size of 64 subjects (including a 10% dropout rate) was calculated as sufficient for the study design. The results indicated that Azithromycin tablets 500 mg from ACI HealthCare Limited were bioequivalent to ZITHROMAX® tablets 500 mg under fed conditions.***

Keywords: Bioequivalence; Azithromycin; Zithromax; C_{max}, AUC, LC-MS MS, Bioavailability

1. Introduction

Azithromycin dihydrate is recognized as a BCS Class III compound due to its high solubility across the physiological pH range and low permeability, characteristics that impact its absorption kinetics. [1]

In pharmaceutical formulation and development, the dissolution behavior of azithromycin dihydrate is crucial as it influences its bioavailability. Despite rapid dissolution, absorption is not limited by dissolution rate but by permeability. To ensure consistency and compliance, the dissolution method recommended by the Office of Generic Drugs (OGD) and detailed in the United States Pharmacopeia (USP) monograph was employed. [2-3]

Understanding the dissolution kinetics of azithromycin dihydrate aids in ensuring consistent drug performance and bioavailability, guiding decisions in pharmaceutical development and regulatory compliance.

Azithromycin is widely prescribed to combat a variety of infections affecting the respiratory system, gastrointestinal tract, and genitourinary system. It is often favored over other macrolides for its efficacy in treating specific sexually transmitted infections and enteric diseases. Structurally, azithromycin is a derivative of erythromycin, belonging to the *Azalide* subclass of macrolides. This subclass is characterized by a 15-membered lactone ring with a nitrogen atom at the 9a position, which is methyl-substituted instead of having a carbonyl group. This modification enhances the drug's stability and bioavailability by inhibiting its metabolism, distinguishing it from traditional macrolides. [4]

Azithromycin exhibits broad-spectrum activity due to its ability to penetrate tissues deeply and its extended half-life, making it suitable for once-daily dosing. The drug is primarily absorbed from the gastrointestinal tract, although its absorption can be affected by food. Its absolute oral bioavailability is approximately 37%. Peak plasma concentrations are reached within 2 to 3 hours post-administration, but it is the extensive distribution into tissues that maintains therapeutic levels well beyond those found in the bloodstream. In the liver, a small portion of azithromycin undergoes demethylation, leading to metabolites that are excreted primarily in bile. These metabolites are considered inactive against microbes. About 6% of the oral dose, representing roughly 20% of the systemic circulation, is excreted unchanged in urine. The drug's terminal elimination half-life averages around 68 hours, contributing to its prolonged therapeutic effect and allowing for less frequent dosing schedules. [5-9]

Azithromycin's pharmacological profile includes enhanced activity against gram-negative bacteria, notably those of the Enterobacteriaceae family, and coverage of numerous gram-positive organisms. [10-11] Despite its established efficacy in bacterial infections, its role in treating viral infections such as respiratory syncytial virus and SARS-CoV-2 remains uncertain and subject to ongoing research. [12-16]

Dosage regimens for azithromycin typically involve a single daily dose of 250 mg or 500 mg for 3 to 5 days. In severe infections, higher doses may be administered. Single doses at 30 mg/kg are occasionally used for otitis media, while adults with Chlamydia infections may receive a single dose of 1 g. [17]

The study adhered strictly to ethical principles rooted in the Declaration of Helsinki, as well as Good Clinical Practice guidelines from the International Council for Harmonization. Regulatory standards, including those outlined in the New

Drugs and Clinical Trials Rules by the Ministry of Health and Family Welfare, Government of India, and CDSCO Guidelines for Bioavailability & Bioequivalence Studies, were rigorously followed. The study design also incorporated guidance from the USFDA regarding orally administered drug products and food-effect bioavailability studies, ensuring compliance with international standards for pharmaceutical research. [18-24] Pharmacokinetic evaluations were conducted based on protocols aligned with USFDA guidelines, WHO guidelines for causality assessments, and the CTCAE (Common Terminology Criteria for Adverse Events) from the U. S. Department of Health and Human Services, emphasizing thorough monitoring of drug effects and patient safety throughout the study. [25-29]

2. Formulations, Subjects, Materials and Methods

2.1 Formulation

The Quality by Design approach guided the formulation development process. Initially, the quality target product profile was established based on the API properties, physicochemical characterization of the reference listed drug (RLD), and considerations from the RLD label. During pharmaceutical development, emphasis was placed on critical quality attributes such as assay, uniformity of dosage units, dissolution, and degradation products, as well as physical attributes susceptible to realistic changes in formulation or manufacturing processes.

Azithromycin dihydrate, known for its cohesive and poorly flowing nature with poor compressibility characteristics, made direct compression impractical due to its high API content. Therefore, the wet granulation method was preferred to enhance the flowability and compressibility of the powder mixture.

Formulation and process optimization studies were conducted to pinpoint the key process parameters and quality attributes for each manufacturing step. Critical process parameters (such as impeller speed, chopper speed, granulation time, etc.) and quality attributes identified during process characterization for each unit operation were instrumental in establishing a robust control strategy for commercial batch production.

According to the results of the drug-excipient compatibility study, it can be concluded that the chosen excipients demonstrate chemical compatibility with Azithromycin dihydrate under controlled conditions (40 ± 2°C and 75 ± 5% RH, 28 days) as well as under open conditions (40 ± 2°C and 75 ± 5% RH, 14 days). The formulation details for Azithromycin are outlined in Table 1 for this investigation.

Table 1: Exhibit Batch Manufacturing Formula for Azithromycin Tablets USP, 250 mg

S/N	Materials Name	Specification	Grade/Brand	Functional Category	Quantity (mg/units)	% (w/w to the tablet core)
Dry Mixing						
1	Azithromycin dihydrate (1)	USP	-	API	524.059	62.277
2	Dibasic Calcium Phosphate Anhydrous	USP	Fujicalin	Diluent and Stabilizer	84.150	10.000
3	Partially Pregelatinized Maize Starch (2)	NF	Starch 1500	Diluent and Binder	190.093	22.590
4	Colloidal Silicon Dioxide	USP	CAB-O-SIL M-5P	Glidant	2.667	0.317
Binder Solution						
5	Sodium Lauryl Sulfate	USP	Kolliphor SLS Fine	Solubilizer	2.663	0.316
6	Purified Water#	USP	-	Solvent	QS	-
Blending and Lubrication						
7	Partially Pregelatinized Maize Starch	NF	Starch 1500	Diluent and Binder	12.623	1.500
8	Croscarmellose Sodium	USP	Ac Di Sol SD-711	Disintegrating Agent	8.415	1.000
9	Magnesium Stearate	NF	Veg	Lubricant	16.830	2.000
Total Core Tablet Weight (mg)					**841.500**	**100.00**
Coating						
10	Opadry II White[at]	In house	85F18422	Film Coating Agent	25.245	-
11	Hypromellose 2910, 6 mPa. s[at]	USP	Pharmacoat 606	Film Coating Agent	10.098	-
12	Purified Water#[at]	USP	-	Solvent	QS	-

Not a part of final product as it is eliminated during drying;[at]20% overage considered to compensate the process loss
Note:
(1) Calculate and dispense the Azithromycin dihydrate USP based on the assay as Azithromycin (µg/ mg) on anhydrous basis.
(2) Adjust the quantity of Partially Pregelatinized Maize Starch NF based on the actual quantity of Azithromycin Dihydrate USP taken, to maintain the constant tablet weight

2.2 Study Subjects

All participants screened for the study were provided with information in both verbal and written formats, either in English or in their respective vernacular languages (Marathi or Hindi), regarding the study's objectives and procedures. Screening procedures commenced only after obtaining written consent from the participants.

For the fasting study, a total of 64 subjects were initially planned and enrolled. During the study, one subject was terminated, and two subjects withdrew, with an additional subject dropping out during period II. Consequently, 60

subjects completed the study, and samples from 61 subjects were analyzed for plasma concentration determination. Data from these 60 subjects who completed both study periods were utilized for pharmacokinetic (PK) and statistical analyses.

In the fed study, a total of 66 subjects (including 64 planned subjects and 2 additional standby subjects*) were enrolled. Three subjects discontinued during period II (subjects 02, 26, and 40), resulting in 61 subjects completing the study. Plasma samples from all 61 subjects were analyzed for concentration determination. Data from the 61 subjects who completed both study periods were included in the PK and statistical analyses.

None of the enrolled subjects had a history of drug abuse, alcoholism, drug dependence, or significant medical conditions.

Throughout the study, the safety of the participants was closely monitored. Adverse events (AEs), any serious adverse events (SAEs), vital signs, and general well-being were assessed during the subjects' in-house stays.

*Subjects S1 (number 65) and S2 (number 66) served as two additional standby subjects and were available until the administration of the drug in Period I. After dosing the initial 64 subjects, subjects S1 and S2 were discharged from the study.

2.3 Study Drugs

For this study, Azithromycin tablets 500 mg from ACI HealthCare Limited (Batch # QY005A, Manufactured in June 2020, Expiry: May 2021) and ZITHROMAX® (azithromycin) tablets 500 mg from Pfizer Labs Division of Pfizer Inc, NY, NY 10017 (Batch # DT2758, Expiry: March 31, 2023) were selected. Samples were physically verified and stored under recommended storage conditions until they were used in the study.

2.4 In-Vitro Analysis

Azithromycin dihydrate is classified under the Biopharmaceutics Classification System (BCS) as a Class III compound, characterized by high solubility across the physiological pH range but low permeability. As a result, drug release is not a limiting factor for absorption. The dissolution method recommended by the Office of Generic Drugs (OGD) and specified in the US Pharmacopeia (USP) monograph for this product was employed. This method involves using 900 mL of pH 6.0 phosphate buffer in USP Apparatus II at 75 RPM.

This dissolution method was utilized during formulation and process development and was subsequently adopted for quality control (QC) release testing and dissolution profile studies at multiple time points: 5 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, and 45 minutes.

2.5 Study Design

This study was conducted as an open-label, randomized, two-treatment, two-period, two-sequence, balanced, single oral dose, two-way crossover bioequivalence trial. A total of 64 subjects under fasting conditions and 64 subjects (including 2 standby) under fed conditions participated. All subjects were normal, healthy adults aged 18 to 45 years, with a body mass index (BMI) ranging from 18.50 to 29.99 kg/m².

Each subject participated for 29 days, which included a 21-day washout period between consecutive dosings for the fasting study. For the fed study, subjects participated for 36 days, including a 28-day washout period between dosings.

Subjects were randomized into two treatment sequence groups. Prior to the study, they underwent pre-study examinations within 21 days of the first dosing period to confirm eligibility based on inclusion and exclusion criteria. Upon check-in to the clinical pharmacology unit (CPU), subjects were provided with a standardized dinner. For the fasting study, subjects fasted for at least 10 hours prior to dosing, while for the fed study, subjects fasted for at least 10 hours before receiving a high-fat, high-calorie breakfast 30 minutes prior to dosing.

On dosing days, subjects were administered one tablet of either the test product or the reference product orally, with 240 ± 2 mL of water, at ambient temperature, while seated upright, under either fasting or fed conditions according to the randomization schedule.

2.6 Blood Sampling

A total of 25 blood samples were obtained from each participant, with each sample amounting to 6.0 mL. The samples were collected at specific time points: 0.00 (within 2.00 hours prior to dosing), 0.50, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.50, 4.00, 4.50, 5.00, 6.00, 7.00, 8.00, 10.00, 12.00, 16.00, 24.00, 48.00, 72.00, 96.00, 120.00, and 144.00 hours after dosing. These samples were collected in pre-labeled vacutainers containing K3EDTA as an anticoagulant throughout the study period.

Blood samples collected at 48.00, 72.00, 96.00, 120.00, and 144.00 hours after dosing were obtained on an outpatient basis.

Following collection, the blood samples underwent centrifugation at 4000 RPM for 10 minutes at 5°C±3°C to separate the plasma. After centrifugation, plasma samples were aliquoted into pre-labeled polypropylene tubes in duplicate (Analytical and Replicate) and stored in a deep freezer maintained at-20°C (-15°C to-25°C) within 60 minutes of each blood collection time point at Raptim Clinical Facility (A-226) until transportation to Raptim Bioanalytical Facility.

At the conclusion of the clinical phase, both sets of plasma samples (Analytical and Replicate) were transported to the bioanalytical facility and stored in a deep freezer maintained at-20°C (-15°C to-25°C) until analysis.

The Analytical plasma samples were shipped to the bioanalytical facility first, followed by the Replicate samples after confirmation of receipt of the first shipment. Both shipments were transported at temperatures of-20°C or lower,

packed with dry ice to ensure sample stability. Temperature was monitored during transport using data loggers. Upon arrival, the samples were stored at the bioanalytical facility in a deep freezer at-20°C (-15°C to-25°C) until analysis.

Participants were discharged 24.00 hours after dosing in each study period and returned to the clinic for outpatient blood sample collections at 48.00, 72.00, 96.00, 120.00, and 144.00 hours post-dose.

After the final blood sample collection at 144.00 hours post-dose in Period II, participants underwent safety assessments as part of the post-study evaluation.

The total blood volume withdrawn from any participant did not exceed 345.0 mL throughout the study period.

2.7 Drug Level Bioanalysis

The concentration of Azithromycin in plasma was determined using LC-MS/MS with a method employing a Phenomenex Luna 5µm CN (100 x 4.6 mm) column. The mobile phase consisted of Pump A: Acetonitrile (60%) and Pump B: 5 mM Ammonium formate containing 0.2% formic acid (40%), flowing at a rate of 0.8000 mL/min. The column temperature was maintained at 50°C, while the autosampler temperature was set to 5°C.

A 5 µL injection volume of the plasma sample was used, with a rinsing volume of 1000 µL using a Methanol: Water (50: 50 v/v) solvent mixture. The total run time per sample was 3.8 minutes, as indicated by the reference chromatogram (Fig-1).

Azithromycin eluted at approximately 2.56 minutes, while the internal standard Azithromycin 13C d3 eluted at about 2.55 minutes under these conditions. The method was validated over a concentration range of 5.05 ng/mL to 3005.59 ng/mL for Azithromycin.

Throughout the analysis, the bioanalysts conducting the measurements were blinded to the randomization schedule until all analyses were completed, ensuring unbiased results.

2.8 Pharmacokinetics and Statistical Analysis

Concentration data from plasma samples collected at each sampling time during each study period for each subject underwent pharmacokinetic (PK) and statistical analyses. PK parameters including Cmax, AUC0-t, AUC0-inf, Tmax, t1/2, Kel, AUC Ratio, and AUC Extrapolated for Azithromycin were computed using Phoenix® WinNonlin® Software, Version 8.3.1, and utilized for subsequent statistical evaluations.

Statistical analysis of natural logarithm (Ln)-transformed PK parameters was conducted using SAS® (Statistical Analysis Software, SAS Institute Inc., USA) Version 9.4.

Plasma concentrations of Azithromycin over time and the PK parameters Cmax, AUC0-t, AUC0-inf, Tmax, t1/2, Kel, AUC Ratio, and AUC Extrapolated were individually listed and summarized using descriptive statistics within each treatment group. Descriptive statistics included the number of observations, arithmetic mean, standard deviation, range (minimum-maximum), median, and coefficient of variance for untransformed data of all PK parameters. Geometric least square means and inter-subject coefficient of variation (ISCV, %) were provided for Ln-transformed data of primary PK parameters Cmax, AUC0-t, and AUC0-inf for both the test and reference products.

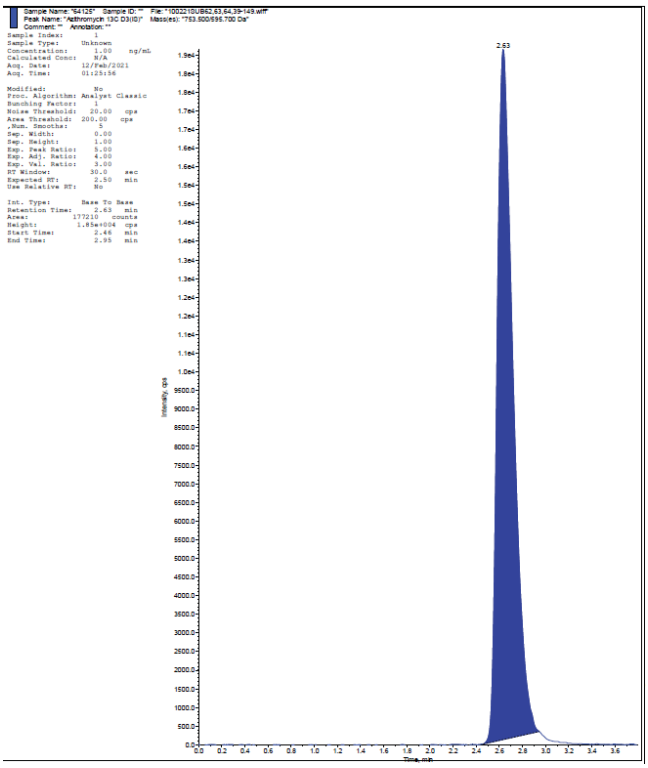

Figure 1.0: Reference Chromatogram

Graphical representations of mean and individual subject's plasma concentrations of Azithromycin over time were presented using linear plots for untransformed data and semi-log plots for Ln-transformed data.

Ratio calculations for primary PK parameters using untransformed data of the test and reference products were provided. PROC GLM was utilized to estimate the differences in geometric least square means (LSM) between the test and reference products for Ln-transformed primary PK parameters of Azithromycin, including computation of standard errors. Ninety percent confidence intervals (CIs) were constructed for these LSM differences. Exponentiating the limits derived from Ln-transformed data provided the 90% CI for the ratio of geometric LSM between the test and reference products.

The statistical analysis comprised:

a) Two one-sided ANOVA tests for bioequivalence assessment,
b) Power analysis,
c) Ratio analysis,
d) Acceptance criteria for bioequivalence.

Following Schuirmann's two one-sided tests procedure, ANOVA was conducted at a 5% significance level on Ln-transformed primary PK parameters (Cmax, AUC0-t, AUC0-inf) to assess equivalence of LSM values between the test and

reference products. This involved calculating a 90% CI for the ratio of population geometric LSMs for each parameter. The ANOVA model included fixed effects factors such as sequence, subjects nested within sequence, period, and treatment. Sequence effects were tested at a 10% significance level using nested subjects as the error term. Treatment and period effects were tested at a 5% significance level against the residual error from the ANOVA model.

Each ANOVA test included computation of LSMs, adjusted mean differences between formulations, and associated standard errors. Geometric LSM values were reported for Ln-transformed primary PK parameters of Azithromycin, upon which the 90% CI calculations were based. Ratios of LSMs were expressed as percentages of the test product compared to the reference product.

Bioequivalence between the test and reference products was concluded if the 90% CI for the ratio (test/reference) of geometric LSMs based on Ln-transformed primary PK parameters (Cmax, AUC0-t, AUC0-inf) fell within the acceptable bioequivalence limits of 80.00% to 125.00% for Azithromycin.

3. Results

Azithromycin exhibits high solubility as indicated by the results of the in-vitro dissolution study conducted in release media, where more than 80% of the drug was released within 30 minutes (Figure 2.0). This demonstrates compliance with the high solubility criteria set by the US FDA.

The maximum intra-subject coefficient of variation (ISCV) observed for Cmax was 27.42%. Based on this ISCV not exceeding 28%, and considering a ratio estimate (Test/Reference) of 93-107%, a significance level (α-error) of 5%, a power of 90%, and the acceptable bioequivalence criteria of 80.00%-125.00%, the estimated sample size for a proposed two-treatment, two-period, two-sequence, two-way crossover, single oral dose bioequivalence study was determined to be 58. Considering a calculated sample size of 58 and accounting for a 10% dropout rate, 64 subjects were deemed sufficient for establishing bioequivalence between the test and reference products in the proposed study design.

No serious adverse events (SAEs) were reported during the fasting period of the study. Additionally, no adverse events (AEs) were reported during the study periods themselves. Four AEs were reported during post-study safety assessments, all of which were of mild to moderate intensity and completely resolved, except for subject number 03 who was lost to follow-up for post-study safety assessment.

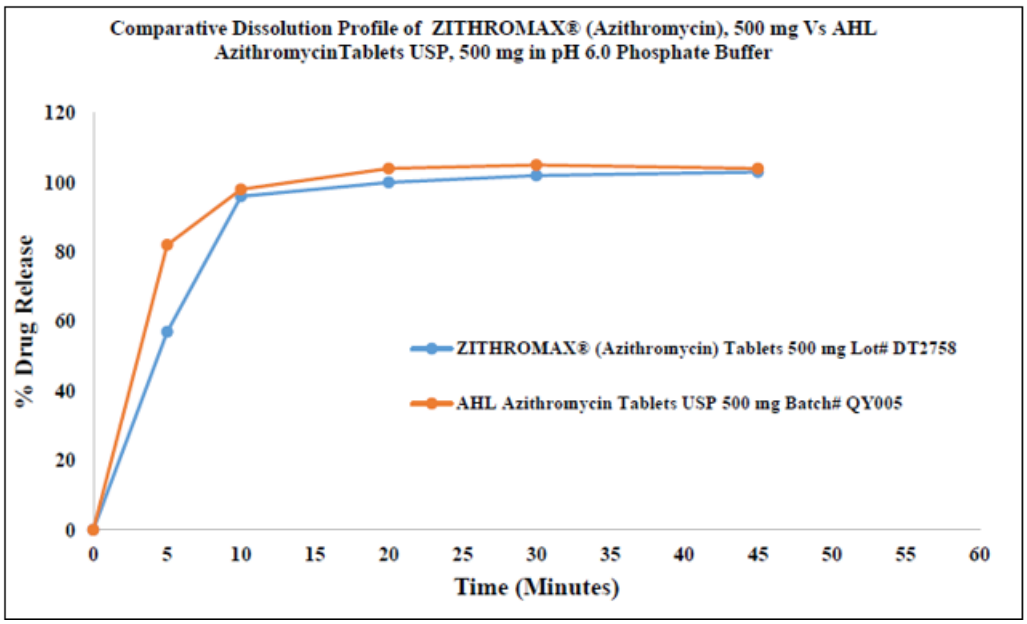

Figure 2: Comparative dissolution profile for ZITHROMAX® (Azithromycin) Tablets 500 mg and AHL's Azithromycin Tablets USP, 500 mg (Batch# QY005) in pH 6.0 Phosphate Buffer (Release Medium)

During the fed study, no serious adverse events (SAEs) were reported. Similarly, there were no adverse events (AEs) noted during the study periods themselves. Seven AEs were reported during post-study safety assessments. All reported AEs were mild to moderate in intensity and resolved completely, except for subject number 37 who was lost to follow-up for post-study safety assessment.

Bioequivalence between the test and reference products for Azithromycin was determined based on whether the 90% confidence intervals (CI) for the ratio (test/reference) of geometric least square means fell within the acceptable bioequivalence (BE) limits of 80.00% to 125.00%.

The 90% CIs for the ratio of geometric least square means based on Ln-transformed primary PK parameters Cmax (fasting: 91.33-104.86, fed: 89.64 – 109.64), AUC0-t (fasting: 93.13-102.76, fed: 90.97-104.79), and AUC0-inf (fasting: 93.37-102.67, fed: 90.70-105.00) were found to be within the acceptable bioequivalence limits for Azithromycin. Detailed results are presented in Tables 1.1 to 2.2.

Mean plasma concentration versus time plots of Azithromycin for both the test and reference products (linear plots) are illustrated in Figure 3.0 (fasting) and Figure 5.0 (fed). Correspondingly, mean Ln-transformed plasma concentration versus time plots (semi-log-linear plots) are

presented in Figure 4.0 (fasting) and Figure 6.0 (fed). These graphical representations provide visual insights into the pharmacokinetic profiles of Azithromycin under fasting and fed conditions.

Table 1.1: Mean Pharmacokinetic Parameters of Azithromycin (N=60) at Fasting Conditions

Pharmacokinetic parameters (units)	Mean ± SD (CV %)	
	Test Product (A)	Reference Product (B)
Cmax (ng/mL)	669.29 ± 264.97 (39.59)	676.48 ± 206.85 (30.58)
AUC0-t (hr. ng/mL)	5119.03 ± 1307.39 (25.54)	5278.95 ± 1473.42 (27.91)
AUC0-inf (hr. ng/mL)	5761.24 ± 1445.08 (25.08)	5915.79 ± 1589.77 (26.87)
Tmax (hr) *	2.33 (1.00-5.00)	2.17 (1.00-5.00)
Kel (hr-1)	0.01 ± 0.00 (24.68)	0.01 ± 0.00 (27.00)
t1/2 (hr)	55.02 ± 17.85 (32.45)	53.98 ± 15.93 (29.51)
AUC Ratio (%)	88.87 ± 3.74 (4.20)	88.99 ± 4.16 (4.68)
AUC Extrapolated (%)	11.13 ± 3.74 (33.57)	11.01 ± 4.16 (37.82)
N-Number of evaluated subjects; *Median (Range) is provided		

Table 1.2: Bioequivalence Assessment of Azithromycin (N=60) at fasting conditions

Parameter (Units)	Geometric Least-Squares Means [1]		Test-to-Reference Ration (%) [2]	ISCV (%) [3]	90% Confidence Interval Limits[4]		Power (%)
	Test	Reference			Lower	Upper	
LnC_{max} (ng/mL)	626.50	640.18	97.86	22.91	91.33	104.86	99.94
$LnAUC_{0-t}$ (hr. ng/mL)	4957.47	5067.71	97.82	16.22	93.13	102.76	100.00
$LnAUC_{0-inf}$ (hr. ng/mL)	5582.90	5702.05	97.91	15.64	93.37	102.67	100.00

1. For loge-transformed results (Ln), value is the least-squares geometric mean.
2. Ratio% of geometric least-squares means for loge-transformed results.
3. ISCV%=Intra-subject CV% calculated from the mean square term of the ANOVA.
4. Confidence interval on ratio.

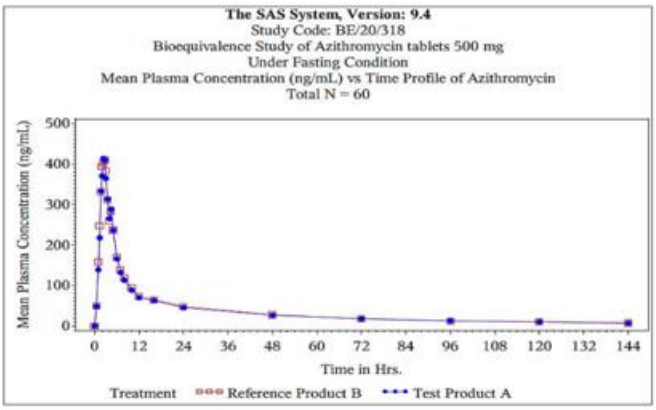

Figure 3: Mean plasma concentration (ng/mL) versus time plot of Azithromycin for the test product and the reference product (Linear plot)

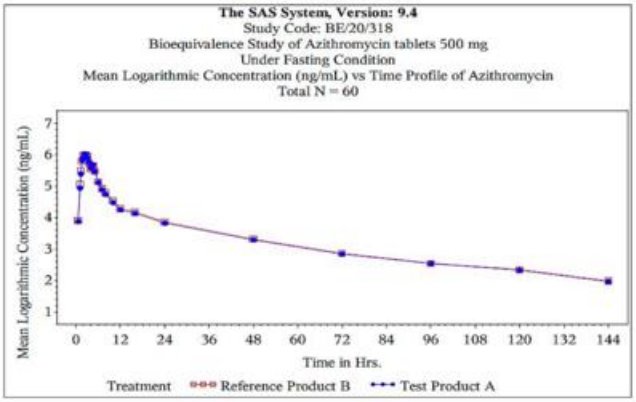

Figure 4: Mean Ln-transformed plasma concentration (ng/mL) versus time plot of Azithromycin for the test product and the reference product (Semi-log-Linear plot)

Table 2.1: Mean Pharmacokinetic Parameters of Azithromycin (N=60) at Fed Conditions

Pharmacokinetic parameters (units)	Mean ± SD (CV %)	
	Test Product (A)	Reference Product (B)
Cmax (ng/mL)	840.49 ± 409.05 (48.67)	857.09 ± 443.63 (51.76)
AUC0-t (hr. ng/mL)	5093.27 ± 1509.92 (29.65)	5268.84 ± 1667.91 (31.66)
AUC0-inf (hr. ng/mL)	5817.96 ± 1651.68 (28.39)	6074.79 ± 2076.69 (34.19)
Tmax (hr) *	2.33 (1.33-7.00)	3.00 (1.33-5.00)
Kel (hr-1)	0.01 ± 0.00 (35.52)	0.01 ± 0.00 (32.57)
t1/2 (hr)	57.97 ± 22.85 (39.41)	61.58 ± 40.73 (66.13)
AUC Ratio (%)	87.30 ± 6.09 (6.98)	87.31 ± 6.12 (7.01)
AUC Extrapolated (%)	12.70 ± 6.09 (47.95)	12.69 ± 6.12 (48.23)
N-Number of evaluated subjects; *Median (Range) is provided		

Table 2.2: Bioequivalence Assessment of Azithromycin (N=61) at Fed Conditions

Parameter (Units)	Geometric Least-Squares Means[1]		Test-to-Reference Ration (%) [2]	ISCV (%) [3]	90% Confidence Interval Limits[4]		Power (%)
	Test	Reference			Lower	Upper	
LnC_{max} (ng/mL)	749.14	756.26	99.06	33.88	89.64	109.46	95.58
$LnAUC_{0-t}$ (hr. ng/mL)	4835.02	4951.92	97.64	23.66	90.97	104.79	99.91
$LnAUC_{0-inf}$ (hr. ng/mL)	5550.14	5687.30	97.59	24.51	90.70	105.00	99.85

1. For loge-transformed results (Ln), value is the least-squares geometric mean.
2. Ratio% of geometric least-squares means for loge-transformed results.
3. ISCV%=Intra-subject CV% calculated from the mean square term of the ANOVA.
4. Confidence interval on ratio.

The study results confirmed that the test product, Azithromycin tablets 500 mg, exhibited bioequivalence to the reference product ZITHROMAX® (azithromycin) tablets 500 mg under fed conditions.

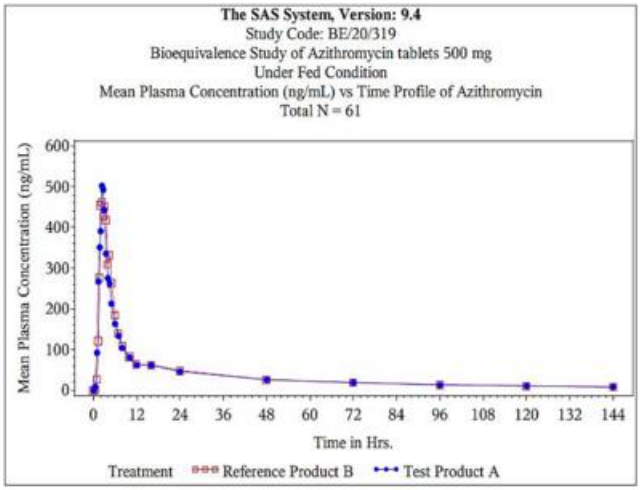

Figure 5: Mean plasma concentration (ng/mL) versus time plot of Azithromycin for the test product and the reference product (Linear plot)

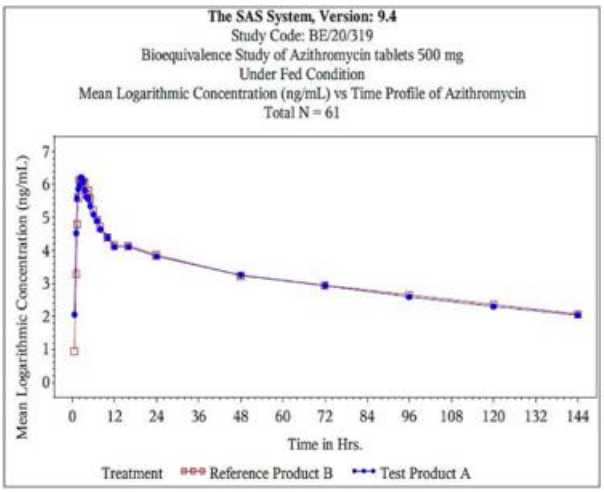

Figure 6: Mean Ln-transformed plasma concentration (ng/mL) versus time plot of Azithromycin for the test product and the reference product (Semi-log-Linear plot)

4. Discussions

The study findings indicated that the 90% confidence intervals for the ratio (test/reference) of geometric least square means, based on Ln-transformed primary PK parameters Cmax, AUC0-t, and AUC0-inf, fell within the acceptable bioequivalence limits of 80.00% to 125.00% for Azithromycin. This confirms that the test product, Azithromycin tablets 500 mg, demonstrated bioequivalence to the reference product ZITHROMAX® (azithromycin) tablets 500 mg under fasting conditions.

Throughout the study, there were no serious adverse events (SAEs) reported, and no adverse events (AEs) were observed with either the test product or the reference product. Both formulations were well tolerated by the study participants, further supporting their safety and equivalence in clinical use.

Acknowledgement

Azithromycin Tablets were developed by ACI HealthCare Limited, located in Songargaon, Narayangonj, Bangladesh. The study conducted at Raptim Research Pvt. Ltd., India, was sponsored by ACI HealthCare Limited and technically supported by USP PQM+, indicating their involvement and support in the research and development of Azithromycin tablets.

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