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Bioequivalence of Two Brands of Omeprazole 20 mg Gastro-Resistant Capsules in 18 Healthy Algerian Volunteers: A Pilot Study

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Abstract: A randomized, two-way, crossover study was conducted in 18 fasting, healthy, algerian volunteers to compare the bioavailability of two brands of Omeprazole 20 mg Gastro-Resistant Capsules where MOPRAL (Astra Zeneca) was the reference product. The study was performed at the bioequivalence center of the national control laboratory for pharmaceuticals product. The drug was administered on two treatment days separated by one week washout period. After dosing, serial blood samples were collected for a period of 12 h. A reliable and robust LC-MS/MS (liquid chromatography-tandem mass spectrometry) method has been developed and validated for the estimation of Omeprazole in human plasma. The assay was found to be linear over the range of 5-1,000 ng/mL. The pharmacokinetical and statistical analysis was conducted with Kinetica 4.4.1. AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were tested for bioequivalence. No significant difference was found based on ANOVA; 90% confidence interval ([97.14%-117..85%] for AUC_{0-t}, [97.17%-117.67%] for $AUC_{0-\infty}$) of test/reference ratio for these parameters were found within bioequivalence acceptance range of 80%-125%. But for the C_{max}, it was not in this acceptance range [73.5%-100.54%]. The results of PK analysis suggested that the reference and test formulations of Omeprazole 20 mg Gastro-Resistant Capsules were not bioequivalent during fasting state in these healthy Algerian volunteers.

Key words: Omeprazole, bioequivalence, LC-MS/MS, pharmacokinetics.

1. Introduction

Bioequivalence of two formulations of the same drug includes equivalence with respect of the rate and extent of their absorption. The area under concentration time curve (AUC) generally serves as the characteristic of the extent of absorption while the peak concentration (C_{max}) and the time of its occurrence (T_{max}), reflect the rate of absorption [1]. In bioequivalence studies, the exposure profile of a test drug product is compared to that of a reference drug product [2].

Omeprazole (Fig. 1) belongs to a class of antisecretory compounds, the substituted benzimidazoles that suppress gastric acid secretion by specific inhibition of the H^+/K^+ ATPase enzyme system at the secretory surface of the gastric parietal cell. This effect is dose-related and leads to inhibition of both basal and stimulated acid secretion irrespective of the stimulus [3]. Omeprazole is a very well tolerated drug, widely used in doses of 20 mg up to 80 mg in duodenal and gastric ulcers, reflux oesophagitis and in the Zollinger-Ellison Syndrome [4].

As Omeprazole is destroyed in an acid medium, it is administered orally in the form of gastroresistant microgranules into gastroresistant capsules. The absorption of Omeprazole is rapid with a peak plasma obtained approximately in 1 to 2 hours. The absolute bioavailability of an oral dose of Omeprazole is approximately 40%. The volume of distribution of Omeprazole in healthy subjects is approximately 0.3 L/kg body weight. The rate of binding of Omeprazole to plasma proteins is 97%. The majority of its metabolism depends on CYP2C19, which is responsible for the formation of hydroxy-omeprazole.

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The plasma elimination half-life of omeprazole is usually less than one hour after a single oral dose or repeated once daily. Approximately 80% of an oral dose of Omeprazole is excreted in the urine as metabolites, the 20% is excreted in the feces from biliary secretion [3, 5].

It is reported that the time for appearance of Omeprazole in plasma seemed to be prolonged when given after food intake. The total amount of drug absorbed, however, was not affected [6]. Thus, concomitant food intake does not affect bioavailability [5].

Because of the low bioavailability and high inter and intra individual variability in the absorption of Omeprazole, it is necessary to perform comparative bioavailability studies [3, 7]. The aim of this study was to evaluate, in healthy Algerian volunteers, the rate and extent of absorption of a local generic Omeprazole formulation against that of the innovator product MOPRAL® from ASTRA ZENECA laboratories in order to evaluate the intra-subject variability of Omeprazole (Coefficient of variation intra-subject: CVintra of C_{max} and AUCs) and to validate the application of developed LC-MS/MS Omeprazole quantification method.

2. Materiel and Methods

2.1 Study Products

Two formulations of Omeprazole 20 mg were evaluated:

Reference formulation: MOPRAL® 20 mg gastro-resistant capsules (batch number YBZR, expiry date 12/2018) manufactured by ASTRA ZENECA.

Test formulation: Omeprazole 20 mg gastro-resistant capsules (batch number 038, expiry date 09/2018).

2.2 Study Subjects

Eighteen healthy Algerian subjects (18 male), suitable for a pilot study, were enrolled, but only 15 subjects completed the study—one voluntary withdrawal before the study—with mean (SD) age, 28.78 (3.62) years (range: 21-37); mean (SD) body weight, 72.78 (12.72) kg (range: 52-92 kg); mean (SD) height, 173.33 (4.45) cm (range: 1.64-1.80 m) and mean (SD) body mass index (BMI), 24.17 (3.79) kg/m^2 (range: 18.16-29.36 kg/m^2).

The volunteers were screened by a complete clinical examination and laboratory tests (hematological, biochemical and urinary analysis and serological test) and were requested to be abstained from taking any medication for two weeks before and during the study, from taking vitamins two days prior the study, from taking grapefruit seven days before the study and from smoking, as well as consuming caffeine or drinks or foods containing xanthines related for 48 h prior to the study drug administration.

2.3 Ethical Consideration

This research was carried according to the Declaration of Helsinki (Seoul, 2008) and GCP (Good Clinical Practice) Guidelines.

The study was conducted at National Control Laboratory for Pharmaceuticals Products (Algiers, Algeria) according to a protocol approved by Research Ethics Committee of University Hospital Center Issad Hassani (Beni Messous, Algiers) and by Ministry of Health.

All the subjects are provided written information consent before entering the study.

2.4 Study Design

The study was based on a randomized, single dose, two-way crossover design under fasting condition with a washout period of one week.

In the morning of phases I and II, after an overnight fast (10 h) volunteers were given a single dose of either formulation (reference or test) of Omeprazole 20 mg with 240 mL of water. No food was allowed until 4 h after dose administration, lunch, snack and dinner were given to all volunteers according to a time schedule. The volunteers were continuously monitored by a medical staff throughout the confinement period of the

study.

2.5 Blood Sampling

Approximately, 5 mL of blood samples for Omeprazole assay was obtained through a heparin-locked catheter before (0 h) and at 15', 30', 45', 60', 75', 90', 105', 120', 135', 150', 165', 180', 210', 240', 5 h, 6 h, 7 h, 8 h, 10 h and 12 h after dosing. The blood samples were collected in tubes containing heparin, and centrifuged at 1,800 rpm for 10 min at 20 °C; plasma was separated and kept frozen at -80 °C in properly labeled tubes. After a period of 7 days, the study was repeated in the same manner to complete the crossover design.

2.6 Optimization of MS Parameters and Chromatographic Conditions

An LC-MS/MS method was developed and validated, for Omeprazole analysis in plasma samples. All solvents were of HPLC grade, other chemicals and reagents were of analytical grade. Omeprazole and Sildenafil (internal standard Fig. 1) are used as reference standards.

The LC-MS/MS system was composed of: an HPLC Perkin Elmer SER 200 (which contains an auto-sampler SER 200 and a binary pump (LC-200Q)) and a mass spectrometer AB Sciex Instruments; 4000 Q Trap triple quadruple instrument equipped with an ESI source. Analyst 1.5.1 software was used for data interpretation.

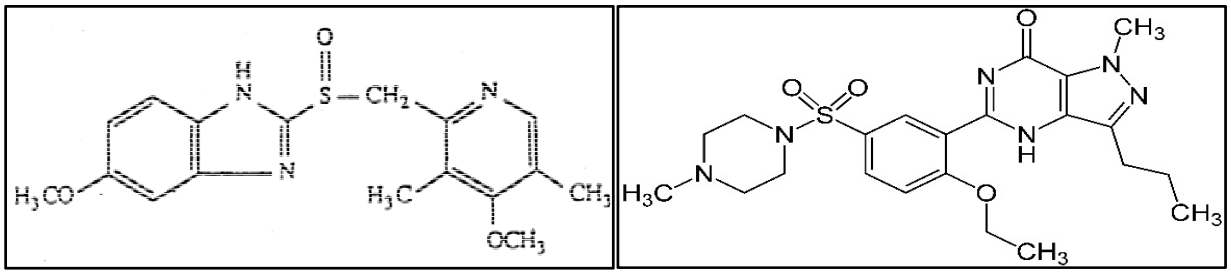
The method was developed in positive mode with turbospray source (ESI) by infusion of 0.1 μg/mL aqueous solutions of Omeprazole and Sildenafil reference standards. The ion transitions m/z 346.1 → 198.1 and 475.2→ 100.0 were selected for the MRM of Omeprazole and Sildenafil respectively. The compound parameters were optimized as follows: Declustering potential: 47 V and 120 V, entrance potential: 6 V and 12 V, collision cell exit potential: 16 V and 16 V, and collision energy: 19 V and 42 V for Omperazole and Sildenafil respectively. The source/gas parameters were optimized as follows: Curtain gas: 25, CAD: High, ion source gas—1:50, ion source gas—2:50, ion spray voltage: 4,500 V and temperature: 600 °C [9-11].

Chromatographic separation was performed using SUPELCO C18 (150 × 4.6) mm, 5 μm, 100 Å column at 40 °C. The mobile phase consisted of methanol of grade HPLC. The mobile phase was eluted at a flow rate of 1.2 mL/min in isocratic mode, each analysis required 5 min. The retention time was 1.6 min for both Omeprazole and Sildenafil. Quantitation was achieved by measurement of the peak area ratio of the drug to the internal standard, using Analyst 1.5.1 software.

The method was validated by following international guideline [12]. The calibration curves were validated over the concentration range of 5-1,000 ng/mL for Omeprazole in human plasma in the low limit of quantification LLOQ of 5 ng/mL.

2.7 Sample Preparation

A 50 μL internal standard (Sildenafil, 50 μg/mL) was



(a) Omeprazole (b) Sildenafil

Fig. 1 Chemical structures of Omeprazole; 5-methoxy-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl)lmethyl]sulphinyl}-1H -benzimidazole and *Sildenafil*; 1-[4-éthoxy-3-(6,7-dihydro-1-méthyl-7-oxo-3-propyl-1H >-pyrazolo[4,3-d]pyrimidin-5-yl) (phénylsulfonyl]-4-méthylpipérazine [4, 8].

added to 200 μL plasma sample and vortexed for 30 seconds, then 600 μL of acétonitrile was added and vortexed for 30 seconds and then centrifuged for 10 min at 4,000 g. A 100 μL of the supernatant was transferred to a vial and was added 900 μL of methanol and vortexed for another 30 seconds. Then 20 μL of each sample was injected into the LC-MS/MS for analysis [13].

The procedure described here was applied not only to subject's samples, but also to the extraction of samples for calibration curve and QC (quality control) process.

2.8 Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by a model independent method using a Kinetica 4.4.1 computer program [14]. The elimination rate constant (lZ) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. The elimination half-life ($T_{1/2}$) was calculated as 0.693 /lZ. The area under the curve to the last measurable concentration AUC0-t was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity AUC0-∞ was calculated as AUC0-t+C_t/lZ, where C_t is the last measurable concentration.

2.9 Statistical Analysis

For the purpose of bioequivalence analysis AUC0-t, AUC0-∞ and C_{max} were considered as primary variables. The bioequivalence of the two products was assessed by means of an analysis of variance (ANOVA GLM procedure; Kinetica 4.4.1 Computer program) for crossover design and calculating standard 90% confidence intervals of the ratio T/R (test/reference) using log-transformed data. The products were considered bioequivalent if the difference between the two compared parameters was found statistically insignificant ($p \geq 0.05$) and 90% confidence intervals for these parameters fell within 80%-25% [1, 2, 15].

3. Results and Discussion

All 18 participants successfully completed both phases of the study with no protocol violations. No serious adverse drug reactions or side effects were reported by the participants or observed by investigators during the study periods.

The relationship between concentration and peak area ratio was found to be linear within the range of 5-1,000 ng/mL with LLOQ of 5 ng/mL. As shown in Table 1, the intraday accuracy of the method ranged from 91.37% to 103.08% while the intraday precision ranged from 2.24% to 10.47%. The inter-day accuracy ranged from 96.8 % to 102.13 % while the inter-day precision ranged from 2.57 % to 14.29 %.

This reproductibility of Omeprazole was able to increase assay sensitivity. Therefore, simple protein precipitation procedure using methanol and acetonitrile has been successfully applied to the extraction of Omeprazole from human plasma. The developed method using the centrifugation technique offers the advantages of a simple and a safe sample preparation procedure without the matrix effect and high throughput with uniformity of extraction which is a critical challenge in LC-MS/MS method development.

Table 1 Precision and accuracy of Omeprazole in human plasma.

Concentration ng/mL	Precision (CV%)		Accuracy (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
5 (LLOQ)	10.47	14.29	91.37	97.16
15	8.20	5.92	97.05	102.13
350	6.01	5.19	103.08	98.82
700	2.24	2.57	94.95	96.8
Mean ± SD	6.355 ± 3.50	6.9925 ± 5.07	96.6125 ± 4.91	98.7275 ± 2.43

LLOQ=lower limit of quantification. CV=coefficient of variation = (SD/mean)*100. All the data were presented as arithmetic means.

Stability studies showed that Omeprazole was stable in plasma for 30 days when stored at -80 °C. The method had a total analysis time of 5 min, which is favored to analyze the samples on a large scale.

Both formulations were rapidly absorbed from the gastrointestinal tract. The peak concentration of 412.44 ng/mL and 366.94 ng/mL for Omeprazole was attained at 2.04 h and 2.02 h after administration of reference and test products respectively, and then declined and remained detectable up until 12 h. The half life elimination was 1.00 h and 1.02 h for reference and test products respectively. These values agree with the bibliographic data [3, 5]. Table 2 shows the pharmacokinetic parameters of Omeprazole for the two brands.

The relative bioavailability of Omeprazole test was 106.999% for AUC0-t, 106.931% for AUC0-∞, and 86.114% for C_{max}.

The 90% confidence limits for AUC0-t, AUC0-∞, and C_{max} as well as the results of the Schuirmann's two one-sided t-tests are also shown in Table 3.

Mean drug plasma concentration-time profiles of Omeprazole (Fig. 2) were nearly identical, suggesting an equal *in vivo* performance of the two products.

Ratio AUC0-t/AUC0-∞ of the two formulations was > 80%, suggesting that the duration of sample collection was appropriate, covering > 80% of the complete drug profile.

Table 2 Pharmacokinetic parameters of Omeprazole gastro-resistant capsule (arithmetic mean ± standard deviation, n = 18).

Pharmcokinetic parameter	Test	Reference
C_{max} (ng/mL)	366.94 ± 220.46	412.44 ± 212.15
SSC_{0-t} (ng. h /mL)	738.34 ± 529.38	546.02 ± 456.82
$SSC_{0-\infty}$ (ng. h/mL)	751.46 ± 537.00	555.96 ± 460.88
t_{max} (h)	2.02 ± 0.98	2.04 ± 1.04
t½ (h)	1.02 ± 0.55	1.00 ± 0.45

Table 3 The statistical evaluation of bioequivalence after oral dosage of 20 mg Omeprazole of each formulation.

	Geometric mean ± SD		CI	CV_{intra}	t-Test	
	Test	Reference			Lower	Upper
AUC_{0-t} (ng/mL·h)	6.41	6.35	[97.14%-117.85%]	16.71%	2.80	5.25
$AUC_{0-\infty}$ (ng/mL·h)	6.43	6.37	[97.17%-117.67%]	16.55%	2.84	5.29
C_{max} (ng/mL)	5.75	5.90	[73.75%-100.54%]	27.09%	0.83	4.20

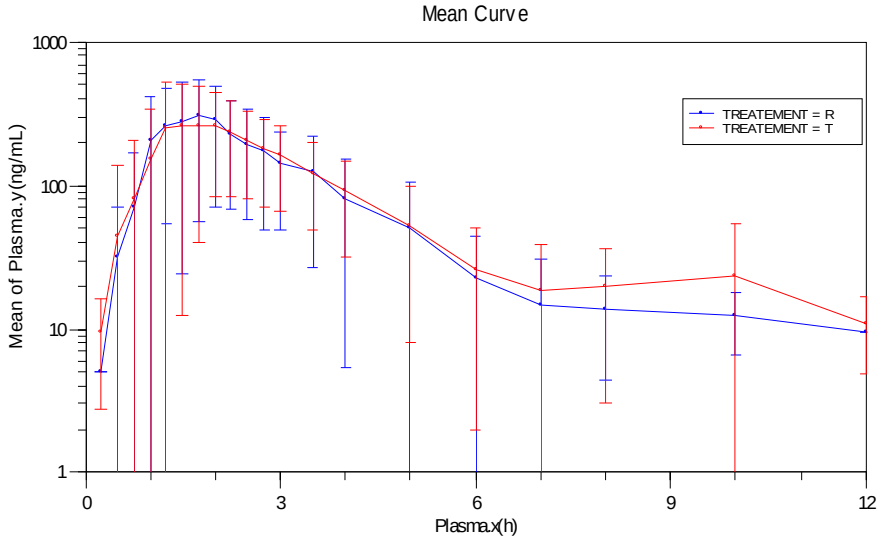
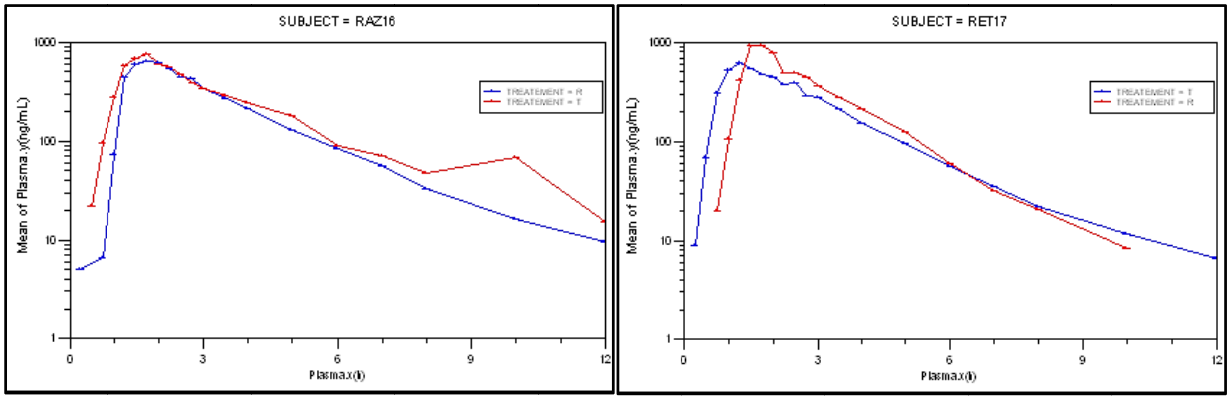


Fig. 2 Mean drug plasma concentration-time profiles of Omeprazole test and reference (ln transformation).



(A) (B)

Fig. 3 Mean drug plasma concentration-time profiles of Omeprazole test and reference in volunteer No. 16 (A) and No. 17 (B) (ln transformation).

The mean and standard deviation of AUC0-t, AUC0-∞ and C_{max} of the two products did not differ significantly, suggesting that the blood profiles generated by Omeprazole test are comparable to those produced by MOPRAL.

Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations either in sequence or formulations, having *p* value greater than 0.05. Same while, statistically significant difference has been found between the two formulations in periods and subjects ($p < 0.05$).

The chosen subjects present physiological differences/variability and they are not identical clones or twins which may explain the subject's effect.

The period's effect cannot be due to a residue's effect as the wash-out was widely sufficient and the C_{P0} at the second period was less than 5% of the C_{max}. Otherwise, the physiological status of the volunteers and/or the environmental changes between the two periods may affect the biodisponibility and so may explain the period's effect. This last is not a bias for our study.

The 90% confidence intervals of AUC0-t and of AUC0-∞ were within the acceptable bioequivalence range of 80% to 125%, but the 90% CI of the C_{max} ratio did not fall within the regulatory range of 80% to 125%. The intra-subject variations for AUC0-t, AUC0-∞ and C_{max} were calculated as 16.71%, 16.55% and 27.09% respectively.

The pharmacokinetic findings in this study are well in agreement with published data for earlier trials [5, 16], in which [16] the confidence interval of C_{max} for their products fell outside the FDA accepted range (0.8%-1.25%). The differences that they have found in C_{max} may be the results of having some subjects who are poor metabolizers since these authors did not exclude them from their data same as us.

Furthermore, it is reported [17] that the $t_{1/2}$ and mean AUC value were approximately 3 times longer and 10 times greater in poor metabolizers than in extensive metabolizers. The deficient metabolizers are known to build up high plasma concentrations over longer periods. Our results showed that the mean AUC values were approximately 3-4 times greater in two subjects No. 16 and 17 (Fig. 3), which is in concordance with the frequency of occurrence of the poor metabolizer phenotype of S-mephenytoin in Caucasian populations (3%-6%) [18]. This might be due to the number of subjects that participated in this study; the sample sizes required to generate > 80% power with error rate of 0.05 and high intra subject variability (30%), if the true difference is equal or less than 20%, it is calculated as 52 subjects [19].

4. Conclusions

In this study, a simple, selective, accurate and

reproducible LC-MS/MS method is positive ESI mode was developed and validated for the estimation of Omeprazole in human plasma. The method shows good performance with respect to all the validation parameters tested. In addition, the present method uses the protein precipitation extraction method and offers high throughput because of a shorter run time. This approves the applicability of the method in our bioequivalence study.

The results of PK analysis suggested that the reference and test formulations of Omeprazole 20 mg gastro-resistant capsules were not bioequivalent during fasting state in these healthy Algerian volunteers.

This pilot study led to evaluate the variability of Omeprazole. Therefore, it is necessary to do a pivot study with an adequate sample size to overcome the intra-subject variability.

In conclusion, the pharmacokinetic results of this study confirm earlier findings [4, 7, 16, 20, 21] and emphasize that it is advisable in future researches to assess the metabolic status by phenotyping subjects with an adequate test prior to conducting pharmacokinetic studies.

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