Acta Poloniae Pharmaceutica – Drug Research, Vol. 71 No. 3 pp. 463–468, 2014 ISSN 0001-6837
Polish Pharmaceutical Society

STUDY OF COMPARATIVE BIOAVAILABILITY OF OMEPRAZOLE PELLETS

SABIHA KARIM[1]*, YUEN KAH HAY[2], SARINGAT H. BAIE[2], NADEEM IRFAN BUKHARI[1] and GHULAM MURTAZA[3]

[1]University College of Pharmacy, University of Punjab Lahore, Allama Iqbal Campus, Lahore-54000, Pakistan
[2]School of Pharmaceutical Sciences, Universiti Sains Malaysia, Pulau Penang-11800, Malaysia
[3]Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan

Abstract: The objective of this study was to assess the bioequivalence between the omeprazole laboratory based formulation and the commercial formulation, Zimor® Rubio, Spain, considered as reference formulation. The experiment was carried out according to a 2-period, 2-sequence crossover design with a two week washout period. A validated high performance liquid chromatographic method was applied for *in vivo* experiments. It was observed that omeprazole contents were comparable in all formulations. To establish bioequivalence, 90% confidence intervals (CI) for the differences of total AUCs of the test and reference formulations were calculated. The 95% CI ratio of the AUC within 0.80 to 1.25 was considered as bioequivalent. The carryout effect was investigated prior to assessing the bioequivalence of the two formulations.The test formulation of omeprazole was found to be comparable with the reference formulation (Zimor®) with regard to bioavailability.

Keywords: pellets, omeprazole, HPLC method, bioequivalence, Zimor®.

In vitro and *in vivo* characterization of a prepared multiparticulate formulation in terms of size distribution, dissolution and drug release is valuable during the formulation developmental stage of pharmaceutical products (1, 2), since absorption of drug is directly affected by the size of formulation. A number of variables affecting the *in vitro* and *in vivo* performance can be investigated. These, in turn, provide the basis for formulating a product with the required drug characteristics. However, *in vitro* studies, such as dissolution, cannot directly predict the *in vivo* performance of formulated product. Therefore, it is essential for a formulation to be verified through *in vivo* testing after satisfactory *in vitro* release profile has been obtained (3).

The development of delayed release omeprazole pellets with satisfactory *in vitro* release profile has been evaluated in our previous presentation (4). On the basis of their satisfactory *in vitro* dissolution tests, this bioavailability study was aimed to evaluate the test formulation in comparison with reference capsule formulation, Zimor® 20 mg in rabbits.

EXPERIMENTAL

Chemicals, solvents and materials

Omeprazole powder was purchased from Cornileus. Chloramphenicol (internal standards for omeprazole) was obtained from Hangzhou Garden Enterprise, China. Most of the chemicals and solvents used in this study were of analytical grade. Methanol, acetonitrile, glacial acetic acid, dichloromethane, crospovidone, polyvinylpyrrolidone (PVP) K90 and K30, lactose monohydrate, sodium lauryl sulfate, Avicel PH 101 and disodium hydrogen orthophosphate were purchased from Merck (Germany).

Preparation of omeprazole pellets

The sieving-spheronization and extrusion-spheronization approaches were used to prepare pellet formulations of omeprazole (F1 to F21) (Table 1) as described previously (4). Variable concentrations of microcrystalline cellulose, lactose and polyvinylpyrrolidone were employed keeping the amount of

* Corresponding author: e-mail: sabihakarim@yahoo.com; phone: +92-334-4309300; fax: +92-992-383441

Table 1. Composition of test formulation of omeprazole (F21) pellets [microcrystalline cellulose (MCC), polyvinypyrrolidone (PVP)].

Formulation Code	Drug (omeprazole)	MCC	Lactose	PVP (K30)	Sodium lauryl sulfate	Polyethylene glycol Grade (6000)	Water	Buffer
21	20 g	24 g	97.75 g	3.75 g	0.6 g	3.90 g	1 mL	7 mL

Table 2. Recovery, intraday and inter-day precision and accuracy of omeprazole from test formulation from standard solutions.

	Amount	Recovery (n = 3)		Intraday (n = 6)		Inter-day (n = 6)	
	(ng/mL)	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
From standard solution	120	100	1.85	100	1.55	100.76	1.35
	80	101	1.15	100.63	1.80	99.32	1.65
	50	99.8	3.85	98.60	2.87	98.25	3.35
	20	100	5.85	100	5.05	100.21	4.68
From rabbit plasma	120	98.32	3.59	97.35	4.54	96.98	5.43
	80	97.61	4.58	97.39	4.39	95.25	5.56
	50	95.87	4.89	96.06	4.79	95.16	5.59
	20	95.24	6.44	95.46	5.96	95.3	6.78

drug at a fixed level i.e., 20 g of omeprazole. The granulating liquid consisted of a mixture of water and phosphate buffer pH 8. The obtained dried pellets were stored in air-tight containers for further study.

In vitro dissolution test of pellets

In vitro dissolution test on 1 g of pellets from each batch was conducted in 1000 mL of phosphate buffer with pH 6.8 stirred at a rate of 100 rpm at 37.0C ± 0.5°C involving the withdrawal of samples at 10, 20, 30 and 45 min, followed by analysis using a UV/VIS spectrophotometer (Hitachi, Japan) at a wavelength of 300 nm (4). Each experiment was repeated in triplicate. The dissolution data were evaluated by applying different kinetic models (5–10). Based on *in vitro* dissolution data, the optimum formulation (F21) was opted (Table 1) and used for bioequivalence study.

HPLC method for omeprazole analysis

Preparation of omeprazole standard solutions

Stock solution of omeprazole was prepared in methanol in a concentration of 1 mg/mL. Working standard solutions of concentrations ranging from 2.5, 5.0, 10.0, 20, 40, 80, 160, 320, and 640 ng/mL were prepared by further diluting the stock solution with the mobile phase (5).

Mobile phase

The mobile phase to validate omeprazole method was prepared by mixing 0.05 M of disodium hydrogen orthophosphate and acetonitrile in the ratio of 65 : 35 (v/v). The pH of the mobile phase was adjusted to 6.5 with glacial acetic acid. The mobile phase was filtered and degassed using the same procedure as mentioned above (5).

Chromatographic conditions

The samples of omeprazole were eluted with isocratic mobile phase comprising of 0.05 M Na_2HPO_5 and acetonitrile (65 : 35 v/v) adjusted to pH 6.5. Elution time was 10 min. Flow rate was fixed at 1 mL/min. The volume of sample injected was 15 µL and detection was carried out at 302 nm (5).

Recovery, accuracy and precision of HPLC method

Standard solution of omeprazole in a concentration of 20, 50, 80 and 120 ng/mL were used to evaluate the recovery, intraday, inter-day accuracy and precision of the method. All the samples were analyzed using chromatographic conditions mentioned above.

For recovery of omeprazole from rabbit plasma, one mL aliquot of the plasma was taken in a glass tube with teflon lined screw cap, followed by the addition of 100 µL of 0.5 M disodium hydrogen phosphate, 100 µL of the internal standard solution (3 µg/mL of chloramphenicol in methanol) and 5 mL of dichloromethane. The mixture was vortexed for 30 s before centrifuging at 2000 rpm for 10 min. The organic layer was transferred into a reaction vial and evaporated to

dryness at 35°C under a gentle stream of nitrogen gas. The residue was reconstituted with 100 µL of mobile phase and 15 µL was injected into HPLC system.

The extraction recovery values were calculated by comparing the peak height of the standard after extraction with that of its standard solution at similar respective concentration. For recovery, the samples were analyzed in triplicate, while for intraday accuracy and precision each standard was analyzed 6 times in a single day and quantified at 4 data points calibrations, while for inter-day accuracy and precision each standard was analyzed 6 times for 5 consecutive days. Accuracy was expressed as a percentage of the drug while precision was expressed as relative standard deviation (RSD) (5). The validation parameters are presented in Table 2.

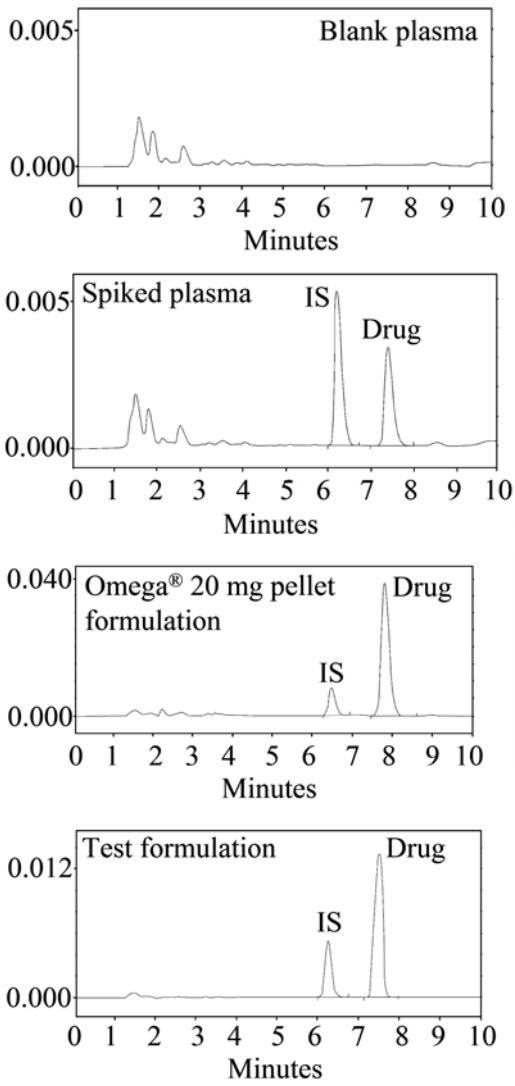

Figure 1. Chromatograms of blank plasma, spiked plasma, market formulation, and test formulation with 0.08 µg of omeprazole/mL, 3 µg internal standard (IS = chloramphenicol)

Preparation of samples of omeprazole test and market formulations for analysis

The 150 mg of omeprazole pellets (F21) equivalent to 20 mg omeprazole and a commercial formulations 150 mg granules equivalent to 20 mg omeprazole (Zimor®) were separately dissolved in 1000 mL of methanol followed immediately by further 50 times dilution with 0.01 M of disodium hydrogen orthophosphate (Na_2HPO_5) adjusted to pH 9.3. Chloramphenicol solution 20 µL of 3 µg/mL was used as an internal standard. Working samples were filtered through 0.55 µm syringe filter (Whatman, Maidstone, England) and kept in HPLC vials. The samples were analyzed in triplicate.

***In vivo* experiments**

Experimental animals

Twelve healthy white albino male rabbits weighing 3.45 ± 0.61 kg were used in the Animal House of the Universiti Sains Malaysia, Pulau Penang, Malaysia. Standard pellet diet (Gold Coin, Penang, Malaysia) and tap water were supplied *ad libitum*. The study protocol was approved by the Animal Ethics Committee, Universiti Sains Malaysia (USM/PPSF/50(014)Jld.2).

Study design

The bioavailability and pharmacokinetic studies were performed for the assessment of bioequivalence between the omeprazole laboratory based formulation, taken as test formulation (F21), and the commercial formulation, Zimor® Rubio, Spain, considered as reference formulation. The animals were randomly divided into two groups, each having six animals. The experiment was carried out according to a 2-period, 2-sequence crossover design with a two week washout period. The animals were fasted for 24 h prior to the administration of drug but had free access to water. After drug administration, no food was allowed for further 6 h but free access to water was allowed *ad libitum*.

In vivo experiments

The pellets of the laboratory formulation were filled into the hard gelatin capsules of size 4 containing 150 mg of pellets (20 mg of drug). The laboratory and the commercial preparations were administered orally to the respective animal group in each study period. The capsules were administered with the help of a 10 mL syringe. The tip of the

syringe was cut off so as to expose a hole big enough to fit a capsule. The rabbit mouth was opened with plastic probe and the syringe containing the capsule was inserted until it reached the back of the mouth. The capsule was pushed into the pharynx with the syringe plunger followed by 3 mL of water. The rabbit was observed for 20 min to ascertain that the capsule was swallowed.

Blood samples of 0.5 mL were withdrawn from the marginal ear vein into vacutainer tubes containing sodium heparin as a anticoagulant at zero min (pre dose), and at 0.5, 1.0, 2.0, 4.0, 6.0, 10.0 and 18.0 h. Blood samples were immediately centrifuged at 2500 rpm at 10°C for 10 min to separate the plasma. The plasma samples were then stored in plain vacutainer tubes at –80°C until analyzed. The drug concentrations in blood samples were determined using the validated HPLC method as reported previously (4).

The plot between drug concentration *versus* time was used to calculate the pharmacokinetic parameters, such as maximum plasma concentration (C_{max}), time to achieve C_{max} (T_{max}), area under plasma drug concentration curve (AUC) and elimination rate constant (K_e) (6).

Statistical analysis

All the samples were analyzed in triplicate and the results were presented as the mean ± SD calculated using SPSS version 13.0. The level of significance was set at 0.05.

RESULTS AND DISCUSSION

In vitro dissolution test of pellets

In this study, sieving-spheronization and extrusion-spheronization were found to be successful to formulate omeprazole pellets with high percent yield, narrow particle size distribution, and the achievement of the required release of drug i.e., greater than 80% within 45 min at pH 6.8. The dissolution profiles followed the first order equation with diffusion as prominent mode of drug release (4, 11–19).

HPLC method for omeprazole analysis

For the analysis of omeprazole, standard solutions were used to evaluate the linearity of the method. Standard curves were constructed between peak area *versus* concentration and linearity was evaluated by linear regression with correlation coefficient, $R^2 = 0.997$. The method was found linear in a range 2.5–640 ng/mL.

The chromatograms of the standard omeprazole and formulation (laboratory pellets) by using the present HPLC method are shown in Figure 1. The chromatograms show well-resolved peak without any interference. The average retention time for omeprazole was found to be 7.55 min and average retention time for internal standard (chloramphenicol) was 6.27 min. The recovery of omeprazole from excipients was 99.8–101.0% and relative standard deviation was ranged from 1.15 to 5.85%. The values for the intraday accuracy and precision were 98.60–100.63% and the relative standard deviation 1.55–5.05%. The omeprazole recovery from plasma was 95.24–98.32% with RSD of 3.59–6.44%. Interday accuracy values were 98.25–100.76% with relative standard deviation 1.35–4.68%.

The findings of the study indicated the reliability and reproducibility of the HPLC method used in this study. The recovery (n = 3), intraday and interday accuracy and precision (n = 6) were determined

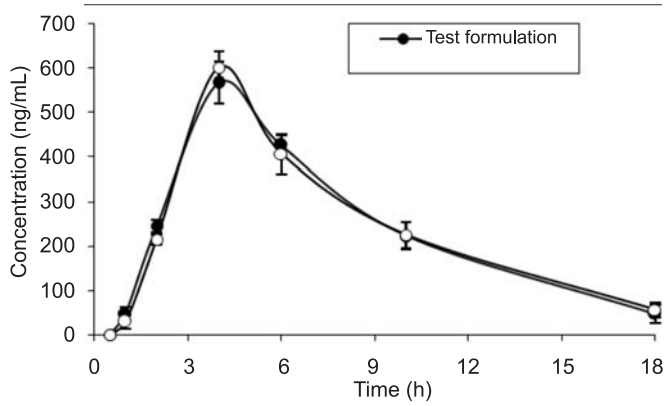

Figure 2. Plasma concentrations *versus* time profiles of test formulation of omeprazole (F21) and market formulation (Zimor®) (mean ± SD, n = 6)

using standard curves with linear regression. The recovery of extraction procedure for omeprazole and internal standard (chloramphenicol) were determined by comparing the peak heights obtained from extraction with that of aqueous drug solution of corresponding concentration without extraction. The recovery values of omeprazole from plasma were found to be 95.24–98.32%. The intraday and interday accuracy was 95.46–97.39 and 95.3–96.98%, respectively, with relative standard deviation less than 10%, hence within the acceptable limits (14, 15). The results are shown in Table 2. The results of the study indicated that the method was repeatable and accuracy was not compromised in within day and between day analyses.

Percentage contents of the developed and market formulations

A validated method was successfully applied to quantify omeprazole in different formulations. The percentage content of omeprazole was similar in the test formulation (98.87 ± 3.13) and the market products (98.21 ± 5.87). It means that the excipients and coating with Kollicoat 30DP of the pellets is suitable and produce comparable efficacy.

***In vivo* experiments**

In the present study, *in vivo* study was carried out to evaluate bioavailability and pharmacokinetics of the laboratory formulation. The laboratory formulations containing pellets of omeprazole were prepared and coated according to the optimum formulative ingredients and processing conditions. The laboratory formulation was characterized by their physicochemical properties. The laboratory formulation was found to be with appropriate characteristics and was suitable for further evaluation of *in vivo* studies. The laboratory formulation was compared for its bioequivalence to the commercial formulation, Zimor® available as granules filled in hard gelatin capsules.

Plasma drug level-time curve

The plasma concentration *versus* time profiles of test formulation and market product are shown in Figure 2. At pre-dose sampling time interval, the drug was not detectable in all the animals in both of the formulations. The concentration of the drug was undetectable at 2 h indicating a delayed release of the formulations. The rising curves for the test and reference formulation are superimposable. The declining curves were found to be similar. The products having similar pharmacokinetic profiles are called bioequivalent. The similarity in profiles of both the formations is suggestive of equivalence of the test formulation to the reference product. It means that the excipients used to prepare test formulation and method for pelletization were appropriate and could be used to prepare pelletized dosage form successfully.

Pharmacokinetic parameters

The plasma drug level-time curves of test and commercial preparations were the basis for computing the pharmacokinetic parameters of above formulations. Table 3 shows the pharmacokinetic parameters of these formulations, respectively. The extrapolated AUC was less than 15% indicating the reliable computation of $AUC_{0-\infty}$.

Bioequivalence testing

The C_{max} and $AUC_{0-\infty}$ were used to assess bioequivalence of laboratory (test) and reference formulations. Before proceeding for the bioequivalence, the carryover effect was investigated for C_{max} and $AUC_{0-\infty}$ (7). The carryover effect for both of the parameters, C_{max} and $AUC_{0-\infty}$ was not statistically significant ($p > 0.01$). The lack of carryout effect in C_{max} and $AUC_{0-\infty}$ validated the bioequivalence testing. The test formulation was bioequivalent to reference formulation as indicated by 95% CI ratios of 0.92 to 1.09 for C_{max} and 0.81 to 1.07 for AUC values of the formulations within the stipulated range

Table 3. Pharmacokinetic parameters of test and reference formulation of omeprazole (F21).

Parameter	Test formulation		Zimor®	
	Mean	SD	Mean	SD
C_{max} (ng/mL)	567.00	47.36	599.6	35.62
T_{max} (h)	4	0	4	0
AUC_{0-t} (ng.h/mL)	4345.45	212.75	4345.41	269.64
$AUC_{0-\infty}$ (ng.h/mL)	4687.015	233.77	4683.03	344.74
K_e (h^{-1})	0.189	0.036	0.168	0.03

of 0.80 to 1.25. The plasma level time curves and the pharmacokinetic parameters further support the bioequivalence of the two formulations under study.

CONCLUSION

It was observed that omeprazole contents were comparable in all formulations elaborating their similar quality. On the basis of above results, test formulation (F21) of omeprazole was found to be comparable with the reference formulation (Zimor®) regarding bioavailability.

Acknowledgments

This study was supported by the grant of School of Pharmaceutical Sciences University Sains Malaysia. Sabiha Karim is thankful to University of the Punjab, Lahore for the grant of Ph.D. study leave.

REFERENCES

1. Akhtar M., Ahmad M., Khan S.A., Murtaza G.: Afr. J. Pharm. Pharmacol. 6, 2613 (2012).
2. Mohammad S., Shah S.N.H., Nasir B., Khan Q., Aslam A., Riaz R. et al.: Afr. J. Pharm. Pharmacol. 6, 2629 (2012).
3. Murtaza G., Ahmad M., Akhtar N., Rasool F.: Pak. J. Pharm. Sci. 22, 291 (2009).
4. Karim S., Baie S.H., Hay Y.K., Bukhari N.I.: Pak. J. Pharm. Sci. 27, 425 (2014).
5. Yuen K.H., Choy W.P., Peng CY., Tan H.Y., Wong J.W., Yap, S.P.: J. Pharm. Biomed. 24, 715 (2001).
6. Shahzad M.K., Ubaid M., Murtaza G.: Tropical J. Pharm. Res. 11, 695 (2012).
7. Murtaza G., Ahmad M, Asghar M.W., Aamir M.N.: DARU J. Pharm. Sci. 17, 209 (2009).
8. D'Angelo G., Potvin D., Turgeon J.: J. Biopharm. Stat. 11, 35 (2001).
9. Sher M., Hussain G., Hussain M.A., Akhtar T., Akram M.R., Paracha R.N., Murtaza G.: Afr. J. Pharm. Pharmacol. 6, 2424 (2012).
10. Khan S.A., Ahmad M., Murtaza G., Shoaib H.M., Aamir M.N., Kousar R., Rasool F., Madni A.: Latin Am. J. Pharm. 29, 1029 (2010).
11. Murtaza G., Ahmad M., Madni M.A., Asghar M.W.: Bull. Chem. Soc. Ethiop. 23, 1 (2009).
12. Khan S.A., Ahmad M., Murtaza G., Aamir M.N., Kousar R., Rasool F., Zaman S.U.: Acta Pharm. Sin. 45, 772 (2010).
13. Ahmad M., Iqbal M., Akhtar N., Murtaza G., Madni M.A.: Pak. J. Zool. 42, 395 (2010).
14. Rasool F., Ahmad M., Khan H.M.S., Akhtar N., Murtaza G.: Acta Pol. Pharm. Drug Res. 67, 185 (2010).
15. Ahmad M., Ahmad R., Murtaza G.: Adv. Clin. Exp. Med. 20, 599 (2011).
16. Khiljee S., Ahmad M., Murtaza G., Madni M.A., Akhtar N., Akhtar M.: Pak. J. Pharm. Sci. 24, 421 (2011).
17. Khan M.I., Murtaza G., Awan S., Iqbal M., Waqas M.K., Rasool A., Asad M.H.H.B. et al.: Afr. J. Pharm. Pharmacol. 5, 143 (2011).
18. Aamir M.F., Ahmad M., Murtaza G., Khan S.A.: Latin Am. J. Pharm. 30, 318 (2011).
19. Ahmad M., Murtaza G., Akhtar N., Siddique F., Khan S.A.: Acta Pol. Pharm. Drug Res. 68, 115 (2011).

Received: 24. 07. 2013