ORIGINAL ARTICLE

PHARMACOKINETIC EVALUATION OF CLARITHROMYCIN ORAL CONTROLLED RELEASE MATRIX TABLETS FOR DESIRED BIOAVAILABILITY AND IMPROVED PATIENT COMPLIANCE

Muhammad Ramzan[1], Muhammad Akhlaq[1], Muhammad Zeeshan Danish[2], Satar Bakhsh[1], Haroon Khan[1], Abid Hussain[3]

[1]Faculty of Pharmacy, Gomal University, D.I.Khan, Pakistan

[2]Faculty of Pharmacy, University of Punjab, Lahore, Pakistan

[3]Department of Pharmacy, University of Poonch, Rawlakot, Azad Jammu & Kashmir, Pakistan

ABSTRACT

Background: Controlled release drug delivery systems release drug at controlled rate over desired period of time. It helps to maintain constant drug concentration in plasma and improves the patient compliance.

Material & Methods: The study aims to design, formulate and evaluate controlled release matrix tablets of Clarithromycin were formulated at different drug-to-polymer (D:P) ratios (10:3, 10:4 and 10:5) using Eudragit RS 100, Methocel® polymers as release retarding agents. The matrices were prepared by direct compression and wet granulation techniques. Dissolution studies were performed in phosphate buffer (PH 7.4) using Pharma Test Dissolution Apparatus. Different preformulation parameters (diameter, thickness, hardness, friability and weight variation tests), drug release kinetics (kinetics model; 1st-Order, Zero-Order, Higuchi, Hixon Crowell's and Power Law) and dissolution profiles comparison with reference standard tablets.

Results: It was observed that the matrices having polymers with more concentrations extended the drug release rates as compared to the matrices having smaller amounts. The matrices released the drug by anomalous Fickian diffusion mechanism and reference standard Clarion® XR tablets does not follow Power Law ($n=0.125$). The tests matrices showed no similarity with reference standard Clarion® XR tablets dissolution profiles. There was no significant difference between T_{max} values of test and reference formulations ($p \geq 0.05$) found for in-vivo studies. Similarly, no significant difference was observed between the values of $AUC_{0-\infty}$ ($p=0.19$) and the values of C_{max} ($p=0.06$) of the two preparations.

Conclusion: The once daily controlled release matrix tablets of Clarithromycin can successfully be formulated using hydrophilic polymers.

KEY WORDS: Clarithromycin; Macrolide; Pharmacokinetics; Drug Evaluation; Patient Compliance.

This article may be cited as: Ramzan M, Akhlaq M, Danish MZ, Bakhsh S, Khan H, Hussain A. Pharmacokinetic evaluation of clarithromycin oral controlled release matrix tablets for desired bioavailability and improved patient compliance. Gomal J Med Sci 2015; 13: 217-22.

INTRODUCTION

Clarithromycin is a macrolide, erythromycin derivative and broad spectrum antibiotic. It is widely used in a standard treatment of gastric H. pylori infection combined with a second antibiotic and an acid-suppressing agent.[1] It has improved bioavailability and half-life and reducing the side effects.[2,3] Advancements in the pharmaceutical technology have made possible, the development of more refined and complicated drug delivery systems, which are most convenient, safe and extensively accepted.[4,5] On the shelves of pharmacies, the oral dosage forms are increasing exponentially and this will continue in the

Corresponding Author:
Dr. Muhammad Akhlaq
Assistant Professor
Faculty of Pharmacy, Gomal University
D.I.Khan, Pakistan
E-mail: akhlaqhayat@hotmail.com

years to come. The growth is due to several factors including patient convenience and compliance, unmet medical needs, health care cost reduction and market exclusivity and the manufacturing at a very large scale.[1,2] All the products that are able to reduce dosing frequency can provide expediency and contentment which has a very positive impact on patient's reliability and more optimistic response. It is observed that the management of few diseases is now possible that have historically been difficult to treat because of appropriate amount of drug can now be delivered to the right site and at the right time by means of diverse controlled release polymeric approaches. Advancements in the pharmaceutical technology have made possible, the development of more refined and complicated drug delivery systems to be manufactured on a large scale and to meet the present challenges in the whole world. The most convenient, safe and extensively accepted means of administration of drugs is considered to be the oral route.[4] The oral route of drug delivery is considered to be safe and is preferred for those drugs that are distinct for life-threatening circumstances. The oral route of drug delivery system provide market exclusivity and broaden a products life cycle that promote the product novelty and is of commercial interest and attention of pharmaceutical industries. The oral route is the most frequent route of drug administration for majority of mild to moderate diseases and is considered to be the most favored and user friendly means of drug administration. It is also considered the most compliance route of drug administration, considering these benefits; more efforts are aimed to identify orally active candidates that would provide effective blood plasma concentrations.[5]

The objective of the sustained/controlled release drug delivery systems are the constant drug release, minimizing the plasma drug fluctuations, improving the efficacy and to reduce toxicity or side effects.[8]

MATERIAL AND METHODS

Clarithromycin was received as gift from Ferozsons Laboratories (Pvt) Ltd, Nowshera. Sodium hydroxide, Monobasic potassium Phosphate (KH_2PO_4) and Disodium Hydrogen Phosphate (Na_2HPO_4), (Merck, Germany), lactose, talc and magnesium stearate (BDH chemical limited, Pool England), Eudragit RS 100, Methocel® (Dow Chemical Co., Midland USA). Drug (500 mg) was mixed with polymer and lactose, mixed thoroughly and passed through sieve # 30. Then mixed and kneaded with water to form dough mass, passed through sieve # 10 and dried at 50C°. After drying the powder was passed through mesh # 30. Then added talc and magnesium stearate and thoroughly mixed. Compressed the final granules using single punch Tableting Machine (Erweka, AR 400 GMBH, Germany).

Funnel method was used to determine the angle of repose, while cylinder method determines the compressibility index and Hausner's ratio (HR) according to USP. Hardness of the tablets was determined by Hardness Tester (Erweka Apparatus TB-24, Germany) and dimensional tests were performed using Vernier Caliper. The friability of the tablets was determined using Friability Tester (Erweka TA-3R, Germany).

An eight station Dissolution Apparatus (PharmaTest Dissolution Apparatus PTWS-11/P, TPT, Hamburg, Germany) was used for in vitro dissolution studies containing 900 mL dissolution medium maintained at 37±0.1°C and stirred at 100 rpm. The optimized tablets were placed in dissolution medium and stirred at 100 rpm according to USP Method-I. The total concentration of Clarithromycin released after specific time intervals at (2, 4, 6, 8, 10, 12, 16, 18, 20, 22 and 24 h). The Chromatographic system (Model: LC) with UV detector at 210 nm, C-18 column (4.6-mm x 15-cm; packing L 1) maintained at 50°C with a flow rate of 1 ml/min and having injection volume 20-50 μL. The sample and standard solutions were run and the percentage concentration of clarithromycin was calculated using the following formula.

$$\text{Percent Concentrtion} = (r_u/r_s \times (C_s/C_u) \times 100 \quad \text{(Eq. 1)}$$

Where, "r_u" and "r_s" are the Peak responses from sample and standard solution respectively. C_s and C_u are drug concentrations in standard and sample solution (μg/ml) respectively, while the acceptance range was kept at 90.0% – 110.0%.

In order to determine the rate and drug transport mechanism of Clarithromycin from controlled release tablets, the dissolution profiles were fitted in various kinetic/mathematical models given as under Zero-Order Kinetics *(W=K1t),* First-Order Kinetics [*In (100–W)=In100–K2t*], Higuchi Kinetics *(W=K4* $t_{1/2}$*),* Hixson Crowell Kinetics *(100–W) 1/3 =1001/3–K3t),* Korsmeyer's Peppas's Kinetics *(Mt/M∞ = K5t*^{n}*).*[7] In Korsmeyer's Peppas Kinetic model an *(n)* value which is a diffusional exponent defines the mechanism of drug transport from matrix tablets. When n = 0.5 then drug diffuses with aquasi-Fickian diffusion mechanism from a matrix tablet. When the value of

$n > 0.5$ then anomalous or non-Fickian diffusion mechanism of drug occurs and when $n = 1$ then non-Fickian, Zero order or case-II release kinetics occurs.

Similarity factor f2 was used to check the similarities and dissimilarities between the formulations from the test and reference standard formulation which was adopted by US Food and Drug Authority 15 (Eq.1).

$$f2 = 50Log \{[1+1/n\ Wt\Sigma nt=1(Rt-Tt)2]-0.5 \times 100\} \quad \text{(Eq.2)}$$

Where *"n"* is a pull point, *"Wt"* is an optional weight factor, *"Rt"* is reference release profile at point *t* and *"Tt"* is test release profile at point *"t".*

In *vivo* studies were conducted on twenty-four healthy male albino rabbits[8], weighing about 3.0 kg, in accordance with the standard protocol by the research and Ethical Committee of Faculty of Pharmacy, Gomal University, D.I. Khan, KPK, Pakistan. The *in vivo* research proposal was approved by research and ethical committee. These experiments were conducted in accordance with the animal scientific procedure Act, 1986. The optimized test-tablets were compared with respective reference tablets Clarion® XR (Market Brand) in a parallel study design. The tablets were given orally, using a 3ml syringe.

Rabbits were fasted for 24 hours. First batch was fed with 500 mg Clarithromycin test tablets while the second batch was given 500 mg Clarithromycin market brand Clarion®XR. Blood samples (0.7ml each time) were collected from the marginal ear vein into heparinized centrifuge tubes just before dosing and at intervals of 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 30, 36 & 42 hours, for Acorbose test and market brands, during the study after dosing.

The collected blood was allowed to clot for about 30 minutes. The resulting clot was remixed with a sterile wooden stick and placed still in the original collection tube in the refrigerator for about one hour. Blood samples were centrifuged at 1500 rev/min and the plasma was separated. One un-dosed plasma sample was kept as blank. To 1ml each of the plasma samples, 5ml of diethyl ether was added and the tubes were then centrifuged at 2500 rev/min for 15 minutes. About 4ml of the supernatant was pipetted out which was evaporated at room temperature. The residue was reconstituted with 5ml of acetonitrile and drugs concentrations were determined by a rapid HPLC method.[9]

Pharmacokinetic parameters, such as AUC, C_{max}, T_{max} and $t_{1/2}$ were derived from the plasma concentration versus time data using Kinetica ver 5.0. For the computation of the above pharmacokinetic parameters, a non-compartmental approach implemented in Kinetica was used. The values of the rate constant for elimination (k_{el}) were used to calculate the absorbed and unabsorbed fractions of drugs using Wegnar-Nelson method given in Pk-Fit ver 2.01. One-way ANOVA at $P < 0.05$ was used to compare the release rate (k) of different formulations. For this purpose the Statistical Package for Social Sciences SPSS was used.

RESULTS

The mix powder of all the formulations showed good flow properties and compressibility as shown in Table 2. The bulk and tapped densities were found to be 0.512±0.02 and 0.64±0.09 for Clarithromycin powder, while the hausner's ratio, angle of repose and the compressibility index was found to be 1.25±0.02, 30.0±0.02 and 20.02 respectively. The above said parameters for the formulation powders (F1-6 & control) were found to be ranged from 0.38±0.02 to 0.48±0.05, 0.41±0.02 to 0.64±0.09, 1.08±0.04 to 1.25±0.02, 14±0.03 to 30±0.03 and 9.09±0.04 to 14.29±0.01, respectively. According to physical dimensions tests, the tablets of all the batches were found to be nearly similar in all as-

Table 1: The composition of Matrix Tablets by Wet Granulation Technique

Formulation Code	Drug-to-Polymer Ratios	Clarithro-mycin	Eudragit® RS 100 / Methocel®	Lactose	Talc	Magnesium Stearate
F1	5:1	500	100	360	30	10
F2	5:2	500	200	260	30	10
F3	5:3	500	300	160	30	10
F4	5:1	500	100	360	30	10
F5	5:2	500	200	260	30	10
F6	5:3	500	300	160	30	10

Table 2: Pre-formulation parameters of various formulation granules and drug

Formulation	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Hausner's Ratio	Angle of Repose (0)	Compressibility Index (%)
Clarithromycin	0.512±0.02	0.64±0.09	1.25±0.02	30±0.03	20±0.02
F1	0.40±0.01	0.44±0.03	1.1±0.032	15±0.02	9.09±0.04
F2	0.48±0.05	0.56±0.01	1.17±0.01	22±0.05	14.29±0.01
F3	0.39±0.02	0.44±0.03	1.13±0.06	16±0.01	11.36±0.02
F4	0.40±0.03	0.46±0.01	1.15±0.03	20±0.04	13.04±0.02
F5	0.38±0.02	0.41±0.02	1.08±0.05	14±0.03	7.32±0.01
F6	0.40±0.06	0.43±0.05	1.08±0.04	15±0.01	6.98±0.05
Control 1	0.39±0.02	0.45±0.04	1.15±0.02	30±0.03	13.37±0.02

Table 3: Post compression tests of tablets formulated by wet granulation technique

Formulation	Hardness (kg) n = 10, Mean ± SD	Friability (%) n = 3, Mean ± SD	Thickness (mm), n=10, Mean±SD	Weight Variation (mg) n=20, Mean±SD	Content Uniformity (%) n=10, Mean±SD
F1	7.6±0.11	0.6±0.02	4.6±0.06	104±0.12	102±0.6
F2	7.2±0.04	0.4±0.04	4.5±0.13	997±0.15	104±0.4
F3	7.5±0.11	0.3±0.05	4.5±0.11	998±0.13	99±0.3
F4	8.2±0.06	0.5±0.11	4.6±0.08	103±0.09	95±0.43
F5	6.9±0.03	0.4±0.03	4.5±0.09	106±0.12	98±0.15
F6	7.4±0.12	0.2±0.05	4.5±0.12	996±0.16	96±0.35
Control 1	7.8±0.25	0.6±0.12	4.6±0.13	987±1.30	103±0.26

Table 4: Pharmacokinetic parameters of Clarithromycin after oral administration in albino rabbits, Mean ±S.D

Pharmacokinetic Parameter	Test Tablets	Reference Tablets
$t_{1/2}$ (hours)	11.48±0.03	6.42±0.19
T_{max} (hours)	8.33±0.11	2.20±1.32
C_{max} (ug/ml)	18.66±1.43	17.24±1.08
AUC_0 (μg. hour/ml)	23.19±3.00	22.09±2.41
$AUC_{0\text{-inf}}$ (μg. hour/ml)	4443.7±2.02	4358.3±2.11
$MRT_{0\text{-}48\ hrs}$ (hours)	12.10±1.05	7.41±1.04
(Cl) (ml/min)	0.05±0.04	0.44±0.11

pects including thickness, diameter and physical appearance. The average friability and hardness of the formulations were in USP acceptable range and fulfilling the requirements of dosage uniformity (table 3). The hardness ranged from 4.5±0.09 to 4.6±0.13 while friability ranged from 0.2±0.05 to 0.6±0.12, thickness ranged from 4.5±0.12 and 14.9±0.01 to 4.6±0.13 and 16.8±0.01 while weight variation and content uniformity ranged from 987±1.30 and 95±0.43 to 106±0.12 and 104±0.4.

The drug release from the matrix tablets based on Eudragit® RS 100 and Methocel® were evaluated in *in-vitro* for 24 hours at different time intervals (2, 4, 6, 8, 10, 12, 18, 20, 22, & 24 hrs). The matrices having Eudragit® RS 100 (5:1, 5:2 and 5:3) released 98.54%, 98.98% and 97.33% drug in 24 hours. The matrices based on Methocel® (5:1, 5:2 and 5:3) released 97.85%, 98.33% and 85.42% of the drug in 24 hours (Fig. 1 & 2). The reference standard Clarion® XR tablets released 99.87% of the drug in 24 hours thereby releasing 60% of the drug in first 2 hrs (Fig. 2).

Different formulations having different concentrations of polymer gave optimized controlled release profiles, and by increasing the amount of polymer, drug release was found to be reduced. The values of R^2 of the formulation F6 (D:P ratio5:3) were highly fit to Korsmeyer Pappas's and Zero Order Equation. In Korsmeyer Pappas equation, the values of *'n'* were found to be $1<n>0.5$ indicating nearly Zero Order/

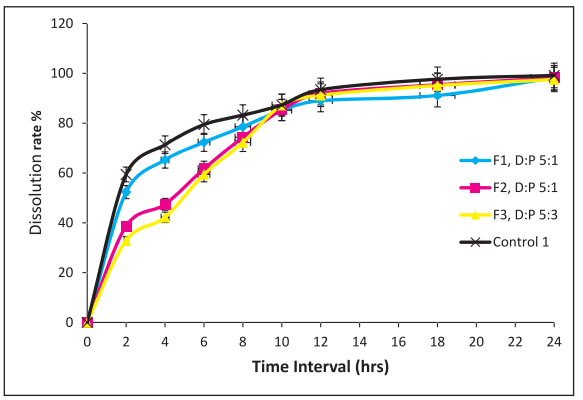

Figure 1: Drug Release Pattern from Matrix tablets using Eudragit as Release Controlling Agent

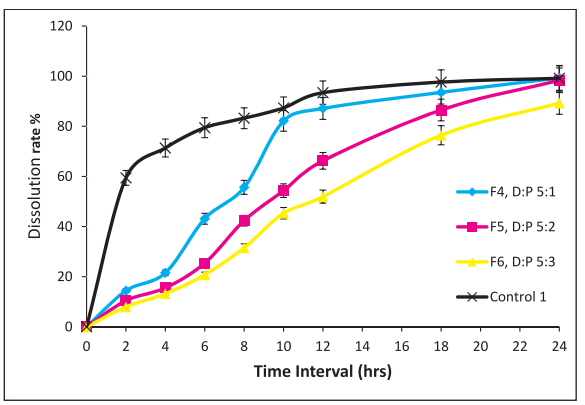

Figure 2: Drug Release Pattern from Matrix tablets using Methocel as Release Controlling Agent

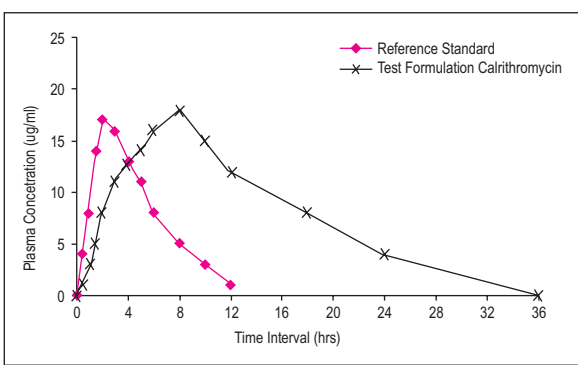

Figure 3: Clarithromycin plasma concentration verses time test product verses reference product

Anomalous non-Fickian release kinetics. Significant differences were found in release rates of all the formulations ($P<0.05$).

Figure 3 is typical chromatograms of standard solution containing 5μg/mL of Clarithromycin rabbit plasma spiked with 10μg/mL of Clarithromycin, rabbit plasma collected after 4 hours of administration of dose. The retention time of 5.5 min was observed. The mean absolute recovery from six aliquot samples was >90% at 0.25 to 10 μg/ml. The mean plasma concentration curve was found to be linear with correlation coefficient R2 of 0.9998.

The *two tailed t-test* was applied on results using SPSS 12.0 software to test for the treatment effect i.e. test tablets vs reference tablets. The K_{el} of reference and test tablets were 0.371 ± 0.310 h^{-1} and 0.0754 ± 0.016 h^{-1}, respectively showing significant difference ($P<0.05$). The $t_{1/2}$ of reference and test tablets were found to be 11.48 ± 0.03 to 6.42 ± 0.19, respectively and significant difference was observed ($P<0.001$). The mean T_{max} for reference and test tablets of 2.20 ± 1.32 and 8.33 ± 0.11 hours respectively and C_{max} were 17.24 ± 1.08 and 18.66 ± 1.43 μg/mL. Statistical analysis showed significant difference between Tmax values of reference and test tablets ($P<0.05$).

DISCUSSION

All preformulation parameters of the granulations prepared by various formulation techniques were in the acceptable limits.[10] The preformulation studies fell in the acceptable limit and were found to be reasonable for the preparation of Clarithromycin controlled release tablets. It was observed from drug release profile that by increasing the amount of polymer the drug release was reduced (Figure 2 & 3). The polymer concentration played a vital role in the drug retarding from the polymer matrix. Polymer concentration might have increasing effect upon the size of tablet which might affect the hardness of compressed tablet. Therefore, to omit the effect of hardness and compressibility variation upon drug release profiles other parameters than the above said variables were kept constant. Thickness and diameter might affect the internal stress of the tablet and could be considered during handling. Friability, thickness, and diameter were also kept constant. Aging did not reveal any sort of degradation and reduction in the drug content so it might prove the physical stability of the prepared matrix tablets when stored for a longer time.

Swelling of the tablets might be occurred due to the absorption of water by formulation ingredients. This water uptake might result in an increased weight and volume. Water absorption by the tablet might be due to the saturation of capillary spaces within the particles and hydration of macromolecules. Water is absorbed through the small pores, binds to the large molecules, breaking the hydrogen binding which ultimately lead to swelling of particles.

The values of R^2 of the formulations (D:P ratio 5:2) were highly fit in Korsmeyer Pappas equation

and Zero Order equation. In Korsmeyer Pappas equation, the values of 'n' were ($1 < n > 0.5$) indicating nearly zero order or anomalous non-Fickian release kinetics. Significant differences were found in release rates of all the formulations ($P < 0.05$).[11,12]

To demonstrate *IVIVC*, fraction of percent drug absorbed (Fa, Y-axis) was plotted against fraction of percent drug released (Fr, X-axis). Good in-vitro and in-vivo correlation of level A was achieved for test formulation with R^2 0.976, which indicate that the formulations were successful enough for further clinical evaluation and promotion. However, the reference SR formulation showed less linearity with R^2 0.8761 and 0.7876, respectively. Moreover, test formulations showed good linear relationship between *In-vitro* drug released and *In-vivo* drug absorption, prolonged $MRT_{0\text{-}t}$ and $t_{1/2}$ values as compared to reference formulations.

CONCLUSION

The once daily controlled release matrix tablets of Clarithromycin can successfully be formulated using hydrophilic polymers.

REFERENCES

1. Rajinikanth PS B Mishra. Floating in situ gelling system for stomach site-specific delivery of clarithromycin to eradicate H. pylori. J Control Rel 2008; 125: 33-41.
2. Alvarez-Elcoro S, MJ Enzler. The Macrolides: Erythromycin, Clarithromycin, and Azithromycin. Mayo Clinic Proce 1999; 74: 613-34.
3. Ana PFerreira, D Olusanmi, O Sprockel, A Abebe, F Nikfar, M Tobyn. Ferreira AP Use of similarity scoring in the development of oral solid dosage forms. Int J Pharm. Available online at http://www.sciencedirect.com/science/article/pii/S0378517315302477.
4. Goyanes A. Fabrication of controlled-release budesonide tablets via desktop (FDM) 3D printing. Int J Pharm 496: 414-20.
5. Alvaro G, H Chang, DS Grace, B Hatton, J Wang, A Buanz, S Gaisford, AW Basit. Formulation development of nifedipine controlled-release coated tablets. Asia J Pharm Sc Nov 24, 2015.
6. ViridÃ©n A, B Wittgren, A Larsson. Investigation of critical polymer properties for polymer release and swelling of HPMC matrix tablets. Eu J Pharm Sc, 2009; 36: 297-309.
7. Khan KA, KG Majid, ZD Muhammad, M Akhlaq, K Haroon, R Fazalm, S Mehsud. Formulation and in-vitro evaluation of directly compressed controlled release matrices of Losartan Potassium using Ethocel Grade 100 as rate retarding agent. Int J Pharm 496: 759-65.
8. Abdelbary GA, MI Tadros. Design and in vitro/in vivo evaluation of novel nicorandil extended release matrix tablets based on hydrophilic inter-polymer complexes and a hydrophobic waxy polymer. Eur J Pharm Biopharm 2008; 69: 1019-28.
9. Joseph NJ, S Lakshmi, A Jayakrishnan. A floating-type oral dosage form for piroxicam based on hollow polycarbonate microspheres: in vitro and in vivo evaluation in rabbits. J Control Rel 2002; 79: 71-9.
10. Sharma S. New Generation of Tablet: Fast Dissolving Tablet. Latest Reviews: Pharmainfo 2008. 6.
11. Lachman L, HA Lieberman JL Kanig. The Theory and Practice of Industrial Pharmacy. Philadelphia, PA: Lea and Fabiger 1987; 317-8.
12. Abdelkader H, Abdalla O, Salem H. Formulation of controlled-release baclofen matrix tablets: Influence of some hydrophilic polymers on the release rate and in vitro evaluation. AAPS Pharm Sci Tech 2007; 8: 156-66.

CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.