ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Formulation Development of Losartan Potassium Microspheres Using Natural Polysaccharides and Their *In-Vitro* Evaluation

Aswartha umakanthareddy M*[1], Sreeramulu J [2], Satyanarayana Punna [1]

[1]Dept of Organic Chemistry, Rayalaseema university, Kurnool-518002, A.P. India
[2]Dept of Analytical Chemistry, Sri Krishnadevaraya University, Anantapur- 515002, A.P. India

ABSTRACT

The present work is regarding formulation and development of Losartan potassium microspheres using chitosan and guar gum as release retarding natural polysaccharides and their in vitro evaluation. Losartan potassium is used as antihypertensive belonging to angiotensin antagonist. Losartan potassium was successfully encapsulated into chitosan and guar gum microspheres. Totally nine formulations were prepared by varying the ratio of chitosan and guar gum using span-85 as an emulsifier and glutaraldehyde as a chemical cross linking agent. The microspheres were evaluated for particle size, encapsulation efficiency, drug loading capacity, mucoadhesion studies, stability studies, in vitro drug release studies. Particle sizes, as measured by the optical microscopic technique, were of an average size in the range 30.2 mm (LCG1) to 36.5 mm (LCG3). The swelling index was in the range 0.45-0.78. The SEM study showed that microspheres have smooth surfaces. Microspheres were characterised by differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy to confirm the absence of chemical interactions between drug and polymer and to know the formation of microspheres structure. The optimised batch LCG 1 released 97.45 % at 10 h Phosphate buffer pH7.4 as dissolution medium.With regard to release kinetics, the data of the optimized formula were best fitted with the Higuchi model (r^2= 0.671) and showed zero order release (r^2= 0.980) with non-Fickian diffusion mechanism. The findings of the present study conclusively state that chitosan (1:2 of drug to polymer ratio) microspheres of Losartan potassium are potential for the sustained drug delivery of the drug in hypertension.

Keywords: Losartan potassium, chitosan, dissolution medium, drug entrapment efficiency, particle size.

Corresponding author
Email: maukreddy@gmail.com

ISSN: 0975-8585

INTRODUCTION

The efficiency of any drug therapy can be described by achieving desired concentration of the drug in blood or tissue, which is therapeutically effective and non toxic for a prolonged period. This goal can be achieved on the basis of proper design of the dosage regimen. Microspheres have potential to deliver drug in a controlled fashion. Losartan potassium is an effective antihypertensive drug but is extensively bound to plasma proteins and also causes gastrointestinal disorders, neutropenia, acute hepatotoxicity, migraine and pancreatitis. It may therefore be more desirable to deliver this drug in a sustained release dosage form. The present study was focused on development of sustained release Losartan microspheres using emulsification solvent evaporation method. Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 µm. They are made of polymeric, waxy or other protective materials, that is, biodegradable synthetic polymer and modified natural products. Such as starches, gums, proteins, fats and waxes [1].

The development of new delivery systems for the controlled release of drugs is one of the most interesting fields of research in pharmaceutical sciences. Microparticles can be used for the controlled release of drugs, vaccines, antibiotics, and hormones. For example, by taking advantage of the characteristics of microspheres, beyond the basic benefits, the microspheres could provide a larger surface area and possess an easier estimation of diffusion and mass transfer behaviour also the encapsulated small molecules could diffuse out of the barrier with precise kinetics modelling and control-release of drugs to the body fluid [2]. Among the polymer systems employed, the Ethyl cellulose, a weak cationic polysaccharide, has many advantages for developing micro-particles in drug release applications. Chitosan is a derivative of chitin, the second most abundant polymer in nature, which is a supporting material of crustaceans, insects, and fungal mycelia [2]. Among the different species of crustaceans, shrimp and crab shells have been widely used for the isolation of chitin.

The use of controlled release systems has certain advantages compared with conventional dosage forms, as they can minimize side effects, and prolong the efficacy of the drug. These release forms regulate the drug release rate and can reduce the frequency of administration of the drug, thus assuring better patient compliance. Pulsatile delivery systems based on chitosan have also been described, which are interesting with regard to adjusting drug release to physiological needs of the body, as in the case of hormone release [3]. The potential of chitosan as a novel excipient which might yet receive extensive application in pharmaceutical products has been highlighted in several reports [3].

MATERIALS AND METHODS

Chitosan (Indian Research Products., Chennai) and Losartan potassium was procured as a gift sample from Macleod's Pvt. Ltd, Mumbai, India). Glutaraldehyde (Paxmy, Chennai) All other reagents used were of analytical grade.

Methodology

For optimizing the polymer concentration nine formulae were prepared by taking drug polymer ratio of 1:1, 1:2 and 1:3. The drug and chitosan microspheres were prepared by making 2% w/v chitosan solution in aqueous acetic acid (1% v/v); 2% w/v guar gum in double distilled water and the drug was added and then this dispersed phase was added with stirring to continuous phase consisting of liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing 1% w/v span 85 to form a w/o emulsion. Stirring was continued at 4000 rpm using a 3 blade propeller stirrer. Solution of measured quantity of (2.5 ml each) of toluene saturated glutaraldehyde (2.5% v/v) was added in drop wise at 15, 30, 45, 60, 75, 90,105, 120 minutes. Stirring was continued for 1 h to obtain microspheres which were separated by filtration under vacuum and washed first with petroleum ether and then with distilled water to remove the adhered liquid paraffin and glutaraldehyde respectively [4]. The microspheres were then finally dried in a dessicator. The final preparation was free flowing powder consisting of spherical micron sized particles. Out of nine formulae LPM1 to LPM3 were prepared by taking drug polymer ratio of 1:1, 1:2 and 1:3 with 2%w/v chitosan LPM 4 to LPM 6 were prepared with 2%w/v guar gum; LPM 7 to LPM 9 were prepared with 2%w/v chitosan and 2%w/v guargum. The drug and chitosan and guargum microspheres were prepared by emulsification solvent evaporation method.

Compatibility studies:

IR studies

In the preparation of drug and polymer may interact as they are in close contact with each other, which could lead to the instability of drug preformulation studies regarding the drug – polymer interaction are therefore very critical in appropriate polymer [5]. FT – IR Spectroscopy was employed to ascertain the compatibility between Losartan Potassium and the cellulose polymer. (Perkin Elmer Jasco FTIR- 401, Japan).

Differential Scanning Calorimetry

The output of a DSC is a plot of heat flux (rate) versus temperature at a specified temperature rate [6]. DSC provides information about the physical properties of the sample as crystalline or amorphous nature and demonstrates a possible interaction between drug and polymers in formulations. According to the thermogram.

Percentage yield [7]

The yield was calculated as the weight of the microspheres recovered from each batch divided by total weight of drug and polymer used to prepare that batch multiplied by 100.

ISSN: 0975-8585

$$\text{Percentage yield} = \frac{\text{Weight of microspheres}}{\text{Weight of drug + weight of polymer}} \times 100$$

Drug content estimation

Drug loaded microspheres (100mg) were powdered and suspended in 100 ml methanol. The resultant dispersion was kept for 20 min for complete mixing with continuous agitation and filtered through a 0.45 µm membrane filter [8]. The drug content was determined spectrophotometrically at 208 nm using a regression equation derived from the standard graph.

Drug Entrapment efficiency [9]:

The drug entrapment efficiency (DEE) was calculated by the following formula

$$DEE = (Pc / Tc) \times 100$$

Here Pc is practical content,
Tc is the theoretical content.

Particle size analysis [10]:

The microsphere size distribution was determined by the optical microscopy method using a calibrated stage micrometer (µm) and was calculated by using following equation;

$$\text{Eye piece division} = \frac{Y}{X} \quad \text{X least count}$$

Here Y= number of stage micrometer division
X = number of eye piece divisions

***In-Vitro* drug release:**

In vitro drug release study was carried out in USP paddle type dissolution test apparatus using Phosphate buffer pH7.4 as dissolution medium, Volume of dissolution medium Was 900 ml and bath temperature was maintained at (37±1) °C throughout study. Paddle speed was adjusted to 50 rpm [10]. An interval of 1 hour, five ml of sample was withdrawn with replacement of five ml fresh medium and analyzed for Losartan potassium content by UV-Visible spectrophotometer at 208 nm. .

ISSN: 0975-8585

Scanning electron microscopy (SEM)

Scanning electron microscopy was carried out to study the morphological characteristics of losartan potassium microspheres [11]. The size of microspheres ranged from 30 to 35µm. The drug was sustained for more than 8 hrs. The strong gel matrix formed due to action of glutaraldehyde was sufficient enough to sustain the drug release for 8hrs.

RESULT AND DISCUSSION

Compatability studies

IR studies

The IR spectrum of the pure Losartan Potassium sample recorded by FTIR spectrometer is shown in Fig. This was compared with standard functional group frequencies of Losartan Potassium as shown in Table. 1.

From FTIR study, the characteristic peaks of drug such as of OH (3130.57), CH Stretching Aromatic (3003.27 cm^{-1}), CH Stretching Aliphatic (2963.12 cm^{-1}), C=O(1749 cm^{-1}), Al-CH-bend(1454.3 cm^{-1}), Ar-CH In plane Bending(1091.75 cm^{-1}), Ar-CH Out plane Bending (920.08 cm^{-1}), c-o-c Ether inkage (1193.98 cm^{-1}) appeared for the pure drug Losartan Potassium. For SLN all peaks which have been obtained for the pure drug were available at same wave length for OH (3130.57), CH Stretching Aromatic (3003.27 cm^{-1}), CH Stretching Aliphatic (2963.12 cm^{-1}), C=O(1749 cm^{-1}), Al-CH-bend(1454.3 cm^{-1}), Ar-CH In plane Bending(1091.75 cm^{-1}), Ar-CH Out plane Bending (920.08 cm^{-1}), c-o-c Ether inkage (1193.98 cm^{-1}) remaining peaks also either shifted or replaced in the IR spectrum of formulation shown in Fig. 1 &**2**

COMPARISON OF FT-IR SPECTRA OF LOSARTAN POTASSIUM AND FORMULAE

Table 1: IR Interpretations for Pure drug and Polymer

Functional groups	Losartan potassium	Losartan potassium+ chitosan
OH	3130.57	3140.12
CH Stretching (Aromatic)	3003.27	3020.34
CH Stretching (Aliphatic)	2963.12	2862.10
C=O	1749	1630
C=C	1600	1560
Al-CH-bend	1454.3	1334.2
Ar-CH (In plane Bending)	1091.75	1082
Ar-CH (Out plane Bending)	920.08	900.21
c-o-c (Ether inkage)	1193.98	1082.23

FOURIER TRANSFORM INFRARED SPECTROSCOPY
Fig No.1 FT – IR of Losartan Potassium

ISSN: 0975-8585

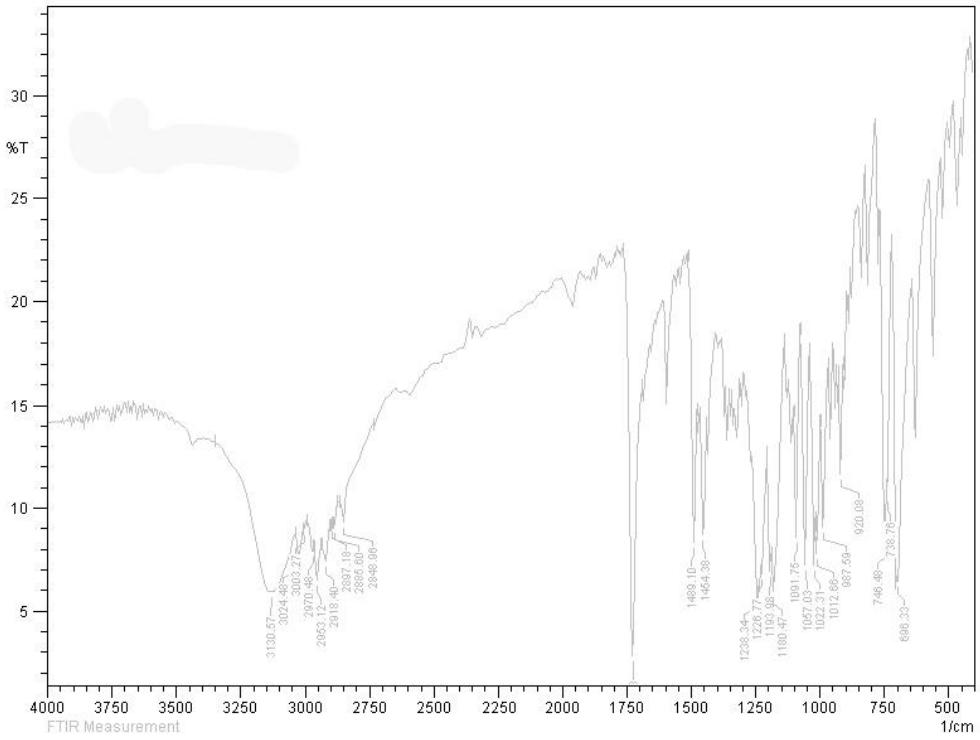
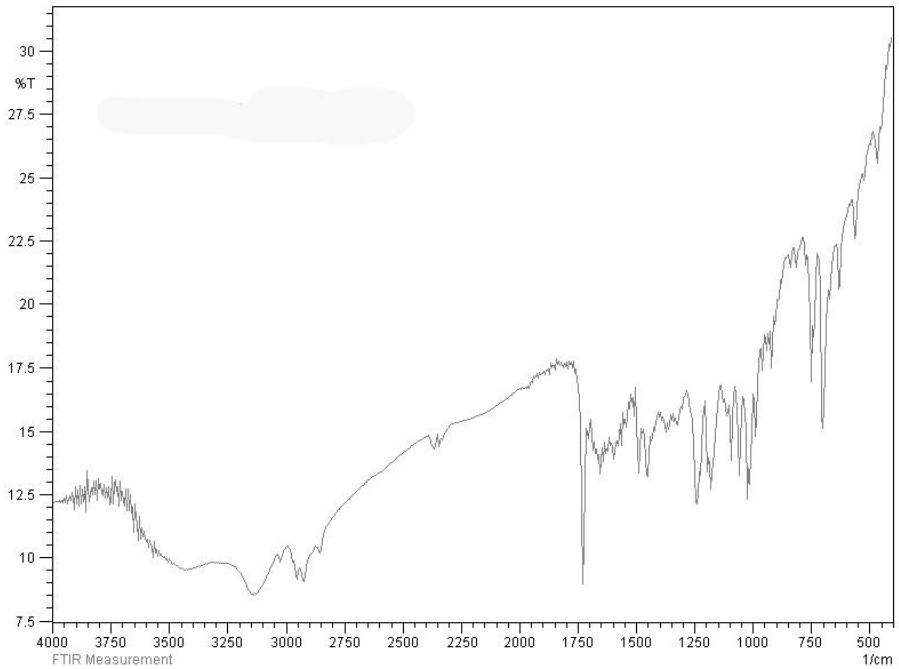


Fig No. 2 FT –IR of Losartan Potassium + Chitosan



ISSN: 0975-8585

DSC studies

The pure drug Losartan Potassium shown as an endothermic peak at 260.91°C. The peak neither is nor shifted in the case of DSC of the Losartan Potassium microspheres formulation containing Losartan Potassium. The DSC of physical mixture of the Losartan Potassium and chitosan as showed an endothermic peak at 260.91°C. The DSC spectra as shown in Fig 3 & 4

Fig 3: DSC of Losartan Potassium

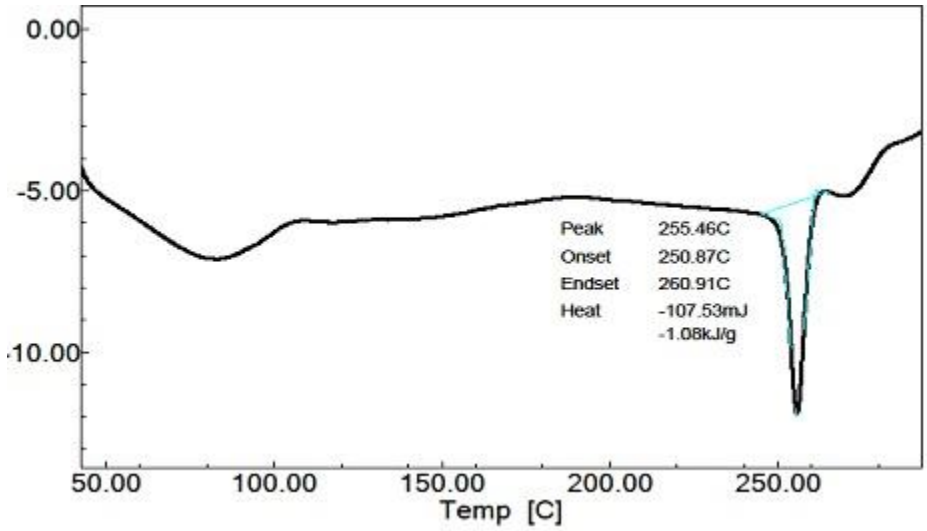
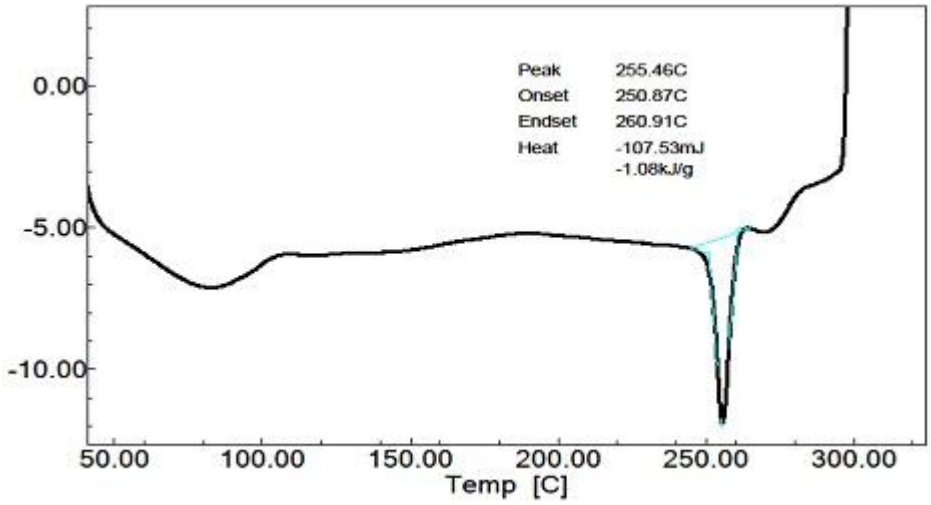


Fig 4: DSC of Losartan Potassium + Chitosan



MORPHOLOGY OF THE PARTICLES:

The following methods are used to determine particle size, size distribution, and morphology of Chitosan microspheres.

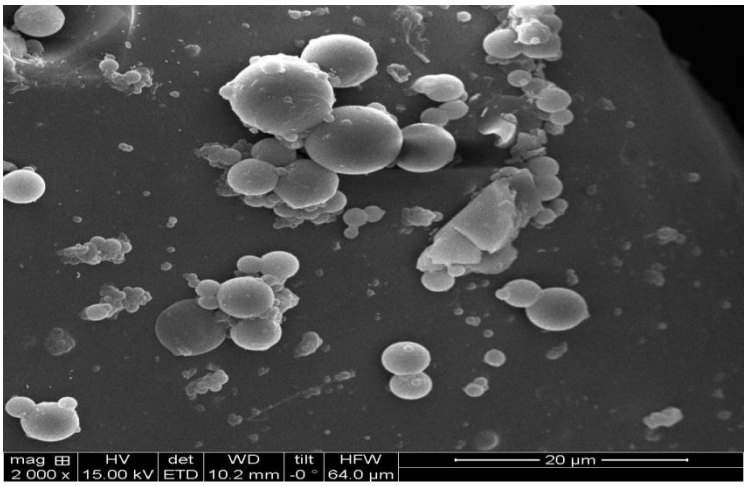
ISSN: 0975-8585

SEM

Morphology and structure of stealth Microspheres were determined using scanning electron microscopy (SEM) (Hitachis- 2600N Japan), and photomicrographs were taken at suitable magnifications. The photographs of the optimized AM6 formulation taken by Scanning electron microscopy are shown in the Fig. 5.

SHAPE AND SURFACE MORPHOLOGY

Fig 5: SEM Picture of Losartan Potassium Microspheres



SEM PICTURES OF FORMULATION LPM 1

EVALUTION OF LOSARTAN POTASSIUM MICROSPHERES

PERCENTAGE YIELD

The production yield of microspheres of Losartan Potassium using chitosan. The LPM 1 (75 %) to LPM 9 (87.4 %) results as shown in Table 2

Encapsulation efficiency

Drug entrapment efficiency (%EE)

Percentage entrapment efficiency of LPM 1 (42.4 %) to LPM 9(38.72 %) respectively. The LPM 1 shows the good formulation & high efficiency. Results as shown in Table 2

ISSN: 0975-8585

Entrapment Loading (%EL)

Percentage entrapment loading of LPM 1 (78 %) to LPM 9 (41%) respectively. The LPM 1 shows the good formulation & high efficiency. Results as shown in Table 2.

Particle size

Particle size distribution of Microspheres represented it indicated that particles are in nanometric range suitable for over active bladder. LPM 1 (36.5%) to LPM 9 (187.46 %) Formula as shown in given Table 2

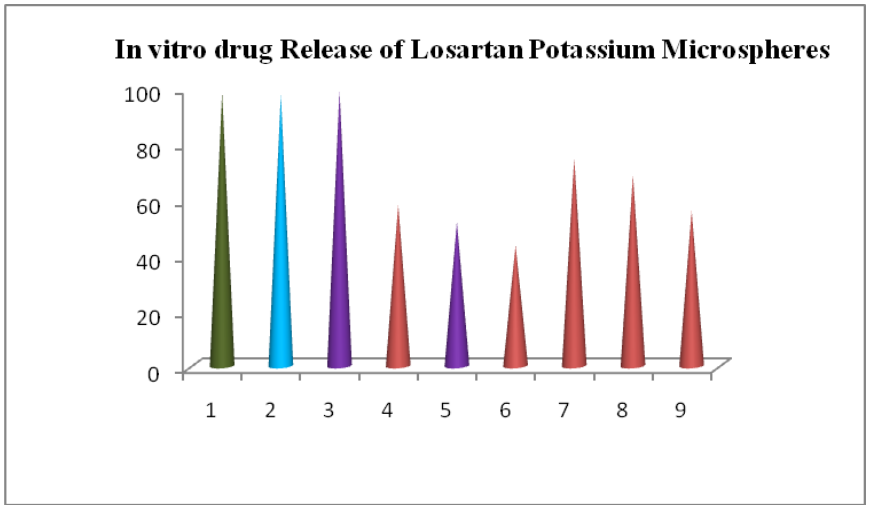
Table 2: Characterization of losartan potassium microspheres

Formulation code	% Yield	% drug content	Drug entrapment efficiency (%)	Particle size (µm)	Cumulative percentage release (%/hrs)
LPM1	**75**	**42.4**	**78**	**36.5**	**98.14**
LPM2	85.6	94.3	85	32.6	97.45
LPM3	74	90.12	82	33.2	98.8
LPM4	81	71.23	50	202.02	57.84
LPM5	77	67.19	58	232.15	51.48
LPM6	80	55.14	65	256.04	43.31
LPM7	82.3	43.17	30	156.71	74.47
LPM8	86.4	40.19	34	172.85	68.45
LPM9	87.4	38.72	41	187.46	55.69

In vitro drug release kinetics

For understanding the mechanism of drug release rate kinetics of the drug from dosage forms. The values are complied in Table . The % drug release with data to various kinetic models for different microspheres formulations is presented in **Fig.8 and Table. 7**

Table: 7 Cumulative % drug releases of Losartan Potassium microspheres



ISSN: 0975-8585

REFERENCES

[1] Shovarani KN. Ind J Pharm Sci 1994; 56(4):45-50.
[2] Prasanth K Rout, Bhabani S Nayak. AJPCR 2009; 2(4).
[3] Wang J, Flanagan DR. J Pharm Sci 1999; 88(7):731-38.
[4] Higuchi T. J Pharm Sci 1963; 52(11):1145-49.
[5] Umadevi SK, Thiruganesh R, Suresh S and Bhaskar Reddy K. Drug Dispos. 2010; 31:407–427.
[6] Encapsulation of griseofulvin in wax /fat Microspheres preparation, characterization and release kinetics of microspheres. Indian drugs 2005; 42(7):453-60.
[7] Shovarani KN, Goundalkar AG. Ind J Pharm Sci 1994; 56(4):45-50.
[8] Ghosh A, Nayak UK, Roy P. Int J Pharmacy 2007; 6:52-7.
[9] Gohel MC, Parik RK, Amin AF, Surati AK. Ind J Pharm Sci 2005; 67(8):575-81.
[10] Morkhade DM, Fulzele SV, Satturwar PM, and Joshi SB. Ind J Pharm Sci 2006; 68(1):53-58.
[11] Wang J, Flanagan DR. J Pharm Sci 1999; 88(7):731-38.