Original Article

DESIGN DEVELOPMENT AND EVALUATION OF MATRIX TABLETS OF AMBROXOL HYDROCHLORIDE: *IN VITRO – IN VIVO* STUDY

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ABSTRACT

Objective: The aim of the present study was to develop oral controlled release once a day matrix tablet of Ambroxol hydrochloride using hydrophilic polymer matrix to maintain the drug plasma concentration and increase patient compliance .

Methods: The granules were formulated by non-aqueous granulation technique. The granules were evaluated. Various physicochemical parameters of the tablets were tested. *In vitro* dissolution studies were carried out by pH change method. A suitable HPLC method to determine the drug plasma concentration was developed. Further, in *vivo* studies were carried out to determine the pharmacokinetic parameters. Also optimized formulation was subjected to stability study.

Results: The results of dissolution studies indicated that formulations containing a combination of matrix showed the desired drug release profile. The drug release followed first order release kinetics with high Higuchi regression value indicating diffusion to be the predominant drug release mechanism. Korsmeyer's plot also indicated that the drug release followed Fickian mechanism. The optimized formulation showed no change in their *in vitro* dissolution profiles after storage at 25°C/60%RH and 40°C/75%RH for a period of six months indicating good stability. Pharmacokinetic parameters were estimated and showed that the developed formulation maintained plasma level for a period of 24 hrs. The AUC for developed formulation was found to be higher as compared with marketed sample.

Conclusion: Thus the developed formulation showed stability and release profile. The formulation showed controlled release *in vivo* and therefore can reduce the frequency of dosing leading to improved patient compliance.

Keywords: Ambroxol hydrochloride, Hydrophilic matrix, HPMC K100M, Drug release Pharmacokinetics.

INTRODUCTION

Ambroxol is a metabolite of bromhexine with similar actions and uses. It is chemically described as trans-4-[(2-Amino-3,5-dibromobenzyl)amino]-cyclohexanol. It is an expectorant improver and a mucolytic agent used in the treatment of acute and chronic disorders characterized by the production of excess or thick mucus. It has been successfully used for decades in the form of its hydrochloride as a secretion-releasing expectorant in a variety of respiratory disorders. Its short biological half life (3- 4 h) calls for frequent daily dosing (2 to 3 times) and therapeutic use in chronic respiratory diseases necessitates its formulation into sustained release dosage form [1]. The development of oral controlled release dosage forms has attracted much attention in the recent years and hydrophilic matrix tablets are among the most widely used for controlled release dosage forms currently available. The most important variable in hydrophilic matrix systems is the rate at which the drug substance is released. The release of drug is controlled by the formation of a hydrogel layer around the matrix following exposure to aqueous fluid [2,3]. Controlled-release (CR) formulations have been introduced into drug therapy with two main purposes: to reduce the number of single doses per day improving patient compliance and to decrease the fluctuations of plasma levels, in order to obtain better therapeutic efficacy and lower toxicity. There are many controlled-release pharmaceutical systems currently known, ranging from monolithic matrices, membrane reservoirs, erodible polymers, to the more technologically complex and sophisticated pH independent formulations, ion exchange resins, osmotically and geometrically modified systems. Many of these systems are not produced in a form that is amenable to large-scale manufacturing processes and the cost of formulation development, raw materials and manufacturing technology are among the principal factors in CR delivery systems for oral dosing [4].

The matrix tablet by direct compression has attracted much attention due to its technological simplicity in comparison to other controlled release systems. It requires fewer unit operations, less machinery, reduced number of personnel and processing time, increased product stability and production rate [5]. Hydrophilic polymer matrix systems are widely used in oral controlled drug delivery because of their flexibility to obtain a desirable drug release profile, cost effectiveness and broad regulatory acceptance. The purpose of controlled release systems is to maintain drug concentration in the blood or in the target tissues at desired value as long as possible [6].

HPMC is the dominant hydrophilic vehicle used for the preparation of oral controlled drug delivery [7]. While HPMC could potentially swell (and therefore control) the release of a soluble drug, it could also facilitate the release of sparingly water soluble drugs i.e. Ambroxol Hydrochloride. The net result is controlled drug delivery for a prolonged period of time [8]. HPMC; a semi synthetic derivative of cellulose, a swellable and hydrophilic polymer was selected to use as release retardant material in sustained release matrix tablets, as it is nontoxic and easy to handle

The present study was designed to formulate matrix tablets of Ambroxol Hydrochloride using a binary hydrophilic polymer mixture containing various concentrations of Methocel K15M, Methocel K100M for once a day release of the drug and to elucidate the release kinetics and conduct stability studies on optimized formulation as per ICH guidelines. *In vivo* studies to determine the various pharmacokinetic parameters were also conducted.

MATERIALS AND METHODS

Chemicals

Ambroxol Hydrochloride was obtained as a gift sample from Ajanta Pharma., Aurangabad, India, HPMC K15M and HPMC K100M were purchased from Colorcon Asia Pvt. Ltd., India, Microcrystalline Cellulose-PH 101 (Mingtai Chemical Co. Ltd., Taiwan), Magnesium Stearate and Talc were procured from Chemical Management Co.,

Germany, Polyvinyl Pyrollidon K-30 and Isopropyl Alcohol were obtained from S.D.Fine chemicals., Mumbai, India. All chemicals and reagents used were of analytical grade.

Methods

Preparation of controlled release tablets

Drug, polymers and other excipients were weighed separately for 150 tablets for each formulation as shown in Table-1. The proposed formulations were coded as A1, A2, A3, A4, A5, A6 and A7. The tablets were prepared by wet granulation method [9]. Active ingredient (Ambroxol Hydrochloride) and polymers (Methocel K15M and Methocel K100M) and Avicel pH 101 were passed through #60 and granulated using PVP K30 in IPA solution. The granules were then dried and passed through a mesh (1190 μm) and these granules were lubricated with a mixture of talc and magnesium stearate (2:1). The lubricated granules were compressed using 8-mm round, flat bevelled punches on a single-station tableting machine (M/s Cadmach Machinary Co Pvt Ltd, Ahmedabad, India). Prior to compression the granules were evaluated for several tests.

Physical characterization of tablets

The prepared tablets were evaluated for hardness, friability, weight variation, thickness and drug content uniformity. Hardness of the tablets was tested using Monsanto hardness tester (Campbell electronics, Mumbai). Friability of the tablets was determined in a Roche friabilator (Campbell Electronics, Mumbai). The thickness of tablets was measured using Vernier callipers. Weight variation test was performed according to official method specified in IP [10]. Content of Ambroxol Hydrochloride was estimated by measuring the absorbance of samples at 244 nm using UV- Visible 9100 spectrophotometer (Jasco, Japan)

In vitro release Studies

The prepared matrix tablets were subjected to *in vitro* dissolution study using USP type II dissolution apparatus (Labindia, Disso). The dissolution studies were carried out in 1.2 pH for 2 hrs and in pH 7.4 Phosphate buffer for remaining period of 12 hrs at 37± 0.5°C at 100 rpm. At regular time intervals, 5 ml of sample was withdrawn from the dissolution medium and replaced with equal volume of fresh medium to maintain sink conditions. After filtration and appropriate dilution, the samples were analyzed at 244 nm for Ambroxol Hydrochloride against blank using UV-Visible spectrophotometer. The amount of drug present in the samples was calculated using standard curve [11].

Elucidation of release kinetics

The mechanism of Ambroxol HCl release from the CR matrix tablets was determined by fitting the release data to different release kinetic models. Zero order (equation 1) as the cumulative percentage of drug release vs time, first order (equation 2) as the log of the amount of drug remaining to be released vs time and Higuchi model (equation 3) as the cumulative percentage of drug release vs square root of time. [12, 13].

$$C = K_o t \ldots \ldots \ldots \ldots \ldots \text{(equation 1)}$$

$$Log\ C = Log\ C_o - K_1\ t\ /2.303 \ldots \ldots \ldots \ldots \text{(equation 2)}$$

$$Q = K_h\ t^{1/2} \ldots \ldots \ldots \ldots \text{(equation 3)}$$

Mechanism of drug release

To evaluate the mechanism of drug release from sustained release tablets, data of drug release was plotted in Korsmeyer et al [14]. A log of cumulative % of drug released vs. log time, and the exponent n value (equation 4) was calculated through the slope of the straight line.

$$M_t\ /\ M\infty = Kt^n \ldots \ldots \ldots \ldots \text{(equation 4)}$$

Accelerated stability studies

Accelerated stability study was conducted by storing tablets in amber coloured bottles at 25°C/60%RH and 40°C/75%RH. The tablets were evaluated for physicochemical parameters and in *vitro* drug release profiles for six months [15]

In vivo study protocol

The *In vivo* studies were conducted on Wistar male rats weighing 250-300 gm. They were housed in polypropylene cages and had free access to food and water. The dose of Ambroxol hydrochloride was calculated as per the body weight of animals and developed tablets were formulated considering the calculated dose [16]. The animal protocol was approved by the Animals Ethical Committee of Bombay Veterinary College, Mumbai, India. The optimized controlled release matrix tablets were administered intragastrically. Blood samples were collected for over 24hrs according to a predetermined sample collection schedule. Various pharmacokinetic parameters like C_{max}, T_{max}, AUC and $t_{1/2}$ were determined. The pharmacokinetic parameters were determined from the individual plasma concentration Vs time data.

Sample treatment and analysis

All samples were collected in ethylene diamine tetracetic acid (EDTA) blood tubes, and centrifuged at 3000 x g for 10 minutes at room temperature. The supernatant plasma samples were transferred to screw top polypropylene tubes. These were capped and immediately stored at –20°C ± 5 until analysis. The drug was extracted from the plasma using a suitable extraction method. To 0.5 ml of plasma, 100μl 0.1N NaOH solution was added in a stoppered test tube. The drug was extracted by further adding 250 μl of 20 % perchloric acid. The solution was further centrifuged at 3000 rpm to obtain clear supernatant solution.

The plasma concentration of Ambroxol was determined using a validated high-performance liquid chromatography (HPLC) method. In the present work, the chromatographic system consisted of stationary phase [μ bonda pak C_{18} column (300 x 3.9 mm, 10μ)]. The mobile phase comprised of a filtered and degassed mixture of acetonitrile and phosphate buffer (0.1M) in the ratio of 20:80 (v/v) was used, an injection loop of 100μl. The flow rate was set at 1.0 (ml/min). The detector was based on U.V absorption at wavelength of 254 nm.

RESULTS AND DISCUSSION

Formulation development and physical characterizations

Oral controlled release once a day tablets were prepared using swellable polymers as release retardants. The main objective of this study was to enhance therapeutic performance of Ambroxol HCl by developing matrix tablets. Methocel K100M and Methocel K15M were selected as the matrix formers in this investigation. Formulations were optimized using different concentrations and ratios of polymer (Table 1). The physical attributes (angle of repose, bulk density, tapped density; compressibility index and total porosity) of the prepared powder blend were found to be satisfactory. Results are shown in Table 2. The physical attributes (weight variation, thickness, hardness, and friability) of the prepared tablets were found to be satisfactory. Typical tablet defects such as capping, chipping and picking were not observed. All these properties complied with the official limits as shown in Table 3.

In vitro release profile

Ideally, an extended release tablet should release the required quantity with pre-determined kinetic pattern in order to maintain an effective drug plasma concentration. To achieve this, the tablet should be formulated so that it releases the drug in a pre-determined and reproducible manner [17].

It was observed (Fig 1) that for batches A1, A2 and A3 formulated using HPMC K4M as release retarding polymer, almost 93% - 97% of drug was released within 6-10 hrs of administration indicating that a sufficient polymer concentration in the hydrophilic matrix system was required to form a uniform gel barrier around the tablet upon hydration. Therefore batches A4, A5 and A6 were formulated using HPMCK100M as release retarding polymer which was more viscous then HPMC K4M. An inverse relationship was seen to exist between the amount of polymer and the release rate of Ambroxol HCl from matrix tablets. The percent drug release for formulations A4, A5 and A6 was 91.4%, 93.84% and 95.87% at the end of 10, 12 and 16 hrs

respectively. The release of Ambroxol HCl from the matrix tablets was found to be slow but extended only up to a period of 16 hrs at 25% polymer concentration Formulation A7 which comprised of a mixture of both the polymers in a 1:1 ratio and a 1:25 drug: polymer ratio showed the desired controlled release profile of up to 98.8% drug release at the end of 24 hrs. This controlled release could be attributed to the formulated polymer matrix which upon contact with the aqueous medium leads to hydration of the hydrophilic matrix system from the peripheral region to the centre, to form a gelatinous mass, through which the drug was diffused in a controlled manner for 24 hrs.

From the data obtained it was observed that all the formulations showed higher regression coefficient values for first order release of the drug (r^2 value; 0.9721 to 0.9928) as compared to zero order release kinetics (r^2 value; 0.8793 to 0.9267) indicating that the release of drug followed first order release kinetics for all the formulations (Table 4). The drug release mechanism from the developed hydrophilic tablets was also determined. The drug release mechanism for hydrophilic tablets usually follows diffusion mechanism. To evaluate this parameter plots of percent drug release vs. square root of time as per Higuchi's equation were constructed. It was observed that all the plots were linear for all formulations (r^2 value; 0.9921 to 0.9981). This confirmed that the drug release from the prepared formulations was diffusion controlled. To further confirm the diffusion mechanism, the data were fit into Korsmeyer-Peppas equation [18]. The formulations A4, A5, A6 and A7 all

showed good linearity (r^2 values: 0.9913 – 0.9983). The slope values (n) also were in the range of 0.5517 to 0.7678. Non- Fickian diffusion (0.5 < n < 1) was the dominant mechanism of drug release with these formulations. It was observed that as the drug: polymer ratio increased the n value also increased for formulations A4, A5 and A6. The highest slope (n) value was found for formulation A7 which consisted of a 1:1 mixture of polymers of different viscosity grades. However this high value of (n) also indicates that diffusion along with erosion could be the mechanism for drug release for formulation A7. This anomalous diffusion could be attributed to the complexity of the hydrophilic polymer matrix formed in formulation A7. Hence, diffusion coupled with erosion may be the mechanism of drug release from formulation A7.

In vivo **studies**

In vivo studies carried out for 24 hours showed that Ambroxol HCl once a day formulation released drug constantly till 24 hrs and thus maintained its concentration for a day, where as conventional tablet was not able to maintain drug concentration for more than 6 hrs. The conventional tablet achieved maximum concentration within four hours and drug release rapidly declined to zero at the end of 12th hr. Controlled release developed formulation maintained desired plasma concentration for a period of 24 hrs. The AUC of developed controlled release formulation was higher than that obtained from equivalent marketed conventional dosage form (Table 5).

Table 1: Composition of controlled release formulations of Ambroxol HCl matrix tablets

Ingredients(mg)	A1	A2	A3	A4	A5	A6	A7
Ambroxol HCL	120	120	120	120	120	120	120
HPMC K100M	-	-	-	25	37.5	62.5	31.25
HPMC K4M	25	37.5	62.5	-	-	-	31.25
Avicel pH 102	88	73.5	46.5	88	73.5	46.5	46.5
PVP	8	10	12	8	10	12	12
Magnesium stearate	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Talc	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Total	250	250	250	250	250	250	250

Table 2: Micromeritic properties of the prepared granules (n=3)

Parameters	A1	A2	A3	A4	A5	A6	A7
Angle of repose (º)	21.5 ±0.27	23 ±0.63	24.8±0.02	21.2 ±0.24	24 ±0.03	25.86±0.02	28.9±0.01
Hausner's ratio± S.D	1.24 ±0.07	1.23 ±0.07	1.17 ±0.12	1.18 ±0.01	1.14 ±0.02	1.19 ±0.07	1.21 ±0.07
Bulk density (g/ml)	0.586±0.03	0.569 ±0.01	0.578±0.04	0.592 ±0.07	0.576 ±0.08	0.592 ±0.07	0.692 ±0.07
Tap density (g/ml)	0.690±0.04	0.654±0.06	0.650±0.03	0.636 ±0.01	0.743 ±0.05	0.612 ±0.01	0.776 ±0.09
Carr's index	13.5±0.02	12.59±0.03	13.90 ±0.11	14.5 ± 0.01	12.7 ± 0.02	12.3 ± 0.04	12.2 ± 0.01

Table 3: Post Compressional parameters of Ambroxol Hcl matrix tablets (n=3)

Parameters	A1	A2	A3	A4	A5	A6	A7
Weight variation± S.D (mg)	251.1±1.2	253.14±12.3	248.1±1.2	256.1±2.4	250.6±2.1	252.4±1.1	251.7±2.3
Hardness± S.D (Kg/cm²)	5.2±0.45	5.3±0.42	5.6±0.45	5.2±0.21	5.3±0.34	5.5±0.17	5.6±0.36
Friability± S.D (% wt loss)	0.64±0.04	0.62±0.02	0.66±0.02	0.68±0.06	0.62±0.04	0.64±0.02	0.59±0.03
Thickness± S.D (mm)	3.5 ± 0.02	3.54 ± 0.01	3.6 ± 0.02	3.52 ± 0.02	3.55 ± 0.02	3.57 ± 0.02	3.5 ± 0.02
Drug content± S.D (%)	97.14±0.04	96.15±0.02	98.23±0.06	97.60±0.04	96.54±0.05	98.14±0.04	98.26±0.02

Table 4: *In-Vitro* drug release kinetic data of prepared matrix tablets of Ambroxol Hcl

Formulations	Regression coefficient (r^2 values)			Korsmeyer-Peppas plots	
	Zero order	First order	Higuchi equation	Slope (n)	Regression coefficient (r^2)
A4	0.8793	0.9721	0.9921	0.5517	0.9913
A5	0.8681	0.9816	0.9935	0.5792	0.9926
A6	0.8951	0.9843	0.9948	0.5847	0.9958
A7	0.9267	0.9928	0.9981	0.7678	0.9983

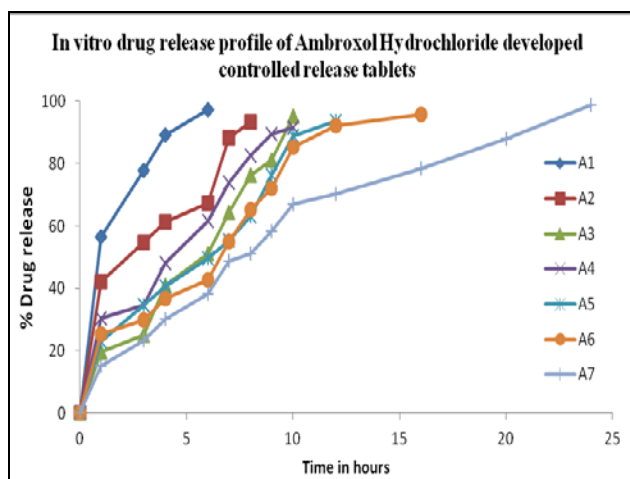Each sample was analyzed in triplicate (*n* = 3)

Fig. 1: *In vitro* release profile of Ambroxol Hcl from the prepared hydrophilic matrix tablets (n=3)
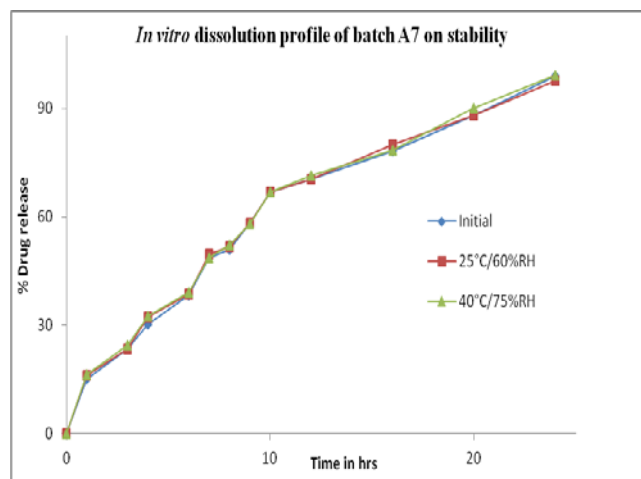

Fig. 2: *In vitro* release profile of Ambroxol Hydrochloride from hydrophilic matrix tablets (A7) after stability studies (n=3)

Table 5: Comparative Pharmacokinetic Data for the Developed Hydrophilic Once a Day Formulation and Marketed Conventional Formulation of Ambroxol HCl

Parameters	Conventional formulation	OD formulation
T_{max} (h)	3.00	4.0
C_{max} (ng/ml)	46.36	41.15
$T_{1/2}$ (h)	1.46	12.09
AUC (ng.h/ml)	48.07	64.60

Each sample was analyzed in triplicate (*n* = 3)

Accelerated stability studies

Stability studies revealed that no visible changes in the appearance of the matrix tablets were observed at the end of the storage period. The drug content was found to be 98.3% ± 1.9%. There was no significant difference in the mean amount of ambroxol hydrochloride released from A7 matrix tablets after storing for 6 months at 25°C/60%RH and 40°C / 75% RH, indicating the stability of the developed formulation (Fig 2).

CONCLUSION

A once-a-day oral controlled release dosage form was successfully developed using swellable polymers. The use of polymeric matrix devices to control the release of variety of therapeutic agents has become increasingly important in development of the modified release dosage forms [19]. In the present research work a combination of hydrophilic polymeric matrix tablets were prepared using Methocel K15M and Methocel K100M as release retarding polymers. The drug release followed first order kinetics and Higuchi controlled release matrix diffusion mechanism. It was observed that the mechanism of release changed with the nature and contents of polymers in the matrix Accelerated stability studies showed no visible or significant changes in the matrix tablets at the end of storage period indicating that the formulation was stable. From *in vitro* drug release profile the formulation A7, it was concluded to be the best formulation showing desired release pattern at higher concentration and combination of polymers.

The drug plasma concentration was found to be maintained upto 24 hours during *in vivo* studies. Therefore as desired, once a day formulation of Ambroxol HCl giving controlled release was successfully developed. The developed formulation would reduce the frequency of dosing and increase the patient compliance.

REFERENCES

1. Sweetman, C. Martindale. The complete drug reference. The Pharmaceutical press. London 2006;1084.
2. Balasubramaniam J, Kumar G. M.T. Srinivas. HPMC-based matrix tablets of atenolol and cisapride: effect of viscosity of polymer and drug solubility on in-vitro release. Ind J Pharm Sci 2005;67:414-21.
3. Robles L. V, A.M.E. Campos. Influence of the viscosity grade and the particle size of HPMC on metronidazole release from matrix tablets. Eur J Pharm Biopharm 1997;43:173-8.
4. Bayomi M, Al-Suwayeh ARM. A, S.A. El-Helw. Excipient-excipient interaction in the design of sustained release theophylline tablets: in-vitro and in-vivo evaluation. Drug Dev Ind Pharm 2012;7:499-506.
5. MM Rahman, S Hasan, MA Alam, S Roy, MK Jha, MH Rahman. Int. J. Pharm. Biomed. Res 2011;2(1):7-12.
6. GD Gothi, BN Parikh, TD Patel, ST Prajapati, DM Patel, CN Patel. Journal of Global Pharma Technology 2010;2(2):69-74.
7. Salsa T, Veiga F, Pina ME. Oral controlled-release dosage forms. I. Cellulose ether polymers in hydrophilic matrices. Drug Dev Ind Pharm 1997;23:929–38
8. Mohammad S.I, Mizanur R.M. Effect of physicochemical properties on the release profile of diltiazem HCl and naproxen from HPMC and Kollidon SR based matrix tablets. Indian Jour of Pharma Edu and Res 2009;43(1):46-54.
9. Leon Lachman, A. Herbert Lieberman, Joseph L Kanig. The Theory and Practice of Industrial pharmacy. 3rd Edition. New York: Varghese publishing house. 1987;296-300.
10. Ganesan, V, D.L. Jayachandran. Design and evaluation of matrix tablets of ambroxol hydrochloride using guar gum. Research J. Pharm. and Tech 2008;1:507-12.
11. United States Pharmacopeia 30 and National Formulary 25. The United States Pharmacopeial Convention 2007.
12. CR Reichal, JB Lakshmi, TK Ravi. Studies on formulation and in vitro evaluation of glimepride floating tablets. *J. Chem. Pharm. Res* 2011;3(3):159-64.
13. RW Korsmeyer, R Gurny, E Doelker, P Buri, NA Peppas. Mechanism of solute release from porous hydrophilic polymers. *Int. J. Pharm* 1983;15:25-35.
14. Sunil, R, P. Pavan kumar, K. Rajanarayana. Formulation and release characterization of a bilayer matrix tablet containing glimepride immediate release component and metformin hydrochloride as sustained release component. Int. J. Pharm. Sci. and NanoTech 2010;3:851-59.

15. Sanjay Bajaj, Dinesh Singla, Neha Sakhuja. Stability testing of pharmaceutical products. Journal of applied pharmaceutical science 2012;02(03):129-38
16. Jang-woo shin, In-chan seol, Chang-gue son. Interpretation of animal dose and human equivalent dose for drug development. The Journal of Korean Oriental Medicine 2010;31(3):1-7
17. Suravanan M, Kataray KS, Ganesh KS. Hydroxy propyl methyl cellulose based cephalexin extended release tablets: influence of tablet formulation, hardness and storage on in vitro release kinetics. Chem. Pharm. Bull 2003;51:978-83.
18. Peppas NA. Analysis of Fickian and non-Fickian drug release from Polymers. Fitting controlled release and dissolution data. Pharma.Acta. Helv 1985;60:110-11.
19. Mukesh R. Patel, K. R. Patel, N. M. Patel, T. J. Mehta, A. D. Patel Development and optimization of colon targeted compression coated tablet of Methotrexate. J. Chem. Pharm. Res 2011;3(2):786-91.