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FORMULATION AND OPTIMIZATION OF RUPTURABLE MEMBRANE COATED PULSATILE DRUG DELIVERY SYSTEM OF ZALTOPROFEN USING BOX-BEHNKEN DESIGN

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ABSTRACT

The present study was aimed at design and development of a time programmed rupturable membrane coated pulsatile drug delivery system for poorly soluble drug, Zaltoprofen for bedtime dosing and pulsed release of drug to encounter symptoms of rheumatoid arthritis (RA) in the early morning. The present dosage form comprising fast dissolving core tablet coated with two consecutive layers, inner swellable layer and external rupturable membrane (water-insoluble but-permeable). In an attempt to enhance the bioavailability of zaltoprofen (BCS class II) by improving rate and extent of solubility and dissolution rate, solid dispersion of drug was prepared by solvent evaporation method using drug, PVP VA68 and Lutrol F127in the ratio of 1:1.6:0.4. Fast dissolving core tablets (FDT) were prepared by direct compression technique using co-processed disintegrating agents. Solid dispersion has shown remarkable enhancement in drug's aqueous solubility by 18.95 folds and FDTs prepared with 8% of co processed super disintegrating agents rapidly released 95% of drug in 30 min with very short disintegration time (47±1.91 Sec). The fast dissolving core tablets coated with swelling layer and further flexible rupturable membrane with plasticized Konjac glucomannan triacetate and Eudragit L100-50. In order to get desired lag time of 4 hrs - 6 hrs, the influence of different kind and amount of swelling materials i.e., HPMC E15, HPMC E50, HPME K4M, L-HPC and Na CMC, quantity of pore forming agent and coating weight were further screened and optimised on trial and error basis. The optimization of Pulsatile tablet formulation and identifying the factors influences the lag time was done by response surface methodology employing Box-Behnken design. The design was developed by selecting an amount of swelling layer (A), pore former (B), outer coat (C) as independent variables and Lag Time (R1) and Time for 75% drug release -$T_{75\%}$ (R2) as dependent variables. The obtained responses were analysed by ANOVA and polynomial equations were derived to optimize the formulation. P- value (<0.0001) F-value (0.4754 &0.3390) of responses confirmed the suitability of quadratic model.

INTRODUCTION:

Chrono-pharmacotherapy, the drug regime design based on circadian rhythm, is recently gaining much attention globally. several diseases like hypercholesterolaemia, asthma, cancer, duodenal ulcer, arthritis, diabetes, neurological disorders, cardio-vascular diseases show circadian variation in 24hr cycle [2,3], that demand time-Program -med drug release to encounter the physio-pathological changes. For example, pain and inflammations in rheumatoid arthritis and trauma, severity of asthma, heart attack, Ischemic heart diseases such as angina pectoris and myocardial infarction are manifested more frequently during early hours of the day [4,5]. In comparison with conventional drug delivery systems, pulsatile systems are very effective and gained much attention with better patient compliance in chrono-pharmacotherapy because it delivers the drug at specific site at the right time in the right amount after predetermined lag time. Pulsatile drug delivery system can be broadly classified into 4 classes.,1. Time controlled 2. Stimuli induced 3. Externally regulated 4. Multi particulate systems. Among various types in each class, rupturable coating systems gained much attention now a days due to production simplicity [6,7].

Rupturable systems consist of a drug-containing core usually fast dissolving tablet, a swelling layer, and an external water insoluble, but permeable polymer coat [6]. Aqueous medium or gastrointestinal (GI)-fluids penetrate through the polymer coat, the swelling layer expands until the outer polymer coat ruptures and releases the drug rapidly. Pressure developed by the swelling layer, water permeability and the mechanical strength of the outer coat were the main factors controlling the lag time of pulsatile tablets [8,9,10].

Zaltoprofen (ZPF), is a derivative of 2-aryl propionic acids (2-APA), chemically it is 2-(10,11-dihydro-10-oxodibenzo (b,f) thiepin -2-yl) propionic acid, an important group of non-steroidal anti-inflammatory drugs (NSAID) and has powerful inhibitory effects on acute and chronic inflammation with less adverse reactions on the gastrointestinal tract than other NSAIDs [8]. ZPF exerts anti-inflammatory actions and analgesic effects by inhibiting prostaglandin synthesis and through a peripheral mechanism by inhibition of bradykinin B2 receptor-mediated bradykinin responses in primary afferent neurons [11-14]. It is a unique compound that not only a cyclo- oxygenase-2 inhibitor but also brady-kinin-induced 12-lipoxygenase inhibitor. Zaltoprofen is an important drug mainly used in the treatment of rheumatoid arthritis and osteoarthritis as well as to relieve pain and inflammation during post-surgery or post trauma and tooth extraction [14-18]. The present study designed., 1). to prepare solid dispersion of zaltoprofen by solvent evaporation method using PVP VA68 and Lutrol F127 to improve drug solubility, 2). to prepare Fast dissolving tablets (FDT's) to enhance dissolution rate in an attempt to enhance its bioavailability, 3). to prepare rupturable membrane coated tablets for rapid release of drug after lag time (6 hrs).

The major objective of the present study is to investigate and optimise the factors effecting lag time (6 hrs) and development of rupturable membrane pulsatile tables of zaltoprofen using factorial design. Further intended to determine the pharmacokinetic parameters for optimized formulation using rabbit as model animal.

MATERIALS AND METHODS:

Materials:

The materials used were as follows: Zaltoprofen (ZPF, gift sample from Ipca labs, India); PVP VA68, Lutrol F127, Microcrystalline Cellulose (MCC), spray dried Lactose, Croscarmellose Sodium, Crospovidone, Sodium starch glycolate (SSG), Hydroxy propyl methyl cellulose (HPMC), Low-substituted Hydroxypropyl Cellulose(L-HPC), Triethyl citrate (TEC), Magnesium stearate, Eudragit L100, Konjac glucomannan triacetate (KGM Tac, Talc. All chemicals were of HPLC or analytical grade.

EXPERIMENTAL METHODS:

Preparation of Calibration curve: 50 milligrams of Zaltoprofen (ZPF) pure drug was accurately weighed and dissolved with minimum quantity of methanol in a 100 mL volumetric flask and then the volume was made up to 100 mL with methanol to get standard stock solution (500µg/mL of ZPF).

1,2,3,4,5, 6 mL of standard stock solutions were transferred into different 100 mL volumetric flask, diluted with buffer pH 6.8 or distilled water up to 100 mL to get final concentration ranging from 5-30 µg/mL and analysed for absorbance at 243nm by using UV Double beam Spectrophotometer [19, 20]

Solubility studies: Solubility of zaltoprofen in various dissolution media was determined by equilibrium solubility method. Sufficient excess amount of zaltoprofen was added to 10mL screw-capped glass vials containing 5 mL of distilled water, 0.1N HCl pH 1.2, phosphate buffer pH 6.8 and pH 7.4. The vials were shaken mechanically for 12 hr on mechanical shaker (Lab India, Mumbai, India) at 37 ± 2∘C, allowed to equilibrate for another 24-hr. centrifuged for 5 min at 2000 rpm. The supernatant liquid was filtered and analysed for drug content by UV visible spectrophotometer at 243nm [21-23].

Formulation and evaluation of Zaltoprofen Solid Dispersion: 10 grams of the finely milled mixture of ZPF: PVP VA64: Lutrol F127 (ratio 1:1.6:0.4) was dissolved in 25 ml methanol with continuous stirring. The solvent was then completely evaporated with stirring at 40˚c to obtain dry granules. The resulting solid dispersion was further dried in a vacuum oven at 40°C for 12hr, milled and analysed for drug solubility [24].

Formulation and evaluation of fast Dissolving core Tablets: Solid dispersion equivalent to 80mg of ZPF and excipients i.e. Spray dried lactose (SDL), Micro-crystalline Cellulose (MCC) Croscarmellose Sodium, Crospovidone Sodium starch glycolate (SSG), were accurately weighed, passed through 60# sieve separately, mixed in geometrical order, glidant and lubricant were added and finally compressed with 8mm sizes flat and round punches using Rimek compression Machine. (**Table no:1**). The prepared fast dissolving core tablets were subjected *in vitro* quality control tests like weight variation, hardness, friability, disintegration time, wetting time and drug content [25,26].

Formulation of Zaltoprofen Rupturable membrane Coated pulsatile Tablets: The core tablets were coated with double layer i.e., inner swelling layer and outer rupturable membrane coat in a conventional coating pan (**Table no:2**). The Inner coat applied with 10% w/v clear aqueous solution of swelling agents (10% wt/wt HPMC E15**,** HPMC E50, HPMC K4M, L-HPC, Na CMC)8% aqueous solution of talc up to desired weight gain. The process and pan variables were given in **Tables no:3.** After coating, the tablets were dried for 12 hr at 40°c - 45°c to remove residual solvent. The

outer coating solution was prepared by dissolving film former (KGMTac), pore former (Eudragit L100-50) and plasticizer (Triethyl citrate) in a solvent mixture (Acetone: Isopropyl alcohol, 70:30) stirred for 24 hrs to get clear solution and applied at spray rate of 1.0 mL/min on swelling layer coated tablets up to desired weight gain, finally dried at 45°c for 4 h to remove residual solvent [27,28].

Investigation of Factors Influencing Lag time of RMCPTs: Rapture of membrane which decides RMCPTs lag time, was obtained by exerting Pressure on membrane by swelling layer upon penetration of fluids through the membrane coat. To screen the influence of swelling agent type (HPMC E15**,** HPMC E50, HPMC K4M, L-HPC, Na CMC),amount of swelling agent (10%-30%) quantity of pore former (15% -35 % w/w), membrane coating weight (5% - 15% w/w), various batches of RMCPTs were prepared and subjected to *invitro* dissolution study and rapture test to determine lag time. The details of screening variables and constant variables were given in **Table no:4**.

***In vitro* dissolution study of RMCPTs:** The drug release studies for RMCPTs were performed using the USP dissolution apparatus II (Lab India, India) at 37 ± 0.5°C and 100 rpm for 2 h in pH 1.2 HCl (900 mL) proceeded by phosphate buffer pH 6.8 for another 7hr. At predetermined time intervals (0.5,1,1.5,2,2.5,3,3.5,4,4.5,5,5.5,6,6.5,7,7.5, 8,8.5,9hrs) 5mL of aliquots were withdrawn and replaced with same volume of fresh medium to maintain sink conditions. The withdrawn samples were then analysed spectrophotometrically at 243 nm [29,30]**.**

Box-Behnken Design:

The Design Expert software (version 10.1, Stat-Ease Inc., Minneapolis, USA) was used for design study, response surface analysis of the model and generation of second order polynomial equations. The BBD very simplest compared to central composite design (CCD) and 3^3 full factorial design (FFD) and derives useful data with lesser number of runs (BBD-17, CCD-21, FFD-27 runs)[3,32].The Box-Behnken design (BBD) with three factors and three levels with 17 runs was employed for the optimization study. Based on preliminary trials, weight of swelling layer - HPMC E15 (A), quantity of pore former - Eudragit-L100-50 (B) and membrane coating weight (C) were identified as independent variables. The responses lag time in drug release (R1) time for 75% drug release- $T_{75\%}$ (R2) were selected as dependent variables. The three levels (−1, 0 and +1) of independent variables A, B, C used are 20–30–40% w/w, 15–25–35% w/w and 6–10.5–15% w/w respectively. The actual and coded values of independent variable were given in **Table:5**. The polynomial equation generated for the model as below.

$$R = b_0 + b_1A + b_2B + b_3C + b_4AB + b_5AC + b_6BC + b_7A^2 + b_8B^2 + b_9C^2$$

Where R is the studied response, b_0–b_9 are the regression coefficients and A, B and C are the factors studied.

Pharmacokinetic study:

In a single-dose, open-label, crossover study, nine rabbits bisected randomly into 3 groups, 3 rabbits in each group. Rabbits weighing 1.4-1.6 kg were pre-treated normal chow diet and water for one week, then anesthetized with diethyl ether, central ear vein was cannulated with polyethylene tubing for blood sampling. The cannula was flushed and filled with heparinized isotonic saline (100 IU/mL) to prevent the blood from clotting.

Table no 1: Composition of zaltoprofen fast dissolving core tablet.

S. No	Ingredient	Quantity (mg)
1	S.D* (≈80mg of Zaltoprofen)	242
2	Cross-povidone	21
3	sodium starch glycolate	3.5
4	Crossmellose Sodium	3.5
5	Spray dried lactose	43
6	Micro crystalline Cellulose	30
7	Talc	3.5
8	Magnesium Stearate	3.5
Total weight of tablet (mg)		**350**

S.D* Solid dispersion of zaltoprofen

Table no 2: Composition of different parts of RMCPT tablet.

S. No	Tablet portion	Ingredients	Quantity
1	Core Tablet	Solid Dispersion + Others	350 mg
2	First Layer - Swellable Layer	HPMC E15	20- 40 % w/w
		Talc	Q. S
		Water	Q. S
3	Second Layer - Membrane coat	KGM Triacetate	45 – 75 %
		Eudragit L100-50	15-35 %w/w
		Triethyl citrate	10%
		Talc	Q. S
		Acetone + IPA (70:30)	Q. S

Table no:3: Pan coating - Process & pan parameters.

S. No	Process or Pan parameter	Set Value
1	Bed temperature	40^{o} C
2	Spray rate	5mL / min
3	Spray time	Up to weight gain
4	Spray nozzle aperture size	1 mm
5	Spray pressure	1.2 bars
6	Pan speed	20 rpm
7	Drying in equipment	10 min

Table no 4: Screening and optimisation of formulation: Composition of RMCPTs

Screening Variable	Name of Excipient	Qty	Constant Variables
Swelling layer material	HPMC E15	20% w/w	1) swelling layer coat-20%w/w 2) MC-Pore former- 20 %w/w 3) Mem coat weight- 5% w/w 4) Plasticizer conc.: 10% w/w
	HPMCE50	20% w/w	
	HPMC K4M	20% w/w	
	L-HPC	20% w/w	
	Na- CMC	20% w/w	
Con. Of swelling layer	HPMC E15	10% w/w	1) MC-Pore former-20 %w/w 2) Mem coat weight- 5% w/w 3) Plasticizer conc.: 10% w/w
		20% w/w	
		30% w/w	
Membrane coating weight	KGMTACT+ Eudragit L100-50 + TEC	5% w/w	1) Swelling layer coat-20%w/w 2) MC-Pore former-20 %w/w 3) Plasticizer conc.: 10% w/w
		10% w/w	
		15% w/w	
Con. of Pore former	Eudragit L100-50	15% w/w	1) Swelling layer coat-20% w/w 2) Mem coat weight- 5% w/w 3) Plasticizer conc.: 10% w/w
		25% w/w	
		35% w/w	

Table no:5: Box-Behnken Design for formulation RMCPT: List of independent variable, dependent variable and experimental results.

Run	Batch code	Independent variable						Dependent variable	
		Coded values			Actual values			R1: (Hr))	R2: (hrs)
		A	B	C	A (%w/w)	B (%w/w)	C (%w/w)		
1	ZM1	-1	-1	+1	20	25	15	7.181	7.538
2	ZM2	+1	+1	0	40	35	10.5	4.236	4.52
3	ZM3	0	0	-1	30	15	6	4.25	4.534
4	ZM4	+1	0	0	40	15	10.5	5.764	6.007
5	ZM5	0	+1	+1	30	35	15	5.903	6.055
6	ZM6	-1	0	0	20	15	10.5	6.778	7.031
7	ZM7	0	+1	-1	30	35	6	3.306	3.501
8	ZM8	+1	-1	-1	40	25	6	3.681	4.014
9	ZM9	+1	-1	+1	40	25	15	4.706	4.995
10	ZM10	0	0	+1	30	15	15	6.694	7.016
11	ZM11	0	-1	0	30	25	10.5	4.764	5.136
12	ZM12	0	-1	0	30	25	10.5	4.722	5.029
13	ZM13	-1	+1	0	20	35	10.5	6.153	6.541
14	ZM14	0	-1	0	30	25	10.5	5.236	5.542
15	ZM15	0	-1	0	30	25	10.5	5.194	5.526
16	ZM16	0	-1	0	30	25	10.5	4.903	5.051
17	ZM17	-1	-1	-1	20	25	6	3.708	4.014

Table no:6: Post compression test results of ZPF fast dissolving tablets.

Post compression Parameter	Results (Mean±SD)
Avg.weight(mg)*	349.96 ± 1.90
Thickness (mm)**	3.46 ± 0.118
Hardness (Kg/cm^2) ***	3.92 ± 0.186
Friability (%) **	0.51 ± 0.206
Disintegration time (Sec)***	47 ± 1.915
wetting Time (Sec)***	38 ± 2.749
Drug Content (%) ***	99.98 ± 0.124

Mean ± standard deviation. Where *n =20, ** n = 3, *** n = 6

Table no:7: Model Summery of statistics.

Source	Std. Dev.		R^2		Adjusted R^2		Lack of Fit (F-Value)		PRESS	
	R1	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**
Linear	0.5568	0.5675	0.8079	0.8047	0.7636	0.7596	7.37	6.64	8.09	8.39
2FI	0.4819	0.4817	0.8893	0.8918	0.8229	0.8268	6.09	5.22	10.47	10.37
Quadratic	**0.2107**	**0.2169**	**0.9852**	**0.9846**	**0.9661**	**0.9649**	**0.4754**	**0.339**	**1.67**	**1.48**
Cubic	0.2394	0.2562	0.9891	0.9877	0.9563	0.951	Aliased			

Table no:8: ANOVA for Quadratic model

Source	Sum of Squares		df	Mean Square		F-value		p-value	
	R1	**R2**		**R1**	**R2**	**R1**	**R2**	**R1**	**R2**
Model	20.67	21.11	9	2.3	2.35	51.72	49.84	< 0.0001	< 0.0001
A	3.69	3.9	1	3.69	3.9	83.08	82.94	< 0.0001	< 0.0001
B	1.89	1.97	1	1.89	1.97	42.55	41.88	0.0003	0.0003
C	11.37	11.38	1	11.37	11.38	256.11	241.79	< 0.0001	< 0.0001
AB	0.2039	0.2485	1	0.2039	0.2485	4.59	5.28	0.0694	0.0552
AC	1.5	1.62	1	1.5	1.62	33.74	34.35	0.0007	0.0006
BC	0.0059	0.0013	1	0.0059	0.0013	0.1318	0.0275	0.7273	0.8729
A^2	0.3181	0.42	1	0.3181	0.42	7.16	8.93	0.0317	0.0203
B^2	1.03	0.8606	1	1.03	0.8606	23.15	18.29	0.0019	0.0037
C^2	0.7415	0.7872	1	0.7415	0.7872	16.7	16.73	0.0047	0.0046
Residual	0.3109	0.3294	7	0.0444	0.0471				
Lack of Fit	0.0817	0.0668	3	0.0272	0.0223	0.4754	0.339	0.7163	0.7996
Pure Error	0.2292	0.2626	4	0.0573	0.0657				
Total	20.98	21.44	16						

Table no:9: Independent and dependent variables constraints for optimisation of RMCPTs

Name	Goal	Lower Limit	Upper Limit
A: A: HPMC E15	is in range	20	30
B: B: Eudragit-L100-50	is in range	15	25
C:C-Membrane Coat Weight	is in range	6	15
R1: Lag Time	is target = 6	3.306	7.181
R2: T75	is target = 6.2	3.501	7.538

Table no 10: *In vitro* evaluation of optimised formulation. Comparison of results.

Dependent variable	Predicted Value	Observed Value	Relative error
R1: Lag Time (Hrs)	5.94	5.78	0.0269
R2: $T_{75\%}$ (Hrs)	6.2	6.11	0.0145

Table no 11. Pharmacokinetic parameters of zaltoprofen marketed IR tablet and optimised formulation

Parameter	Marketed Tablet (Mean±SD) n=3	Optimised Tablet (Mean±SD) n=3
KE (1/h)	0.2329 ± 0.022	0.2565 ± 0.038
$t_{1/2}$ (Hrs)	3.0053 ± 0.308	2.758 ± 0.377
T_{max} (Hrs)	1.5 ± 0	8 ± 0
C_{max} (µg/ml)	15.02 ± 1.308	17.443 ± 2.207
AUC $_{0-t}$ (µg/ml*h)	80.51 ± 6.737	103.05 ± 15.44
AUC $_{0-\infty}$ (µg/ml*h)	81.12 ± 6.854	105.68 ± 16.78
MRT (Hrs)	5.2842 ± 0.186	10.81 ± 0.547
V_d (µg/ml)	0.4284 ± 0.034	0.305 ± 0.045
CL ((µg/ml)/h)	0.0994 ± 0.009	0.0775 ± 0.011

Fig.no 1: Zaltoprofen calibration curve in different dissolution media.

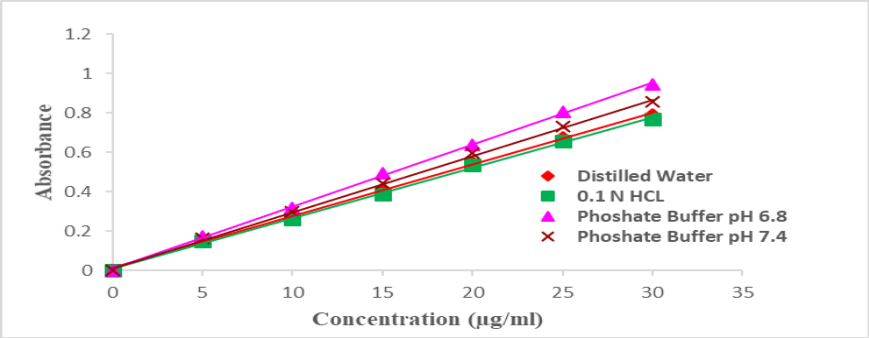


Fig.no 2: Dissolution profiles of zaltoprofen rupturable membrane coated tablets with different type of swelling agent s (HPMC E15, HPMC E50, HPMC K4M, L-HPC, Na-CMC)

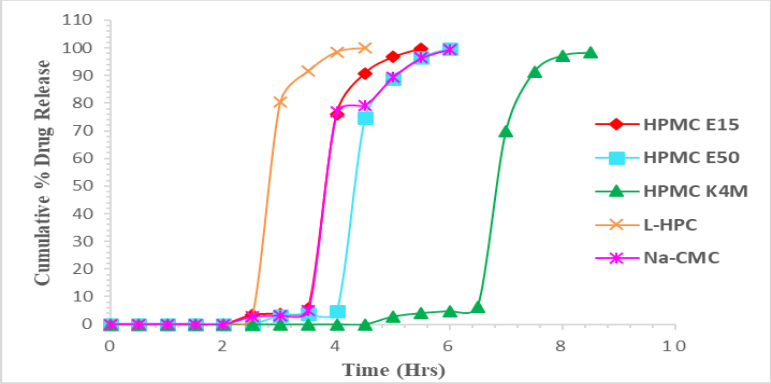


Fig.no 3: Dissolution profiles of zaltoprofen rupturable membrane coated tablets with different amount of swelling agent HPMC E15 (10%, 20%, 30% w/w).

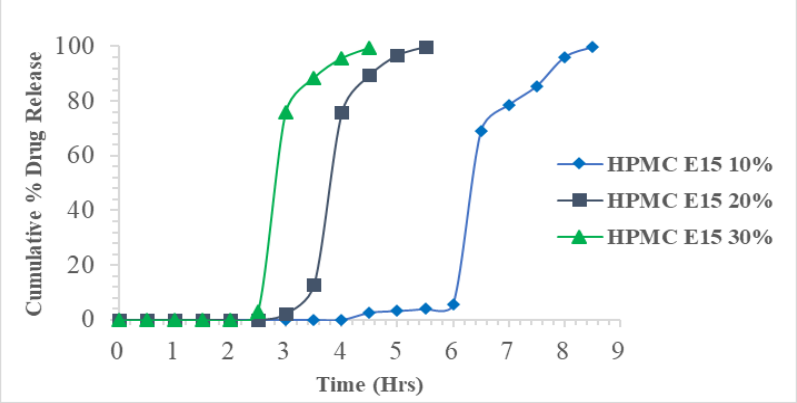


Fig.no 4: Dissolution profiles of zaltoprofen rupturable membrane coated tablets with different amount of pore former (15%, 25%, 35%) in polymeric membrane.

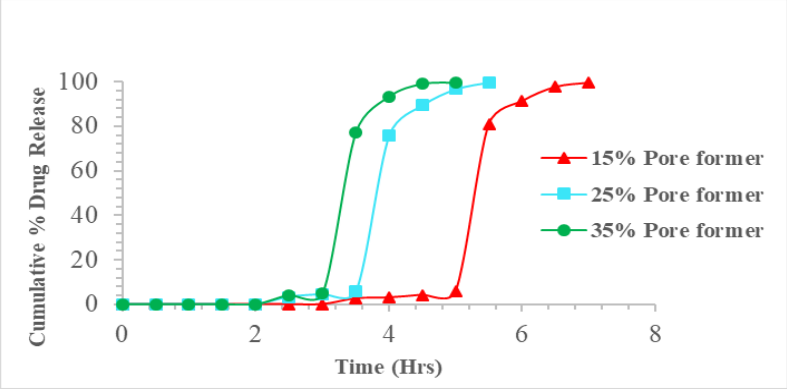


Fig.no 5: Dissolution profiles of zaltoprofen rupturable membrane coated tablets with different coating weights (5%,10%,15%).

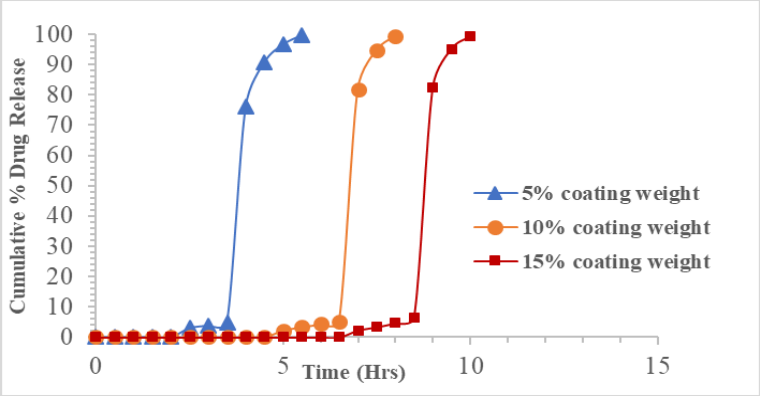


Fig.no 6:2-D Contour plots showing the effect of two (2) independent variables concentration on Dependent variables Lag Time (R1) and $T_{75\%}$ (R2) at a fixed third independent variable.

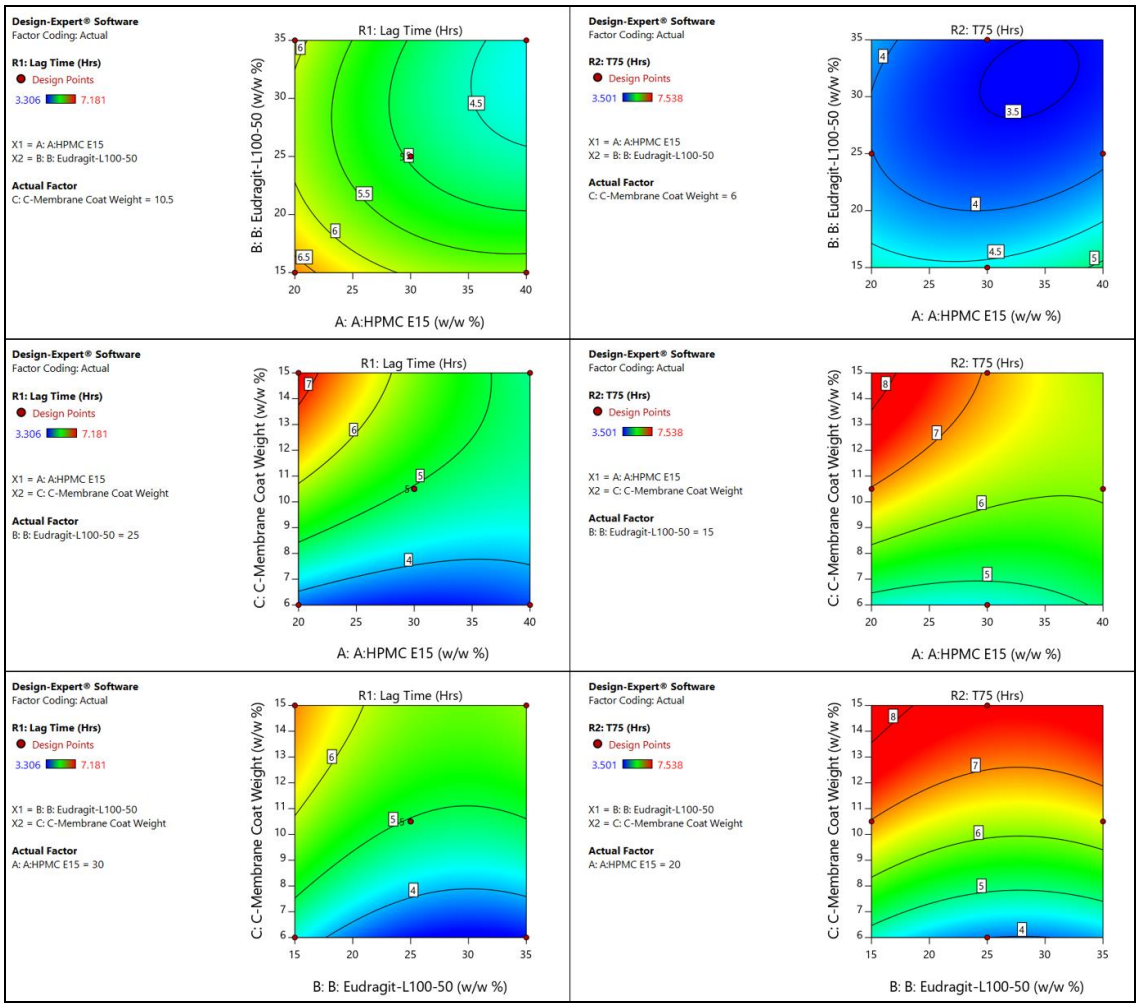


Fig.no 7: 3-D Response surface plots showing the effect of two (2) independent variables concentration on dependent variables Lag Time (R1) and $T_{75\%}$ (R2) at a fixed third independent variable.

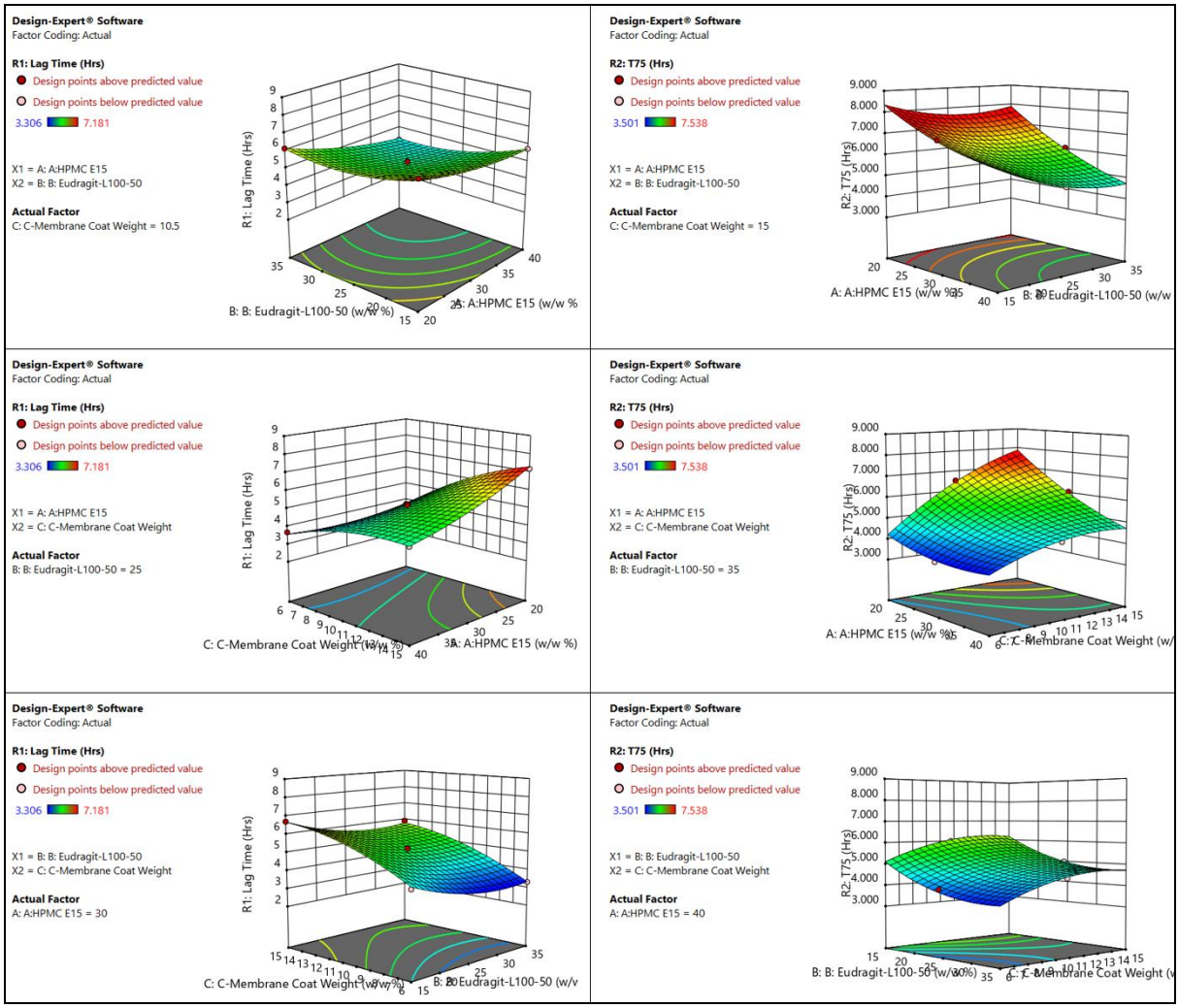
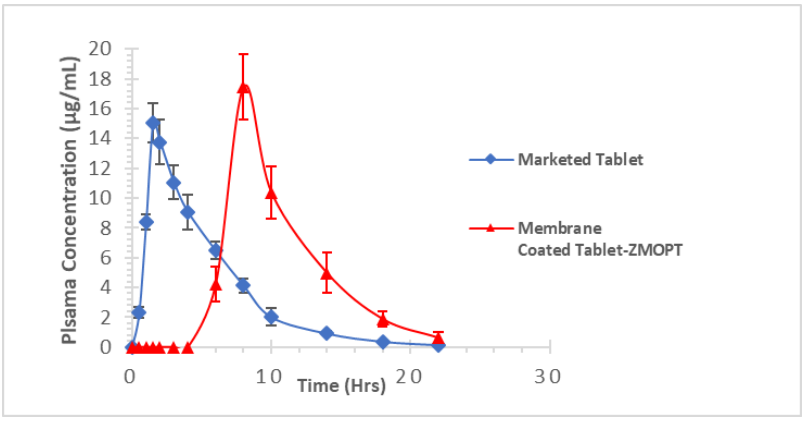


Fig.no 8: Comparison of mean plasma concentration profile of zaltoprofen marketed Immediate release tablet with Optimised tablet



The rabbits were fasted at least 6 hr before the experiment, with free access to water. Optimised RMCPT formulation and marked formulation containing drug equivalent to 8 mg of zaltoprofen was administered with 5 mL of distilled water to help them swallow. 1 mL of blood was collected from each rabbit at 0,0.5,1,1.5,2,3,4,6,8,10,14,18, 22hr after the oral administration of the drug. About 0.2 mL of heparinized 0.9% NaCl solution was added to each blood sample, centrifuged at 2500 rpm for 40min immediately, the plasma was collected and stored at -20^{o}c until HPLC analysis. The pharmacokinetic parameters (AUC_{0-22h}, $AUC_{0-\infty}$, K_E, Vd, $T_{1/2}$, C_{max}, T_{max}, CL) of optimised and marketed formulation were determined by non-compartmental analysis using PK Solver software. [33,34]

RESULTS AND DISCUSSION

Calibration curve:

The UV absorption data at the wavelength 243 nm in distilled water, HCl, phosphate buffer pH 6.8 and phosphate buffer pH 7.4 were plotted against concentrations (**Fig.1**). It was observed that zaltoprofen showed good linearity (R2 values in distilled water, HCl, PB pH 6.8, PB pH 7.4 were 0.9987, 0.9989, 0.9994, 0.9991 respectively) and obeyed Beer-Lambert's law in the conc. range of 5-30 µg/mL.

Solubility Studies:

The solubility of zaltoprofen in distilled water, 0.1N HCl, phosphate buffer pH 6.8 was found to be 0.015 mg/mL, 0.013mL, 0.851mg/mL respectively. The solid dispersion prepared by using PVP VA64 & Lutrol F127 shown remarkable improvement in drug's solubility by 18.83-fold,19.31-fold, 18.77-fold respectively. Hence the solubility problem of poorly soluble drug ZPF was overcomed by employing solid dispersion of drug.

Evaluation of Zaltoprofen fast dissolving core tablets:

The prepared fast dissolving core tablets were subjected *invitro* quality control tests like weight variation, hardness, friability, disintegration time, wetting time and drug content, results were tabulated (**Table no:6**). The core tablets shown rapid disintegration and all other values were in accepted limits.

Influence of swelling layer type on lag time of RMCPTs:

To examine the influence of different swelling material on membrane rupture and lag time, different batches of RMCPTs were prepared by using HPMC E15**,** HPMC E50, HPMC K4M, L-HPC, Na CMC and studied for tablet rapture property and lag time. The were results shown in **Fig no:2.** It revealed that hydrostatic pressure exerted on membrane to rapture was depends on swelling rate, which primarily depended on swellable material type, viscosity, quantity and its porosity. The lag time obtained with different swellable materials is in the following order., HPMC K4M > HPMC E50 > HPMC E15 > Na CMC > L-HPC. Based on lag time produced, HPMC E15 HPMC E50 are the right candidates to produce lag time from 4 hrs to 6.5 hrs.

Influence of the amount of swelling layer on lag time of RMCPTs

Extent of swelling is the critical parameter which influences the rupture of outer polymer coat. Different batches of RMCPTs were prepared by using 10%,20%,30% w/w of HPMC E15 and were studied for tablet rapture property and lag time. **Fig.no:3**. The results evident that the lag time before drug release decreased with increase in amount of swelling layer. As the amount of swelling agent increased, it exerted more pressure over the outer layer, resulting in rapid rupturing of the tablet. The expanded

swelling layer facilitated the entry of dissolution medium in to the core tablet containing disintegrating agent, which further synergize the effect of swelling agent. It shows that <10% w/w HPMC E15 layer might not be enough for the complete rupture of the outer layer, whereas >30% w/w HPMCE15 produce lesser lag time. Therefor the optimum quantity of swellable layer coating was between 10% -30% w/w.

Influence of the amount of Pore forming agent on lag time of RMCPTs:

The membrane permeability of coated tablets depends on formation of pores by solubilisation of pore former Eudragit L100-50 in polymer membrane. In pH >6 medium, Eudragit L100 dissolved and a large number of micro-pores were produced in the membrane, promotes the swelling of HPMC E15 layer which rapture outer membrane. Different batches of RMCPTs were prepared by using 15%, 25%, 35% w/w of Eudragit L100-50 in membrane coat and studied for tablet rapture property and lag time. According to **Fig.no:4**, The higher the Eudragit L100-50 concentration, the greater the number of micro-pores created, fasten the swelling which reduced the lag-time.

Influence of coating weight on lag time of RMCPTs:

To screen the influence of coating weight on lag time of RMCPTs different batches of tablets were prepared with 5%,10%,15% w/w membrane coating weights, examined for tablet rapture property and lag time. **Fig no: 5** shows that, as the coating weight increased, the lag time of the tablets was linearly increased.

Statistical analysis and Optimization:

As per design study, the values for dependent factors, lag time (R1) and $T_{75\%}$ (R2) were calculated from rapture test and dissolution studies shown in **Table:5**. The multiple regression analysis was performed by using Design Expert software (version 10.1), significant model which fits data was selected based on highest regression coefficient (R^2), insignificant Lack-of-Fit test, smallest PRESS value, lesser standard deviation between predicted and actual data points. Based model summary statistics (**Table no:7**), In comparison with all other models, quadratic model showing lesser PRESS value (1.67 for R1,1.48 for R2) (lesser the PRESS value, better the data points fit model)**,** highest adjusted R^2 for responses (Lag time $R^{2=}$ 0.9661, $T_{75\%}$ R^2 =0.9649). Based on lack-of-fit test results (**Table no:7**) quadratic model has lowest F-value (0.4754 for R1, 0.339 for R2) which is insignificant. Non-significant lack of fit is good for model to fit the data generated accurately. Based on PRESS value, Lack of fit results, S.D value and R^2value quadratic model selected as best model and subjected to ANOVA analysis for finding significant and insignificant model terms in polynomial equations for the responses (if p value<0.05, the model term was significant) (**Table no:8).** Generated polynomial equation for dependant variables as follows.,

R1: Lag Time = 4.96 - 0.6791 A - 0.486 B + 1.19 C - 0.2258 AB - 0.612 AC + 0.0382 BC + 0.2748A^2 + 0.494 B^2 - 0.4196 C^2

R2: $T_{75\%}$ = 5.26 - 0.6985 A - 0.4964 B + 1.19 C - 0.2493AB - 0.6358 AC + 0.0180 BC +0.3159 A^2 + 0.4521 B^2 - 0.4324 C^2

To study the effect of factors on responses 2-D contour plot and 3-D response surface plots were generated using the software Design expert 10.1. These plots will provide the information about effect of two factors on one response at fixed concentration of third factor.

From the 2-D contour plots (**Fig no:6**) it can be seen that curved or circular response lines which indicates each factor shows significant influence on responses (Lag Time, $T_{75\%}$). The factor C: Membrane coating weight shown highest of influence on responses when comparing other factors. The 3-D response surface plot (**Fig.no:7**) shows the effect of factors A, B and C on responses (Lag Time, $T_{75\%}$). It was found that responses are very sensitive to factor C-membrane coating weight. Based on the **Fig no:6&7**, the desired responses i.e., Lag Time (5.5 to 6.3hr), $T_{75\%}$ (5.8- 6.5hr) were obtained by using independent variable in the following range A- 20-25% w/w, B-12-18%w/w, C- 8-11% w/w.

Optimization of RMCPT:

BBD was utilised to predict the formulation with targeted lag time and T75%. Constraints were set to produce tablets with a target of 6hr lag time and 6.2 hr T75% (**Table no:9**) with highest desirability value. The suggested formula with 27.43% w/w, 15 w/w and 10.04 % w/w of A, Band C, a batch of tablets prepared, evaluated for responses. The predicted and observed responses were given in **Table no:10**. The optimised formulation showing similar responses with an acceptable relative error 0.0145 -0.0268.

Pharmacokinetic studies:

The plasma drug concentration time profile of the marketed and optimised formulation was shown in **Fig.8**. The pharmacokinetic parameters ($AUC_{0\text{-}22h}$, $AUC_{0\text{-}\infty}$, K_E, Vd, $T_{1/2}$, C_{max}, T_{max}, CL) of optimised and marketed formulation were calculated by non-compartmental analysis using PK Solver software. **Table no:11**.

The obtained C_{max}, T_{max}, $AUC_{0\text{-}\infty}$ & MRT values for the optimised tablet were 17.443±2.207 µg/mL, 8h,105.68±16.78 µg h/mL & 10.81±0.547 hr respectively, were compared with the same values of marketed tablet. (C_{max}, T_{max}, $AUC_{0\text{-}\infty}$ & MRT;15.02 µg/mL, 1.5 h and 81.12 ± 6.854 µg h/ mL & 5.2842±0.186hr respectively). The statistical analysis of the pharmacokinetic parameters shown a significant difference in the value of MRT, Cmax, T_{max} with lag time between optimised and marketed formulation.

CONCLUSION:

Chrono-pharmacology based diseases like Rheumatoid arthritis were effectively treated by using pulsatile drug delivery systems. The study has proven that Box-Behnken Design is easy and convenient for optimisation of zaltoprofen RMCPTs with an initial lag time of 6 hr. The initial lag time of drug release from the RMCPTs can also be modulated by varying concentrations of HPMC E15, Eudragit L-100-50 and Coating weight as per the needs.

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest.

REFERENCES:

1. Lemmer B. Chrono pharmacokinetic Implications for drug treatment. J Pharm Pharmacology.1999; 51:887.
2. Arvidson, Gudbjornson B. Circadian rhythm of secum interleucin-6 in rheumatiod arthritis. Ann Rheum Dis. 1994;53: 521–524.
3. Barnes JP. 1985.Circadian variation in airway function.Am J Med 9:5–9.
4. Fox KM, Mulcahy DA.Circadian rhythms in cardiovascular diseases. Postgrad Med J 1991;67: S33–S36.
5. Millar-Craig MW, Bishop CN, Raftery EB. Circadian variation of blood pressure. Lancet.1978;1:795.
6. Gupta NitanBharti. Pulsatile drug delivery as modified release dosage form. a review. Journal of Drug

Delivery & Therapeutics. 2012; 2(6):102-110
7. Chourasia, M.K. and Jain, S.K. J. Pharm. Pharm. Sciences. 2003, 6,33.
8. Bussemer, T. Dashevsky. Journal of Controlled Release 2003; 93:331–339.
9. Bussemer T, Bodmeier R. In Journal of Pharmaceutics.2003; 267:59–68.
10. Sangothjeen. Journal of Controlled Release. 2004; 95:147-159.
11. Tsurumi K, Kyuki K, Niwa M, Pharmacological investigations of the new anti-inflammatory agent 2-(10,11-dihydro-10-oxodibenzo thiep -in-2-yl) propionic acid. 1stcommu: inhibitory effect of rat paw edema. Arzneimittel forschung. 1986; 36: 1796 -800.
12. Tang HB, Inoue A, Oshita K. Zaltoprofen inhibits bradykinin-induced responses by blocking the activation of second messenger signalling cascades in rat dorsal root ganglion cells. Neuropharmacology. 2005; 48:1035-42.
13. Matsumoto M, Inoue M, Ueda H. NSAID zaltoprofen possesses novel anti-nociceptive mechanism through blockage of B2-typebradykinin rece--ptor in nerve endings. Neurosci Lett 2006; 397: 249-53.
14. Hirate K, Uchida A, Ogawa Y, Arai T, Yoda K. Zaltoprofen, a non steroidal anti-inflammatory drug, inhibits bradykinin-induced pain responses without blocking bradykinin rece- -ptors. Neurosci Res2006; 54: 288-94.
15. Shigeru F, Nobuyoshi A, Emiko K, Akio H, Yoshihiro K, Nobuhiko K, Atsuhiro I, Anne Matthews, Michael Hall, Tadashi K. Involvement of CYP2C9 and UGT2B7 in the metabolism of zaltoprofen, a non-steroidal anti-inflammatory drug, and its lack of clinically significant CYP inhibition potential. J Clin Pharmacol 2002; 54:295-303.
16. Japanese Pharmacopoeia XV. Society of Japanese Pharmac.15 Ed.2006.1242-4.
17. Kameyama T, Nabeshima T, Sato M. Analgesic and anti-inflammatory effects of 2-(10,11-dihydro-10-oxo-dibenzo [b,f]thiepin-2-yl)propionic acid in rat and mouse. Drug Res. 1987;37: 19-26.
18. Muratani T, Doi Y, Nishimura W, Nishizawa M, Minami T, Ito S. Primitive analgesia by zalto that inhibits bradykinin action and cyclooxygenase in a post-operative pain model. Neurosci Res.2005; 51:427-3.
19. Kiran B. Aher, Girija. Economical spectrophotometric method for estimation of zaltoprofen in pharma -ceutical formulations. Pharm Methods. 2011; 2(2): 152–156.
20. Sridhar Rao. K, Zaltoprofen fast disintegrating tablets: formulation and in-vitro evaluation studies. International Journal of Pharmacy and Bio Sciences.2016;6(2):122-129.
21. Higuchi, T. and Connors, K.A. Phase-solubility techniques. Adv. Anal. Chem. Instrum. 1965;4: 117-212.
22. F. Veiga a, J.J.C. Teixeira-Dias b. Inclusion complexation of tolbutamide with cyclodextrin and hydroxy propyl-beta-cyclodextrin: Int Journal of Pharmaceutics. 1996; 126: 63-71.
23. Shailendra Singh Solanki. Study on Mixed Solvency Concept in Formn Development of Aqueous Injection of Poorly Water-Soluble Drug. Journal of Pharmaceutics.2013;1:1-8.

24. M.Najmuddin. Enhancement of disso -lution rate of ketoconazole by solid dispersion technique. International journal of pharmacy and pharm. sciences.2010;2(3):132-136.
25. N. G. Raghavendra Rao. Develop. of fast dissolving nimodipine tablets: effect of functionality of super disintegrants. Journal of Pharmcy Research. 2011;4(9):2957-2962.
26. Rajesh S. Jadon, Swadesh Nayak. Taste masking of Lornoxicam by polymer carrier system and formulation of oral disintegrating tablets: International Journal of Drug Delivery. 2009:1:27-31.
27. Meijuan Zou, Yue Wang. Wax-Matrix tablet for time-dependent colon-specific delivery system of so phoraflave scensaiton: preparation and *in vivo* evaluation. Drug Dev. and Industrial Pharmacy. 2009; 35: 224–233.
28. BK Garg1. Formulation and Eval., of pulsatile drug delivery system of rosuvastatin calcium using different swelling polymers. The pharma innovation. 2012:1(7):61-67.
29. Om Prakash Ranjan et al.,Optimiza -tion of Chrono-modulated Delivery System Coated with a Blend of Ethyl Cellulose and Eudragit L100 by Central Composite Design: In Vitro and In Vivo Evaluation. PharmInnov.2014;9:95–105.
30. Om Prakash Ranjan. Osmotically controlled pulsatile release capsule of montelukast sodium for chrono therapy. Statistical optimization, in vitro and in vivo evaluation. Drug Deliv, 2014; 21(7): 509–518.
31. Nirav v. Patel, jayvadan k. Patel. Box-Behnken experimental design in the development of pectin-compritol ATO 888 compression coated colon targeted drug delivery of mesalamine. Acta Pharm.2010; 60:39–54.
32. G. E. P. Box and D. W. Behnken. Some new three level designs for the study of quantitative variables, Technometrics.2ed. 1960; 455–475.
33. Hari Krishna E, Rama Mohan Gupta V. Pharmacokinetic study of zaltoprofen spherical agglomerated dense compacts canvassed with commercial product. Clinic Pharma -col. Biopharm. 2013;2(1):1000107.
34. Hyun Kyoung Yanga Seo Young Kim. Application of column-switching HPLC method in evaluating pharmacokinetic parame -ters of zaltoprofen and its salt. Biomed. Chromatogr. 2009; 23: 537–542.