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STUDY ON ENHANCEMENT OF MEAN RESIDENCE TIME OF REPAGLINIDE FLOATING DRUG DELIVERY SYSTEM BY RABBIT MODEL

ISPITIVANJE UNAPREĐENJA SREDNJEG VREMENA ZADRŽAVANJA REPAGLINIDIN PLUTAJUĆEG SISTEMA OSLOBAĐANJA LEKOVA NA MODELU KUNIĆA

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poorly water soluble drug,
floating drug delivery system,
guinea pigs

Ključne reči
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u vodi slabo rastvorni lek,
plutajući sistem oslobađanja leka,
kunići

Apstrakt

The present work is aimed to study the pharmacokinetic parameters of optimized repaglinide floating drug delivery system (FDDS) by 2^4 factorial designs, followed by comparison with a commercially available formulation. The main effects and interactions of formulation variables were studied by using normal and Pareto charts. The formulation of releasing the drug was optimized by fickian diffusion mechanism. Pharmacokinetic parameters were evaluated in rabbits for floating drug delivery system. Mean while a simple, specific high performance liquid chromatographic method was developed and validated as per biopharmaceutical specifications. The linearity was reached at the range of 110-550 ng/ml (r^2= 0.999) whereas repaglinide retention was at 5.2 min by using methanol: phosphate buffer pH 2.5 (70:30 % v/v) as mobile phase at the flow rate of 1.0 ml/min. The validated method was used for pharmacokinetic profile of repaglinide in the existing products on the market and FDDS. The pharmacokinetic parameters tmax, elimination half-life, area under the curve, mean resistance time were significantly increased compared with a commercial products, except cmax and elimination rate which are stayed constant.

INTRODUCTION

The type II diabetes mellitus is characterized by insulin deficiency and insulin resistance; the former can manifest the post prandial hyperglycemia and latter was fasting hyperglycemia. Higher failures were reported from long term monotherapy with sulphonylureas and biguanidines [1], post prandial hyperglycemia [2] and microvascular complications can be reduced by non-sulfonylureas like repaglinide [3].

Repaglinide, a non-sulfonylurea oral anti-diabetic agent, chemically, it is S (+) 2-ethoxy-4-(2-((3-methyl-1-(2-(1-piperidinyl) phenyl) butyl) amino)-2-oxoethyl) benzoic acid, it is not yet official in any pharmacopoeia and suitable for first-line monotherapy in the management of type II diabetic patients, who were failed to respond adequately to diet alone. Addition of insulin secretogoggue can be helpful, and it is rapidly absorbed and shows a short duration of action. It is poorly soluble in water and belongs to biopharmaceutical classification system (BCS) class II, low solubility and high permeability; it has two pka values due to its zwitterionic behavior at 3.96 and 6.2 [4].

Hydrophilic systems[5] with $NaHCO_3$ was chosen to fabrication of effervescent floating drug delivery system (FDDS). These systems absorb water, causing to swell. The evolved CO_2 gas will be entrapped in hydrated gel causing them to float, promotes gastric retention and allows the drug to dissolve and followed by diffuse and erode the system. The amount of drugs to diffuse and erode depends upon the water penetrability and energy required to relax the polymeric chains. Polymeric chain length, chain cross linking influences the gel strength of the polymer, higher the viscosity of the gel system, retards the drug release. The aqueous

solubility of the polymers will decide the drug release mechanism. Both diffusion and erosion contribute in controlling the drug release from a hydrophilic matrix. Contribution of a drug release mechanism influenced by the combination of physicochemical, mechanical properties of gel layer, and biochemical factors surround the gastric environment. Estimation of repaglinide by methods like electrochemical [6], Spectrofluorimetric [7], UV-visible spectrophotometric [8-9], reverse phase-high pressure liquid chromatography [10, 11] (RP-HPLC) with ultraviolet (UV) detection in pharmaceutical formulations and human plasma [12] were reported.

The present objective is to determine the pharmacokinetic parameters for optimized formulation by factorial design and followed by in vivo study, rabbits as an animal model and comparative to a marketed formulation.

MATERIALS AND METHODS

Repaglinide (98.3% purity), gift sample was received from aurobindo Pharma ltd (Hyd, India). Methanol and water were Hplc grade from RFCL ltd (Mumbai, India), Ammonium dihydrogen orthophosphate, Orthophosphoric acid, ethyl acetate, isoamyl alcohol. Sodium hydroxide was from SD fine chemicals ltd (Mumbai, India). Bath sonicator was used for degassing (PCI analytics, Mumbai, India). LWL precision instruments used for sample weighting (Contech, Mumbai, India). Elico® pH meter was used for adjustment. Ten station rotatory tablet punching machine used for compression of tablets with 8mm flat shaped punch (Chamunda Pharma, Mumbai, India). Hydroxy propyl methyl cellulose K4M (HPMC K4M), hydroxyl ethyl cellulose (HEC), sodium bicarbonate ($NaHCO_3$), acetyl alcohol, magnesium stearate (Mg. stearate) were purchased from yarrow chemicals ltd (Mumbai, India). REMI R-2 research centrifuge and REMI CM-101 cyclomixer were used for extraction of drug from plasma (REMI® Electrotechnik ltd, Vasai, India).

2.1 Formulation of floating drug delivery system

Accurately weighted drug, HPMC K4M, HEC and $NaHCO_3$ were mixed by geometric mixing in a laboratory blender until the homogenization was attained. Followed by adding of liquefied acetyl alcohol at 60°C, ensure the proper mixing until the uniform damp mass was formed and screened on 22#, then the screened granules were dried at 45°C. Granules were tabletted by using 8mm flat shaped punch and die set in 10-station rotary punching machine at the compression forces of 5 ton. Before compression the dried granules were lubricated by adding of magnesium stearate. The formulation compositions were shown in table 1.

Table1. Composition of optimized formulation by 2^4 factorial design.

Duration of floating (min)	Lag time (min)	$t_{50\%}$	Total weight (mg)	Mg stearate (mg)	$NaHCO_3$ (mg)	Cetyl alcohol (mg)	HEC (mg)	HPMC K4M (mg)	Run order/ formulation
>12	0.2	4.40	143.07	8.07	25	30	30	50	Optimized

Table 2. Drug release kinetics for optimized formulation

Diffusion exponent	Drug release mechanism		Korsmeyer–peppa's		first order Higuchi		zero order	
Fickian diffusion	Diffusion exponent	r2	Slope	r2	Slope	r2	Slope	r2
	0.4603	0.9964	23.9613	0.9973	-0.0627	-0.9900	6.2304	0.9608

2.1.1 In vitro drug release study

Dissolution studies were conducted by using USP dissolution test apparatus II (paddle) and a 900 ml of 0.1N Hcl pH 1.2 as a dissolution medium was used, maintained at 37±0.5°C, rotations at the speed of 50 rpm, aliquots of 1 ml was withdrawn at 0.5,1,2,4,6,8,10,12 hours and replace of same volume of a fresh dissolution medium. Then the aliquots were diluted to 10 ml, further analyzed and relative concentrations were found by using best fitting of the least square regression analysis of peak area versus concentration.

2.1.2 Factorial design

The hydrophilic matrix system was designed by selecting of HPMC K4M, HEC, Cetyl alcohol, $NaHCO_3$; the effects of all formulation variables will influence the drug release. 2^4 factorial studies were designed to determine the interactions of four variables at two levels (low and high concentrations). MINITAB® 15, English version was used to study the factorial plots and investigates the one, two, three, four way interactions upon $t_{50\%}$ of drug release as a response factor. The interactions were interpreted by normal and Pareto charts for study of significance effect.

CHROMATOGRAPHIC SYSTEM

The shimadzu Hplc system was consisted of CBM-20A, prominence® series, two pumps of LC-10 AT vp with the SPD-10A vp detector, installed with Class-vp 6.13 software for data acquisition and quantification of peaks. BDS Hypersil® C_{18} (150×4.6mm: i.d) 5µm (thermoscientific, India) and symmetric® C_{18} (250×4.6mm) 5µm (waters, Mumbai, India) used for chromatographic separations and method validation. Solvent and sample filtrations were done by using Ultipor® $N®_{66}$ 0.2 µm and 0.45 µm membranes, respectively.

3.1 Chromatographic separation

The mobile phase was composed of methanol: Phosphate buffer pH 2.5 (70:30) prepared daily with freshly prepared deionizer water. The flow rate was set at 1.0 ml/min and ambient temperature maintained for column. Allow the mobile phase to equilibrate the column for 45min. Detector was pre-configured to a specific wavelength at 245nm. The run time was 8min.

3.2 Preparation of stock solutions

Stock solution (220µg/ml) of repaglinide was prepared by dissolving 22mg of drug in 100ml of methanol. The stock solutions were stored at 4°C for until analysis. The aliquot solutions were

110,220,330,440,550ng/ml prepared by serial dilution with mobile phase.

3.3 Extraction procedure

The repaglinide solutions were added to blank plasma samples of rabbits, 5ml of ethyl acetate and 50µl of iso amyl alcohol were added and adjusted the pH to 7.4 with 0.2M NaoH for absolute recovery followed by centrifuge at 2500rpm for 40min. The ethyl acetate phase was separated and evaporated under the nitrogen stream, reconstituted with 2ml of mobile phase and injected to Rp-Hplc system. The plasma samples were stored at -20°C, until for analysis.

3.4 Analytical method validation

The calibration curve was analyzed on consequent three days and slope, linearity, regression for intra-day; inter-day and accuracy, recovery, LOQ (limit of quantification) were found out.

STUDY DESIGN

The study was approved by the Institutional Animal Ethical Committee (IAEC) licensed no: 1035/ac/09/cpcsea. Twelve New Zealand white female rabbits, weighting 2.5-3.0 kg, were housed individually in standard cages on a 12 h light-dark cycles. The rabbits were fasted for 24 h before drug administration but allowed to free access of water. The animals were divided randomly into two groups (six animals each) and under randomized study design, first group of the animals were received each, one uncoated marketed tablet formulation (n=6) and second group received each, one optimized FDDS (best formulation in invitro studies, F16) corresponding to a dose of 2mg. The formulations were administrated by the oral lavage.

About 2 ml of blood sample was collected through the peripheral ear vein prior to drug administration (0 h) and 0.5,1,2,4,6,8,10,12,16,20 and 24hrs after oral administration. Samples were transferred immediately into heparin containing test tubes. After centrifugation at 5000 rpm for 30 minutes, plasma samples were harvested and stored at -20°C until analysis.

4.1 Pharmacokinetic Analysis

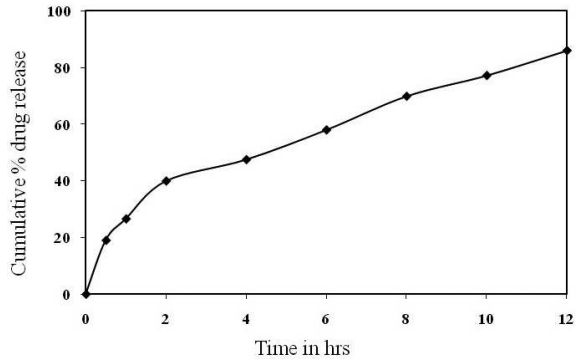
The area under the plasma drug concentration-time curve (AUC_{0-t}), area from zero to infinity ($AUC_{0-\infty}$), cmax, tmax were primary parameters and half-life, elimination rate constant were secondary parameters. The possible pharmacokinetic parameters were determined by using Pk1, Pk2 functions are Microsoft® Excel add-in programs. All results were expressed in the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

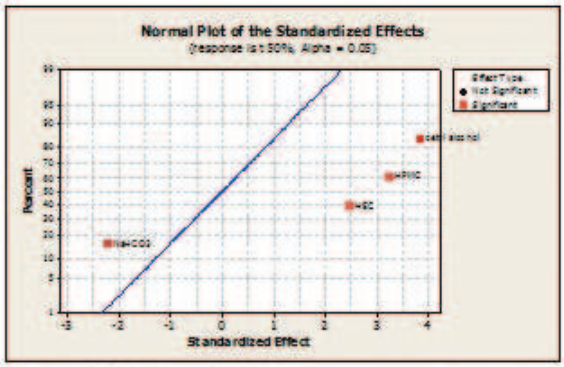
5.1 In vitro drug release study

HPMC K4M on expose to water becomes hydrate, swell and forms gel layer around the tablet. In FDDS, the CO_2 gas is released from the system and slowly trapped by the porous gel layer which helps to retain the dosage form extended to float. After the sufficient hydration of gel layer, the polymeric chains tend to disentangle and allow the drug to dissolution and further diffuse throughout the polymeric chains, poorly water soluble drugs which is possibly done by erosion of the matrices. The previous studies have shown that the higher the molecular weight the highest the medium uptake proportional to square to the time and is prone less to erosion. The lag time to float was depending on hydration, gelling of polymer and the amount of released and trapped CO_2 gas. The faster the retention of released CO_2 gas lowers the lag time.

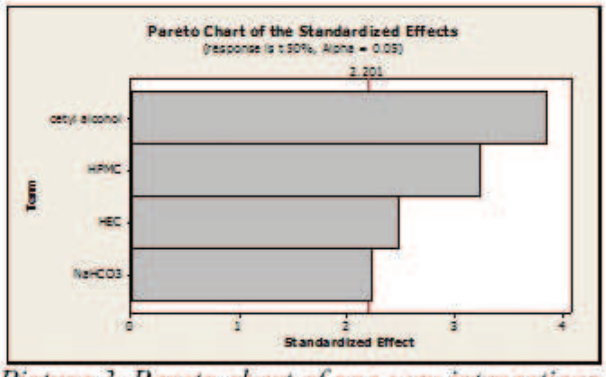
HEC is slowly soluble in water, but the degree of hydration of matrices was higher which relates to erosion. Drug release mechanism by HEC was mostly done by non-fickian transport. The higher the concentration of $NaHCO_3$, the dispersibility of dosage form was faster. The lower the $NaHCO_3$ concentration, the higher the lag time to float. The cumulative % drug release of optimized formulation was fitted to higuchi (cumulative % drug release vs. square root of time), korsmeyer-peppa's (log cumulative percentage drug release vs. log time). The diffusion exponent was 0.4603, can be seen in table 2. The in vitro drug release for optimized formulation was depicted in picture 1.



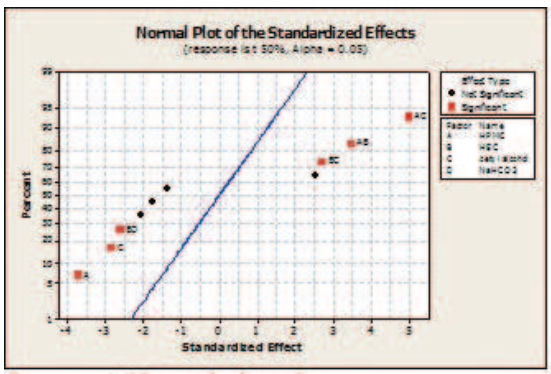
Picture 1. Invitro drug release for optimized formulation



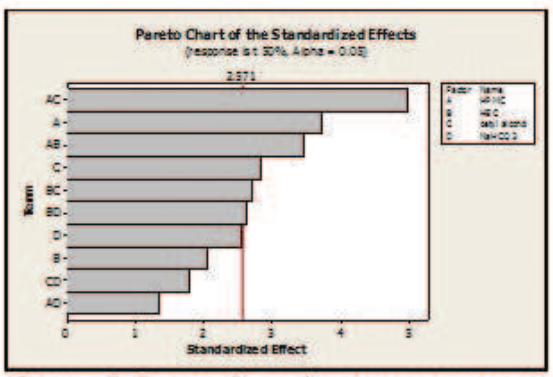
Picture 2. Normal plot of one- way interactions



Picture 3. Pareto chart of one-way interactions



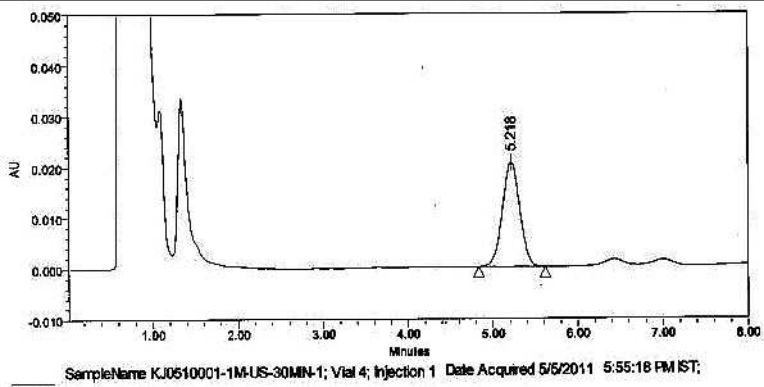
Picture 4. Normal plot of two-way interactions



Picture 5. Pareto chart of two-way interactions

Table 3. Significances of interactions

Interactions P- value ($\alpha = 0.05$)	P- value
0.025	One way
0.013	Two way
0.113	Three way
0.235	Four way



Picture 6. Standard Chromatogram was obtained from rabbit plasma.

Table 4. Intra-day assays (n=5)

Waters symmetric® column C18				BDS Hypersil® column C18				
Accuracy (%)	Precision (% RSD)	SD	Actual concentration (ng/ml)	Accuracy (%)	Precision (% RSD)	SD	Actual concentration (ng/ml)	Nominal concentration (ng/ml)
96.36	5.8	6.18	106	101.81	0.9	1.04	112	110
98.63	3	6.68	217	97.72	1.69	3.66	215	220
100.9	4.6	15.3	333	98.48	2.12	6.90	325	330
98.18	4.54	19	432	97.95	1.17	5.00	431	440
99.63	2.5	13.9	548	98.36	0.8	4.54	541	550

Table 5. Inter-day assays (n=2)

Nominal concentration (ng/ml)					Inter-day assays
550	440	330	220	110	
Actual concentration (ng/ml)					Day
538	431	325	215	112	1
534	428	315	210	101	
504	418	305	196	84	2
513	407	310	190	75	
492	385	296	198	72	3
485	397	293	189	76	
18.28	16.911	10.11	9.5	15.52	SD
3.57	4.11	3.29	4.75	17.91	Precision(% RSD)
92.9	93.4	93.03	90.45	78.18	Accuracy (%)

5.2 Study of interactions by factorial plots

The retardation of drug release was observed in the order of acetyl alcohol (D) >HPMC K4M (A) > HEC (B) > $NaHCO_3$ (C) in one-way interactions can be seen in the normal plot, Pareto chart was depicted in pictures 2 & 3. All variables were showed a significant effect individually in all formulations (95% confidence, α = 0.05). The acetyl alcohol was more retardants to release the drug whereas $NaHCO_3$ has a negative standardized effect, the more the $NaHCO_3$ use, the quick to burst release. By the time HPMC K4M polymer is on continuous hydration, polymeric chains will relax, and swell within the less time than HEC polymer.

The two-way interactions were in the order of AC>AB>BC>BD>CD>AD. The additive effects of two variables were in combination of polymers as AC, AB, BC which shows positive standardized significant effect and combination of BD (HEC and acetyl alcohol) illustrate the negative significant effect along with A & C in the drug release, shows in the picture 4 & 5. Pareto chart A & C represents the minimum standardized effect. The additive effect of three-way, four-way combination, was resulted in the insignificant effect. The fitted means were resembled the optimized formulation. All significances were shown in table 3.

5.3 Method development

BDS Hypersil® column was equilibrated and tested by methanol and water at various combinations and at the flow rate of 1ml/min, results in broad peaks with poor resolution, then switched to methanol with phosphate buffer pH 2.5 gives the sharp peak at 5.20min. Optimum separation was obtained by using 70% methanol: 30% phosphate buffer pH 2.5 and the injection volume was 20µl; chromatogram was shown in picture 6.

5.4 Recovery, linearity, accuracy, precision

Recovery studies were conducted for extracted samples by applying the slope derived from the calibration curve found to be 102.02%.The standard solutions were covering the linearity between 110-550ng/ml. each sample injected for five times (R2=0.999). Accuracy was determined by injecting the same concentrations for five times, by the same operator, same day, and same equipment and by the different column to check specificity of analytical method. The intra-day, inter-day results were shown in Table 4 & 5. The values obtained for the accuracy (%) and precision (%RSD) were in the range of 10-20%, acceptable for study of biopharmaceutical estimations [13]. The LOQ was 110ng/ml based on five-time replication of samples.

5.5 Pharmacokinetic analysis

The following pharmacokinetic parameters were Cmax, tmax, elimination rate constant, half-life, AUC0-t, $AUC_{0-\infty}$, $AUMC_{0-t}$, $AUMC_{0-\infty}$, and MRT (mean residence time) determined with the help of PK1 and PK2 functions. All data were shown in table 6. The elimination half-life was extended from 1.7 to 4.5 hours for marketed and optimized formulation respectively. The mean residence time of the dosage forms showed extension to 7.45 from 2.81 followed by sustained action in the body. The continuous drug release from the delivery system, followed by absorption to the systemic circulation by upper intestinal area is the absorption window for repaglinide.

The increased values were observed for tmax, half-life, AUC0-t, $AUC_{0-\infty}$, AUMC0-t, $AUMC_{0-\infty}$, and MRT in the optimized formulation comparative to a marketed formulation, except for cmax where elimination rate stayed constant.

SD	Optimized formulation	SD	Marketed product	Pharmacokinetic parameters
0.89	29.09	0.77	34.88	Cmax (ng/ml)
0.00	4.00	0.00	1.00	Tmax (h)
0.01	0.15	0.01	0.39	Elimination rate constant (h^{-1})
0.20	4.53	0.04	1.77	Half-life (h)
12.13	236.08	1.53	107.34	AUC0-t (ng*h/ml)
12.51	245.01	1.52	108.73	AUC0-∞ (ng*h/ml)
99.78	1761.11	5.48	302.35	AUMC0-t ($ng*h^2/ml$)
131.49	2034.03	6.46	322.63	AUMC0-∞ ($ng*h^2/ml$)
0.12	7.46	0.03	2.82	MRT (h)

Table 6. Comparision of pharmacokinetic parameters for marketed and optimized formulation.

SUMMARY

Good quality of effervescent floating drug delivery system was successfully optimized by using 2^4 factorial design and HPMC K4M, HEC, acetyl alcohol as retarding materials for controlling the drug release. The $NaHCO_3$ was used for maintaining the density of a delivery system to float. The reproducible drug release kinetic values were indicating that, this method of preparation is acceptable.

The optimized formulation showed that fickian diffusion of drug release is due to the high impact of HPMC K4M. By the rabbits study design, controlling the peak plasma concentrations in optimized levels may overcome the side effects associated with a conventional formulation of long term monotherapy. Further studies are needed to be evident.

Apstrakt

Prikazani rad imao je za cilj da odredi farmakokinetičke parametre optimizovanog repaglinidin plutajućeg sistema oslobađanja leka (PSOL) preko 2^4 faktorijalnog dizajna, kao i poređenje sa komercijalno dostupnim formulacijama leka. Glavni efekti i interakcije izmena u formulaciji su ispitivani uz korišćenje normalnih i Pareto grafikona. Optimizovana je formulacija oslobađanja leka Fickian-ovim difuzionim mehanizmom. Farmakokinetički parametri su izračunati nakon primene plutajućeg sistema oslobađanja leka na kunićima.

U cilju praćenja biofarmaceutskih parametara, razvijena je i validovana jednostavna i specifična metoda visokoefikasne tečne hromatografije. Linearnost je postignuta u opsegu koncentracija od 110 – 550 ng/mL (r^2= 0.999). Retenciono vreme za repaglinidin iznosilo je 5,2 min. uz korišćenje mobilne faze metanol:fosfatni pufer pH 2,5 (70:30 % v/v), čiji je protok bio 1 mL/min. Validovana metoda je primenjena za dobijanje farmakokinetičkog profila repaglinida u postojećim proizvodima na tržištu i PSOL-u. Farmakokinetički parametri tmax, poluvreme eliminacije, površina ispod krive, srednje vreme zadržavanja, bili su značajno veći u poređenju sa komercijalnim proizvodima, osim cmax i stepena eliminacije koji su bili isti.

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