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In vitro, in vivo and stability assessment of optimised nanoparticulate matrix tablet of nebivolol hydrochloride

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***Abstract*---**The objective of the present study was to define the effects on the antihypertension activity and pharmacokinetics of an optimised nanoparticulate matrix tablet containing nebivolol hydrochloride. The nanoparticulate matrix tablet was formulated using Nebivolol nanoparticles which are prepared by a solvent evaporation method using varying concentrations of drug and polymer Eudragit RL 100 and optimised by two factorial designs using a quadratic model and the nanoparticulate matrix tablet was prepared by using a varying concentration of drug nanoparticles and ethyl cellulose. The sustained release ability formulation was further demonstrated in an in-vivo study in Wister rats. The prepared matrix tablets showed an improved bioavailability. The T_{max}, Cmax, and the AUC were increased and extended, respectively. The antihypertensive activity showed a distinct change in SBP in DOCA salt-induced hypertensive rats as compared with pure drug. A significant reduction of DBP and HR were also observed after the treatment with nanoparticulate matrix tablet. There was no significant degradation and change in drug release rate in formulations during six months of stability testing. The results suggest that nanoparticulate matrix tablets can act as a promising carrier for nebivolol hydrochloride which offers an alternative approach for regular delivery of nebivolol.

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***Keywords*---**pharmacokinetics, antihypertensive activity, stability studies, matrix tablet.

Introduction

Raised blood pressure is among the most important risk factors for cardiovascular diseases.[1] Beta-blockers are used ranging from lowering blood pressure to improving heart failure and can significantly reduce the incidence of myocardial infarction and strokes. Betablockers are an ideal candidate for incorporation into modified release dosage forms such that once a daily dosing regimen can be used to optimize therapy. [2]

Nebivolol hydrochloride chemically α, α'-(iminobis[methylene]) bis (6- fluoro- 3,4-dihydro- 2H-1-benzopyran-2-methanol). It is a novel third-generation β-blocker that has demonstrated a higher degree The β-adrenergic receptor blockers play an important role in the management of cardiovascular disease, including hypertension and chronic heart failure. Nebivolol has both a greater degree of selectivity for β_1-adrenergic receptors than other agents in this class and an ability to stimulate endothelial nitric oxide production, leading to vasodilation and other potential clinical effects. [3]

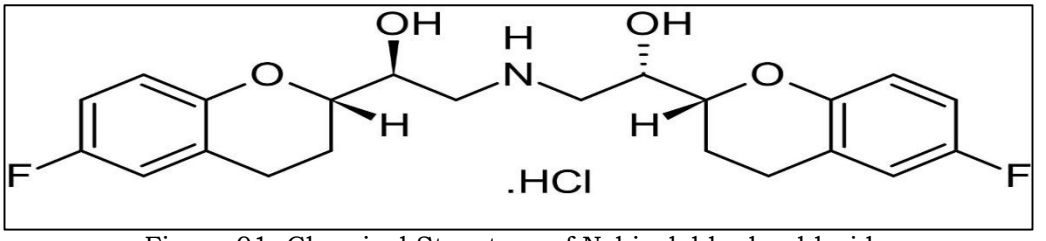

Figure 01: Chemical Structure of Nebivolol hydrochloride

After oral administration, nebivolol has peak plasma concentration within 0.5–2 h and Oral bioavailability of Nebivolol is only 12 % in substantial metabolizers (maximum of the population) as it undergoes considerable first-pass hepatic metabolism due to cytochrome P450 2D6 (CYP2D6) enzymes. The half-life of the drug also varies substantially from 10.3 to 31.9 hrs. in presence of vast and fewer metabolizers respectively. As Nebivolol has low aqueous solubility and high membrane permeability, it is categorized as a Class II drug according to BCS System.[4]

Due to poor water solubility, low bioavailability, substantial first-pass effect and variable elimination half-life of nebivolol hydrochloride, There is a need to use a sustained-release drug delivery system like matrix tablets to deliver a drug at a constant rate which can improve the pharmacokinetic and pharmacodynamic properties of the drug, The main purpose in the treatment of cardiovascular diseases is to provide the drug in higher concentrations in early morning hrs when greatest need, and in fewer concentrations in the late evening when the need is less, and it can be achieved by administration of the nebivolol hydrochloride in the nanoparticulate matrix system which releases a loaded dose

immediately after its administration and maintains drug plasma concentration and maintains it for a prolonged time.[5-7]

Material and Methods

Nebivolol Hydrochloride was obtained from the college drug library. Eudragit RL 100, Ethyl Cellulose, Microcrystalline Cellulose, Magnesium Stearate, Calcium Carbonate and other chemicals were purchased from Thermosil fine chem Industries, Khed, Pune India. All other chemicals used were of analytical grade.

Analytical Method Development [8-11]

Construction Calibration Curve of Nebivolol

Stock solution (100 mg/mL) of Nebivolol HCl was prepared in buffer solutions (phosphate buffer solution pH 6.8 and 0.1 N HCl) which was then assayed in the range of 200–400 nm using a UV double-beam spectrophotometer (UV-1700, Shimadzu, India) for the determination of λmax. Then Standard dilutions of nebivolol were prepared in the range of 10 to 100 µg/ml by using phosphate buffer pH 6.8 and absorbance was determined at λmax 281nm against phosphate buffer pH6.8 as blank, similarly, standard dilutions of nebivolol were prepared in the range of 10 to 60 µg/ml in 0.1N HCl and Absorbance was noted at 282 nm against 0.1 N HCl as a blank solution.

Method of Preparation of Nebivolol Nanoparticles [12-14]

Solvent Evaporation Method

The solvent evaporation method is used for the preparation of all batches of nanoparticles. Firstly, the required quantity of drug and polymer was dissolved in 10 ml ethanol and then 50 mg of sodium dodecyl sulphate was dissolved in 10 ml of water, this mixture was well dissolved. Then, the sodium dodecyl sulphate solution was mixed with the drug, polymer mixture by syringe. By using a vortex mixture, the mixture was homogenized for 5 min and then sonicated for size reduction. By using a flash evaporator solvent get evaporated and nanoparticles were collected.

Optimisation of Nanoparticles[15-17]

A randomized response surface design was used for the optimization of nebivolol loaded eudragit NPs, Mathematical modelling and evaluation of the ability to fit the selected model were performed with statistical analysis through Design-Expert® Software 11.1 (Stat-Ease, Inc). By using a central composite design using a two-level fractional factorial design using a quadratic model. In these 2 factorial studies and 1 factor was evaluated at randomised 2 levels (5 centre points and 8non-centre points). The selected variables and their levels are shown in Table 1.

Table 1: Randomised Response Surface design parameters indicating the levels of variables

Variable	Levels	
	-1	+1
Eudragit RL 100 (mg)	10	80
SDS (mg)	30	50

The Eudragit RL 100(mg)and SDS (mg)were selected as independent variables. Entrapment efficiency was a dependent variable. The batches thus prepared by factorial design are evaluated and the effect of the individual variable was studied according to the central composite randomized design. Analysis of Variance (ANOVA) was performed, and P-value with a 95% confidence interval was evaluated to determine the significance of each coefficient term. To determine the fitting extent of experimental data, regression coefficient R2 along with predicted and adjusted determined.

Formulation of Nanoparticulate Matrix Tablet [18-20]

Nebivolol Nanoparticulate matrix tablets were prepared by direct compression method using a 10-station tablet compression machine (Mini Press I, superman, India) using a 10 mm punch. The corresponding amount of drug and excipients were accurately weighed and mixed properly and the matrix tablets were compressed.

Table 2: Formulation of nanoparticulate matrix Tablet

Ingredients in mg	F1	F2	F3	F4	F5	F6
Nebivolol HCl Loaded Nanoparticle	100	100	100	100	100	100
Microcrystalline Cellulose	16	14	12	-	-	-
Ethyl Cellulose	-	-	-	16	14	12
Magnesium Stearate	2	3	4	2	3	4
Talc	2	3	4	2	3	4
Total	120	120	120	120	120	120

Comparison of Optimised formulation with marketed tablet and pure drug

Weight variation, hardness and friability:

Uniformity of weight

The weight of Optimised nanoparticulate matrix tablets and marketed Nebivolol HCl tablets were determined with the help of an electronic balance and the observed results have been included in table 3. (Mean values ± SD, n=20). From the results, it was obvious that the weight variation limit values of tablets were less than 7.5 which is within the limit.

Hardness and friability of tablets

Oral tablets normally have a hardness of 4 to 8 kg. As the hardness of the tablets was increased gradually there was a notable decrease in the percent friability in all formulations. the observed results have been included in table 3.

Uniformity of content

Drug content was determined according to the method given in IP. The % drug content was found to be between 97 to 99%. The result shows that the nebivolol present in both optimised formulation and marketed tablets are within a specific limit. (Table 4)

Comparative In vitro drug release study

Comparative in vitro drug release studies were performed on the pure drug, marketed product and optimised formulation(F5). The dissolution studies were performed using the USP-II (paddle) dissolution apparatus at 50 rpm. Dissolution media was phosphate buffer pH 6.8 and temperature was maintained at 37±0.5^{o}C. A 5ml was withdrawn at specific time intervals and the same volume of fresh medium was replaced. The withdrawn samples were diluted with pH 6.8, filtered and analysed on a UV spectrophotometer at 282 nm using pH 6.8 as a blank. Percentage cumulative drug release was calculated.

In vivo study [21-26]

A bioavailability study was conducted to determine the plasma drug concentration profile of the optimised nanoparticulate matrix tablet of Nebivolol HCl in Wistar rats. All the experiments and protocols were approved by the Animal Ethical and Welfare Committee of the S.N. Institute of Pharmacy and with the approval number (SNIP/IAEC/2022/14). The ethical guidelines of the Institutional Animal Ethics Committee were strictly followed while performing experiments.

Rat dose calculation

Rat dose was calculated based on Human dose and Km values. The correction factor Km was estimated by dividing the average body weight of species by its body surface area. The Km values are constant for each species.

Rat dose (mg/kg) = Human dose (mg/kg) X (Human Km / Rabbit Km)

= (10/60) X (37/6) = 1.02≈1 mg/kg

The optimised nanoparticulate matrix tablets of nebivolol HCl were crushed into powder by mortar and pestle and then the powder was suspended in 0.1% Carboxy methyl cellulose solution. The drug was then administered to rats orally (1 mg/kg of nebivolol) using intragastric gavage at the dose levels of 10 mg/kg body weight. The suspensions of nanoparticulate matrix tablets of nebivolol HCl were freshly prepared every day for 28 d. The control animals were administered vehicle only.

Analytical method development for estimation of Nebivolol in rat plasma by HPLC

The HPLC method reported by Punna Rao et al. (Punna Rao et al., 2015) was adapted and used for the estimation of nebivolol in plasma samples obtained in the in vivo study. In this study, plasma samples were processed by protein precipitation method and protein-free plasma samples were directly injected into the HPLC column. A standard calibration graph was plotted with different concentrations of nebivolol HCl in plasma samples by the High-pressure Liquid Chromatography (HPLC) method run at 280 nm.

Chromatographic conditions

An HPLC system Shimadzu, Japan with SIL-HTC autosampler and for this study Mobile Phase of acetonitrile & potassium dihydrogen orthophosphate buffer (pH 3.5 ± 0.1) in the ratio of 37:63 v/v is used and separation was carried out on Phenomenex C18 (150×4.6 mm, 5µ) Column with flow rate 1 mL/min and detected by SPD UV-Visible absorbance detector at 280nm wavelength.

Method of Nebivolol HCl extraction from rat plasma samples

Blank plasma sample, drug suspension and optimized test formulation F5 in rat plasma samples were extracted using precipitate proteins. 100 µL of Plasma sample was taken and 300 µl of acetonitrile was added by vertexing and sonicated for 5 minutes then it is Centrifuged for 5 minutes at 3000 rpm and then clear supernatant was collected in a labelled vial and a volume of 150 µL was injected into HPLC system. The flow rate was maintained at 1.0 ml/min and the measurements were made at 280 nm. The column was maintained in ambient conditions using a thermostat. The amount of nebivolol in the sample was determined from the peak area ratio correlated with the standard curve prepared under the same condition.

Pharmacokinetic analysis:

Healthy Wistar rats (weighing 180–220 gm), were used for the study. The animals were randomly divided into two groups each containing six rats
Group 1: Received pure nebivolol HCl suspension
Group 2: Received optimised nanoparticulate matrix tablet
Before the dosing with the test drug, the rats fasted for 12 hours. During fasting, the drinking water has abstained. The nebivolol suspension and optimised formulation were administered in Both groups of rats that were administered a 10 mg/kg dose. Blood samples (~0.5 ml) were taken from the postorbital venous plexuses at 1, 2, 3, 4, 5,6, 12, 24, and, 72 h following oral administration. Blood samples will be collected into heparin tubes. Plasma samples were obtained immediately by centrifuging blood samples at 7000 rpm for 10 min, after which plasma samples were transferred to new individual microcentrifuge tubes and stored at 20 ℃ until further analysis by HPLC. The parameters needed were determined from plasma concentration data by a non-compartmental model. The parameters such as The maximum plasma concentration (C_{max}), the area under the plasma concentration-time curve (AUC) and the time taken to reach the

maximum plasma concentration (T_{max}) were calculated directly from the plasma concentration-time curve.

Antihypertensive Study

Wistar rats weighing between 150 and 200 g were housed in groups of five under standard laboratory conditions of temperature 25°C±1°C with free access to food (The standard rat chow was available ad libitum to the rats) and water. The experiments were performed during the light portion (9–14 h). The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India, and approved by the Institutional Animal Ethical Committee.

Induction of DOCA- Salt induced hypertension

Induction of DOCA- Salt induced hypertension involves anaesthesia by ketamine (75 mg/kg; i.p) and xylazine (7.5 mg/kg; i.p) and uninephrectomy via the left flank incision. A week after unilateral nephrectomy, DOCA (25mg/kg, once a week; s.c; for 4 weeks) dispersed in cottonseed oil was injected into uninephrectomised rats. 1% saline and 0.2% KCl ad libitum were given throughout the experiment instead of drinking water.

Experimental protocol for optimized formulation

Group I: Normal, Received vehicle 1 ml/day
Group II: Disease Control, Unilateral nephrectomized animals receive DOCA injection (25mg/kg/week, s.c.) for 4weeks, dissolved in sterilized cottonseed oil subcutaneously and 1% saline and 0.2% KCl ad libitumas drinking water.
Group III: Unilateral nephrectomized animals receive DOCA injection (25 mg/kg/week, s.c.), orally native nebivolol (10 mg/kg) for 4 weeks and 1% saline and 0.2% KCladlibitumas drinking water.
Group IV: Unilateral nephrectomized animals receive DOCA injection (25 mg/kg/week, s.c.), orally nanoparticulate matrix tablet of nebivolol (10 mg/kg) for 4 weeks and 1% saline and 0.2% KCl ad libitumas drinking water.

Measurement of blood pressure

Measurement of blood pressure by non-invasive (indirect) method for formulation

The rats were trained for at least one week until the BP is steadily recorded with minimal stress and restraint. The first cardiovascular parameters were discarded and the mean of five or six subsequent measurements was recorded. Systolic blood pressure is measured weekly for four weeks by an indirect non-invasive tail-cuff method using Power Lab.

Measurement of blood pressure by the invasive (direct) method

After completion of the treatment schedule rats from each group were anaesthetized with urethane (120 mg/100gm). The femoral vein is cannulated

with a fine polyethylene catheter for administration of the drug. Tracheostomy is performed and blood pressure is recorded from the left common carotid artery using a pressure transducer by direct method on Chart data system. Heparinized saline (100 IU/ml) is filled in the transducer and in the fine polyethylene catheter cannulated to the carotid artery to prevent clotting. After 30 min of stabilization, heart rate and basal blood pressure was recorded.

Stability Studies [27-29]

Optimised Nanoparticulate matrix tablets of Nebivolol hydrochloride were subjected to stability studies as per ICH (Q1A (R2), 2003) guidelines, an Intermediate Stability study was carried out in a climatic chamber (Thermo lab, Mumbai, India) at30±2°C/65±5% relative humidity for 06 months and an Accelerated stability study was carried out at 40 ± 2°C/75 ± 5% relative humidity for 6 months. In this stability study, Nanoparticulate matrix tablets were evaluated for weight variation, thickness, hardness, friability, content uniformity and tablet dissolution rate.

Result and Discussion

Analytical Method Development

From the data obtained in the UV spectrophotometer, The maximum (λmax) of Nebivolol was found at 281 nm in phosphate buffer pH 6.8 and 282 nm in 0.1N HCl.

Calibration curve of Nebivolol in Phosphate Buffer

The calibration curve of Nebivolol in phosphate buffer pH 6.8 showed a linear relationship over the range of 10-100 µg/ml. Beers law is obeyed over the concentration range of 10-100 µg/ml, with a correlation coefficient of 0.9965. (Table 3 and Figure 1)

Table 3: Calibration Curve of Nebivolol in Phosphate Buffer pH 6.8

S.NO	Concentration (µg/ml)	Absorbance at 281 nm
1	0	0
2	10	0.102
3	20	0.149
4	30	0.241
5	40	0.319
6	50	0.392
7	60	0.462
8	70	0.532
9	80	0.601
10	90	0.672
11	100	0.712

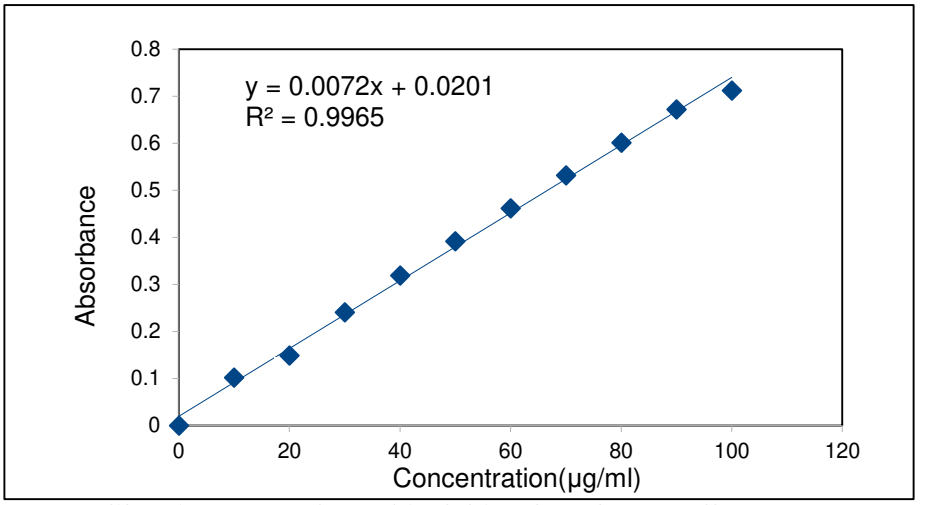

Figure 1: Calibration Curve for Nebivolol in Phosphate Buffer pH 6.8

Calibration curve of Nebivolol in 0.1N HCl

The calibration Curve of Nebivolol in 0.1 N HCl is linear over the concentration of 10-60 µg/ml (Table 4) with a mean correlation coefficient found to be 0.9977. (Table 4 and Figure 2)

Table 4: Calibration curve of Nebivolol in 0.1N HCl

Sr. No.	Concentration(µg/mL)	Absorbance(282nm)
1	0	0
2	10	0.116
3	20	0.225
4	30	0.342
5	40	0.444
6	50	0.557
7	60	0.658

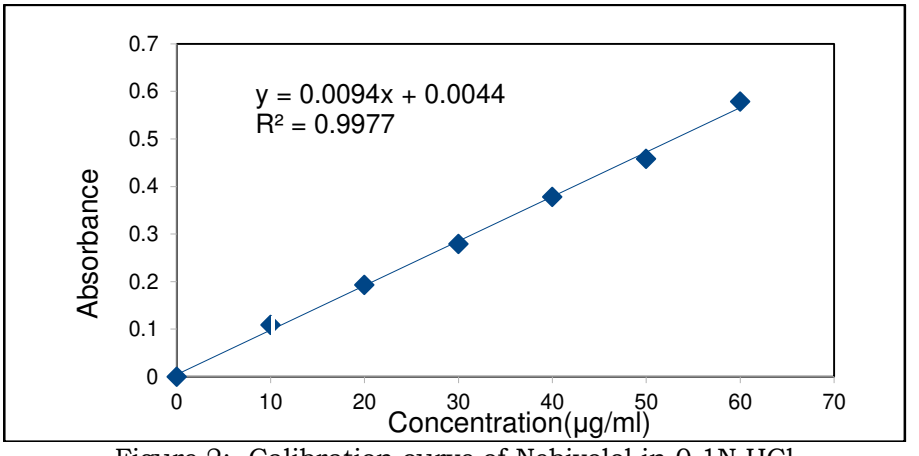

Figure 2: Calibration curve of Nebivolol in 0.1N HCl

Optimisation of Nebivolol HCl Nanoparticles:

The response surface quadratic models were generated using Expert Design Software. These were subjected to multiple regressions in quadratic order to yield polynomial equations. Experimental trials were performed in the least 13 possible combinations prepared consistent with the model and therefore the results got in Table 5.

Table 5: Randomised Response Surface design parameters indicating the levels of variables

Run	Eudragit RL 100 (mg)	SDS (mg)	Entrapment Efficiency (%)
1	-4.49747	40	34.18
2	94.4975	40	91.15
3	80	30	89.08
4	80	50	95.04
5	10	50	60.12
6	45	40	78.12
7	10	30	52.23
8	45	25.8579	69.017
9	45	40	76.32
10	45	40	77.14
11	45	54.1421	82.11
12	45	40	75.11
13	45	40	77.24

ANOVA results for the optimization of entrapment efficiency were summarized in Table 3. The contour plot and the 3D surface response graph for particle size were given in Figure 3. Statistical data of the dependent variables obtained were subjected to ANOVA and were found to be significant at ($p<0.001$) indicating a good fit.

Table 6: ANOVA responses for the factorial design for entrapment efficiency of NPs

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3281.35	5	656.27	67.96	< 0.0001	significant
A-Eudragit RL 100	2900.85	1	2900.85	300.39	< 0.0001	
B-SDS	130.95	1	130.95	13.56	0.0078	
AB	0.9312	1	0.9312	0.0964	0.7652	
A^2	234.80	1	234.80	24.31	0.0017	
B^2	2.85	1	2.85	0.2947	0.6041	
Residual	67.60	7	9.66			
Lack of Fit	62.46	3	20.82	16.21	0.0105	significant
Pure Error	5.14	4	1.28			
Cor Total	3348.94	12				

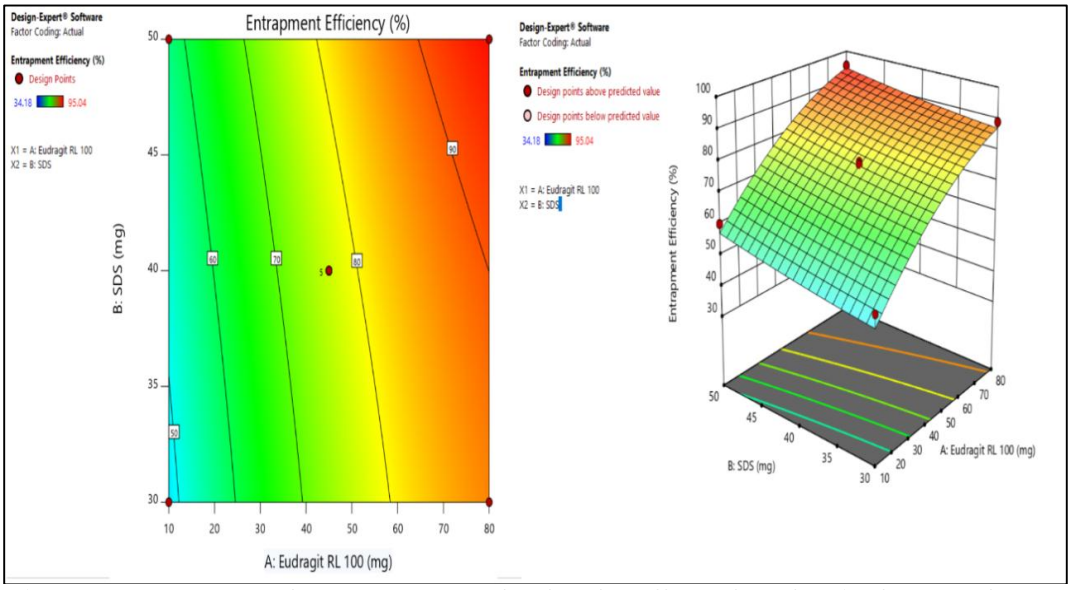

Figure 3: Contour and Response 3D plot for the effect of Eudragit rl100 and SDS on entrapment efficiency of the NPs

Table 7: Coefficient of estimate values for the Entrapment efficiency of NPs

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	76.79	1	1.39	73.50	80.07	
A-Eudragit RL 100	19.04	1	1.10	16.44	21.64	1.0000
B-SDS	4.05	1	1.10	1.45	6.64	1.0000
AB	-0.4825	1	1.55	-4.16	3.19	1.0000
A^2	-5.81	1	1.18	-8.60	-3.02	1.02
B^2	0.6396	1	1.18	-2.15	3.43	1.02

The main effects A and B represent the average result of changing variables at a time from their low level to a high level. The interaction terms (AB, A2and B2) show how the responses change when 2 variables are simultaneously changed. All the coefficients of estimates (Table 7) are negative both for main effects and for interactive effects. This indicates all the independent variables have a favourable effect on entrapment efficiency.

Table 8: Regression analysis of the full factorial design of the NPs

Response	Entrapment efficiency	Response	Entrapment efficiency
Std. Dev.	3.11	R^2	0.9798
Mean	73.60	Adjusted R^2	0.9654
C.V. %	4.22	Predicted R^2	0.8650
		Adeq Precision	26.5181

Regression analysis of variance for both particle size and drug loading was given in Table 8. The correlation coefficient for the models was also calculated which is found to be > 0.9 indicating a good fit. Predicted R2 was calculated as a measure of how good the model predicts a response value. The adjusted R2 and predicted R2 should be within approximately 0.20 of each other to be in reasonable agreement. Present model fit values indicate a difference of less than 0.2, indicating an agreement with the adjusted R2 value in all responses.

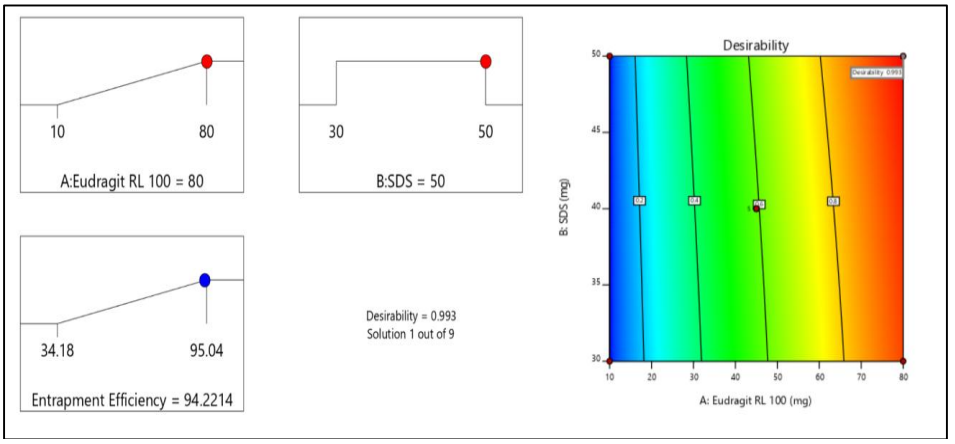

Figure 4: Optimized levels of independent variables for the maximum drug Entrapment efficiency shown in ramp plot and contour plot

By using this factorial design (Figure 4), it was found that the maximum concentration of Eudragit RL 100 at 80mg and sodium dodecyl sulphate at 50 mg with entrapment efficiency of 95.04 % with the desirability of 0.993. This combination is used for the formulation of nanoparticles and these NPs are used to formulate Nanoparticulate Matrix Tablet.

Comparison of Optimised matrix tablet (F5) with marketed tablet

Weight variation, hardness and friability

From the results, it was found that the weight variation limit values of tablets were less than 7.5 % which is within the limit. hardness and friability were also found within specification limits for both optimised formulation and marketed tablets.

Table 9: Weight variation, hardness and friability

Formulation	Weight variation (mg)	Hardness (kg/cm^2)	Friability (%)
Optimized Formulation (F5)	120.4 ±1.31	4.7 ± 0.03	0.39 ± 0.02
Marketed Tablet	198 ± 4.0	4.15 ± 0.36	0.50 ± 0.01

Uniformity of content

The % drug content was found to be between 97 to 99%. The result shows that the nebivolol present in both optimised formulation and marketed tablets are within a specific limit. (Table 10)

Table 10: Percentage of drug content, Disintegration time, and drug release at 90 mins

Formulation	% Drug content	Disintegration time (min)	(%) Drug release at 90 min
Optimized Formulation (F5)	99.5 ± 0.03	NA	45 ± 2.4
Marketed Tablet	97.6±1.4	6.16± 0.40	76 ± 1.8

Comparative In-vitro drug release studies

The initial burst release was 39 % and a slow-release was observed for optimized nanoparticulate matrix tablets for up to 24 hrs. due to the formation of a matrix around the drug by the polymer. It is expected that the initial burst release from the tablet is due to dissolution and diffusion and after 12 hrs. the constant drug release was identified and this could be a result of polymer erosion. So, it suggests that the combination of dissolution, diffusion and erosion is the possible mechanism for drug release from the nanoparticulate matrix tablet. The drug release from the marketed formulation was found to be 96 % in 08 hr. Pure drug nebivolol shows the release of about 39 % in the first hour. The results found are given in table 11 and Figure 5.

Table 11: Comparative Invitro drug release data

Sr. No.	Time (Hr.)	Optimized matrix tablet (F5)	Pure Drug	Marketed Formulation
01	0.30	15.25± 1.14	34.21 ±1.42	38.23 ± 1.32
02	0.45	32.27 ± 1.36	37.45±1.52	45.38 ± 0.78
03	1	39.23 ± 2.26	43.63 ± 0.92	52.91 ± 0.93
04	2	45.46 ± 1.90	46.27 ± 1.07	76.44 ± 0.72
05	4	47.68 ± 1.68	51.85 ± 1.12	84.26 ± 1.08
06	6	55.87 ± 2.77	57.14 ± 0.79	91.48 ± 1.13
07	8	61.56 ± 1.20	64.77 ± 0.81	93.18 ± 0.91
08	10	65.38 ± 1.63	69.75 ± 0.58	96.21 ± 1.47
09	12	73.85 ± 2.06	76.12 ± 1.41	
10	15	82.64 ±2.12	84.72 ± 0.81	
11	18	91.82 ± 1.14	92.65 ± 0.58	
12	21	94.26± 1.48		
13	24	96.58 ± 2.92		
S.D.(n=3)				

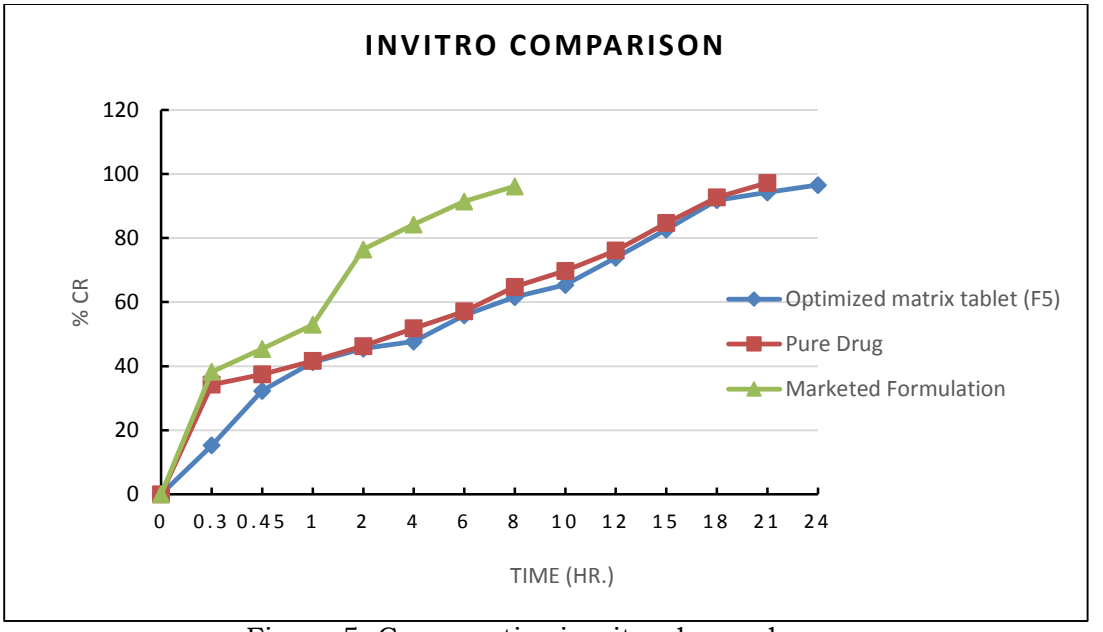

Figure 5: Comparative in-vitro drug release

In Vivo study

Analytical method for estimation of Nebivolol in rat plasma

The HPLC method reported by Punna Rao et al. was adapted and used for the estimation of nebivolol in plasma samples obtained in the in vivo study. Nebivolol HCl showed linearity between 100-500 ng/mL concentration and the calibration curve showed a good coefficient of determination of 0.989 (Figure 6). Nebivolol retention time was observed to be about 7.97 minutes whereas that of sildenafil citrate (IS) was about 4.16 minutes. The IS was separated from the drug. The retention times for both Nebivolol and IS were highly precise.

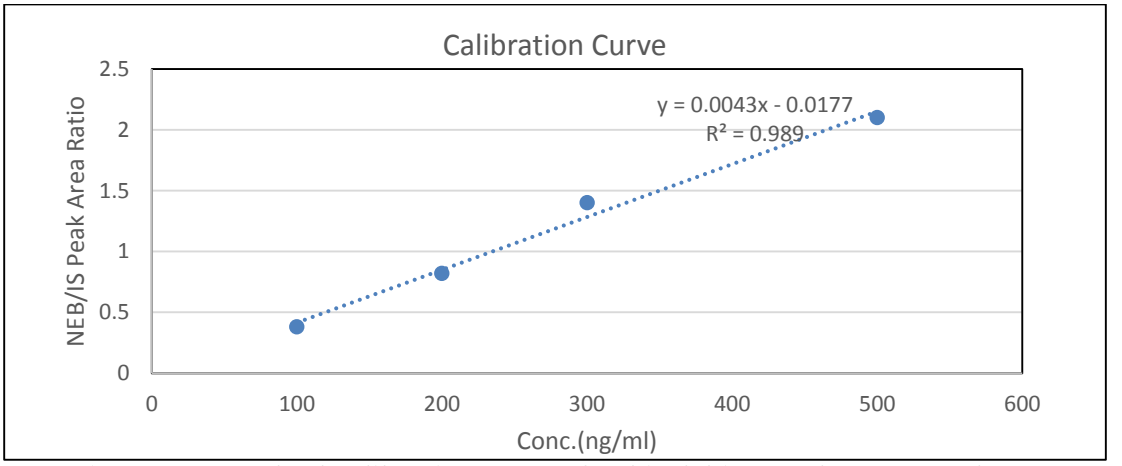

Figure 6: Standard calibration curve of Nebivolol in rat plasma samples

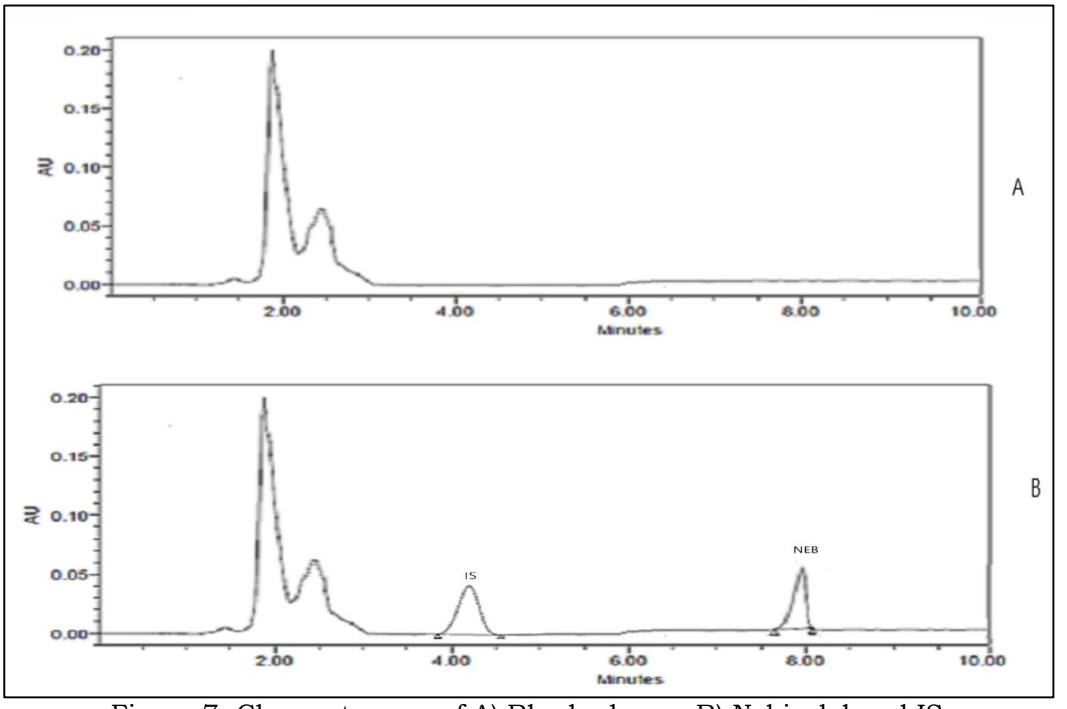

Figure 7: Chromatogram of A) Blank plasma B) Nebivolol and IS

Pharmacokinetic Study

The plasma drug concentration-time profile of nebivolol HCl was constructed after the oral administration of pure nebivolol suspension and nanoparticulate matrix tablet at a dose of 10 mg/kg to Wister rats. The plasma concentration-time profile is shown in Figure 8 and the pharmacokinetic parameters are listed in Table 12.

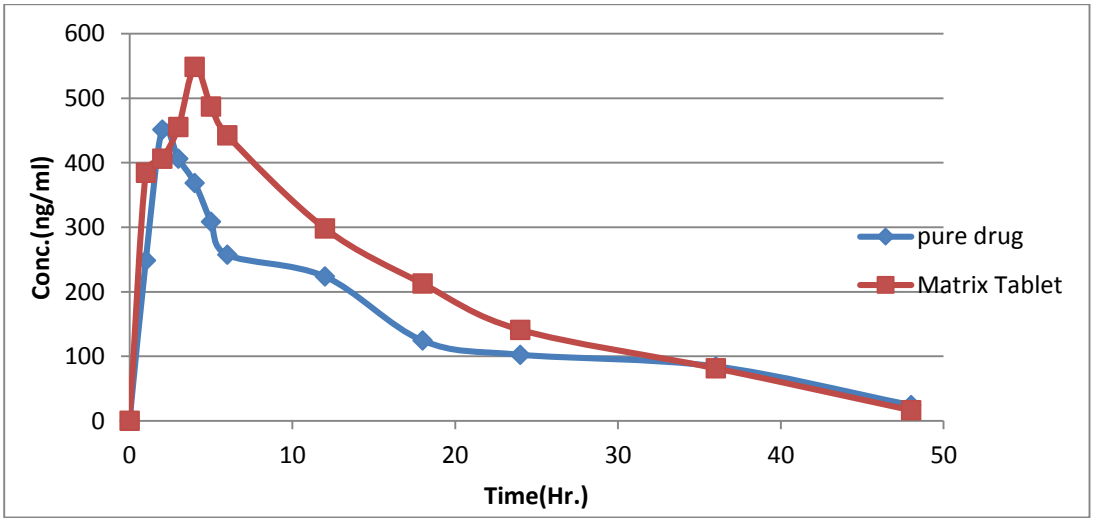

Figure 8: Comparison curves of Plasma Concentration Vs time of pure drug suspension and optimized matrix tablet formulation

The results of pharmacokinetic profiles of nanoparticulate matrix tablets of nebivolol HCl and pure nebivolol suspension show a significant difference. The prepared matrix tablets showed an improved bioavailability of 123.8. The peak plasma concentration (C_{max}) of nebivolol in rats treated with nanoparticulate matrix tablet was (528 ± 37 ng/ml), which was significantly improved compared with that of pure nebivolol suspension (451 ± 21ng/ml). The value of AUC for rats treated with nanoparticulate matrix tablets was enhanced compared with a pure drug suspension. The improved AUC of nebivolol nanoparticles is due to the sustained release of the drug from the nanoparticulate matrix tablet.

Table 12: Pharmacokinetic parameters after oral administration of the free drug and nanoparticulate matrix tablet) (10mg/kg)

Parameters	Pure Drug Suspension	Nanoparticulate Matrix Tablet
T_{max} (Hr)	1.6 ± 0.21	4.0 ± 0.28
C_{max} (ng/ml)	451 ± 21	548 ± 37
AUC (ng.h/ml)	6847 ± 140	9235 ± 214

The values are represented as Mean ± SD (n = 4)

Antihypertensive Study:

Measurement of blood pressure by the non-invasive (indirect) method after administration of the formulation

Administration of DOCA for 4 weeks in unilateral nephrectomized rats produced a significant elevation ($p<0.05$) in systolic blood pressure (SBP) as measured by tail-cuff method on II, III and IV weeks when compared to normal rats. Unilateral nephrectomized rats which received nanoparticulate matrix tablet for 4 weeks along with DOCA significantly ($p<0.05$) reduced SBP on III and IV weeks as compared with SBP of unilateral nephrectomized DOCA-salt hypertensive rats, thus implying an antihypertensive effect.

Table 13: Effect of formulation (mg/kg/day, p.o., for 4 weeks) on SBP in DOCA-salt hypertensive rats

Treatment groups (10mg/kg)	Mean SBP (mm Hg)				
	0 week	I week	II week	III week	IV week
Group I	126.5	125.9	126.2	126.1	126.5
Group II	128.2	172.4	176.6	177.6	181.5
Group III	126.3	137.1	131.2	129.4	127.7
Group IV	125.8	137.5	141.3	137.5	134.5

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * $p<0.05$ when compared to sham control and # $p<0.05$ when compared to DOCA group.

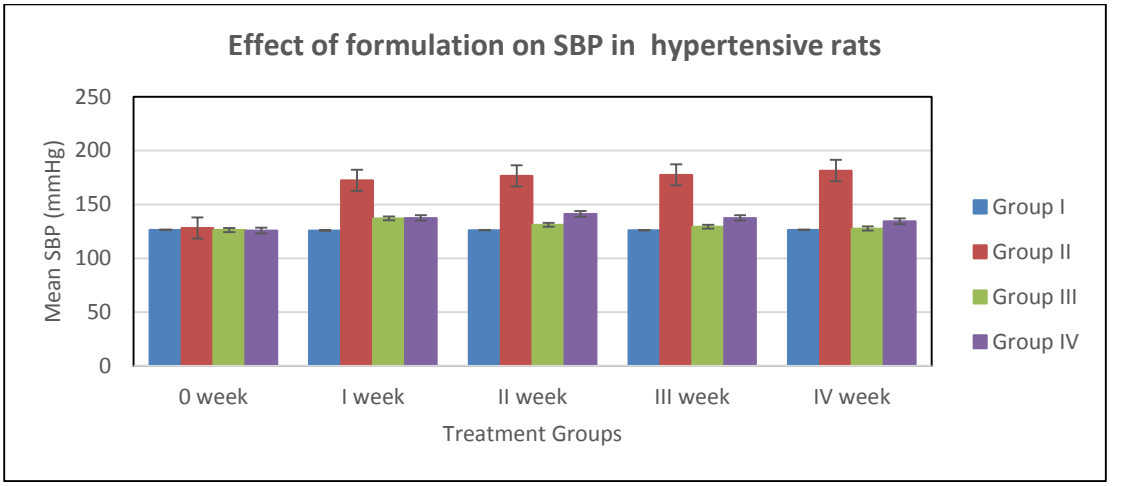

Figure 9: Effect of formulation on SBP in DOCA-salt hypertensive rats

Measurement of blood pressure by the invasive (direct)method

The heart rate, basal arterial blood pressure and pressure responses to NA, Adr, PE, 5-HT and AngII were significantly ($p<0.05$) increased in unilateral nephrectomized DOCA-salt hypertensive rats as compared to normal rats. The heart rate, basal arterial blood pressure and pressure responses to NA, Adr, PE, 5-HT and AngII were significantly ($p<0.05$) reduced in the case of unilateral nephrectomized DOCA-salt hypertensive rats that received nanoparticulate matrix tablet of nebivolol for 4 weeks as compared to unilateral nephrectomized DOCA-salt hypertensive rats.

Table 14: Effect of formulation on heart rate (BPM) and basal arterial blood pressure in DOCA-salt hypertensive rats

Treatment groups (10mg/kg)	Basal arterial blood pressure (mm Hg)	Heart rate (BPM)
Group I	87.51 ± 5.27	228 ± 7.17
Group II	145.3± 7.24	317± 9.38*
Group III	98.5 ± 3.54	256 ± 7.54
Group IV	103 ± 5.26	275± 6.38

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * $p<0.05$ when compared to sham control and # $p<0.05$ when compared to DOCA group.

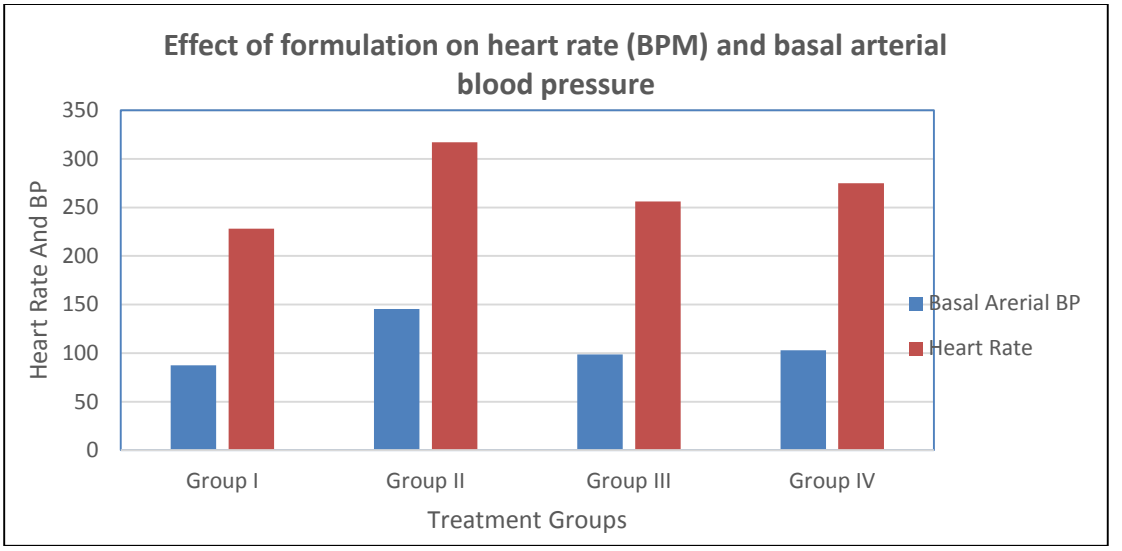

Figure 10: Effect of formulation on heart rate (BPM) and basal arterial blood pressure

Stability Studies

physicochemical parameters

Optimised Nanoparticulate matrix tablets were evaluated for weight variation, thickness, hardness, friability, content uniformity and tablet dissolution rate at various time intervals (0,1,3 and 6 months) and the data for stability studies showed no considerable differences in physical parameters of the optimized formulation. (Table 15)

Table 15: Summary of physical parameters of Optimized matrix tablet (F5)

Time (months)	Temperature (°C) and Relative Humidity RH (%)	Appearance	Weight (mg)	Thickness (mm)	Hardness (kg /cm^2)	Friability (%)	Content Uniformity	Drug Released (%) (24 h) NLT
t=0	30±2°C/65±5%	White Appropriate	120.4±1.31	3.99±0.10	4.7± 0.03	0.39 ±0.02	99.5 ± 0.03	97.39 ± 1.92
	40±2°C/75 ± 5%	White Appropriate	119.6±1.22	3.89±0.17	4.9± 0.02	0.34 ±0.01	96.7 ± 0.08	97.39 ± 1.92
t=01	30±2°C/65±5%	White Appropriate	121.1±1.41	3.96±0.12	5.1± 0.04	0.35 ±0.03	99.5 ± 0.06	95.58 ± 2.48
	40 ± 2°C/75 ± 5%	White Appropriate	120.8±1.61	3.89±0.21	4.8± 0.06	0.41 ±0.04	99.5 ± 0.07	97.58 ± 2.92
t=03	30±2°C/65±5%	White Appropriate	121.3±1.42	3.92±0.18	5.2± 0.07	0.37 ±0.05	97.3 ± 0.09	94.64 ± 1.71
	40 ± 2°C/75 ± 5%	White Appropriate	119.4±1.35	3.97±0.14	5.3± 0.04	0.31 ±0.05	95.6 ± 0.02	96.78 ± 1.88
t=06	30±2°C/65±5%	White Appropriate	120.7±1.84	3.96±0.18	4.9± 0.08	0.36 ±0.04	98.7 ± 0.01	96.84 ± 0.35

	40±2°C/75 ± 5%	White Appropriate	119.6±1.21	3.89±0.23	5.4± 0.02	0.42 ±0.07	97.3 ± 0.04	95.20 ± 2.01

In vitro drug release during stability study

In vitro release studies were also performed during stability studies and In the optimised nanoparticulate tablets, the release rate of nebivolol was found to be ≈94-96% at the end of 24 h (Table 16 and Fig.11).

Table 16: Summary of In vitro drug release results of Optimized matrix tablet (F5)

Time (months)	Temperature (°C) and RH (%)	Dissolution Time (Hrs.)						
		02	04	08	12	16	20	24
Beginning (t=0)	30±2°C/65±5 %	45.46 ± 1.90	47.68 ± 1.68	61.56 ± 1.20	73.85 ± 2.06	82.64 ±2.12	94.26 ± 1.48	97.39 ± 1.92
	40±2°C/75±5 %							
1 month (t=1)	30±2°C/65±5 %	39.87 ± 1.82	46.36 ± 2.25	65.28 ± 1.36	71.56 ± 2.14	80.32 ±1.34	92.25 ± 2.31	95.58 ± 2.48
	40±2°C/75±5 %	37.84 ± 1.48	48.41 ± 2.46	62.31 ± 1.53	69.26 ± 2.35	77.57 ±1.15	93.32 ± 1.45	97.58 ± 2.92
3 months (t=3)	30±2°C/65±5 %	40.25 ± 1.36	47.52 ± 2.32	61.87 ± 1.61	68.37 ± 1.87	81.48 ±1.42	91.65 ± 1.85	94.64 ± 1.71
	40±2°C/75±5 %	36.89 ± 2.32	45.38 ± 1.53	64.37 ± 2.56	72.48 ± 1.64	84.47 ±1.75	94.37 ± 1.47	96.78 ± 1.88
6 months (t=6)	30±2°C/65±5 %	38.24 ± 1.46	44.65 ± 2.86	59.45 ± 2.17	67.62 ± 2.38	79.38 ±1.38	93.85 ± 1.36	96.84 ± 0.35
	40±2°C/75±5 %	37.56 ± 2.71	47.36 ± 2.23	59.63 ± 2.45	68.32 ± 2.68	78.56 ±1.65	91.45 ± 2.56	95.20 ± 2.01

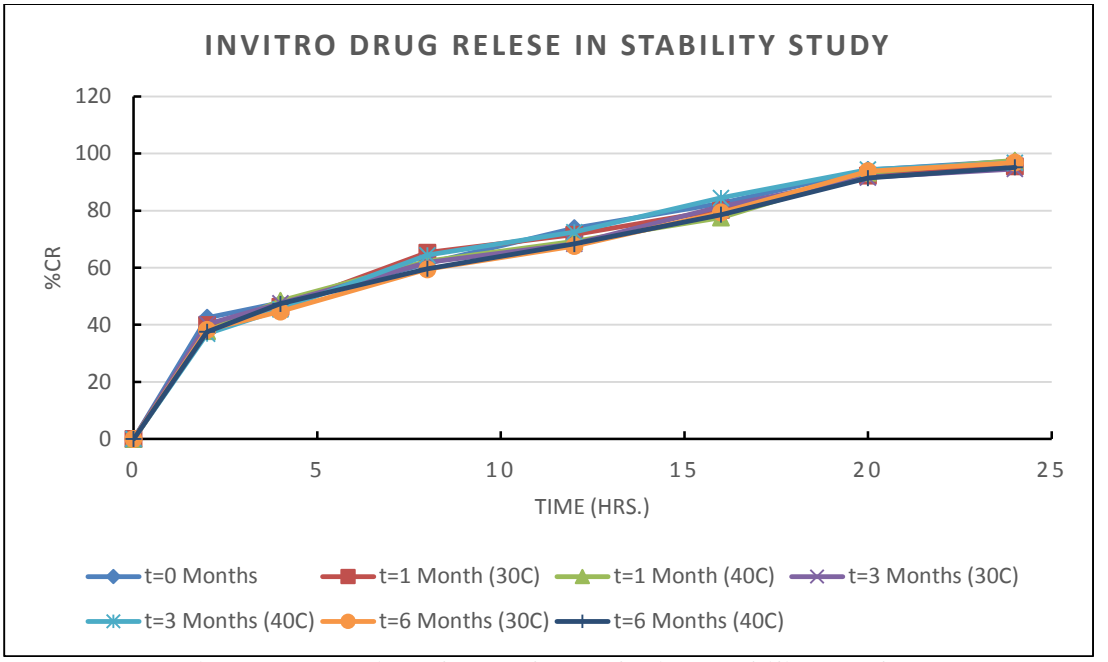

Figure 11: In-vitro drug release during stability study

The data for stability studies revealed no considerable differences in physical parameters and in-vitro release of drug from optimized formulation (F5).

Conclusion

Our work has led us to the conclusion that optimised nanoparticulate matrix tablets of nebivolol hydrochloride were successfully designed by using the solvent evaporation method and varying concentrations of polymer and nanoparticles. Based on results obtained from comparative in-vitro studies The formulated nanoparticulate matrix tablet can extend the release of contents up to 24 hrs. and can overcome the disadvantages associated with conventional tablets. The in vivo pharmacokinetic study showed delayed Tmax, Improved Cmax and AUC indicating slow and prolonged in-vivo release and increased absorption and oral bioavailability. The antihypertensive activity showed a distinct change in SBP in DOCA salt-induced hypertensive rats after the treatment with matrix tablet as compared with pure drug. A significant reduction of DBP and HR were also observed after the treatment with nanoparticulate matrix tablet. The developed nanoparticulate matrix tablet of nebivolol HCl has a greater potential for effective antihypertensive activity. Stability studies revealed no significant changes when optimised matrix tablets were evaluated for various physical parameters, in-vitro release profile and drug content, after one three and six months period.

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