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Formulation of Polymeric Nanoparticles of Lercanidipine by Two Bottom Down Techniques Optimized by Design of Experiment

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Abstract: *Lercanidipine has found to be effective in lowering blood pressure among the potent calcium channel blockers, through its action on L- type calcium channels. However, the major disadvantage associated with Lercanidipine is, it is a BCS class II drug having low solubility, bioavailability is around 10% through oral route due of extensive first pass metabolism. The present study is aimed to prepare and evaluate polymeric nanoparticles of Lercanidipine using a combination of two bottom down techniques, High speed homogenizer and Probe sonication. Preformulation studies like, DSC, FTIR using surfactants such as Tween 80, Sodium Lauryl sulphate, Polyvinyl Alcohol, singely and in combination were used. A full factorial method was utilized to study the effect of various factors such as surfactant concentration, homogenization speed, sonication amplitude and sonication time on Lercanidipine nanoparticles in two levels. Optimized nanoparticles (with PVA as surfactant) showed an average particle size of 141 nm, PDI 0.248 and zeta potential +6.46. Formulation was further optimized using Design Expert 10 software. Optimized formulation was found to be stable during 3 months stability studies as per ICH guidelines.*

Keywords*: lercanidpine, polymeric nanoparticles, bioavailability, Design of Expert, optimization*

1.Introduction

Hypertension is a silent invisible killer, which rarely causes symptoms [1]. It has led to stroke and heart diseases which is the leading cause of death in the world today [2]. About 32% of the American adults have high blood pressure, that is, 1 in every 3 adults [3]. Hypertension is a plague which causes serious cardiovascular diseases including peripheral heart disease, myocardial infarction, stroke and heart failure. Indian statistics have shown that 20 to 40% of urban and 12 to 17% of the rural population are suffering from this disease.

Oral route is the most preferred route of drug delivery because of greater ease, compliance and adherence of the patient to the therapy. A large number of conventional antihypertensive drugs are available in market but the major drawback with this route of drug delivery is low bioavailability, low solubility, substrates for p-glycoprotein enzymes (P-gp) and therefore exhibit extensive high first pass metabolism. They also have high dosing frequency and short half life. These problems can be prevented by delivering the drug in the form of nanoparticles which will reduce the dosing frequency as well as circumvent the hepatic metabolism, P-gp efflux and helps in delivering the drug in the systemic circulation [4].

About 10% of the drugs have poor aqueous solubility and more that 60% of the drugs entering the market with potential pharmacological activities fail because of this problem. Therefore it is essential for a New Molecular Entity (NME) to have adequate aqueous solubility to dissolve in the contents of the GI tract as well as sufficient lipophilicity to induce partition between the lipoidal membrane and systemic circulation [5].

One of the approach to enhance solubility of the drug molecule is converting it into a polymeric nanoparticle. Particles in the range of 1 - 1000 nm are called as nanoparticles. Nanotechnology is a

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technology to improve the solubility of drugs by reducing them to nano size ranges, increasing the surface area of the particle and improving the solubility by allowing greater contact with the surrounding dissolution medium. When such approach is extrapolated to polymers it is termed as a novel technology for drug delivery system in the form of polymeric nanoparticles.

Polymeric nanoparticles systems are able to entrap both hydrophilic and hydrophobic drug. They have gained interest in the recent past because of their site specificity and sustained drug release. Because of site specificity toxicity to the peripheral organs is also reduced to a greater extent.

Lercanidipine is a BCS class II drug having low solubility because of which the bioavailability is around 10% and has food-dependent absorption. In comparison to other calcium channel blockers such as Felodipine, Amlodipine and Nicardipine, Lercanidipine has chiral centers, with two enantiomers, where S (+)-Lercanidipine has higher antihypertensive activity. It is highly bond to plasma proteins >98% and no unchanged drug is found in urine or faeces owing to its first pass metabolism when given by oral route [6].

Various formulations of Lercanidipine have been developed to solve this problem like buccoadhesive controlled release tablets [7] and fast dissolving oral films using HPMC [8] which significantly increases the solubility, bioavailability and overcame the erratic absorption patterns observed with marketed formulations. The concept of Polymeric nanoparticles has been applied to many drugs such as Ibuprofen, Celecoxib and Felodipine to enhance their solubility.

However, no published literature is available on development of polymeric nanoparticles of

Lercanidipine to improve solubility and bioavailability. Therefore in this study, polymeric nano-particles of Lercanidipine have been prepared using a synthetic polymer aimed to enhance the solubility and bioavailability of Lercanidipine.

2.Materials and methods

Lercanidipine and Eudragit RL 100(Mol wt-3200g/mol) was given as a gift from Lupin (Pune, India), Polyvinyl alcohol (PVA) (degree of polymerization P_n = 1700, 88 mol% hydrolyzed) was obtained from HIMedia Laboratories (Mumbai, India), Tween 20, Tween 80 and Sodium Lauryl Sulphate (SLS) were obtained from Loba Chemie Pvt Ltd, India. Milli Water was obtained from Millipore SAS system (Mosheim, France). All other chemicals were of analytical grade.

2.1. Method of preparation of Lercanidipine polymeric nanoparticles

In a beaker, 10 mL of water was taken, to which 1% of PVA was added under constant magnetic stirring. The solution was filtered and was then placed in an ice bath and set aside to attain 4^0C. In another beaker a mixture of 20 mg of the drug and 60 mg of Eudragit RL 100 was dissolved in Methanol to make 2mL of solution. The above organic solution was added to the aqueous solution containing PVA and the resulting solution was homogenized using High speed homogenization at 17000-18000 rpm for 10 min. The solution was then sonicated using Probe- Sonication method for 70 Hz amplitude, pulse 10s for 10 min.

2.2. Preformulation studies

Identification of the drug

The wavelength of Lercanidipine was analyzed using UV-Visible Spectrophotometer (UV-1601PC, Shimadzu Corporation, Japan). Further, the obtained sample was also analyzed using FTIR (Fourier Transform Infrared) Spectrophotometer (FTIR 8300, Shimadzu Corporation, Japan) and DSC (Differential Scanning Calorimeter, DSC-60 Plus, Shimadzu Corporation, Japan).

Drug –Excipient compatibility Studies

Fourier Transform Infrared Spectroscopy (FTIR)

Compatibility of the drug along with the excipients was determined using FTIR Spectrophotometer (FTIR 8300, Shimadzu Corporation, Japan), by recording the spectrum in the range of 4000 – 400 cm^{-1}.

The sample was compressed into a disc with the excipients in the ratio 1:1 in 2% potassium bromide (KBr). The pressure every 5 min was around 5 tons in a hydraulic press. Then the spectrum was recorded using solution v.1.2 software by placing the disc in the path of the light [9].

Differential scanning calorimeter (DSC)

DSC study was performed for Lercanidipine, PVA, Eudragit RL 100, Lercanidipine HCl+ PVA,

Lercanidipine HCl + Eudragit RL 100, Lercanidipine HCl + PVA to confirm the compatibility of the drug with the excipients by using the DSC (DSC- 60 Plus with TA- 60WS Thermal analyser, Shimadzu Corporation, Japan). The samples were then placed in a sealed aluminium pan, keeping an empty aluminium pan as reference before heating under nitrogen flow (30 mL/min) at a scanning rate of 5°C/ min from 30 to 250°C. The heat flow as a function of temperature was measured for the drug and excipients mixture [10].

Screening of Aqueous phase

To find a suitable aqueous phase for the formulation, surfactants were screened i.e Tween 20, tween 80, Sodium Lauryl Sulphate (SLS), either individually or in combination. The formulation was prepared with these surfactants in the aqueous solution and evaluated using Malvern Zeta Sizer (Malvern Zetasizer NanoZS, Malvern Instruments, UK) for Particle size, PDI (Poly Dispersity Index) and Zeta potential.

Analytical method for estimation of Lercanidipine (UV method)

The ultraviolet spectrophotometric method was used for estimation of Lercanidipine by using UV-Visible Spectrophotometer (UV-1601PC, Shimadzu Corporation, Japan). Scanning of the drug was done in the range of 200-400 nm. The ʎmax obtained after scanning the drug in each solvent is selected to prepare standard plot of the drug in that particular solvent.

Optimization of the nanoformulation

Optimization of Lercanidipine polymeric nanoparticles was done using full factorial method of Design Expert® version 10 software. Four independent variables, concentration of surfactant (%), homogenization speed (rpm), sonication amplitude (W) and time (min) were chosen with two levels and three parameters, particle size, PDI and Zeta potential as response factors.

Independent variables and levels for optimization of the Nano formulation

Independent variable	**Levels**	
Factors	**-1**	**+1**
Surfactant concentration (%w/v)	0.5	1.5
Homogenization speed (rpm)	12000	15000
Sonication amplitude(W)	40	60
Time (min)	4	8

2.3. Characterization of nanoparticles

Particle size and Poly Dispersity Index (PDI)

The two parameters were analyzed using Malvern zeta sizer Nano ZS (Malvern Instruments, UK). The system was fitted out with a 4 mW Helium/Neon laser at 633 nm wavelength and processed at a detection angle of 173°. The formulation was diluted 10x with Milli-Q water. All the analysis work was carried out at 18°C.

Zeta potential [11]

The sample was placed in gold platted electrodes, which works on the principle of electrophoretic mobility. Cations move towards the anode and anions move towards the cathode, which will determine the net charge on the particle. All the analysis was carried out at 18°C.

Entrapment efficiency [12]

10 mL of the formulation was centrifuged using an Ultra centrifuge (Sigma, Mumbai) at 22000 rpm for 30 min. The pellet was redispersed in methanol, sonicated for 10 min to obtain a clear solution. Absorbance of the solution was checked at 245 nm against blank formulation prepared in the same manner.

$$\%\text{Entrapment Efficiency} = \frac{T_d - F_d}{T_d} x100$$

T_d - Total drug content

F_d - Free drug taken

Stability testing of the optimized formulation

A stability study of the optimized polymeric nanoparticles was done by keeping the sample at 4° and room temperature for duration of 60 days. The solution was diluted with Milli- Q water and analysed using Malvern Zeta Sizer for particle size, PDI and Zeta potential at 15, 30, 45 and 60 days.

***In vitro* release study [11]**

In vitro dissolution study of the optimized polymeric nanoparticles was carried out in a dialysis bag. United States Pharmacopoeia type II dissolution apparatus was used for the study, 4 mL of the nanoparticles solution was taken in a sigma membrane, fastened on both the ends with a thread and suspended in dissolution medium. The same procedure was used for preparation of standard drug solution for dissolution with 8 mg of the pure drug (Lercanidipine) dispersed in distilled water to make up the volume to 4 mL. Dissolution was carried out using two different buffers (0.1N HCl *p*H 1.2 and phosphate buffer *p*H 6.8) in 500 mL of dissolution medium with 50 rpm maintained at 37°C. 2mL of the sample was withdrawn at 0.05, 0.15, 0.3, 0.45, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48 and 60 h and replenished with blank to maintain sink conditions. The collected samples were then filtered using syringe filter and analyzed spectroscopically.

***In vivo* Pharmacokinetics studies [13]**

Wistar rats (180- 200 g) used for the pharmacokinetic study were obtained from Animal house, Manipal. They were kept at 25°C and 50% RH for 12 h light dark cycle in polypropylene cages filled with sterile paddy husk. Well balanced diet and water ad libitum were provided. Institutional Ethical Committee, Kasturba Medical College, Manipal University, Manipal (IAEC/KMC/01/2016) approved the protocol of the study.

Pharmacokinetic study of optimized formulation

The Wistar rats were divided into 2 groups (n=3). Dose 3mg/Kg were given. Blood was withdrawn from retro orbital flex puncture (0.5mL of each rat at predetermined time intervals (0.5, 1, 2, 4, 6, 12, 24 h). Lercanidipine as extracted from the plasma was centrifugation at 6000 rpm for 10 min and stored at -72°C until further analysis.

Bio analytical method for analysis

High performance liquid chromatography system (HPLC LC- 2010 CHT, Shimadzu Corporation, Kyoto, Japan) having a low pressure quaternary gradient pump along with dual wavelength detector, column oven and auto sampler was used.

Extraction of Lercanidipine from Rat plasma

Protein precipitation method was selected for extraction of Lercanidipine from the rat plasma. 500 µL of chilled Acetonitrile was added to 250 µL of rat plasma and vortexed for 3 min. It was then cold centrifuged for 10 min at 14000 for 10 min. The supernatant was extracted and analyzed with HPLC.

Preparation of calibration curve

Different concentration of Lercanidipine were spiked in blank plasma, vortexed for 3 min and cold centrifuged for 10 min at 14000 rpm. The supernatant was removed and analysed with HPLC. Calibration curve was constructed in concentration range of 2, 4, 6, 8, 10, 50, 100 µg/mL of Lercanidipine.

Chromatographic conditions for analysis of Lercanidipine

Parameters	Description
Column	Phenomenex ® Gemini (250.0×4.6 mm 5µ)
Mobile Phase	Acetonitrile and 25 nM phosphate buffer pH 3.5
Detection wavelength	242 nm
Flow rate	1.0 ml/min
Injection Volume	100 µL
Temperature	25°C
Sample temperature	4±0.5 °C

3. Results and discussions

3.1. Physical parameter of drug for identification

Standard plot of the drug in various solvents

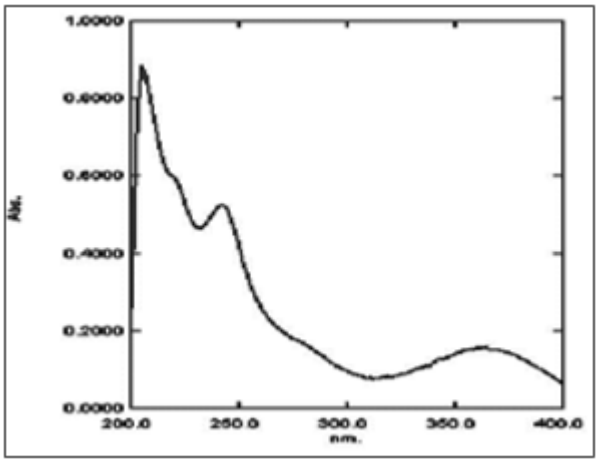


Figure 1. UV- Vis Spectroscopy of Lercanidpine in 0.1N HCl *p*H 1.2

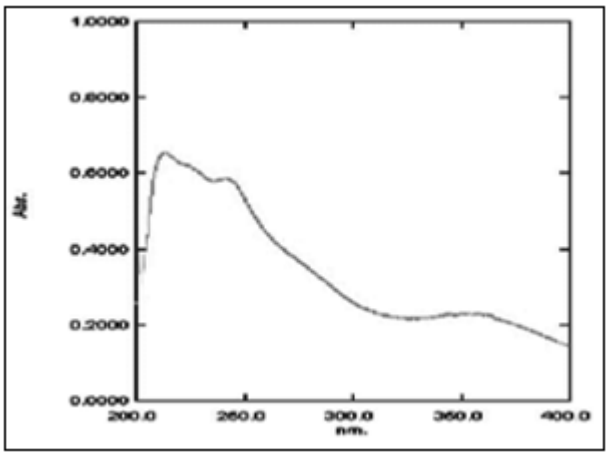


Figure 2 UV- Vis Spectroscopy of Lercanidpine in Phosphate buffer *p*H 6.8

Purity of the drug was found by determining the wavelength using UV Visible spectrophotometer. The wavelength was found to be at 242.2nm for 0.1 N HCl and 244.4nm for phosphate buffer *p*H 6.6.

Differential scanning calorimetry (DSC)

Drug excipient compatibility studies were obtained for pure drug and in combination with excipients. No shift in the peak when compared to the pure drug, indicates that the drug and the excipients are compatible.

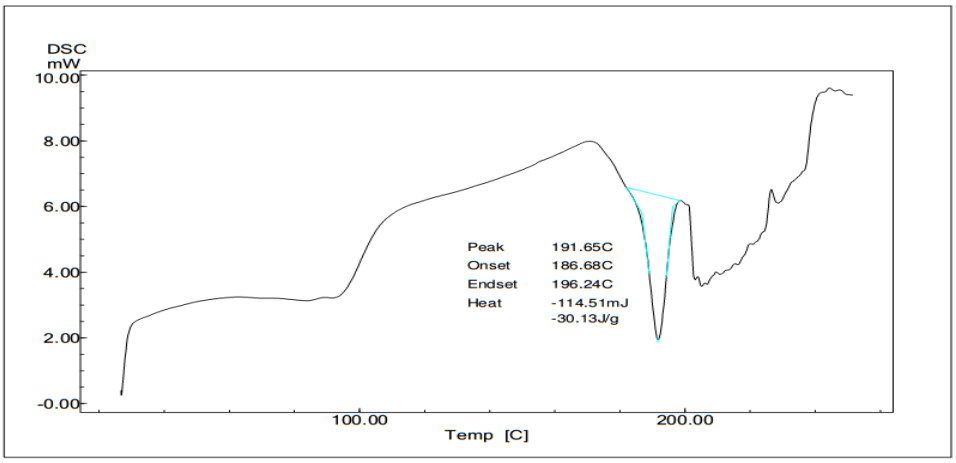


Figure 3. DSC thermogram of Lercanidipine

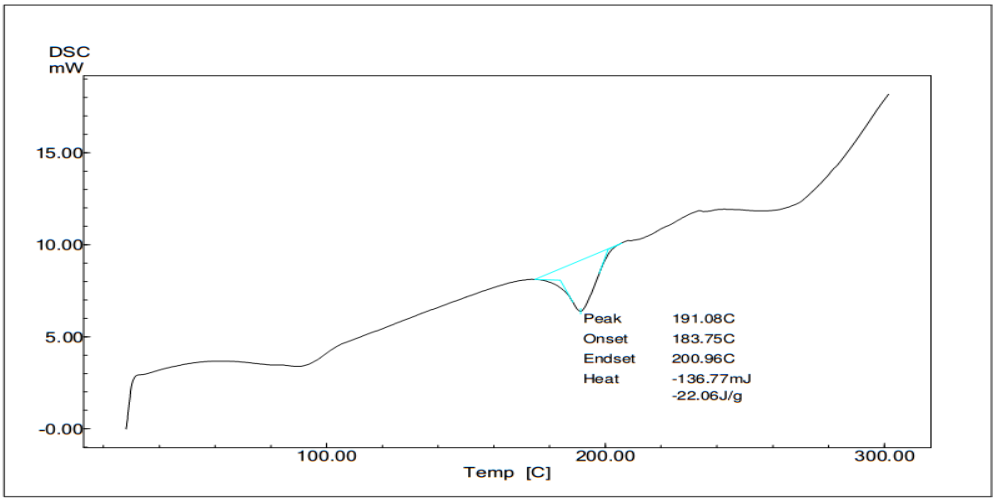


Figure 4. DSC thermogram of Lercanidipine +PVA

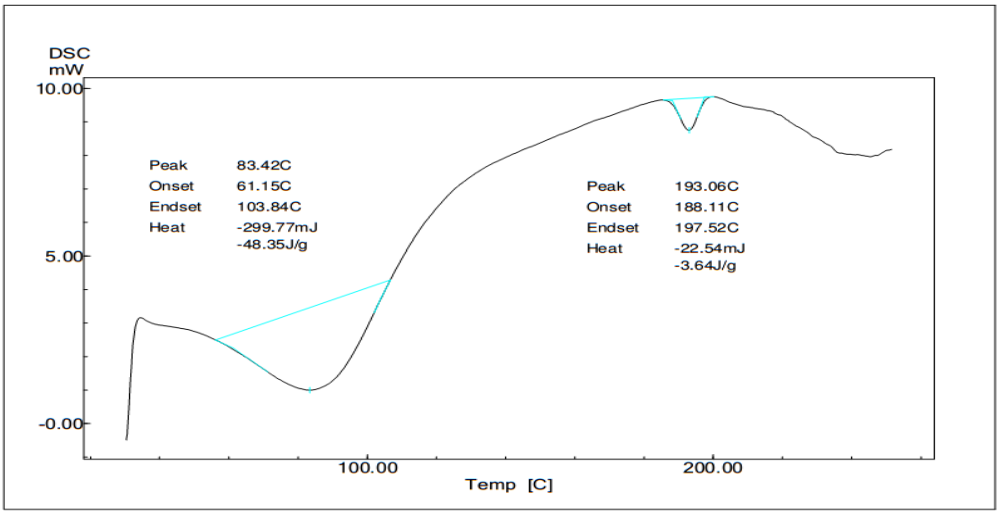


Figure 5. DSC thermogram of Lercanidipine + Eudragit RL 100

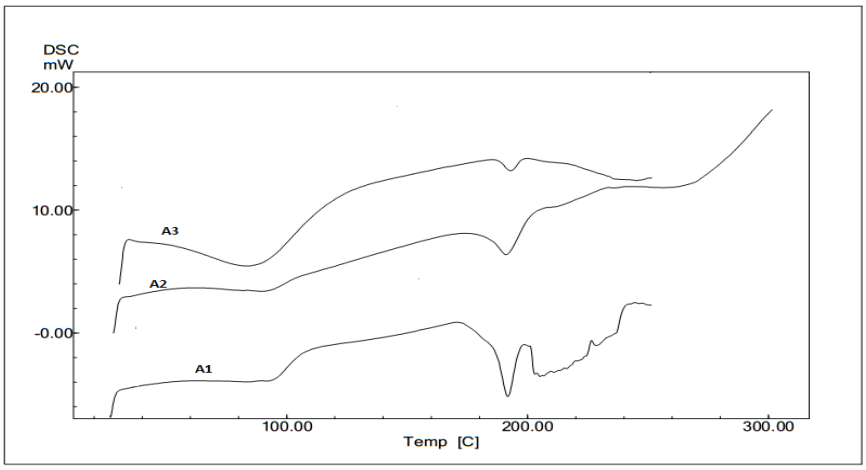


Figure 6. DSC thermogram of A1, A2, A3

Table 1. List of DSC samples

Sample ID	Sample name	Melting point(°C)
A1	Lercanidipine	191.65
A2	Lercanidipine + PVA	191.08
A3	Lercanidipine + Eudragit RL 100	193.06

From Figure 3 the endothermic peak of drug was found to be at 191.65°C which was also present when the drug is present in a mixture with PVA and Eudragit RL 100 with slight shift of +2°C. Since the endothermic peak between the pure drug and in combination with excipients (PVA and Eudragit RL 100) showed no significant changes, it was concluded that they are compatible.

A.**Fourier transform infrared spectroscopy**[14]

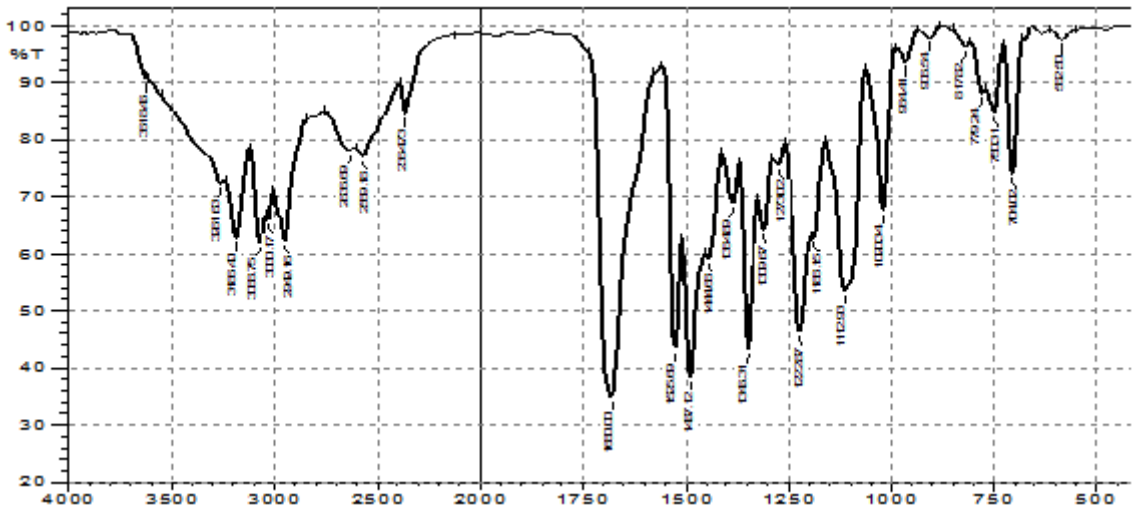


Figure 7. IR spectra of Lercanidipine HCl

Table 2. Major peaks of Lercanidipine HCl

Functional groups	Chemical group	Type	Absorption peak (cm^{-1})
Carboxylic acid	C=O	Stretching	1680
Amine and amino compounds	N-H	Stretching	3186.40
Aromatic nitro compounds	CH-NO_2	Stretching	1525.69
Aromatic Ring	C=C-C	Stretching	1487.12 (1510- 1450)
	C-H	Stretching	3068.75

			(3130–3070)
	C-H	In plane bending	1225–950(several)
	C-H	Out of plane bending	900–670(several)
	CH 1,2-disubstitution (meta)		906.54 (900-860)
	CH 1,6 disubstitution (ortho)		750.31
Saturated aliphatic group frequencies	Methyl ($-CH_3$) Methyl CH	asym./sym Bending	1444.68, 1384.89 (1470–1430/1380–1370)

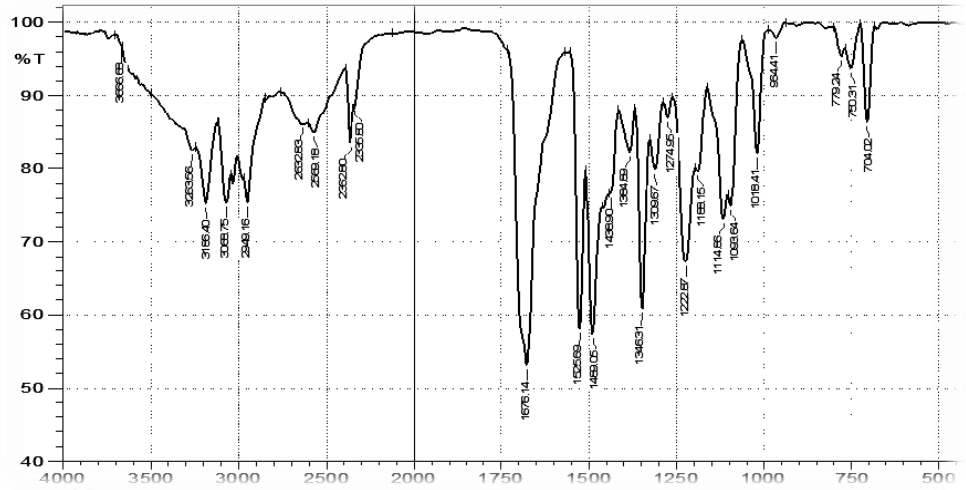


Figure 8. IR spectra of Lercanidipine + PVA

Table 3. Major peaks of Lercanidipine + PVA

Functional groups	**Chemical group**	**Type**	**Absorption peak (cm^{-1})**
Carboxylic acid	C=O	Stretching	1676.12
Amine and amino compounds	N-H	Stretching	3186.40
Aromatic nitro compounds	$CH\text{-}NO_2$	Stretching	1525.69
Aromatic Ring	C=C-C	Stretching	1489.90 (1510- 1450)
	C-H	Stretching	3068.75 (3130–3070)
	C-H	In plane bending	1225–950(several)
	C-H	Out of plane bending	900–670(several)
	CH 1,6 disubstitution (ortho)		750.31
Saturated aliphatic group frequencies	Methyl ($-CH_3$) Methyl CH	asym./sym Bending	1438.90,1384.89 (1470–1430/1380–1370)

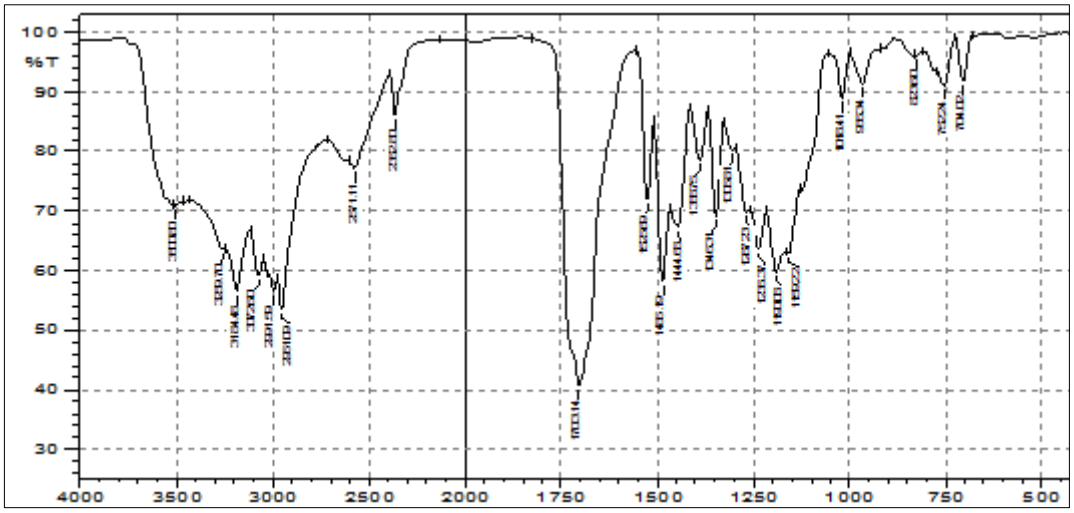


Figure 9. IR spectra of Lercanidipine + Eudragit RL 100

Table 4. Major peaks of Lercanidipine + Eudragit RL 100

Functional groups	**Chemical group**	**Type**	**Absorption peak (cm^{-1})**
Carboxylic acid	C=O	Stretching	1703.14
Amine and amino compounds	N-H	Stretching	3184.48
Aromatic nitro compounds	CH-NO_2	Stretching	1525.69
Aromatic Ring	C=C-C	Stretching	1485.19 (1510- 1450)
	C-H	Stretching	3072.60 (3130–3070)
	C-H	In plane bending	1225–950(several)
	C-H	Out of plane bending	900–670(several)
	CH 1,6 disubstitution (ortho)		750.31
Saturated aliphatic group frequencies	Methyl (−CH_3) Methyl CH	asym./sym Bending	1444.68,1388.75 (1470–1430/1380–1370)

Physical features and chemical properties of the drug and excipients are important features that influence the design and preparation of a dosage form. In the IR spectra of Drug and Eudragit RL 100 there was slight decrease in the intensity of the aromatic nitro group and the intensity of the carboxyl group is increased with slight broader peak. Since there are no significant changes in comparison with the pure drug it indicates absence of interaction. The above obtained peaks in the IR spectra confirm the purity and authentication of the drug. No significant changes in IR spectra of Drug+Excipient ((PVA and Eudragit RL 100) were observed in comparison to pure drug. This concludes that the drug and excipients are chemically compatibility.

3.2. Selection of surfactant

Surfactants were screened for their suitability in aqueous solution depending on the surface size, PDI and Zeta potential

Table 5. Selection of surfactant

Surfactants	After Homogenization			After Sonication		
Parameters	Size	PDI	Zeta potential	Size	PDI	Zeta potential
1 % PVA	220.7	0.363	-8.60	126.6	0.227	-4.27
0.25 % SLS	918.4	0.455	-47.2	252.2	0.314	-65.3
0.25% PVA+ 0.25% SLS	592	0.412	-13.6	317.4	0.486	-26.2
0.5% SLS	250.7	0.306	-39.4	210.3	0.221	-62.1
1% Tween 80	193	0.436	-16.5	181	0.389	-4.54
1 % Tween 20	166.9	0.384	-5.67	150.7	0.406	-3.9

Four different types of Surfactants: PVA, SLS, Tween 80, Tween 20 singly or in combination were studied to see the influence on average size, PDI and Zeta potential. Size of the polymeric nanoparticle is an important property that helps in adhesion and interaction with the biological cells. PDI will determine the homogeneity of the preparation and Zeta potential is a function of the charge of the particle, which is responsible for the stability of the formulation. Adhesive agents like PVA help in adhesion and absorption, and it also by-passes from being recognized by P-glycoprotein/cytochrome P450 enzymes. From results obtained from Table 5 it is evident that the particle size has reduced considerably by using combination using combination of homogenization and sonication. Particle size obtained from PVA is the smallest, with good PDI of 0.227 with appreciable Zeta potential in comparison with other surfactants. Therefore PVA solution has been finalized as surfactant in aqueous phase for preparation of the polymeric nanoparticles. From the screening data, 1% PVA solution was used for further designing.

3.3. Design of experiment for optimization of nanoformulation

The following runs as shown in the Table 6 were suggested by Design Expert® software v 10.0.4.0 for 4 independent variables and 2 levels. Accordingly the responses such as average size, PDI and Zeta potential were noted for the 16 runs suggested by the software.

Table 6. The following data represents the results of 16 runs suggested by the software

Sample No	Surfactant conc (%w/v)	Homogenization speed (rpm)	Sonication amplitude (W)	Sonication time(min)	Average size(nm)	PDI	Zeta Potential
LPN 1	0.5	12000	40	4	100.063	0.2766	1.612
LPN 2	0.5	12000	40	8	112.626	0.2446	4.339
LPN 3	0.5	12000	60	4	125.23	0.3616	5.32
LPN 4	0.5	12000	60	8	105.90	0.3116	6.616
LPN 5	0.5	15000	40	4	130.73	0.309	5.65
LPN 6	0.5	15000	40	8	107.08	0.3233	10.01
LPN 7	0.5	15000	60	4	154.53	0.313	7.52
LPN 8	0.5	15000	60	8	96.70	0.2243	7.71
LPN 9	1.5	12000	40	4	121.73	0.324	9.7
LPN 10	1.5	12000	40	8	167.266	0.425	10.523
LPN 11	1.5	12000	60	4	148.63	0.323	13.13
LPN 12	1.5	12000	60	8	141.25	0.339	11.5

LPN 13	1.5	15000	40	4	140	0.325	11.056
LPN 14	1.5	15000	40	8	138.3	0.329	12.23
LPN 15	1.5	15000	60	4	154.5	0.3545	5.43
LPN 16	1.5	15000	60	8	167.8	0.329	10.48

Table 7. ANOVA value of Response : Particle size

Factorial	Coefficient Estimate	F Value	P value	R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision	Significance
Model	132.21	9.73	0.0013	0.7796	0.6995	0.5337	10.281	Significant
A-surfactant conc	15.60	*24.15*	*0.0005*					
AC	8.44	*7.06*	*0.0223*					
BC	-6.14	*3.74*	*0.0792*					
CD	-6.31	*3.95*	*0.0723*					

F Value of 9.73 obtained from Table 7 indicates the significance of the model. As the concentration of the surfactant increases, the particle size increases but as the sonication time increases, the particle size decreases.

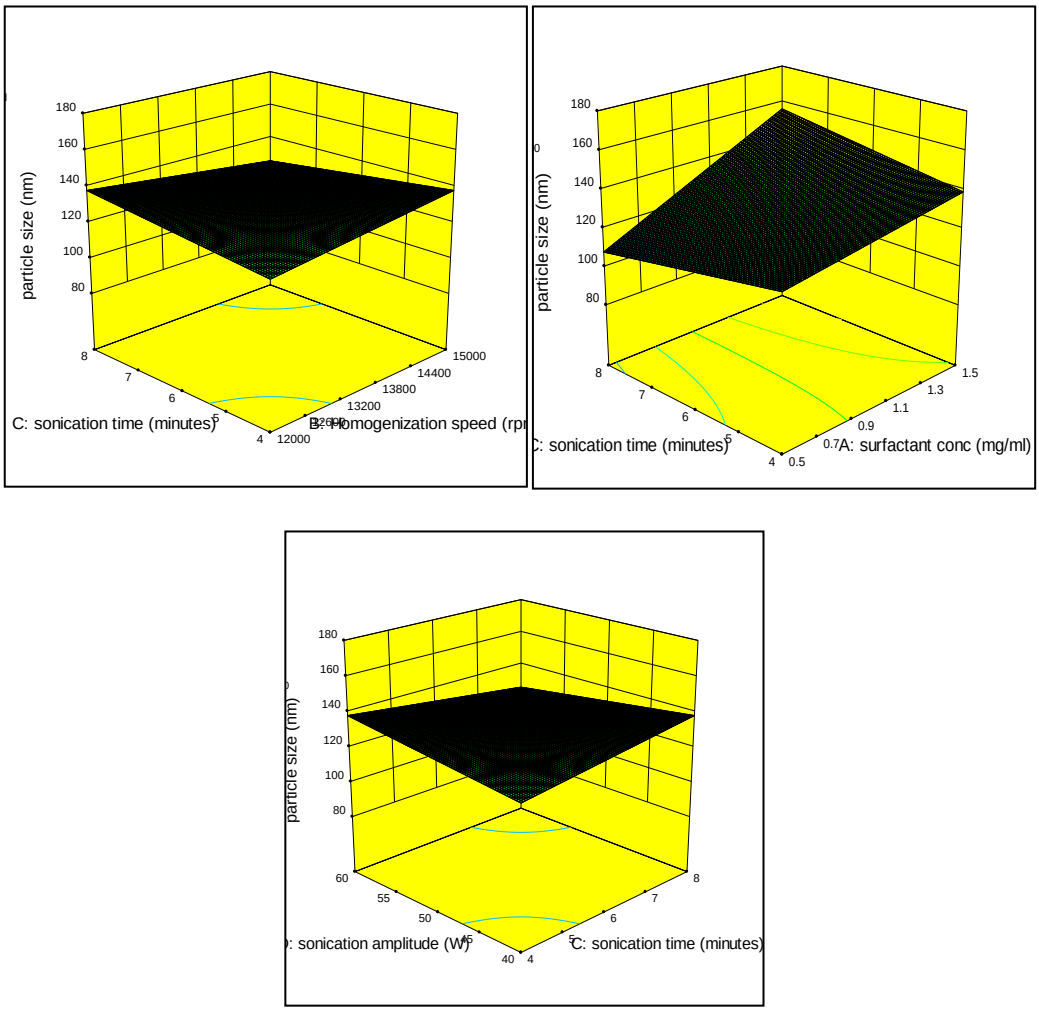


Figure 10. 3D graph depicting the effect of independent factors on particle size

Final equation for particle size:

Particle size = + 132.21 + 15.60 * A + 8.44 * AC - 6.14 * BC

Table 8. ANOVA response for PDI

Factorial	Coefficient Estimate	F Value	P value	R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision	Significance
Model	0.32	10.35	0.0010	0.7900	0.7900	0.7900	0.7900	Significant
A-surfactant conc	0.024	*15.30*	*0.0024*					
AC	0.016	*6.55*	*0.0266*					
CD	-0.015	*5.72*	*0.0358*					
ABD	0.023	*13.82*	*0.0034*					

F Value of 10.35 indicates the significance of the model. As the homogenization speed increases the particle size decreases, but as the concentration of the surfactant increases, the particle increases.

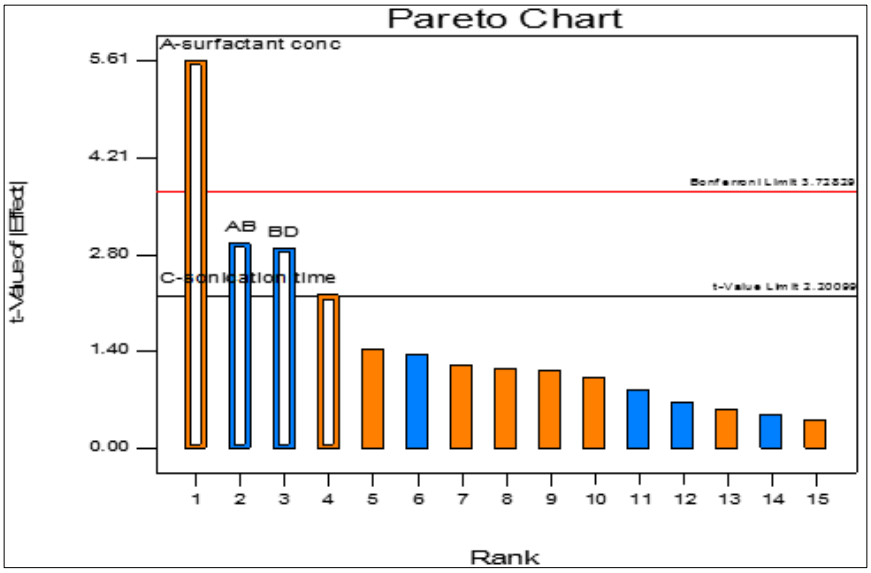
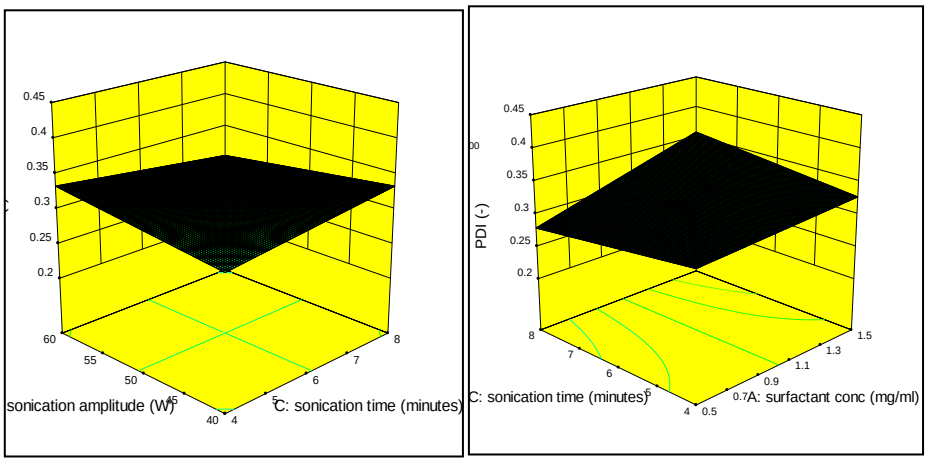


Figure 11. Pareto chart for PDI



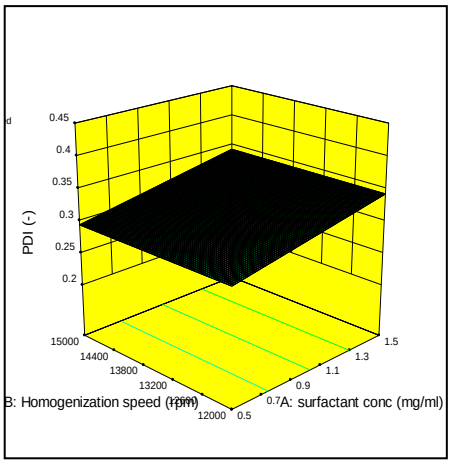


Figure 12. 3D graph depicting the effect of independent factors on PDI

Final Equation for PDI
PDI = +0.32 + 0.024 * A + 0.016 * AC - 0.015 * CD + 0.023 * ABD

Table 9. ANOVA response for Zeta Potential

Factorial	Coefficient Estimate	F Value	P value	R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision	Significance
Model	8.30	13.39	0.0003	0.8297	0.8297	0.8297	0.8297	Significant
A-surfactant conc	2.20	*31.44*	*0.0002*					
C-sonication time	0.87	*4.93*	*0.048*					
AB	-1.16	*8.80*	*0.0128*					
BD	-1.14	*8.41*	*0.0145*					

The F value of 13.3 indicates the significance of this model. As the sonication amplitude increases, the zeta potential increases.

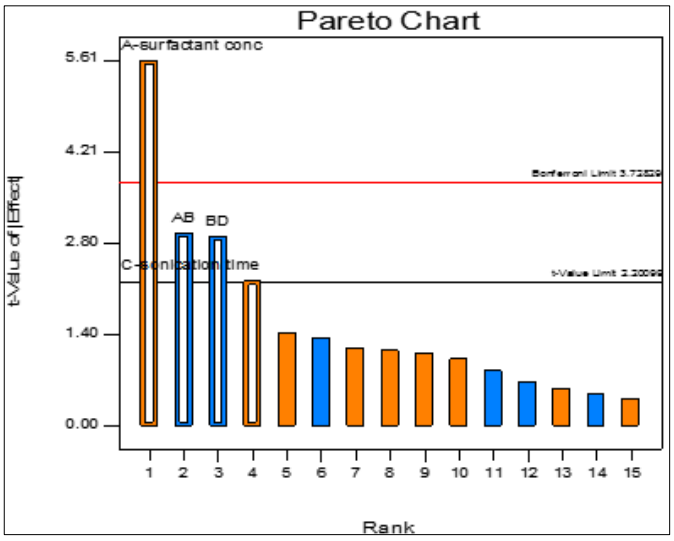


Figure 13. Pareto chart for Zeta potential

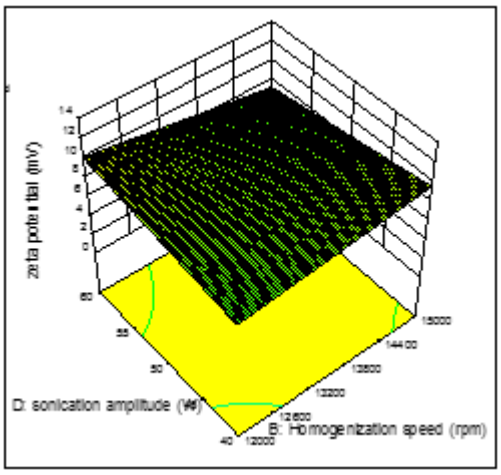


Figure 14. 3D graph depicting the effect of independent factors on Zeta Potential

Final Equation for Zeta potential

Zeta potential = +8.30 + 2.20 * A + 0.87 * C - 1.16* AB - 1.14 * BD

3.4 Characterization of the optimization formulation

Optimized nanoparticles (with PVA as surfactant) showed an average particle size of 141 nm, PDI 0.248 and zeta potential +6.46.

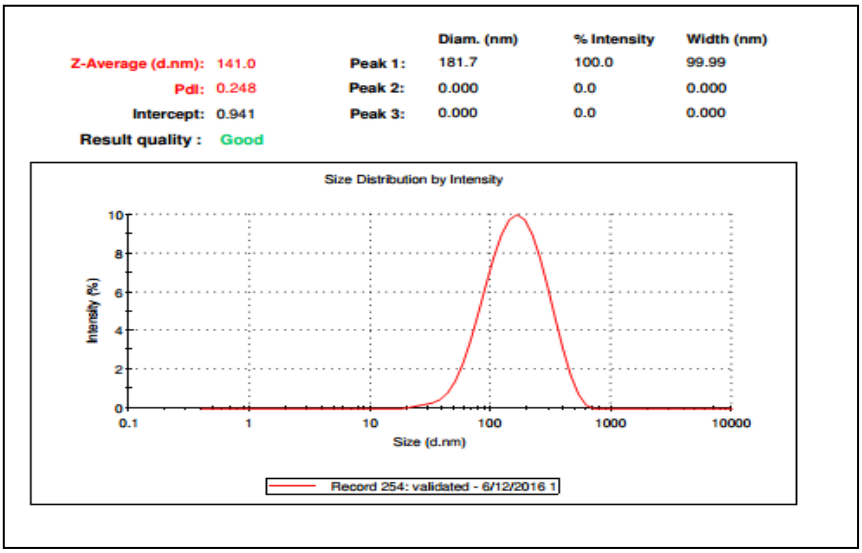


Figure 15. Size Distribution of the optimized formulation

Percentage residual error

It can be obtained by substituting the actual and predicted values of optimized formulation from software, in the following equation:

Percentage residual

$$\%\text{Residual} = \frac{P_s - A_c}{P_s} x100$$

P_s - Predicted value of optimized formulation from software

A_c - Actual value of the optimized formulation obtained

Table 10. Actual and predicted values of the optimized formulation

Response	PVA (%)	Homogenization speed (rpm)	Sonication time(min)	Sonication Amplitude (W)	Average Size	PDI	Zeta Potential
Actual	1.50	12000	4	40	141	0.248	1.45
Software Predicted	1.50	12000	4	40.0	139.202	0.290	4.670

Entrapment efficiency

Table 11. Concentration of dispersed pellet

Absorbance	Dilution	Conc (µg/mL)	Conc (mg/mL)
0.536	10	13.63867684	0.136387

$$\%\text{Entrapment Efficiency} = \frac{19.045}{20} x100 = 95.225\%$$

The entrapment efficiency of the drug depends on the lipophilic nature of the drug. As Lercanidipine is lipophilic in nature, it justifies the high entrapment of 95.225%

Stability study data

Table 12. Stability data of the optimized formulation

Days	Room Temperature			4°C		
	Average Size(nm)	PDI	Zeta Potential(mV)	Average Size(nm)	PDI	Zeta Potential(mV)
15 days	112.7	0.255	14.8	130.7	0.208	11.5
30 days	112.7	0.232	16.1	135.1	0.225	12.8
45 days	107.4	0.228	10.2	146.8	0.350	4.62
60 days	112.6	0.236	14.5	140	0.30	13.4
90 days	112.8	0.224	13	148.8	0.340	13.0

In- vitro Dissolution data

Table 13. *In vitro* dissolution data of the drug and the formulation

Time (h)	Percentage Cumulative Drug Release (%CDR)			
	Standard		Formulation	
	pH 1.2	pH 6.8	pH 1.2	pH 6.8
0.15	8.38±0.76	3.15±0.07	10.99±2.05	9.81±1.95
0.3	10.94±0.79	3.34±0.17	20.09±0.87	9.59±1.91
0.45	13.80±2.29	4.44±0.01	23.30±3.61	9.62±1.90
1	18.73±0.69	6.62±1.38	24.013±4.100	9.034±2.76
2	29.073±2.56	8.022±0.03	27.44±0.29	10.32±1.31
4	33.52±1.18	8.53±0.4285	35.50±6.90	14.41±0.76
6	37.03±1.80	8.70±0.2844	38.19±3.78	17.08±1.93
8	41.81±2.00	9.3290±0.59	41.12±1.66	19.57±1.88
10	43.21±1.17	9.51±0.44	59.91±1.86	20.96±2.64
12	47.34±2.62	11.20±0.617	73.23±3.45	22.06±3.13
24	49.38±3.23	12.08±0.41	81.49±7.67	36.742±3.98
36	51.23±2.46	16.84±3.580	90.40±6.80	61.28±1.65
48	53.54±1.60	17.65±3.96	95.14±6.47	82.01±3.85
60	-	23.10±2.102	-	95.60±5.81

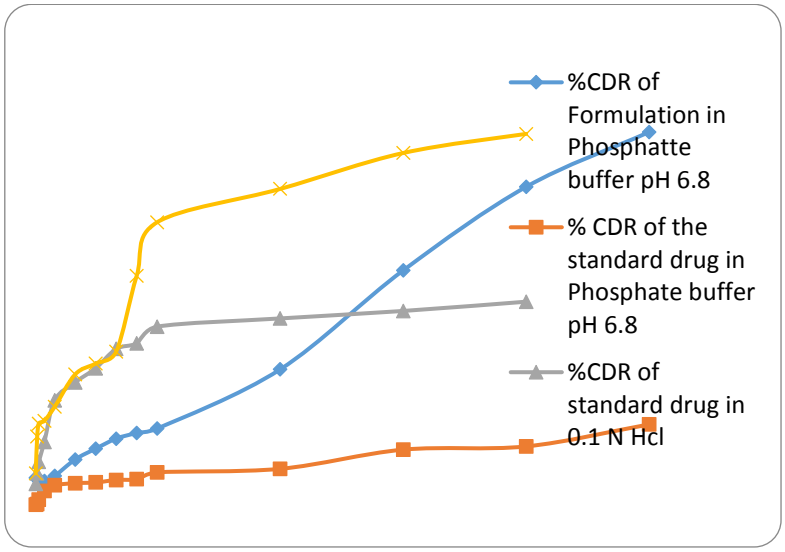


Figure 16. Dissolution profile of the pure drug and Formulation

Bioanalytical estimation of Lercanidipine by HPLC

HPLC method was used to determine the Lercanidipine in rat plasma. The standard calibration curve was generated. Lercanidipine showed good Linearity in all systems in the range of 2- 100µg/ mL. standard plot of Lercanidipine is shown in the Figure 17.

Table 14. Peak area of Lercanidipine in various concentrations

Conc (µg/ml)	Peak area
0	0
4	3922
6	5202
8	5901
10	15490
50	40648
100	70729

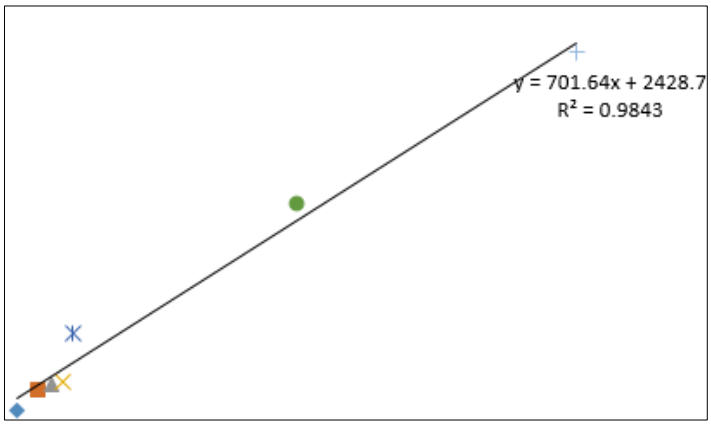


Figure 17. Bioanalytical standard plot of Lercanidipine

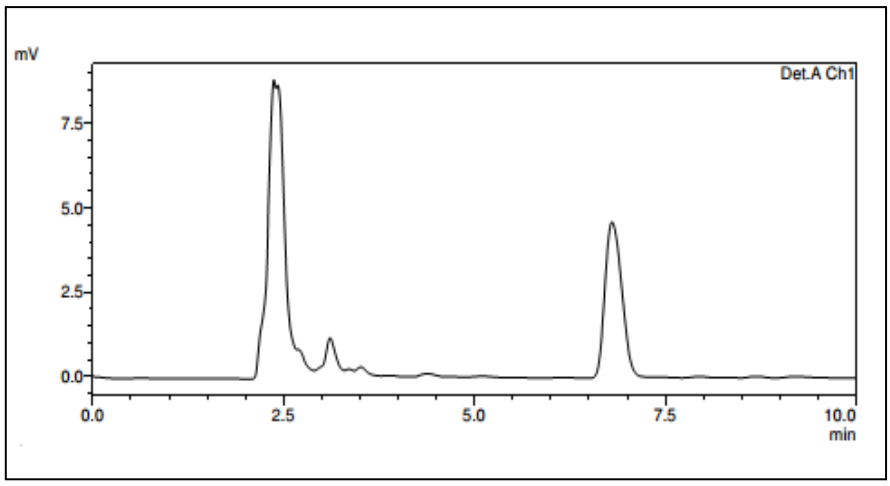


Figure 18. Chromatogram of Lercanidipine in Plasma

Pharmacokinetics studies were conducted on 2 groups of Wistar rats (blank and formulation) where n= 3 for a duration of 48 h. But the pharmacokinetics studies failed to detect the drug peak, indicating drug was not in the detectable limit.

The reason for not detecting the peak may be the following:

- The oral dose administered may be too low for detection
- The drug may have converted into in -active metabolites

- The protein precipitation method used for processing of plasma samples may not be efficient in extraction of drug bound to the plasma proteins, indicating a more robust method such as nitrogen evaporation might help in complete extraction of the drug from the plasma protein.

4. Conclusions

-FT-IR and DSC studies revealed that there was no interaction between the drug and the excipients used in the formulation

-The formulation was optimized using Design expert software 10 and polynomial equations were calculated using ANOVA

-The average particle size, PDI and Zeta potential were all within the predicted range

-The *in vitro* release studies conducted in the optimized formulation showed 91% release in 0.1 N HCl at 48th hour whereas in Phosphate buffer *p*H 6.8 the release was 95% at 60th hour.

-Pharmacokinetics studies conducted on Wistar rats was not able to detect the drug peak because the amount of drug in the plasma was very low to be in the detectable range.

-Stability studies conducted on optimized formulation for a duration of 3 months in room temperature and 4◦C showed that the formulation is stable

The prepared polymeric nanoparticles showed increased solubility in comparison to the pure drug. Based on the results it can be concluded that Polymeric Nanoparticles of Lercanidipine developed can be considered as a successful alternative to the other drug.

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