7. METHODOLOGY

7.1. Drug Excipient Compatibility Studies

To evaluate the Stability of LER in presence of excipients like PEG, HPMC, PVA, Chitosan and TPGS; compatibility study was performed for combination of lercanidipine with excipients in 1:1 ratio. Mixed samples of drug and excipient were analyzed by Differential Scanning Calorimetry (DSC) and Fourier Transform Infra Red (FTIR) spectroscopy. All samples were stored at accelerated (40 ± 2 °C and 75 ± 5% RH) and ambient (25 ± 2 °C and 60 ± 5% RH) conditions protected from light for 6 months and DSC and FTIR studies were repeated [135].

7.1.1. Differential Scanning Calorimetry (DSC)

DSC curves were obtained in a Shimadzu DSC-60 cell using aluminum crucibles with about 4 mg of samples under inert nitrogen atmosphere (flow rate: 10 mL min⁻¹) and at a heating rate of 10°C min⁻¹ in the temperature range from 25 to 300°C.

7.1.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was performed using Shimadzu FTIR 8300 Spectrophotometer and the spectrum was recorded in the region of 4000 to 400 cm⁻¹. In this study, FTIR spectra for the pure API and API along with the excipients (1:1) were obtained. The sample dispersed in the Potassium Bromide (200-400mg) and compressed into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet of each active ingredient was placed in the light path and the spectrum was obtained.

7.2. Solubility Studies

Solubility of lercanidipine was determined in 0.1N HCl and several buffer solutions covering entire pH range of GIT i.e. 1-8. Solubility studies were carried out by placing excess amount of drug in volumetric flasks containing 10 ml of vehicle. The dispersions were continuously agitated on rotary shaker for 48 hours. After reaching equilibrium the samples were filtered through 0.45 µm membrane filter. The filtrates were suitably diluted as needed and analyzed UV spectro-photometrically for dissolved drug. [136].
7.3. **Formulation and Development of Fast Dissolving Oral Films containing LER Nano-particles**

7.3.1. **Preformulation studies**

Several polymers were evaluated for their film forming ability. Polymeric films without drug were prepared by dissolving different polymers and plasticizers (PEG 400/ TPGS 1000) compositions in distilled water. Resulting solution was casted on the glass surface of different areas (25-60 cm\(^2\)) and dried in the oven at 45 °C for 24 hr. Resulting films were examined visually for homogeneity, transparency and smoothness. Disintegration and flexibility properties of these films were evaluated. Flexibility of these films was assessed by determining folding endurance value. Based on the results, polymers were selected for further formulation.

7.3.1.1. **In-vitro disintegration time**

*In vitro* disintegration time of all the formulations was analyzed by adopting visual method. Film of 4 cm\(^2\) was placed in a petri dish (internal diameter 5 cm) containing 10 ml of simulated saliva. Disintegration time was considered as a time when film starts to disintegrate. All the measurements were done in a triplicate and average value was reported [137].

7.3.1.2. **Folding endurance**

Folding endurance is an important quantitative parameter to determine the flexibility of the oral strip. Folding endurance is obtained by repeated folding of the strip at the same place till the strip breaks. The number of times the film is folded without breaking is figured as the folding endurance value [138].

7.3.2. **Formulation of fast dissolving oral films containing LER nano-particles**

As discussed in introduction, Leranidipine has water solubility of only 5µg/ml. Therefore it is difficult to incorporate leranidipine in fast dissolving oral films in its native form. Therefore nano-suspension of LER was prepared and incorporated in fast dissolving films.

In current novel methodology, process-integration of drug nanonization and its incorporation into the polymeric film is achieved without freeze drying or spray drying of nano-suspensions. Such processes (freeze drying or spray drying) are otherwise inevitable in converting nanosuspension in solid dosage forms. Methodology of preparation of nano-suspension and its incorporation in fast dissolving films is described in following flowchart 1.
7.3.2.1. Preparation of nanosuspension and optimization

Lercanidipine nanoparticles were prepared by evaporative antisolvent precipitation technique. Preliminary studies were carried out to investigate the solubility of lercanidipine in various organic solvents such as methanol, DMSO, DCM etc. Lercanidipine (25 mg) was dissolved in sufficient volume (approx 250 µl) of methanol while the polymers/surfactants (stabilizers) were dissolved in water (5 ml) separately. Selected polymers/ surfactants in the preparation of nanosuspension were those which also can be used as plasticizer or polymers in film formulation (PEG 400, hypromellose E15, PVA, sodium alginate, methyl cellulose and TPGS 1000). The resulting mixture was kept under probe sonication (Sonics Vibracell, USA) for 15 min at amplitude 30% with 10 sec pulse. The temperature of the stabilizer solution was maintained at 10ºC. The drug solution was added all at once in stabilizer solution kept under probe sonicator. After complete addition of the drug solution, sonication was continued for 15 min [139]. Two nanosuspension formulations, one containing PEG 400 as a stabilizer and the other one containing TPGS 1000, were further selected for incorporation into oral films. Important parameters like drug:stabilizer ratio and probe sonication amplitude, involved in the preparation of nanosuspension were optimized by using $2^2$ factorial design (Design-Expert® Software).
7.3.2.2. Particle size determination

The average particle size and polydispersity index (PDI) of nanosuspensions were measured by Zetasizer (NanoZS, Malvern Instruments, UK) using laser dynamic light scattering technique.

7.3.2.3. Addition of nanosuspension in polymeric solution

As the quantities of polymers and plasticizer required for the film formation were determined by preliminary studies, part of the amount of this plasticizer/polymer used as a stabilizer in nanosuspension and remaining amount of plasticizer/polymer and sweetener were dissolved separately in 2.5 ml of water.

5 ml of nanosuspension (containing 25 mg of drug) was added to 2.5 ml of polymeric solution and stirred for 4 hr. Resulting 7.5 ml solution was casted on the surface area of 25 cm² and allowed to dry in vacuum oven at 45°C, 15 inHg pressure for 24 hr to get film. Volume and surface area of glass mould plays important role in increasing the drug loading per unit area of the oral film.

7.3.3. Drug content and content uniformity

7.3.3.1. Analytical method for estimation of lercanidipine HCl

Separation of lercanidipine was achieved under optimized chromatographic condition on a Kromasil (100-5c18 250×4.6 mm) column using Shimadzu HPLC (High-performance liquid chromatography) system. The mobile phase consisted of mixture of ammonium acetate buffer (20 mmol pH 4.5) and acetonitrile in the ratio of 10:90, v/v. It was pumped through the chromatographic system at a flow rate of 1 ml min⁻¹. The detection was carried out at 240 nm using UV-VIS detector.

7.3.3.2. Drug recovery from formulation

Prepared films were cut into 3 different sizes (2 cm², 4 cm² and 6 cm²) from different regions of the film and transferred to 10 ml volumetric flasks separately. Volume was made up with methanol and mixed thoroughly using vortex mixer to extract the drug completely. These solutions were filtered through 0.45 µm cellulose membranes. Appropriate dilutions were made to get the concentrations of 5, 10 and 15 µg/ml (50, 100, and 150% of assay concentration). These solutions were prepared in triplicate. Active ingredient was assayed by HPLC.
7.3.3.3. FTIR (Fourier transform infrared) Imaging

FTIR images are chemical pictures providing information which is highly complementary to images obtained from scanning electron microscopy (SEM). FTIR spectroscopic imaging with Focal plane array Detector was used to analyze the spatial distribution of lercanidipine in oral films. Chemical images of films were oral films. Chemical images of films were acquired using the 3000 Hyperion Microscope with Vertex 80 FTIR System (Bruker, Germany). The images were obtained in transfectance mode by placing the film over a white ceramic disk. Spatial resolution with 15x objective was 2.7 µm which provides images of an area of approximately 300 µm × 300 µm. The area of 4 cm² of the film was considered suitable for administration and therefore the uniformity of drug content in each sample was analyzed at different locations and typical image was reported in results & discussion. The spectra were obtained with 1 scan in the spectral range of 4000–900 cm⁻¹. The data collected was analyzed using the integrated OPUS operation and evaluation software. The chemical images were obtained in spectral range 1700–1300 cm⁻¹ as this spectral range provides the greatest differences between excipients and drug.

7.3.4. Evaluation Physical Properties of fast dissolving oral films

7.3.4.1. In Vitro Disintegration Time.

In vitro disintegration time of all the formulations was analyzed by adopting visual method. Film strip of 4 cm² was placed in a petri dish (internal diameter = 5 cm) containing 10 ml of simulated saliva. Disintegration time was considered as a time when film starts to disintegrate. All the measurements were done in a triplicate and average value was reported [137].

7.3.4.2. Weight Variation

Film of 4 cm² area was cut from different batches of the formulations and weights were noted on electronic balance. The estimations were carried out in triplicate.

7.3.4.3. Thickness

The thickness of different films was measured using a digital micrometer with an accuracy of 0.001 mm. Thickness was measured at 4 different locations of the film and the average thickness was reported [137].
7.3.4.4. Scanning Electron Microscopy (SEM) Analysis

A small piece of the film was fixed on the carbon tape and the samples were mounted on the SEM sample stab using a double-sided adhesive tape. The mounted samples were sputter coated with gold (200 Å) under reduced pressure (0.001 torr) for 5 min to improve the conductivity using an Ion sputtering device and analyzed with SEM (SEM-JEOL, JSM-7600F, Japan).

7.3.4.5. X-ray diffraction analysis

X-ray diffraction patterns were obtained using Rigaku miniflex 600 X-ray diffractometer (Rigaku Co., Tokyo, Japan) using cu-kα X-radiation. Instrument was operated at 600 watts (X-ray tube), with a fixed tube current of 15 mA and a voltage of 40 kV. The diffracted X-ray beam was monochromated by a graphite monochromator and a standard scintillation counter was used as the detector. X-ray diffraction patterns of individual polymers and oral films were recorded over the 2θ range of 4–80° (4-50° for drug loaded films; 4-80° for polymers and blank films) at a scan rate of 4°/min.

7.3.4.6. Differential Scanning Calorimetry (DSC) studies

DSC thermograms of the pure lercanidipine, blank films and lercanidipine containing films were recorded on a thermal analyzer (Shimadzu DT-60, Kyoto, Japan) by heating the samples from 30 to 300°C at a rate of 10°C/min in an inert nitrogen atmosphere.

7.3.5. Mechanical Properties of films

7.3.5.1. Folding endurance

Folding endurance value was obtained by repeated folding of the strip at the same place till the strip breaks. The number of times the film is folded without breaking is figured as the folding endurance value [138].

7.3.5.2. Tensile strength, Young’s modulus and percent elongation

Tensile strength, Young’s modulus and percent elongation of film were evaluated using Universal testing machine (INSTRON 3366-10kN) equipped with 10 kN load cell. The film strip (5 × 1 cm²) was held in between the two clamps. Both clamps were positioned at the distance of 3 cm. Film was pulled by the upper clamp at the rate of 5 mm min⁻¹ until it tears, to determine the above parameters [138].
7.3.6. Re-dispersion of nano-particles from films

Rectangular films with an area of 4 cm$^2$ were placed in approximately 10 ml of de-ionized water. The particle size of this dispersion (nanoparticles re-dispersed from films) was measured after 10 min of magnetic stirring using Zeta sizer as explained above.

7.3.7. In-vitro dissolution studies

7.3.7.1. Preparation of simulated saliva

Simulated saliva was prepared by dissolving sodium chloride (8 g), potassium phosphate monobasic (0.19 g) and sodium phosphate dibasic (2.38 g) in 1 L of distilled water and pH was adjusted to 6.8 [140].

7.3.7.2. In-vitro dissolution studies

The dissolution study of fast dissolving film was carried out in a beaker containing 30 ml of simulated salivary fluid (pH 6.8) as a dissolution medium, maintained at 37 ± 0.5°C. Fast dissolving films equivalent to 4 mg of lercanidipine were used for dissolution studies. The medium was stirred at 100 rpm. Aliquot of 1 ml was withdrawn from dissolution medium at different time intervals between 0.5 and 5 min and the same amount were replaced with the fresh medium. Samples were analyzed by using HPLC [141].

In order to predict and correlate the release behavior of lercanidipine from different formulations and different dissolution media, in vitro drug release data was evaluated kinetically using various mathematical models like zero-order, first-order and Higuchi’s model [142].

7.3.8. Ex-vivo permeation studies

Permeation studies were performed using the Franz diffusion cell of internal diameter of 2.5 cm. Porcine oral mucosa was used as the model membrane. The buccal pouch of the freshly killed pig was procured from the local slaughter house. The buccal mucosa was excised and trimmed evenly from the sides and washed in isotonic phosphate buffer (pH 7.4). The mucosa was mounted between the donor and receptor compartments. The receptor compartment was filled with 30 ml of isotonic phosphate buffer of pH 7.4 + 40% v/v PEG 400, which was maintained at 37 °C ± 0.5 °C and stirring was achieved with a magnetic bead at 100 rpm. A film of area equivalent to 4 mg of drug was placed in intimate contact with the mucosal surface of the membrane that was previously moistened with 2 ml of simulated saliva.
Samples were withdrawn at suitable intervals up to one hour, replacing the same amount with the fresh medium.

7.3.9. In vivo pharmacokinetic studies

7.3.9.1. Dosage administration to rabbits

In vivo studies were conducted on 8 New Zealand albino male rabbits weighing between 1.5 and 2 kg. The animals were kept in individual metal cages for 10 days prior to the experiment. They were provided with standard diet and water ad libitum. The approval of the Institutional Animal Ethics Committee was obtained before starting the study, and it was conducted according to the institutional guidelines. The rabbits were kept in fasting condition for 24 h before the experiment commenced. The rabbits were grouped into two, each group containing four rabbits. Group I was administered with oral lercanidipine. To study the oral pharmacokinetics of drug, 5 mg of lercanidipine was filled in gelatin capsule and capsule was administered to rabbits \( (n = 4) \) using an oral catheter. For buccal administration of film, orodispersible film containing 5 mg of lercanidipine was administered to group II. No anaesthesia was used for the study. Blood samples of 1 ml were collected periodically through the marginal ear vein. After centrifugation, 0.6 ml of the plasma was separated and analyzed by the developed and validated HPLC method in laboratory [135].

7.3.9.2. Analysis of lercanidipine HCl in plasma

A validated HPLC method was used to analyze the lercanidipine HCl in plasma. The analysis was carried out on a Phenomenex HyperClone™ 5μm BDS C18 (130A° 250 × 4.6 mm) reversed-phase column, using a mixture of acetonitrile, and ammonium acetate buffer pH 4.5 (70:30) as the mobile phase. Felodipine was used as an internal standard. Lercanidipine was extracted from rabbit plasma using acetonitrile as a protein precipitating agent.

7.3.10. Accelerated Stability Studies.

Stability testing was carried out as per the ICH guideline Q1A (R2). LR-FDO 2 was selected as optimized formulation based on the physical properties, release profile and permeation properties. Formulation was wrapped in aluminum pouch and was sealed. It was stored at accelerated \( (40 ^\circ C \pm 2 ^\circ C/ 75\% \text{ RH } \pm 5\% \text{ RH}) \) condition for a period of 6 months. Films were evaluated for drug content, in vitro disintegration time, in vitro dissolution, X-ray diffraction studies and SEM analysis.
7.4. Formulation and Development of Thermo-Responsible Nasal Gel Containing Lercanidipine Nano-Particles

For nasal drug administration dosage volume should be maintained in between 25-200 µl otherwise anterior leakage of drug takes place [10]. Therefore nanosuspension containing lercanidipine 20 mg/ml was prepared and further transformed into nasal gel without lyophilizing or spray drying. Incorporation of nanosuspension in nasal gel assists in delivering higher amount of drug in a lower volume thereby completely eliminating possibility of anterior leakage. Also Leronidipine has water solubility as low as 5µg/ml. Therefore nanosuspension of lercanidipine also helps in incorporating lipophilic drug in aqueous gel matrix. Methodology of preparation of nano-suspension and its incorporation in thermo-responsive nasal gel is described in following flowchart.

### 7.4.1. Preparation and optimization of lercanidipine nanosuspension

Lercanidipine nanosuspension was prepared by evaporative antisolvent precipitation technique. Preliminary studies were carried out to investigate the solubility of lercanidipine in various solvents. 80 mg of lercanidipine was dissolved in sufficient volume (approx 800 µl) of methanol while the polymers/surfactants (stabilizers) were dissolved in water (4 ml) separately. Poloxamer and/or TPGS were used as a stabilizer in the preparation of nanosuspension. The resulting stabilizer solution was kept under high speed homogenizer (Polytron PT 1300D) at homogenization speed 10000 rpm. The temperature of the stabilizer solution was maintained at 10°C. The drug solution was added all at once in stabilizer solution kept under high speed homogenizer. After complete addition of the drug solution,
homogenization was continued for 15 minutes [139]. Nanosuspensions were kept for low speed stirring for 4 hours to evaporate residual amount of methanol.

7.4.2. Preparation of thermo-responsible poloxamer-TPGS gelling composition

Several poloxamer 407-TPGS 1000 in-situ gelling compositions were made. First poloxamer 407 solution of desired concentration (15-20 % w/v) was prepared by cold technique as described by Schmolka [143]. In this clear solution of poloxamer desired amount of TPGS 1000 (0.75-1.25% w/v) was added. After complete dissolution of TPGS, in-situ gelling temperature of prepared compositions was measured.

7.4.2.1. In-situ gelling temperature

Above in-situ gel formulation (2 ml) was transferred to a test tube placed in the isothermic water bath of 15°C. The temperature of water bath is allowed to increase gradually in increments of 2°C and sample was allowed to equilibrate at each new temperature. However, in the region of Tsol–gel temperature was allowed to raise slowly in increments of 0.5°C. The formulation was examined for gelation which is said to have occurred when the meniscus no longer moved upon tilting through 90° [144]. Prepared in-situ gelling compositions and their gelling temperatures were shown in results and discussion.

It was observed that 14.5% poloxamer with 1% TPGS possesses gelling temperature of 29.5 C therefore further selected for addition of mucoadhesive agent.

Short nasal residence time of drug formulation in nasal cavity due to mucocilliary clearance is one of the drawbacks of nasal drug delivery system. To enhance the retention of formulation in nasal cavity mucoadhesive polymeric approaches have to be used. Poloxamer is considered as weak mucoadhesive agent due to its non-ionic nature and low molecular weight [145]. Therefore there is need for addition of mucoadhesive agent in poloxamer-TPGS solution. Selection of mucoadhesive agent was performed based upon 2 methods. (a) Retention on agar plate method, (b) Measurement of Zeta potential.

7.4.2.2. Retention on agar plate

14.5% poloxamer with 1% TPGS prepared by cold dispersion method as described above. In that desired quantity (0.1-0.5%) of mucoadhesive polymer added by magnetic stirring. Mucoadhesive polymers like Sodium Alginate high viscosity, HPMC E-15,HPMC K-15,Sodium Carboxy methyl cellulose, Pullulan, carbopol 934, chitosan lactate, Gelatin, Hydroxy propyl
cellulose (HPC) were selected for addition in poloxamer-TPGS solution the range of 0.1-0.5% respectively.

Mucoadhesion parameter was determined by a method modified from that of Suzuki et al. (1985). An agar plate (containing agar at 1.5% w/v) of 8.7 cm in diameter was prepared with saline pH 7.4 phosphate buffered. 200 µl formulation was taken in the syringe and syringe was placed in the vacuum oven at 34±2°C to convert the solution in semisolid gel. This gel sample was placed/injected on the center of the agar plate and made a circle. The plate was slanted at 30 °, and the longest movement distance of the sample at 34°C was measured [146].

7.4.2.3. Zeta potential

Nanoparticles with a zeta potential between -10 and +10 mV are considered approximately neutral, while nanoparticles with zeta potentials of greater than +30 mV or less than -30 mV are considered strongly cationic and strongly anionic, respectively [147]. Therefore zeta potential of 1% solution of above mentioned each mucoadhesive polymer was measured to predict type of its interaction with mucin.

0.1% of chitosan lactate was selected as mucoadhesive agent for addition in poloxamer-TPGS solution based upon the result obtained.

7.4.3. Preparation of in-situ nasal gel incorporated with lercanidipine nanoparticles

To incorporate the lercanidipine nanoparticles in thermo-responsive nasal gel, nanosuspension containing definite amount of poloxamer 407 and TPGS 1000 as a stabilizer was added with appropriate amount of poloxamer 407 granules to attain the final poloxamer concentration of 14.5%. The mixture was then stirred with the magnetic stirrers in an ice bath until all the poloxamer granules were completely dissolved and clear solution was obtained. TPGS is used as a nanosuspension stabilizer in a concentration of 1.25%. Therefore volume was made up to attain final TPGS concentration of 1% and 0.1% of chitosan lactate was then added.

7.4.4. Characterization of in-situ nasal gel

7.4.4.1. Drug content in-situ nasal gel

Specific amount of ‘sol’ equivalent to 10 mg of drug was taken in 10 ml of volumetric flask. Volume was made up with methanol and mixed thoroughly using vortex mixer to extract the
drug completely. Sufficient dilutions were made to get the appropriate concentration of 10 μg/ml. Such six solutions were injected continuously and the active ingredient was assayed by using validated High-performance liquid chromatography (HPLC) method.

7.4.4.2. Analytical method for estimation of lercanidipine HCl

Analysis was carried out under optimized chromatographic condition on a Kromasil (100-5c18 250×4.6 mm) column using Shimadzu HPLC system. The mobile phase consisted of mixture of ammonium acetate buffer (20 mmol ~ pH 4.5) and acetonitrile in the ratio of 10:90, v/v. It is pumped through the chromatographic system at a flow rate of 1 ml min⁻¹. The detection was carried out at 240 nm using UV-VIS detector [141].

7.4.5. Rheological behavior

Viscosity was measured on MCR 102Rheometer (Anton Paar India Pvt. Ltd.). Viscosity measurements were made at variable temperatures and shear rate 5s⁻¹. For temperature dependency study, formulation was subjected to temperature from 15 to 35°C. During this testing the temperature was raised gradually and viscosity of the sample was measured after attaining the set temperature. Measurements were done in triplicate. Rotation speed of spindle was kept at 1.53 rpm [144].

7.4.6. Mucoadhesive strength

The mucoadhesive potential of the developed formulation was determined by measuring the force required to detach the formulation from goat nasal mucosal tissue using a modified method described by Majithiya et al [11]. Adhesion test was performed using TA.HD.plus Texture Analyzer (Stable Micro Systems). An analytical probe of area 0.5 cm² was depressed into each sample to a defined depth (15 mm), at a defined rate (0.1 mm/sec). A trigger force of 3 g was applied. Data collection and calculation were done with ‘Exponent Software’ [144].

7.4.7. SEM Studies

Morphology of the particles produced via evaporative antisolvent precipitation technique and their distribution in in-situ gel was observed by Field emission scanning electron microscopy (SEM-JEOL, JSM-7600F, Japan). A drop of thermo-responsible gel was spread on the coverslip and left overnight in vacuum oven to dry. This coverslip placed on top of carbon tape and analyzed.
7.4.8. *In vitro release studies*

Lercanidipine release studies were carried out in USP Dissolution test apparatus Type II (TDT 06T, Electrolab, India). A dialysis bag (M. Wt. cutoff: 12,000–14,000, Sigma-Aldrich, India) was filled with 2.5 ml of lercanidipine *in situ* gel formulation and nanosuspension equivalent to 30 mg of drug. Also 30 mg plain drug was dispersed in 2.5 ml of water and filled in dialysis bag. Test was carried out at 35 ± 1°C in 300 ml of Simulated Nasal Electrolyte Solution (SNES) + 2.5% tween 80 as a dissolution medium using agitation speed 100 rpm (n = 3). The SNES was composed of 7.45 mg/ml NaCl, 1.29 mg/ml KCl and 0.32 mg/ml CaCl$_2$.2H$_2$O and pH adjusted to 5.5 [12]. Aliquot (2 ml) of the dissolution medium was withdrawn at different time intervals. The amount of lercanidipinereleased was determined by previously validated HPLC method [144].

7.4.9. *Ex-vivo permeation studies*

The freshly excised goat nasal mucosa, except for the septum part, was collected from a local slaughter house in phosphate buffer saline (PBS), pH 7.4. The membrane was kept in PBS pH 7.4 for 15 min to equilibrate. The superior nasal conche was identified and separated from the nasal membrane. The excised superior nasal membrane from turbinate region was then mounted on Franz diffusion cells. The Franz diffusion cells used for in vitro diffusion studies has an internal diameter of 2.5 cm and the thickness of the mucosa is 1 mm. The temperature of the receiver chamber containing 100 ml of phosphate buffer of pH 7.4 + 2.5 % v/v tween 80 was controlled at 37 °C±1. Diffusion media were continuously stirred with a Teflon-coated magnetic bar at a constant rate, in such a way that the nasal membrane surface just flushes the diffusion fluid. A volume of 2 ml of gel was placed in the donor compartment of the Franz diffusion cell. Samples from the receptor compartment were withdrawn at predetermined time intervals and analyzed using a previously developed and validated HPLC method. Each removed sample was replaced by an equal volume of diffusion media. Each study was carried for a period of 8.0 h, during which the drug in receiver chamber (mg/ml) across the goat nasal membrane was calculated at each sampling point [144].

7.4.10. *Nasal Cilia Toxicity*

Freshly excised goat nasal mucosa, except for the septum, was collected from the slaughter house in saline phosphate buffer pH 7.4. Two goat nasal mucosa pieces with uniform thickness were selected and mounted on Franz diffusion cells. One was treated with 1 ml of saline phosphate buffer pH 7.4 (negative control). Another mucosa piece was treated with 1
ml of formulation for 8h. After 8 h, the mucosa was rinsed with saline phosphate buffer solution pH 7.4. Mucosa pieces were fixed in 10% formalin solution and sectioned with a rotary microtome. The sectioned tissues were stained with hematoxylin and eosin and subjected to histological studies and photographed by microscope [144].

7.4.11. In vivo pharmacokinetic studies

7.4.11.1. Administration of dosage to rabbits

In vivo studies were conducted on 8 New Zealand albino male rabbits weighing between 1.5 and 2 kg. The animals were kept in individual metal cages for 10 days prior to the experiment. They were provided with standard diet and water ad libitum. The approval of the Institutional Animal Ethics Committee was obtained before starting the study, and it was conducted according to the institutional guidelines. The rabbits were kept in fasting condition for 24 h before the experiment commenced. The rabbits were grouped into two, each group containing four rabbits. Group I was administered with oral lercanidipine. To study the oral pharmacokinetics of drug, 5 mg of lercanidipine was filled in gelatin capsule and capsule was administered to rabbits (n = 4) using an oral catheter. For nasal administration of thermosensitive gel, 0.6 ml of sol was instilled into one nostril of the rabbit with the help of a micropipette. No anaesthesia was used for the study. Blood samples of 1 ml were collected periodically through the marginal ear vein. After centrifugation, 0.6 ml of the plasma was separated and analyzed by the developed and validated HPLC method in laboratory [148].

7.4.11.2. Analysis of lercanidipine HCl in plasma

A validated HPLC method was used to analyze the lercanidipine HCl in plasma. The analysis was carried out on a Phenomenex HyperClone™ 5µm BDS C18 (130A° 250 × 4.6 mm) reversed-phase column, using a mixture of acetonitrile, and ammonium acetate buffer pH 4.5 (70:30) as the mobile phase. Felodipine was used as an internal standard. Lercanidipine was extracted from rabbit plasma using acetonitrile as a protein precipitating agent.

7.4.12. Stability studies

Stability testing was carried out as per the ICH guideline Q1A (R2). LR-FDO 2 was selected as optimized formulation based on the physical properties, release profile and permeation properties. Formulation was packed in plastic tubes. It was stored at accelerated (25 °C ± 2 °C/ 60% RH ± 5% RH) condition for a period of 6 months. Thermo-responsible gel was
evaluated for appearance (clarity), drug content, in vitro disintegration time, in- vitro dissolution and SEM analysis.