ABSTRACT

Analytical & Bioanalytical Method Development: A specific, accurate and precise reversed phase high performance liquid chromatographic method was developed and validated for the quantification of lercanidipine in nanoproliposomes and polymeric nanoparticles. The developed method mobile phase comprised of acetonitrile and potassium dihydrogen phosphate buffer (25 mM; pH 3.5) at the ratio of 70:30% v/v at a flow rate of 1.0 ml/min. The separation of lercanidipine was carried out on a Phenomenex® Gemini C_{18} (250 × 4.6 mm, 5µ) column using UV-visible detector set at 242 nm. The method was found to be specific for the analysis of lercanidipine in the developed novel carrier systems. The calibration curve was linear over concentration range 0.5 to 25.0 µg/ml with coefficient of determination $r^2 > 0.999$. The limit of detection and limit of quantification was found to be 0.05 µg/ml and 0.1 µg/mL, respectively. The method meets validation criteria in accordance with ICH Q2(R1) guidelines indicating its usefulness in quantification of lercanidipine in novel carriers. Also, a sensitive, accuate and precise high performance liquid chromatographic method was developed for quantification of lercanidipine in rabbit plasma. Protein precipitation method was utilized to extract lercanidipine in rabbit plasma using 0.2% v/v HCl in methanol as protein precipitating agent. Valdecoxib was used as an internal standard. The chromatographic separation was achieved by Hichrom Kromosil 100-5C_{18} (250 × 4.6 mm id) column and the effluent was monitored by an UV-visible detector set at 242 nm. The mobile phase consisted of mixture of acetonitile: 25mM potassium dihydrogen phosphate buffer (pH 3.5) at a ratio of 50:50% v/v at a flow rate of 1 mL/min. The developed method was linear over the range of 25-2000 ng/ml with coefficient of determination of greater than 0.99. The method was validated as per USFDA guidelines. The developed method was successfully used for the preclinical pharmacokinetic studies of novel carrier systems in rabbits.

Formulation of Lercanidipine Loaded Nanoproliposomes: Lipid based nanomedicines have received greater attention recently to deliver poorly water soluble and lipophilic compounds. Proliposomes, one such drug delivery system, finding multiple applications beacuse of their notable advantages such as protection of drug molecules from gastrointestinal degradation, improve drug solubilization, permeation across the gastrointestinal barrier and there by enhances bioavailability of the compounds. Lercanidipine, a calcium channel blocker, is practically insoluble in water and undergoes extensive first-pass metabolism with absolute bioavailability of

10%. Therefore, to overcome these poor biopharmaceutical properties, in the present study, an attempt was made to develop freeze dried, free flowing nanoproliposomes of lercanidipine using modified thin-film hydration method. The formula optimization of lercanidipine nanoproliposomes was achieved by evaluating various types of lipids and cryoprotectants. Amongst all the screened batches, the optimized batch (B. No. PL-06) comprised of SPC, cholesterol and trehalose. This formulation showed a particle size of 174.7 nm and entrapment efficiency of 85.35%. The AFM and TEM image analysis results displayed smooth, spherical to oval shaped vesicles with homogeneous size distribution. DSC studies suggest amorphization of drug in the nanoproliposomes whereas XRD diffractograms denoted amorphous state of encapsulated drug in the lipidic matrix, which is in agreement with DSC results. The formulation exhibited a biphasic release pattern characterized by an initial rapid release of 19.33% within 1 h followed by a sustained release profile up to 14 h releasing 88.37% of the encapsulated drug. The *in situ* single-pass perfusion studies demonstrated a significant increase ($p < 0.05$) in absorption with new formulation across rat intestinal membrane. The pharmacokinetics of novel form signified 2.75-fold increase in the absolute bioavailability as compared to free lercanidipine. The efficacy of formulated lercanidipine nanoproliposomes in treating hypertension as well as its role in maintaining the therapeutic levels for extended periods of time was observed in DOCA-Salt induced hypertensive rats. These findings suggest nanoproliposomes as a promising approach in improving oral bioavailability and bioactivity of lercanidipine.

Formulation of Lercanidipine Loaded Nanoparticles: Aim of the present study was to investigate the potential of polymeric nanoparticles in improving the biopharmaceutical properties of lercanidipine. Lercanidipine loaded nanoparticles were prepared by modified emulsion-diffusion-solvent evaporation technique. The resulting nanoparticles were freeze dried and then characterized for particle size, zeta potential, encapsulation efficiency, DSC, XRD, AFM, TEM and *in vitro* drug release. The absorption behaviour of lercanidipine loaded nanoparticles was assessed by *in situ* single-pass perfusion technique across the rat intestine. The novel nanoparticles were also evaluated for pharmacokinetics in rabbits and *in vivo* antihypertensive efficacy in DOCA-Salt induced hypertensive rats. Amongst all the screened batches, optimized batch (B. No. PN-05) exhibited a particle size of 200.2 nm, PDI of 0.130, zeta potential, -20.6 mV and encapsulation efficiency of 77.41%. The AFM analysis illustrated spherical, non-aggregated, smooth particles whereas TEM images showed smooth, regularly

spherical homogeneous mass of the particles. DSC studies suggested amorphization of the drug in nanoparticles and same was also observed in XRD diffractograms as reflected by the amorphous humps. The optimized batch exhibited a significantly different drug release profile from that of lercanidipine. It showed an initial rapid release of 17.96% in 1 h and followed an extended release profile releasing 82.74% in 24 h. The *in situ* single-pass perfusion studies showed a significant increase ($p < 0.05$) in absorption of nanoparticles across the rat intestinal membrane. The novel polymeric lercanidipine nanoparticles demonstrated 2.12-fold increase in absolute bioavailability as compared to free lercanidipine. Acute administration of lercanidipine loaded nanoparticles in DOCA-Salt induced hypertensive rats exhibited a significant decline ($p < 0.05$) in blood pressure immediately from initial hour and effect was prolonged until 24 h. In conclusion, encapsulation of lercanidipine in nanoparticles forms a sound basis for improving its bioavailability and for better management of hypertension.