SOLUBILITY ENHANCEMENT AND DISSOLUTION METHOD DEVELOPMENT OF SOME POORLY SOLUBLE DRUGS

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Content

Abstract

According to BCS classification system, drugs are classifies in 4 different classes. Drugs belonging to BCS Class II are characterized by low solubility and high permeability. The dissolution and thus the *in vivo* performance of this class of drugs widely depend on their solubility and hence their behaviour in dissolution medium.

Lercanidipine Hydrochloride (LER) is L-type calcium channel blocker widely used in the management of hypertension. Oral bioavailability of LER is limited to 10 % only. LER is not official in any pharmacopeia and no official dissolution method is available for the same. The work on LER involves development and validation of dissolution method and also attempts are made to increase solubility to improve its bioavailability.

The present work is focussed on development and validation of a dissolution test that can be used as a quality control test for Lercanidipine hydrochloride tablets and formulations. Saturation solubility and sink conditions that can be achieved in different medium suggested that 0.1 N HCl, Acetate buffer pH 4.5 and Phosphate buffer pH 6.8 can be used as dissolution medium. Dissolution tests of Lercanidipine hydrochloride tablets were carried out in different media at different rotation of paddle apparatus. The most suitable dissolution conditions were 0.1 N HCl buffer (900 mL at 37 ± 0.5 ° C) as dissolution medium and USP type II (paddle) apparatus at100 rpm for 60 min. The analysis of released Lercanidipine hydrochloride was done by UV spectrophotometric method. The developed method was validated according to ICH guidelines. Method showed linearity with r^2 of 0.999 within the concentration range of 2-20 µg/ml. The method was found to be accurate with recoveries ranging from 98.50 % to 103.72 %. The interday and intraday precision was below RSD 2%. The developed method can effectively be used for quality control evaluation of Lercanidipine hydrochloride tablets.

Solid dispersions of LER were made to form a molecular dispersion of LER into hydrophilic polymer and to increase its water solubility. Hydrophilic polymer such as PVP K30, PEG6000 and PEG 8000 were preliminary screened for selection. Finally, solid dispersions of LER with PEG6000 were prepared by solvent evaporation and melt fusion techniques in different drug to polymer ratio. The solid dispersion formed demonstrated increased solubility. Solid dispersion obtained by solvent evaporation and fusion techniques showed improved release i.e. 93.7% and 57% respectively as compared to pure LER and physical mixture 37.2% and 38.9% respectively in 1hour min. Formation of amorphous solid dispersion in ratio of 1:6 by solvent evaporation method was confirmed by DSC and PXRD studies.

Further effect of cyclodextrin derivatives (CDs) on solubility and dissolution of Lercanidipine Hydrochloride (LER) was studied by preparing inclusion complexes with β-Cyclodextrin (βCD) and Hydroxy Propyl β-Cyclodextrin (HPβCD) using kneading and freeze drying techniques in different molar ratios. Interactions between CDs and LER in solutions were studied with the help of phase solubility. Comparison of dissolution profile in 0.1 N HCl suggested that the maximum interactions were observed when drug to CDs molar ratio was 1:1.5. Solid state characterization of inclusion complexes was done using Differential Scanning Calorimetry (DSC), powder X-ray diffractometry (XRD), ^{1}H Nuclear Magnetic Resonance (NMR) and Fourier transformation-infrared (FT-IR) studies to investigate types of interaction,. The results obtained of XRD and DSC demonstrated that crystalline structure of drug was changed to amorphous in inclusion complexes formulated using freeze drying techniques. In the NMR studies, shifts of signals relating to LER or CDs were found in inclusion complexes suggesting binding between LER and CDs. The inclusion complexes showed improved solubility and dissolution behavior compared to pure drug. Freeze dried inclusion complex of HPβCD and LER releasing 85% of drug in 30 min was considered optimized compared to inclusion complexes of LER with βCD which showed only 74% release in 30 min. These findings concluded that dissolution and in turn bioavailability of LER can be improved when an inclusion complex with HPβCD is formed in 1:1.5 molar ratios.

Both optimized formulations of solid dispersions and inclusion complexes were subjected to accelerated stability studies as per ICH guidelines. No significant change in % release and content was observed in case of both the formulations of LER.

A pharmacokinetic study for LER was performed to compare bioavailability of pure drug and optimized formulation of solid dispersion and inclusion complex by HPLC method. Pharmacokinetic parameters of optimized formulation of solid dispersion and inclusion complex were better than that of obtained with pure drug and marketed formulations.

Cilnidipine is relatively new antihypertensive agent of category calcium channel blocker. It belongs to BCS Class II and is practically insoluble in water and thus in gastrointestinal tract. It has a limited oral bioavailability of 13%. Attempts had made to use solubility enhancement technique to formulate cilnidipine in a solid dosage form with enhanced water solubility.

Liquisolid compacts of cilnidipine (CLN) After screening liquisolid compacts of CLN were prepared using Transcutol HP as non-volatile liquid, neusilin as a carrier material , cab-o-sil as a coating material, cross carmellose sodium as a disintegrant and magnesium stearate as lubricant . Amount of Transcutol HP and ratio of carrier to coating ratio was optimized using

3^2 full factorial designs. All the liquisolid systems obtained were analysed for pre compression and post compression parameters as per pharmacopeia and results were found to be in agreement with the limits specified. All the liquisolid systems showed faster release of CLN as compared to compressed tablets. Obtained optimized batch was subjected to characterization by FTIR, DSC, PXRD and SEM. DSC and PXRD results suggested loss of crystallinity of CLN in liquisolid compacts which is due to solubilisation of drug followed by dispersion at a molecular level which in turn lead to enhanced drug dissolution properties.

Cilnidipine nanosuspension was prepared in order to increase dissolution rate and in turn oral bioavailability of the drug. Precipitation of CLN from organic phase followed by ultrasonication process was used to formulate nanosupension. The effect of seven important process parameters namely concentration of poloxamer188 in aqueous phase, solvent to antisolvent ratio, concentration of drug, speed of agitation, amplitude of sonication, time of sonication and concentration of Tween 80 were investigated by applying placket and Burmann design. Out of all the parameters, concentration of drug in organic phase, solvent to antisolvent ratio and time of sonication was found to be most significant from the pareto chart. Further Box Behnken design was employed to optimize the process using these significant factors. All the batches of nanosuspension showed higher release of CLN than pure drug. The particle size obtained for Box Behnken design was found in range of 232.1-923.3nm. Entrapment efficiency of formulated nanosuspension batches were found to be in between 51.39 % to 96.12 %. Optimized formulation of nanosuspension was found to be stable in freeze dried form as well as in liquid form with zeta potential of -23.31 mV. The morphology of freeze dried nanosuspension was analysed by Transmission Electron Microscopy. PXRD and DSC analysis indicated that significant change in crystal structure of CLN was observed as compared to raw drug.

in vivo performance of optimized liquisolid compacts and nanosuspension was investigated by pharmacokinetic studies. Optimized formula of liquisolid compacts released 87 % CLN in 30 min and optimized nanosuspension released 85 %which is higher than that of pure drug.

Brief description on the state of the art of the research topic

Drug absorption, sufficient and reproducible bioavailability and/or pharmacokinetic profile in humans are recognized today as one of the major challenges in oral delivery of new drug substances[1]. Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility of drug molecules[2, 3]. Solubility is one of the important parameter to achieve desired concentration of drug in systemic circulation for pharmacological response to be shown. As a matter of fact, more than one-third of the drugs

listed in the U.S. Pharmacopoeia fall into the poorly water-soluble or water-insoluble categories. By many estimates up to 40 per cent of new chemical entities (NCEs) discovered by the pharmaceutical industry today are poorly soluble or lipophilic compounds[4]. It was reported a couple of decades ago that more than 41% of the failures in new drug development have been attributed to poor biopharmaceutical properties, including water insolubility[5,6].

The solubility issues complicating the delivery of these new drugs also affect the delivery of many existing drugs. Poorly soluble compounds also present many *in vitro* formulation obstacles, such as severely limited choices of delivery technologies and increasingly complex dissolution testing with limited or poor correlation to the *in vivo* absorption [7]. These *in vivo* and *in vitro* characteristics and the difficulties in achieving predictable and reproducible *in vivo/in vitro* correlations are often sufficiently formidable to halt development on many newly synthesized compounds due to solubility issues[8].

Many new drugs can be categorized as Class II or IV drugs according to the Biopharmaceutics Classification System Guidance (BCSG). BCS class II drugs are characterized by high membrane permeability, slow dissolution rate (due to low aqueous solubility), and high peroral dose. These drugs are poorly water soluble however they rapidly pass biological membranes once dissolved. The solubility or dissolution rate of a drug in this category is therefore a key factor in determining the rate and extent of its absorption. Since the absorption of these drugs is dissolution rate-limited their bioavailability can only be increased by solubility enhancement.

To enhance the solubility of BCS Class II drugs various techniques such as Particle Size Reduction [9], solid dispersions [10], Nanosuspension [11], Supercritical Fluid (SCF) Process [12], Cryogenic Techniques [13], Inclusion Complex Formation-Based Techniques [14], Cosolvency [15], Hydrotrophy [16]. Microemulsion [17], Nanocrystal [18], Self-emulsifying drug delivery systems [19], Liquisolid Compacts [20].

The marketed formulation of cyclodextrin based pharmaceutical product, nanotechnology based pharmaceutical product, solid dispersion etc are available showing better bioavailability [21].

Definition of problem

Solubility is an important determinant in drug liberation and absorption and hence plays a key role in its bioavailability [22]. For a drug to be absorbed, it must be present in the form of an aqueous solution at the site of absorption. Aqueous solubility of the drug can be regarded as a key factor responsible for low oral bioavailability of poor water soluble drugs thereby limiting their therapeutic potential. Other issues related to low oral bioavailability for a

sparingly soluble drug are lack of dose proportionality, substantial food effect, and high intra & inter subject variability, gastric irritancy and slow onset of action.General view on the solubility problem of various BCS Class II drugs suggests that their bioavailability is limited by drug solubility/dissolution and thus it is formulation dependent [23-25].

Developing a new chemical entity with effective pharmacological activity costs minimum of 1.3 billion dollars. Also, the growing percentage of NCEs displaying solubility issues demands technologies for enhancing drug solubility be developed to reduce the percentage of poorly soluble drug candidates eliminated from development. Hence the efforts made to increase the bioavailability of existing molecule by increasing their solubility can give effective and economic drug formulation.

Hypertension is a major contributor to global disease burden, occurring as an insidious accompaniment to aging populations. It is estimated to have caused 7.1 million premature deaths in 2002 and is an ever-increasing worldwide problem. It is a well-recognized risk factor for cardiovascular disease. A substantial majority of hypertensive patients require long-term drug therapy for appropriate blood pressure control. Although there are many classes of antihypertensive drugs for clinical use, calcium channel blockers (CCBs) have a special role in the management of hypertension owing to their well-established safety and efficacy [26]. Lercanidipine (LER) and Cilnidipine (CLN) are new generation lipophilic, dihydropyridine calcium antagonists with a long receptor half-life[27]. Both the agents belong to BCS Class II drugs having low solubility and high permeability. LER and CLN are available in dosage form of 10 mg and have mean half-lives of 2.8 h and 2.5 h respectively. After oral administration although LER and CLN is absorbed from the gastrointestinal tract, their absolute bioavailability is approximately 10% and 13 %.These pharmacokinetic parameters suggest that formulation with better bioavailability of Lercanidipine hydrochloride and Cilnidipine can be obtained if its solubility is enhanced.

Lercanidipine Hydrochloride is not official in any pharmacopeia and currently no quality control or discriminatory dissolution method is available for raw material and tablets [28]. And hence no dissolution method is available for the quality control testing of LER dosage forms.

Objectives

The objective of present study is to develop and validate a dissolution method for LER tablets that can be used as routine quality control test for evaluation of dissolution behavior of LER.

The objective of present work is to enhance the solubility of poorly water soluble drug Lercanidipine and Cilnidipine using solid dispersion, inclusion complex, liquisolid compacts and nanosuspension approaches. It would then be subjected to optimization and

characterization followed by *in vitro* and *in vivo* studies. This may result in rapid drug delivery, maximized therapeutic index, reduced side effects and reduced dose/frequency of dosing and perhaps cost of therapy.

Scope of Work

Dissolution method development and validation of Lercanidipine Hydrochloride tablets

Determination of sink conditions and selection of dissolution medium

Selection and Optimization of dissolution test parameters

Development of dissolution test method and validation according to guidelines

Formulation, optimization and characterisation of solid dispersion of Lercanidipine Hydrochloride

Selection of method of preparation and ratio of LER to polymer

Evaluation of physicochemical properties and *in vitro* drug release profile of prepared solid dispersions

Selection of optimized solid dispersion based on evaluation parameters and solid state characterisation of same

Formulation, optimization and characterisation of Inclusion complex of Lercanidipine Hydrochloride

Phase solubility of LER with β-Cyclodextrin (βCD) and Hydroxy Propyl β-Cyclodextrin (HPβCD)

Preparation of inclusion complex in different molar ratio of LER to βCD and HPβCD by kneading method and freeze drying method

Evaluation of saturation solubility and *in vitro* release

Solid state characterisation of inclusion complexes by FTIR, DSC, PXRD and ^{1}H NMR

Stability studies and photo stability study of LER, optimized solid dispersions and inclusion complex

in vivo studies for optimized Lercanidipine Hydrochloride solid dispersions and inclusion complexes

Formulation, optimization and characterisation of Liquisolid compacts of Cilnidipine

Screening of various carrier and coating materials according to their liquid loading capacity

Use of mathematical model to calculate required amounts of powder excipients and non-volatile liquid for the formulation of liquisolid compacts

Use of 3^2 factorial design to study the effect of independent variables

in vitro evaluation of prepared liquisolid compacts and selection of optimized formula

Solid state characterization of optimized liquisolid system

Formulation, optimization and characterisation of nanosuspension of Cilnidipine

Selection of excipients for the preparation of nanosuspension

Use of Placket and Burmann design to screen the significant process variable

Use of Box Behnken design to optimize the formulation of nanosuspension

Preparation and evaluation of check point batch based on results of Box Behnken design

Solid state characterisation of optimized nanosuspension

Stability and accelerated stability studies of optimized liquisolid compacts and nanosuspension

in vivo evaluation of optimized liquisolid compacts and nanosuspension

Original contribution by thesis

For solubility enhancement of LER, nanotechnology approach is much explored. Nanosuspensions of LER containing PEG 400 and TPGS 1000 as stabilizers were incorporation in Fast Dissolving Oral Films, nanoproliposomes using SPC and cholesterol, HPMC containing nanoparticles and solid self-emulsifying nanoparticles of LER has been reported [29-35]. However no literature has revealed use of solid dispersion technique and inclusion complex formation to enhance solubility of LER. Being a dispersion of drug in hydrophilic polymer at molecular lever, solid dispersion is a promising approach to improve solubility. Inclusion complex can also be explored as the preferred manufacturing process with high probability of increase in solubility of the drug in complex form.

Cilnidipine has been explored by scientists for solubility enhancement to a limited extent. Approaches such as solid dispersion with hydrophilic polymer, inclusion complex with HPβCD and microemulsion prepared by water titration has been reported [36-38]. However, till date approaches such as liquisolid compacts and nanosuspension has not been tried. Thesis work will emphasize on exploring novel techniques such as liquisolid compact using transcutol HP, neusilin and cab-o-sil along with nanosupension stabilized by poloxamer and tween 80. Liquisolid is one technique which will produce apparently dry, free flowing, and compressible powder. Liquisolid compacts of cilnidipine dissolved in a transcutol as a vehicle can provide enhanced drug release due to an increased drug solubility, a high surface area of the drug, and an improved wettability of the drug particles[39, 40]. Moreover liquisolid compacts has simple manufacturing process and can be commercialized to produce cilnidipine dosage form with improved bioavailability. In nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an

increased dissolution rate and therefore improved bioavailability. An increase in the dissolution rate of micronized particles (particle size < 10 μm) is related to an increase in the surface area and consequently the dissolution velocity. Preparation of nanosuspensions is more cost effective and technically more simple alternative, particularly for poorly soluble drugs and yield a physically more stable product [41].

Methodology of Research and Results

Part I

Pre formulation studies

Literature review to select BCS Class II Drugs

i] Characterization of drugs was done as per the details provided by supplier.

ii] Analytical methods were developed to facilitate analysis of LER and CLN.

iii] Analytical method development: UV and HPLC methods were developed to facilitate quantification of the active.

iv] Drug-excipient compatibility studies was performed to study interactions between drugs and excipients used during formulation.

v] Bioanalytical methods were developed to quantify drugs in plasma for *in vivo* studies.

Part II (Lercanidipine Hydrochloride)

Part II (a): Development and validation of dissolution method for Lercanidipine Hydrochloride tablets

For development of dissolution method the objective was set to achieve dissolution profile showing < 50% drug release in 15 minutes and > 85% drug release in 30 min for immediate release dosage form of Lercanidipine hydrochloride. Saturation solubility of LER was performed in 0.1 N HCl (pH 1.2), Acetate buffer (pH 4.5) and Phosphate buffer (pH 6.8) to determine sink condition status. LER has pKa 6.38 and hence it shows pH dependent solubility with minimal increase in the solubility beyond pH 6. Result suggested that acceptable sink condition can be maintained with the 0.1 N HCl and acetate buffer pH 4.5 than with phosphate buffer pH 6.8. Effect of different pH and different rotation speed was studied for dissolution of LER tablet in USP type II apparatus using 900 ml volume of dissolution media at 37 ± 0.5 °C. Sampling time of dissolution test was kept 10 min for total duration of 60 min. Aliquots collected were filtered and analysed by validated UV spectroscopic method. Dissolution profiles of Lotensyl® 10 tablets were generated with 0.1 N HCl (pH 1.2), Acetate buffer (pH 4.5) and Phosphate buffer (pH 6.8) with the rotation speed of 50, 75 and 100 rpm. Obtained dissolution profiles suggested that the set objective of % drug release and a plateau shaped dissolution release profile was obtained when dissolution

test was carried out using 0.1 N HCl (pH 1.2) as dissolution medium and with rotation speed of 100 rpm. Specificity study shows that at the detection wavelength of the analytical method, no interference of the excipients used in tablet was observed. Method was found to be linear in the range of 2-20 µg/ml. Accuracy was studied (n=9)by performing recovery studies of standard LER solution from the dissolution medium after stirring at appropriate speed for 60 min. Recovery so obtained was found to be in range of 98.50 % to 103.72 %. Interday and intraday precision results of dissolution method signifies that method is precise as NMT 2% RSD was observed for all the determinations. The validated method was successfully applied to observe the release pattern of LER from tablet dosage form and was found to follow first order release kinetics suggesting that the release of drug is directly proportional to the concentration.

Part II (b): Formulation and evaluation of Solid dispersion containing Lercanidipine Hydrochloride

Hydrophilic polymers such as PVP K30, PEG 6000 and PEG 8000 were preliminary tried to prepare solid dispersion of LER. Attempts were made to disperse LER into the hydrophilic polymer to increase its solubility and dissolution. Two conventional methods namely melt technique and solvent evaporation were tried to select one with more potential of dispersing LER into hydrophilic polymers. From preliminary studies it was observed that PVP K30 can form solid dispersion with the LER. However, the solid dispersion thus prepared showed processing problems upon storage and hence was not considered for further development. In comparison of PEG 6000 and PEG 8000, it was found that PEG 6000 shows better result in terms of solubility enhancement.Three different ratios of LER to PEG6000 1:3, 1:6 and 1:9 were explored to see the extent of solubility enhancement using melt fusion method and solvent evaporation method. Physical mixtures of LER and PEG6000 were compared with prepared solid dispersion with respect to saturation solubility and *in vitro* dissolution release. Saturation solubility of solid dispersions so prepared was found to be higher than the LER pure drug. Maximum increase in solubility was achieved with LER to PEG6000 ratio of 1:6. About 6.35 fold increase was seen in solid dispersion prepared by solvent evaporation method and 2.96 fold increase in solubility was obtained with solid dispersion prepared by melt technique. LER raw material showed release of nearly 40 % in 60 min, whereas solid dispersion prepared showed more than 80 % release of drug in only 30 min in 0.1 N HCl. Fastest and maximum release of LER was observed with solid dispersion prepared in 1:6 ratio by solvent evaporation method and was considered optimized and further evaluated for solid state characterization.

Solid state characterisation of the optimized solid dispersion was performed by FTIR, DSC and PXRD. An FTIR spectrum of LER shows characteristic sharp peaks confirming no interaction between PEG6000 and LER. However FTIR scan of solid dispersion shows disappearance of few peaks of FTIR suggesting incorporation of LER in PEG6000 which might be due to dispersion of LER at molecular level in PEG6000. DSC curve of LER showed a sharp endothermic peak corresponding to its melting point. A noticeable reduction in peak height and heat of fusion was observed in DSC curve of physical mixture and further more reduction was observed in DSC curve of solid dispersion. Physical state of solid dispersion was confirmed by PXRD studies in which LER showed many sharp peaks and the peak heights as well as number of peaks were reduced in the solid dispersion. All the observations of solid state characterisations suggested that the physical state of LER changed from crystalline to amorphous.

Part II (c): Formulation and evaluation of inclusion complex of Lercanidipine Hydrochloride and Cyclodextrin(s)

Two types of inclusion complexes were formed with β- cyclodextrin (βCD) and Hydroxy Propyl β- cyclodextrin (HPβCD). Phase solubility diagrams were plotted for LER and Cyclodextrins in distilled water at room temperature. From the slope of phase solubility diagram apparent stability constant was calculated for both βCD and HPβCD and the values obtained were within 100-1000 M^{-1} suggesting that proper interaction takes place between cyclodextrins and LER in solution state. Based on result of phase solubility, inclusion complex of LER with both βCD and HPβCD were prepared in different molar ratio (1:1, 1:1.5 and 1:2) using kneading method and freeze drying method. Prepared inclusion complexes were screened for their saturation solubility and *in vitro* release in 0.1 N HCl. Solubility and % release of LER was increased in the complexes with βCD/HPβCD. Upon comparing the results, it was observed that solubility of complexes formed in molar ratio 1:1.5 was higher than that was formed in molar ratio 1:1 and 1:2. Similar observations were seen for % release of LER also. Moreover effect of method of preparation was also clearly seen as inclusion complex formed by freeze drying method showed better solubility and release than the complexes prepared by kneading method. Saturation solubility of inclusion complex formed in 1:1.5 ratio by freeze drying method showed 2.79 fold increase with βCD and 4.16 fold increase with HPβCD. Compared to pure drug, βCD and HPβCD complexes (1:1.5, freeze dried) showed better *in vitro* release. Freeze dried βCD complex formed in 1:1.5 molar ratio showed more than 70% release in 30 min and that of HPβCD complex showed more than 85 % release in 30 min. Evaluation of inclusion complex for saturation

solubility and *in vitro* release concluded that inclusion complexes prepared by freeze drying method in a molar ration 1:1.5 is optimized for both βCD and HPβCD and were subjected to solid state characterisation.

βCD inclusion complex (1:1.5 molar ratio)

FTIR studies of LER, βCD, Physical mixture and inclusion complex suggested no interaction taking place in physical mixture as prominent peak of LER is still present. However in inclusion complexes, peaks of LER at 1520 cm $^{-1}$, 1485 cm $^{-1}$, 1344 cm $^{-1}$and 1232 cm $^{-1}$ showed shifting to 1524 cm $^{-1}$,1487 cm $^{-1}$,1347 cm $^{-1}$ and 1215 cm $^{-1}$,which suggests strong interaction taking place between LER and βCD in inclusion complex. DSC studies showed decrease in peak height and shifting of LER in inclusion complexes suggesting change in the crystalline form of LER. This conclusion were well supported by PXRD studies wherein number of crystalline peaks and their intensities were reduced in the inclusion complex as compared to pure LER and physical mixture. Thus results of DSC and PXRD confirm decrease in the crystalinity of LER upon complexing with the βCD. Inclusion of LER at molecular level in cavity of βCD was confirmed with ^{1}H NMR spectra. ^{1}H NMR spectra of inclusion complex showed downfield shift of aromatic protons of LER which suggests that prominent non covalent interaction is taking place between protons of LER and βCD.

HPβCD inclusion complex (1:1.5 molar ratio)

FTIR spectra of inclusion complex showed absence of LER peaks at 1982 cm^{-1}, 2564cm^{-1} 2612 cm^{-1} and 3081 cm^{-1} along with shifting of many other peaks , which were present in FTIR of physical mixture. This suggests that physical mixture of LER and HPβCD does not change physical properties of LER but inclusion complex shows that there is a change in LER environment takes place. DSC curve of physical mixture shows decrease in LER peak height and peak area, whereas that of inclusion complex shows total absence of peak of LER. This confirms that LER has converted from crystalline state to amorphous state in inclusion complex with HPβCD. These observations were confirmed by the PXRD pattern which shows disappearance of all the peaks of LER in the inclusion complex. ^{1}H NMR spectra of HPβCD inclusion complexes shows downfield shift of LER protons along with disappearance /merging of few of the peaks which is the additional observation to that of obtained with ^{1}H NMR spectra of LER and βCD inclusion complex. This concludes that formation of inclusion complex of LER is more prominent with HPβCD than βCD as more strong interaction is being observed with HPβCD inclusion complex. Accelerated stability studies was performed same as that of solid dispersion and no significant change was observed in % release and physical state of inclusion complex after 6 months.

Part III Cilnidipine formulations

Part III (a) Liquisolid compacts of Cilnidipine

Solubility of Cilnidipine was measured in different non-volatile liquids such as Poly Ethylene Glycol, PEG200, PEG400, PEG600, Tween 20, Tween 40, Tween 80 and transcutol. Out of all the non-volatile liquids, transcutol showed maximum solubility of 192.6 mg/ml and hence it was selected as non-volatile liquid to solubilize Cilnidipine. Similarly, angle of slide was measure to screen best carrier material amongst dicalcium phosphate, Avicel PH 102, Avicel PH 101 and neusilin and coating material from Aerosil, Aerosil 200 and cab-o-sil. From the result obtained, it was found that neusilin has maximum loading capacity as a carrier material and cab-o-sil has maximum loading capacity as a coating material. Compatibility study of selected excipients was performed by FTIR and no interaction was observed for any of the excipients. Hence liquisolid system was prepared using transcutol as non-volatile liquid, neusilin as carrier material and cab-o-sil as a coating material. To prepare liquisolid compacts, pure cilnidipine powder was dispersed in the non-volatile liquid vehicle Transcutol to form a liquid medication. Then, carrier (Neusilin) and coating (Cab-o-sil) materials were added to the liquid medication under constant mixing using a mortar and pestle, to produce a dry and free-flowing powder. Lastly 5 % w/w Croscarmellose sodium was added as a disintegrant and 1% w/w Magnesium stearate was added as lubricant into the liquisolid systems. 3^2 full factorial design was employed to obtain final optimized formula for liquisolid compact with maximum solubility and enhanced dissolution. For factorial design carrier to coating ratio (R) and weight of non-volatile liquid (W) was considered as independent variables and each at three levels. Angle of repose, disintegration time and cumulative drug release at 30 min was taken as dependent variables. Along with angle of repose, carr's index and hausner's ration of all the factorial batches were carried out. Post compression parameters such as friability, hardness, weight variation and content uniformity. 3^2 full factorial design so developed was validated using Design-Expert® Software (version- 9.0.6, Stat-Ease).

To check the reliability of applied mathematical model, check point batches covering the range of experimental domain with optimized results were prepared and evaluated. A close agreement was observed with the predicted and actual values of dependent variables of check point batches. This confirms that the mathematical model used was successfully validated.

Solubility of optimized batch was found to be 0.021 mg/ml and more than 80 % release was obtained in 30 minutes. Optimized batch obtained from the factorial design, was subjected to solid state characterization. FTIR spectra of liquisolid compact showed characteristic peaks

cilnidipine ruling out possibilities of any type of interactions. PXRD of liquisolid compact showed only peak corresponding to neusilin, whereas clear absence was noted for any crystalline peak of cilnidipine. This result is attributed to solubilisation of drug in non-volatile liquid followed by adsorption on neusilin and amorphisation of cilnidipine. DSC thermogram of physical mixture showed endothermic peak with low intensity as compared to pure drug. However DSC thermogram of liquisolid compact didn't show any peak which supports the conversion of crystalline cilnidipine to amorphous drug in liquisolid compacts. SEM was performed to study the surface characteristic of carrier molecule before and after adsorption of liquid medication. The images obtained showed successful adsorption of liquid cilnidipine onto neusilin.

Part III (b) Nanosuspension of Cilnidipine

Nanosuspension of Cilnidipine was prepared by precipitation-ultrasonication method. Formulation of nanosuspension is affected by many factors; screening of these formulation and process related factors by trial and error technique is time consuming and can be inaccurate at times. Hence, Plackett–Burman design was employed as the screening technique to determine the most significant factors that affected the formulation of microsponges using Design-Expert® software. The factor screened by the design were Conc of poloxamer 188 (X_1), solvent to antisolvent ratio (X_2), Concentration of drug (X_3) Speed of agitation (X_4), Sonication amplitude (X_5), Time of sonication (X_6), Concentration of Tween 80 (X7). The effect of these independent variables was checked on dependent variables (% average particle size and % release at 30 min). Pareto charts revealed that Concentration of drug (X_3) Speed of agitation (X_4), Sonication amplitude (X_5), Time of sonication (X_6), Concentration of drug, Time of sonication and Concentration of Tween 80 significantly affects the nanosuspension characteristics. Hence these factors were considered as critical.

Final optimization of cilnidipine nanosuspension was done using Box-Behnken design. A total of 17 batches were prepared as given by design expert. The effect of independent variables, selected from the screening design, was determined on the particle size, % release at 30 min and entrapment efficiency. The prepared nanosuspensions were evaluated for saturation solubility, *in vitro* release and solid state characterisation. The results indicated that the optimized formulation had entrapment efficiency of 81.31 ± 1.4%, solubility of 0.0288 mg/ml and drug release of more than 90 % in 45 minutes. Size of the optimized formulation was found between 389 to 401 nm with zeta potential of -23.31mV.

The optimized formulation was characterized by FTIR, DSC, XRD and TEM. FTIR of mixture of drug and polymer showed no interaction. XRD studies showed crystallinity of

Cilnidipine has reduced in nanosuspension form. DSC spectra supports the result obtained in XRD studies.

Part IV: Accelerated stability studies

The following samples were subjected to stability studies and photostability studies

LER – Solid dispersion prepared by solvent evaporation in 1:6 ratio with PEG6000

LER-Inclusion complex prepared by freeze drying method in 1:1.5 molar ration with cyclodextrin

CLN-Optimized liquisolid compact

CLN- Optimized freeze dried nanosuspension

Conditions: 1] 40 °C ± 2 °C Temperature and 75% ± 5% Relative humidity (RH) 2] Ambient conditions (all for 6 months)

Samples were inspected for saturation solubility, *in vitro* release, drug content and physical state. No significant changes in characteristics of formulations were observed.

Photo stability study suggests that LER and CLN in solution state are sensitive to sunlight and in solid form sensitive to UV light. Hence, the production processes has to be performed in a light protected environment.

Part V: *in vivo* studies

Pharmacokinetic study (C_{max}, t_{max}, AUC) for LER

The protocol (BIP/IAEC/2014/18 and BIP/IAEC/2015/03) for *in vivo* study was approved by the Institutional Animal Ethical Committee (IAEC) in accordance with guidance of committee for the purpose of control and supervision of experiments on animals (CPCSEA). Wistar/Sprague Dawley rats of either sex were divided into 03 groups as follows: Positive control (drug) and Test formulation (Optimized formulations of LER and CLN). The optimized formulations were given orally and blood was withdrawn at different time intervals from femoral arteries of the animals into heparinized vials, centrifuged and diluted appropriately for analysis by developed bioanalytical methods (HPLC for LER and HPLC MS/MS for CLN).

The analysis of plasma treated with optimized formulation showed improved t_{max} and c_{max} as compared to plain drug and marketed formulations.

Achievements with respect to objectives

1. Solid dispersion and inclusion complex of LER was prepared by solvent evaporation and freeze drying techniques respectively.
2. Optimized solid dispersion and inclusion complex were subjected to solid state characterization.

3. Solubility of solid dispersion was increased by 6.35 fold and that of inclusion complex was increased 4.16 fold than the pure drug
4. Liquisolid compacts of cilnidipine were prepared using transcutol, neusilin and cab-o-sil.
5. Formula of liquisolid was optimized using 3^2 full factorial design and optimized formulation of liquisolid compacts was subjected to *in vitro* and solid state characterization.
6. Nanosuspension of cilnidipine was prepared by precipitation-ultrasonication method. Placket and Burmann and box Behnken design was used to optimize the variables.
7. Optimized batch of nanosuspension was subjected to *in vitro* and solid state characterization.
8. Increase in solubility of Cilnidipine was obtained with liquisolid compacts (5.07 fold) and with nanosuspension (6.89 fold).
9. All the four prepared formulation showed better bioavailability than pure drug and marketed formulations.

Conclusion

Various techniques can be used to increase solubility and dissolution rate to increase bioavailability of BCS Class II drugs LER and CLN. **For LER**, Solid dispersion technique is proved to be most efficient in achieving manifold increase in saturation solubility and bioavailability. The method being simplest can be used for commercial production of LER dosage forms with better bioavailability. Cmax obtained for LER was 401.48 ng/ml, for Solid dispersion 1131.84 ng/ml and for inclusion complex 1316.08 ng/ml. Also t max was achieved in 0.5 h in case of solid dispersion and inclusion complex whereas pure drug took 0.75 h to show the maximum drug in circulation. Nanosuspension by bottom up technology was proved to be better in comparison with liquisolid compacts for **CLN**. Cmax obtained for CLN was 6.2 ng/ml in 1 h, for Liquisolid compact 9.7 ng/ml in 0.5 h and for Nanosuspension 23.63 ng/ml in 0.5 h. Thus it can be concluded that increase in solubility, dissolution showed better pharmacokinetic parameters compared to pure drug.

List of Publication

A) Oral Presentation

Title: Enhancement of dissolution of Lercanidipine Hydrochloride using solid dispersion technique

At 4th International Science Congress held at Pacific University, Udaipur on 8th and 9th December 2014.

B) Poster Presentation

Title: Use of cyclodextrin derivatives to enhance dissolution of Lercanidipine Hydrochloride.

At International Conference on Challenges in Drug Discovery and Delivery (ICCD#-2017) held at BITS, Pilani on March 2- 4, 2017.

C) Paper Published

1. Shaikh, FI, Patel VB, Enhancement of dissolution of Lercanidipine Hydrochloride using Solid Dispersion Technique. Research Journal of Recent Sciences,2015, 4, 299-307.
2. Shaikh FI, Patel MB, Surti NI, Patel VB. Preparation and Characterization of Lercanidipine Hydrochloride Inclusion complex with β-cyclodextrin and effect of Complexation on Solubility and Dissolution. Research Journal of Pharmacy and Technology, 2017(in press).
3. A research article titled (Dissolution method development and validation for Lercanidipine Hydrochloride) has been submitted to Research Journal 'Dissolution Technologies' and it's under revision.

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