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PREPARATION OF LERCANIDIPINE SOLID DISPERSIONS BY VARIOUS POLYMERS, *IN VITRO* AND *IN VIVO* COMPARATIVE PHARMACOKINETICS STUDY IN RABBITS

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ABSTRACT

This research aimed to prepare Lercanidipine solid dispersions with polymer blend equal portion of poly vinyl pyrrolidine (PVP) K-30, poloxamer-188, and hydroxy propyl methyl cellulose (HPMC) K4M. Various ratios of Lercanidipine Polymer blend in the ratios (1:3 and 1:7) were fabricated as solid dispersions by melting and solvent evaporation methods, later compressed into tablets. The solid dispersions were tested for physicochemical, and release constraints impressed with the increase in the solubility. Among them formulation with a 1:7 ratio found to be the best proportion for enhancing the solubility and release rate of Lercanidipine from the solid dispersions. LSD-4 was selected for the In vivo study Cmax was increased by 3.37 times, T max values of the formulations LSD-4 was equivalent, the AUC (0-8h) was ~4 folds more and the AUC (0-∞) was marginal increase i.e., 4.12 folds more than LCD pure drug. These fallouts suggest that the absorption rate and bioavailability of SD formulation is more when compare to pure

INTRODUCTION

The oral route is preferred, as they are easy to handle and take by patients of all age groups. Lercanidipine (LCD) is an antihypertensive drug (Talluri MK, et al 2012) belongs to BCS-class II drug and low solubility results in low bioavailability (~45%) (Yang L, et al 2014) Among the various techniques of solubility enhancing, solid dispersion (SD) technique (LeunerC, et al 2000). stands on the top priority as it is a simple, easy and efficient approach.

Water-soluble polymers viz., Poly Vinyl Pyrrolidone (PVP) K-30 (BhiseS, et al., 2011) Poloxamer-188 and Hydroxy Propyl Methyl Cellulose (HPMC) K4M (Zhong L, et al 2013) were employed with a promising role in increasing the solubility of drugs. In the present examination, the SD were prepared by melting and solvent evaporation techniques. The pharmacokinetic parameters of the prepared systems were

calculated after oral administration to rabbits as model animal

MATERIALS AND METHODS

Materials: Lercanidipine was gifted by Cipla Ltd, Bengaluru. PVP K30, Poloxamer-188, HPMC K4M, Microcrystalline Cellulose, Talc, and Magnesium stearate were procured from SD Fine chemicals India. Double distilled water was used whenever appropriate.

Methods

Preparation of solid dispersions

Melting: The polymers were melted based on their decreasing melting points (HPMC-K4M, PVP K-30 then Poloxamer-188) in a china dish, then LCD was dispersed in the molten mass with continual thrilling. The mixture was permitted to solidify at room temperature. The product was stored in a desiccator (ABG Initiatives, Hyderabad, Telangana) for 24 h and then crushed in a mortar (Aruna Scientific, Hyderabad, Telangana). Later the powder was allowed through # 60 sieve (ASTM E 11, Hyderabad, Telangana) to get uniform particle size (Mahmah O, et al., 2013).

Ingredients	Quantity per tablet
Solid dispersions equivalent to 40 mg of Lercanidipine	150
Lactose	75
Starch	15
Micro Crystalline Cellulose	50
Magnesium stearate	5
Talc	5
Weight of the tablets	300

Preparation of solid dispersion tablets: The SD equivalent to 40 mg of LCD was made by direct compression into tablet form, after blending (Liberman HA,1980) with constituents as described in table 2 by using 8 station tablet compression machines (Karnavati, India).

Evaluation:

Melting point: The purity of LCD pure sample was confirmed by measuring melting point. The temperature at which LCD melts was recorded using melting point apparatus (kaur M, et al.,2014). (MT934).

Solubility studies: Lercanidipine (LCD) pure drug was tested for solubility in 0.1N HCl, water, pH 4.5 Acetate buffer, pH 6.8 and pH 7.4 Phosphate buffers (Fraczek J, et al.,2007)

Drug-excipients compatibility studies: The DSC and FTIR studies were performed to find the interaction among the LCD and carriers used in the study.

Differential Scanning Calorimetry (DSC): Pure LCD and 1:1 ratio of LCD: Polymer mix were exposed to the analysis. About 10 mg sample was taken in a DSC crucible and scanned at 50-300°C (DSC-50, Shimadzu, Japan).

Fourier-transform infrared (FTIR) spectroscopic study: The interactions between components of the SD were investigated using FTIR spectroscopy. The FTIR spectra of the LCD alone and in combination with carriers were documented by the FTIR spectrometer (Bruker) by scanning at 4000-400 cm^{-1} range.

Scanning Electron Microscopy: The surface topography of SD was confirmed by scanning the surface of SD by scanning electron microscopy [25] (Perkin Elmer, USA). An accelerating voltage of 20KV was used and the images obtained at the magnification of x500.

Evaluation Lercanidipine Solid Dispersions

The subsequent results were examined for LCD-SD.

Flow properties

The LCD-SD were evaluated for flow constraints (Wong AC. Teal.,2000). (Bergstrom CA et al., 2003). viz., angle of

repose, true and tapped densities, Carr's Index, Hausner's ratio.

Yield: The % recovery (Juppo AM, et al., 2003). Comprises the weight of dried SD to the total weight of LCD and polymers used in making the SD.

Actual weight of the SD

% Yield = Actual weight of the SD/ Total weight of drug and excipients × 100

Evaluation Lercanidipine Solid Dispersion tablets

The LCD-SD tablets were exposed to the following assessments.

Uniformity in size and shape: The tablets were examined under a dissection microscope (DM-100) for their morphology.

Thickness: The LCD-SD tablets were firmed between the jaws of Vernier Calipers (Qumos Enterprises, India) and thickness is examined in triplicate. (Patel S, et al.,2016).

Uniformity in weight: Every batch of LCD-SD tablets (20 quantity) was individually weighed with an electronic digital balance (Citizen, CY-104, Mumbai, India) and mean measured and related to the individual tablet weights. The deviation in weights was premeditated and then crisscross with IP specifications (Limit ± 7.5% of mean weight) (Cruz J et al.,2011).

Hardness

LCD-SD tablets were pressed with the spindle of Monsanto tablet hardness tester (Vinsyst Technologies, Mumbai). The force needs to break the tablets were recorded in triplicates (Patel S, et al.,2016).

Friability

Surface erosion may happen while tablet handling can be elucidated by Roche Friabilator. Pre-weighed (10 tablets) (W initial) and placed and rotated for 4 min at 25 rpm and the final weight of tablets (W final) was dogged. The loss on friability was calculated by the following equation Bushra R et al,.2008).

Calibration curve

100mg of LCD dissolves in pH 1.2 of 0.1 M HCl. A series of dilutions (2, 4, 6, 8 and 10µg/ml) were prepared scanned spectrophotometrically at 291nm then the measured the absorbance vs. concentrations which gives a calibration curve (Pandey A, et al., 2011).

Uniformity of drug content: 5 tablets of each batch weighed and powdered. 40mg of LCD dissolved in 100ml of 0.1 N HCl (pH 1.2). From this 0.5ml was diluted to 5ml with pH 1.2 of 0.1 N HCl. The absorbance was estimated at 291nm using a double beam UV-Visible spectrophotometer (Lab India, Mumbai). The content uniformity was calculated from Lercanidipine standard calibration graph (Chaudhari P et al., 2013).

***In-vitro* drug release studies:** The dissolution specifications were as below Patei B et al., 2012).

- Apparatus used: USP-II dissolution test apparatus
- Dissolution medium: 0.1M HCl
- volume of dissolution medium: 900ml
- Temperature: 37±0.5°C
- Speed of basket paddle: 50rpm
- Sampling intervals: 5 min
- Sample withdraws: 10ml
- Absorbance measured: 291nm

***In vivo* studies**

All the experimental procedures used in the present study were conducted according the protocol for utilization of experimental animals set has been approved by the **IAEC/ANCP/2018-19** and **CPCSEA.** Healthy rabbits of either sex (weighing 1.5 – 2.5 kg) fasted overnight. LCD and its SDs were administered at a dose equivalent to 1.4mg/kg of LCD. Each product was repeated 6 times (n = 6). The in vivo experiments were conducted as a crossover study.

Estimation of Lercanidipine in serum samples: LCD in serum samples were estimated according to High Performance Liquid Chromatographic (HPLC) method.

Preparation of standard solution and plotting calibration curves

The Stock solutions of LCD and Nifedipine were prepared in acetonitrile at a free base strength of 1000 µg/ml. Secondary and working standard solutions were made with water: acetonitrile (50:50 v/v). Blank rabbit plasma was screened before spiking to ensure it was free of endogenous interference at retention times of LCD and the internal standard Nifedipine. The calibration curve ranged from 5.0-250.0 ng/ml. Quality control samples were prepared at 20, 100 and 200 ng/mL for LCD. The samples were vortexed and stored at –70±2^{o}C until processing.

Sample preparation

A 0.5 ml aliquot of animal plasma sample was mixed with 0.1 ml of internal standard working solution (2000.0 ng mL-1 of Nifedipine) and 500 µL of 10 % Perchloric acid (precipitating agent). Later vortexed for 5 min and centrifuged at 4000 rpm for 10 min. Supernatants from the above solutions were separated and used for the analysis. 20µL of the eluent was injected into the HPLC system.

Preparation of spiked plasma sample

250µl of rabbit plasma, 50µl of internal standard, a 10µl of LCD was pipette into 10ml centrifuge tube and to this 2ml of Acetonitrile was added. 10µl of the supernatant layer was collected (after centrifugation at 3200 rpm for 10min) and injected into HPLC. A typical chromatogram is achieved from a sample solution.

RESULTS AND DISCUSSION

Melting point: The melting point of pure LCD was observed as 197.1 ±0.5^{0}C, indicates the purity of the LCD sample. The LCD shown good solubility in 0.1N HCl (0.325±0.001µg/ml) compared in contrast to Water, Acetate buffer (pH4.5), Phosphate buffer (pH6.8) and Phosphate buffer (pH7.4).

Drug-excipients compatibility studies: The DSC thermograms of LCD with polymer mix were lifted to lesser temperature representing good impregnation of LCD with polymers used (figure 1). The FTIR study discovered that the typical peaks and stretches of LCD pure drug were also found in LCD – polymer blend, indicates no negative incompatibility of LCD with carriers used. The FTIR spectra of LCD pure and polymers were shown in figure 2 and

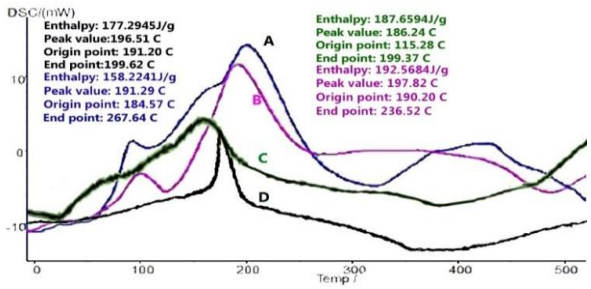


Figure 1: DSC thermograms of LCD A) Pure drug B) with PVP K C) with Poloxamer 188 D) with HPMC K4M

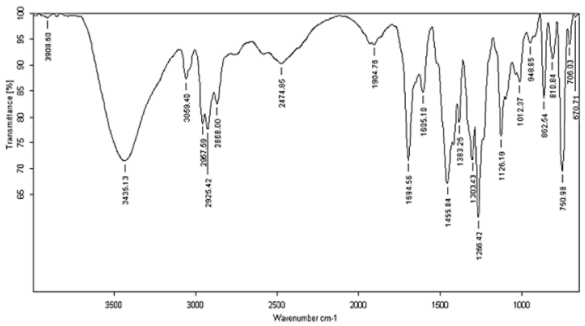


Figure2: FTIR spectrum of Lercanidipine pure drug

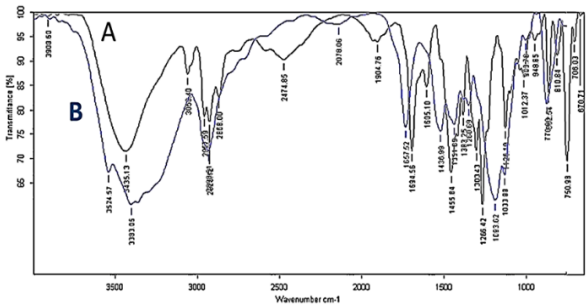


Figure 3. FTIR spectrum of Lercanidipine with a polymer blend

Formulation	Flow properties				
	Angle of repose (^{0})	Bulk Density	Tapped Density	Carr's Index	Hausner Ratio
LSD-1	25.45±0.45	0.549	0.563	2.4866	1.0255
LSD-2	26.08±0.14	0.598	0.632	5.3797	1.0568
LSD-3	26.95±0.25	0.562	0.599	6.1769	1.0658
LSD-4	25.51±0.84	0.528	0.549	3.8251	1.0397

	Physical parameter					
Formulation	Uniformity of weight (mg)	Hardness (cm^2)	Thickness (mm)	Friability (%)	Yield (%)	Assay (%)
LSD-1	302.5±3.25	6.3±0.02	4.50±0.04	0.18±0.01	94.65±1.59	98.06±1.65
LSD-2	300.2±1.23	6.2±0.04	4.51±0.03	0.44±0.02	95.79±1.62	97.69±1.12
LSD-3	301.8±1.45	5.8±0.11	4.52±0.06	0.65±0.03	96.65±1.53	95.46±1.06
LSD-4	301.1±2.16	7.4±0.15	4.50±0.02	0.49±0.02	97.15±1.16	98.51±1.71

Table 4: Physical Characteristics (Optimized formulation with Lercanidipine)

Values in mean ±SD; trials made (n=3)

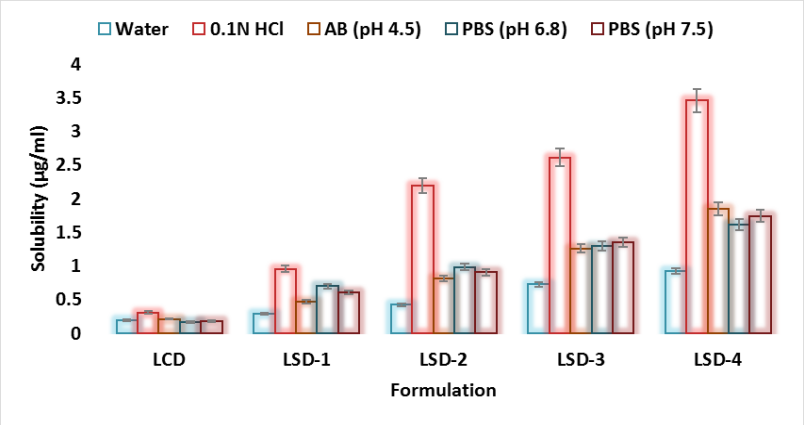


Figure 4. Solubility of optimized formulations of

Table 5: Solubility studies of LCD optimized formulation

Solvent	Solubility in different media (µg/mL)				
		Solid dispersion			
	LCD	LSD-1	LSD-2	LSD-3	LSD-4
Water	0.195±0.002	0.295±0.01	0.426±0.01	0.728±0.01	0.925±0.01
0.1N HCl	0.311±0.001	0.957±0.03	2.195±0.03	2.610±0.05	3.459±0.02
AB (pH 4.5)	0.212±0.002	0.469±0.01	0.821±0.01	1.259±0.01	1.848±0.04
PBS (pH 6.8)	0.167±0.006	0.702±0.03	0.984±0.03	1.298±0.03	1.615±0.03
PBS (pH 7.5)	0.182±0.001	0.606±0.04	0.907±0.01	1.349±0.01	1.745±0.01

Values in mean ±SD; trials made (n=3)

Table 6: Dissolution data of LSD-1 to LSD-4

		% LCD released			
Time (min)	**LCD**	**LSD-1**	**LSD-2**	**LSD-3**	**LSD-4**
0	0.00	0	0	0	0
5	4.48±0.03	29.98±0.04	36.25±0.09	39.75±0.12	40.95±0.03
10	9.62±0.02	71.04±0.05	69.47±0.08	71.67±0.09	70.17±0.12
20	12.65±0.26	79.11±0.01	75.49±0.07	79.08±0.12	83.15±0.09
30	22.11±0.48	88.54±0.09	88.85±0.48	89.05±0.05	90.59±0.15
45	30.32±0.51	91.09±0.07	94.72±0.09	92.97±0.11	95.07±0.45
60	36.97±0.11	95.88±0.02	96.49±0.14	97.74±0.23	98.13±0.23

Values in mean ±SD; trials made (n=3)

The kinetic data of optimized LCD formulations

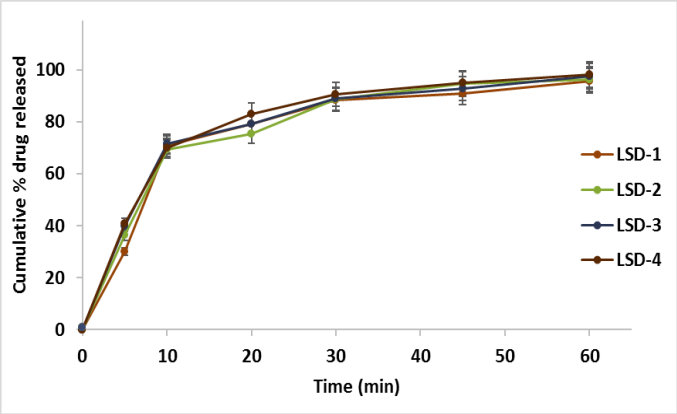


Figure5. Zero-order plot of Lercanidipine optimized formulation (LSD-1 to LSD-4)

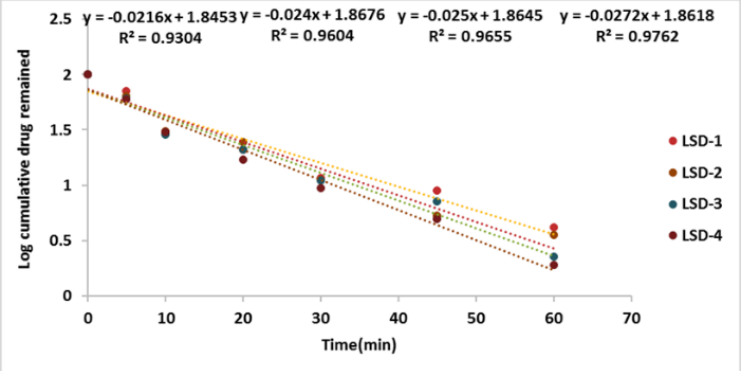


Figure 6. First-order plot of optimized formulation LSD-1 to LSD-4

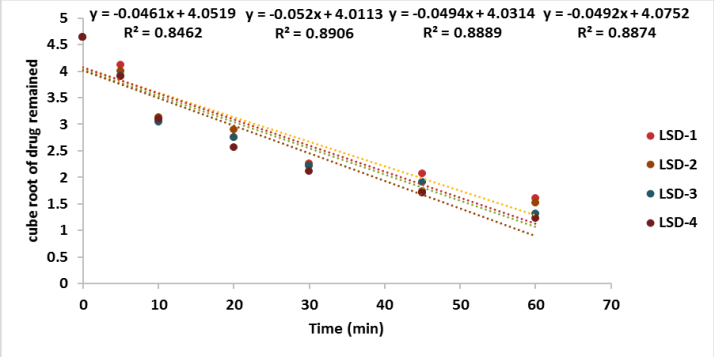


Figure 7. Hixson Crowell's plot of optimized formulation LSD-1 to LSD-4

Table 7: Correlation coefficients (r^2) for different release kinetics of optimized LCD solid dispersions

Formulation	Correlation (r)		
	Zero-order	First order	Hixson Crowell's
LSD-1	0.6847	0.9304	0.8462
LSD-2	0.6965	0.9604	0.8906
LSD-3	0.6355	0.9655	0.8889
LSD-4	0.6742	0.9762	0.8874

Values in mean ±SD; trials made (n=3)

Table 8: Standard calibration data of LCD

Lercanidipine concentration (µg)	Peak area (mean ±SD)
1	233948±34.84
2	353628±41.58
3	466127±15.68
4	586327±17.15
5	695109±10.28

Values in mean ±SD; Number of trials (n=3)

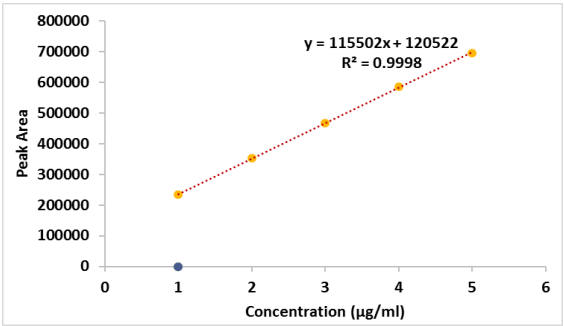
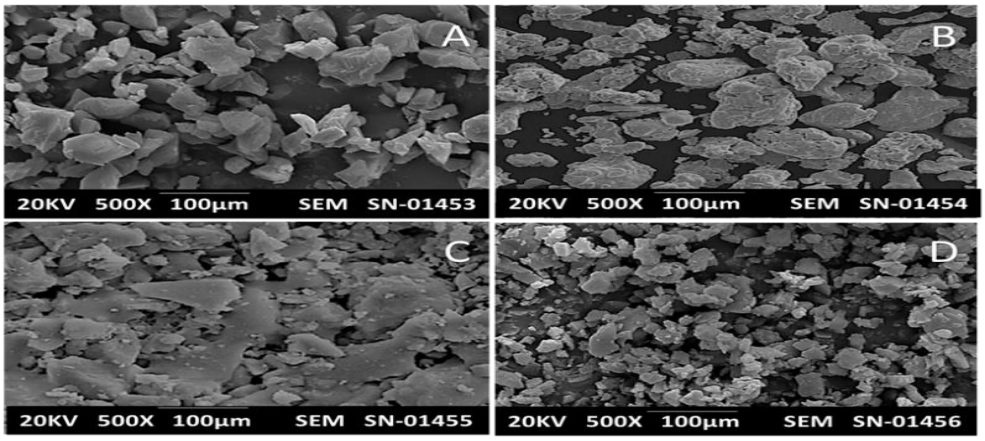


Figure 9: Calibration curve for the estimation of LCD in serum by HPLC

Figure 8: SEM Images of LSD



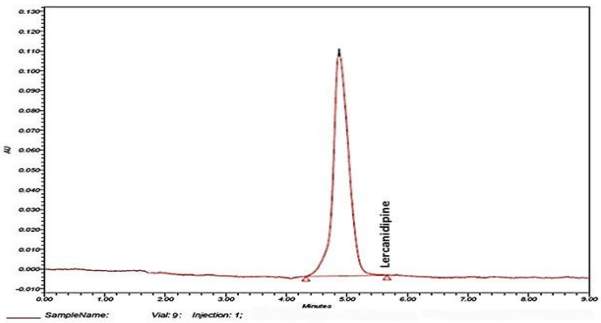


Figure 10: HPLC chromatogram of the serum sample (TSD-4) showing retention time of (4.894 min). Estimation of drugs by RP-HPLC

Table 9: Concentration of LCD in Serum with pure LCD and SDs (LSD-4) by p.o

Time (h)	Concentration of LCD in Serum	
	Pure drug	SDs (LSD-4)
0	0.00	0.00
0.5	0.29±0.01	1.56±0.01
1.0	0.52±0.01	2.95±0.01
2.0	1.06±0.04	4.87±0.08
4.0	0.62±0.03	2.28±0.07
6.0	0.29±0.01	0.88±0.02
8.0	0.11±0.01	0.32±0.01

Values in mean ±SD; trials made (n=3)

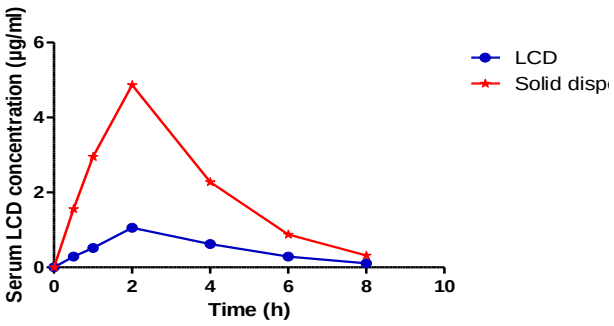


Figure 11: Serum concentration of LCD and its SDs (LSD-4) in Rabbits (p.o)

Table 10: Pharmacokinetic parameters of LCD in serum (pure drug and SDs (LSD-4)

Pharmacokinetic parameter	Concentration of LCD in Serum	
	Pure drug	**SDs (LSD-4)**
C_{max} (µg/mL)	1.29±0.01	4.35±0.14
t_{max} (h)	02.00±0.00	02.00±0.00
$K_{el}(h^{-1})$	0.44±0.05	0.46±0.01
$t^{1/2}$ (h)	01.687±0.02	01.654±0.02
$(AUC)^{0-8}$ (µg.h/mL)	3.98±0.02	16.55±0.01
$(AUC)^{0-\infty}$ (µg.h/mL)	4.27±0.01	17.58±0.19
$K_a(h^{-1})$	01.05±0.01	01.85±0.02
AUMC (µg.h/mL)	11.07	44.07
MRT	3.49	3.85

Values in mean ±SD; trials made (n=3)

The flow properties as the angle of repose was between (25 to 30o) i.e., 25.45±0.45 to 26.95±0.25 whereas the compressibility Index was less than 10 and Hausner ratio less than 1.09, representing good compression possessions while tableting. The flow properties of fabricated LCD-SD were summarized in table 3

The yield of LCD-SD was observed to be good (>90%), The LCD-SD tablets were seeming to have uniform in surface. The tablets were found to have a uniform in thickness (5 mm) and weight. The loss on friability was < 1% and the hardness was >4 Kg/cm 2 (5.8±0.08 to 7.3±0.05) indicating that the tablets bearing considerable mechanical strength and the LCD content was also found to be uniform. All these values were illustrated in table 4.

LCD

The solubility of LCD was found to be good in 0.1N HCl and decrease with an increase in pH of media. Among the tablets, LSD-4 showed good solubility in 0.1 N HCl. The detailed description of solubility was shown in figure 4. LCD released from the tablet was initial burst within 10 min and the end of 1h the LCD was completely released. The dissolution of prepared tablets was found good in formulations containing LCD: polymer mix at the ratio of 1:7 (figure 5).

Pharmacokinetics parameters were calculated by kinetics software (version 5.0). The statistical significance of observed different groups assessed by ANOVA test by using Graph and prism software**.**

After a single dose of formulation LSD- was 1.29±0.01 µg/mL, which is higher than that of pure. The C_{max} of SDs (LSD-4) was3.37 times more than LCD. The T_{max} values of the formulations LSD-4 was equivalent to the pure drug. The $AUC_{(0-8h)}$ of SDs (LSD-4) was ~4 folds more than LCD. The $AUC_{(0-\infty)}$ of SDs (LSD-4) was marginal increase i.e., 4.12 folds more than LCD. These fallouts suggest that the absorption rate and bioavailability of SD formulation LSD-4 are remarkably quicker and greater than that of pure drugs.

CONCLUSION

In the examination HPMC-K4M, PVP K-30 and Poloxamer-188 combination was a good grouping as a carrier for making solid dispersions by taking Lercanidipine as a model drug. The formulation LSD-4 in the ratios 1:7 prepared by solvent evaporation was observed to have good solubility and drug dissolution constrains In vivo animal trials in rabbits shown good levels of LCD SDs in serum related to pure LCD

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Conflict of interest: No conflict of interest was declared by the authors.

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