Research Article

Self-microemulsifying drug delivery system for enhancement of oral bioavailability of losartan

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ABSTRACT

Objective: The objective of the present study was to formulate self-microemulsifying drug delivery system (SMEDDS) to enhance the oral bioavailability of losartan. **Experimental:** The solubility of losartan was determined in various vehicles, and pseudoternary phase diagrams were used to assess the micro-emulsification boundary. The losartan SMEDDS was prepared using Capmul MCM EP (oil), tween 80 (surfactant), and polyethylene glycol (PEG) 400 (cosurfactant). **Results:** The SMEDDS was tested for microemulsifying properties, and the resultant microemulsions were evaluated for droplet size, zeta potential, transmission electron microscopy, and *in vitro* dissolution. The formulation development and screening were done on the basis of results obtained from phase diagrams and characteristics of resultant microemulsions. The selected formulation of Capmul MCM EP with surfactant and cosurfactant mixture (Smix) ratio of 1:1 (Tween-80: PEG-400) subjected to *in vivo* bioavailability study using rabbit. The results of drug release and oral bioavailability of losartan SMEDDS were compared with marketed formulation. The selected formulation enhanced the oral bioavailability of losartan by 1.49 folds than the marketed formulation.

KEY WORDS: Bioavailability, Losartan, Psudoternary phase diagram, Self-microemulsifying drug delivery system, Zeta potential

INTRODUCTION

Approximately 40% of new chemical entities have poor water solubility, and oral delivery of such drugs is associated with implications of low bioavailability, high intra and inter subject variability, and lack of dose proportionality.[1,2] Different types of buffers, surfactants or complex forming excipients have been used to increase the water solubility of drug.[3] Lipid-based formulation approaches, particularly the self-microemulsifying drug delivery system (SMEDDS) are popular for their potential as alternative strategies for delivery of poorly soluble drug.[4] The SMEDDS is isotropic mixtures of an oil, surfactant, cosurfactant or solubilizer and a drug. The basic principle of this system is its ability to form fine oil-in-water (o/w) microemulsion under gentle agitation following dilution by aqueous phases.[4,5] Losartan was used as a first line agent to treat uncomplicated hypertension, isolated systolic hypertension and left ventricular hypertrophy. Losartan may be also used as a second line agent in the treatment of congestive heart failure, systolic dysfunction, myocardial infarction, and coronary artery disease in those intolerant of ACE inhibitors. Losartan is well absorbed following oral administration and undergoes significant first pass metabolism to produce 5-carboxylic acid metabolite, designated as EXP3174.[6] About 14% of an oral dosage is converted to this metabolite, which is long-acting (6-8 h) and a noncompetitive antagonist at the AT1 receptor, contributing to the pharmacological effects of losartan.[7,8]

In this study, the solubility behavior of losartan was tested in different lipid solvents and SMEDDS was formulated. The prepared SMEDDS was characterized by zeta potential and particle size. *In vitro* dissolution profile of drug was studied to observe the drug release from SMEDDS. The oral bioavailability of drug was also investigated and compared with a commercially available tablet (Losar 50, Unichem Laboratories).

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MATERIALS AND METHODS

Materials

Losartan was obtained as a gift sample from Unichem Laboratories Ltd., Mumbai, India. Capmul MCM received as a gift sample from Abitec Corporation, Janesville, USA. The following materials were purchased from Loba Chemicals Pvt., Ltd., Mumbai, and were used as received. Tween-20, Tween-80, Span 20, Span 80, polyethylene glycol (PEG)-200, PEG-400 and PEG-600, isopropyl myristate, and propylene glycol were purchased from Molychem Pvt., Ltd., Mumbai, India. Oleic acid was purchased from Ozone international, Mumbai. The deionized water was prepared by a Milli-Q purification system from Millipore (Molsheim, France).

Animals

New Zealand white male rabbits (2.0 ± 0.2 kg) were used for the oral bioavailability study and *in vivo* pharmacokinetic study. Animals were maintained at a temperature of 25°C ± 2°C and a relative humidity of 70% ± 5% under natural light/dark conditions and were fed with food and water. The experiments were approved by the Institute Committee of Pharmacy Department (Protocol No: 1211/PO/ac/08/CPCSEA) and were conducted as per the guidelines of the committee for the purpose of control and supervision of experiments on animals.

Methods

Solubility study

The solubility of losartan was determined in 5 ml of selected vehicles (oil/surfactant/co-surfactant) (Figure 1). Excess amount of losartan was added to the mixture. The mixture was shaken with magnetic stirrer at 25°C for 24 h. Once the equilibrium was reached, the mixture was centrifuged at 3000 rpm for 5 min and the excess insoluble drug was discarded by filtration using membrane filter (0.45 µm, Whatman).[9] The concentration of free drug was then quantified by ultraviolet (UV) spectroscopy(JASCO, V-630, Japan).

Pseudoternary phase diagrams

Pseudoternary phase diagrams of oil, surfactant/cosurfactants (Smix) and water were developed using the water titration method.[9,10] The mixtures of oil and Smix at certain weight ratios were diluted with water in a dropwise manner. Surfactant (tween 80) and cosurfactant (PEG 400) were mixed in different weight ratios (1:1, 2:1, and 3:1). Capmul MCM EP oil was optimized as an oil phase based on the solubility study. For each phase diagram, oil (Capmul MCM EP), and specific Smix ratio were mixed thoroughly in different weight ratios, *viz.*, 0.5:9.5, 1:9, 1.5:8.5, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. Each ratio of oil and Smix was taken and titrated with water at 5% increment interval and then mixed on a magnetic stirrer. The solutions were observed visually for phase clarity and flowability. The concentration of water at which turbidity-to-transparency and transparency-to-turbidity transitions occurred was derived from the weight measurements. These values were then used to determine the boundaries of the microemulsion region corresponding to the chosen value of oils, as well as the Smix mixing ratio. Based on the observations phase diagrams were constructed using CHEMIX School v3.51 Software.[5,11]

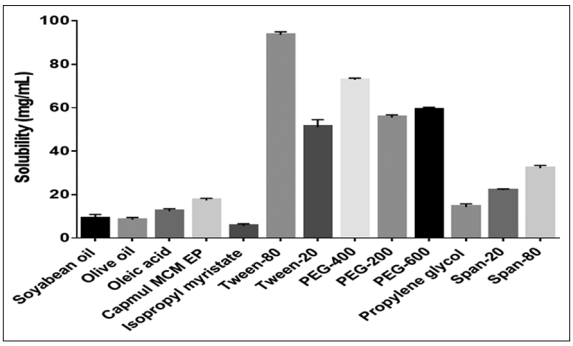

Figure 1: Solubility of losartan in various components

Preparation of SMEDDS formulations

Based on the area of micro-emulsification from the phase diagrams, Smix ratio of 1:1, 2:1, and 3:1 was selected for the formulation development studies. The first three formulations were selected from each ratio of Smix and having the ratio of oil and Smix was 0.5:9.5, 1:9, and 1.5:8.5, respectively. SMEDDS was prepared using tween 80 and PEG 400 as surfactant and cosurfactant with Smix ratio of 1:1, 2:1, and 3:1 (Table 1). The weight of the formulation was kept approx. 10 g. The level of losartan in all the formulations was kept constant (12.5 mg/g). Losartan was accurately weighed and placed in a glass vial with the respective required quantity of Capmul MCM EP oil. The components were mixed by gentle stirring and vortex mixing. Respective quantity of surfactant and cosurfactant was added to the vial and mixed by a magnetic stirrer. The mixture was stored at room temperature.

In vitro characterization of SMEDDS

Self-emulsification assessment

The self-emulsifying properties of SMEDDS were evaluated by visual assessment based on clarity and apparent stability of the resultant emulsion. SMEDDS (10 g) were added into 250 ml of distilled water and stirred magnetically at 100 rpm. The solution was then assessed visually for drug precipitation.[12,13]

Freeze thawing

All developed formulations were subjected to 3-4 freeze thaw cycles, which included freezing at −4°C for 24 h followed by thawing at 40°C for 24 h. The formulations were then centrifuged at 3000 rpm for 15 min and observed for phase separation.

Table 1: Developed E1-E9 formulation with their composition

Components	E1	E2	E3	E4	E5	E6	E7	E8	E9
Losartan (mg/g)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Capmul MCM EP (g)	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Smix ratio*	1:1	1:1	1:1	2:1	2:1	2:1	3:01	3:1	3:01
Tween 80 (g)	4.75	4.5	4.25	6.33	6	5.7	7.12	6.75	6.38
PEG 400* (g)	4.75	4.5	4.25	3.17	3	2.8	2.38	2.25	2.12

*Smix indicates surfactant/cosurfactant; PEG: Polyethylene glycol

Emulsion droplet size analysis and zeta potential measurement

SMEDDS formulation (10 g) was mixed with 250 ml of water in a beaker using a glass rod. The resultant emulsion was then subjected to globule size analysis and zeta potential measurement (Dalsa Micro Beckman Coulter, USA) at a scattering angle of 90° with a particle size measurement range of 0.02-2000 µm. Globule size was calculated from the volume size distribution.

Transmission electron microscopy (TEM)

The morphology of selected microemulsions was observed by TEM using a FEI Tecnai G2 F20 S-twin/EDAX transmission electron microscope fitted with a CCD camera (Fei Corporation, Hillsboro, OR, USA). 2-3 drops of microemulsion samples were placed on the film faces of 200 mesh carbon-coated copper grids without dilution and negative stained with 3 w% phosphotungstic acid (PTA) aqueous solution subsequently. Excess solution was carefully blotted up with filter paper during each step. Samples were air-dried at room temperature for at least 12 h. All steps were conducted at room temperature. The sample was loaded into the TEM for imaging at 200 kv. TEM images were captured at magnifications ranging from low LM ×2100 to SA ×145000 image modes.

In vitro dissolution studies

The dissolution of SMEDDS and standard losartan was carried out using USP XXIV Type-II dissolution test apparatus in 900 ml of 0.1 N HCL solutions at 37°C ± 2°C with 50 rpm rotating speed. Samples of 10 ml were withdrawn at a regular time interval of 5, 10, 15, 20, 25, 30, 45, and 60 min and filtered using 0.45 µm filters. An equal volume of dissolution medium was replaced to maintain the sink condition. The samples were analyzed by UV spectroscopy.

In vivo bioavailability study

Bioavailability of losartan was compared with marketed formulation (MF) and standard losartan. Rabbits were allocated at random to three treatment groups and administered standard losartan, MF and SMEDDS formulation. The standard losartan, MF and SMEDDS formulation equivalent to 0.7 mg/kg dose of losartan were administered orally. Blood samples (0.5 ml) were collected through the marginal ear vein into heparinized tubes at 2, 4, 6, 8, and 10 h after administration. Blood samples were centrifuged at 3000 rpm for 15 min using high-speed centrifuge machine, and plasma samples were withdrawn and stored at −20°C until analysis by high-performance liquid chromatography at 221 nm.

RESULTS AND DISCUSSION

Solubility Study

To achieve optimum drug loading, solubility study was aimed to identify suitable SMEDDS components that possess good solubilizing capacity for losartan. Among the various oils tested Capmul MCM EP (17.35 ± 0.3060 mg/ml) showed higher solubility for losartan and tween 80 (93.6 ± 0.4414 mg/ml) and PEG 400 (72.9 ± 0.24333 mg/ml) exhibited the higher solubility for losartan among the various surfactant and cosurfactant tested. Based on the solubility data, Capmul MCM EP was selected as oil phase, tween 80 as surfactant, PEG 400 as cosurfactant for formulating SMEDDS of losartan as these solvents showed higher solubility (Figure 1). In addition, synthetic oils have been reported to form good emulsification.

Pseudoternary Phase Diagrams

To form self-emulsifying microemulsions, oil, a blend of two surfactants and an aqueous phase were used. These four component systems can be best described by pseudoternary phase diagram. To determine optimum concentration of oil, surfactant and cosurfactant, phase diagrams were constructed. SMEDDS forms microemulsion when titrated with water under agitation condition. Surfactant and cosurfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence.[11] The decrease in the free energy required for the emulsion formation consequently improves the thermodynamic stability of the microemulsion formulation.[5] Therefore, the selection of oil, surfactant and the mixing ratio of oil to surfactant–cosurfactant plays an important role in the formation of microemulsion.

In the present study, both oleic acid and Capmul MCM were tested for phase behavior studies with tween 80 and PEG 400 as the Smix. As seen from the ternary plot (Figures 2 and 3), Capmul MCM gave a wider microemulsion region than oleic acid at all

Smix ratios. Thus, Capmul MCM was selected as the preferred vehicle for the optimized formulation.

Self-emulsification Assessment

The results of self-emulsification studies are given in Table 2. It was observed that an increase in the proportion of oil in the formulation resulted in decreasing self-emulsification time. E1, E4, and E7 were found to be clear dispersion and did not show any drug precipitation and thus were considered as stable. While E2, E3, E5, E6, E8, and E9 were formed clear dispersion but showed drug precipitation and thus were considered unstable. This may be due to the presence of high percentage of cosurfactant in the formulation which being water-soluble, is anticipated to enter the water phase and redistribute mainly between the water phase and the emulsion water interface, resulting in a loss of solvent capacity of the vehicle.

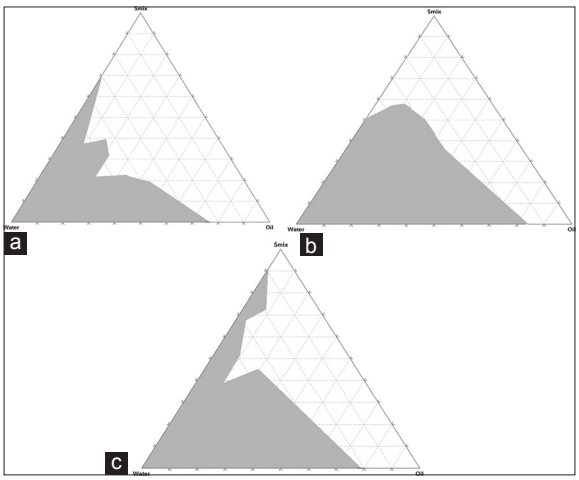

Figure 2: Pseudoternary phase diagram of system with the following components: Oil = Oleic acid, surfactant = Tween 80, and cosurfactant = polyethylene glycol 400. Smix ratio of a is 1:1, b is 2:1, and c is 3:1

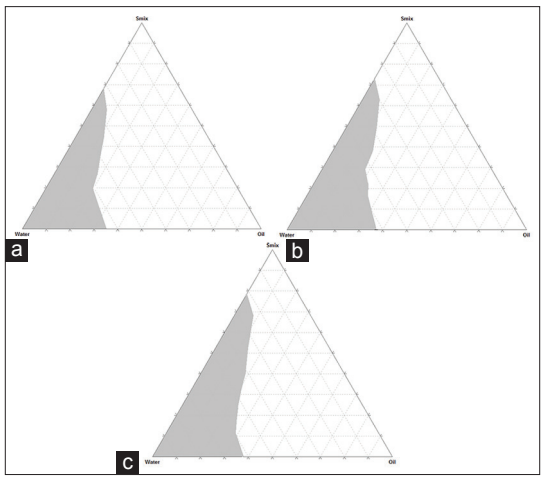

Figure 3: Pseudoternary phase diagram of system with the following components: oil = Capmul MCM, surfactant = tween 80, and cosurfactant = polyethylene glycol 400. Smix ratio of a is 1:1, b is 2:1, and c is 3:1

Freeze Thawing

The formulations were observed for phase separation. Formulations were stable to phase separation and were selected for further studies (Table 3).

Emulsion Droplet Size Analysis and Zeta Potential Measurement

The mean droplet size and polydispersity index (PDI) for all the SMEDDS have been summarized in Table 4. The higher the value of PDI, the lower is the uniformity of the droplet size in the formulation. The formulation E1 and E2 showed the less particle size as compared to the E3 and E4 formulation. The PDI of the E1 and E2 formulation was less as compared to the E3 and E4 formulation.

Formulation E1 reports negative zeta potential value −4.95 mV (Figure 4 and Table 5). The surfactant (tween 80) and cosurfactant (PEG 400) used in this

Table 2: Self-emulsification assessment of E1-E9 formulation

Formulation code	Time (S)*	Observation
E1	55±2.082	Clear dispersion
E2	57±2.082	Slightly clear
E3	71±0.0152	Slightly clear
E4	59±2.64	Clear dispersion
E5	55±3.0	Slightly clear
E6	67±0.0100	Slightly clear
E7	60±0.0 721	Clear dispersion
E8	79±0.0208	Slightly clear
E9	75±0.0208	Slightly clear

*Mean±SD; *n*=3. SD: Standard deviation

Table 3: Screening of formulations on the basis of thermodynamic stability studies

Batch code	Observation based on freeze thaw cycle		Inference
	Freeze throw cycle	Centrifugation	
E1	N	N	Passed
E2	N	N	Passed
E3	N	N	Passed
E4	N	N	Passed
E5	N	Y	Failed
E6	N	Y	Failed
E7	Y	Y	Failed
E8	Y	Y	Failed
E9	Y	Y	Failed

N: No change occurs in formulation, Y: Significant change occurs in formulations

Table 4: Globule diameter and PDI of the formulations

Formulations	Globule diameter (d) (nm)	PDI	Diffusion const. (D) (cm^2/s)
E1	69	0.177	$7.14e^{-08}$
E2	103.9	0.128	$4.73e^{-08}$
E3	185.2	0.218	$2.66e^{-08}$
E4	267.1	0.284	$1.84e^{-08}$

PDI: Polydispersity index

study are nonionic which do not contribute any charge to the microemulsion particle. This indicates that negative charge particle do not affect the stability of microemulsion.

TEM

The shape of particles may have a significant impact on the performance of the formulation. Moreover, the study of droplet shape provides a check on the validity of the size measurement and data analysis which assume spherical droplets.

The TEM micrographs of the microemulsion formulations illustrate their microstructure (Figure 5). These structures were considered to be o/w microemulsion because they have been formed at high water content; the microstructures observed are discrete spherical droplets. In the TEM micrographs, droplets were highly uniform with an average droplet diameter smaller than 50 nm. These diameters of droplet observed in TEM are in good accordance. TEM image exhibited that the particles were discrete, non-aggregated, homogenously dispersed, and nearly spherical in shape.

In Vitro Dissolution Studies

Drug release from the SMEDDS formulation (E1) was found to be significantly higher as compared to that of the other batches (E2 to E9) (Table 6). It was observed that the SMEDDS improved the dissolution rate of losartan significantly as compared to the MF (Figure 6).

In vivo Bioavailability Study

The % cumulative drug concentration versus time profiles in rabbits, administered with SMEDDS formulation (E1), MF and standard losartan shown in Figure 7. The pharmacokinetic parameters are presented in Table 7.

The relative bioavailability was calculated using following equation keeping dose of reference (MF) and SMEDDS (Batch E1) constant,

$$\text{Relative bioavailability}(\%) = \frac{[\text{AUC}]\text{test}}{[\text{AUC}]\text{reference}} \times 100$$

Plasma concentration C_{max} and $AUC_{0\text{-}t}$ are significantly increased for SMEDDS than those for the MF. Relative bioavailability is increased 1.49 folds than the MF. This result might be due to the enhanced intestinal permeability.[14] The main rate-limiting barrier for drug absorption/diffusion is the single layer of intestinal epithelial cell. High content of surfactants in SMEDDS could increase the permeability by partitioning with the cell membrane.[15]

Table 5: Measurement result of formulation E1 zeta potential

Measurements results	Observation
Zeta potential	−4.95 (mV)
Mobility	−3843e-005
Conductivity	0.1411 (mS/cm)
Doppler shift	−3.11
Base frequency	116.7

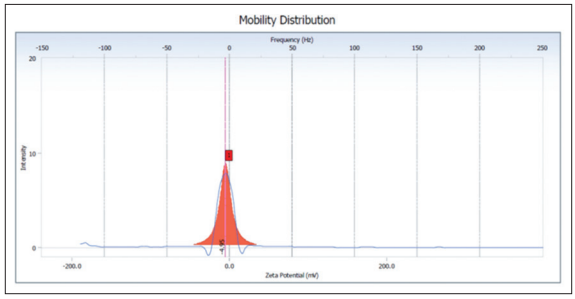

Figure 4: Zeta potential of the formulation E1

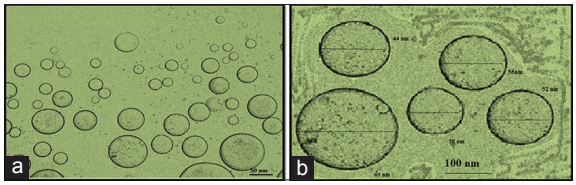

Figure 5: Transmission electron microscopy of E1 formulation (a) at 50 nm, (b) size measurement at 100 nm

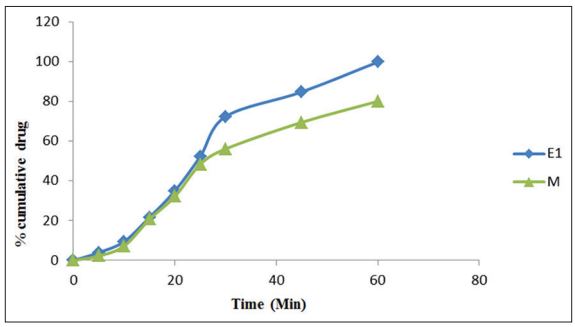

Figure 6: *In vitro* dissolution study of self-microemulsifying drug delivery system of E1 and M (marketed tablet formulation)

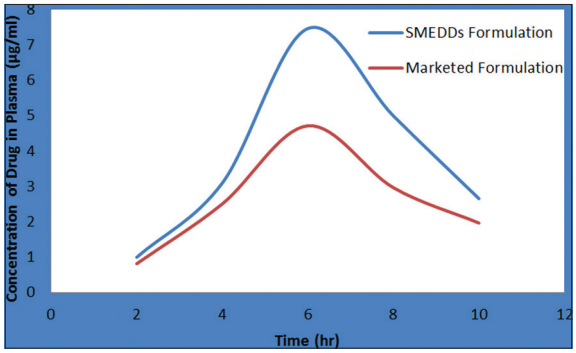

Figure 7: Plasma drug profile of self-microemulsifying drug delivery system formulation (E1) and marketed formulation

Table 6: *In vitro* dissolution study of SMEDDS of E1-E9 and SMEDDS formulation

% Cumulative drug release									
Time in min	**E1**	**E2**	**E3**	**E4**	**E5**	**E6**	**E7**	**E8**	**E9**
5	3.91±0.0152	3.14±0.0152	13.76±0.0208	3.25±0.0100	1.36±0.0100	8.007±0.0281	0.591±0.0010	3.46±0.0305	6.12±0.4996
10	9.45±0.0115	18.3±0.1528	18.75±0.0208	16.42±0.0200	8.34±0.0115	19.52±0.0300	15.31±0.0152	4.24±0.0321	17.64±0.1929
15	21.64±0.0208	24.75±0.0115	24.2±0.0200	21.31±0.0264	20.19±0.0100	27.96±0.0100	17.1±0.2082	15.21±0.0450	24.3±0.3606
20	34.94±0.0152	36.95±0.0152	29.54±0.0100	37.94±0.0200	29.84±0.0100	35.96±0.4239	24.53±0.2631	28.06±0.4500	40.49±0.2916
25	52.25±0.0264	48.72±0.0208	35.33±0.0100	40.64±0.0208	38.73±0.0152	45.85±0.4157	36.18±0.0907	38.72±0.0964	51.16±0.4202
30	72.34±0.0251	63.28±0.0100	42.89±0.0208	56.07±0.0100	51.17±0.0152	60.18±0.4734	57.14±0.0808	60.02±0.5658	67.73±0.2219
45	84.71±0.0230	73.86±0.0208	62.76±0.0100	64.54±0.0152	62.74±0.0100	68.1±0.4509	65.06±0.0360	63.51±0.3017	72.32±0.3765
60	99.75±0.0288	85.79±0.0100	70.698±0.0264	74.47±0.0152	72.77±0.0152	76.15±0.4744	73.44±0.0808	72.77±0.1735	85.46±0.2786

Mean±SD, n=3. SD: Standard deviation, SMEDDS: Self-microemulsifying drug delivery system

Table 7: Pharmacokinetic parameters of SMEDDS (batch E1) and MF

Parameters	SMEDDS formulation	MF
$T_{1/2}$ (h)	2.68	3.17
C_{max} (µg/ml)	7.47	4.72
T_{max} (h)	6	6
AUC_{0-t} (µg/ml*h)	35.804	24.01
MRT_{0-t} (h)	7.98	8.43
Relative bioavailability (%)	149.10	-

$T_{1/2}$: Half-life, C_{max}: Peak of maximum concentration, T_{max}: Time of peak concentration, AUC_{0-t}: Area under the concentration time profile curve until last observation, MRT_{0-t}: Mean residence time, SMEDDS: Self-microemulsifying drug delivery system, MF: Marketed formulation

CONCLUSION

SMEDDS of losartan was prepared and selected using various parameters such as particle size, zeta potential, PDI, *in vitro* drug release, and *in vivo* bioavailability studies. Optimal SMEDDS contains Capmul MCM (Oil), tween 80 (Surfactant), and PEG 400 (Cosurfactant). The combination of all three components (oil/surfactant/cosurfactant) in the ratio of 0.5:1:1 gives SMEDDS with a lower particle size (69 nm), PDI (0.177) and zeta Potential (−4.95 mV). This selected SMEDDS showed good *in vitro* drug release when compared with MF. *In vivo* study revealed significant improvement in extent of absorption of losartan in rabbit to 1.49 folds as compared to MF. Thus, our study confirmed that the SMEDDS formulation can be used as a possible alternative to oral solid formulations of losartan to improve its bioavailability.

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