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ENHANCED ORAL BIOAVAILABILITY OF RAMIPRIL BY SELF EMULSIFYING LIPID DELIVERY SYSTEM

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ABSTRACT

To enhance the solubility and bioavailability of poorly water-soluble Ramipril self-emulsifying lipid delivery system (SEDDS) composed of oil, surfactant and cosurfactant for oral administration was formulated. The solubility of ramipril was determined in various oils and surfactants & co-surfactants. The optimized SEDDS formulation consists of sunflower oil, caprylocaproyl macrogol-8 glycerides & glyceryl monocaprylate was selected basis the ternary phase diagram studies. This formulation were evaluated for visual characters, emulsification time, particle size & charge of dispersed emulsion, drug release profile & physic-chemical initial & accelerated stability. Ternary phase diagrams were used to evaluate the emulsification domain. The pharmacokinetic study in rats for the optimized formulation was performed and compared against marketed product. SEDDS have significantly increased the C_{max} and area under the curve (AUC) of ramipril and reduced T_{max} compared to marketed tablet formulation ($P < 0.05$). Thus, this self-micro emulsifying drug delivery system should be an effective oral dosage form for improving oral bioavailability of ramipril.

KEYWORDS: Ramipril, Self-emulsifying lipid delivery system, Solubility, Bioavailability.

INTRODUCTION

Ramipril, an angiotensin-converting enzyme (ACE) inhibitor, is a prodrug which is rapidly hydrolysed after absorption to the active metabolite ramiprilat. Ramipril, dosed in the range of 2.5 mg - 10 mg/day, has reduced the relative risk of myocardial infarction (MI) and other ischaemic events by 14 to 23%, in patients who are not known to have low ejection fraction

or heart failure but are at increased risk for developing cardiovascular events. Interestingly, among the pre-diabetics, ramipril significantly reduced the development of diabetes mellitus.[1][2]

Approximately 56% of an oral administered dose of ramipril is absorbed and converted to the active metabolite ramiprilat. After a single 5mg dose of ramipril, bioavailability values of ramipril and ramiprilat were 28 and 44%, respectively with the protein binding of ramiprilat about 56%.[1]

In recent years much attention has been focused on lipid based formulations with particular emphasis on SEDDS to improve oral bioavailability of poorly water-soluble drugs.[3] A few other studies have reported of an enhancement in the bioavailability of poorly soluble drugs when formulated as SEDDS.[4] In this study, we have developed an optimized formulation using a SEDDS in order to improve the solubility and bioavailability of ramipril.

MATERIALS AND METHODS
Materials
Ramipril was provided as gift sample by Sword & Shield Pharma (India); sunflower oil, castor oil,soybean oil, olive oil were supplied by SNN(India); caprylocaproyl macrogol-8 glycerides & propylene glycol dicaprylocaprate were from Gattefosse (France);sorbitan monoleate,polysorbate 20,polysorbate 60,polysorbate 80 were from Croda (UK); polyoxyl 40-hydrogenated castor oil,polyoxyl 35-hydrogenated castor oil were from Corel pharma (India);glyceryl monocaprylate & propylene glycol dicaprate were from Abitec (USA);empty hard gelatin capsule were from Capsugel(India) & gelatin bloom 200 was from Sterling Gelatin (India).All other chemicals and solvents were of reagent grade and were used without further purification.

Solubility studies
Solubility studies were conducted by placing an excess amount of ramipril (approximately 400 mg) in 10 ml amber colour vial containing 5 gm vehicle. Semi-solid excipients should be fully melted at 20°C above their melting points. Sonicate the mixture for 10 minutes and kept for 24 hours at 25 °C to equilibrate, followed by centrifugation at 3000g for 15min.[5]

The supernatant was taken and diluted with mobile phase acetonitrile: sodium perchlorate (43:47 v/v) with pH adjusted 3.1.Quantification of alitretinoin by RP-HPLC equipped with a

UV-Visible detector, manual injector with 20 ml loop, LC-solution software and Phenomenex C_{18} column (250 mm x 4.6 mm, 5 µm particle size).The solvents were filtered through nylon 0.45 µm membrane filter and was eluted at a flow rate of 1.8 ml/min. Effluents were monitored at 210 nm.[6] The inter- and intra-day variance of this HPLC method were within the acceptable range of less than 2% (R^2 = 0.996).

Construction of ternary phase diagram

Phase diagrams were constructed by identifying the good self-emulsifying region.The selection of co-surfactants and its ratio with surfactant will be done basis series of self-emulsifying systems prepared with varying concentrations of oily vehicle (sunflower oil) & three shortlisted surfactants basis solubility studies, surfactant 1(polyoxyl 40-hydrogenated castor oil),surfactant 2 (polysorbate 60) & surfactant 3(caprylocaproyl macrogol-8 glycerides) & co-surfactants (glyceryl monocaprylate).Micro emulsions were prepared by increasing the oil phase concentration from 10-90% & simultaneously decreasing the surfactant-co-surfactant/co-solvent from 90-10% to decide the maximum uptake of water by LDS up to which they remained transparent.[7] All studies were repeated thrice, with similar observations being made between repeats.

To investigate the effects of ramipril on the self-emulsifying performance of SEDDS, the 5mg ramipril was added to the boundary formulation of the self-emulsifying domain of the ternary phase diagrams. The self-emulsifying performance was visually assessed after infinite dilution using purified water.

Preparation of liquid SEDDS formulations

The innovator product Cardace® is commercially available strengths of 5mg film coated tablets.[8] Basis solubility and phase diagram studies,ramipril test formulation of 5mg strength ramipril were vortexed till all the drug component gets dissolved in oil and surfactant-cosurfactant blend (Table 1).[9] This were then filled using an eppendorph micro-pipette in size 00 hard gelatine capsules. To prevent the leakage, band-seal the filled capsules using sealing solution, gelatine bloom 200 (5%) and polysorbate 80 (0.5%) in water. All the measurements were done on w/w basis.

Visual characterization of SEDDS

The finished product appearance and any form of change or physical instability are captured in this section. Formulation was melted at 60°C centrifuged 10000 rpm for 1 min at room temperature and observed for any change in for phase separation, flocculation or precipitation.[10] Self-emulsification time estimation was performed in an United States Pharmacopeia (USP) type II dissolution apparatus. 300 mg of each formulation added drop wise to 500ml purified water at 37° at 50 rpm. Emulsification time was assessed visually.[11] For transmission studies, 1 ml of formulations was diluted with 50 ml & 100 ml distilled water. % transmittance was then measured spectrophotometrically at 650 nm using distilled water as a blank by UV-spectrophotometer and for each sample three replicate assays were performed.[12]

Droplet size & zeta potential

Optimized SDS formulation was diluted with excess (100 times) water and particle size of the system was determined by Zetasizer Nano ZS (Malvern Instruments, UK) dynamic light scattering particle size analyzer at a wavelength of 635 nm and at a scattering angle of 90° at 25 °C.The *z*-average diameter of the emulsions was derived from cumulated analysis by the Automeasure software (Malvern Instruments, Malvern, UK).[13]

Drug release studies

Drug release studies from SEDDS capsules were performed using USP Apparatus II with sinkers in 500 ml water with 50 mg/L pancreatin at 37 ± 0.5 °C with the paddle speed adjusted to 50rpm.Ramipril 5mg capsules and marketed ramipril 5mg tablets (Cardace® by Sanofi Aventis) were placed in a dissolution tester (Sotax,Switzerland).At predetermined time intervals(0, 10,15,30,45,60 minutes),aliquot (0.5 ml) were collected, filtered and analysed for the content of ramipril as mentioned above. An equivalent volume (0.5 ml) of fresh dissolution medium was added to compensate for the loss due to sampling.[14] All studies were repeated six times.

Indicative stability studies

Optimised ramipril capsules were packed in amber type I glass vials, sealed with a butyl rubber cap and aluminium flip-off seal closures and stored at 40 ± 2°C/75 ± 5% RH.[15] The vials were withdrawn at 1 month and 3 month time intervals & analysed for above physicochemical parameters.[15]

***In vivo* study**

The *in vivo* performance of the test and control formulations of ramipril were compared in rats at 0.52 mg/kg.[16] Male Wistar rats (twelve rats were divided into two groups) were fasted for 10 hours (h) prior to the experiments but allowed free access to water. All animals care and procedures were conducted according to the guidelines issued by Committee for Purpose of Control and Supervision of Experiments on Animals, Government of India.

Samples were collected from the eye at 0, 30, 60, 120, 240, 480, 720 minutes time intervals after administration from alternate rat. The blood samples were collected into heparinized tubes and 100 µL of plasma was collected by centrifuging blood samples at 3000g for 15 min. Plasma samples were stored with photo protection at -20 °C until further analysis.[17]

300 µL plasma was pipette into a 2 mL polypropylene tube and 3 ml mixture containing diethyl ether and dichloromethane (70:30, v/v) was added. The mixture was vortex mixed for 10 min and centrifuged at 3000g for 10 min. The organic layer containing ramipril was separated, transferred to a separate test tube and evaporated to dryness under a stream of N2 at 40 °C. The residue obtained on drying was reconstituted with the 250 µl of mobile phase(0 mM ammonium formate–methanol (3:97, v/v)).The reconstituted samples was injected into HPLC system.[18]

Pharmacokinetic parameters C_{max} , T_{max} and AUC were calculated using PK Solver Software.[19] Student's *t*-tests were performed to evaluate the significant differences between the two formulations. Values are reported as mean ± S.D. and the data were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Solubility study

The self-emulsifying formulations consisted of oil, surfactants, cosurfactant, and drug should be a clear and monophasic liquid at ambient temperature when introduced to aqueous phase and should have good solvent properties to allow presentation of the drug in solution. The solubility of ramipril in various vehicles is presented in figure 1. Among the oil & co-surfactant shortlisted were sunflower oil & glyceryl monocaprylate with 17.80 ± 2.97 mg/gm & 33.71± 2.42 mg/gm respectively showed highest drug solubility.Among surfactants three most promising candidates,polyoxyl 40-hydrogenated castor oil(20.69 ±2.40 mg/gm), polysorbate 60(14.45±   2.01 mg/gm) & caprylocaproyl macrogol-8 glycerides

(29.66±3.12mg/gm) were selected for developing optimal SEDDS formulation resulting in improved drug loading and spontaneous emulsion forming capabilities.
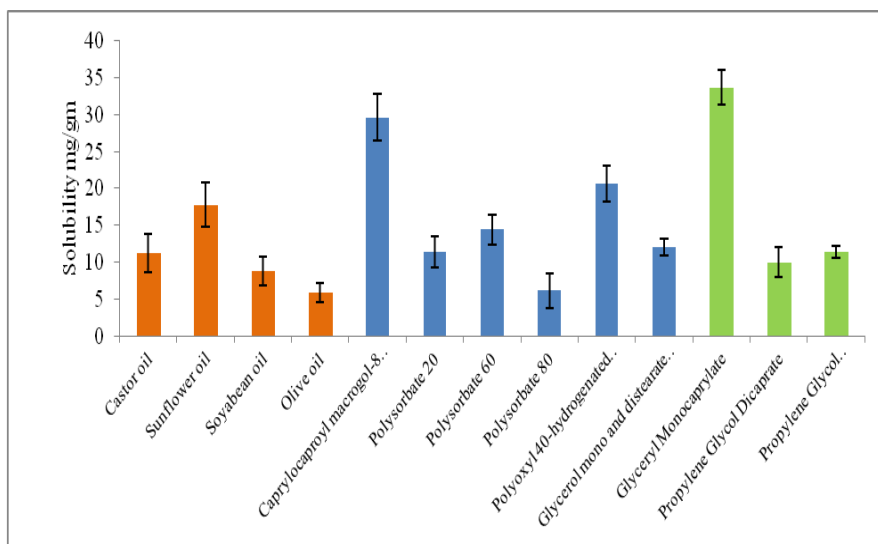

Figure 1. Solubility studies of ramipril in various oils(orange columns),surfactants(blue colums) & co surfactant(green columns). Each value represents the mean ± RSD (n = 3)

Construction of pseudo-ternary phase diagrams

The existence of self-emulsifying oil formulation fields that could self-emulsify under dilution and gentle agitation was identified from ternary phase diagrams of systems containing oil & surfactant- cosurfactant (Smix).A series of SEDDS were prepared and their self-emulsifying properties were observed visually. Pseudo-ternary phase diagrams were constructed in the absence of ramipril to identify the self-emulsifying regions and to optimize the concentration of oil, surfactant and cosurfactant in the SEDDS formulations. The phase diagrams of the systems containing sunflower oil, caprylocaproyl macrogol-8 glycerides as surfactant, and glyceryl monocaprylate as cosurfactant (Fig. 2) as the composition with surfactant: co-surfactant ratio at (1:1) had shown highest self-emulsification region.This may be attributed to the fact that the addition of cosurfactant may lead to greater penetration of the oil phase in the hydrophobic region of the surfactant monomers thereby further decreasing the interfacial tension,which will lead to increase in the fluidity of the interface thus increasing the entropy of the system.[20], [21]

It has been reported that the drug incorporated in the SEDDS may have some effect on the self-emulsifying performance.[22] In our study, 5mg of ramipril could be safely incorporated in to formulations without affecting other properties of SEDDS.
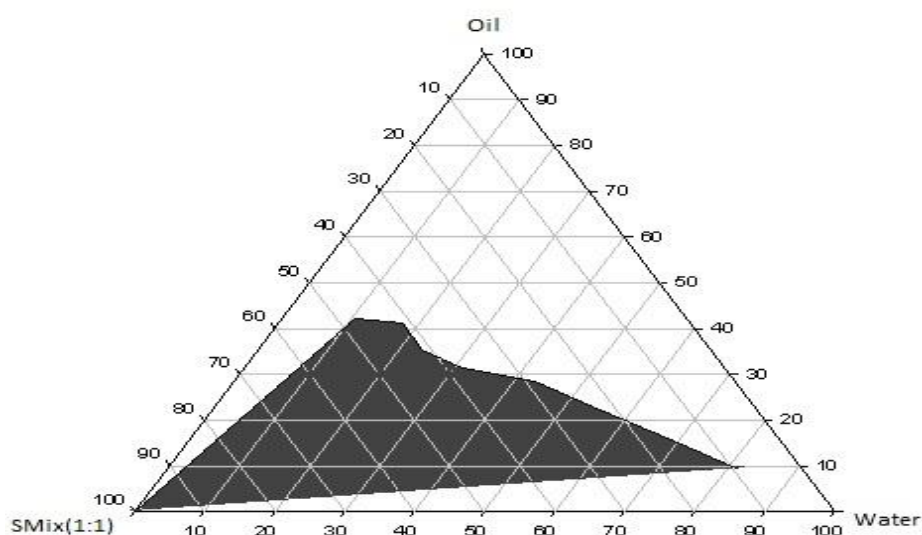
Figure 2. Pseudo-ternary phase diagram of optimised placebo SEDDS formulation.

Table 1: Composition of the optimised SEDDS formulations basis ternary phase diagram studies.

Ingredients	Batch B1	
	mg/Unit	% w/w
Ramipril	5.00	2.38
Sunflower oil	96.74	46.07
Caprylocaproyl macrogol-8 glycerides	54.13	25.78
Glyceryl monocaprylate	54.13	25.78
Total	210.00	-

Visual characterization, droplet size, zeta potential, dissolution release & indicative stability studies

In SEDDS, the primary means of self-emulsification assessment is visual evaluation, centrifugal stability, efficiency of self-emulsification, transmission and droplet size distribution(Table 2). The droplet size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption.[23][24][25]

Table 2: Physical characterisation parameters ramipril SEDDS formulations.

Batch B1	Physical Appearance	Centrifugal Stability	Grade	Transmittance (%) ± RSD		Droplet size (nm)	PDI	Zeta potential (mv)
				50 times dilution with	100 times dilution with			

				water	water			
Initial	Off-white liquid	NPS	I	99.12 ± 0.08	99.46 ± 0.015	181.2	0.241	-6.35
1M 40°C/75%RH	Off-white liquid	NPS	I	98.34 ± 0.008	98.67 ± 0.009	246.1	0.136	-6.01
3M 40°C/75%RH	Off-white liquid	NPS	I	97.76 ± 0.098	98.10 ± 0.004	333.8	0.114	-6.9

NPS: No phase separation observed

The above compilation of physical performance and stability data suggest that the test ramipril SEDDS formulation had shown satisfactory performance across the period of evaluation. Zeta potential values between -6.01 to -6.90 indicated a stable emulsion system. Although a marginal shift was observed the SEDDS formulations emulsion in mean droplet size, poly dispersity index (PDI) values were close to zero, an indication of spherical globules.
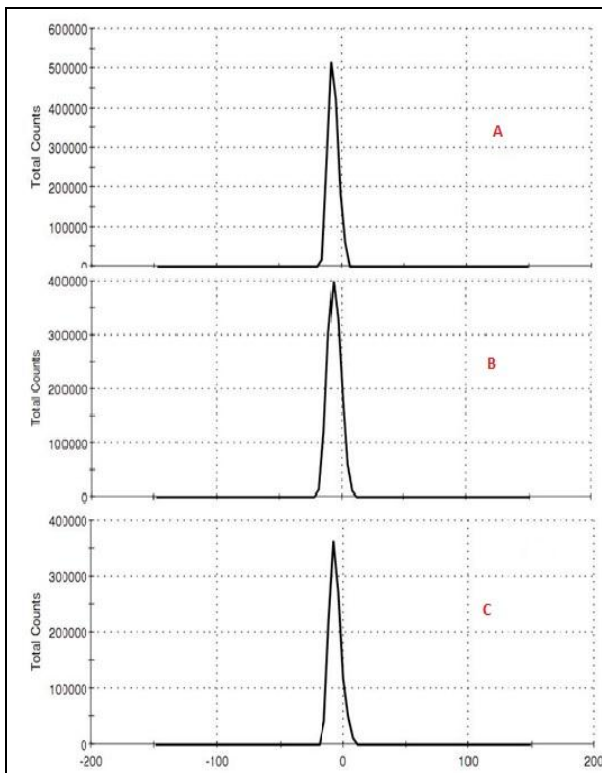

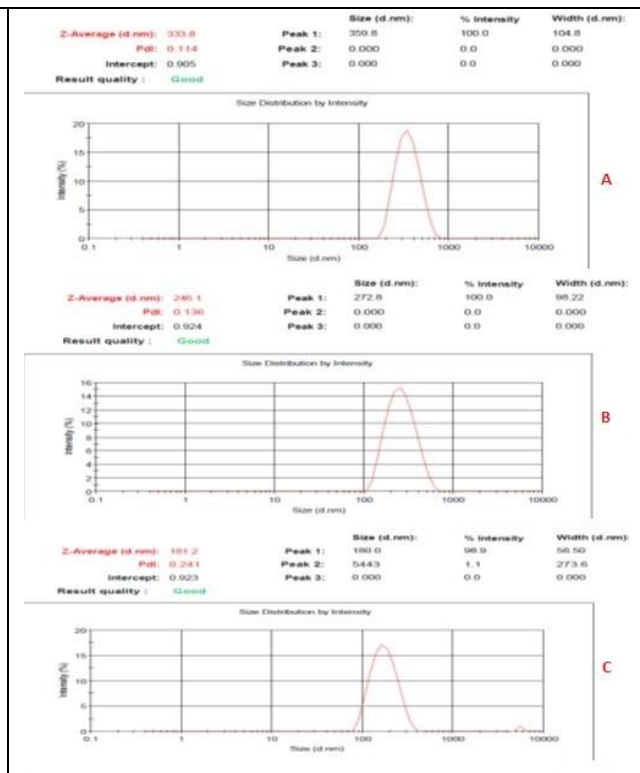| **Figure3. Zeta potential values for ramipril SEDDS: A) 3 month-40°C/75%RH B) 1-month-40°C/75%RH C)Initial** | **Figure 4: Droplet size & PDI values for ramipril SEDDS: A) 3 month-40°C/75%RH B) 1-month-40°C/75%RH C)Initial** |

The drug content for ramirpil SEDDS capsules & were Dissolution studies were performed for the SEDDS formulations and the results are presented in figure 5 & figure 6 respectively. No significant change in ramipril content and the release profile from the ramipril SEDDS formulation was observed. When compared to marketed product; release from ramipril

SEDDS release has shown a consistent improvement across the all time points. In fact, variability profile for all the SEDDS system at all stability time-points were found to be lower against the conventional format. It is worthwhile to mention that as the formulations were filled in hard gelatin capsules, the initial lag was observed for 1st 15 minutes release profile attributed to the generally observed opening time for hard gelatin capsule in dissolution media. Basis the above physic-chemical evaluation and indicative stability studies, ramipril SEDDS formulation has shown a satisfactory and stable performance profile.
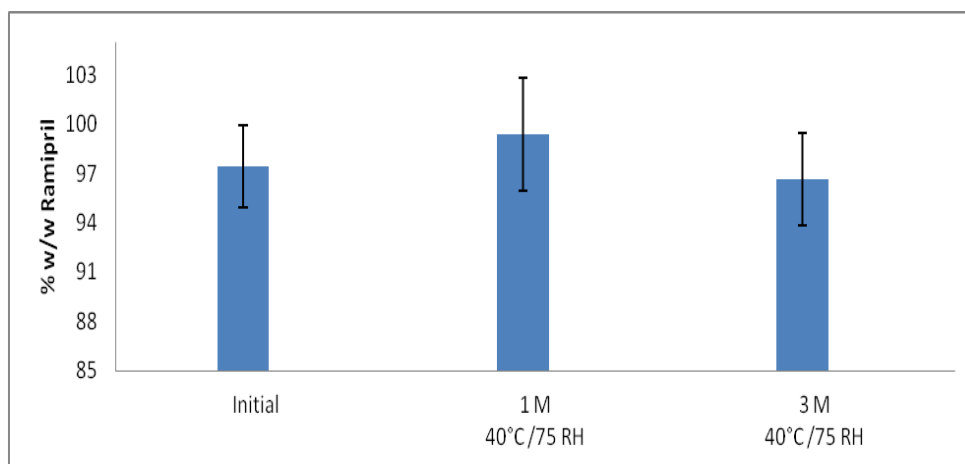

Figure 5. Ramipril content in optimised test SEDDS formulations against. Each value represents the mean ± RSD (n = 6).
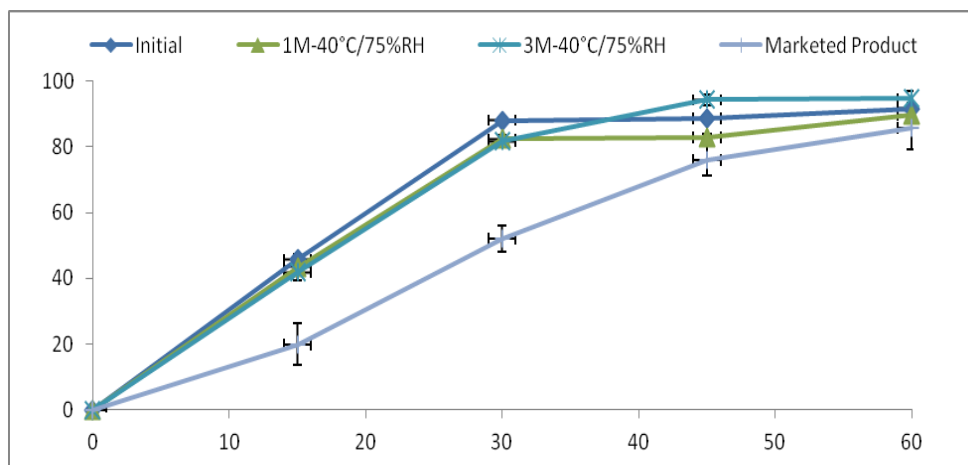

Figure 6. Drug release profile study for ramipril SEDDS 5 mg formulations against ramirpil conventional 5 mg tablet. Each value represents the mean ± RSD (n = 6).
***In vivo* study**

As pointed out in earlier section, being a Class IV BCS drug, the improvement in dissolution cannot be the sole guarantor of superior *in vivo* performance. Therefore, *in vivo* pharmacokinetic evaluation of the optimized ramipril SEDDS formulation (B2) and marketed

product using suitable animal model using suitable animal model was performed, basis which AUC, T_{max} and C_{max} were calculated (Table 3).
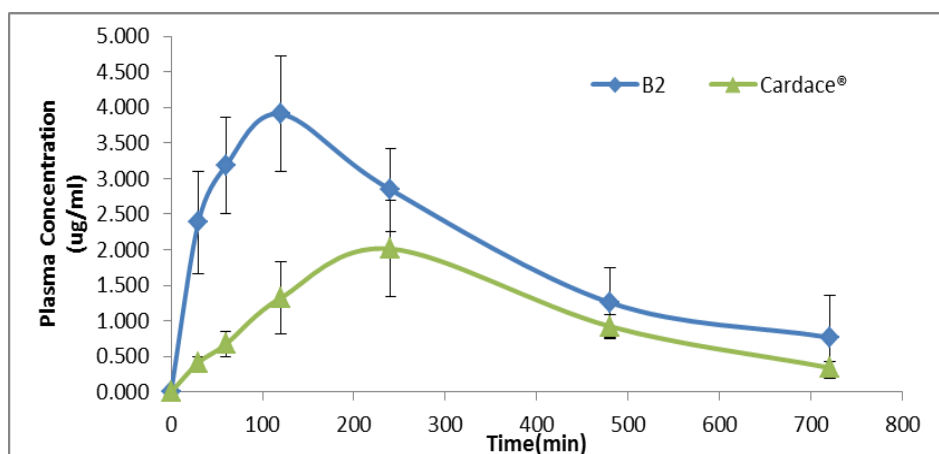

Figure 7. Plasma profiles of ramipril from SEDDS and marketed formulations in rats. Each value represents the mean ± S.E. (n = 6).

Table 3: Pharmacokinetic parameters of ramipril from SEDDS and marketed formulations in rats. Each value represents the mean ± S.E. (n = 6).

Parameter	Ramipril SEDDS	Marketed Ramipril Tablet
T_{max} (min)	120.0 ± 0	240.0 ± 0
C_{max} (µg/mL)	3.907 ± 0.810	2.014 ± 0.481
AUC (µg min/mL)	1472.265± 419.17	741.855 ± 230.966

The pharmacokinetic values indicated a statistically significant improvement ($P < 0.05$) in bioavailability for test ramipril formulation. Typically type II & III lipid formulation undergo shorter gastric transit and will be in colloidal state earlier than type I lipid formulation leading to more rapid absorption and higher peak concentration of drug.[26] The test ramipril capsules significantly increased ramipril plasma levels and 2-fold jump was observed in the relative oral bioavailability compared to innovator composition.In fact, time to C_{max} was reduced by 120 minutes in test formulation when compared against the control product. The improved oral bioavailability of ramipril indicating its poor bioavailability can be increased by improving its solubility properties in the finished product.

CONCLUSION

Ramipril was formulated as a SEDDS in an attempt to increase its solubility and bioavailability. An optimized formulation of SEDDS containing ramipril was developed through solubility studies, construction of pseudo-ternary phase diagram, visual

observational, droplet particle size analysis, zeta potential and drug release studies. Following oral administration in rats, SEDDS provided significant increase in the bioavailability.Still conspiring the limited sample size, the results are at best indicative and further clinical work can be undertaken to develop & commercially viable supra-bioavailable format of ramipril liquid-in- hard gelatin capsules for human use. Overall, the study has indicated that it is indeed possible to produce reasonably stable SEDDS via a simple one-step process for drugs that are poorly soluble and/or poorly permeable to achieve a significant improvement in the bioavailability.

ACKNOWLEDGEMENTS

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