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Preparation and *in-vitro* evaluation of self emulsifying drug delivery system of antihypertensive drug valsartan

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Abstract

The present study deals with formulation of a valsartan based Self emulsifying drug delivery system of a poorly water soluble drug. SEDDS are the isotropic mixtures of oil, surfactant, co surfactant and drug that form oil in water microemulsion when introduced into aqueous phase under gentle agitation. The present research work describes a Self Emulsifying Drug Delivery System (SEDDS) of valsartan using Castor oil, Tween-80, PEG-600. Valsartan is an angiotensin converting enzyme (ACE) inhibitor with limited water solubility, which accounts for a low and variable oral bioavailability (19-25%). Hence, the main objective of study was to formulate SEDDS of valsartan in order to achieve a better dissolution rate which would further help in enhancing oral bioavailability. Pseudo-ternary phase diagrams were plotted to check for the micro-emulsification range and also to evaluate the effect of valsartan on the emulsification behavior of the phases. The mixtures consisting of oil (castor oil) with surfactant (tween 80), co-surfactant (PEG 600) were found to be optimum formulations. Prepared SEDDS formulations were tested for microemulsifying properties and the resultant microemulsions were evaluated for robustness to dilution, assessment of efficiency of self emulsication, emulsification time, turbidity measurment, viscosity, drug content and *in-vitro* dissolution. The optimized SEDDS formulation further evaluated for heating cooling cycle, centrifugation studies and freeze thaw cycling, particle size distribution, zeta potential were carried out to confirm the stability of the formed SEDDS. The formulation was found to show a significant improvement in terms of the drug release with complete release of drug within 60 minutes. Thus, Self microemulsifying formulation of valsartan was successfully developed.

Key-Words: Self Emulsifying Drug Delivery System, Valsartan, Bioavailability

Introduction

The oral route is the preferred route for chronic drug therapy. Numerous potent lipophilic drugs exhibit low oral bioavailability due to their poor aqueous solubility properties. For this class of compound defined as "low solubility/ high permeability class II, dissolution in the environmental lumen is the rate controlling step in the absorption process. Efforts are ongoing to enhance oral bioavailability of lipophilic drug in order to increase their clinical efficacy[1].

In recent years, the formulation of poorly soluble compounds presented interesting challenges for formulation scientists in the pharmaceutical industry. Up to 40% of new chemical entities discovered by the pharmaceutical industry are poorly soluble or lipophilic compounds, which leads to poor oral bioavailability, high intra- and inter-subject variability, and lack of dose proportionality.

To triumph over these problems, various formulation strategies are exploited such as use of surfactants, lipids, permeation enhancers, micronisation, salt formation, cyclodextrins, nanoparticles and solid dispersions. Each and every method for bioavailability enhancement is having its own merits and demerits. In salt formation, the salts that are formed may convert back to their original acid or base forms and lead to aggregation in the gastro-intestinal-tract (GIT). Particle size reduction may not be desirable in situations where handling difficulties and poor wettability are experienced for very fine powders. For compounds in which the primary limitation to absorption is poor aqueous solubility and slow dissolution rate, where intestinal permeability is not a limiting factor, (e.g., BCS Type II drugs) and for which conventional formulation approaches (e.g. salt or crystal form selection, particle size reduction, solid dispersions or the addition of surfactants) have failed, a lipid-based

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formulation should be considered[2]. Thus, in recent years, much attention has focused on lipid based formulations to improve the oral bioavailability of poorly water-soluble drug compounds. The lipid formulation classification system (LFCS) was introduced as a working model in 2000. Typical properties of Type I, II, III A and III B lipid formulations. The main purpose of the LFCS is to enable *in vivo* studies to be interpreted more readily, and subsequently to facilitate the identification of the most appropriate formulations for specific drugs, i.e. with reference to their physicochemical properties[3].

Self emulsifying drug delivery system are the isotropic mixtures of oil, surfactant, co surfactant and drug that form oil in water microemulsion when introduced into aqueous phase under gentle agitation. SEDDS are among the methods used to improve the oral bioavailability of poorly soluble drugs by presenting and maintaining the drug in a dissolved state, in small droplets of oil, all over its transit through the GIT[4]. These formulations spread readily in the GIT, and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification. In a good self-emulsifying system (SES), small emulsion droplets containing dissolved drug are formed on contact with gastrointestinal fluid. The drug in the fine emulsion droplets is exposed to a large interfacial area thus allowing for greater diffusion through the membrane to take place[5].

Valsartan is ACE inhibitor used as an adjuvant in treatment of hypertension. However, the low aqueous solubility and poor dissolution of this molecule in gastric fluid affects its rate of absorption, resulting in a low and variable oral bioavailability. It is absolute bioavailability of 19-25%, when 80 mg of oral valsartan is compared with the same dose 80 mg marketed tablet.

The dose of valsartan varies between 80-320 mg and frequently prescribed dose is 80 mg for the adult. Therefore, for the present study, 80 mg dose was selected for the development of microemulsion formulation. Primary objective is to enhance the solubility, dissolution rate and avoid intra and inter subject variability of valsartan by self emulsifying drug delivery system. The main objective of the study was to develop and evaluated an optimal SEDDS formulation containing valsartan.

Material and Methods

Materials for component selection

Valsartan base was a gift sample from Torrent pharmaceutical limited, Ahmadabad (Gujarat, India). Castor oil, tween-80, PEG-600 were gift samples from sd fine chem. Ltd Mumbai (Maharashtra, India). Butyrate hydroxyl toluene (BHT) was a gift sample from Renchem Mumbai (Maharashtra, India). All other chemicals were of analytical grade.

Solubility studies

The most important criterion for the screening of components for microemulsion is the solubility of poorly soluble drug in oils, surfactants and co surfactants. The solubility of valsartan in various oils was determined by adding an excess amount of drug in 2 ml of selected oils (castor oil, soya bean oil, arachise oil, sheesham oil, sunflower oil, cod-liver oil, olive oil, oleic acid) and surfactants (tween-20, tween-40, tween-80, span-20, span-80, non-ionic phenyl ethyl oxalate, PEG-400, PEG-600, Propylene-glycol) in 5 ml capacity stopper vials, and mixed using a vortex mixer (Spinix, Japan). The mixture vials were then kept at $25\pm1.0^{O}C$ in an ultra-sonicator (Pci, Japan) for 72 h to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 µm membrane filter. The concentration of valsartan was determined in oils using UV Spectrophotometer (Shimadzu 1700, Japan) at 251nm.

Pseudo-Ternary Phase Diagram

On the basis of the solubility study of drug, oil, surfactants, co-surfactants and aqueous phase were used for construction of phase diagram. Oil, surfactant, and co-surfactant are grouped in four different combinations for phase studies. Surfactant and co-surfactant (Smix) in each group were mixed in different weight ratio (1:0, 1:1, 1:2, 2:1, 1:3, 3:1, 1:4, 4:1 etc). These Smix ratios are chosen in increasing concentration of surfactant with respect to co-surfactant and in increasing concentration of co-surfactant with respect to surfactant for detail study of the phase diagram for formulation of nano/micro emulsion. For each phase diagram, oil, and specific Smix ratio are mixed thoroughly in different weight ratio from 1:9 to 9:1 (0.5:9.5, 1:9, 1.5:8.5, 2:8, 2.5:7.5, 3:7, 3.5:6.5, 4:6, 4.5:5.5, 5.0:5.0, 5.5:4.5, 6:4, 6.5:3.5, 7:3, 7.5:2.5, 8:2, 8.5:1.5, 9:1, 9.5:0.5) in different glass vials. Different combination of oils and Smix were made so those maximum ratios were covered for the study to delineate the boundaries of phase precisely formed in the phase diagrams. Pseudo-ternary phase diagram was developed using aqueous titration method. Slow titration with aqueous phase is done to each weight ratio of oil and Smix and visual observation is carried out for transparent and easily flowable o/w nano/micro emulsion. The physical state of the nano/micro emulsion was marked on a pseudo-three-component phase diagram with one axis representing aqueous phase, the other representing oil and the third representing a mixture of surfactant and co-surfactant at fixed weight ratios (Smix ratio)[6].

Preparation of Self Emulsifying Formulation

Pseudo ternary phase diagram were constructed in the presence of drug to contain optimum concentration of oil, surfactant and co-surfactant. SEDDS forms fine o/w emulsion with only gentle agitation, upon its introduced into aqueous media[6].

Thermodynamic Stability Studies

Heating cooling cycle

Six cycles between refrigerator temperatures 4[O]C and 45[O]C with storage at each temperature for not less than 48 hours was studied. Those formulations which are stable at these temperatures were subjected to centrifugation test.

Centrifugation test

Passed formulations were centrifuged at 3500 rpm for 30 min (Remi-12C, Japan) Those formulations that did not show any phase separation were taken for freeze thaw stress test.

Freeze thaw cycle

Three freeze thaw cycles between -21[O]C and +25[O]C with storage at each temperature for not less than 48 hours was done for the formulations[6].

Robustness to dilution

These systems when diluted with excess of water, standard phosphate buffer (pH 6.8) and 0.1N HCl (500-900 ml) and were stored for 12 hours give no precipitation or phase separation and are thus, said 'robust to dilution'[7].

Assessment of efficiency of self emulsification

The efficiency of self emulsification was assessed using a standard US pharmacopoeia XXIII dissolution apparatus type II. One g of each formulation was added dropwise to 200 ml of either 0.1N HCl or distilled water at 37[O]C. Gentle agitation was providing by a standard stainless steel dissolution paddle at 60 rpm. The lipid based formulations were assessed visually according to the rate of emulsification and final appearance of the emulsion. The *in-vitro* performance of the formulation was visually assessed using the following grading system[8].

Grade A: Rapidly forming emulsion having a clear or bluish appearance.

Grade B: Rapidly forming, slightly less clear emulsion, having a bluish white appearance.

Grade C: Fine milky emulsion that formed within 2 minutes.

Grade D: Dull, greyish white emulsion having slightly oily appearance that is slow to emulsify longer than 2 minutes.

Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.

Emulsification time

The emulsification time of SEDDS was determined according to USP XXIII, dissolution apparatus II. 0.5 g of the SEDDS formulations were introduced into 250 ml of 0.1N HCl or distilled water in 500 ml conical flask under action of magnetic stirrer rotating at constant speed. Emulsification time was done at room temperature[9].

Turbidity measurement

Turbidity of the resultant emulsion given a nephlometric turbidity unit (NTU) was measured using turbidimeter (Esico D-10-model 331, Japan). Turbidity measurements were performed on the emulsion stored in a screw capped sample vials. 0.5 ml of the SEDDS formulation was introduced into 250 ml of 0.1 N HCl or distilled water in 500 ml conical flask under action of magnetic stirrer rotating at constant speed. Emulsification was done at room temperature[9].

Viscosity determination

The viscosity of the system was determined as such before and after dilution using Brookfield viscometer (Japan) DV-E using spindle RV-6 at 100 rpm at 25±0.5[O]C[6].

***In-vitro* dissolution study**

The quantitative *in-vitro* drug release from formulation was studied to assess if self emulsifying properties remain consistent. The USP XXIV, dissolution apparatus used to study the release of the drug from the oil in the aqueous system. Hard gelatin capsule containing SEDDS was tied to paddle using para film spring to prevent the capsule from floating 900 ml dissolution media were used standard phosphate buffer solution pH 6.8[10].

To compare different SEDDS, dissolution studies were done at 37±0.5[O]C, using paddle rotating at 75 rpm, 1ml sample was withdrawn at 5, 15, 30, 45, 60 min. the reason for running the dissolution test for 60 min is that curve reaches a steady state after 15 min. the sample volume of fresh media replaces the withdrawn sample. Sample was filter whatmann filter paper and analyzed spectrophotometrically (Shimadzu 1700, Japan) at 250 nm of valsartan content[11]. The drug release from the SEDDS formulation was found to be significantly higher as compared with that of marketed valsartan tablet.

Droplet size analysis

The droplet size of the emulsion was determined by photon correlation spectroscopy (which analyses the fluctuations in light scattering due to Brownian motion of the particles) using a zetasizer able to measure sizes between 10 and 5000 nm. Light scattering was monitored at 25[O]C at an angle a 90[o] angle, after external standardization with spherical polystyrene beads. The nanometric size range of the particles is

retained even after 100 times dilution with water which proves the system compatibility with excess water[12].

Photon correlation spectroscopy (Malvern instrument, Australia) using laser light scattering was employed to measure particles sizes of preconcentrate generated emulsion/ microemulsion. The samples were loaded onto $1cm^2$ cuvettes in a thermostated chamber.[7].

Zeta potential determination

The emulsion stability is directly related to the magnitude of the surface charge. The zeta potential of the diluted SEDDS formulation was measured using a zeta meter system. The SEDDS were diluted with a ratio o 1:2500 (v/v) with distilled water and mixed with magnetic stirrer[13].

Zeta-potential of the resulting microemulsion was determined using the Zetasizer (Malvern instrument, Australia)[14].

Results and Conclusion

Solubility studies

The solubility of the drug was tested in different oils phases and maximum solubility was determined in castor oil 143.63±1.51 mg/g and was selected as oily phase for SEDDS formulation.
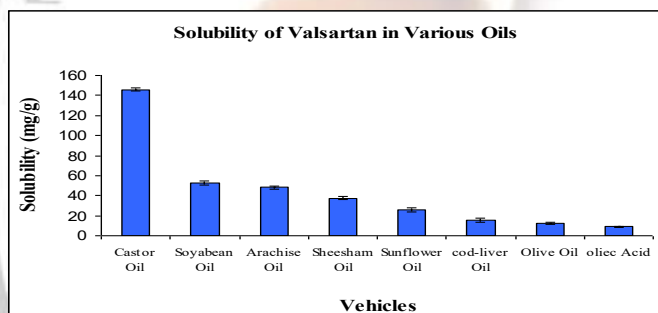

Fig 1: Solubility of drug in various Oils

The solubility of the drug was tested in different surfactant and co-surfactant and maximum solubility determined 122.83±2.56 mg/g of tween-80 as a surfactant phase and 178.26±2.05 mg/g of PEG-600 as a co-surfactant phase. It was selected as surfactant for SEDDS formulation.
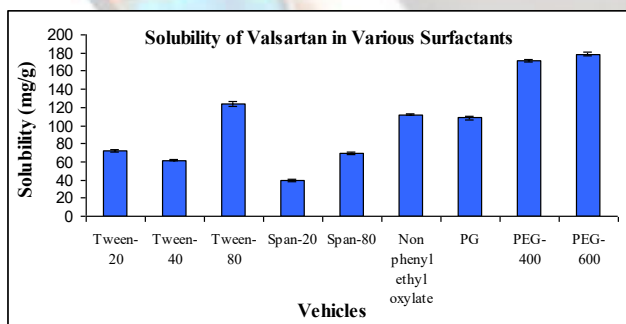

Fig 2: Solubility of drug in different Surfactants

Pseudo-Ternary Phase Diagram

The construction of pseudo ternary phase diagram of 1:1 Smix ratios maximum area covered by particular Smix was selected which indicates that the area covers the maximum numbers of formulation.
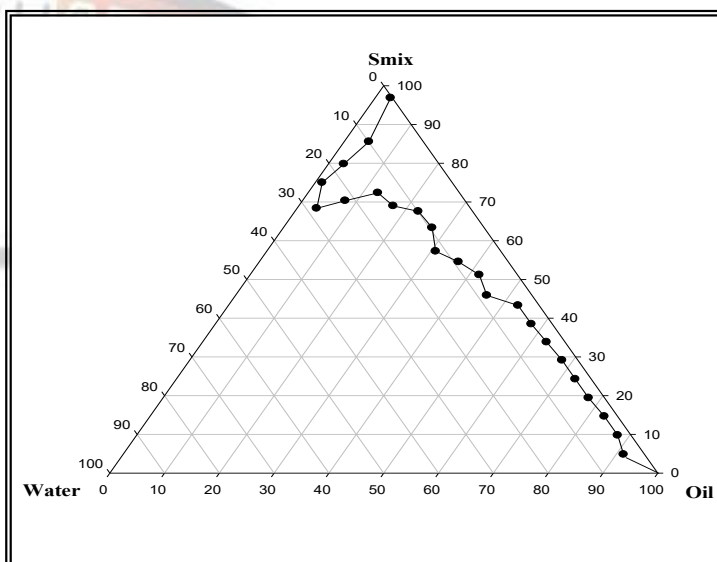

Fig 3: Pseudo ternary phase diagram of Smix ratio 1:1

Preparation of Self Emulsifying Formulation

After the construction of pseudo ternary phase diagram of 1:1 Smix ratios maximum area covered by particular Smix was selected and also which indicate that the area covers the maximum numbers of formulation and further subjected to spontaneous emulsification formation and percent transmittance test[6].

Thermodynamic Stability Studies

The thermodynamic stability study was performed three studies like heating cooling cycle, freeze thaw cycle, centrifugation. On the basis of the mentioned three studies six formulations were selected out of nine formulations and results were shown in Table 1

On the basis of the thermodynamic stability studies it was found that 6 formulations were passed and selected for further characterization. Note- S. No. 2, 3, 4, 5, 7, and 9 was passed thermodynamic studies and it mentions F1, F2, F3, F4, F5, and F6 respectively.

Table 1: Thermodynamic stability studies

Heating cooling cycle	Centrifugation	Freeze thaw cycle
√	X	X
√	√	√
√	√	√
√	√	√
√	√	√
X	√	X
√	√	√
√	X	X
√	√	√

Where √ Formulation passed
X Formulation failed

Robustness to dilution
Robustness to dilution was performed diluted with excess of water, standard phosphate buffer pH 6.8 and 0.1N HCl (500-900 ml) and was stored for 12 hours gives no precipitation or phase separation was found and result were shown in Table 2

Table 2: Robustness to dilution

Vehicles	F1	F2	F3	F4	F5	F6
Distilled water	√	√	√	√	√	√
0.1N HCl	√	√	√	√	√	√
Phosphate buffer pH 6.8	√	√	√	√	√	√

Where √ Stable formulation

Assessment of efficiency of self emulsification
Assessments of efficiency of self emulsification were performed for all the formulations. The *in-vitro* performances of the formulation were visually assessed using the grading system and the results were shown in Table 3

Emulsification time
The emulsification time of SEDDDS was determined as per procedure and maximum emulsification time was found in F2 formulation and the results were shown in Table 3

Turbidity measurement
The turbidity of SEDDS was performed determined as per procedure and maximum turbidity was found in F6 formulation and the results were shown in Table 3

Viscosity determination
The viscosities of the system were determined as such before and after dilution using Brookfield viscometer

DV-E using spindle RV-6 at 100 rpm at 25±0.5°C and results were shown in Table 3

Table 3: Results of parameters

Parameters		F1	F2	F3	F4	F5	F6
Assessment of self emulsification		Grade A	Grade A	Grade B	Grade B	Grade B	Grade C
Emulsification time (Second)		20±4.04	11±1.52	21±3.60	15±2.51	24±1.73	30±2.00
Turbidity NTU	1000 NTU	005	011	018	028	048	068
Viscosity (cps)	Before dilution	22	20	24	21	28	31
	After dilution	10	9	12	9	13	15

Where- value expressed as Mean ± SD, (n=3)

***In-vitro* dissolution study**
Drug release from the SEDDS formulation (F2) was found to be significantly higher as compared with that of marketed valsartan tablet. It could be suggested that the SEDDS formulation resulted in spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of marketed valsartan tablet. Thus, this greater availability of dissolved valsartan from the SEDDS formulation could lead to higher absorption and higher oral bioavailability. The maximum drug release was found to be F2 formulation 98.19±0.6 and results were shown in figure 4

Droplet size analysis
Photon correlation spectroscopy (Malvern instrument, Australia) using laser light scattering was employed to measure particles sizes of preconcentrate generated emulsion/ microemulsion. The samples were loaded onto 1cm^2 cuvettes in a thermostated chamber. For microemulsion particles size ≤150 nm and coarse emulsion particles size >150 nm (Li *et al*., 2004). But some scientist the nano emulsion showed fairly similar mean globule size with in range of 150-170 nm (7).
The best release result of *in-vitro* dissolution study F2 formulation was determined by photon correlation spectroscopy for zetasizer on droplet size analysis and the results are shown in Figures 5.

Zeta potential determination
The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles have a large negative or positive zeta potential they will repel each other and there is dispersion stability. If the particles have low zeta

potential values then there is no force to prevent the particles coming together and there is dispersion instability. A dividing line between stable and unstable aqueous dispersions is generally taken at either +30 or -30 mV. Particles with zeta potentials more positive than +30 mV are normally considered stable. Particles with zeta potentials more negative than -30mV are normally considered stable. Zeta potential of the system negative (-) mV, which indicated the droplets of microemulsion having negative charge, which is closer to range. The best release results of *in-vitro* dissolution study F2 formulation was determined by photon correlation spectroscopy for zeta potential meter on surface charge of the emulsion and the results are shown in Figures 6.
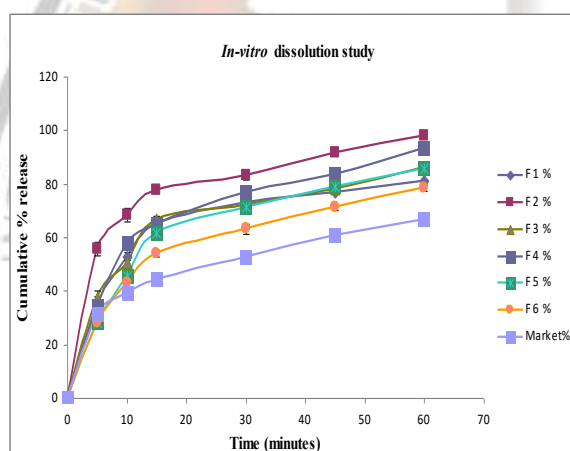

Fig 6: Zeta potential of F2 formulation
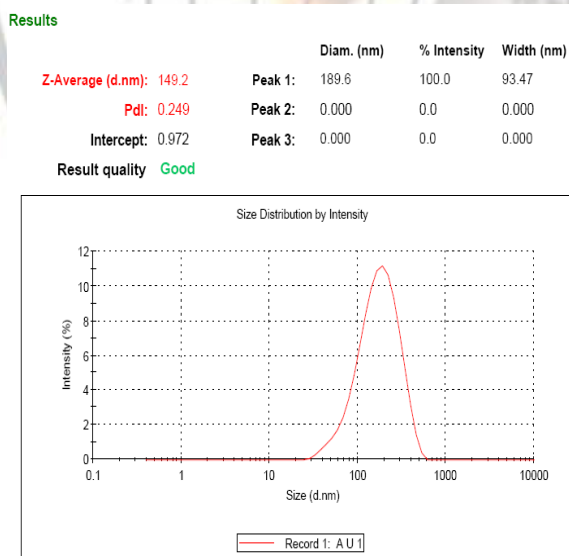

Fig 4: *In-vitro* dissolution studies

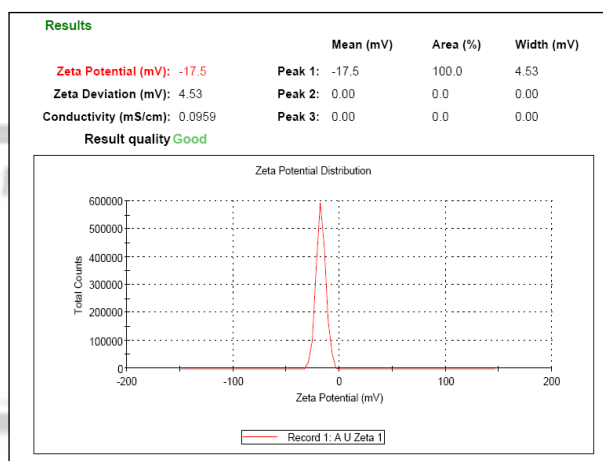**Fig 5: Droplet size analysis of F2 formulation**

In SEDDS formulation consist of oil, surfactant and co-surfactant. Oil, surfactant and co-surfactant were selected on the basis of solubility and emulsification ability. Castor oil, tween-80 and PEG-600 were selected on the basis of solubility and emulsification ability for the SEDDS formulation.

Valsartan was formulated as a SEDDS in an attempt to increase its solubility. An optimized formulation of SEDDS containing valsartan was developed through the construction of pseudo-ternary phase diagram, *in-vitro* dissolution study, particle size analysis and zeta potential and other evaluation study. SEDDS provided significant increase in the solubility compared to a marketed formulation. SEDDS appeared to be an interesting approach to improve problems associated with oral delivery of valsartan. Valsartan SEDDS formulation was superior to marketed formulation with respect to *in-vitro* dissolution profile activity. Thus, SEDDS can be regarded as novel and commercially feasible alternative to current valsartan formulations.

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