Research Article

Development and Evaluation of Atazanavir Solid SEDDS: *In vitro - in vivo* Evaluation Studies

Karunakara Reddy T*, Yajaman Sudhakar[1], N Devanna[2]
*Research and Development department, JNTU, Ananthapur, A.P, India.
1. S.V. Govt. Polytechnic College, Tirupathi, A.P, India.
2. Dept. of Chemistry, JNTU, Aanthapur, A.P, India.
***Corresponding author's E-mail:** tkarunakareddy@gmail.com

Accepted on: 12-09-2016; **Finalized on:** 30-11-2016.

ABSTRACT

The objective of present study was to develop solid self-micro emulsifying drug delivery system (S-SEDDS) with Neusilin for enhancement of dissolution rate of model drug atazanavir. Atazanavir SEDDS was prepared using oils - Captex 35, Capryol 90, Castor oil, Olive oil, surfactants - Gelucire 44/14, Kolliphor HS 15, Kolliphor RH 40, Labrasol, Tween 80, Lauroglycol, co-surfactants - PEG 400, PEG 600, Propylene glycol. Solid SEDDS were prepared using adsorbing agent Neusilin US2. A pseudo ternary phase diagram was constructed to identify the self-micro emulsification region. Further, the resultant formulations were investigated for clarity, phase separation, globule size, effect of pH and dilutions and freeze-thaw stability and found to be within the limits. The optimized SMEDDS (F6) formulation of atazanavir contained Castor oil (Oil), Kolliphor RH 40 (Surfactant) and PEG 400 (Co-surfactant). The prepared liquid SEDDS was thermodynamically stable with good self-emulsification efficiency and having globule size in nanometric range which may be physiologically stable. On the basis of different evaluation parameters F6 was found to be optimized formulation. S-SEDDS of atazanavir prepared with optimized SEDDS (F6) using adsorbing agent Neusilin US2 by adsorption technique have good flow property and drug content. This optimized formulation (F6) was converted in to solid SEDDS by adding required quantity of Neusilin US2 as adsorbing agent used for in vitro dissolution and bioavailability assessment. Results of SEM demonstrate that spherical S-SEDDS can be obtained without agglomeration. *In vivo* studies revealed that the oral bioavailability of atazanavir from solid SEDDS was 2.3-fold higher compared to that of atazanavir suspension in rats, suggesting a significant increase ($p < 0.05$) in oral bioavailability of atazanavir from solid SEDDS. Hence it was concluded that S-SEDDS can be efficiently formulated by adsorption technique using Neusilin US2 as solid carrier to enhance dissolution rate of poorly soluble drug such as atazanavir.

Keywords: Atazanavir, solid SEDDS, Neusilin US2, Castor oil.

INTRODUCTION

About 40% of the drug candidates identified via combinatorial screening programmes are poorly water soluble. The aqueous solubility for poorly water soluble drugs is usually less than 100 µg/ml[1]. Especially poorly soluble, highly permeable active pharmaceutical ingredients (BCS Class II drugs) represent the technological challenge, as their poor bioavailability is solely caused by poor water solubility resulting in low drug absorption[2]. Self-emulsifying drug delivery systems (SEDDs) have gained exposure for their ability to increase solubility and bioavailability of poorly soluble drugs[3].

Self-emulsifying drug delivery systems (SEDDs) are mixtures of oils and surfactants, ideally isotropic, and sometimes containing co-solvents, which emulsify spontaneously to produce fine oil-in-water emulsions when introduced into aqueous phase under gentle agitation. Recently, SEDDS have been formulated using medium chain tri-glyceride oils and non-ionic surfactants, the later being less toxic[4,5].

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[6].

SEDDS are generally encapsulated either in hard or soft gelatin capsules. Lipid formulations however may interact with the capsule resulting in either brittleness or softness of the shell. To overcome this problem SEDDS need to convert into Solid SEDDS. Numerous reports states that, the major techniques for converting SEDDS to SSEDDS are spray cooling, spray drying, adsorption onto solid carriers, melt granulation, melt extrusion, super-critical fluid based methods and high pressure homogenization. But adsorption process is simple and involves simply addition of the liquid formulation to solid carriers by mixing in a blender[7,8].

MATERIALS

Atazanavir pure drug, Lauroglycol, Labrasol & Neusilin US2 was generous gift from Hetero drugs limited, Hyderabad, India. Castor oil, Capryol 90, Captex 355 and Olive oil were obtained from Granules India limited, Hyderabad. Gelucire 44/14, Kolliphor HS 15, Kolliphor RH 40, Labrasol, Lauroglycol, were gifted from BASF, Mumbai. Tween 80, Propylene glycol, PEG 400 and PEG 600 were obtained from SDFCL, Mumbai. All other chemicals used were of analytical grade.

METHODS

Solubility Studies

The solubility study was used to find out the suitable oil, surfactant and co-surfactant that possess good solubilizing capacity for atazanavir. An excess amount (250 mg) of atazanavir was added into 2 ml of each excipient (oils - Captex 35, Capryol 90, Castor oil, Olive oil, surfactants - Gelucire 44/14, Kolliphor HS 15, Kolliphor RH 40, Labrasol, Tween 80, Lauroglycol, co-surfactants - PEG 400, PEG 600, Propylene glycol) and kept in mechanical shaker for 24 hrs and centrifuged at 10,000 rpm for 20 min using a centrifuge. Supernatent was filtered through membrane filter using 0.45µm filter disk. Filtered solution was appropriately diluted with methanol, and UV absorbance was measured at 299nm. Concentration of dissolved drug was determined spectrophotometrically[12].

Construction of pseudo ternary phase diagram

Pseudo ternary phase diagram is used to map the optimal composition range for three key excipients according to the resulting droplet size following self-emulsification, stability upon dilution and viscosity. On the basis of the solubility studies of drug in oil, surfactants and co-surfactants were used for construction of phase diagram. Surfactant and co-surfactant (Smix) in each group were mixed in different weight ratio (1:1, 1:2, 2:1, 3:1). 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 microemulsion. For each phase diagram, oil and specific Smix ratios are mixed thoroughly in different weight ratio from 1:9 to 9:1 (1:9, 2:8, 3:7, 4:6, 5:5, 6:4,7:3, 8:2, 9:1) in different glass vials. 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 micro emulsion. The physical state of the micro emulsion was marked on a pseudo-three-component phase diagram with one axis representing oil, the other representing surfactant and the third representing co-surfactant at fixed weight ratios.

Development of SEDDS formulation

A series of SEDDS formulations for atazanavir were prepared based on solubility studies, pseudo ternary phase diagram and visual observation. Here, Castor oil was used as oil phase and Kolliphor RH 40 and PEG 400 were used as surfactant and co-surfactant respectively. The compositions were given in the **Table 1**. In brief, atazanavir (100mg) was added in accurately weighed amount of oil into screw-capped glass vial and heated in a water bath at 40ºC. The surfactant and co-surfactant were added to the oily mixture using positive displacement pipette and stirred with magnetic bar. The formulation was further sonicated for 15mins and stored at room temperature until its use in subsequent studies.

Table 1: Formulation trials of liquid SEDDS

Smix (Surfactant: Co-surfactant)	Oil:Smix	Formulation code	Drug (Atazanavir) (mg)	Oil (Castor oil) (ml)	Surfactant (Kolliphor RH 40) (ml)	Co-surfactant (Peg-400) (ml)
3:1	1:5	F1	100	0.640	2.488	0.828
	1:6	F2	100	0.568	2.572	0.856
	1:7	F3	100	0.500	2.624	0.872
	1:8	F4	100	0.444	2.664	0.888
	1:9	F5	100	0.400	2.700	0.900
2:1	1:8	F6	100	0.444	2.368	1.184
	1:9	F7	100	0.400	2.400	1.200
	1:6	F8	100	0.568	2.284	1.140
	1:5	F9	100	0.664	2.220	1.108

Freeze thawing

Freeze thawing was employed to evaluate the stability of formulations. The formulations were subjected to 3 to 4 freeze-thaw cycles, which included freezing at – 4 °C for 24 hours followed by thawing at 40 °C for 24 hours. Centrifugation was performed at 3000 rpm for 5 minutes. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected for further studies.

% Transmittance

% Transmittance of Atazanavir SEDDS was measured by U.V spectroscopy at wavelength of 400 to 500nm. A graph for %particle range vs. formulations was plotted.

Determination of drug content

SEDDS equivalent to 100mg of Atazanavir were weighed accurately and dissolved in 100 ml of phosphate buffer pH 6.8. The solution was filtered, diluted suitable and drug content was analyzed at λ_{max} 299 nm against blank

by UV spectrometer. The actual drug content was calculated using the following equation as follows:

$$\text{\% Drug content} = \frac{\text{Actual amount of drug in SEDDS}}{\text{Theoretical amount of drug in SEDDS}} \times 100$$

In-Vitro Dissolution studies

The release of drug from liquid SEDDS formulations and pure drug was determined using a US Pharmacopoeia Type II dissolution apparatus. The liquid SEDDS formulations were directly placed into the medium[13]. The dissolution media is phosphate buffer pH 6.8, and temperature of the dissolution medium was maintained at 37^{0}C operated at 50 rpm. An aliquot of 5 ml was withdrawn at predetermined intervals 2, 5, 10, 15, 20, 25, 30, 45, and 60mins and filtered through 0.45-µm pore size membrane filters. The removed volume was replaced each time with 5 ml of fresh medium. The concentrations were assayed spectrophotometrically at 299 nm.

Oil adsorption study

The objective of this study was to select adsorbent for preparing free flowing solid Self emulsifying formulation for Atazanavir. Microcrystalline cellulose, colloidal silicon dioxide, Neusilin US2, dicalcium phosphate and tricalcium phosphate were used as adsorbents. They were added separately to the optimized liquid SEDDS formulation i.e F6 under stirring. Adsorbents were added to the formulation until free flowing blend was formed. Neusilin US2 showed higher oil adsorption capacity when compared to other adsorbents[14].

Conversion of SEDDS to SOLID SEDDS

The optimized liquid SEDDS formulation (F6) based on droplet size and dissolution study was converted into free flowing powder by adsorption onto solid carriers and the composition was shown in Table 2. The solid carrier used for adsorption comprised of materials that provided a high surface area with good disintegration characteristics. Neusilin US2 (Magnesium aluminum silicate) was used as a solid carrier. It can adsorb at high levels up to 70% (w/w). The conversion process involved addition of liquid formulation onto carriers under continuous mixing.

Table 2: Composition of SEDDS and solid SEDDS

Components	SEDDS (F6) (g)	Solid – SEDDS (gms)
Atazanavir	0.02	0.02
Castor oil	0.426	0.426
Kolliphor RH 40	2.429	2.429
PEG 400	1.133	1.133
Neusilin US2	-	1.300

Determination of drug content of solid SEDDS

Solid SEDDS equivalent to 100mg of Atazanavir were weighed accurately and dissolved in 100 ml of phosphate buffer pH 6.8. The solution was filtered, diluted suitable and drug content was analyzed at λ_{max} 299 nm against blank by UV spectrometer. The actual drug content was calculated using the following equation as follows:

$$\text{\% Drug content} = \frac{\text{Actual amount of drug in Solid SEDDS}}{\text{Theoretical amount of drug in Solid SEDDS}} \times 100$$

In Vitro Dissolution studies of SEDDS

The dissolution test was performed using USP type 2 dissolution apparatus (paddle method) with 900 ml of pH 6.8 phosphate buffer containing various concentrations of Atazanavir at 37 ^{0}C with a paddle speed of 50 rpm. The liquid SEDDS containing 100 mg of Atazanavir was filled into hard gelatine capsules (Capsule No. 00), samples were collected at appropriate time intervals 2, 5, 10, 15, 20, 25, 30, 45 & 60min and filtered through 0.45-µm pore size membrane filters. 5 ml of the sample was withdrawn and replaced with same volume of dissolution medium and the concentration of Atazanavir was measured at 299nm by UV-VIS spectroscopy.

Characterization of SEDDS:

Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectra of pure drug, excipients and optimized formulations were recorded using FT-IR (Shimadzu 8400-S) with diffuse reflectance principle. Sample preparation involved, drying of potassium bromide (KBr), drug and excipients in the oven to get rid of any moisture content then mixing the sample with KBr by triturating in glass mortar. Finally preparing of pellet and placing in the sample holder. The spectrum was scanned over a frequency range 4000 – 400cm^{-1} [15].

Determination of droplet size

The droplet size of the micro emulsions is 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 2 nm and 5000 nm. Light scattering is monitored at 25°C at a 90° angle.

Determination of zeta potential

The emulsion stability is directly related to the magnitude of the surface charge. In conventional SEDDS, the charge on an oil droplet is negative because of the presence of free fatty acids. The zeta potential of the diluted SEDDS formulation was measured using a zeta meter system. The SEDDS were diluted with a ratio 1:2500 (v/v) with distilled water and mixed with magnetic stirrer. Zeta-potential of the resulting micro emulsion was determined using a Malvern Zetasizer[16].

Characterization of solid SEDDS

Micromeritic properties of solid SEDDS

Prepared S-SEDDS was evaluated for micromeritic properties such as angle of repose, bulk and tapped density, compressibility index and Hausner's ratio.

Drug entrapment efficiency of solid SEDDS

The quantities of the drugs theoretically contained in the SEDDS were compared with the quantity actually obtained, from the drug content studies i.e. the quantity loaded into the SEDDS formulated. To get the drug encapsulation efficiency, following equation is used for calculation.

$$EE\ (\%) = ADC/TDC \times 100$$

Where

ADC is the actual drug content.

TDC is the theoretical drug content

Scanning electron microscopy

The surface and shape characteristics of pellets were determined by scanning electron microscopy (SEM) (HITACHI, S-3700N). Photographs were taken and recorded at suitable magnification.

Stability studies

The SEDDS formulations were put into empty hard gelatin capsules and subjected to stability studies at 25°C/60% relative humidity (RH), 30°C/65% RH, and 40°C/75% RH. Samples were charged in stability chambers (Thermolab, Mumbai, India) with humidity and temperature control. They were withdrawn at specified Accelerated conditions and 3months for long-term conditions. Drug content of the capsules was analyzed using a previously developed and validated stability-indicating UV method.

***In vivo* bioavailability studies**

Twelve healthy Wister rats weighing 250 ± 20 g were used for this study. The protocol of animal study was approved by the institutional animal ethics committee. The Wister Rats were divided in to two groups at random and each group contains six animals. First group was administered with pure Atazanavir made suspension with 0.5% methocel and second group was administered with solid SEDDS diluted in 0.5% methocel by oral route at an equivalent dose of 20 mg/kg body weight. About 500 µl of blood was withdrawn from retro orbital plexus at different time intervals such as 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00 & 24.00h. During collection, blood sample has been mixed thoroughly with heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5min to 10 minutes and stored frozen at −20°C until analysis. The concentration of Atazanavir from plasma was measured by using reversed phase HPLC. The chromatographic system consisted of a C18 chromatographic column, Phenomenex (150 mm × 4.6 mm ID) and a mobile phase consisting of Acetonitrile and ammonium acetate buffer (pH-3.5) in the ratio of 55: 45. The flow rate was 1.0 ml/min and effluents were monitored at 252 nm[17]. Plasma samples (250 µl) were transferred into a test tube to which internal standard (Hydrochlorothiazide 50µl) and 1 ml of acetic acid was added and vortexed for 3 min. To this, 1ml of chloroform was added and vortexed. The samples were centrifuged at 10,000 rpm for 10 min. The organic phase was transferred into another test tube and the solvent was evaporated to dryness. The residue was redissolved in 200 µl of mobile phase, of which 20 µl of the supernatant was injected for analysis[18].

Pharmacokinetic analysis

The pharmacokinetic parameters, peak plasma concentrations (C_{max}) and time to reach peak concentration (t_{max}) were directly obtained from concentration time data. In the present study, AUC_{0-t} refers to the AUC from 0 to 24 hrs, which was determined by linear trapezoidal rule and $AUC_{0-\alpha}$ refers to the AUC from time at zero hours to infinity.

The $AUC_{0-\alpha}$ was calculated using the formula $AUC_{0-t} + [C_{last}/K]$ where C_{last} is the concentration in µg/ml at the last time point and K is the elimination rate constant.

Various pharmacokinetic parameters like area under the curve [AUC], elimination half life (t½). Volume of distribution (V_d), total clearance (Cl_T) and mean residence time for each subject using a non compartmental pharmacokinetic programme. The pharmacokinetic parameters were performed by a non compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean ±SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

Solubility studies

The Atazanavir pure drug solubility was found to be 19.4 mg/ml. The solubility of the Atazanavir pure drug was tested in different oils phases and maximum solubility was found in Castor oil 36.55 mg/ml**.** The solubility of the drug was tested in different surfactants and co-surfactants, maximum solubility was found in Kolliphor RH 40, and PEG 400 as 42.63 mg/ml and 41.54 mg/ml was in PEG-400 respectively. Castor oil, Kolliphor RH 40, and PEG 400 were used for the formulation of Atazanavir SEDDS.

Construction of ternary phase diagram

From the solubility studies, Castor oil, Kolliphor RH 40, and PEG 400 were selected as oil, surfactant and co-

surfactant respectively. Diagram shown in **Figure 1**, it was observed that self-emulsifying region was enhanced with increasing concentrations of surfactant and co-surfactant with oil. Efficiency of self-emulsification was good when the surfactant concentration increased. With the use of visual observation method, the tendency of formation of emulsion was observed. Visual observation test was performed for different ratios by keeping the surfactant and co-surfactant ratio (Smix) as 2:1 and 3:1. Grades were given to the ratios based on the tendency of formation of micro-emulsion. Ratios 1:5, 1:6, 1:8, and 1:9 of Smix 2:1 and 1:5, 1:6, 1:7, 1:8, 1:9 of Smix 3:1 showed rapid formation of micro emulsion within a minute having a clear appearance. Therefore these ratios were selected for the formulation of SEDDS. From the phase diagram, it was observed that self-emulsifying region increased with increasing concentrations of surfactant or combination of surfactant and co-surfactant. Efficiency of self-emulsification was good when the surfactant concentration was increased.

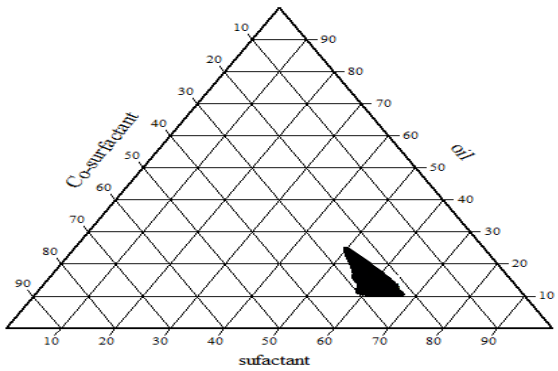

Figure 1: Ternary phase diagram of Castor oil, Kolliphor RH 40 and PEG 400

Preparation of Atazanavir SEDDS

SEDDS of Atazanavir were prepared by using Castor oil (oil), Kolliphor RH 40 (surfactant), and PEG 400 (co-surfactant). In the present study, nine formulations were prepared and their complete composition was shown in **Table 1**. All the formulations prepared were found to be clear and transparent.

Freeze thaw method

In thermodynamic stability study, no phase separation and no change of temperature variations on prepared formulations were observed. There was no change in the visual description of samples after centrifugation freeze-thaw cycles.

% Transmittance measurement

The clarity of micro emulsions was checked by transparency, measured in terms of transmittance (%T). SEDDS forms o/w microemulsion since water is external phase Formulation F6 has % transmittance value greater than 99%.These results indicate the high clarity of microemulsion. In case of other systems %T values were less than 99% suggesting less clarity of microemulsions. This may be due to greater particle size of the formulation. Due to higher particle size, oil globules may reduce the transparency of microemulsion and thereby values of %T.

Drug content of SEDDS

The drug content of the prepared SEDDS was found to be in the range of 91.43 - 97.76 %. Maximum % drug content i.e. 97.76% was found in the formulation F6.

***In-Vitro* Dissolution studies of SEDDS**

The results of in vitro dissolution comparisons of SEDDS formulations are shown graphically in **Figure 2.** The faster dissolution from SEDDS may be attributed to the fact that in this formulation, the drug is a solubilized form and upon exposure to dissolution medium results in small droplet that can dissolve rapidly in the dissolution medium. The % release from liquid SEDDS formulation F6 was highest (95.42) and faster than other SEDDS formulations and pure drug substance indicating influence of droplet size on the rate of drug dissolution.

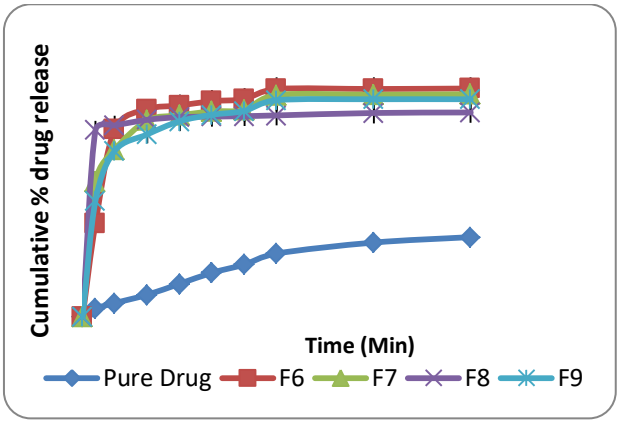

Figure 2: Dissolution profiles of Atazanavir pure drug and different formulations

When compared to % transmittance, emulsification test and in vitro drug release studies for all formulations, F6 formulation was found to be best and optimized formulation and further characterized for other studies.

Oil Adsorption Study

Oil adsorption study was performed for Atazanavir by using different adsorbents**.** Neusilin US2 showed higher oil adsorption capacity when compared to other adsorbents. From oil adsorption study Neusilin US2 was used for the preparation of Solid SEDDS for adsorbing the optimized SEDDS formulation (F6).

SEDDS to Solid SEDDS

On the basis of visual observation test and faster dissolution rate, formulation F6 was selected as optimized formulation and this formulation was converted in to solid SEDDS by adding required quantity of Neusilin US2 as adsorbing agent.

Micromeretic properties of Atazanavir solid SEDDS

The micromeretic properties angle of repose, LBD, TBD, Carr's index and Hausner's ratio of the solid SEDDS was found to be 26.65, 0.22, 0.25, 12.31and 1.13 respectively, which shows good flow properties of the powdered blend.

Drug Content of solid SEDDS

The drug content of solid SEDDS was carried out. The % drug content of the optimized formulation was found to be 97.89%.

Dissolution sstudies of solid and liquid SEDDS

The release of Atazanavir from solid, liquid SMEDDS formulations, pure drug and marketed product (Atazor) - Atazanavir 100mg tablets was determined.. The concentrations were assayed spectrophotometrically at 257nm. The comparative dissolution profiles of solid and liquid SEDDS formulation (F6) with pure drug and Marketed product are shown in **Fig 3.** Solid and liquid SEDDS have shown better release profiles when compared with the pure drug, SEDDS and the innovator product.

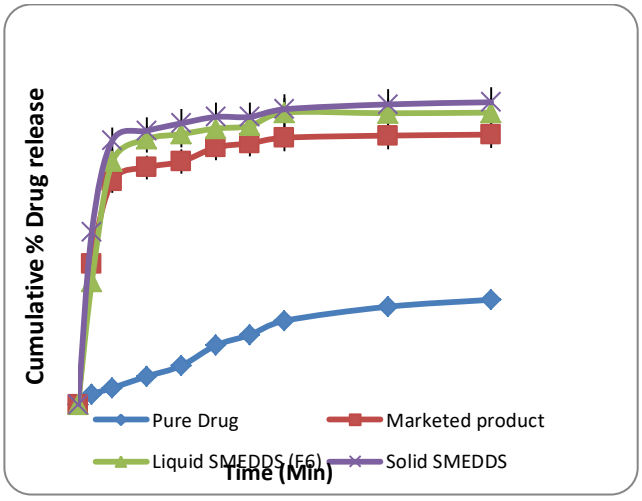

Figure 3: Comparative results of drug release from SEDDS, Solid SEDDS, Pure drug and Marketed product

Particle size analysis of SEDDS

Droplet size of Atazanavir emulsion decreased with reducing the oil content in SEDDS. The smaller the droplet size, the larger the interfacial surface area will be provided for drug absorption. The size of F6was found to be below range of 24.2 nm which indicated that formulation F6 was SEDDS. The Polydispersity index of the optimized Atazanavir formulation (F6) was found to be 0.484.

Zeta potential of SEDDS

Zeta potential has got practical application in the stability of emulsion since it governs the degree of repulsion between adjacent, similarly charged and dispersed droplets. In general, the zeta potential value of ±30 mV is sufficient for the stability of a micro emulsion. The zeta potential of the optimized SEDDS formulation was found to be -33.0 mV which comply with the requirement of the zeta potential for stability.

FT-IR Studies

Interpretation of FTIR data

FT-IR spectrums are mainly used to determine if there is any interaction between the drug and any of the excipient used. The FTIR spectra of pure Atazanavir displayed bands at 3398 cm^{-1} due to N-H stretch, at 1707 cm^{-1} due to C=O stretching, at 1631 cm^{-1} due to heterocyclic C=C stretching. The spectra also showed bands at 1224 cm^{-1} due to C-N bending. The FTIR Spectrum of Atazanavir Pure drug + Castor oil and Atazanavir with excipient (Kolliphor RH 40) are respectively. The FTIR spectrum of SEDDS containing Atazanavir exhibited characteristic bands consistent with the molecular structure of Atazanavir such as bands at 3396 cm^{-1} due to N-H stretch, at 1710 cm^{-1} due to C=O stretching, at 1631 cm^{-1} due to heterocyclic C=C stretching, at 1251 cm^{-1} due to C-N bending**.** Thus, the presence of characteristic absorption bands of Atazanavir and the SEDDS containing Atazanavir suggest that there was no interaction between the drug and excipients used in the formulation.

Scanning electron microscopy (SEM) For Solid SEDDS

Micrographs of Solid SEDDS shows Liquid SEDDS adsorbed onto the surface of Neusilin US2 particles. Since the formulation process involved facilitating adsorption through physical mixing, partially covered Neusilin US2 is also visible in the field of vision. Crystalline structures characteristic of Atazanavir are not seen in solid SEDDS micrographs suggesting that the drug is present in a completely dissolved state in the Solid SEDDS. **Figure 4** represents the morphology of solid SEDDS. This indicates that solid SEDDS appeared as spherical particles having an even and a smooth surface.

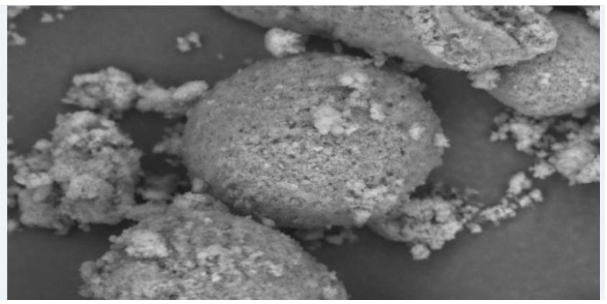

Figure 4: Scanning Electron Microscopy of Solid SEDDS

Stability studies

The Atazanavir SEDDS are put into hard gelatin capsules as the final dosage form. The developed formulation was subjected to stability studies to evaluate its stability and the integrity of the dosage form. There was no significant change in the drug content, drug release. It was also seen that the formulation was compatible with the hard gelatin capsule shells, as there was no sign of capsule

shell deformation. There was no significant change in the appearance, or microemulsifying property. Thus, these studies confirmed that the formulation was stable and its compatibility with hard gelatin capsules.

Bioavailability studies

As can be seen from the **Table 3**, the solid SMEDDS shows the increased AUC values of about 513.62±4.56 ng.hr/ml and 648.94±5.43 ng.hr/ml when compared with pure drug. C_{max} of the solid SMEDDS 52.92±2.43ng/ml was significant ($p<0.05$) as compared to the pure drug suspension formulation 41.46±1.23ng/ml. T_{max} of both solid SMEDDS formulation and pure drug suspension was 3.00±0.22and 2.50±0.12h, respectively. AUC is an important parameter in evaluating bioavailability of drug from dosage form, as it represents the total integrated area under the blood concentration time profile and represents the total amount of drug reaching the systemic circulation after oral administration. $AUC_{0-\infty}$ infinity for solid SMEDDS formulation was higher (829.05±4.76 ng.hr/ml) than the pure drug suspension formulation 723.85±4.76 ng.hr/ml. Statistically, AUC_{0-t} of the solid SMEDDS formulation was significantly higher ($p<0.05$) as compared to pure drug suspension formulation. Higher amount of drug concentration in blood indicated better systemic absorption of atazanavir from solid SMEDDS formulation as compared to the pure drug suspension formulation.

Table 3: Pharmacokinetic Parameters of Atazanavir solid SMEDDS formulation and pure drug

Pharmacokinetic Parameters	Atazanavir pure drug	Atazanavir solid SMEDDS
C max (ng/ml)	41.46±1.23	52.92±2.43
AUC 0-t (ng.hr/ml)	513.62±4.56	648.94±5.43
AUC 0-inf (nng.hr/ml)	723.85±4.76	829.05±4.76
T max (h)	2.50±0.12	3.00±0.22
t 1/2 (h)	5.00±0.15	5.32±0.15

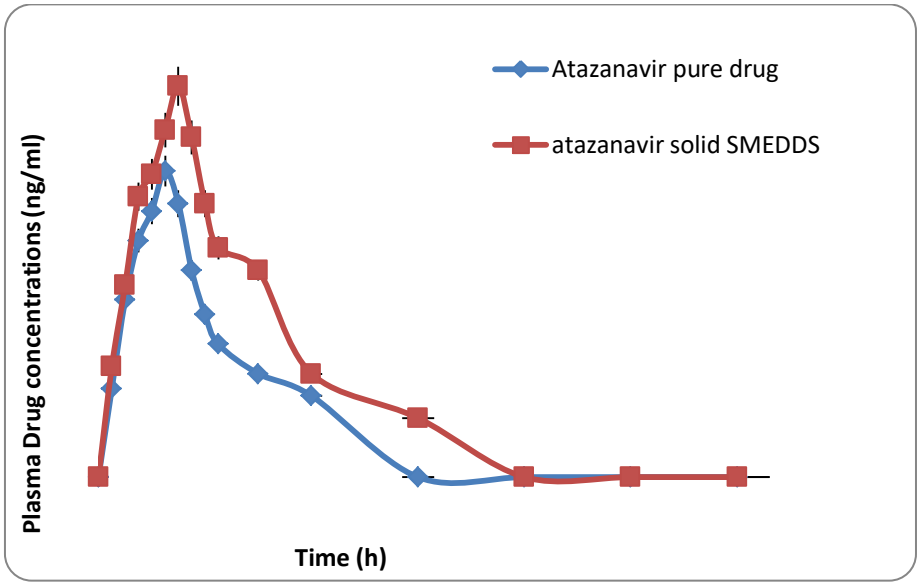

Figure 5: Plasma concentration profiles of Atazanavir solid SEDDS and pure drug

SUMMARY AND CONCLUSION

Atazanavir, being a lipophilic drug, was formulated as SEDDS based on the oil solubility studies and ternary phase diagrams. From this study it was concluded that, prepared liquid SEDDS was thermodynamically stable with good self-emulsification efficiency and having globule size in nanometric range which may be physiologically stable. On the basis of different evaluation parameters F6 was found to be optimized formulation. Study also concluded that, S-SEDDS of Atazanavir prepared with optimized SEDDS (F6) using adsorbing agent Neusilin US2 by adsorption technique have good flow property and drug content. SEDDS formed clear microemulsion with micrometric size. Results of SEM demonstrate that spherical S-SEDDS can be obtained without agglomeration. In-vitro drug release of S-SEDDS was much higher than that of pure Atazanavir, SEDDS and marketed formulation. Hence it was concluded that S-SEDDS can be efficiently formulated by adsorption technique using Neusilin US2 as solid carrier to enhance dissolution rate of poorly soluble drug such as Atazanavir.

The oral bioavailability study of Atazanavir solid SMEDDS showed improvement by a factor of 2.3 compared to the pure drug suspension in rats. Thus Atazanavir solid SMEDDS may be used for improvement of oral bioavailability of drugs with poor water solubility and low oral bioavailability.

REFERENCES

1. Ngiik T, Amal AE. Effects of liquid solid formulations on dissolution of naproxen. Eur j pharma and bio pharma 73, 2011, 373-84.
2. Hentzschel CM, Alnaief M, Smirnova I, Sakmann A, Leopold CS. Enhancement of griseofulvin release from liquisolid compacts. Eur j pharma and bio pharma 73, 2011, 1-6.
3. Sachan R, Khatri K, Kasture SB. Self-Eumlsifying Drug Delivery System: A Novel Approach for enhancement of bioavailability. Int J Pharm Tech Res 2, 2010; 1738-45.
4. Patel PA, Chaulang GM, Akolkotkar A, Mutha SS, Hardikar SR, Bhosale AV. Self-emulsifying drug delivery system: A Review. Research J. Pharm. and Tech 1, 2008, 313-23.
5. Patel NN, Rathva SR, Shah VH, Upadhyay UM. Review on self-emulsifying drug delivery system: Novel approach for solubility enhancement. International Journal of Pharmaceutical Research & Allied sciences 1, 2012, 1-12.
6. Katteboina S, Chandrasekhar PVSR, Balaji S. Approaches for the development of solid self-emulsifying drug delivery systems and dosage forms. Asian J Pharma Sci 4, 2004, 240-53.
7. Shah SP, Shah MD, Agrawal YK, Self Micro Emulsifying Drug Delivery System: A Novel Approach for Enhancement of Oral Bioavailability of Poorly Soluble Drugs. Ame J Pharm Tech Research 2, 2012, 193-215.
8. Singh MK, Chandel V, Gupta V, Ramteke S, Formulation development and characterization of micro emulsion for topical delivery of Glipizide. Der Pharmacia Lettre 2, 2010, 33-42.
9. Shailesh T, Prajapati, Harsh A. Joshi, Chhaganbhai N. Patel. Preparation and Characterization of Self-Micro emulsifying Drug Delivery System of Olmesartan medoxomil for Bioavailability Improvement., Journal of Pharmaceutics 2, 2013, 01-09.
10. Chirag R, Neha J, Jitendra P, Upadhyay U M. Enhanced oral bioavailability of Olmesartan by using novel Solid Self Emulsifying Drug Delivery System., International Journal of Advanced Pharmaceutics., 2, 2012, 82-92.
11. Prabagar B, Beom Jin L, Dong Hoon O, Jong Oh K, Young Im L, Chul Soon Y. Enhanced oral bioavailability of Coenzyme Q_{10} by self-emulsifying drug delivery systems. Int J Pharma 2009; 58-73.
12. Anand U, Kyatanwar K, Jadhav R, Vilasrao J K. Self-micro emulsifying drug delivery system (SEDDS): review. J Pharmacy Res 3(1), 2010, 75- 83.
13. Jessy S, Digambar J. Newer approaches to self-emulsifying drug delivery system, International Journal of Pharmaceutical Sciences 2(1), 2010, 57-65.
14. Ahmed Abd E, Mahfouz A, Kassem M, Abou Samra M. Formulation and hypoglycemic activity of Pioglitazone-cyclodextrin inclusion complexes, Drug Discovery 2(2), 2008; 94-107.
15. Eman A, Albert A. Belmonte, Formulation and in vitro and in vitro characterization of a phenytoin self-emulsifying drug delivery system (SEDDS); European journal of pharmaceutical sciences 35, 2008, 257-63.
16. Craig DQM, Barket SA, Banning D, Booth SW. An investigation into the mechanisms of self-emulsification using particle size analysis and low frequencies dielectric spectroscopy. Int J Pharm 114, 1995, 103-10.
17. Amudhavalli V, Lakshmi K. S, Karthick M. development and validation of a reversed phase hplc method for simultaneous estimation of olmesartan and hydrochlorothiazide in combined tablet dosage form. IJRPC 1(3), 2011, 474-75.
18. Mohan G, Ramesh B, Kishan V. Development of sustained release floating drug delivery system for norfloxacin: *In vitro* and *in vivo* evaluation. PDA J Pharm Sci Tech 65(3), 2011, 198-206.

Source of Support: Nil, **Conflict of Interest:** None.