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Enhancement of Solubility and Oral Bioavailability of Poorly Soluble Drug Rilpivirine by Novel Self Emulsifying Drug Delivery System

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ABSTRACT

Aim of present study was to develop self emulsifying drug delivery system (SEDDS) for enhancement of solubility, dissolution rate and oral bioavailability of model drug Rilpivirine. Fifteen formulations were prepared using different oils, surfactants and co-surfactants. A pseudo ternary phase diagram was constructed to identify the self-micro emulsification region. Further, the resultant formulations were investigated for clarity, phase separation, drug content, % transmittance, globule size, freeze-thaw stability and in vitro dissolution studies. On the basis of dissolution profile and other above mentioned studies, F5 was found to be the best formulation of Rilpivirine SEDDS which contains Captex 355(Oil), Kolliphor RH 40 (Surfactant) and PG (Co-surfactant). In vivo studies revealed that the oral bioavailability of Rilpivirine from SEDDS was 2.2-fold higher compared to that of pure Rilpivirine suspension in rats, suggesting a significant increase ($p<0.05$) in oral bioavailability of Rilpivirine from SEDDS formulation.

Keywords: Rilpivirine, SEDDS, Captex 355, Solubility, Bioavailability studies.

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INTRODUCTION

Oral route has been the major route of drug delivery for the chronic treatment of many diseases. However, oral delivery of 50% of the drug compounds is hampered because of the high lipophilicity of the drug itself. 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]. To overcome these drawbacks, various other formulation strategies have been adopted. Among them, one formulation is self-emulsifying drug delivery systems (SEDDS)[3]. Self-emulsifying drug delivery systems (SEDDs) have gained exposure for their ability to increase solubility and bioavailability of poorly soluble drugs[4]. 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[5, 6]. 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[7]. Oral absorption of several drugs has been reported to be enhanced by SEDDS by one of the several mechanisms. Main mechanisms include increasing membrane fluidity to facilitate transcellular absorption, opening tight junction to allow paracellular transport, inhibiting P-gp and/or Cytochrome P450 (CYP450) enzymes to increase intracellular concentration and residence time by surfactants, and stimulating lipoprotein/chylomicron production by lipid. Also specific components of SEDDS promote the intestinal lymphatic transport of drugs which would be very useful in reducing the first pass of the drugs[8]. Human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) is a spectrum of conditions caused by infection with the human immunodeficiency virus (HIV)[9]. HIV is transmitted primarily via unprotected sexual intercourse, contaminated blood transfusions, hypodermic needles, and from mother to child during pregnancy, delivery, or breastfeeding[10]. Rilpivirine is the most recently approved NNRTI. It is a second-generation non-nucleoside reverse transcriptase inhibitor for the treatment of HIV infection with higher potency, longer half-life and reduced side-effects[11]. The aim of the present study is to formulate and evaluate a stable self micro emulsion (SEDDS) of poorly water-soluble drug rilpivirine to enhance the solubility and oral

bioavailability, which provide better effect with decrease dosing frequency and hence produces better patient compliance.

MATERIALS AND METHOD

EDURANT (Rilpivirine 25mg) film coated tablets were purchased from Janssen-Cilag Pvt Ltd, Australia. Rilpivirine pure drug, Lauroglycol, Labrasol was generous gift from Aurobindo Pharma 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.

Solubility studies

It was carried out to determine solubility measurements of Rilpivirine according to the published method[12]. The solubility study was used to find out the suitable oil, surfactant and co-surfactant that possess good solubilizing capacity for Rilpivirine. An excess amount (250 mg) of Rilpivirine was added into 2 ml of each excipient (Oils - Captex 355, Capryol 90, Castor oil, Oliec acid and Miglnoly 812), (Surfactants – Capmul MCM, Cremophor RH 40, Kolliphor EL, Kolliphor ELP, Kolliphor HS 15, Kolliphor RH 40,Kolliphor PS 80, Labrasol, Labrafac, Labrafil M 1944, Labrafil 2125,Tween 80, tween 20, Transcutol-P, 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 280nm. Concentration of dissolved drug was determined spectrophotometrically.

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, 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, 1:8, 1:7, 1:6, 1:5, 1:4,1:3, 1:2, 1:1) and (9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, and 2:1) w/w 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 Rilpivirine were prepared based on solubility studies, pseudo ternary phase diagram and visual observation. Here, Captex 355 was used as oil phase and Kolliphor RH 40 and PG were used as surfactant and co-surfactant respectively. The composition was tabulated in Table 1. In brief, Rilpivirine (25mg) 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	Oil (Captex 355) (ml)	Surfactant(Kolliphor RH 40)(ml)	Co-surfactant (PG) (ml)
1:1	1:9	F1	0.500	2.250	2.250
	1:8	F2	0.555	2.220	2.220
	1:7	F3	0.625	2.187	2.187
	1:6	F4	0.714	2.142	2.142
2:1	1:9	F5	0.500	3.000	1.500
	1:8	F6	0.555	2.960	1.480
	1:7	F7	0.625	2.916	1.458
	1:6	F8	0.714	2.856	1.428
	1:5	F9	0.833	2.776	1.388
3:1	1:9	F10	0.500	3.375	1.125
	1:8	F11	0.555	3.330	1.110
	1:7	F12	0.625	3.281	1.093
	1:6	F13	0.714	3.213	1.071
	1:5	F14	0.833	3.123	1.041
	1:4	F15	1.000	3.000	1.000

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 Rilpivirine SMEDDS was measured by U.V spectroscopy at wavelength of 600 to 660nm. A graph for %particle range vs. formulations was plotted.

Determination of drug content

SMEDDS equivalent to 25mg of Rilpivirine was weighed accurately and dissolved in 100 ml of 0.01N HCL containing Tween 20 at pH 2.0.The solution was filtered, diluted suitable and drug content was analyzed at λ_{max} 280 nm against blank by UV spectrometer. The actual drug content was calculated using the following equation as follows:

% Drug content = Actual amount of drug in SEDDS/ Theoretical amount of drug in SEDDS X 100

***In-Vitro* dissolution studies**

The release of drug from liquid SMEDDS formulations and pure drug was determined using a US Pharmacopoeia Type II dissolution apparatus. The liquid SMEDDS formulations were directly placed into the medium[13]. The dissolution media is 0.5% polysorbate 20 in 0.01N HCL at pH 2.0, and temperature of the dissolution medium was maintained at 37^0C operated at 75 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 280nm.

Characterization of SEDDS:

Drug-excipient compatibility studies:

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 – 400 cm-[1]. [14]

Differential scanning calorimetry (DSC)

Differential scanning calorimetric (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Accurately weighed samples were placed on aluminium plate, sealed with aluminium lids and heated at a constant rate of 5ºC /min, over a temperature range of 0 to 250ºC[15].

Determination of droplet size

The average droplet size of Rilpivirine SMEDDS formulations were determined by Photon correlation spectroscopy (Malvern Instrument UK) able to measure sizes between 10 and 5000 nm. The selected formulations were diluted with deionized water and placed in an electrophoretic cell for measurement[16].

Determination of Zeta potential

The emulsion stability is directly related to the magnitude of the surface charge. In conventional SMEDDS, the charge on an oil droplet is negative because of the presence of free fatty acids. The zeta potential of the diluted SMEDDS formulation was measured using a zeta meter system. The SMEDDS 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[17].

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 SMEDDS 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**

Animals

Healthy Wistar rats were (Weighing 150-180 g) selected for this study, all the animals were healthy during the period of the experiment. All efforts were made to maintain the animals under controlled environmental conditions (Temperature 25^0C, Relative Humidity 45% and 12 h alternate light and dark cycle) with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply. Rats were fed with standard diet and water ad libitum. The protocol of this study was approved by the institutional animal ethics committee.

Study design

Healthy Wistar rats were divided in to two groups at random containing six animals each. The rats were fasted for 24 hours prior to the experiments. After 4 hours of dosing, foods were reoffered. First group was administered with pure Rilpivirine (as such) made suspension with 0.5% methocel

and second group was administered SMEDDS diluted in 0.5% methocel by oral route at a dose of 25mg/kg equivalent to animal body weight. Then, 500 µL blood samples were collected from the retro-orbital vein using a heparinized needle (18-20 size) at 0, 1, 2, 4, 8, 12, 16 and 24 hrs post dose and transferred into Eppendorf tubes containing heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5min to 10minutes and stored frozen at −20°C until analysis[18].

Determination of Rilpivirine in rat plasma by HPLC method

Determination of Rilpivirine and internal standard tenofovir disoproxil fumerate by high performance liquid chromatography using a RP-C18 chromatographic column, Phenomenex Kinetex (150 mm × 4.6 mm i.d) and a mobile phase consisting of Acetonitrile and phosphate buffer PH 3 in the ration of 55:45 at a flow rate 0.35 mL/min and the wavelength detection was 265 nm[19].

Pharmacokinetic analysis

The pharmacokinetic parameters employed to evaluate were maximum plasma concentration (C_{max}), time to attain C_{max} i.e., T_{max} and $t_{½}$ values, area under plasma concentration–time curve from zero to the last sampling time (AUC_{0-t}), area under plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$). AUC_{0-t} was calculated by the linear trapezoidal rule and $AUC_{0-\infty}$ from the following formula.

$AUC_{0-\infty} = AUC_{0-t} + C_t / K_E$

RESULTS AND DISCUSSION

Solubility studies

The Rilpivirine pure drug solubility in water was found to be 0.0116 mg/ml. The solubility of the Rilpivirine pure drug was tested in different oil phases and maximum solubility was found in Captex 355 as 38.52 mg/ml (Figure 1). The solubility was tested in different surfactants and co-surfactants, maximum solubility was found in Kolliphor RH 40, and PG as 42.44 mg/ml and 42.52 mg/ml was in PG respectively (Figure 2 and 3). Captex 355, Kolliphor RH 40, and PG were used for the formulation of Rilpivirine SEDDS.

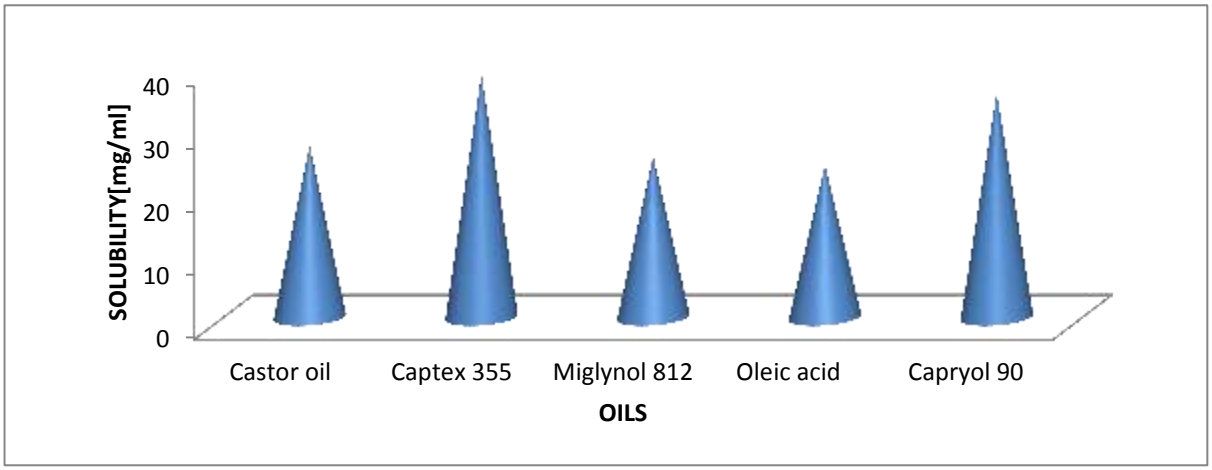

Figure 1: Solubility studies of Rilpivirine in oils

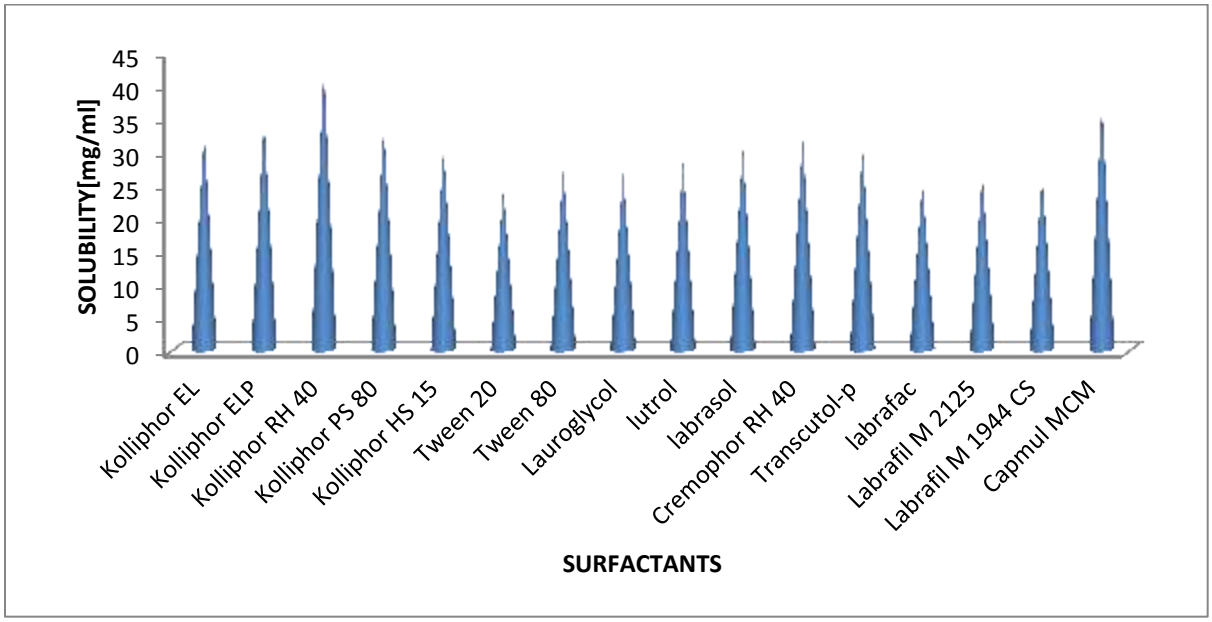

Figure 2: Solubility studies of Rilpivirine in surfactants

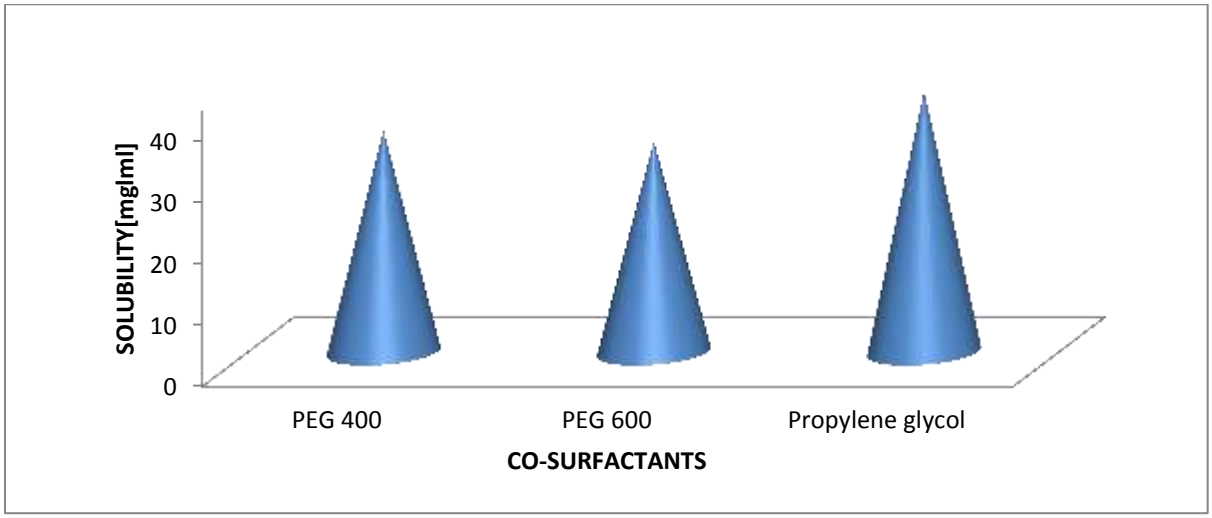

Figure 3: Solubility studies of Rilpivirine in co-surfactants

Construction of ternary phase diagram:

From the solubility studies, different oils like Captex 355, Castor oil and Capryol 90 and Kolliphor RH 40, and PG were selected as surfactant and co-surfactant respectively and phase diagrams are depicted in Figure 4, 5 and 6 respectively. From the phase diagram with Captex 355, Kolliphor RH 40, and PG which shown in Figure 4, 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 1:1, 2:1 and 3:1. Grades were given to the ratios based on the tendency of formation of micro-emulsion. Ratios 1:9, 1:8, 1:7, and 1:6 of Smix 1:1 and 1:9, 1:8, 1:7, 1:6, 1:5 of Smix 2:1 and 1:9, 1:8, 1:7, 1:6, 1:5 and 1:4of 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 SMEDDS. 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.

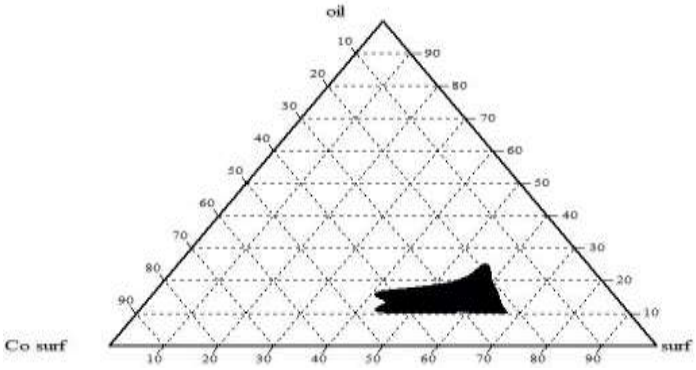

Figure 4: Ternary phase diagram of Captex 355, Kolliphor RH 40, and Propylene Glycol

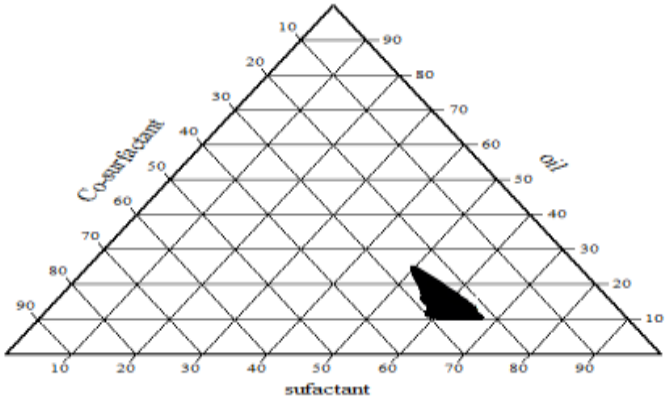

Figure 5: Ternary phase diagram of Castor oil, Kolliphor RH 40 and Propylene Glycol

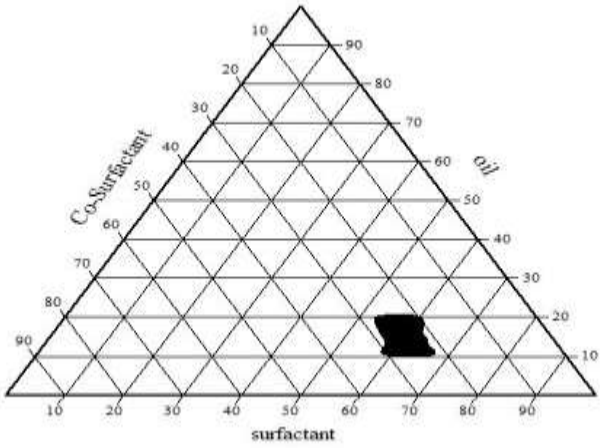

Figure 6: Ternary phase diagram of Capryol 90, Kolliphor RH 40 and Propylene Glycol

Preparation of Rilpivirine SMEDDS

SEDDS of Rilpivirine were prepared by using Castor oil (oil), Kolliphor RH 40 (surfactant), and PG (co-surfactant). In the present study, fifteen formulations were prepared and their complete composition was shown in Table 1. All the formulations prepared were found to be clear and transparent. Rilpivirine optimized SEDDS formulation (F5) is shown in Figure 7.

Figure 7: Optimized Rilpivirine SMEDDS formulation (F5)

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 and Drug content

The clarity of micro emulsions was checked by transparency, measured in terms of transmittance (%T). SMEDDS forms o/w micro emulsion since water is external phase Formulation F5 has % transmittance value 100%.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 micro emulsion and thereby values of %T. The drug content of the prepared SMEDDS was found to be in the range of 90.47 - 98.82 %. Maximum % drug content i.e. 98.82% was found in the formulation F5. The results of visual observation % Transmittance and drug content were shown in Table 2.

Table 2: Visual observation % Transmittance & Drug content of different SEDDS formulations

S. No.	Formulation Code	Visual observation	% Transmittance	% Drug content
1	F1	Transparent	99.97	95.99
2	F2	Slightly clear	98.37	92.36
3	F3	Turbid	96.90	92.50
4	F4	Slightly clear	97.45	95.90
5	F5	Transparent	100.05	98.82
6	F6	Transparent	99.16	93.33
7	F7	Slightly clear	97.81	92.26
8	F8	Slightly clear	98.62	93.64
9	F9	Slightly clear	97.10	90.47
10	F10	Slightly clear	98.88	91.45
11	F11	Transparent	99.01	92.52
12	F12	Transparent	99.26	95.98
13	F13	Transparent	99.93	93.46
14	F14	Slightly clear	97.82	94.25
15	F15	Slightly clear	98.27	93.31

***In-Vitro* Dissolution Studies of SMEDDS**

The results of *in vitro* dissolution comparisons of SMEDDS formulations are summarized in Table 3 and 4 and Figure 8 and 9. The faster dissolution from SMEDDS 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 SMEDDS formulation F5 was highest (98.93) and faster than other SMEDDS formulations and pure drug substance indicating influence of droplet size on the rate of drug dissolution.

Table 3: Dissolution profile of pure drug, Innovator and F1-F8 SEDDS formulations

Time (mins)	(Cumulative % drug release)									
	Pure drug	Innovator Eudrant (25mg)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0	0	0
2	4.52	42.03	64	38.33	39.26	56.31	35.26	38.08	54.32	32.06
5	7.36	72.34	76.29	50.82	53.37	68.12	73.95	50.84	69.48	54.05
10	10.96	77.94	78.36	64.37	69.82	77.03	89.59	61.01	81.39	67.08
15	13.92	79.88	79.22	74.01	77.48	82.29	91.76	69.09	82.99	72.94
20	17.81	81.71	80.10	75.88	81.29	84.44	92.66	70.72	84.02	74.08
25	21.22	82.96	80.25	76.48	81.48	85.12	93.14	71.93	84.96	76.50
30	24.68	84.40	81.16	78.30	82.33	85.56	93.84	73.68	85.50	76.61
45	31.47	85.38	82.24	79.96	83.76	85.96	95.72	75.83	86.92	78.04
60	34.95	85.43	82.33	80.25	83.99	87.24	98.93	75.91	88.73	78.93

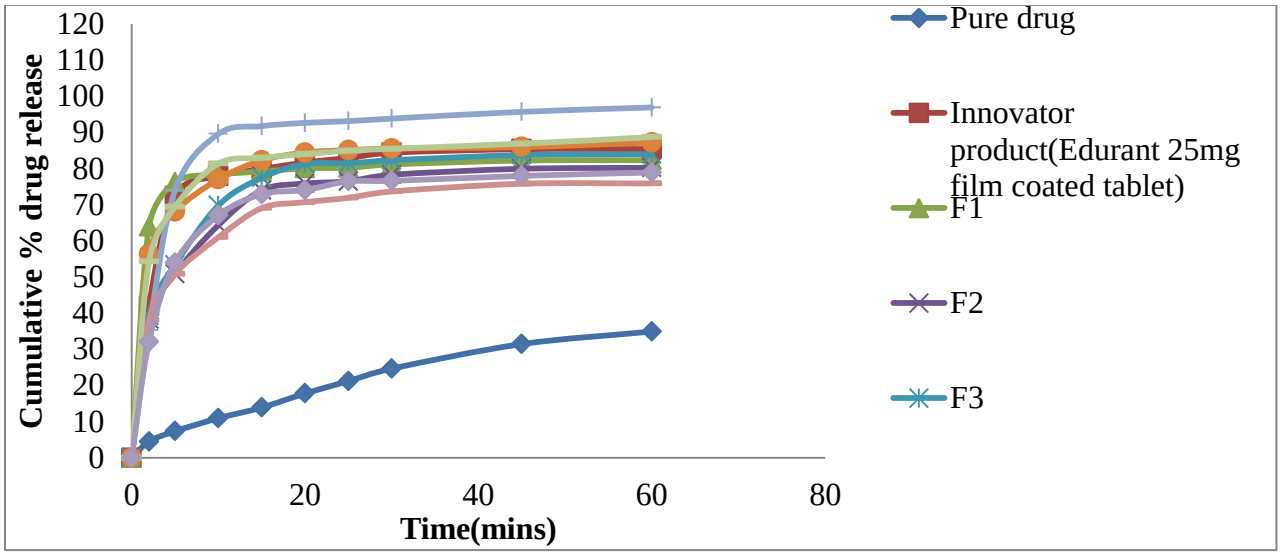

Figure 8: Dissolution profiles of Pure drug, Innovator and F1-F8 SEDDS formulations

Table 4: Dissolution profile of pure drug, Innovator and F9-F15 SEDDS formulations

Time (mins)	(Cumulative % drug release)								
	Pure drug	Innovator Eudrant (25mg)	F9	F10	F11	F12	F13	F14	F15
0	0	0	0	0	0	0	0	0	0
2	4.52	42.03	59.04	43.3	37.78	38.88	43.31	69.00	56.39
5	7.36	72.34	71.48	64.42	53.69	71.40	56.74	77.92	67.43
10	10.96	77.94	76.27	71.89	64.98	79.00	68.32	82.24	74.82
15	13.92	79.88	77.96	77.38	69.93	82.33	74.61	85.29	79.63
20	17.81	81.71	79.52	80.27	71.49	83.34	77.86	87.48	80.01
25	21.22	82.96	79.95	81.93	73.26	83.61	78.92	87.99	81.15
30	24.68	84.40	80.33	82.94	74.92	84.28	79.08	88.21	82.02
45	31.47	85.38	81.36	82.99	76.41	85.39	79.99	88.63	82.26
60	34.95	85.43	81.38	85.08	78.27	85.91	81.66	89.01	83.31

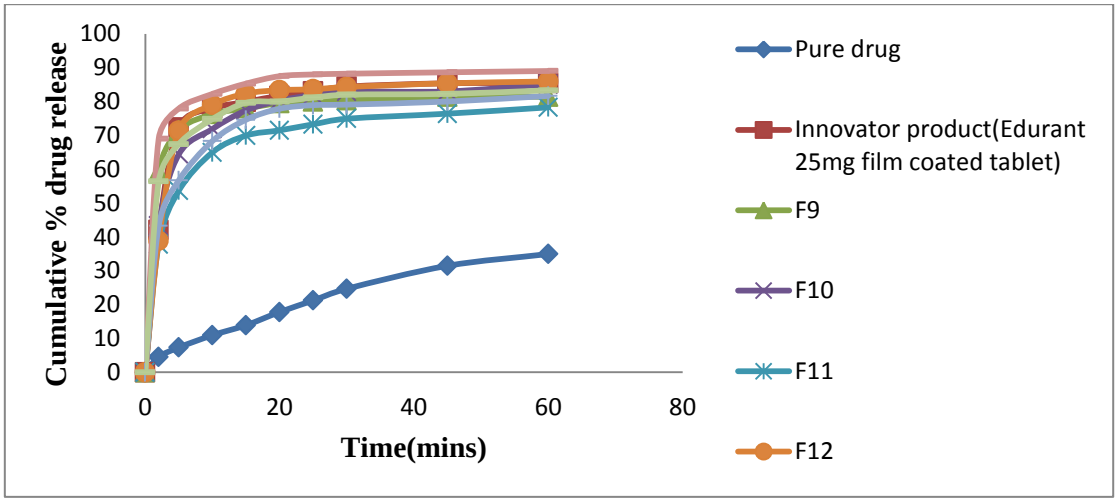

Figure 9: Dissolution profiles of Rilpivirine Pure drug, Innovator and F9-F15 SEDDS formulations

Particle size analysis of SMEDDS

The particle 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. The average particle size of SMEDDS for transparent micro-emulsions should be less than 50nm. The particle size of the optimized SMEDDS formulation was found to be 25.4nm indicating all the particles were in the micrometer range. Figure 10 represents the particle size analysis of optimized SEDDS formulation.

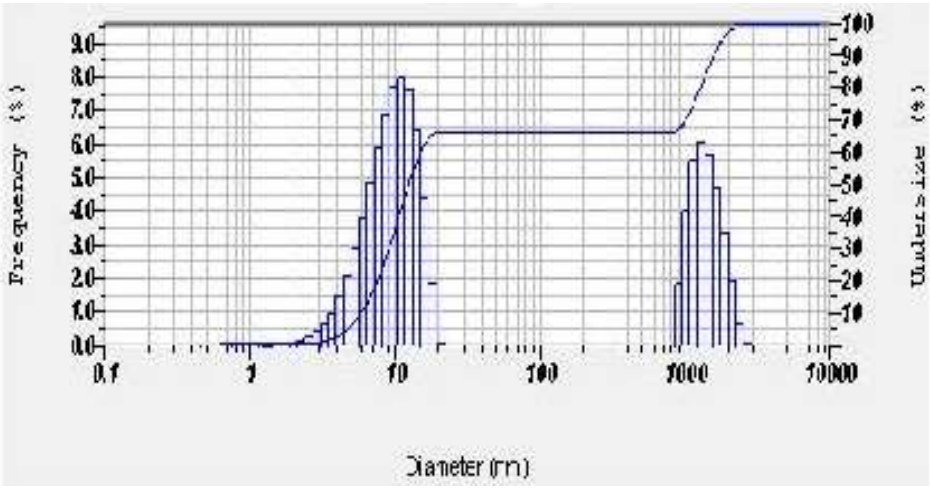

Figure 10: Particle size analysis of Rilpivirine optimized formulation (F5)

Zeta potential of SMEDDS

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 SMEDDS formulation was found to be -35.7mV which comply with the requirement

of the zeta potential for stability. Figure 11 represents the particle size analysis of optimized SMEDDS formulation.

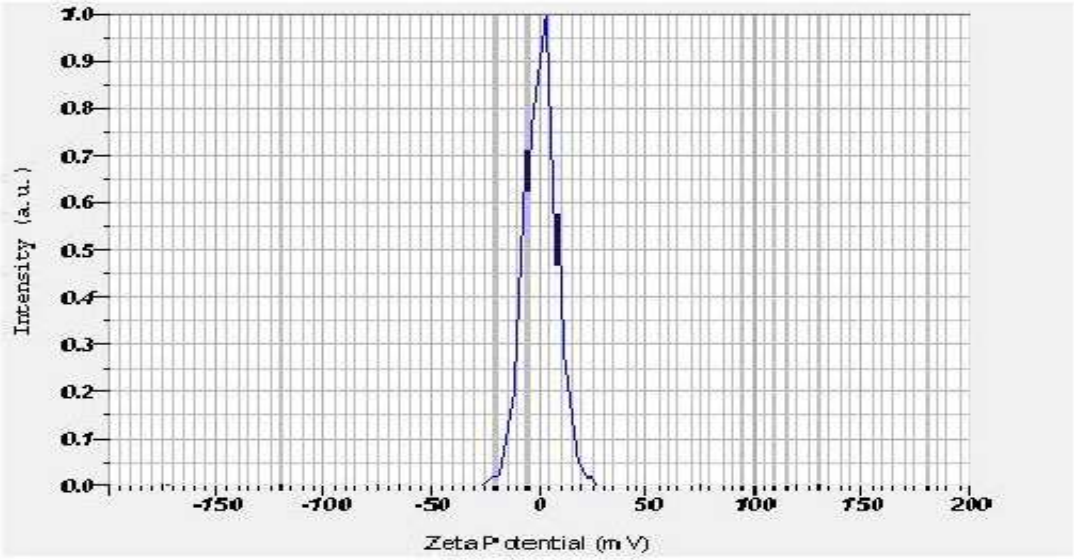

Figure 11: Zeta potential of the Rilpivirine optimized formulation (F5)

Drug excipient interactions by FTIR spectroscopy

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 Rilpivirine (Figure 12) displayed bands at cm^{-1} due to N-H stretch, at cm^{-1} due to C=O stretching, at cm^{-1} due to heterocyclic C=C stretching. The spectra also showed bands at cm^{-1} due to C-N bending. The FTIR spectrum of physical mixture is shown in Figure 13. The FTIR spectrum of SMEDDS containing Rilpivirine (Figure 14) exhibited characteristic bands consistent with the molecular structure of Rilpivirine such as bands at cm^{-1} due to N-H stretch, at cm^{-1} due to C=O stretching, at cm^{-1} due to heterocyclic C=C stretching, at cm^{-1} due to C-N bending. Thus, the presence of characteristic absorption bands of Rilpivirine and the SMEDDS containing Rilpivirine suggest that there was no interaction between the drug and excipients used in the formulation.

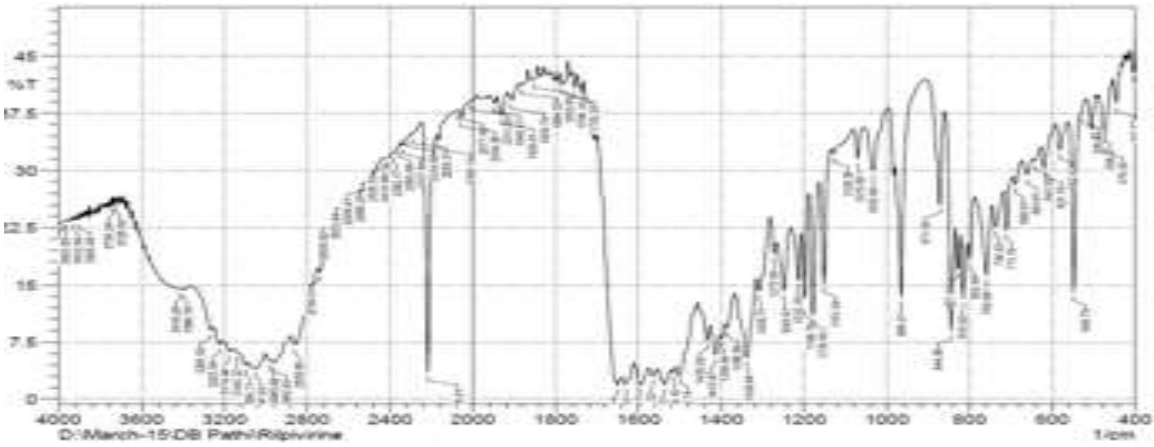

Figure 12: FTIR Spectroscopy of Rilpivirine pure drug

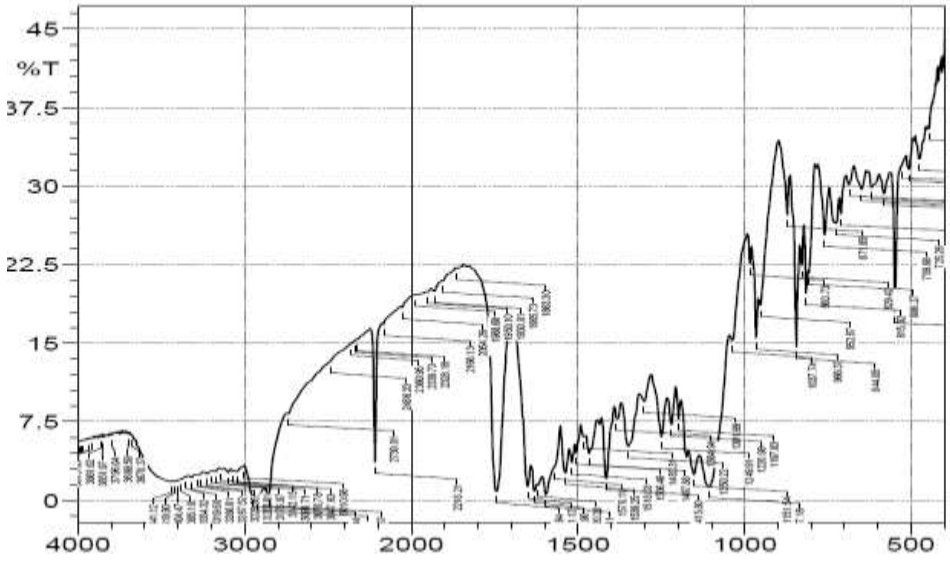

Figure 13: FTIR Spectroscopy of physical mixture

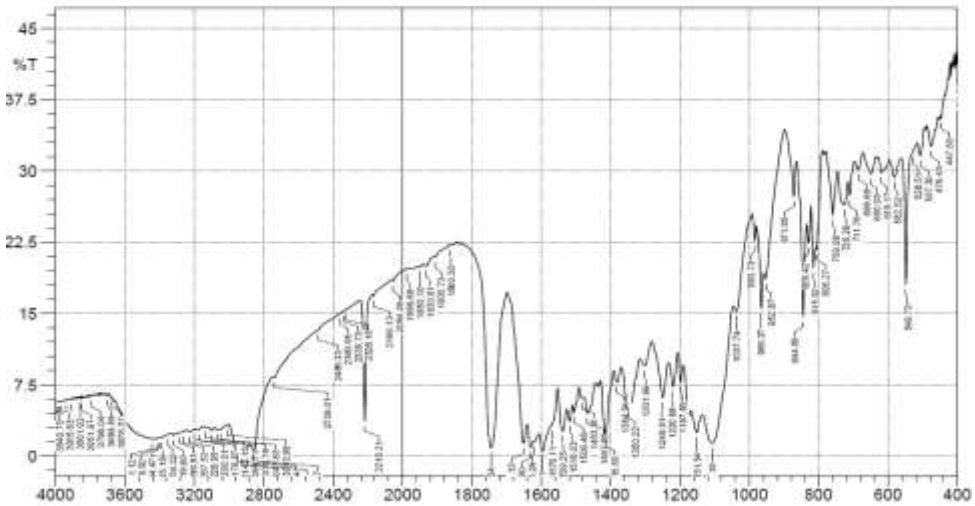

Figure 14: FTIR Spectroscopy of Rilpivirine optimized formulation (F5)

DSC studies

The DSC thermo grams of Pure Rilpivirine showed in Figure 15, sharp endothermic peak at melting point (242^{0}C), indicating that the drug is highly crystalline. The absence of drug peak in the SEDDS optimized formulation F5 indicating the drug was in amorphous form.

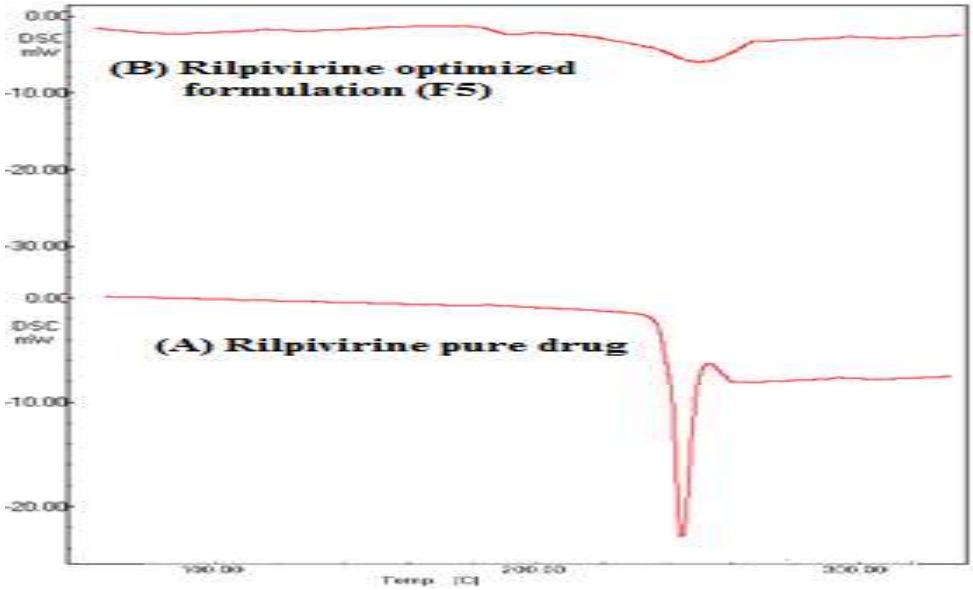

Figure 15: DSC thermo grams of pure drug (A) and SEDDS optimized formulation F5 (B)

Scanning electron microscopy (SEM) for optimized SMEDDS (F5)

SEM photographs show the surface morphology of optimized formulation (F5) as seen in the Figure 16, indicated that the homogeneous and spherical droplets in micro emulsion were observed.

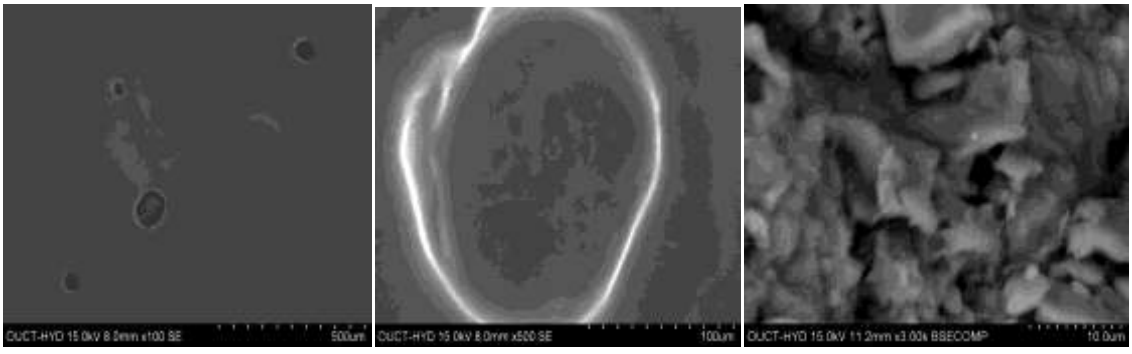

Figure 16: SEM images of optimized formulation (F5)

Stability studies

The optimized Rilpivirine SEDDS (F5) was poured 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 micro emulsifying property. Thus, these studies confirmed that the formulation was stable and its compatibility with hard gelatin capsules.

***In vivo* bioavailability studies**

Pharmacokinetic parameters comparison for pure drug suspension and Optimized formulation (F5)

Figure 17 shows the plasma concentration–time curve in Wistar rats after a single oral dose of Rilpivirine liquid SMEDDS formulation as compared to Rilpivirine pure suspension. At all the indicated time points, the Rilpivirine plasma concentrations in rats treated with liquid SMEDDS formulation was significantly higher than those treated with pure drug. Pharmacokinetic parameters of Rilpivirine after oral administration of the two formulations in Wistar rats are shown in Table 5. From the above table C_{max} of the liquid SMEDDS 0.928µg/ml was significant ($p<0.05$) as compared to the pure drug suspension 0.416µg/ml. T_{max} of both liquid SMEDDS formulation and pure drug suspension was 1.18±0.05hr and 1.70±0.05hr 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 SMEDDS formulation was higher 6.05±0.07µg.hr/ml than the pure drug suspension 2.85±0.04 µg.hr/ml. Statistically, AUC_{0-t} of the SMEDDS formulation was significantly higher 5.94±0.01 µg.hr/ml as compared to pure drug suspension 2.62±0.04 µg.hr/ml. Higher amount of drug concentration in blood indicated better systemic absorption of Rilpivirine from SMEDDS formulation as compared to the pure drug suspension**.** Calculated concentration was found to be more for liquid SMEDDS formulations compared with pure drug of Rilpivirine. The pharmacokinetic data indicated that the Rilpivirine SMEDDS have better *in vivo* absorption compared to pure drug Rilpivirine. The higher bioavailability might be due to the enhanced solubility of Rilpivirine and the composition of the delivery system. After oral administration of Rilpivirine to Wistar rats, the optimized formulation showed superior absorption profile than the suspension of pure drug. The relative bioavailability of liquid SMEDDS formulations was enhanced in comparison with pure drug suspension.

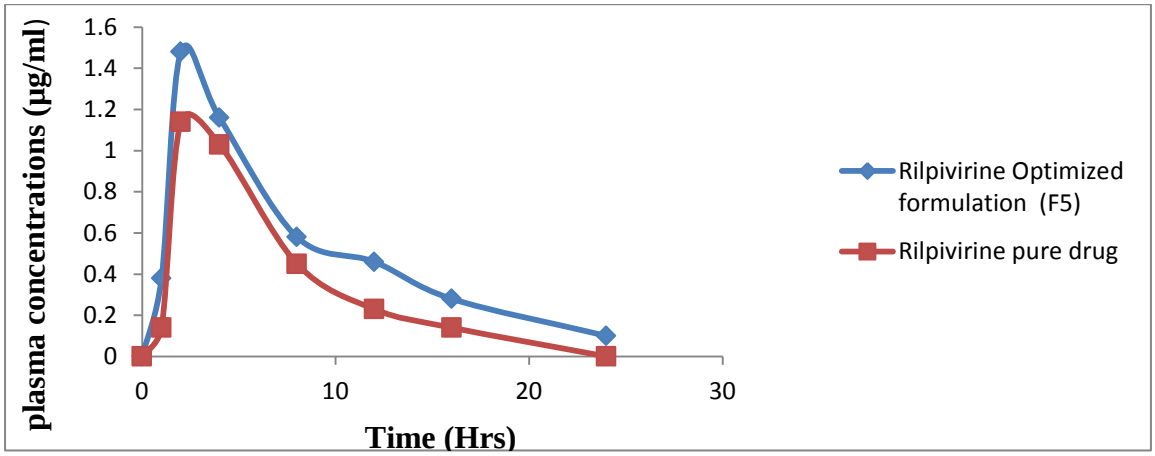

Figure 17: Plasma Concentrations of Rilpivirine SMEDDS and Rilpivirine pure drug at different time intervals (Mean ± SD, n = 6)

Table 5: Pharmacokinetic parameters of Rilpivirine SMEDDS formulation and pure drug

Pharmacokinetic Parameters	Rilpivirine ooptimized formulation (F5)	Rilpivirine Pure drug suspension
C_{max} (µg/ml)	0.928±0.02	0.416±0.05
AUC_{0-t} (µg.hr/ml)	5.94±0.01	2.62±0.04
AUC_{0-inf} (µg.hr/ml)	6.05±0.07	2.85±0.04
T_{max} (hr)	1.18±0.05	1.70±0.05
$t_{1/2}$ (hr)	3.11±0.04	3.52±0.07
K el (hr^{-1})	0.161±0.05	0.264±0.04

CONCLUSION

Rilpivirine, being a BCS class II drug, was formulated as SEDDS based on the oil solubility studies and ternary phase diagrams. From this study it was concluded that, prepared 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 and dissolution studies F5 was found to be optimized formulation which contains Captex 355(Oil), Kolliphor RH 40 (Surfactant) and PG (Co-surfactant). FTIR analysis revealed that, there was no interaction between the drug and polymers. From DSC studies it was concluded that the optimized formulation was in amorphous state, which influenced the enhancement of solubility. Results of SEM indicated that the homogeneous and spherical droplets in micro emulsion were observed. In-vitro drug release of optimized SEDDS (F5) was much higher than that of pure Rilpivirine and marketed formulation. Hence it was concluded that SEDDS can be efficiently formulated to enhance dissolution rate of poorly soluble drug such as Rilpivirine. The pharmacokinetic data indicated that the Rilpivirine SEDDS have better *in vivo* absorption compared to pure drug suspension. The higher bioavailability might be due to the enhanced

solubility of Rilpivirine by SEDDS formulation. The oral bioavailability study of optimized SEDDS (F5) showed improvement by a factor of 2.2- fold compared to the pure drug suspension in rats. Thus Rilpivirine with 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. EJPB 2011; 73: 373-384**.**
2. Hentzschel CM, Alnaief M, Smirnova I, Sakmann A. Leopold CS. Enhancement of griseofulvin release from liquisolid compacts. EJPB 2011; 73: 1-6.
3. Neslihan Gursoy R, Benita S. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed. Pharmacothe 2004; 58: 173-182.
4. Sachan R, Khatri K, Kasture SB. Self-Eumlsifying Drug Delivery System: A Novel Approach for enhancement of bioavailability. Int J PharmTech Research 2010; 2(3): 1738-1745.
5. Patel PA, Chaulang GM, Akolkotkar A, Mutha SS, Hardikar SR, Bhosale AV. Self emulsifying drug delivery system: A Review. Research J. Pharm. and Tech 2008; 1(4): 313-323.
6. Patel NN, Rathva SR, Shah VH, Upadhyay UM. Review on self emulsifying drug delivery system: Novel approach for solubility enhancement. Int J Pharma Res & Allied sci 2012; 1(3): 1-12.
7. Katteboina S, Chandrasekhar PVSR, Balaji S. Approaches for the development of solid self emulsifying drug delivery systems and dosage forms. Asian J Pharma Sci 2004; (4): 240- 253.
8. Kale AA and Patravale VB. Design and Evaluation of Self-Emulsifying Drug Delivery Systems (SEDDS) of Nimodipine. AAPS Pharm SciTech 2008; 9(1): 191–196
9. Sepkowitz KA. "AIDS the first 20 years". N. Engl. J. Med 2001; 344 (23): 1764–72.
10. Markowitz, William N, Steven B. Environmental and occupational medicine 4th Ed. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins. 2007: 745.
11. Stellbrink HJ. "Antiviral drugs in the treatment of AIDS: what is in the pipeline?". Eur. J. Med. Res 2007; 12 (9): 483–95.
12. Higuchi T and Connors KA, Phase solubility techniques. Adv. Anal.Chem. Instrum 1965; 4: 117-122.
13. Prabagar Balakrishnan, Beom-Jin-Lee, Dong Hoon O, Jong Oh Kim, Young-Im-Lee, Chul Soon Yong. Enhanced oral bioavailability of Coenzyme Q_{10} by self-emulsifying drug delivery systems. Int J Pharmaceutics 2009; 374: 58-73.

14. Ahmed Hussein A. Preparation and Evaluation of Liquid and Solid Self-Micro emulsifying Drug Delivery System of Mebendazole. Iraqi J Pharm Sci 2014; 23(1): 89-100.
15. Prasanna Kumari J, Ramarao T, Jayaveera K N, Bhikshapathi D V R N, Madhusudan Rao Y. Design and In vivo evaluation of Metoprolol Tartrate bilayer floating tablets in healthy human volunteers. Int J Drug Delivery 2014; 6:14-23.
16. Jessy Shaji, DigambarJadhav. Newer approaches to self emulsifying drug delivery system. Int j pharmacy and pharma sci. 2010; 2: 37-41.
17. Eman At. Formulation and in vitro and in vivo characterization of a phenytoin self-emulsifying drug delivery system. European journal of pharmaceutical sciences. 2008; 35: 257-263.
18. Chudasama S, Patel V. V, Nivsarkar M, Kamala Vasu K , Shishoo C. J. *In vivo* Evaluation of Self Emulsifying Drug Delivery System for Oral Delivery of Nevirapine. Indian J Pharm Sci 2014; 76(3): 218–224.
19. Kavitha KY, Geetha G, Hariprasad R, Venkatanarayana R, Kaviarasu. Development and validation of RP-HPLC analytical method for simultaneous estimation of emitrilitabine, rilpivirine, tenofovirdisporoxil fumarate and its pharmaceutical dosage forms. Int Res J Pharmacy 2013; 4(1): 150-155.