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Solubility enhancement of poorly soluble antiretrovirals by novel self emulsifying drug delivery system

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ABSTRACT

Rilpivirine is a pharmaceutical drug used for the treatment of HIV infection. The drug is characterized with poor aqueous solubility and dissolution rate leading to low bioavailability of the drug. Hence, there is a need for the improvement of the solubility and dissolution of such drugs. The 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).

Keywords: Rilpivirine, SEDDS, Captex 355, Solubility, Ternary phase diagram.

INTRODUCTION

One of the world's leading causes of death with a major medical and economic impact on a society is AIDS, caused by the HIV virus. Around 36 million people are infected with human immunodeficiency type-1 (HIV-1) worldwide[1]. 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[2]. The aqueous solubility for poorly water soluble drugs is usually less than 100 µg/ml. 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.

To overcome these drawbacks, various other formulation strategies have been adopted. Among them, one formulation is self-emulsifying drug delivery systems (SEDDS). Self-emulsifying drug delivery systems (SEDDs) have gained exposure for their ability to increase solubility and bioavailability of poorly soluble drugs[4].

Recently, SEDDS have been formulated using medium chain tri-glyceride oils and non-ionic surfactants, the later being less toxic[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]. 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[7].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) . HIV is transmitted primarily via unprotected sexual intercourse, contaminated blood transfusions, hypodermic needles, and from mother to child during pregnancy, delivery, or breastfeeding[8].

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 .

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 METHODS

Materials

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.

METHODS

Solubility Studies

It was carried out to determine solubility measurements of Rilpivirine according to the published method . 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[9].

Development of SMEDDS formulation

A series of SMEDDS 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 SMEDDS

Smix (Surfactant: Co surfactant)	Oil: mix	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:

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

In-Vitro Dissolution Studies

The release of drug from liquid 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.

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 280 nm.

Characterization of SMEDDS

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.]

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.

RESULTS AND DISCUSSIONS

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 & 3)**. Captex 355, Kolliphor RH 40, and PG were used for the formulation of Rilpivirine SEDDS.

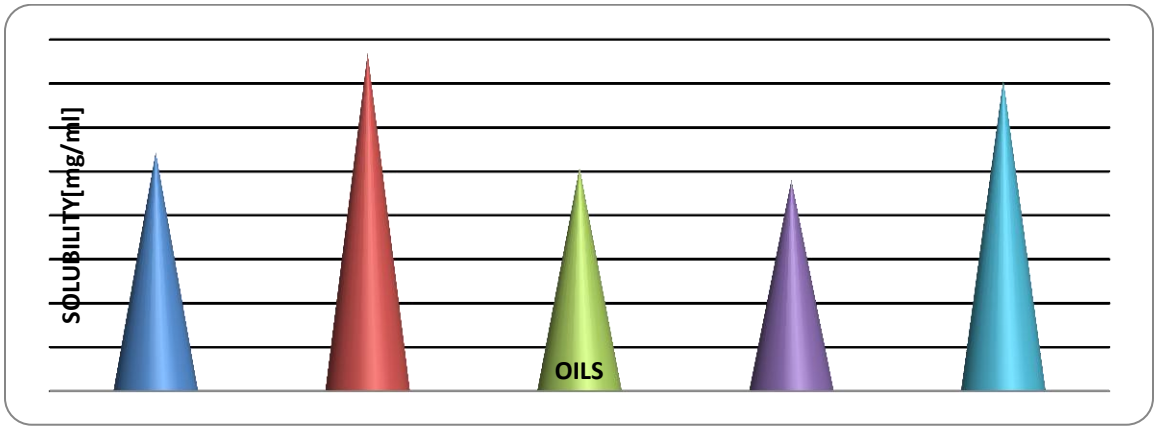

Figure 1: Solubility studies of Rilpivirine in oils

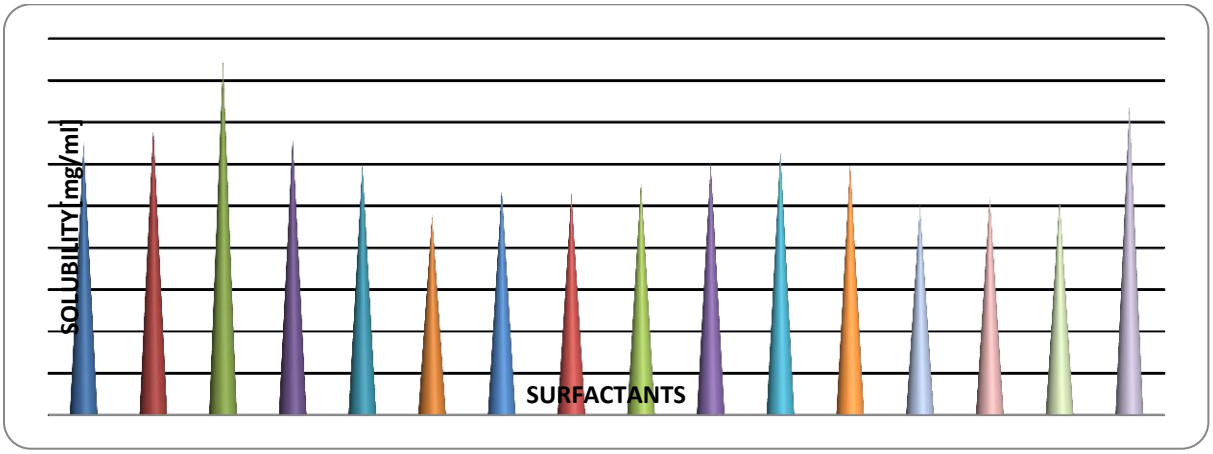

Figure 2: Solubility studies of Rilpivirine in surfactants

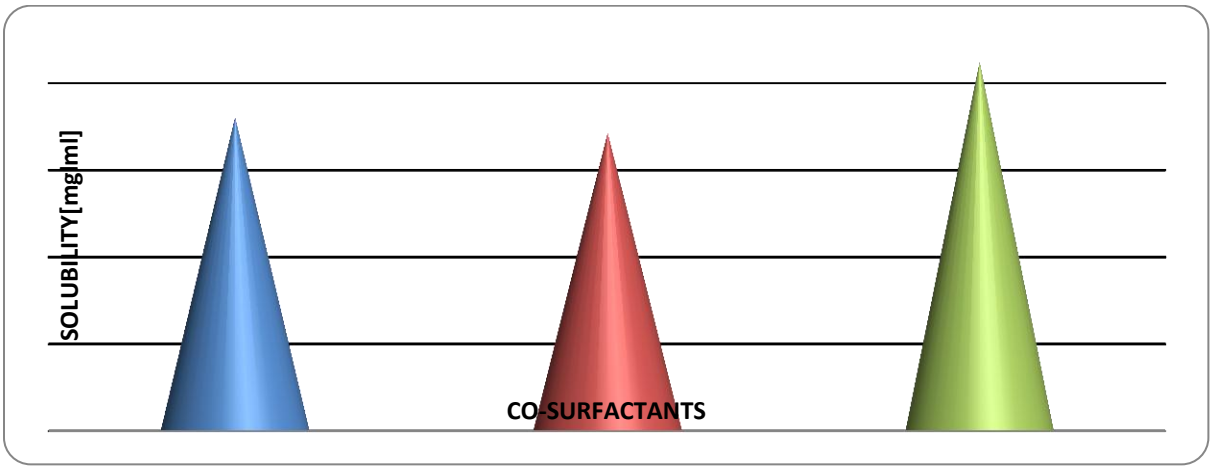

Figure 3: Solubility studies of Rilpivirine in co-surfactants

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 & 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.

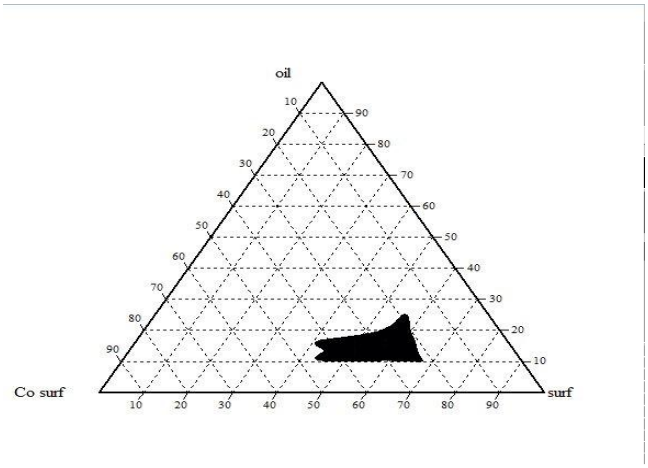

Figure 4: Ternary phase diagram of Captex 355, Kolliphor RH 40, and Propylene Glycol

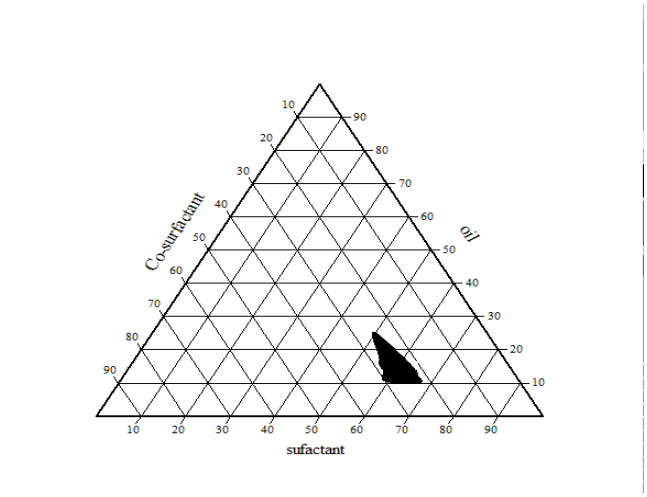

Figure 5: Ternary phase diagram of Castor oil, Kolliphor RH 40 and Propylene Glycol

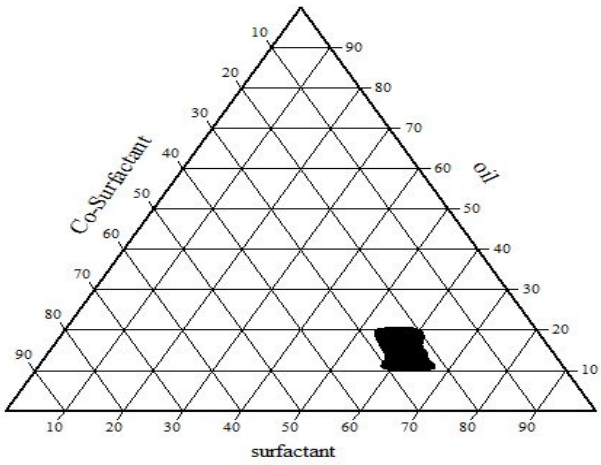

Figure 6: Ternary phase diagram of Capryol 90, Kolliphor RH 40 and Propylene Glycol

Preparation of Rilpivirine SMEDDS

SMEDDS 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 SMEDDS 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 & 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 & 4 and Figure 8 & 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

	(Cumulative % drug release)									
Time (mins)	**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

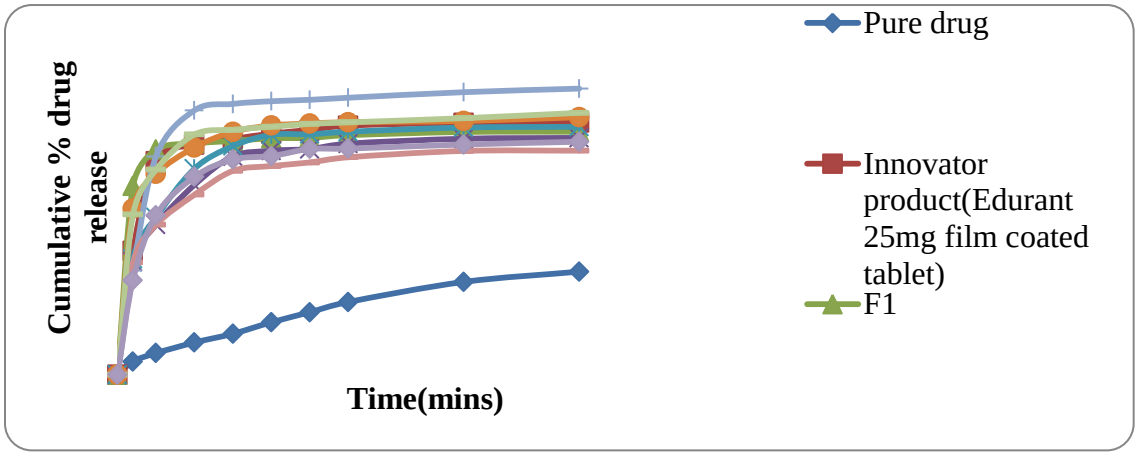

Figure 8: Dissolution profiles of Rilpivirine Pure drug, Innovator and F1-F8 SEDDS formulations

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

	(Cumulative % drug release)								
Time (mins)	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

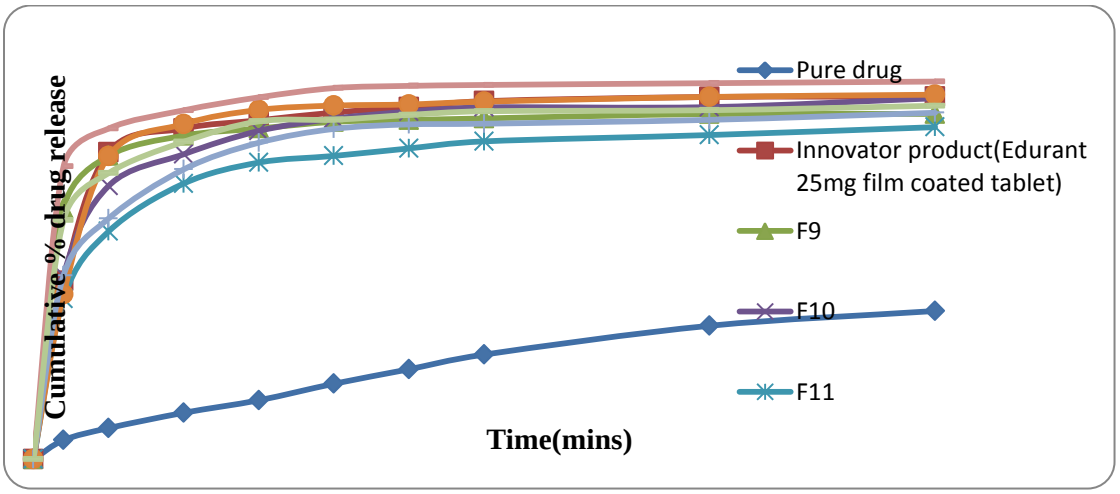

Figure 9: Dissolution profiles of Rilpivirine Pure drug, Innovator and F9-F15 SEDDS formulations

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 10)** displayed bands at 2217cm^{-1} due to C≡N stretch, at 1652cm^{-1} due to C=O stretching, at 1497cm^{-1} due to heterocyclic C=C stretching. The spectra also showed bands at 1199cm^{-1} due to C-N bending. The FTIR spectrum of SMEDDS containing Rilpivirine exhibited characteristic bands consistent with the molecular structure of Rilpivirine such as bands at 2219cm^{-1} due to C≡N stretch, at 1659cm^{-1} due to C=O stretching, at 1503 cm^{-1} due to heterocyclic C=C stretching, at 1204cm^{-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 10 & 11)**.

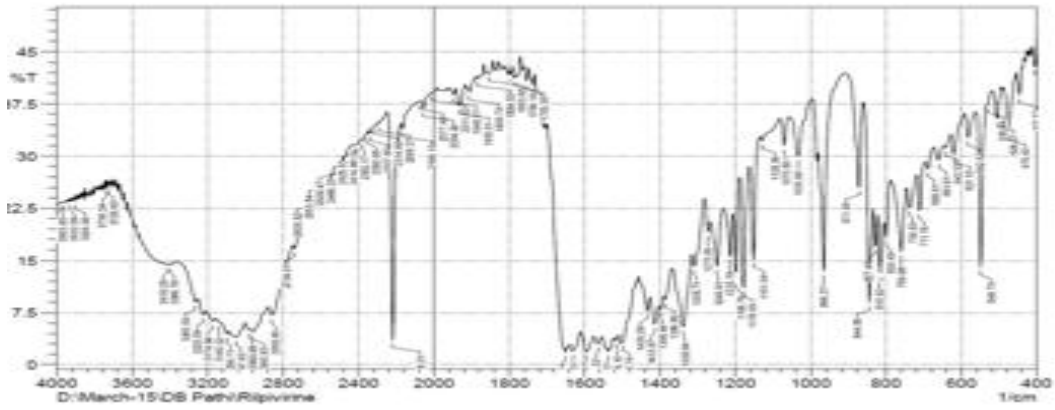

Figure 10: FTIR Spectroscopy of Rilpivirine pure drug

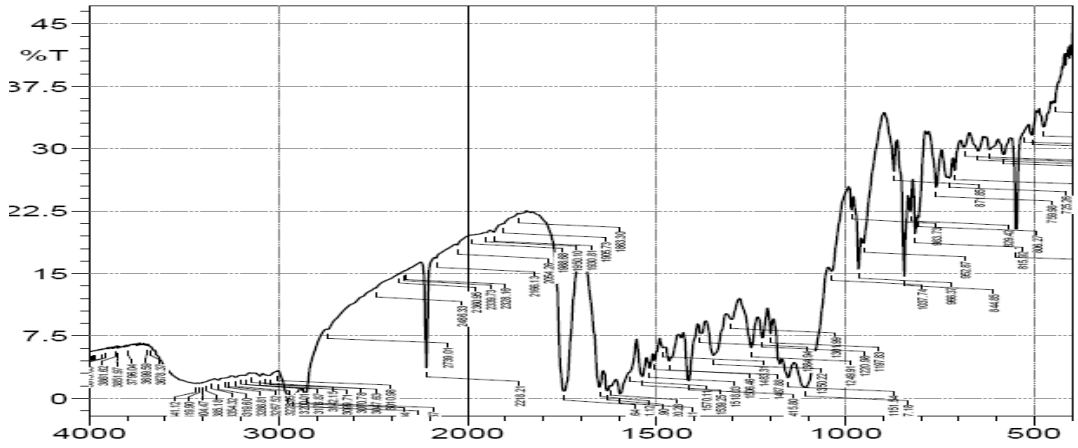

Figure 11: FTIR Spectroscopy of Physical mixture

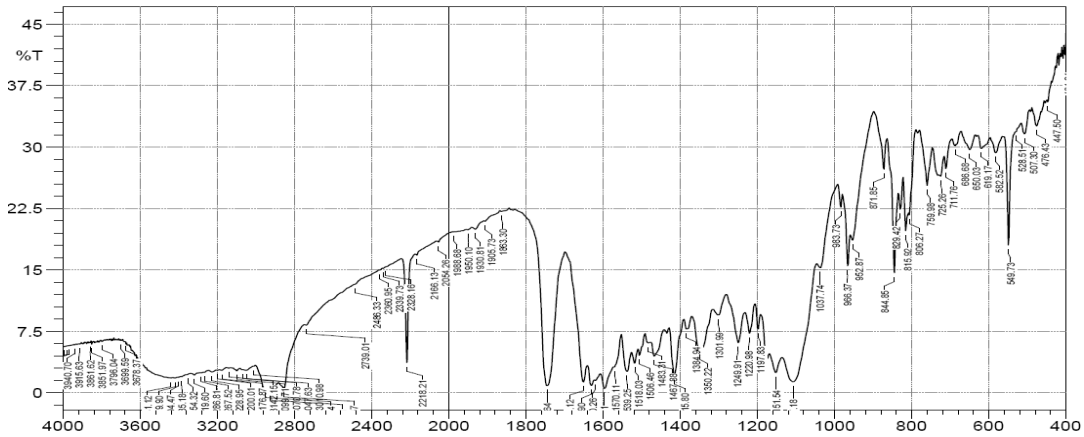

Figure 12: FTIR Spectroscopy of Rilpivirine optimized formulation (F5)

SUMMARY AND 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. 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.

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