IJPSR (2016), Vol. 7, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 December, 2015; received in revised form, 04 June, 2016; accepted, 14 June, 2016; published 01 July, 2016

FORMULATION AND IN-VITRO CHARACTERISATION OF SOLID - SELF NANOEMULSIFYING DRUG DELIVERY SYSTEM (s-SNEDDS) OF RILPIVIRINE

Baddam Sunitha Reddy [1*], G. Harish [1] and Md. Fazal Ul-Haq [2]

Head of Centre Pharmaceuticals Sciences [1], Department of Pharmaceutics CPS [2], Jawaharlal Nehru Technological University, Hyderabad-500085 Telagana, India.

Keywords:

Rilpivirine, Self nanoemulsifying; Pseudo ternary phase diagram; Solubility study

Correspondence to Author:
Baddam Sunitha Reddy

Head of Centre Pharmaceuticals Sciences Jawaharlal Nehru Technological University, Hyderabad-500085 Telagana, India.

Email: harish.pharmacy4@gmail.com

ABSTRACT: Rilpivirine (RILP) is non-nucleoside reverse transcriptase inhibitor. It is indicated for the treatment of HIV-1 infection. The current investigation was aimed to formulation and evaluation of solid self nano emulsifying drug delivery system (S-SNEDDS) for Rilpivirine to enhance solubility and dissolution rate. Solubility of drug was determined in different vehicles. Pseudo ternary phase diagram generated using PEG 400, cremophore RH 40 and Transcutol 90. The S-SNEDDS was prepared by adsorbing the optimized liquid SNEDDS on to neusilin US2 as carrier. The S-SNEDDS characterized by micromeritic properties, differential scanning studies, percentage transmittance, emulsification time and zeta potential. The optimized formulation had shown globule size of 16.27 nm with PDI of 0.276, less emulsification time of 30.2sec, good % transmittance (97.152±0.414) and good *in vitro* release. The *in vitro* dissolution rate of the drug from the s-SNEDDS was three folds than that of the plain drug and its suspension respectively cumulative percentage of drug release was found to 96.9±2.66 in 90 minutes. Accelerated stability studies was performed at 40^0C and 75%RH for s-SNEDDS of Rilpivirine found satisfactory for effect of dilution. Droplet size is 15.37 nm with PDI 0.252. Cumulative percentage drug release of Rilpivirine is 96.9±2.66 % at the end of 1month indicating no change in % drug release after stability study for 1month.

INTRODUCTION: The fact that a large majority of the newly discovered chemical entities and many existing drug molecules are poorly water soluble presents a serious challenge to the successful formulation and marketing of new drugs in the pharmaceutical industry [1]. Since in many cases the dissolution step is the rate limiting step, formulation design can be a useful approach to improve the absorption and thus the oral bioavailability of such drug candidates [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(7).3117-29

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (7).3117-29

Many formulation approaches are presently employed to tackle the formulation challenges of poorly water-soluble drugs, either by means of improving the dissolution rate or via presenting and maintaining the drug in solution throughout its period in the gastrointestinal tract.

Self-emulsifying drug delivery systems (SEDDS) are among the methods used to improve the oral bioavailability of poorly soluble drugs by presenting and maintaining the drug in a dissolved state, in small droplets of oil, all over its transit through the gastrointestinal tract [3]. SEDDS are composed of a mixture of oil and surfactant and they are capable of forming oil-in-water emulsions upon gentle agitation provided by the gastrointestinal motion. The SEDDS properties strongly depend on the selected lipids and

emulsifiers and their mixing ratios. In addition, the characteristics and load of the incorporated drug are critical parameters. The use of lipid mixtures with different polarities and emulsifiers give the possibility to optimize the SEDDS for a particular drug.

The digestion of lipid-based formulations, in the presence of endogenous materials (bile salts, phospholipids and cholesterol), induces a change in lipid composition and result in the formation of different colloidal phases (micelles, vesicles, and liquid crystalline phases) in the intestinal lumen [4, 5]. The change in lipid composition, induced by digestion, plays a major role in the solubilization capacity and consequently the absorption of co-administered drugs [6].

RILP a non-nucleoside reverse transcriptase inhibitor that effectively blocks Replication of DNA and used to patients with HIV1.Logarithm of partition coefficient [log P (octanol/water)] value of RILP is 5.47 and it comes under BCS class II drug. Poor solubility of the drug is associated with poor dissolution rate and thus low oral bioavailability [7]. The absolute bioavailability of RILP cannot be determined, as the compound is virtually insoluble in aqueous media suitable for injection [8].

The objective of the present study was to optimize SNEDDS of rilipivirine using minimum surfactant concentration, to maintain nanosized droplets on dilution by the GI fluids with an aim to increase its solubility and dissolution profile. Formula optimization was based on in vitro assessments. The formulation was tailored to compromise between drug solubility in excipients, ease of emulsification and globule size of the dispersion. Selected formulation exhibiting promising in vitro properties is anticipated to improve oral delivery of the drug [9, 10].

MATERIALS AND METHODS:

Materials:

Rilpivirine was a generous gift from from Mylan Pharma Ltd (hyderabad, India), Labrafac PG, Lauroglycol 90, Labrafac Lipophile WL 1349, Labrafil M 1944 CS, Peceol, Lauroglycol, Plurol Oleique CC 497, Labrasol, Transcutol HP, Capryol PGMC and Capryol 90 were kind gifts from Gatoforese, France. Cremophore RH 40 cremphore EL were kind gifts from from BASF, PEG 400, Span 20 and Tween-20 were purchased from SD Fine Chemicals Ltd., India. Other reagents were of analytical reagent grade and purchased from the merck chemicals.

Solubility analysis: [11, 12]

Apparent solubilities of rilpivirine were determined in different oils, surfactants and co-surfactants at ambient temperature for the selection of appropriate oil and surfactant. About 1gm of each of vehicles was taken to different cap tube, where excess of Rilpivirine was added was added in each vehicle. After sealing, the mixtures were heated at 50°C in a water bath shaker to facilitate the solubilization. Then, the mixtures were agitated with shaker at room temperature for 48hours. After reaching equilibrium, samples were collected and centrifuged at 10,000 rpm for 15min. 100µL of supernatant was collected and suitably diluted with methanol and rilpivirine was quantified by using UV spectrophotometry at 307 nm.

Construction of Pseudo-Ternary Phase Diagrams [13]:

PEG 400 and propylene glycol was selected as the oil CremophorRH40 was used as surfactants Transcutol 90 as the cosurfactant surfactant and cosurfactant (Smix) ratio was PECR40T90 1:3 PECR40T90 1:4 PGCR40T90 1:3 PGCR40T90 A series of 36 formulations were prepared using oil: S_{mix} ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1). A pseudo-ternary phase diagram was constructed by titration of component mixture of oil, surfactant and co-surfactant with water at room temperature. After equilibrium, the mixture was visually observed. The generated sample which was clear or slightly bluish in appearance was determined as nanoemulsion.

Characterization of Sedds:

Globule size analysis and zeta potential [13]: Prepared SNEDDS formulations were added to distilled water in ratio 1:1000 in test tube and mixed for 1 minute using a cyclo mixer. The droplet size, PDI and zeta potential of the emulsions were determined at 25^oC by dynamic light scattering (DLS) technique at 90^0 angle Using a Zeta sizer nano ZS90.

Self-Emulsification Time Determination[13]:
In order to determine the emulsification time (the time needed to reach the emulsified and homogeneous mixture, upon dilution). 100 mg of each formulation was added to 200 mL of 0.1N HCl at 37°C with gentle agitation using magnetic stirrer. The formulations were assessed visually for the rate of emulsification and the final appearance of the emulsion.

Phase separation and stability study of emulsion: [14]
Each SEDDS (100µL) was added to a glass beaker containing 300ml double distilled water and stimulated gastric fluid at 37ºC. Each emulsion was stored for a period of 24 hours and observed for phase separation and precipitation of drug. The observations were made after 4, 6, 8, 12 and 24 hours.

Percentage Transmittance: [14]
Each SNEDDS formulation (100µL) was added to a vial containing 10mL of double distilled water, 0.1 N HCl and phosphate buffer of pH 6.8 at room Temperature and cyclomixed for 1minute. Each sample was observed for %Transmittance at 307 nm.

Drug loading efficiency: [14]
Drug content in formulation was determined UV-Spectrophotometrically. 50mg of Each formulation was accurately weighed and dilute to 100mL with methanol. Resultant solutions were analyzed spectroscopically following suitable dilution.

Drug loading efficiency was calculated by equation

$$\text{Drug loading efficiency} = \frac{\text{Amount of drug in known amount of formulation}}{\text{Initial drug load}} * 100$$

FT-IR Studies: [14]
FT-IR Spectrum of pure drug and drug-excipients were obtained by FT-IR Spectrophotometer. The drug was dissolved in each excipient and kept a side for 72hrs for equilibration. The spectrums of drug and drug-excipients were taken with the accumulation 24 scans and a resolution of $4cm^{-1}$ over the range of 400-4000 cm^{-1}. The spectrum of drug-excipient mixtures so obtained were compared with spectrum of pure drug for any interactions.

Thermodynamic stability studies: [15]
The prepared SNEDDS formulations were subjected to thermodynamic stability studies to study the effect of centrifugation and temperature on stability of nano emulsions.

Centrifugation study: [15]
The formulations were added to deionized water in ratio 1:20 and centrifuged at 3500 rpm for 30minute and observed for phase separation (or) precipitation.

Freeze thaw cycle: [15]
The formulations which are stable under centrifugation were subjected to freeze thaw cycle. In this study, SNEDDS formulations were diluted with deionized water in 1:20 ratio and subjected to two freeze thaw cycles between -20 ^{o}C and +25 ^{o}C by storing at each temperature for 48hrs and after 48hrs samples were observed for phase separation (or) precipitation.

***In-vitro* drug release study:** The *in vitro* drug release of Rilpivirine SNEDDS was performed using USP dissolution Apparatus II (Lab india DS 8000, Mumbai, India). Hard gelatin capsules, size "00" filled with preconcentrate (equivalent to 10 mg Rilpivirine) were put into 500ml of 0.1N HCL, at 37 ± 0.5°C with a 50 rpm rotating speed. Samples (10ml) were withdrawn at regular time intervals (5, 10, 15, 30, 45, 60, 75 and 90 min) and filtered using a whatman filter paper. An equal volume of the dissolution medium was added to maintain the volume constant. The drug content of the samples was assayed using UV visible spectrophotometric method. All measurements were done in triplicate.

Preparation of self nanoemulsifying powder: [16]
S-SNEDDS was prepared by mixing optimized PECR40T90 8:2 liquid SNEDDS containing Rilpivirine with neusilin US2 adsorbent carrier. In brief Neusllin was added over liquid SNEDDS aliquot (equivalent to 10 mg Rilpivirine) contained in broad porcelain dish. After each addition, mixture was homogenized using a glass rod until a uniform distribution, free flowing powder was

obtained of formulation [13]. Resultant damp mass was passed through 60# sieve and dried at ambient temperature. Then, the powder was filled in a "00" size hard gelatin capsule shell and stored for further use.

Characterization of S-SNEDDS:

Angle of repose: [17]

The angle of repose of S-SNEDDS was determined by funnel method. Height of funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap of powder. Accurately weighed sample was allowed to flow through funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose was calculated using the equation

$$\tan \theta = h/r$$

where h and r are height and radius of powder cone.

Bulk density and tapped density: [17]

A quantity of 2gm of S-SNEDDS was introduced into 10mL measuring cylinder. Initial volume was noted and cylinder was allowed to fall under its own weight into a hard surface from a height of 2.5 cm at 2 second intervals. Tapping was continued until no further change in volume was noted. Bulk density and Tapped density were calculated using the following equations;

$$\text{Bulk density (BD)} = \frac{\text{Weight of powder blend}}{\text{Volume of the packing}}$$

$$\text{Tapped density (TD)} = \frac{\text{Weight of powder blend}}{\text{Tapped Volume of the packing}}$$

Compressibility index: [17]

The compressibility index of the blend was determined by Carr's compressibility index given by the equation.

$$\text{Carr's compressibility index (\%)} = \frac{\text{TD - BD}}{\text{TD}} \times 100$$

Hausner's Ratio: [17]Hausner's Ratio is a number that is correlated to the flowability of a powder (or) granular material. Hausner's ratio can be calculated by the equation

Hausner's Ratio = TD/BD

Drug content: [18]

S-SNEDDS of Rilpivirine equivalent to 10mg was accurately weighed and dissolved in sufficient quantity of methanol. The solution was sonicated for 10min in order to extract the drug in methanol and filtered. The absorbance of filtrate was measured at 237.8 nm using UV-Visible Spectrophotometer.

Reconstitution properties of S-SNEDDS: [18]

Effect of dilution on S-SNEDDS

100mg S-SNEDDS was accurately weighed and introduced into 100mL double distilled water in a beaker at 37^{o}C and mixed gently using magnetic stirrer at 100rpm. The property of rapid emulsification was observed. The tendency to form an emulsion is assessed as **"**good" when emulsification occurs rapidly in less than 1 minute with clear (or) transparent appearance. The tendency to form an emulsion is assessed as "bad" when there is less clear emulsion formation.

Droplet size determination:

100 mg of S-SNEDDS formulation was diluted with 100 mL distilled water in a test tube and cyclomixed and filterd. The droplet size and poly dispersibility index of emulsion was determined at 25 ^{o}C by dynamic light scattering (DSC) technique using a zeta sizer ZS90.

FT-IR studies:

FT-IR Spectrum of pure drug, neusilin US2 and Formulation were obtained by FT-IR Spectrophotometer. The spectrums were taken with the accumulation 24 scans and a resolution of 4cm^{-1} over the range of 400-4000 cm^{-1}. The spectrum of formulation so obtained was compared with spectrum of pure drug for any interactions.

***In-vitro* drug release study:**

The *in-vitro* dissolution study of S-SNEDDS which were filled into 0 size capsule and marketed drug were carried out using USP-Type II dissolution test apparatus (DS1800 Lab India) in 500mL buffer of pH 1.2 at 37±0.5^{o}C with 100rpm rotating speed.

Samples were withdrawn at 5, 10, 15, 30, 45, 60,75 and 90 minutes time intervals and filtered through 0.45µ filter. An equal volume of dissolution medium was replenished after every sampling to maintain constant volume. Samples were analyzed using UV-Spectrophotometer at 238.8nm. The concentration of drug was calculated from calibration curve. Amount of drug released was calculated from concentration and absorbance.

Accelerated stability studies:

Accelerated stability studies of S-SNEDDS formulations was carried out by storing the formulation at 40°C and 75%RH for 1 month in stability chamber. Later the formulation was evaluated for parameters such as effect of dilution, droplet size, PDI and in vitro drug release.

RESULT AND DISCUSSION:

Solubility Studies of Rilpivirine:

Solubility of Rilpivirine in various components (oil, surfactant and co-surfactants) was studied for the selection of oil & surfactants. Among all of the vehicles, PEG 400 Propylene glycol are selected as oil phase where as Cremphore RH 40 as surfactant and transcutol 90 as co-surfactant showed maximum solubility for Rilpivirine which was 30±3.87 and 15±2.95 mg/ml show in **Fig.1.**

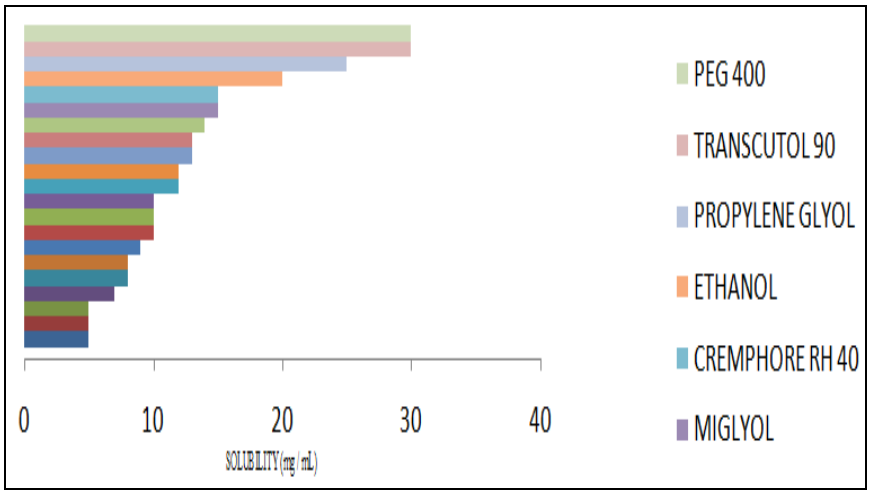

FIG.1: SOLUBILITY STUDIES OF RILPIVIRINE

TABLE 1: SOLUBILITY STUDIES OF RILPIVIRINE

Excipients	mg / mL
Oleic Acid	5
Gelucire	5
Span 80	5
MCT	7
Cappryol 90	8
Tween 20	8
Cotton Seed Oil	9
Castor Oil	10
Cremphore El	10
Glycerine	10
Oilve Oil	12
Transcutol P	12
Soya Bean Oil	13
Lauroglycol 90	13
Labrafac Lipophile	14
Miglyol	15
Cremphore Rh 40	15
Ethanol	20
Propylene Glyol	25
Transcutol 90	30
PEG 400	30

Pseudo – ternary Phase Diagrams:

Pseudo – ternary Phase Diagrams are constructed to identify the nano emulsion regions and to identify suitable composition of oil, surfactant and co-surfactant for formulation of SNEDDS. From Pseudo – ternary phase diagrams it has been found that the systems consisting of PEG 400 and propylene glycol as oily phase, Cremophore RH40 as surfactant and Transcutol HP as co-surfactant all the 36 formulations showed good nano and micro emulsifying property show in **Fig. 2, 3, 4, 5.**

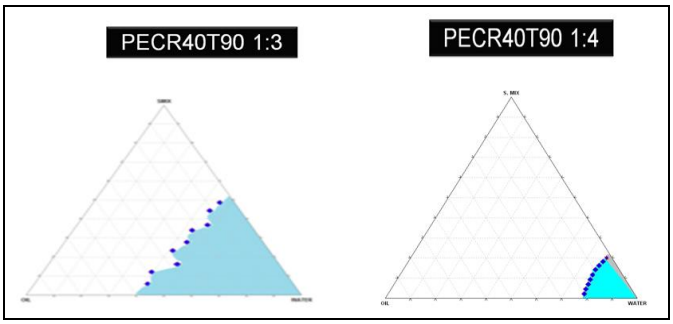

FIG. 2: PECR40T90 1:3

FIG. 3: PECR40T90 1:4

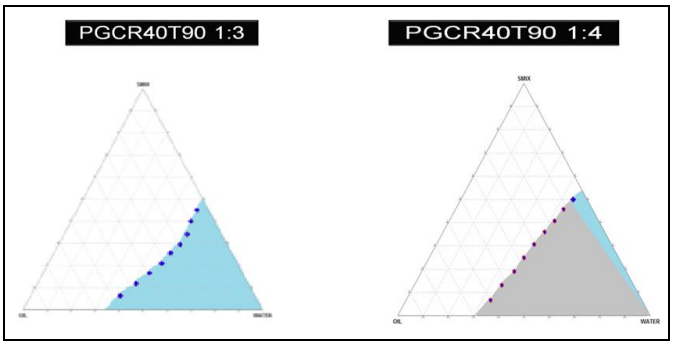

FIG. 4: PGCR40T90 1:3

FIG. 5: PGCR40T90 1:4

Globule size analysis and zeta potential:

The globule 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 [20]. Formulation F7, F8 and F9 was found to have the smallest globule size and least polydispersity index (PDI) (mean± SD, n = 3) **Table 2**. PDI of the developed nanoemulsion formulations in the present study was small, this indicates uniformity in the size distribution of the dispersed oil globules.

Zeta potential signifies degree of repulsion between neighbouring, like charged particles in dispersion; it can be related to the stability of colloidal dispersions. The zeta potential of the optimized formulations was ranging from -24.5 to -18.7 (mean± SD, n = 3). In general, the zeta potential value of±30 mV is sufficient for the stability of a nanoemulsion. Negative values of zeta potential of the optimized formulations showed that the formulations were negatively charged and high values of zeta potential of all the formulations denoted stability of the system. The high negative charge of the prepared nanoemulsion is probably due to the anionic groups of the glycols present in the oil, surfactant and co-surfactan as show in **Table 2** and in **Fig. 6, 7, 8, 9, 10, 11.**

TABLE 2: GLOBULE SIZE ANALYSIS AND ZETA POTENTIAL

Formulation code (OIL:SMIX)	Size of Droplet	Region	Zeta potential
PECR40T90 1:3			
F1 (1:9)	189.3	MICRO	11.0
F2 (2:8)	51.90	NANO	-0.33
F3(3:7)	25.6	NANO	12.3
F4(4:6)	22.35	NANO	-0.87
F5(5:5)	46.46	NANO	-3.6
F6(6:4)	21.55	NANO	-5.6
F7(7:3)	**20.64**	**NANO**	**-21.5**
F8(8:2)	**14.75**	**NANO**	**-24.5**
F9(9:1)	**20.85**	**NANO**	**-18.7**
PECR40T90 1:4			
F10 (1:9)	14.75	NANO	-0.556
F11 (2:8)	16.54	NANO	-0.113
F12(3:7)	31.64	NANO	-11.1
F13(4:6)	16.49	NANO	5.8
F14(5:5)	84.74	NANO	-0.55
F15(6:4)	22.55	NANO	7.38
F16(7:3)	47.78	NANO	-3.8
F17(8:2)	32.74	NANO	-12.5
F18(9:1)	51.90	NANO	-10.3
PGCR40T90 1:3			
F19(1:9)	18.36	NANO	-1.83
F20(2:8)	26.64	NANO	-2.8
F21(3:7)	80.29	NANO	-0.66
F22(4:6)	89.53	NANO	5.94
F23(5:5)	89.07	NANO	-6.34
F24(6:4)	73.08	NANO	-1.8
F25(7:3)	95.28	NANO	3.18
F26(8:2)	23.04	NANO	-7.59
F27(9:1)	31.20	NANO	8.64
PGCR40T90 1:4			
F28(1:9)	71.2	NANO	-6.6
F29(2:8)	116.8	MICRO	-3.6
F30(3:7)	106.9	MICRO	-2.1
F31(4:6)	172.9	MICRO	-0.38
F32(5:5)	123.4	MICRO	-1.67
F33(6:4)	146.5	MICRO	-0.51
F34(7:3)	258.3	MICRO	-0.33
F35(8:2)	234.2	MICRO	1.64
F36(9:1)	150.7	MICRO	5.67

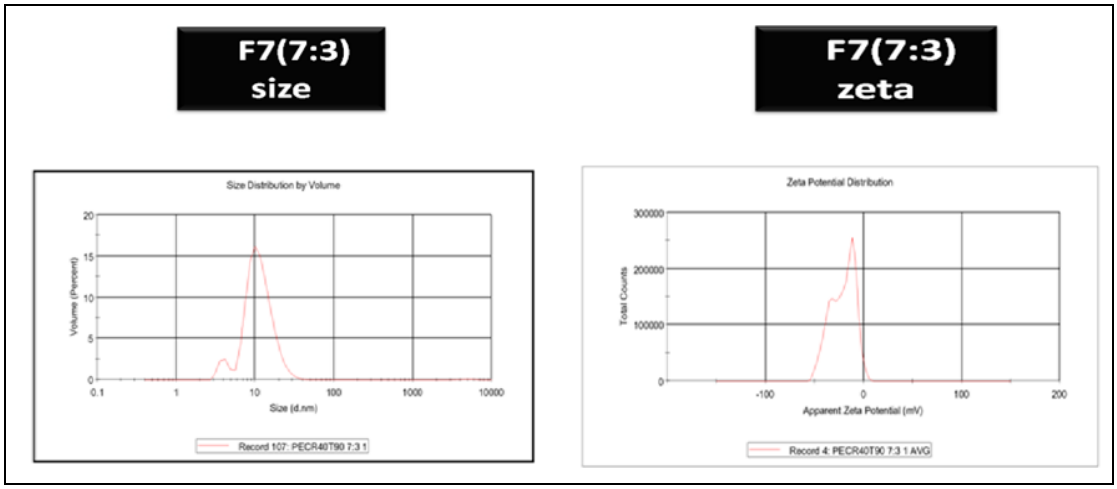

FIG. 6: F7 (7:3) GLOUBLE SIZE **FIG. 7: F7 (7:3) ZETA POTENTIAL**

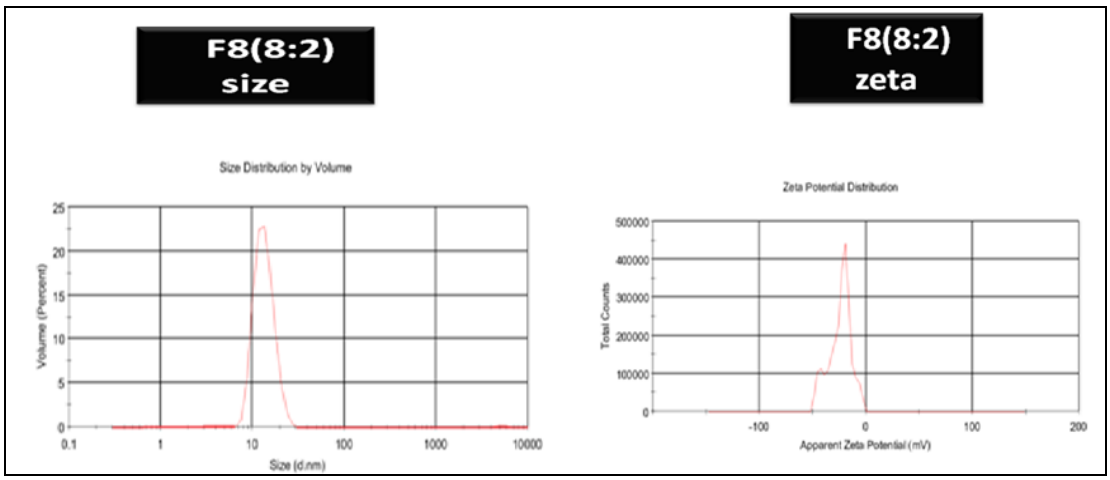

FIG. 8: F8 (8:2) GLOUBLE SIZE **FIG. 9: F8 (8:2) ZETA POTENTIAL**

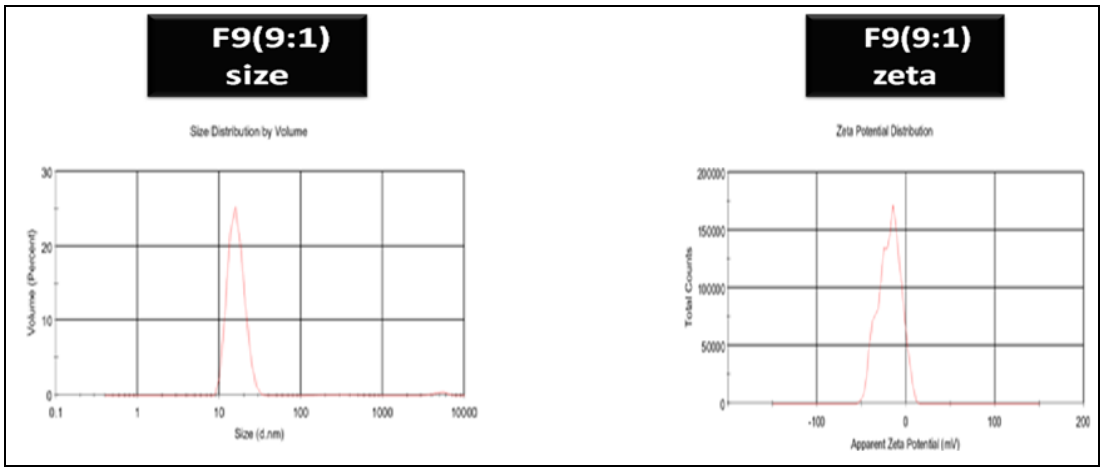

FIG. 10: F9 (9:1) GLOUBLE SIZE **FIG. 11: F9 (9:1) ZETA POTENTIAL**

Emulsifying time and percentage of transmission:

Each diluted sample was observed for %Transmittance at 307nm. All formulations showed %Transmittance more than 95% (97.52 ± 0.4 to 98.313 ± 0.322) indicating clear emulsions show in **Table 3**.

TABLE 3: EMULSIFYING TIME AND PERCENTAGE OF TRANSMISSION

Formulation code	Emulsification time*	Percenrage transmittance*
F7	39.33 ± 1.52	97.93 ± 0.694
F8	30.2 ± 2.1	97.152 ± 0.414
F9	45.33 ± 1.83	96.313 ± 0.922

Drug loading efficiency:

TABLE 4: DRUG LOADING EFFICIENCY

Formulation name	Drug loading efficiency
F7	97.15 ± 0.61
F8	98.283 ± 0.35
F9	95.61 ± 0.85

FT-IR studies:

The spectrums of drug-excipient mixtures and the formulations so obtained were compared with spectrum of pure drug for any interactions. Characteristic peaks observed at3400–3250 cm^{-1} N–H stretch 1°, 2° amines, 2217 cm^{-1}(C≡N), amides 1760–1665cm^{-1} (C=O stretch), 1680–1640cm^{-1} (aromatic C = C), \ 1470–1450cm^{-1} (C-H bending), 1370–1350cm^{-1} (-CH wagging) and 1250–1020cm^{-1} (symmetric C-N stretching). FT-IR spectrum of pure drug, drug-excipient mixtures and the formulations were almost similar because of same functional groups. It indicates there was no interaction between RILPIVIRINE and excipients used in formulation. FT-IR spectrums of pure drug, drug-excipients and the formulations are shown in **Fig.12, 13, 14, 15, 16.**

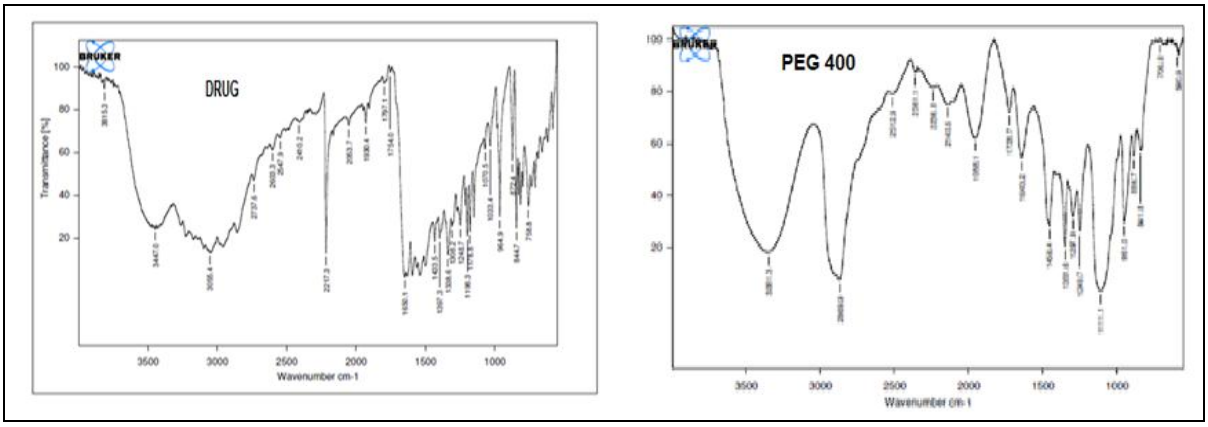

FIG. 12: RILPIVIRINE DRUG **FIG. 13: PEG 400**

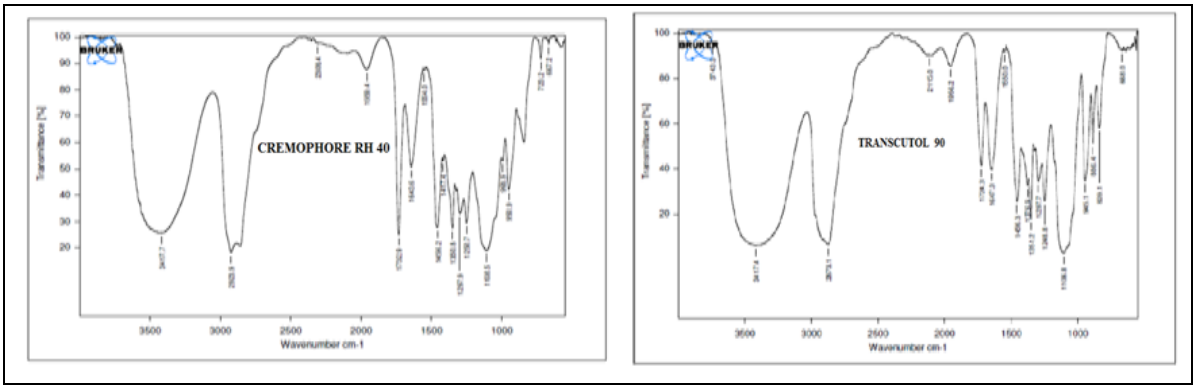

FIG. 14: CREMOPHORE RH 40 **FIG. 15: TRANSCUTOL 90**

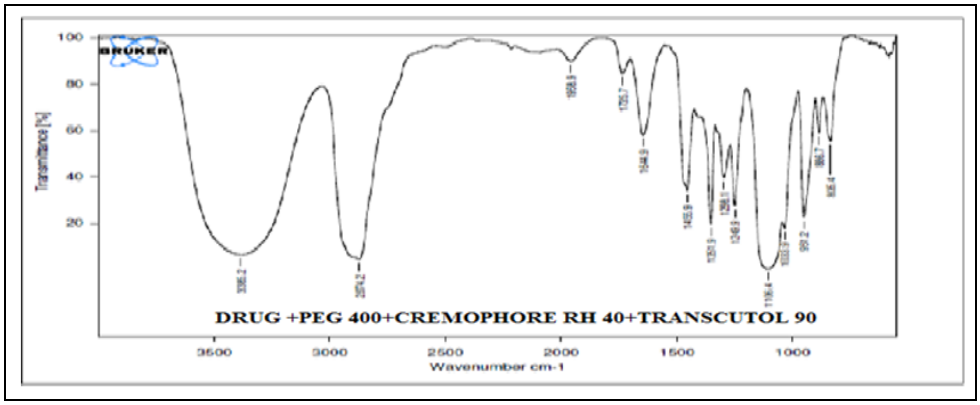

FIG. 16: DRUG+ PEG 400+ CREMOPHORE RH 40+ TRANSCUTOL 90

Thermodynamic Stability Studies:

Thermodynamic stability study is designed to identify Metastable formulation. The SNEDDS are subjected to Centrifugation study and Freeze thaw cycle. The emulsions are stable during centrifugation at 3,500rpm and alternative temperature cycles of -20°C and +25°C. There is no precipitation and phase separation of was observed show in **Table 5**.

TABLE 5: THERMODYNAMIC STABILITY STUDIES

Formulation name	Centrifugation (3,500rpm for 30min)	Freeze thaw cycle (-20°C and +25 °C)
F7	Passed	Passed
F8	Passed	Passed
F9	Passed	Passed

***In – vitro* drug release study:**

The pure drug and optimized SEDDS formulations F7, F8 and F9 was filled in capsules with 10 mg of the drug in formulation and dissolution studies were performed in 0.1 N HCL for 90 mins. The % drug release values formulations was calculated and results were compared with pure drug and **Fig. 16**. It was observed that within 90 mins F8 SEDDS formulation release 97.5±0.9 were as other formulations release less than that of F8 so the F8 is considered as optimized formulae where as pure drug have shown 18.7±4.7% drug release indicating drug release have increased by formulating as SEDDS show in **Table 6, Fig.17.**

TABLE 6: *IN – VITRO* DRUG RELEASE STUDY

Time	Drug Release			
	F7	F8	F9	Pure Drug
5	9.75±1.224	47.5±2.2	22.5±2.1	0
10	30±3.97	60±2.8	31±4.5	0.12±0.15
15	35±1.16	71.2±2.5	35±7.1	1.1±0.1
30	36.25±3.65	81.2±3.0	40±4.2	1.2±0.2
45	37.5±2.61	86.2±2.6	42.5±4.6	6.2±0.6
60	43.75±6.51	93.7±1.4	43±4.3	11.2±1.1
75	46.25±2.34	96.2±0.3	47±2.9	16±3.9
90	47.5±2.91	97.5±0.9	56±4.7	18.7±4.7

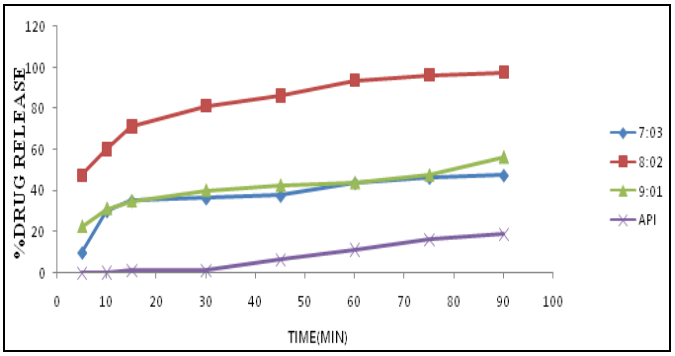

FIG. 17: *IN – VITRO* DRUG RELEASE STUDY GRAPH

Preparation of Solid SNEDDS of Rilpivirine:

Based on evaluation tests done for three liquid SNEDDS formulations the formulation F8 is selected for preparation of solid SNEDDS of Rilpivirine. Compared to other formulations F8 showed good self emulsification property which was emulsified spontaneously in 30 ± 2.1 sec and also droplet size (14.75 nm) was less than other formulations with more uniform distribution of particles (PDI = 0.497). Cumulative percentage of drug release was found to be 97.5±0.9 Hence the optimum composition for preparation of s-SNEDDS was found to be PEG400(80%w/w), Cremophore RH40 (10%w/w), Transcutol – 90 (5%w/w) and Drug (10Mg). With selected optimum formulation s-SNEDDS are prepared using neusllin as carrier in 1:0.25 ratio by adsorption technique.

Evaluation of solid SNEDDS:

Flow properties of s-SNEDDS:

Flow properties such as Angle of Repose, Bulk density, Tapped density, Compressibility Index and Hausner's Ratio are determined and it was found that Prepared s-SNEDDS showed **"**Good" flow properties. Results are given in **Table 7.**

TABLE 7: FLOW PROPERTIES OF S-SNEDDS

Flow Properties	Results
Angle of repose(θ)	25.998 ± 0.722
Bulk density (g/mL)	0.356 ± 0.028
Tapped density (g/mL)	0.31 ± 0.025
Compressibility index (%)	9.35 ± 0.48
Hausner's ratio	1.21 ± 0.004

Drug Content:

Amount of drug present in prepared s-SNEDDS was determined. Drug content of the s-SNEDDS was found to be 96.375 ± 1.9 %

Reconstitution properties of s-SNEDDS:

Effect of dilution on s-SNEDDS:

Effect of dilution on s-SNEDDS was studied and it was found that prepared s-SNEDDS showed spontaneous emulsification i.e. in less than 1min and it was also found that there is no phase separation (or) phase inversion of nano emulsion after 2hrs storage of diluted sample.

Droplet size Determination:

Mean droplet size and Poly dispersibility index of reconstituted s-SNEDDS were found to be

16.27nm and 0.276. The s-SNEDDS showed PDI less than 0.3 i.e. there is distribution of uniform size particles. Results are shown in **Fig. 22**.

FT–IR Studies: FT-IR spectrum of pure drug and s-SNEDDS were almost similar because of same functional groups. It indicates there was no interaction between rilpivirine and excipients used in formulation. FT-IR spectrums of pure drug, neusilin US2 and s-SNEDDS as show in **Fig.19.**

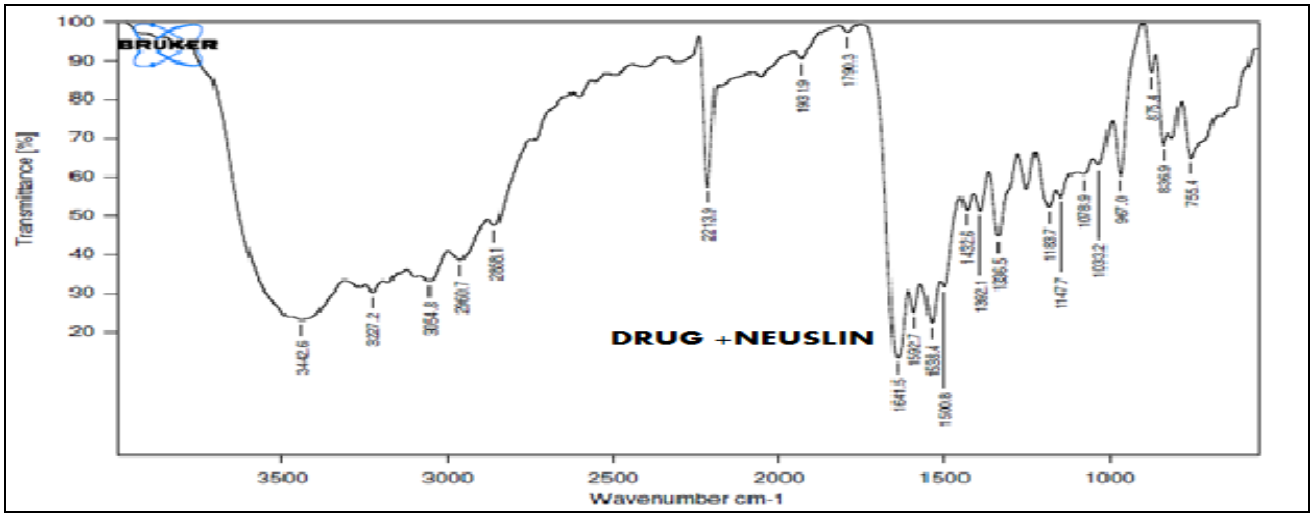

FIG. 18: DRUG+NEUSLIN

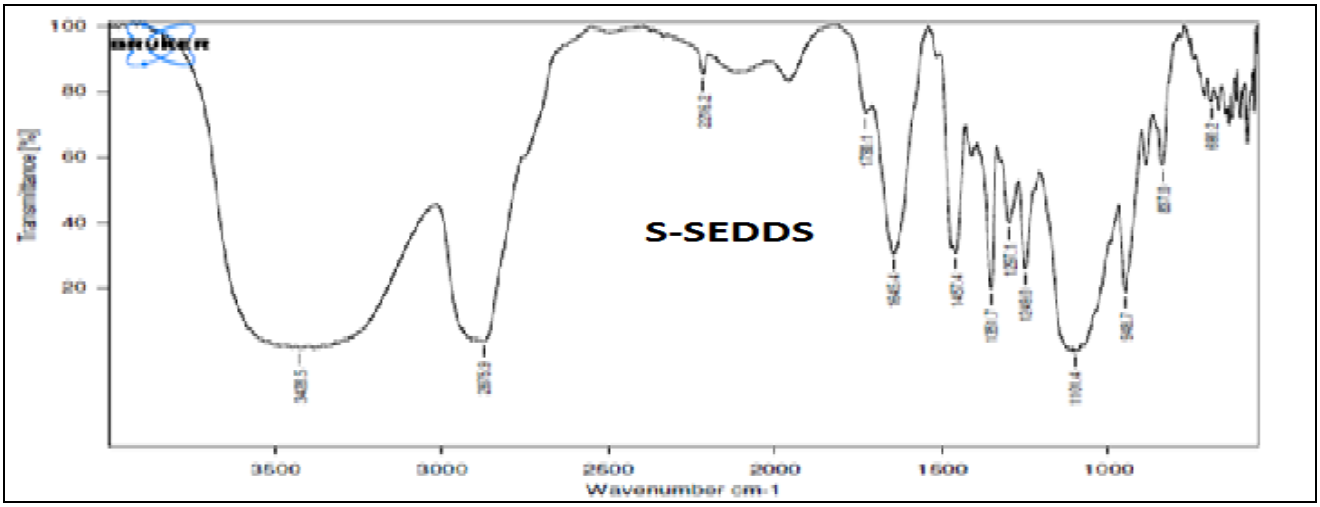

FIG. 19: S-SEDDS

***In–vitro* drug release study:**

In –vitro drug release study was done for marketed formulation, and S-SNEDDS of Rilpivirine. The percentage drug release from S-SNEDDS was found to be higher than that of marketed formulation. Percentage drug release and cumulative percentage drug release were calculated from absorbance and concentration that were obtained with the help of standard graph of Rilpivirine. *In–vitro* drug release study was performed for 90 mins show in **Table 8**.

TABLE 8: *IN – VITRO* DRUG RELEASE STUDY OF S-SEDDS

Time	S-Seeds Cumulative % Drug Release	Marketed Formulation Cumulative % Drug Release
5	45.5±1.56	1.38±0.63
10	59.8±0.98	3.25±1.102
15	71.3±4.68	5.08±2.69
30	83.5±2.36	8.98±3.99
45	85.9±1.57	13.56±4.18
60	91.7±0.79	20.37±2.74
75	95.6±4.23	27.87±6.43
90	96.9±2.66	40.83±3.96

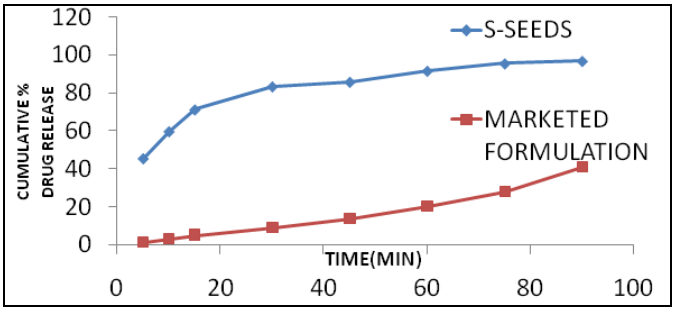

FIG. 20: *IN – VITRO* DRUG RELEASE STUDY OF S-SEDDS

Accelerated Stability Studies:

After 1month storage formulation was evaluated for parameters such as effect of Dilution, Droplet size, PDI and *In–vitro* drug release. s-SNEDDS passed the test of Effect of Dilution. Droplet size was found to be 16.27 nm with PDI 0.276 indicating no effect on Droplet size after 1month stability study. Cumulative percentage of Rilpivirine from s-SNEDDS was 96.9±2.60 % at the end of 1month indicating no change in % drug release after 1month stability study. Results are given in Table and Figure, results of drug release are shown in **Table 9** and **Fig. 21**. From the results it was clear that formulation was stable for 1month accelerated stability study.

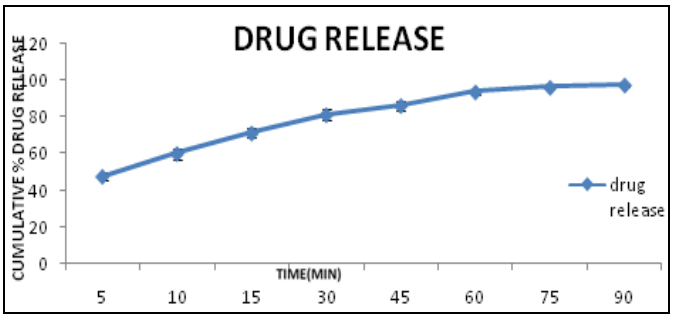

FIG. 21: *IN – VITRO* DRUG RELEASE STUDY OF S-SEDDS

TABLE 9: *IN – VITRO* DRUG RELEASE STUDY OF S-SEDDS

Time	Drug Release
5	43.5±1.56
10	59.8±0.98
15	71.3±2.68
30	82.5±2.36
45	85.9±1.57
60	91.7±0.79
75	94.6±2.23
90	96.9±2.66

TABLE 10: ACCELERATED STABILITY STUDIES S-SEDDS

Formulation	Effect of dilution	Droplet size (d.nm)	PDI	Drug release (AM ± SD for n=3)
s-SNEDDS	Passed	16.27	0.276	96.9±2.66

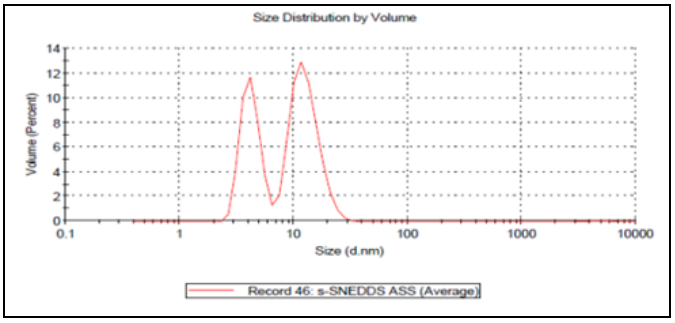

FIG. 22: GLOUBLE SIZE

Summary and Conclusion:

The drug Rilpivirine which is poorly soluble drug is selected for formulation of SNEDDS due to its poor aqueous solubility and its oral bioavailability which is less than 5%. Self nanoemulsifying drug delivery system was developed to improve its solubility.

Solubility of Rilpivirine is determined in various Oils, Surfactants, and C0-surfactants by UV-Spectrophotometric method. Rilpivirine has been shown maximum solubility in oils Oleic acid (31.43533 ± 0.45mg/mL) & Capryol 90 (25.454 ± 0.58mg/mL); in surfactants Labrasol (30.08 ± 0.17mg/mL) & Cremophore RH40 (29.82267 ± 0.32mg/mL) and in co-surfactant Transcutol HP (31.12933 ± 0.23mg/mL).

A series of Pseudo ternary phase diagrams are constructed to identify nanoemulsion region. Various compositions of Oil and S_{mix} are titrated with water to identify nanoemulsion region. From pseudo ternary phase diagrams systems consisting of PEG 400 as oily phase, Cremophore RH40 as surfactant, Transcutol 90 as co-surfactant are selected for formulation. SNEDDS are prepared by selecting oil: S_{mix} ratio 1:3, 1:4.

Three mixtures (PECR40T90 7:3, PECR40T90 8:2 and PECR40T90 9:1) are selected for formulation of SNEDDS by keeping amount of drug constant (10mg) in all formulations. Prepared formulations are evaluated for Self emulsification and visual assessment, Phase separation and precipitation of the drug, Robustness to dilution, Percentage Transmittance, drug loading efficiency, FT-IR Studies, Thermodynamic stability studies, Droplet size, PDI and Zeta potential. All five formulations are emulsified in 30-42 seconds i.e. in less than

1min. No formulation had showed precipitation and phase separation of drug. All formulation are robust to dilution. All formulations shown percentage transmittance more than 95% **(**97.52±0.4 to 98.313±0.322**)** indicating clear emulsions. Thermodynamic stability studies had indicated that all formulations are stable at centrifugation and freeze thaw cycle. Droplet size was found to be in between 14.75 to 20.85 nm and PDI of all formulations was found to be below 0.6 i.e. there is distribution of uniform size particles. Zeta potential was found to be in between – 18.7 to – 24.5 mV. FT–IR spectrums shown no interaction between drug and excipients.

Based on evaluation tests done for three liquid SNEDDS formulations the formulation PECR40T90 8:2 is selected for preparation of solid SNEDDS of Rilpivirine. Compared to other formulations PECR40T90 8:2 showed good self emulsification property with droplet size (14.75 nm), more uniform distribution of particles (PDI = 0.497). and has *in-vitro* cumulative percentage of drug release was found to be 97.5±0.9 Hence the optimum composition for preparation of s-SNEDDS was found to be PEG 400(80%w/w) , Cremophore RH40 (5%w/w), Transcutol – HP (15%w/w) and Drug (10mg). With selected optimum formulation s-SNEDDS are prepared using neuisllin as carrier in 1:0.5 ratio by adsorption technique.

Prepared s-SNEDDS was evaluated for Flow properties, Drug Content, Effect of dilution, Droplet size Determination, FT – IR Studies, In–Vitro drug release study and Accelerated Stability Studies for 1month. Prepared s-SNEDDS showed "Good" flow properties. Amount of drug present in prepared s-SNEDDS was found to be cumulative percentage of drug release was found to be 97.5±0.9

Prepared s-SNEDDS showed spontaneous emulsification i.e. in less than 1min after dilution and also showed no phase separation (or) phase inversion of nano emulsion after dilution. From FT – IR studies it was found no interaction between drug and neusilin US2. Droplet size was found to be 16.27nm with PDI 0.276. From In-Vitro drug release profile it was found that the percentage drug release from s-SNEDDS is 96.9±2.66 in 90minutes. Accelerated stability studies at 40^0C and 75%RH for s-SNEDDS of Rilpivirine found satisfactory for effect of dilution. Droplet size is 15.37 nm with PDI 0.252. Cumulative percentage drug release of Rilpivirine is 96.9±2.66 % at the end of 1month indicating no change in % drug release after stability study for 1month.

The s-SNEDDS formulation clearly shown improved and increased drug dissolution for poorly soluble drug and also keep the drug in soluble state in GIT. So these have capability for delivering poorly water soluble drugs.

REFERENCES:

1. Lipinski et al., 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings.
2. Pouton CW. Formulation of poorly water soluble drugs for oral administration: Physicochemical and Physiological issues and formulation classification system. European Journal of Pharmaceutical Sciences, 2006, 29: 278 – 287.
3. Pouton CW. Lipid formulation for oral administration of drugs, non-emulsifying, self emulsifying drug delivery systems. European Journal of Pharmaceutical sciences 2000; 11 suppl 2: S93 – S98.
4. Patton and Carey, 1979 science class watching fat digestion. 1979 Apr 13; 204(4389):145-8.
5. Staggers et al., 1990 physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. 1990 Feb 27; 29(8):2041-56.
6. Kossena, G. A., Charman, W. N., Boyd, B. J., & Porter, C. J. H. (2005) Influence of the intermediate digestion phases of common formulation lipids on the absorption of a poorly water-soluble drug. J. Pharm. Sci., Vol. 94. pp. 481-492
7. European pharmacopeia fifth edition: 2413 – 2414.
8. Indian pharmacopeia
9. C Srinivas; S Vanitha Sagar. Enhancing the bioavailability of Rilpivirine using Microemulsion Drug Delivery System. Asian journal of Pharmaceutical and Clinical research, 2012; Volume 5, issue 4: 134-139.
10. Bhupinder singh Recent advancements in self emulsifying drug delivery systems(SEDDS) Crtical reviews in therapeutic drug carrier systems 31(2) 2014.
11. Shantanu Bandyopadhyay; O.P. Katare; Bhupinder Singh. Optimized self nanoemulsifying systems of Ezetimibe with enhanced bioavailability potential using long chain and medium chain triglycerides. Colloids and Surfaces B, Biointerfaces 100, 2012: 50-61.
12. M. Sunitha Reddy; P.Srinivas Goud; S.S. Apte. Solubility enhancement of Candesartan cilexetil by self emulsifying drug delivery systems. IJPSR, 2012; Vol.3 (7): 2098-2104.
13. Jyothi B Jeevana ; K Sreelakshmi. Design and evaluation of self nanoemulsifying drug delivery system of

Flutamide. Journal of young pharmacists, 2011; Vol.3 (1): 4 -8.

14. A. Madhu Babu; B. Prakash Rao; P. Sudhakar; K.R.S. Sambasiva Rao. Formulation and evaluation of self nanoemulsifying drug delivery system of low solubility drug Rilpivirine for improved solubility and bioavailability. International journal of biological & pharmaceutical research, 2012; 3(6): 767 – 774.
15. Preeti K Suresh; Sudhanshu Sharma. Formulation and In-vitro characterization of self-nanoemulsifying drug delivery system of Cinnarzine. Pharmacie Globale (IJCP) 2011, 9(08): 1 -6.
16. Shantanu Bandyopadhyay; O.P. Katare; Bhupinder Singh: Optimized self nanoemulsifying systems of Ezetimibe with enhanced bioavailability potential using long chain and medium chain triglycerides. Colloids and Surfaces B, Biointerfaces 100, 2012: 50-61.
17. Durgacharan Arun Bhagwat; John Intru D′ Souza. Formulation and evaluation of solid self micro emulsifying drug delivery system using Aerosil 200 as solid carrier. International current pharmaceutical journal, 2012; 1(12): 414 – 419.
18. Tamer H.hassan, karsten mader novel semisolid SNEDDS based on PEG-30-di-(polyhydroxystearate); progesterone incorporation and in vitro digestion international journal of pharmaceuticals 486(1-2) page no 77-87 march 2015.

How to cite this article:
Reddy BS, Harish G, Md. Ul-Haq F: Formulation and *in-vitro* Characterisation of Solid - Self Nanoemulsifying Drug Delivery System (S-SNEDDS) of Rilpivirine. Int J Pharm Sci Res 2016; 7(7): 3117-29.doi: 10.13040/IJPSR.0975-8232.7(7).3117-29.