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Supersaturated Self Emulsifying Drug Delivery System: A Promising Vector for Efficient Oral Delivery of Rilpivirine

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Jyoti A. Patil.*, Rohit R. Shah[1]

**Research Scholar, Faculty of Pharmacy, Pacific Academy Of Higher Education and Research University, Udaipur 313003, Rajasthan, India*

[1] *Department of Pharmaceutics, Appasaheb Birnale College of Pharmacy, Sangli 416416, Maharashtra, India.*

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ABSTRACT

Rilpivirine, an antiretroviral NNRTI is categorised as a BCS Class II drug having low solubility. The vast majority of people living with HIV/AIDS reside in the developing countries with limited health budgets. Conventional dosage forms of Rilpivirine undergoes first pass metabolism resulting in poor bioavailability. The purpose of this proposed research was to improve the drug release of Rilpivirine, by preventing precipitation and thus reduce its dose through the development of supersaturated self-emulsifying drug delivery systems (SuSEDDS). This in turn shall assist in its transportation through the lymphatic system which ultimately contributes to improvement in bioavailability. The study began with a preliminary screening carried out to select the proper components on the basis of which pseudoternary phase diagrams were constructed using Capryol 90(oil), CremophorRH40 (surfactant) and Transcutol HP (co-surfactant). A ratio of 3:1 (surfactant: co-surfactant) was finalised for formulation of SEDDS. The prototype liquid SEDDS were characterised for zeta potential (-2.5±0.98 mv), particle size (24.5±1.06 nm) and polydispersity index. (0.183±1.04). The optimized prototype formulation was further considered for formulation of SuSEDD, using HPMC E15 and PVP K30 as precipitation inhibitors. The precipitation inhibition ability and drug release were assessed at 3 different levels of inhibitors and were statistically analysed applying 3^2 full factorial design using Design Expert Software (Version 13) The optimized batch was capsulated using Neusilin US2 as adsorbent and drug release and SEM were performed. The absence of interaction with drug components was confirmed by XRD. The successful design of SuSEDDS of Rilpivirine showed a drug release of 99.37 % at the end of four hours. Drug stability studies after a period of three months showed no variation in drug content for liquid SuSEDDS. The limitation of poor solubility of Rilpivirine was successfully overcome by the effective use of precipitation inhibitors, PVP K30 and HPMC E15. The increase in drug release of optimized liquid and capsulated formulations would be expected to contribute for better patient compliance.

INTRODUCTION:

Rilpivirine, a BCS class II drug is a second-generation non-nucleoside reverse transcriptase inhibitor having higher potency, longer half-life and reduced side effects.[4] The first generation agents such as efavirenz and nevirapine although well tolerated and have long term safety have limitations that include tolerability issues, a low genetic barrier and cross resistance between agents. HIV reverse transcriptase is one of the three virally encoded enzymes essential for HIV replication,[1] following entry into CD4-T- lymphocytes, viral RNA is transcribed into DNA by reverse transcriptase enzyme. NNRTIs inhibit this process by binding to an allosteric hydrophobic pocket resulting into a conformational change in the enzymes active site that prevents further viral RNA transcription.[2]

The existing literature till date demonstrates research carried out on simple SEDDS of Rilpivirine. The present work is focussed on supersaturated self-emulsifying drug delivery systems (SuSEDDS) of Rilpivirine. The pharmacokinetic studies of Rilpivirine shows that the drug undergoes first pass metabolism and it is reported that 25% of the administered dose is excreted unchanged in the faeces and 1% excreted unchanged in urine. SEDDS being lipidic systems have miro / nanoemulsified drug which has uptake through the lymphatic drainage. This provides an added advantage of bypassing first pass effect which contributes to lower dose of drug.

Supersaturation is an effective strategy for improving drug release for poorly water soluble drugs to overcome low oral bioavailability. This can be achieved through the most accepted design of lipid based formulations i.e. Su-SEDDS with the effective use of precipitation inhibitors (PIs). [3] Thermodynamic and kinetic inhibitions are two processes that might explain drug precipitation inhibition. PIs can interact with drugs and prevent crystal nucleation and / or growth. Furthermore, the effect of PIs on the characteristics of the medium, such as viscosity and pH, may result in drug precipitation inhibition. Drug precipitation, on the other hand, can be thermodynamically reduced by increasing drug solubility. [4]

Amongst the polymeric precipitation inhibitors HPMC (Eseries>Kseries) showed a greater precipitation inhibition as compared to PVP. This was attributed to hydrophobicity of HPMC than PVP which contributes by stronger crystallisation inhibition effect.[5]

Generally it is accepted that a metastable saturated state of drug in SEDDS possibly causes precipitation of drugs, the reason being insufficient solubilising capability of SEDDS. Also many basic drugs could be precipitated in the acidic environment of the GI fluid leading to decrease in absorption. So to prevent the precipitation of hydrophobic drugs in GI fluid, SEDDS have been formulated with polymers capable of inhibiting drug precipitation, the so called supersaturated SEDDS.[6-7]

Although SEDDS solubilizes hydrophobic pharmaceuticals by incorporating them into colloids, the free drug fraction, which is in equilibrium with the solubilized fraction, is still constrained by its poor water solubility. Supersaturation, on the other hand, aims to raise the drug's thermodynamic activity beyond its solubility limit, resulting in increased free drug concentrations and a strong driving force for passage into and across biological barriers, resulting in a more pronounced effect on the uptake flux. After the formulation is discharged from an adequate dosage form into the aqueous medium of the GIT and on dilution with GI fluid and the breakdown of lipids by lipase, a SuSEDDS with PIs can avoid drug precipitation by producing and sustaining a supersaturated condition *in vivo.* [8]

Drug precipitation is expected to be the principal component that leads to variation in *in vivo* pharmacokinetics associated with conventionally solubilized SEDDSs after emulsification in the GIT. As a result, for stable liquid SEDDSs, modest drug loading is advised, as loading is regulated by drug saturation solubility in the liquid lipid phase; moreover, drug precipitation during and after emulsification should be taken into account. For high-dose BCS class II and IV medicines, the limited drug-loading capacity of typical solubilized SEDDSs is a major drawback. SuSEDDS formulation techniques, on the other hand, can help to improve drug loading by stabilising supersaturation in the formulation and/or in the GIT. [4]

MATERIALS AND METHODS

Materials:

Rilpivirine was received as gift sample from Chiral Biosciences Ltd Hyderabad. Captex 350,Captex 500,Capryol 90, Capryol PGMC, Pecol, Maisine, Acconon, Labrasol, Labrafil M1944 CS, Labrafac, Transcutol HP were obtained from Gattefose (France). Kolliphor RH 40, KolliphorEL,Kollisolv 124, PEG 400, PEG 600, Polyvinyl pyrrolidone K30, PVP K90, HPMC E3,HPMC E5, HPMC E15 were provided by BASF (Mumbai) Castor oil, Tween 20, Tween 80were purchased from Loba Chem. Neusilin US2 was supplied by Gangwal

Chemicals Private Limited, Mumbai. All the solvents employed in the experiment were of high performance liquid chromatography (HPLC) grade.

Solubility testing of Rilpivirine in oils, surfactants and co-surfactants:

The solubility of Rilpivirine in various vehicles, oils, surfactants and co-surfactants was determined by the shake flask method. An excess amount of Rilpivirine was added to each capped vial containing 3 ml of the vehicles. After sealing, the mixture was vortexed at a maximum speed for 10 min in order to facilitate proper mixing of Rilpivirine with the vehicles. Mixtures were then shaken in orbital shaker (Remilabs RS-24) maintained at room temperature until equilibrium (48 h). After 24 h, the vial was examined for drug residue, and an excess amount of drug was put to the vial that showed no residue and the vial was shaken for another 24 h. Then mixtures were then centrifuged at 12000 rpm for 20 min. The supernatants were collected into glass vials and solubility was determined by analysing it spectrometerically on UV-visible spectrophotometer at 304 nm.[9]

Construction of pseudo-ternary phase diagram:

A pseudo ternary phase diagram was used to define the microemulsion existence zone. Water titration method was employed for its determination. This diagram would be best suited for making different possible compositions of oil surfactant/co-surfactant and water. Pseudo ternary phase diagram were constructed to examine the formation of O/W microemulsion using four component system oil, surfactant, co-surfactant, and aqueous phase system. [10,11] The four components consisted of Capryol 90, Cremophor RH 40, Transcutol HP and distilled water (aqueous phase). The mixture of oil and surfactant/co-surfactant (S/ CoS) at specified weight ratios were diluted with water on a drop-by-drop basis. The ratios of surfactant/co-surfactant were designed in a precise way, i.e.1:1, 2:1 and 3:1 (w/w) respectively. All of these proportions were blended with increasing percentage of oil, i.e., 10%, 20%, 30%, 40% up to 90% to get phase diagram. Following that, each combination was titrated with water and agitated with the aid of a magnetic stirrer. The system was inspected for appearance and flow quality after each addition. The point at which solution turns turbid marks the end point of the titration. The oil, surfactant and co-surfactant values were utilised to establish the margin of emulsion region.[12] After the identification of emulsion region in the phase diagrams, the emulsion formulations were selected at desired component ratios. The emulsion area was chosen from the phase diagram because the solution remains clear even after infinite dilution.

Preparation and characterization of SEDDS:

Based on the results of phase diagrams Capryol 90, Cremophor RH 40, Transcutol HP were selected as components for self-micro emulsifying mixture (SME) for drug delivery. Liquid SEDDS formulation were prepared by dissolving 250 mg of Rilpivirine in the optimized SME mixture consisting of Kolliphor RH 40 (75 %v/v) and Transcutol HP (25% v/v) with varying concentrations of oil.(Table 1) A clear solution was formed by vortexing the drug-containing SME combination. For a period of 48 h, these mixtures were monitored for indications of turbidity or phase separation. The prepared mixtures were evaluated for particle size, zeta potential and polydispersity index. [13, 14]

Selection of precipitation inhibitor:

Precipitation inhibitors were used to prevent the drug from precipitating and keep the formulation in its metastable form. Different precipitation inhibitors used for the development process were HPMC E3, HPMC E15, HPMC K4M, HPMC K100, PVP K30 and PVP K90. From previous reports and studies, HPMC and PVP were proven to be effective in preventing drug from precipitation. The precipitation inhibitors were chosen based on the viscosity of the solution. [15-17]

Drug Excipient Compatibility:

Drug excipient compatibility was performed by mixing Rilpivirine with different ratios of oil and surfactant mixture. Prepared formulations were kept at 40˚C ± 2˚C and 70% ± 5% RH for 1 month and analysed for different parameters like precipitation, crystallization, phase separation, colour change and % assay by HPLC.[18] The results for excipient compatibility are shown in table 2.

Formulation of Liquid Supersaturated Self Emulsifying Drug Delivery Systems using 3^2 Full Factorial Design:

Formulation was optimized by using 3^2 Full Factorial Design obtained from Design Expert Software. Formulations were prepared by using Capryol 90 as oil (37.5% v/v), Kolliphor RH 40 as surfactant and Transcutol HP as co-surfactant (Smix Ratio = 3:1) (62.5% v/v). HPMC E15 and PVP K30 were selected as precipitation inhibitors and used in different combination ratios (Table 3). Prepared formulations were kept for 24 h at room temperature for

stabilization. After stabilization formulations were characterised for different parameters. [19-21]

Characterization of Liquid Supersaturated Self Emulsifying Drug Delivery Systems:

Physical Evaluation:

Formulation batches were observed for its colour and phase separation to ensure the physical stability of the formulation.[21]

Drug Content Determination:

A reverse phase HPLC was employed to determine drug content of prepared formulations. Briefly, Rilpivirine was analysed at ambient oven temperature of 30º using Agilent Zorbax Bonus RP (250 x 4.6 mm, 5µ) column. The mobile phase was buffer:acetonitrile, 20:80 (buffer:10Mmol/Sodium dihydrogen phosphate, pH 3 adjusted using ortho-phosphoric acid) and was pumped with 1ml/min flow rate for a run time of 9 min, using a UV detector set at a wavelength of 304 nm.[21]

Drug Precipitation Inhibition Study:

The drug precipitation inhibiting ability of prepared formulations was assessed in simulated gastric fluid to expect changes in the solubility of Rilpivirine in the gastric fluid *in vivo.* Rilpivirine SuSEDDS (formulation F1-F9), and Rilpivirine bulk powders equivalent to 250 mg Rilpivirine were added to 10 ml of the simulated gastric fluid containing 0.1N HCl placed in a round bottom flask. The samples were kept in a shaking incubator at 50 rpm at 37º Aliquots (2 ml) were withdrawn at 15, 30, 60 and 90 min, and the levels of Rilpivirine in the aliquots were analysed using the UV-Visible Spectrophotometer at 304 nm. [22-23]

***In-Vitro* Dissolution Testing:**

The dissolution test was carried out for 4hr at 50 rpm using USP II dissolution apparatus paddle method. The dissolution medium was 900 ml 0.1N HCl. The dissolution medium was kept at 37±0.5 º. The optimized liquid formulations filled in dialysis bag [24] to be equivalent to 25 mg Rilpivirine were used for the dissolution test. At regular intervals, 5ml of dissolution medium was sampled and fresh dissolution medium was replenished in the apparatus to keep the volume constant. The withdrawn sample was passed through whatman

filter paper 41 and the filtrate was assayed by UV spectrophotometer at 304 nm to determine the dissolved drug concentration. [22-23]

Stability study:

Centrifugation:

Final single optimized liquid supersaturated self-emulsifying drug delivery system F7 was subjected to centrifugation at 3000 rpm for 5 min. The formulation was visually observed for phase separation. [24]

Freeze Thawing:

The liquid SuSEDDS pre concentrate of final formulation was subjected for 3 to 4 freeze thaw cycles, which included freezing at 4˚ and thawing at 40˚ for 24 h. The formulation was then centrifuged for 5 min at 3000 rpm. The formulation was visually observed for phase separation. [24-25]

Accelerated stability testing:

Optimised formulation F7 was subjected to accelerated stability testing for 6 m at 40 ± 2^{o}/75 ± 5% RH, 25 ± 2^{o}/60 ± 5% RH. The formulation under study was examined for transparency, phase separation and drug content.

Formulation of Solid Supersaturated Self Emulsifying Drug Delivery Systems:

The optimized batch F7 of liquid SuSEDDS was adsorbed onto NeusilinUS2 by physical mixing in a small mortar and pestle. The resulting solid SuSEDDS was a free-flowing powder that was subsequently subjected to solid state characterization and dissolution studies. [26-27]

Characterization of Solid Supersaturated Drug Delivery Systems:

***In vitro* dissolution testing:**

The dissolution test was carried out for 4 h at 50 rpm by USP II dissolution apparatus paddle method. The dissolution medium was 900 ml 0.1N HCl. The dissolution medium was kept at 37±0.5^{o}The solid SuSEDDS were filled in capsule to be equivalent to 25 mg Rilpivirine were used for the dissolution test. 5ml of the dissolution medium was sampled at appropriate intervals, and in order to maintain a constant volume, fresh dissolution media was replenished

in the device at the same time. The withdrawn sample was passed through whatman filter paper 41 and the filtrate was assayed by UV spectrophotometer at 304 nm to determine the dissolved drug concentration.

Particle Size Determination:

Particle Size determination doneon Equipment Quant 3D FEG, with WD at 10.0 mm, Tilt - 0º, Magnification - 200x and Pressure - 1.45 e^{-5} Torr. Images captured and recorded. [28]

X-Ray Diffraction:

X-rays are electromagnetic waves with a wavelength of approximately 1°A., which is near to the size of an atom. It's utilised for atomic-level examination of crystalline substances. The sample quantity analysed is determined by the sample holder's capacity. The mesh size for coarse samples should be around 200. Using a glass slide or a razor blade, a level compact bed of sample powder should be made on the sample holder. To ensure that the orientation of the lattice is distributed randomly, the sample's upper surface should be flat. Measurements made with an X-ray diffractometer employing X-ray powder scattering (PXRD) were used to determine the physical state of Rilpivirine in solid SuSEDDS (X:Pert PRO with X-Pert Data Collecter, PAN analytical). Using monochromatic CuK-radiation at 40mA and 40kV over a 2° range of 7 to 80° with a continuous scanning speed of 4°/min, the tests were carried out at room temperature. A glass slide was used to pack the evaluated samples tightly into the cavity of an aluminium sample holder. PXRD was performed on samples of Rilpivirine powder, solid SuSEDDS, Neusilin and a physical mixture of Rilpivirine and Neusilin.[28]

Comparative Drug Release Profile of Liquid SuSEDDS, Solid SuSEDDS and market Rilpivirine Formulation:

The drug release characteristics of optimized liquid SuSEDDS was compared with solid SuSEDDS and market Rilpivirine tablet formulation. The results are depicted in table 4 and fig 8.

RESULTS AND DICUSSION

Solubility of Rilpivirine in oils, surfactants and cosurfactants:

The solubility of Rilpivirine in various oils, surfactant and co-surfactants was investigated in order to select the best ingredients for designing SEDDS with the highest Rilpivirine solubilisation capacity. (Fig. 1)

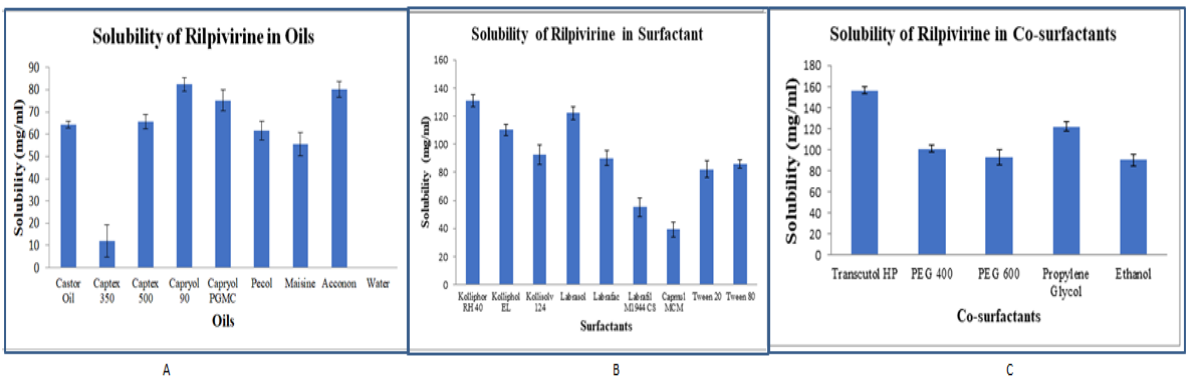


Figure. 1: Solubility of Rilpivirine in excipients (A) Oils, (B) Surfactants and (C) Co-surfactants.

When compared to other oils, Capryol 90 (82.45 mg/ml) had the maximum solubility of Rilpivirine while in water it was 0.0153 ± 0.001 mg/ml. This might be due to the polarity of weakly water soluble medicines, which favours solubilization in small/mid molecular volume oils such medium chain triglycerides, mono or diglycerides. As a result, Capryol 90 was chosen as the oil phase for the formulation development.

The solubility of Rilpivirine was revealed to be highest in surfactant Cremophor RH 90 (82.45 mg/ml) as compared to other surfactants, it had highest solubility.

Rilpivirine was determined to be the most soluble in co-surfactant Transcutol HP (156.43 mg/ml) as compared to other co-surfactants.

Due to weak solubility in water as Rilpivirine is a non-polar molecule, the surfactant phase resulted in higher solubility than oil. For further determinations, Capryol 90 (oil), Cremophor RH 40 (surfactant) and Transcutol HP (co-surfactant) were used.

Cremophor RH40 being non-ionic surfactant is relatively safe and biocompatible and being hydrophilic (HLB 14-16) they are superior in forming fine, uniform emulsion droplets which can empty rapidly from the stomach and provide a large surface area that facilitates rapid

drug release and absorption. Surfactants also form a film around the emulsion droplets, lowering the interfacial energy, and preventing coalescence. This can prevent precipitation of drug within the GI lumen.

Generally, a transient negative interfacial tension and fluid interfacial films are seldom created using a single surfactant and usually require the addition of a co-surfactant. The addition of co-surfactants reduces the interface's bending stress, allowing an interfacial layer to take the many curvatures required to create a nano emulsion across a wide variety of compositions.

Construction of Pseudoternary Phase Diagram:

The propensity of SEDDS is to undergo change when diluted as occurs after administration causing precipitation of drug due to loss in solvent capacity. A phase diagram can be used to determine the link between a mixtures phase behaviour and its composition. Self-emulsifying areas were found using pseudoternary phase diagrams created independently for each group. Thus phase diagrams were plotted as depicted in fig 2, at surfactant/co-surfactant ratios of 1:1, 2:1 and 3:1 The self-emulsifying region was increased with a change in the surfactant and co-surfactant from 1:1 to 3:1In the present study Capryol 90(oil) was tested for phase behaviour studies with Cremophor RH 40(surfactant) and Transcutol HP (co-surfactant) as the S/CoS mixture. As seen from the ternary plot S-Mix 1:1, 2:1 and 3:1 with higher the concentration surfactant higher is the self- emulsification efficiency. When the proportion of co-surfactant in the formulation was higher than that of surfactant, significant phase separations were detected. Also increase in the surfactant concentration resulted in an increase in viscosity of the formulation. Thus fixing the surfactant/co-surfactant ratio at 3:1 is a suitable choice from stability considerations.

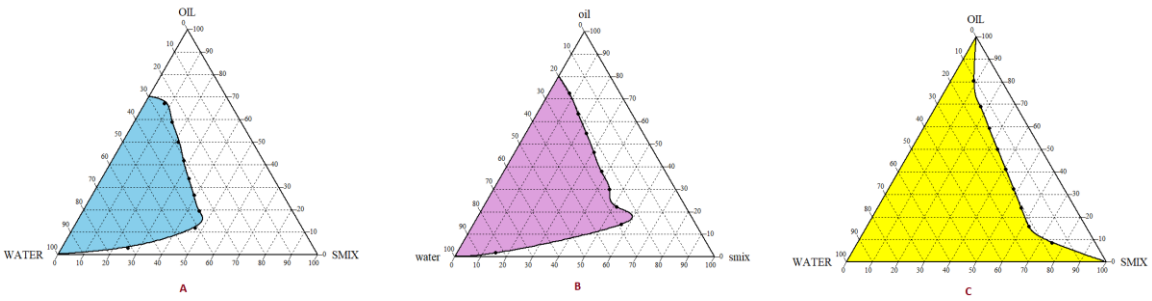


Figure. 2: Pseudoternary phase diagram of (A) Smix 1:1 (B) Smix 2:1 (C) Smix 3:1

Selection of Prototype Formulation:

To understand self-emulsifying behaviour several batches were prepared with different oil: smix ratio to know the characteristics of formulation. Accordingly, six batches (YH-1 to YH-6) were prepared as shown in Table 1. The formulations were analysed for particle size, zeta potential, polydispersity index and viscosity (Table 1). The analysis was performed using Horiba Scientific SZ-100 analyzer.

From Table 1 it was inferred, prototype YH-3 had the best fit of particle size, zeta potential and PDI. Batch YH-3 had the more oil and s-mix proportion. As the drug is soluble in the surfactant and oil, to avoid any precipitation during stability, a precipitation inhibitor was added.

Table 1. Formulation and evaluation parameters for prototype development.

Formulation	Oil (ml)	Smix (ml)	Drug (mg)	Particle size(nm)	ZetaPotential (mv)	Polydispersity Index	Viscosity (mPass)
YH-1	4	6	250	26.5±0.13	-3.8±1.80	0.264±1.22	0.897±0.49
YH-2	2	8	250	32.0±0.26	-1.9±3.20	0.377±1.28	0.896±0.95
YH-3	3.75	6.25	250	24.5±1.06	-2.5±0.98	0.183±1.04	0.897±0.79
YH-4	2.5	7.5	250	813.7±1.34	-3.6±1.36	1.077±1.52	0.896±1.20
YH-5	2.22	7.77	250	7431.7±0.90	-3.6±2.03	2.385±2.42	0.896±1.25
YH-6	5	5	250	148.1±2.47	-4.6±1.10	0.434±3.37	0.896±0.84

Data are shown as average of n=3 ±SD.

Formulation of Supersaturated Self Emulsifying Drug Delivery System (SSEDDS) by using 3^2 Full Factorial Design:

Considering optimization of a formulation as a crucial component of any research, a 3^2 full factorial design was applied using Design Expert Software. Accordingly, the prototype SEDDS formulation YH-3 which was selected on the basis of particle size, zeta potential and PDI, was further considered to formulate supersaturated SEDDS. For this study HPMC E15 and PVP K30 were selected as precipitation inhibitors and the resulting formulations were analyzed for precipitation inhibition and drug release characteristics. The composition of the formulations and the results for the responses obtained are given in Table 3.

Characterization of Liquid Supersaturated Self Emulsifying Drug Delivery Systems:

Physical Evaluation:

The formulation batches of supersaturated liquid self- emulsifying systems of Rilpivirine showed no phase separation and remained colourless (Table 2). [21]

Table 2: Results for excipient compatibility study

Formulation	Oil	Surfactant	Cosurfactant	Drug	Precipitation	Crystallization	Colour Change	% Assay
AT-1	2 ml	5 ml	3 ml	250 mg	√	√	√	99.71
AT-2	1ml	4 ml	5 ml	250 mg	√	√	√	101.33
AT-3	3 ml	4 ml	3ml	250 mg	√	√	√	100.78
AT-4	2 ml	2 ml	6 ml	250 mg	√	√	√	99.64
AT-5	1ml	7 ml	2 ml	250 mg	√	√	√	100.52
AT-6	4 ml	4ml	2 ml	250 mg	√	√	√	101.13
AT-7	2 ml	3 ml	5 ml	250 mg	√	√	√	100.85
AT-8	4 ml	5 ml	1 ml	250 mg	√	√	√	100.99

Drug Content Determination:

The drug content was determined using RP-HPLC. [21] Following are the results of the batches: All the batches had a drug content ranging from of 98-102%. It was revealed that the content of all the formulations was consistent.[29-30] Batch F5 and F7 showed approximately 100% drug content as compared to other batches as shown in Table 3.

Precipitation Inhibition study:

To simulate the drug precipitation inhibition by the SuSEDDS formulation in the stomach *in vivo*, the change in the concentration of Rilpivirine in an artificial gastric fluid was evaluated. As shown in Fig. 3, the concentration of Rilpivirine released from SuSEDDS formulations (F1, F7, and F9) increased quickly at the beginning and remained relatively unchanged during the entire testing period. [30] As a result, it was discovered that the precipitation inhibitors included in the SuSEDDS formulations were successful in preventing drug precipitation. No significant differences in Rilpivirine concentration were found between formulations F1, F7, and F9, which may be attributed to the SuSEDDS formulations'

similar viscosities and consequently similar capacity to suppress precipitation. Rilpivirine bulk powder has a very low drug concentration when compared to formulations tested using Su-SEDDS. The results (Table 3 and fig 3) therefore, implied that the precipitation inhibitors should be used to prevent the precipitation of drug in the stomach and to enhance the delivery of Rilpivirine because the precipitated drug would not be efficiently absorbed.

The equation in terms of coded factors obtained using Design Expert Software (Version13) for precipitation inhibition ability is as follows.

$$Y_1 = 53.06 + 13.17\ X_1 + 14.25\ X_2 - 4.50 X_1 X_2$$

The contour plot Fig 5(A) was plotted to identify the effect of X1 and X2 variable i.e. PVP K30and HPMC E15 on precipitation inhibition. The plot depicted that as the PVP K 30 concentration increased from 0.1 to 0.5 the % precipitation inhibition increased with approximately 60%. Also, as the HPMC E15 concentration went from 0.4 to 0.8 the % precipitation inhibition also increased with approximately 60%. Hence, it was found that PVP K30 and HPMC E15 had similar effect with the increase in the concentration.

Table 3. Formulation and evaluation of SuSEDDS with application of 3^2 full factorial design.

Batch	**Drug (mg)**	**Capryol90 (ml)**	**Smix (ml)**	**Independent variables**		**Dependent variables**		**Drug Content (%)**
				PVPK30 (X_1)(mg)	HPMC-E15 (X_2)(mg)	Precipitation inhibition (Y_1) (%)	Drug Release (Y_2) (%)	
F1	250	3.75	6.25	0.5	0.6	63±0.57	84.78±1.2	99.83±1.11
F2	250	3.75	6.25	0.1	0.6	48±0.43	77±0.58	99.74±0.96
F3	250	3.75	6.25	0.1	0.4	20±0.18	60±1.89	98.52±1.78
F4	250	3.75	6.25	0.1	0.8	53±0.48	85.77±1.88	101.53±2.32
F5	250	3.75	6.25	0.5	0.4	58±0.52	82.14±1.74	100.12±0.99
F6	250	3.75	6.25	0.3	0.6	51±0.46	81.77±1.48	98.73±1.56
F7	250	3.75	6.25	0.3	0.8	76±0.68	98±1.98	100.03±1.07
F8	250	3.75	6.25	0.3	0.4	38±0.34	87.59±1.48	99.49±2.08
F9	250	3.75	6.25	0.5	0.8	75±0.68	88.45±1.56	101.76±1.37

Data are shown as average of n=3 ±SD.

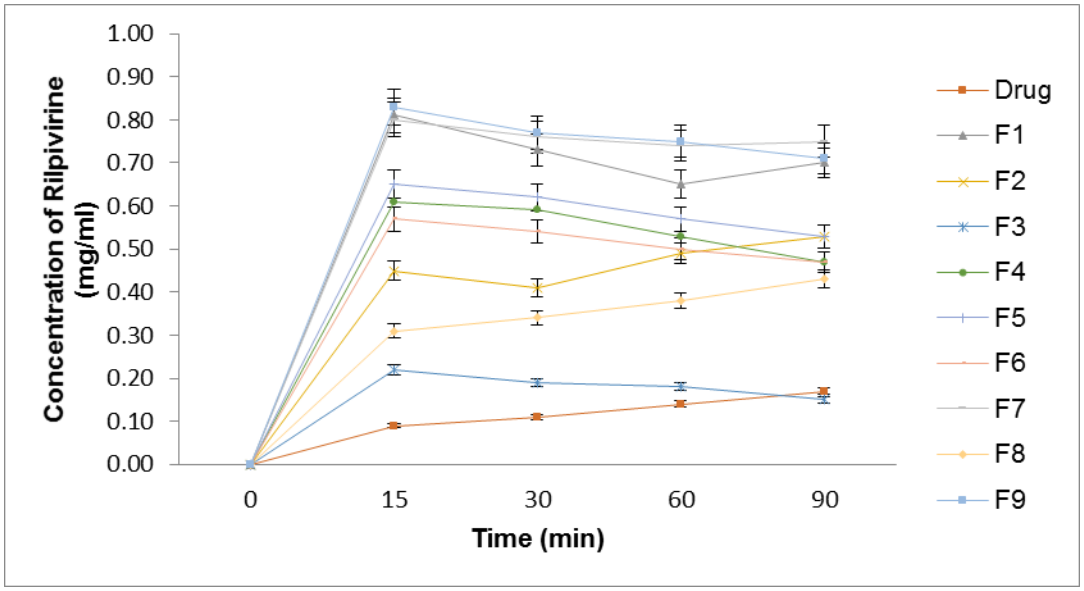


Figure.3 Precipitation inhibition study for liquid SuSEDDS.s

***In-Vitro* Dissolution Study and Drug Release Study:**

The formulations (F1 to F9) were subjected for *in-vitro* dissolution studies using tablet USP dissolution tester II. The dissolution medium pH 0.1N HCl was used to study the drug release. In the self-emulsifying drug delivery system, the amount of free energy necessary to make an emulsion was relatively minimal, allowing the oil droplets and water to spontaneously form an interface.[31] It is suggested that the oil/surfactant/co-surfactant and water phases effectively form micro emulsion, decrease the oil droplet size and eventually increase the release rate.[21] It is evident from the results (table 3 and fig 4) that Rilpivirine SEDDS showed a dramatic improvement in the in vitro dissolution profile. Results revealed that Rilpivirine SEDDS showed more than 70% of Rilpivirine released in 240 min. It is seen that surfactant-co surfactant concentration play pivotal role in the drug release. The factors affecting drug release may be (a) SEDDS with smaller particle sizes give increased surface area for drug release from solvents, increasing drug release rate and (b) oil phase of SEDDS may act as carrier molecules which itself does not diffuse through the barrier but allow drug molecules to get diffused from it. Although the specific mechanism is unknown, it is known that any of these conditions can impact drug release. [32]

The equation in terms of coded factors obtained using Design Expert Software (Version13) for drug release is as follows.

$$Y_2 = 86.76 + 5.39\ X_1 + 7.47\ X_2 - 4.71 X_1 X_2 - 9.72\ X_1^2 + 2.65\ X_2^2$$

The contour plot Fig 5(B) was plotted to identify the effect of X1 and X2 variables, PVP K30 and HPMC E15 on % drug release. The plot depicted that as the PVP K30 concentration went 0.1 to 0.3 the % drug release increased with approximately 40% but when the concentration was further increased there was a decrease in the % drug release by 20%. Also, as the HPMC E15 concentration went 0.4 to 0.8 the % drug release also increased with approximately 40%. Hence, it was found that HPMC E15 had effect with the increase in the concentration the % drug release was also increased.

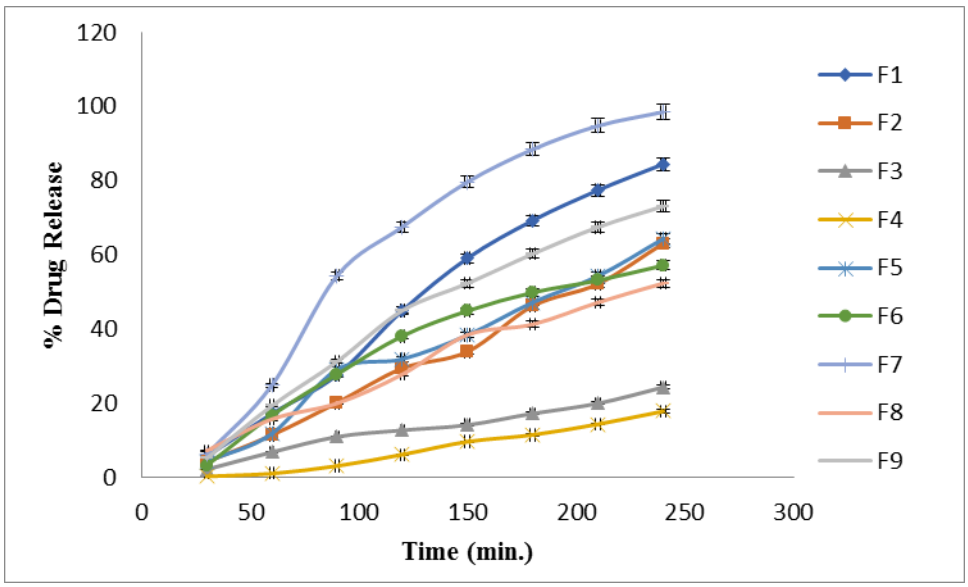


Figure.4 Percent drug release for liquid SuSEDDS.

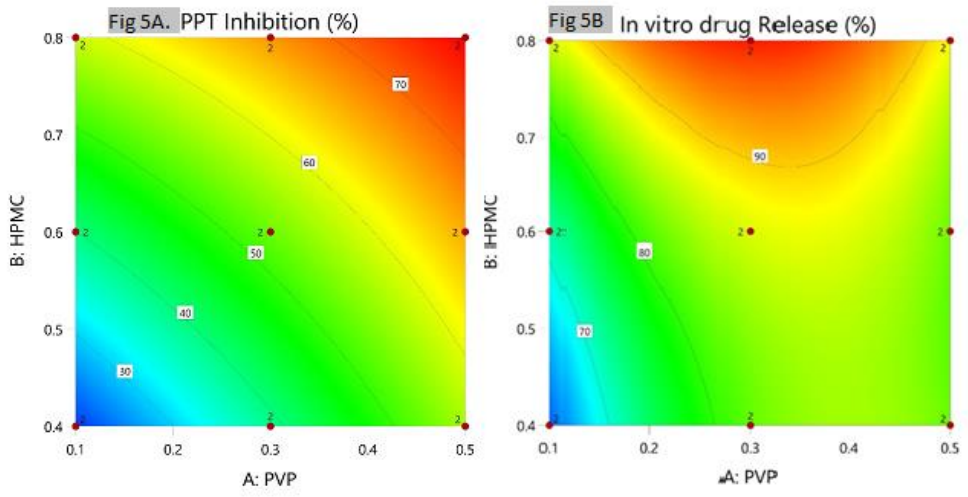


Figure.5 Contour plot for (A) Precipitation Inhibition Study (B) In-Vitro Drug Release Study

Stability Study:

The optimized liquid SuSEDDS formulation F7 prepared when subjected to the centrifugation and freeze thaw cycles survived the test and showed no signs of phase separations. This clearly indicated the physical stability of the prepared formulation. Drug content of the optimized batch was found to be 99.73 % after 6 months.

Formulation of Solid Supersaturated Self Emulsifying Drug Delivery Systems:

To convert Rilpivirine loaded liquid SuSEDDS to solid SuSEDDS, it was adsorbed onto Neusilin US2. Physical mixing produced a mixture of free-flowing powder and adsorbed liquid SEDDS. It was best suited for solid state characterisation. Neusilin US2 has porous amorphous form and gives it a high oil adsorption capability (3.2 mg/ml). The solid SEDDS were also subjected to an emulsification test, and the outcomes were analogous to those of the liquid SuSEDDS. Solid SuSEEDS required around 57s to completely emulsify in comparison to roughly 49s for liquid SuSEEDDS. These findings demonstrated that Rilpivirine was present in the solid formulation in a solubilized state. [33-35] XRD was used to confirm the physical state of Rilpivirine in the solid SuSEDDS. (fig. 6)

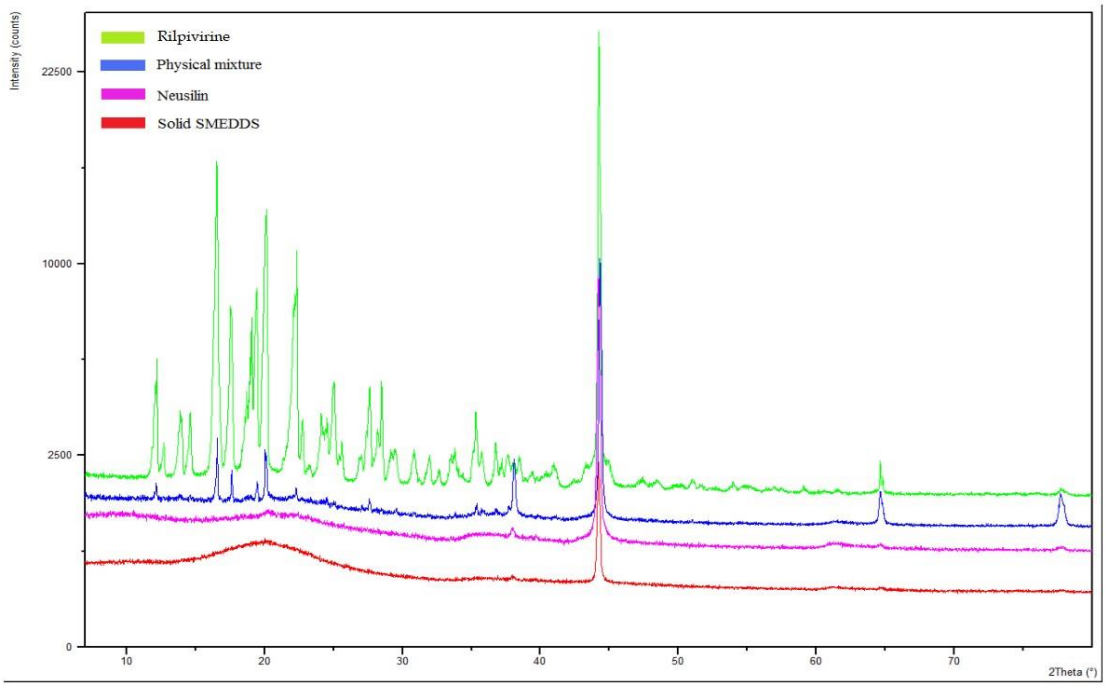
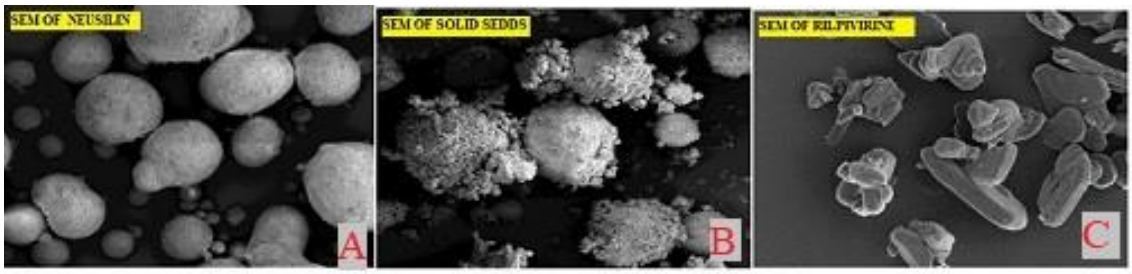


Figure.6 Powder X-ray Diffraction (PXRD) of Rilpivirine, Physical mixture (Rilpivirine & Neusilin US2), Neusilin US2 and Solid SuSEDDS.

Characterization of Solid-SuSEDDS:

***In vitro* Dissolution Study and Drug Release Study:**

The *in vitro* dissolution of the solid SuSEDDS and marketed formulation was performed in 0.1N HCl using a USP Type II dissolution apparatus with a paddle speed of 50 rpm. Solid SuSEDDS and marketed formulations were placed in the dissolution medium and were sampled at time intervals of 30, 60, 90, 120, 150, 180, 210 and 240 min. The amount of drug released was calculated from the calibration curve of Rilpivirine dissolved in 0.1N.



HCl.

Figure.7 Scanning Electron Microscopy images of (A) Neusilin US2, (B) solid SuSEDDS and (C) Rilpivirine. Magnification – 200x

It was found that solid SuSEDDS showed a % drug release of 99.37% and marketed formulation showed 92.13% over a period of 4 h, which is equivalent to liquid SuSEDDS. Hence, the release of liquid SuSEDDS and solid SuSEDDS were quite comparative with marketed formulation. Therefore, NeusilinUS2 does not alter the release kinetics and can be a potential candidate as an inert carrier for liquid SuSEDDS.

Particle Size Analysis:

A highly porous powder with good oil adsorbing ability is required to convert a liquid SEDDS to a solid form. These powders can absorb the oil components of liquid SEDDS and turn them into a free-flowing powder. NeusilinUS2 has a porous structure that can absorb up to three times its own weight in oil. The morphology of solid SuSEDDS is shown by scanning electron microscopy as depicted in fig 7 Smooth rectangular crystalline crystals emerged to make up Rilpivirine. NeusilinUS2 looks to be spherical porous particles with a diameter of around 100 micrometres. Liquid SuSEDDS is adsorbed onto the surface of NeusilinUS2 particles in micrographs of solid SMEDDS. Because the formulation process included physical mixing to aid adsorption, partly coated NeusilinUS2 can be seen in the

field of vision. [36-37] Solid SuSEDDS micrographs do not show the crystalline structures that are characteristic of solid Rilpivirine, implying that the medication is entirely dissolved in the solid SuSEDDS.

Diffraction:

PXRD was used to confirm the drug's physical condition in the solid SuSEDDS. The presence of strong peaks indicates that Rilpivirine is present in its highly crystalline form. The absence of distinct diffraction patterns indicates that NeusilinUS2 is in an amorphous form. The inclusion of Rilpivirine in the physical combination (1:1) of Rilpivirine and NeusilinUS2 resulted in certain crystalline peaks. The solid SuSEDDS showed no significant crystalline peaks, in contrast to the physical mixing of Rilpivirine and NeusilinUS2, confirming the molecularly dispersed condition of Rilpivirine in the formulation. The results for X-ray diffraction are shown in fig 6.

Comparative Drug Release Profile of Liquid SuSEDDS, Solid SuSEDDS and market Rilpivirine Formulation:

Comparative drug release profile (Table 4 and fig 8) showed a significantly higher release for our liquid and solid Su-SEDDS compared to marketed Rilpivirne tablet. Further we can conclude that solidification did not affect the selected responses specially the *in vitro* drug release profile.

Table 4. Comparative drug release for liquid SuSEDDS, solid SuSEDDS and market Rilpivirine tablet product.

Time (Min)	Liquid SuSEDDS	Solid SuSEDDS	Marketed Tablet
30	6.19±0.308	6.26±0.655	9.81±0.670
60	24.88±0.168	25.34±0.485	32.13±0.257
90	54.39±0.689	57.66±2.461	47.34±0.569
120	67.56±0.147	66.93±0.446	57.21±0.482
150	79.67±0.234	80.25±0.440	62.9±1.200
180	88.44±681	86.63±0.342	67.54±1.680
210	94.77±0.377	92.11±1.15	74.33±0.973
240	98.54±1.01	99.37±1.50	82.43±1.234

Data are shown as average of n=3 ±SD.

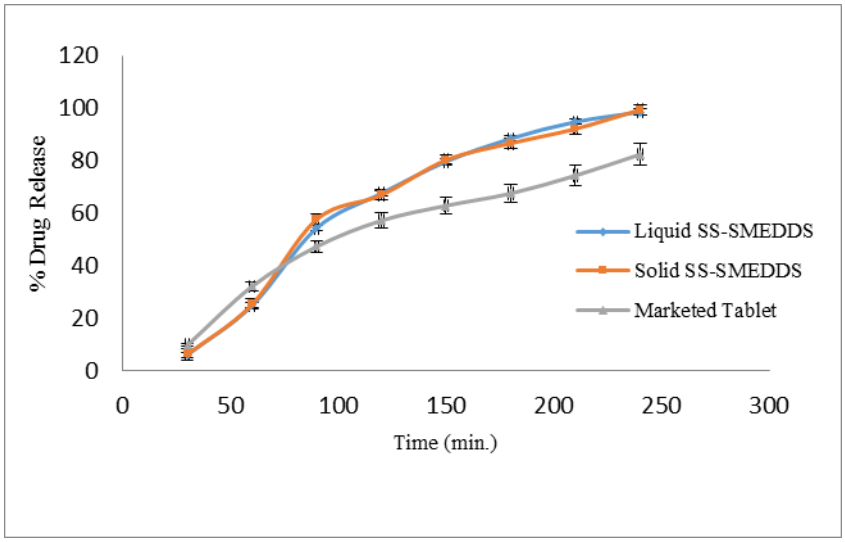


Figure. 8: Comparative drug release study for liquid SuSEDDS, Solid SuSEDDS and Marketed Tablet

CONCLUSION:

The study was designed to prepare SuSEDDS of Rilpivirine to minimize drug precipitation and subsequently enhance drug release. This was achieved through an appropriate choice of oils, surfactants and co-surfactants, coherently guided with an efficient selection of precipitation inhibitors, HPMC E15 and PVP K30.The optimized liquid SuSEDDS was designed to a final capsulated form by adsorption on to Neusilin US2. The solid SuSEDDS showed a drug release of 99.37 % compared to the marketed formulation 92.13%, over a period of 4 h and could emulsify in about 57s.

Better solubility and drug release obtained with SuSEDDS of Rilpivirine demonstrated in this work could thus provide a promising alternative to ensure successful oral delivery of drug with poor biopharmaceutical properties. This may be due to combined effect of lipidic systems, drug presented as nanosized SEDDS and supersaturation confirming superiority over commercial formulation, with respect to *in-vitro* dissolution and drug release.

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