World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
Research Article

FORMULATION AND EVALUATION OF RILPIVIRINE NANOSUSPENSION BY NANO PRECIPITATION METHOD

*A. Vivek Pavan Kumar, [1]Dr. M. Lakshmi Prasanna, [2]Dr. D. Narendra, [3]Dr. N. V. V. Jagan Mohan Reddy.

*M.Pharm, Department of Pharmaceutics, VJ's College of Pharmacy, D.B, V Raju Township, Diwancheruvu, Rajahmundry, Andhra Pradesh, 533103.
[1]M.Pharm, Ph.D. Professor and HOD in the Department of Pharmaceutics, VJ's College of Pharmacy, D.B, V Raju Township, Diwancheruvu, Rajahmundry, Andhra Pradesh, 533103.
[2]M.Pharm, Ph.D. Principal, VJ's College of Pharmacy, D.B, V Raju Township, Diwancheruvu, Rajahmundry, Andhra Pradesh, 533103.
[3]M.Pharm, Ph.D. Secretary & Correspondent, VJ's College of Pharmacy, D.B, V Raju Township, Diwancheruvu, Rajahmundry, Andhra Pradesh, 533103.

Received: 25-10-2024 / Revised Accepted: 15-11-2024 / Published: 13-12-2024

ABSTRACT:

The aim of the present work is to develop oral Nanosuspension of Rilpivirine by Nano Precipitation method using various Stabilizers & Surfactant such as β-cyclodextrin, Soluplus, Poloxamer 407, Polyvinyl alcohol, Sodium lauryl sulfate, Polysorbate 80 Various formulation as well as process parameters were optimized in order to achieve desirable size and saturation solubility. Characterization of the prepared Nanosuspension was done with respect to Drug Content, Percentage yield, Entrapment efficiency, Viscosity, Sedimentation volume, Scanning electron microscopy, Particle's size, zeta potential, dissolution rate, in-vitro dissolution study. Zeta potential value for the optimized formulation (NS12) was found to -4.49 mv which was found to be within the acceptable limits. Average particle size of Nano suspension of optimized formulations (NS12) which is in ratio with Poloxamer 407 was found to be 500.4 nm. From the In vitro studies we can say that formulation NS12 shows best drug release of 98.42±1.27% within 30 minutes whereas all the other formulations didn't release the drug. The drug release from the Nanosuspension was explained by the using mathematical model equations such as zero order, first order, and equation methods. Based on the regression values it was concluded that the optimized formulation NS12 follows first order kinetics with super case-II transport mechanism and the stability studies shows that the formulation was stability upto 3Months of time period.

Keywords: Rilpivirine, Poloxamer 407, Sodium lauryl sulfate, Polysorbate 80, Nanoprecipitation technique, SEM, Zeta potential, PSD.

INTRODUCTION

Anti-Retroviral: For the past 25 years, treating an HIV-1 infection with antiretroviral therapy consisting of three active medications has been accepted as the standard of care. This therapeutic approach, which consists of a combination of two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) as the main agent and a third agent—which could be an integrase strand transfer inhibitor (INSTI),[1] a boosted protease inhibitor (PI), or a non-nucleoside reverse transcriptase inhibitor (NNRTI)—has allowed for progressive immune system restoration, control of HIV-1 infection with efficacy rates exceeding 90% in recent clinical trials,[2] and, consequently, a marked decrease in acquired immunodeficiency syndrome (AIDS) events and other complications related to HIV-1 infection itself. A rise in the CD4+ cell count was considered a sign of immune system restoration enhanced CD4/CD8 ratio >0.9 and a T-cell count returning to normal levels [3].

Rilpivirine: Rilpivirine is an antiretroviral medication that is used to treat HIV. It is a derivative of diarylpyrimidine and is classified as a second-generation NNRTI. Commercially marketed as Edurant®, this medication is manufactured by Janssen Pharmaceuticals (Beerse, Belgium) and was approved in 2011 by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA). NNRTIs work by directly attaching to the HIV reverse transcriptase (RT) allosteric site, which modifies the active site's conformation. As a result, the cDNA elongation process is stopped because the nucleosides are unable to attach to the reverse transcriptase.[4]

Address for Correspondence: A. Vivek Pavan Kumar**,** M.Pharm, Department of Pharmaceutics, VJ's College of Pharmacy, D.B, V Raju Township, Diwancheruvu, Rajahmundry, Andhra Pradesh, 533103, **Mail ID:** vivekpavan123@gmail.com.

How to Cite this Article A. Vivek Pavan Kumar. Formulation and Evaluation of Rilpivirine Nanosuspension by Nano Precipitation Method. World J Pharm Sci 2023; 12(04): 118-137; https://doi.org/10.54037/WJPS.2022.100905

Rilpivirine is a member of BCS Class-II of the Biopharmaceutics Classification System, characterized by its high permeability and poor solubility. Drugs are categorized using the BCS method according to their permeability and solubility. By grouping medications into classes, it is utilized to estimate the in vivo pharmacokinetics of oral immediate release products. Rilpivirine has a terminal half-life of 34-55 hours.

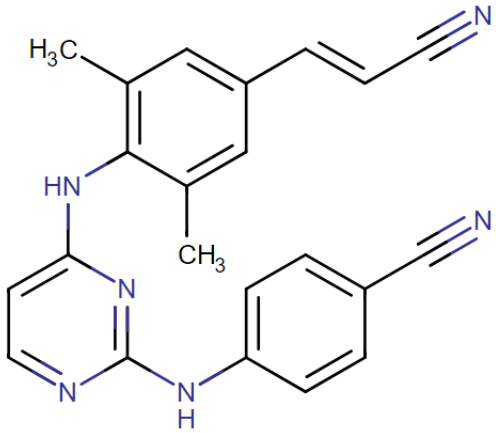

Figure No.1 Structure of Rilpivirine

Nano technology:

might be characterized as the science and engineering dealing with the creation, synthesis, characterisation, and use of materials and devices whose smallest functional organization is on the nanoscale scale, or one billionth of a meter, in at least one dimension. Since it has control over the underlying molecular structure, which permits control over the macroscopic chemical and physical properties, at these scales, consideration of individual molecules and interacting groups of molecules in relation to the bulk macroscopic properties of the material or device becomes significant.[5]

Nanosuspension:

The effective formulation of medications depends on a number of factors, including solubility, stability at room temperature, compatibility with solvent, excipient, and photostability. Currently, approximately 40% of newly created chemical entities resulting from drug development initiatives are lipophilic or poorly soluble in water substances.[6,7] Drugs with limited solubility and low bioavailability can be solved using a variety of formulation techniques. Conventional methods such as micronization, fatty solution application, penetration enhancer or cosolvent application, surfactant dispersion method, salt creation, precipitation, etc., have limited effectiveness in improving the solubility of poorly soluble pharmaceuticals. Other strategies include vesicular systems like liposomes, solids dispersion, emulsion and microemulsion techniques, and inclusion complexes with cyclodextrins. These strategies demonstrate promise as drug delivery systems, but their main drawback is that they are not universally applicable to all medications.[8] Nanoparticle engineering has been researched and reported for use in pharmaceuticals throughout the past few decades.[9] The challenges posed by the previously discussed methods can be resolved via nanotechnology. The study of science and engineering at the nanoscale, or 10–9 m, is known as nanotechnology. Techniques like Bottom-Up Technology and Top-Down Technology are used to transfer the drug microparticles/micronized drug powder to drug nanoparticles.[10] Submicron colloidal dispersions of medication particles that are nanosized and stabilized by surfactants are called nanosuspensions.[11] The weakly water-soluble medication is suspended in a dispersion with no matrix material in nanosuspensions.[12] These can be applied to improve the solubility of medications that have low solubility in lipid and water environments. Increased solubility causes the active ingredient to flood at a quicker pace, reaching the maximum plasma level more quickly. This method works well for compounds that are difficult for formulators to work with because they have poor permeability, poor solubility, or both. Because of the smaller particle size, poorly soluble medications can be administered intravenously without obstructing blood vessels. The suspensions can also be formed into a solid matrix by lyophilization. It also has the benefits of liquid formulations over other formulations in addition to these advantages.[13] The benefits, drawbacks, and pharmaceutical use of these various preparation techniques as a drug delivery mechanism are the primary topics of this review.

Methods of Preparation of Nanosuspensions:

There are primarily two ways to prepare nanosuspensions. "Bottom Up technology" refers to the typical ways of precipitation (Hydrosols [14]). In Bottom Up Technology, the medication is dissolved in a solvent and introduced to a nonsolvent mixture to cause crystals to precipitate. The utilization of inexpensive, basic equipment is the precipitation technique's main benefit.

Precipitation methods:

The medicine is first dissolved in an organic solvent and then mixed with an anti-solvent in the precipitation procedure. A fine crystalline or amorphous solid is produced when a medication solution is quickly added to an anti-solvent, causing a rapid super saturation of a variety of solutions

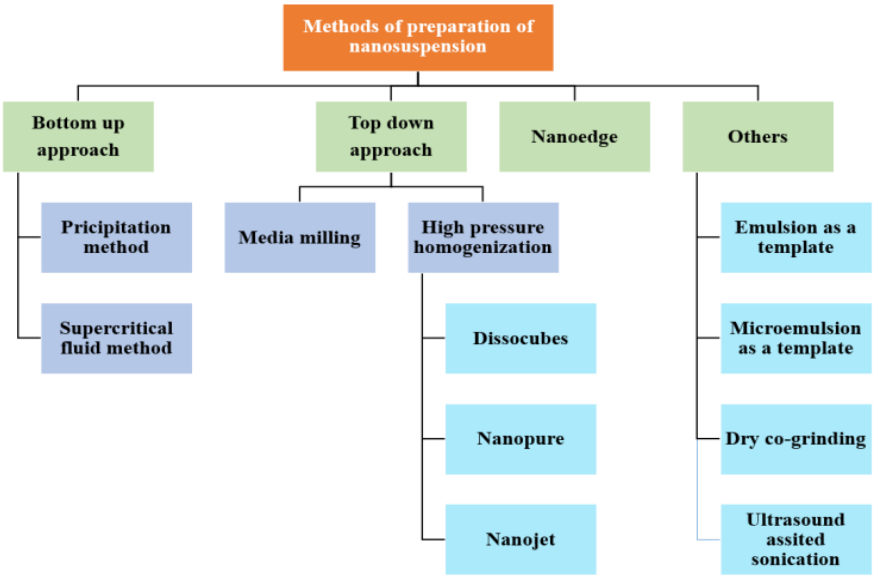

Figure No.2 Approaches for preparation of nanosuspension35

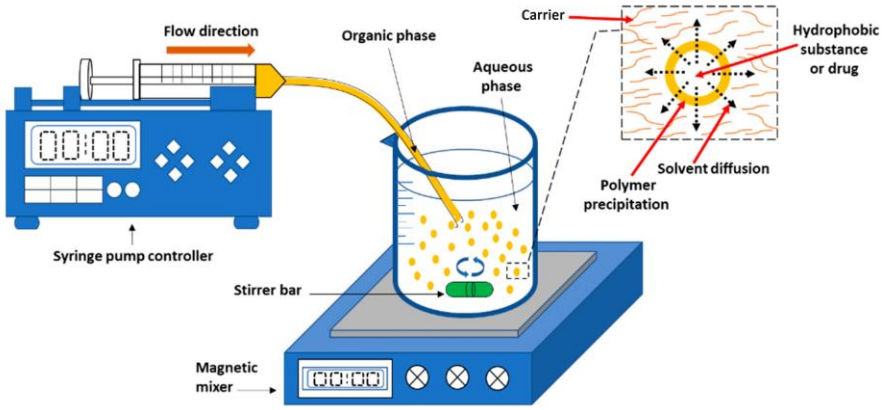

Figure No.3 A schematic representation of Nano Precipitation preparation methods

MATERIALS AND INSTRUMNETS:

- Rilpivirine API was procured from Gift Sample from hetero labs, Stabilizers like β-cyclodextrin, Soluplus, Poloxamer 407, Poly Vinyl Alcohol were procured from Loba chemie, Mumbai, Surfactants like Sodium Lauryl Sulphate, Polysorbate 80 was procured from Loba chemie, Mumbai.

Instruments used:

- Electronic Weighing Balance(Shimadzu Corporation Tokyo, Japan)
- UV-Visible Spectrophotometer(Single Beam Spectrophotometer (YIS-294))
- FTIR (Shimadzu Corporation Tokyo, Japan)
- Dissolution Apparatus (LAB India 8000)
- Ultra sonicator (UP400St, hielscher)
- Magnetic stirrer (Remi industries, Kerala)
- Viscometer (Brookfield viscometer, Choksi Lab)
- Particle Size Analyzer (Malvern Instrument Ltd Particle Size Analyzer)
- DSC (DSC Q20 Universal V4.5A TA Instruments)
- XRD (PW 1729, Philips, Amsterdam)
- Scanning Electron Microscopy (Scanning Electron Microscopes, JEOL)

METHODOLOGY

Determination of Melting Point:[15] Melting point of Rilpivirine was determined by capillary method. Fine powder of Rilpivirine was filled in glass capillary tube (previously sealed at one end). The capillary tube was tied to thermometer and the thermometer was placed in the Thais tube and this tube was placed on fire. The powder at what temperature it melted was noticed.

Solubility studies: [16] Solubility of Rilpivirine was carried out in different solvents like- 0.1N HCl, 7.4 pH Phosphate buffer and 6.8 pH Phosphate Buffer and also in organic solvents like ethanol and methanol. Solubility

studies were performed by taking excess amount of drug in different beakers containing the solvents. The mixtures were shaken for 24 hrs at regular intervals. The solutions were filtered by using whatmann's filter paper grade no.41. The filtered solutions were analyzed spectrophotometrically.

Spectroscopic studies

Determination of absorption maximum (λmax): The wavelength at which maximum absorption of radiation takes place is called as λmax. This λmax is characteristic or unique for every substance and useful in identifying the substance. For accurate analytical work, it is important to determine the absorption maxima of the substance under study. Accurately weighed 10mg Rilpivirine separately was dissolved in 0.1N HCl Buffer in a clean 10ml volumetric flask. The volume was made up to 10ml with the same which will give stock solution-I with concentration 1000µg/ml. From the stock solution-I, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml 0.1N HCl Buffer to obtain stock solution-II with a concentration 100µg/ml. From stock solution-II, 2ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using pH buffer to get a concentration of 20µg/ml. This solution was then scanned at 200-400nm in UV-Visible double beam spectrophotometer to attain the absorption maximum (λ-max). The λ-max of Rilpivirine of 100% solution i.e 20ppm (µg/ml) by using Single Beam Spectrophotometer (YIS-294) was found to be at 280 nm by using 0.1N HCl Buffer.

Construction of calibration curve using 0.1N HCl Buffer: Accurately weighed 10mg Rilpivirine was dissolved in 0.1N HCl Buffer taken in a clean 10ml volumetric flask. The volume was made up to 10ml with 0.1N HCl Buffer which gives a concentration of 1000µg/ml. From this standard solution, 1ml was pipette out in 10ml volumetric flask and volume was made up to 10ml using 0.1N HCl Buffer to obtain a concentration of 100µg/ml. From the above stock solution, aliquots of 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml each was transferred to a separate 10ml volumetric flask and solution was made up to 10ml using 0.1N HCl Buffer to obtain a concentration of 4, 6, 8, 10, 12, & 14µg/ml respectively. The absorbance of each solution was measured at 280 nm.

Drug excipient compatibility study:

Excipients are integral components of almost all pharmaceutical dosage forms. The successful formulation of a stable and effective solid dosage form depends on the careful selection of the excipients, which are added to facilitate administration, promote the consistent release and bioavailability of the drug and protect it from degradation.

Fourier-transform infrared (FTIR):

Infra Red spectroscopy is one of the most powerful analytical techniques to identify functional groups of a drug. The drug and excipient compatibility was observed using (FT-IR). The FT-IR spectra obtained from Bruker FT-IR Germany (Alpha T) was utilized in determining any possible interaction between the pure drug and the excipients in the solid state. The potassium bromide pellets were prepared on KBr press by grounding the solid powder sample with 100 times the quantity of KBr in a mortar. The finely grounded powder was then introduced into a stainless steel die and was compressed between polished steel anvils at a pressure of about 8t/in2.The spectra were recorded over the wave number of 4000 to 400cm-1.

Differential scanning calorimetry (DSC)

DSC was performed utilizing DSC Q20 Universal V4.5A TA Instruments. Samples were allowed to equilibrate for 1 min and then heated in an atmosphere of nitrogen over a temperature range from 0 to 300°C. Thermograms were obtained using TA Instruments universal analysis software 2000.

X-Ray diffraction (XRD)

The samples were recorded on XRD (PW 1729, Philips, Amsterdam,Netherlands). XRD patterns were recorded using monochromatic Cu Kα radiation with Ni filter at a voltage of 40 kV and a current of 30 mA between 10° and 80° 2θ values. The data were processed with the software Diffrac Plus V1.01.

Figure No.4 Optimized formulation of Rilpivirine Nanosuspenion

Preparation of Nanosuspensions:[17-20]

Table No.1 Formulation table of Rilpivirine loaded Nano suspensions using by Nano Precipitation method from NS1-NS8

Ingredients	NS1	NS2	NS3	NS4	NS5	NS6	NS7	NS8
Rilpivirine	100	100	100	100	100	100	100	100
β-cyclodextrin	50	100	150	200	-	-	-	-
Soluplus	-	-	-	-	150	225	300	450
Poloxamer 407	-	-	-	-	-	-	-	-
Polyvinyl alcohol	-	-	-	-	-	-	-	-
SLS(mg)	5	5	5	5	5	5	5	5
Polysorbate 80(ml)	3	3	3	3	3	3	3	3
Methanol	8	8	8	8	8	8	8	8
Water	30	30	30	30	30	30	30	30

Table No.2 Formulation table of Rilpivirine loaded Nano suspensions using by Nano Precipitation method from NS9-NS12

Ingredients	NS9	NS10	NS11	NS12	NS13	NS14	NS15	NS16
Rilpivirine	100	100	100	100	100	100	100	100
β-cyclodextrin	-	-	-	-	-	-	-	-
Soluplus	-	-	-	-	-	-	-	-
Poloxamer 407	150	225	300	450	-	-	-	-
Polyvinyl alcohol	-	-	-	-	150	225	300	450
SLS(mg)	5	5	5	5	5	5	5	5
Polysorbate 80(ml)	3	3	3	3	3	3	3	3
Methanol	8	8	8	8	8	8	8	8
Water	30	30	30	30	30	30	30	30

Method of Preparation of Nanosuspensions by Nano Precipitation method:

Procedure: Nanosuspension of Rilpivirine was prepared by Nano precipitation method with various carriers and drug. At first the weighed amount of Rilpivirine was taken and dispersed into the beaker containing methanol which acts as organic solvent. This drug and methanol solution is termed as organic phase. Now the stabilizers β-cyclodextrin, Soluplus, Polyvinyl alcohol (PVA) and Poloxamer 407 was dissolved in water and add surfactant (SLS) and Polysorbate 80 to the surfactant solution. we can label as aqueous phase. This solution was kept on magnetic stirrer for uniform mixing. Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer/surfactant containing water (aqueous phase). After 1 hour, the solution was kept in sonicator for about 60 mins. Then formed Nanosuspensions were collected by filtration through whatman filter paper and dried.

Note:

- Total Drug content of Rilpivirine in Nanosuspension is 100mg/8ml.
- Label claim is 25mg/2ml.

Evaluation Parameters of Nanosuspension.

Drug content

An accurately measured Nanosuspension equivalent to 10mg of drug was taken in 100ml volumetric flask and diluted to 100ml with 0.1N HCl. (To prepare the stock solution of 100µg/ml). The amount of drug determined spectrophotometrically at 280 nm by using Single Beam Spectrophotometer (YIS-294).

Percentage yield

Percentage practical yield of Rilpivirine Nanosuspensions is calculated to know about percentage yield, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of Rilpivirine Nanosuspensions recovered from each batch in relation to the sum of starting material. The percentage yield of prepared nanosuspensions was determined by using the formula.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Entrapment efficiency

The 100 mg of the Rilpivirine weight equivalent Nanosuspensions was analysed by dissolving the sample in 10ml of methanol. After the drug was dissolved 10ml of clear layer of dissolved drug is taken. There after the amount of drug in the water phase was detected by a UV-Spectrophotometric method at 280 nm. The concentration of the drug is determined with the help of calibration curve. The amount of drug inside the particles was calculated by subtracting the amount of drug in the aqueous phase from the total amount of the drug in the Nanosuspensions. The entrapment efficiency (%) of drug was calculated by the following equation.[21]

$$\% \text{ of Drug entrapment} = \frac{\text{Mass of drug in Nanosuspensions}}{\text{Mass of drug used in formulation}} * 100$$

Viscosity:

The rheologic parameters of the prepared suspensions, in terms of Viscosity, were determined by use of the steady shear method, Measuring the "non-Newtonian viscosity". Rheology of all Nanosuspensions was performed with a RVT Brookfield viscometer from Choksi Lab. (Indore, M. P.) All measurements were performed after Eliminating all thixotropy from the suspension.

Sedimentation volume:

The suspensions were stored individually in a 50ml measuring cylinder for 8hours at room temperature. Observations were made at every hour upto 8hours. The sedimentation volume (F) was then calculated using the following equation:

$$F = \frac{Vu}{Vo} * 100$$

Where, **Vu** is the ultimate volume of the sediment and **Vo** is the original volume of the suspension.

Scanning electron microscopy:

The morphological features of Rilpivirine nanosuspension are observed by scanning electron microscopy at different magnifications.

Particle Size analysis:

The particle size of the formulated Nanosuspension batches was determined by using the Malvern Instrument Ltd Particle Size Analyzer and the particle size of the batches were recorded in Malvern software v2.0. The formulations were diluted with an appropriate volume of 0.1N HCl Buffer. The measurements were carried out three times where the mean value was used.

Zeta potential:[22]

Zeta potential (ZP) is a physical property that controls electrostatic interactions in particle dispersions and is essential in understanding the stability of colloidal dispersions. It is identified as the difference in potential between the particle and its ionic atmosphere surrounding the medium and is measured in the plane of shear.

In-vitro drug release studies:

Procedure:

The in vitro release of various nanosuspension formulations were performed by dialysis bag diffusion technique. Dialysis tubing will act as dialysis sac. (Sigma dialysis membrane MW 12000 Da). Length of dialysis tube is 4 - 5 cm., The sac was then emptied and 1 ml of the formulated liquid nanosuspension was accurately transferred into the sac, which served as the donor compartment. The sac was once again examined for leak and then suspended in the stoppered vessel containing 100 ml 0.1N HCl Buffer, which behave as the receptor compartment. [23,24,25] The Media temperature should be 37º± 0.5ºC at 500 rpm speed. At predetermined time intervals, 3 ml of the sample was withdrawn from the receptor compartment and analyzed for the quantity of drug released. Fresh buffer was used to replenish the receptor compartment at each time point. The samples were withdrawn at 5, 10, 15, 20, 25 and 30 min. The diffusion studies and sample analysis were carried out for all the developed formulations. Collected samples were suitably diluted with 0.1N HCl Buffer and analyzed at 280 nm using 0.1N HCl Buffer as blank by using a UV spectrophotometer. The cumulative percentage drug release was calculated and graphs were plotted against time Vs % cumulative drug release.[26,27,28]

In vitro drug release kinetic studies

Kinetic model had described drug dissolution from nano suspension where the dissolved amount of drug is a function of test time. In order to study the exact mechanism of drug release from the nanosuspension, drug release data was analyzed according to zero order, first order, Higuchi square root, Korsmeyer-Pappas model. The criteria for selecting the most appropriate model were chosen on the basis of goodness of fit test.[29]

Zero-order model:

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation

$$Qt = Q0 + K0t$$

First Order Model:

The first order equation describes the release from systems where the dissolution rate is dependent upon the concentration of the dissolving species. Release behaviour generally follows the following first order equation:

$$\text{Log C= Log Co-kt/2.303}$$

Higuchi model: The first example of a mathematical model aimed to describe drug release from a system was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then sustained to different geometrics and porous systems. This model is based on the hypothesis that.

$$Q = KH - t1/2$$

Korsmeyer-Peppas model: Korsmeyer et al.(1983) derived a simple relationship which described drug release from a polymeric system equation. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model,

$$Mt / M\infty = Ktn$$

Stability studies[30]

The stability study of the optimized formulation of Nanosuspenion were carried out under different environmental conditions. The Nanosuspension was packed in a closed amber-colored glass vial and stored in a stability chamber for stability studies at 2-8°C (45% RH), 40^0C/75%RH, after a period of 1Month and at 40^0C/75%RH after a period of 3 Months. The patches were characterized for the Percentage yield, Drug Content, Entrapment efficiency and In-vitro dissolution study parameters during the stability study period.

RESULTS AND DISCUSSIONS

Determination of Melting point:

The melting point of found to be in range of 242.20 °C, which was determined by capillary method.

Saturation Solubility:

Saturation solubility was carried out at 250C using 0.1N HCl, 6.8 pH Phosphate Buffer, 7.4 pH Phosphate buffer, ethanol and methanol.

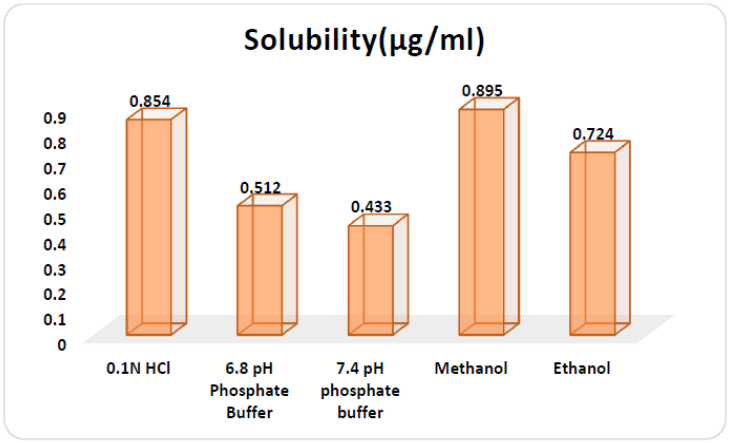

Figure No.5 Solubility studies of Rilpivirine

Discussion: From the above conducted solubility studies in various buffers, we can say that 0.1 N HCl and methanol has more solubility when compared to other buffer solutions. So 0.1 N HCl is used as dissolution medium, based upon the solubility studies on organic solvents methanol has more solubility than others. So methanol was used as organic solvent in the formulation of Nanosuspension.

Determination of absorption maximum (λmax): Determination of Rilpivirine λ-max was done in 0.1 N HCl medium for accurate quantitative assessment of drug dissolution rate.

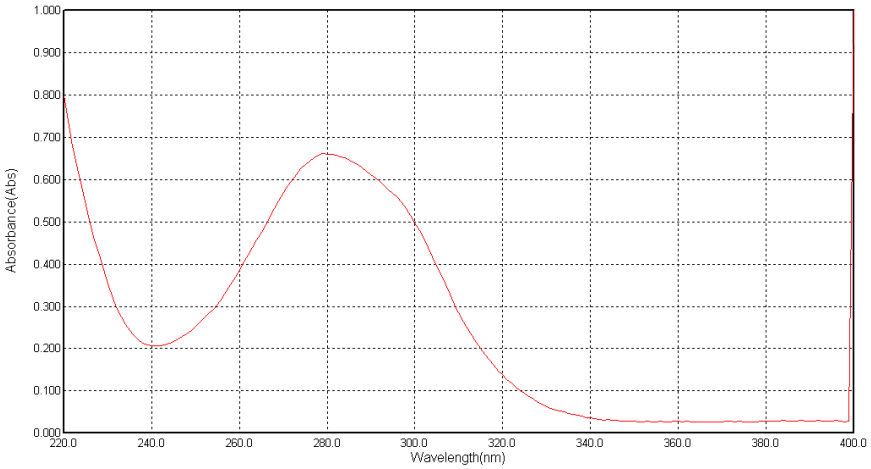

Figure No.6 UV spectrum of Rilpivirine

Discussion: The λ-max of Rilpivirine of 100% solution i.e 10ppm (µg/ml) by using Single Beam Spectrophotometer (YIS-294) was found to be at 280 nm by using 0.1 N HCl buffer.

Calibration curve of pure Drug:

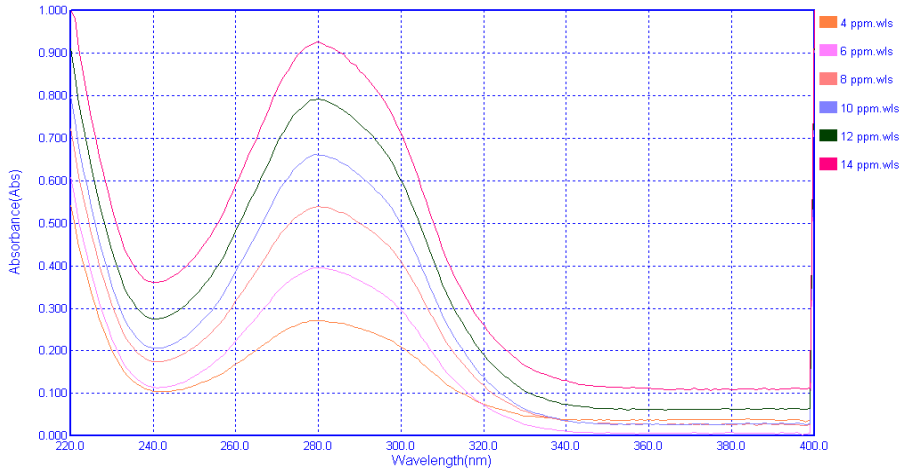

Figure No.7 Spectrum comparison of different concentrations of pure drug

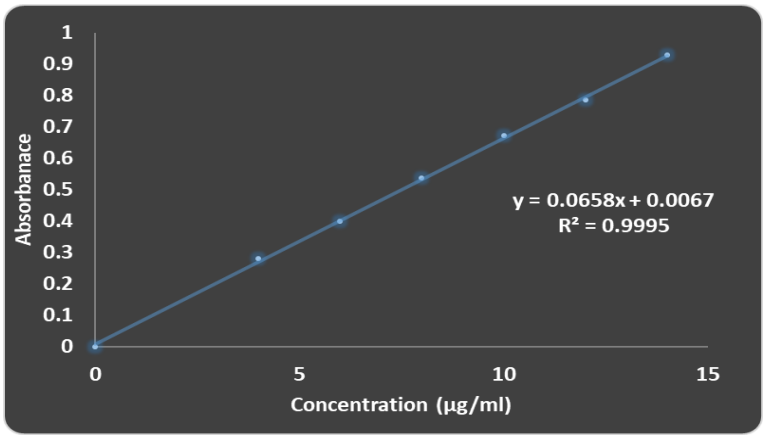

Figure No.8 Standard calibration curve of Rilpivirine in 0.1 N HCl

Discussion: The linearity was found to be in the range of 4-14 µg/ml in 0.1 N HCl. Regression analysis was selected because it minimizes the deviation and correct the variance heterogeneity. The regression line was defined by its slope (m) and its intercept (C) for normal regression analysis was found as 0.0658 and 0.0067, with regression coefficient of 0.9995 respectively. The regression value was closer to 1 indicating the method obeyed Beer-lamberts' law.

Drug excipient compatibility (FTIR): Drug and excipient compatibility was confirmed by comparing spectra of FT-IR analysis of pure drug with that of various excipients used in the formulation.

Pure Drug IR Spectrum:

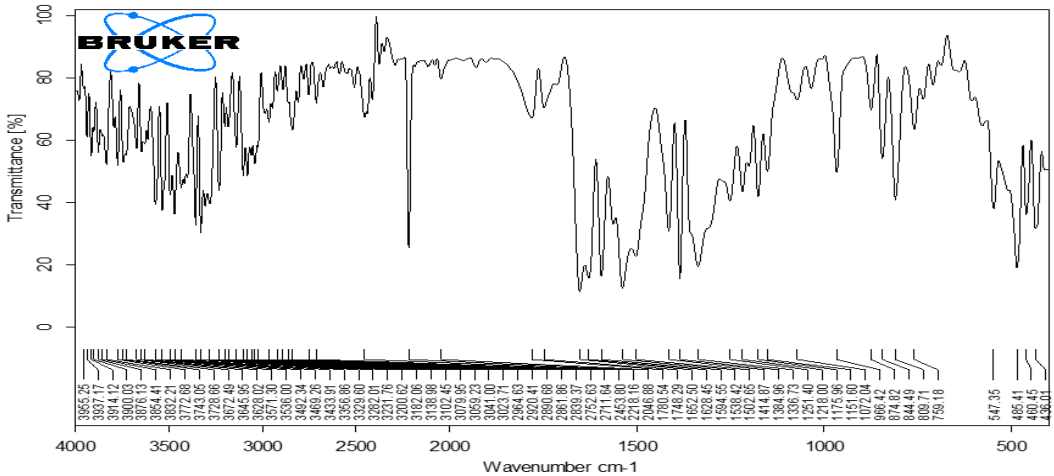

Figure No.9 FTIR spectrum of Rilpivirine

Optimized Formulation IR Spectrum

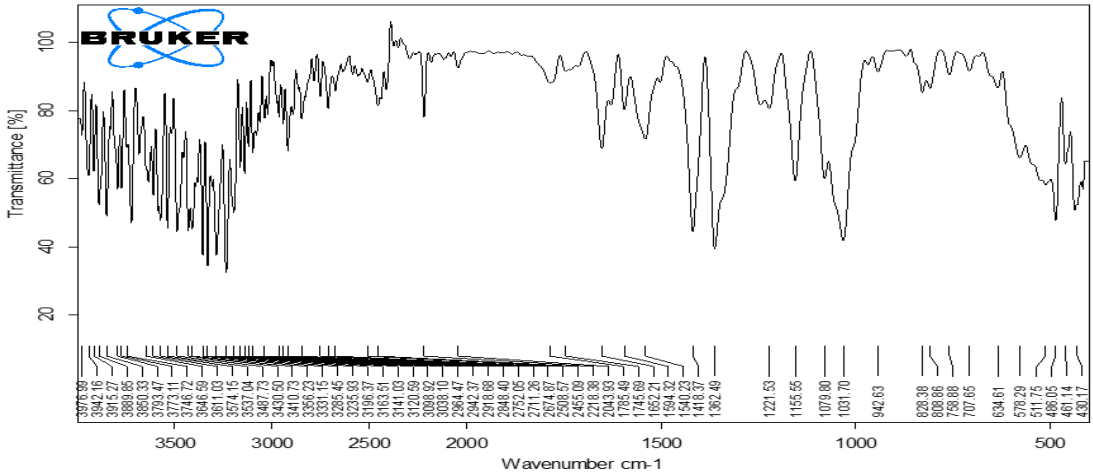

Figure No.10 FTIR spectrum of Rilpivirine Optimized Formulation

FTIR Interpretation Table for Pure and Optimized:

Table No.3 Interpretation table for Pure And Optimized Formulation

Functional groups	Stretching/Bending	Pure drug(cm-1)	Drug + polymers(cm-1)
N-H	Stretching	3492.34	3487.73
C-H	Stretching	3079.45	3038.16
C ≡N	Stretching	2218.16	2043.38
C=N	Stretching	1652.50	1652.21
C=C	Stretching	1628.45	1594.32
N-C	Stretching	1251.40	1221.53

Discussion: Form the drug excipient compatibility studies we observe that there are no interactions between the pure drug (Rilpivirine) and optimized formulation (Rilpivirine + excipients) which indicates there are no physical changes.

Differential Scanning Calorimetry: The DSC curves of pure drug, and optimized trail were obtained using differential scanning calorimeter.

Pure Drug:

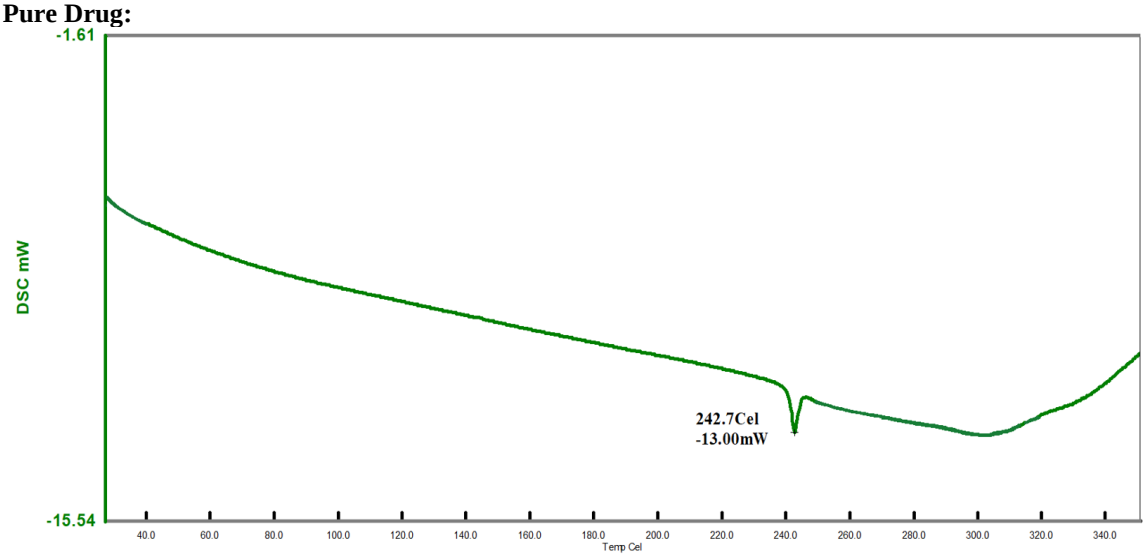

Figure No.11 DSC of the Pure Drug

Optimised :

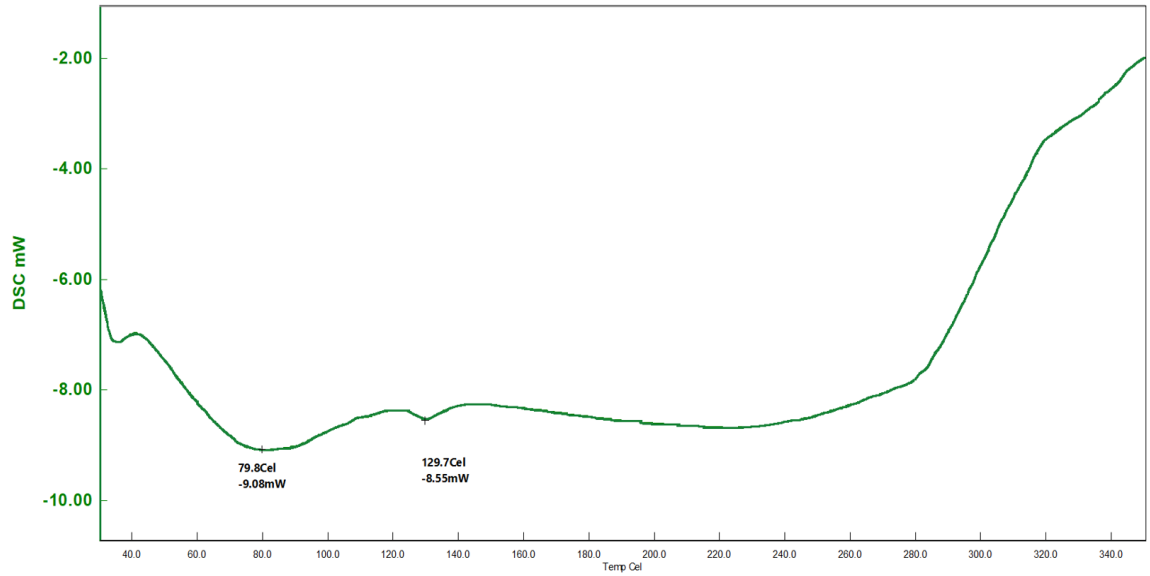

Figure No.12 DSC of the Optimized trail

Table No.4 Results of Differential scanning calorimetry

Component	Endothermic peak
Pure Rilpivirine	242.7 Cel
Optimized Trail	79.8 Cel and 129.7 Cel

Discussion: DSC experiments were carried out on pure Rilpivirine and Optimized Formulation. The melting point of pure drug Rilpivirine was determined to be between 200 and 250 degrees Celsius.The DSC thermograms of Pure Rilpivirine has a noticeable sharp peak at 242.7 Cel, matching to its melting point. The peak disappeared in the improved formulation, indicating 79.8 Cel and 129.7 Cel perfect homogeneity with the film component and the development of an amorphous form of Rilpivirine. The peaks in the DSC thermograms of Rilpivirine, Rilpivirine + Poloxamer 407, mixes correspond to the drug's melting points. Thus, the DSC investigation found no interactions between the chosen medication Rilpivirine and Poloxamer 407 and SLS combinations.

X-Ray Diffraction: The powder XRD pattern of pure drug Figure. No.24. and with the excipients Figure. No.25. showed that drug was highly crystalline in nature as indicated by the distinctive peaks.

Pure Drug:

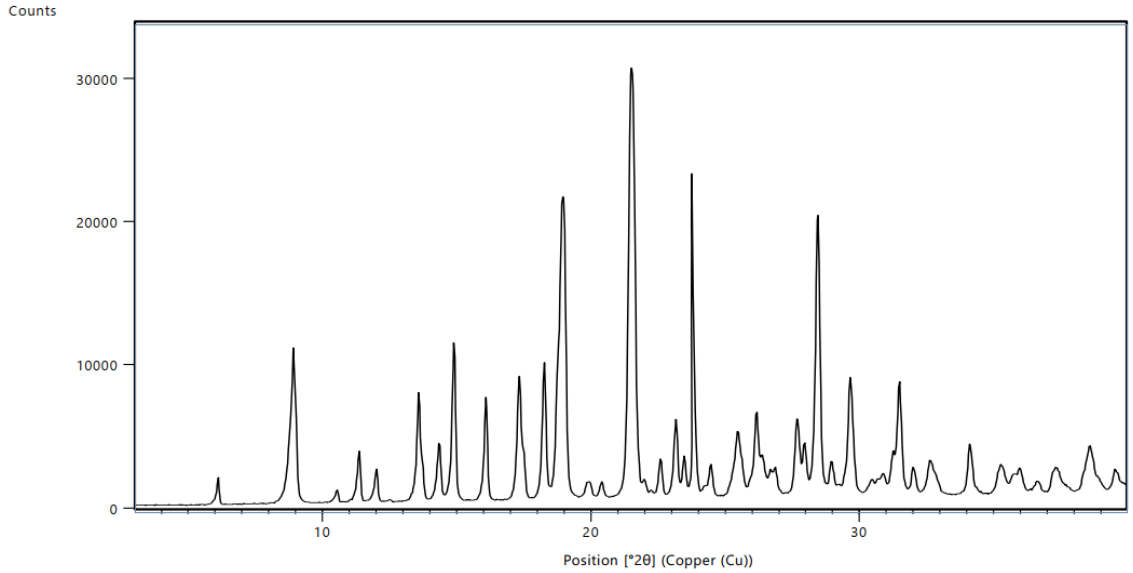

Figure No.13 XRD of Pure Drug

Optimized Trail:

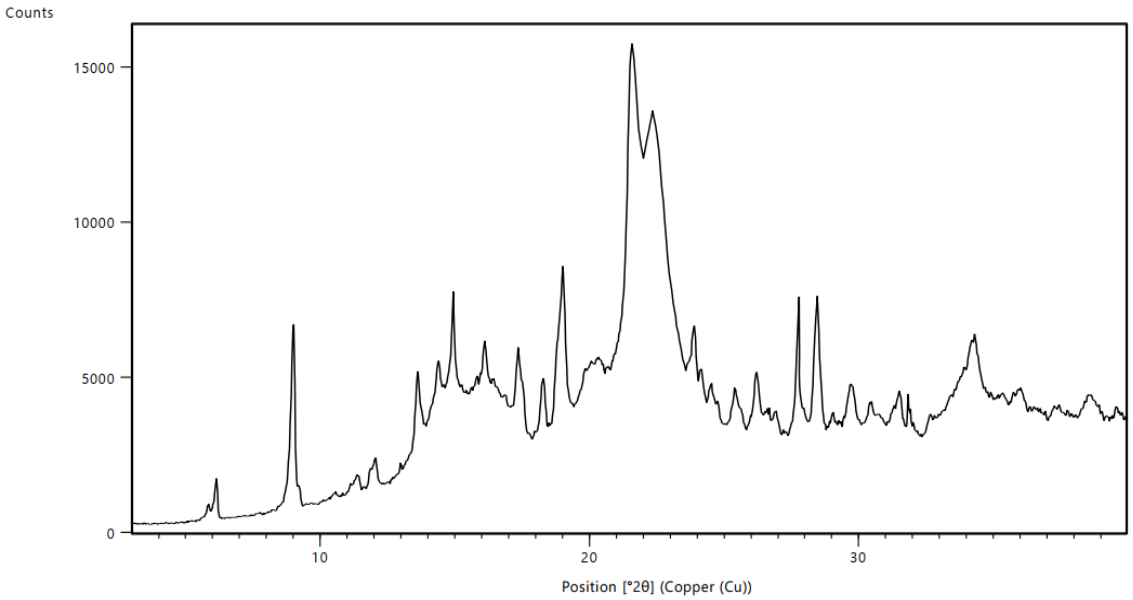

Figure No.14 XRD of Optimized Trail

Discussion: Rilpivirine displayed sharp peaks at different diffraction angles indicating its crystalline shape. The major characteristic peaks of Rilpivirine drug, Poloxamer 407 and SLS polymer were observed in physical mixture with lower intensity, where the X-ray diffractogram of Optimized formulation showed no obvious peaks of Rilpivirine. The X-ray diffraction pattern of Poloxamer 407 not showed any peaks which indicates that the structure is completely amorphous. As the Poloxamer 407 was amorphous, smooth, and free flowing powder and it had got all the characteristics of stabilizer, it was concluded that Poloxamer 407 can be used as stabilizer in the formulation of nanosuspensions.

% **Drug Content**

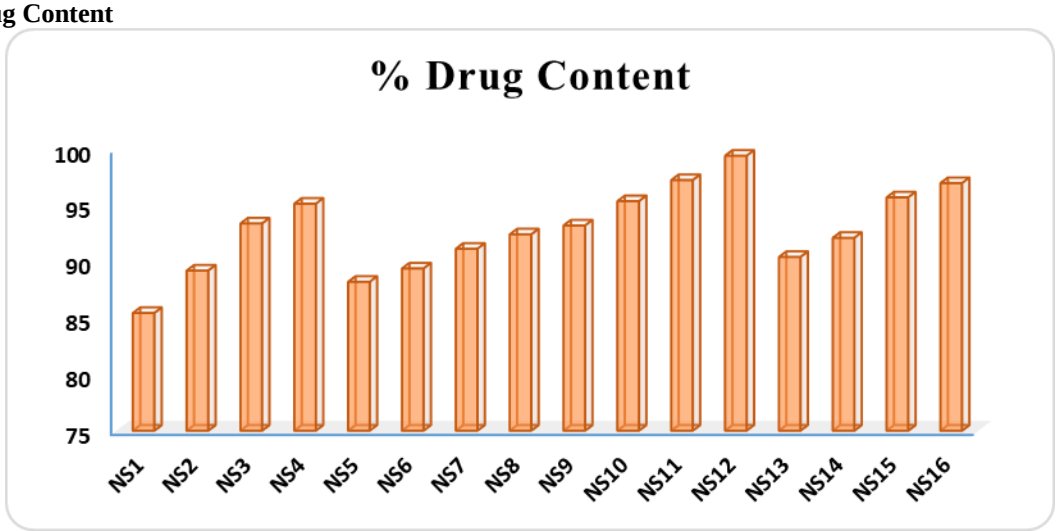

Figure No.15 % Drug content graphs for formulations

Discussion: The Nanosuspension Formulation from NS1-NS12 was found to be in between 85.47±1.14%-99.45±1.42% and the drug content of Nanosuspension NS12 which was having more drug content when compared to the remaining formulations.

Percentage Yield

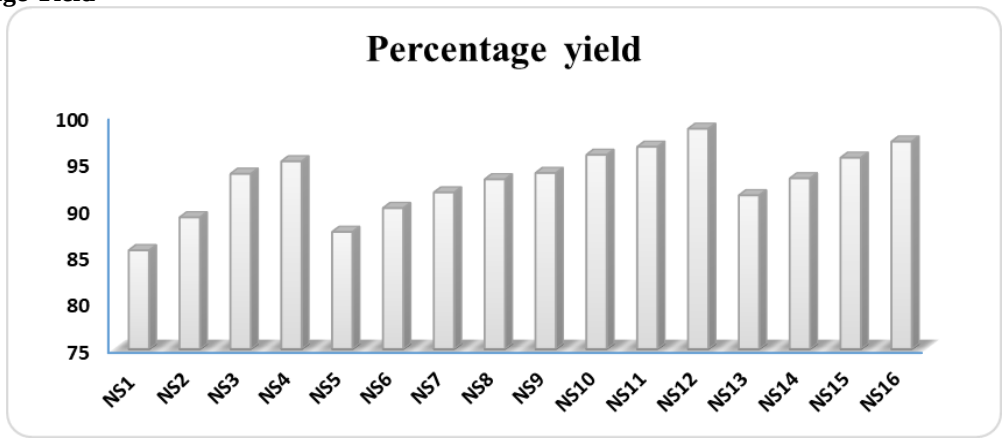

Figure No.16 Percentage yield graphs for formulations

Discussion: The Nanosuspension Formulation from NS1-NS12 was found to be in between 85.47±1.14%-98.58±1.21%% of percentage yield and the Nanosuspension NS12 which was having more yield when compared to the remaining formulations

Entrapment Efficiency: -

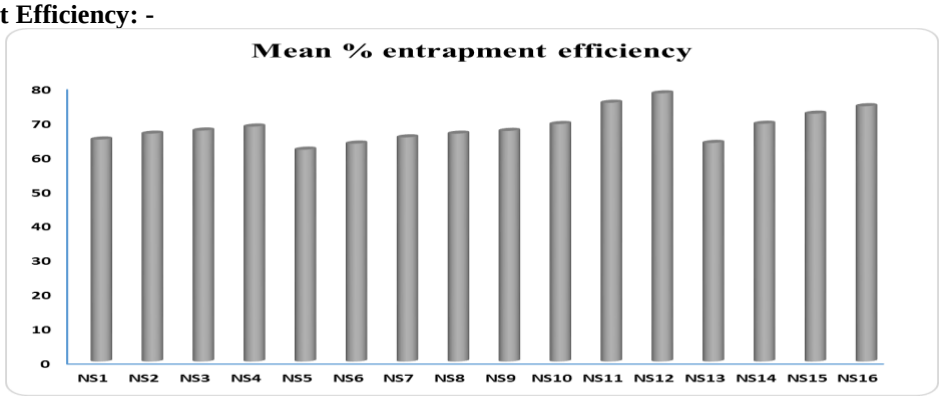

Figure No.17 % Entrapment efficiency graphs for formulations

Discussion: The Nanosuspension Formulation from NS1-NS12 was found to be in between 61.78±1.25%-78.21±1.37%. The concentration of Poloxamer 407 increase then the entrapment efficiency also increase**.** So, The Drug entrapment efficiency of optimized formulation NS12 was found be 78.21±1.37%. When compared to remaining formulation NS12 having more entrapment efficiency that means the loss of drug was less.

Viscosity: The viscosity results are mentioned in Table. The Viscosity of the formulation and NS12 were found to cps 0.54 cps,which was lower compared than other batches. A lower viscosity means that the fluid flows more easily, while a higher viscosity suggests that the fluid is thicker and flows less easily. In the context of nanosuspensions, viscosity plays a crucial role in their behavior and performance.

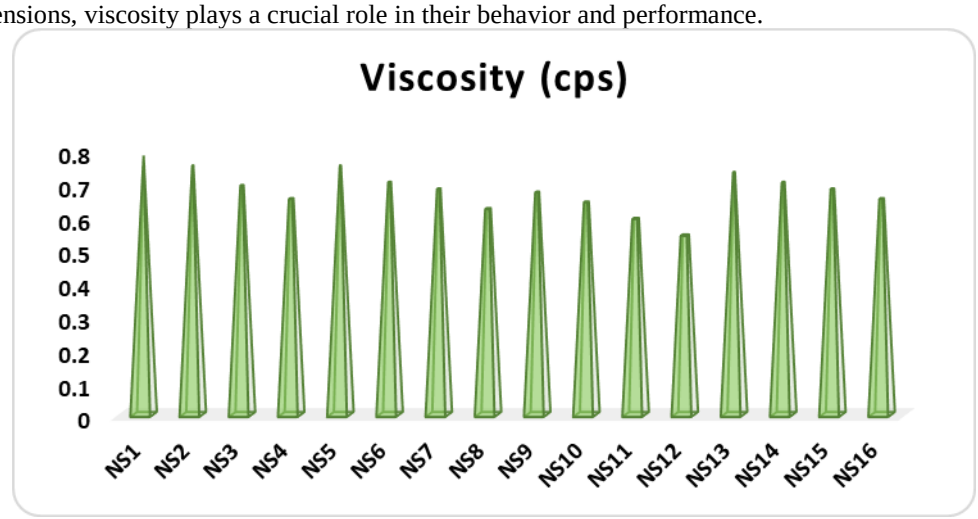

Figure No.18 Viscosity graphs for formulation

Discussion: The viscosity of formulation NS1-NS16 was found in between 0.79 cps-0.54 cps. In this, the optimized formulation shows the low viscosity with 0.54cps.

Sedimentation Volume:

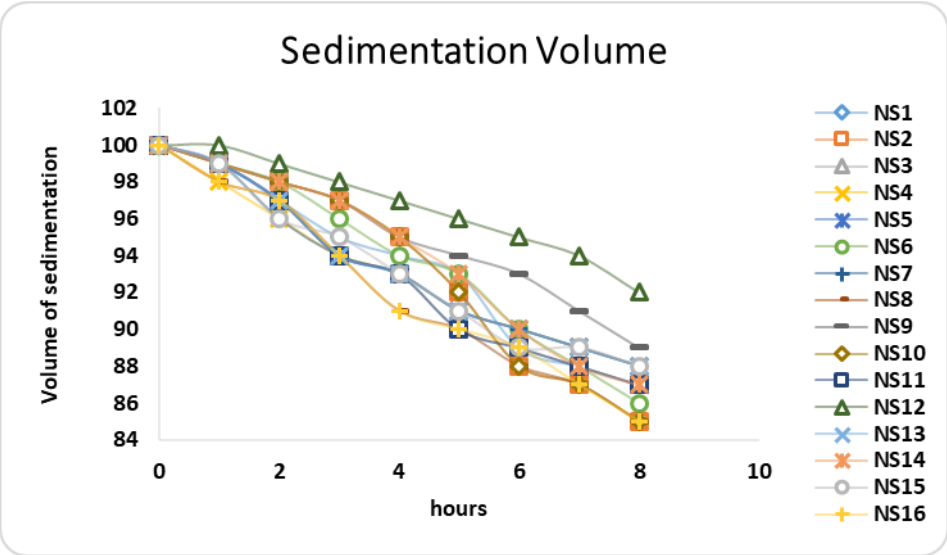

Figure No.19 Sedimentation volume of the Nanosuspensions (NS1-NS16)

Discussion: The Sedimentation volume of formulations NS1-NS16 was found in between 100-80%. The sedimentation volume for the Optimized formulation NS12 with the 450 mg of Poloxamer 407 was found to be 92% at the end of 8th hour shown the good flocculation of particles in the suspension.

Scanning Electron Microscopy:

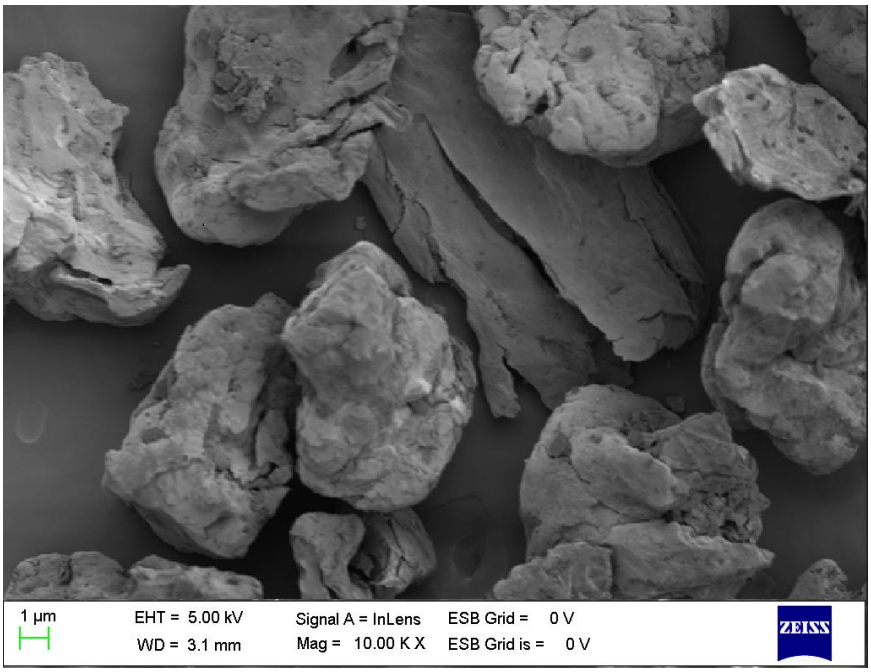

Figure No.20 Surface morphology of NS12 at 1 µm

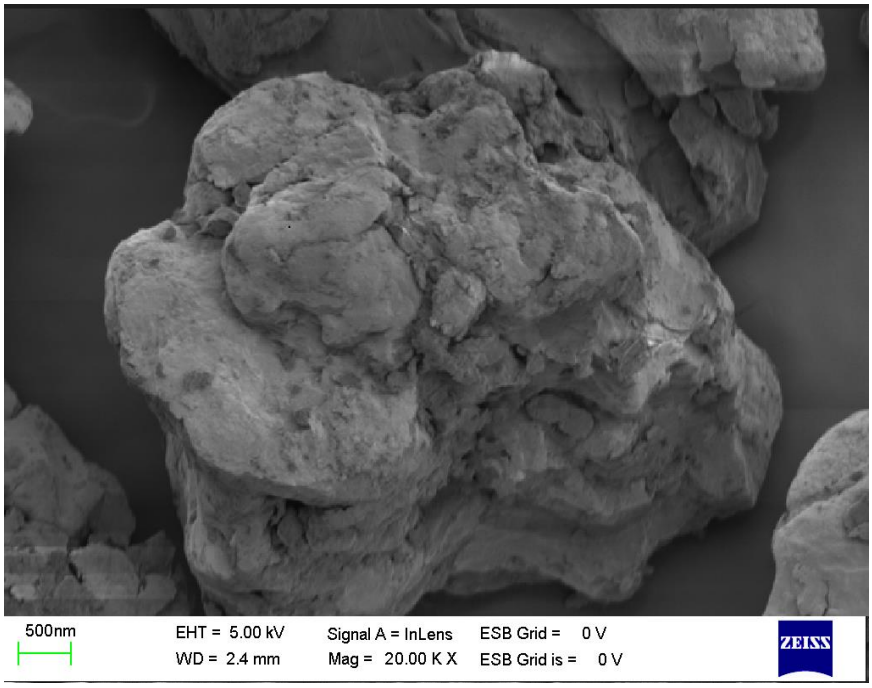

Figure No.21 Surface morphology of NS12 at 500 nm

Discussion: The surface structure of optimized formulation was observed by scanning electron microscopy at different magnifications. In this SEM, the nanosuspension particles are appeared within slightly spherical in shape and particle size was reduced up to 500 nm.

Particle Size Analysis:

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 500.4	Peak 1:	545.2	100.0	493.2
PdI: 0.251	Peak 2:	0.000	0.0	0.000
Intercept: 0.920	Peak 3:	0.000	0.0	0.000

Result quality : Good

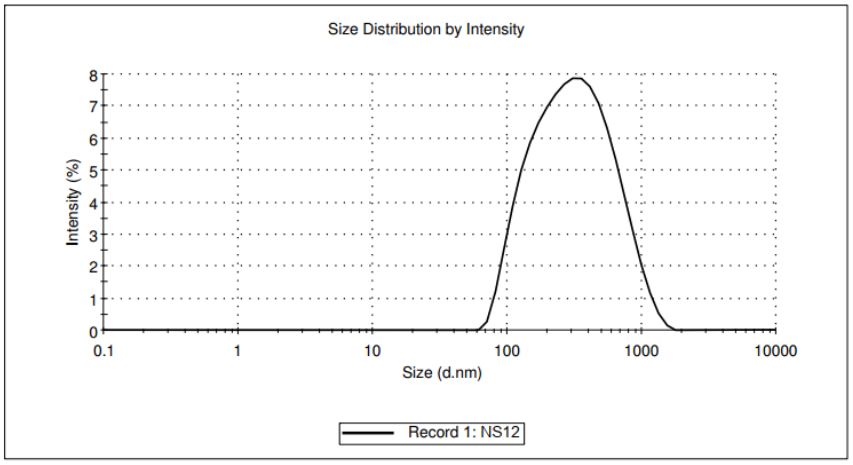

Figure No.22 Particle Size Analysis of Optimized Formulation

Discussion: The optimized Formulations (NS12) nanosuspension of drug Rilpivirine, Poloxamer 407 as Stabilizer and SLS and Polysorbate 80 as Surfactants which show the positive effect on particle size so average particle size of 500.4 nm which was determined by using the Malvern Instrument Ltd Particle Size Analyzers. Therefore, the particle size was decreased by this formulation.

Zeta Potential: The measurement itself is a particle electrophoresis, the particle velocity is determined via the Doppler shift of the laser light scattered by the moving particles. The field strength applied was 20 V/cm. The electrophoretic mobility was converted to the zeta potential in mV using the Helmholtz-Smoluchowski equation. At standard measuring conditions (room temperature of 25.1°C, water) this equation can be simplified to the

multiplication of the measured electrophoretic mobility (μm/cm per V/cm) by a factor of -4.49, yielding the ZP in mV.

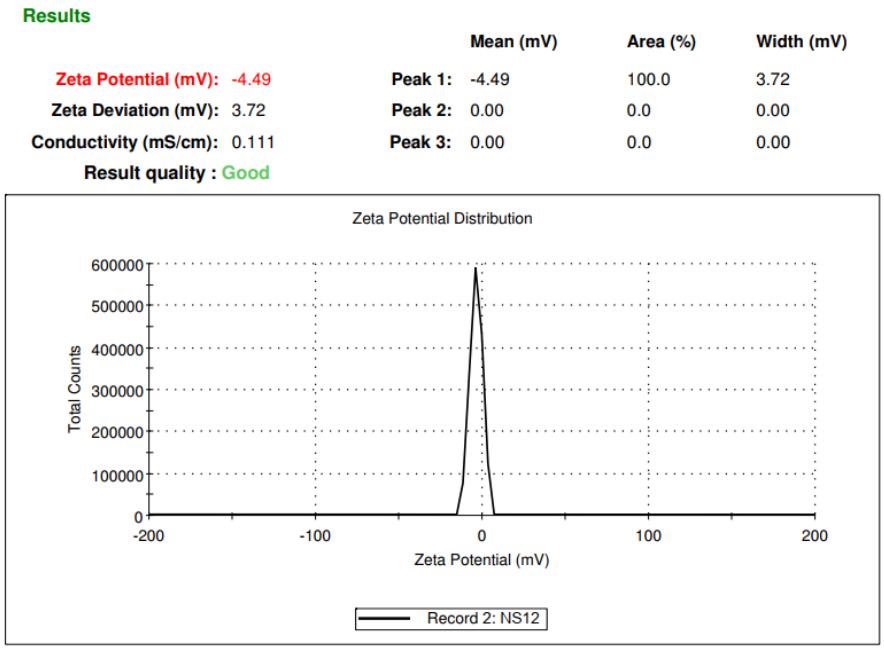

Figure No.23 Zeta potential value for the optimized formulation (NS12)

Discussion: The Optimized Formulations (NS12) nanosuspension of the medication Rilpivirine and the polymers Poloxamer 407 as Stabilizer, and SLS, Polysorbate 80 as surfactants, which demonstrate the Zeta potential value for the optimized Formulation (NS12) was Found to be -4.49 mv that indicates good stability of the formulation. So, the zeta potential was found to be within the acceptable limits.

In Vitro Drug Release:

Table No.5 In vitro drug release data of formulation NS1 to NS4

Time (minutes)	NS1	NS2	NS3	NS4
0	0	0	0	0
5	19.45±1.24%	24.26±1.20%	28.26±1.69%	38.47±1.54%
10	29.26±1.72%	38.47±1.69%	40.45±1.45%	45.43±1.45%
15	37.45±1.44%	46.57±1.37%	52.29±1.37%	56.56±1.69%
20	51.74±1.29%	61.56±1.48%	65.47±1.50%	74.67±1.20%
30	65.20±1.58%	73.97±1.26%	79.34±1.20%	88.54±1.75%
45	82.54±1.69%	85.63±1.75%	88.58±1.74%	98.45±1.35%
60	93.38±1.37%	95.26±1.65%	97.48±1.52%	

***Mean± S.D (n=3)**

Table No.6 In vitro drug release data of formulation NS5 to NS8

Time (minutes)	NS5	NS6	NS7	NS8
0	0	0	0	0
5	15.43±1.74%	22.26±1.20%	39.45±1.45%	45.52±1.20%
10	27.48±1.35%	35.47±1.67%	53.29±1.26%	58.56±1.46%
15	33.37±1.45%	41.57±1.45%	67.47±1.74%	70.67±1.37%
20	48.58±1.06%	60.56±1.29%	82.34±1.26%	86.54±1.84%
30	61.67±1.78%	75.97±1.36%	90.58±1.20%	97.45±1.42%
45	78.21±1.25%	82.63±1.74%	98.48±1.85%	
60	88.48±1.36%	90.47±1.52%		

***Mean± S.D (n=3)**

Table No.7 In vitro drug release data of formulation NS9 to NS12

Time (minutes)	NS9	NS10	NS11	NS12
0	0	0	0	0
5	18.42±1.21%	25.68±1.74%	38.38±1.47%	49.59±1.57%
10	30.61±1.45%	37.20±1.27%	55.45±1.17%	63.56±1.21%
15	35.45±1.27%	43.15±1.45%	68.21±1.20%	75.67±1.37%
20	51.38±1.69%	64.37±1.37%	84.69±1.69%	88.54±1.69%
30	63.72±1.71%	78.42±1.25%	92.74±1.74%	98.42±1.27%
45	86.52±1.20%	88.69±1.74%	99.36±1.36%	
60	95.20±1.69%	96.24±1.21%		

***Mean± S.D (n=3)**

Table No.8 In vitro drug release data of formulation NS13 to NS16

Time (minutes)	NS13	NS14	NS15	NS16
0	0	0	0	0
5	18.43±1.75%	25.26±1.54%	46.39±1.74%	52.23±1.21%
10	25.48±1.26%	37.47±1.21%	57.42±1.28%	64.31±1.57%
15	36.37±1.48%	43.57±1.74%	65.34±1.46%	73.45±1.67%
20	49.58±1.75%	64.56±1.59%	86.51±1.20%	88.20±1.57%
30	64.67±1.27%	76.97±1.24%	93.48±1.84%	96.46±1.29%
45	86.21±1.56%	83.63±1.75%	98.24±1.26%	
60	93.48±1.27%	95.47±1.37%		

***Mean± S.D (n=3)**

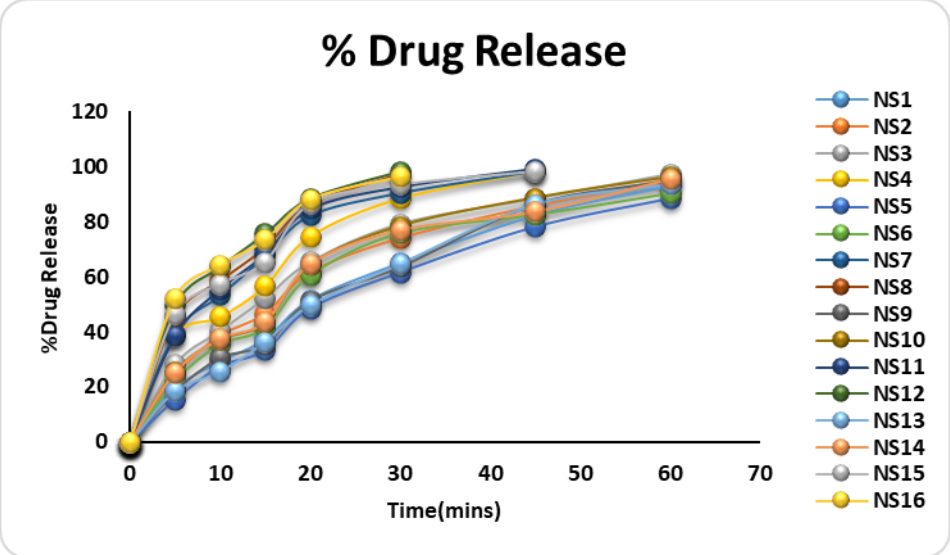

Figure No.24 Dissolution parameters for the formulations NS1-NS16

Discussion: From the above in vitro studies we can say that increase in the polymer concentration of polymers decrease in the dissolution time of all the formulations. Increase in the stabilizer concentration of Poloxamer 407 shows 98.42±1.27% of drug release, so the formulations prepared by using Poloxamer 407 releases more drug release at the end of 30 mins than the other Stabilizers. So NS12 was considered as optimized formulation as it shows drug release with in 30mins.

Drug Release Kinetic Studies: Optimized formulation NS12

Zero Order Release Kinetics:

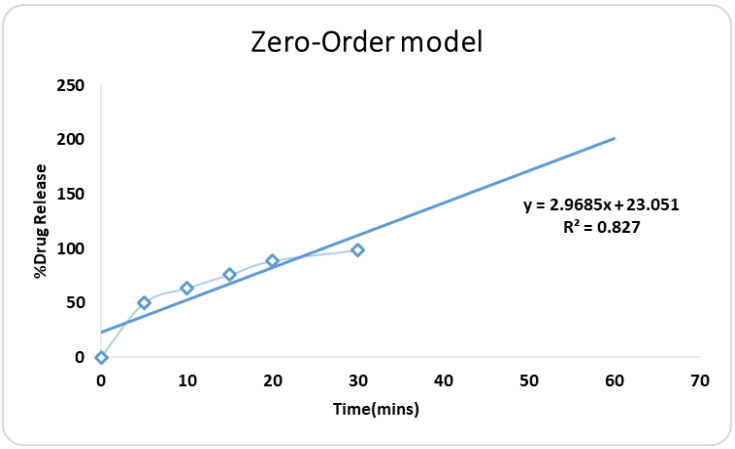

Figure No.25 Zero order release profile of formulation NS12

First Order Release Kinetics:

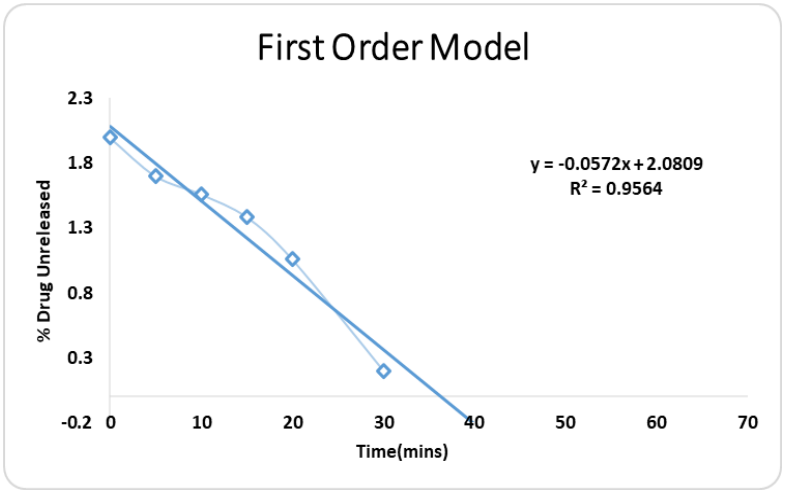

Figure No.26 First order release profile of formulation NS12

Higuchi model:

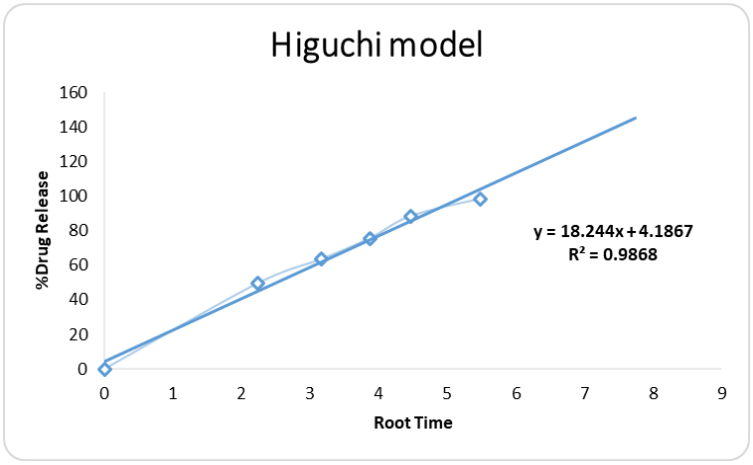

Figure No.27 Higuchi model of formulation NS12

Korsmeyer-Peppas model

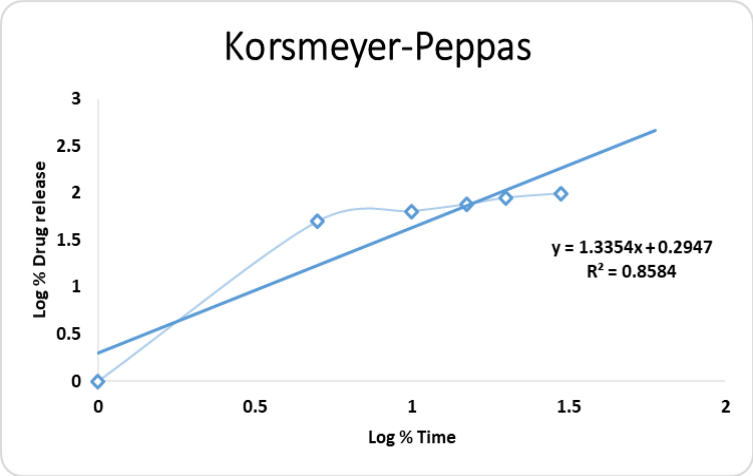

Figure No.28 Korsmeyer-Peppas model of formulation NS12

Discussion: The drug release from the Nanosuspension was explained by using mathematical model equations such as zero order, first order, Higuchi and Peppas model. Based on the regression values it was concluded that the optimized formulation NS12 follows first order kinetics with super case-II transport mechanism.

Stability study was conducted on optimized trail (NS12). The trails were packed in an airtight container and stored in stability chamber at 40 ± 2°C and 75 ± 5% RH for a period of 1st month and 3rd month. The samples were then withdrawn at interval of 30, 90 days and were evaluated for Percentage yield, drug content uniformity, Entrapment efficiency and In-vitro dissolution studies.

%Drug Content:

Table No.9 Drug content of optimized trail NS12

Drug Content Uniformity			
Trail	**Initial**	**1M 40^{0}C/75%RH**	**3M 40^{0}C/75%RH**
NS12	99.45±1.42%	98.92±1.75%	98.49±1.59%

Percentage Yield:

Table No.10 Percentage yield of NS12

Percentage yield			
Trail	**Initial**	**1M 40^{0}C/75%RH**	**3M 40^{0}C/75%RH**
NS12	98.58±1.21%	98.37±1.59%	98.13±1.73%

Entrapment Efficiency

Table No.11 Entrapment efficiency for optimized trail NS12

Entrapment efficiency			
Trail	**Initial**	**1M 40^{0}C/75%RH**	**3M 40^{0}C/75%RH**
NS12	78.21±1.37%	78.65±1.18%	79.27±1.42%

In Vitro Drug dissolution studies

Table No.12 Stability data for In vitro drug release for optimized trail NS12

In Vitro Drug Release				
NS12	**Minutes**	**Initial**	**1Month 40^{0}C/75%RH**	**3Months 40^{0}C/75%RH**
	0	0	0	0
	30	98.42±1.27%	98.27±1.45%	98.13±1.39%

Discussion: The optimized trails (NS12) was the subject of the stability experiments. The trails were stored in a stability laboratory at 40 ± 2°C and 75 ± 5% RH for the first and third months, after being packaged in an

impermeable container. The samples were subsequently withdrawn at intervals of 30, 90, and were assessed for percentage yield, drug content uniformity, entrapment efficiency and In vitro dissolution studies were done and the stability studies concluded that the optimized nanosuspenion was stabled up to 3Months.

SUMMARY AND CONCLUSION

In present investigation Nanosuspensions of Rilpivirine was prepared by Nano Precipitation method. The Nano suspensions are novel promising target and controlled released dosage form which is gaining importance because of ease of manufacturing and diversified applications. The present trend of pharmaceutical research lies in the usage of biodegradable polymer because of its availability and low toxicity. Nanosuspension containing drug was prepared by Nano precipitation method by using combinations of β-cyclodextrin, Soluplus, Poloxamer 407, Polyvinyl alcohol, Sodium lauryl sulfate, Polysorbate 80 and quantity sufficient water and ethanol. Estimation of Rilpivirine was carried out spectrophotometrically at 280.0nm. The Nanosuspension were evaluated for parameters such as drug excipient interactions i.e FTIR, DSC, XRD and Drug Content, Percentage yield, Entrapment efficiency, Viscosity, Sedimentation volume, Scanning electron microscopy, Particle's size and shape, Zeta potential, In-vitro drug release studies, Drug release kinetics studies finally stability studies. The stability data was also subjected to statistical analysis. The melting point of Rilpivirine was Found to be in range of 242.20 ˚C which was determined by capillary method. Saturation solubility was carried out at 25^0C using 0.1N HCl, 6.8 phosphate buffer, 7.4 pH buffer and ethanol, methanol. From the drug excipient compatibility studies we observe that there are no interactions between the pure drug (Rilpivirine) and optimized Formulation (Rilpivirine + excipients) which indicates there are no physical changes. The Evaluation parameters like Percentage yield, Drug content, entrapment Efficiency, viscosity and sedimentation volume for the Nanosuspension formulations are being conducted and in all parameters, the formulation NS12 yields the best results. The morphological studies was conducted by using scanning electron microscopy and it was observed that in slightly spherical in shape and particle size was reduced upto to 1 µm. Zeta potential value for the optimized Formulation (NS12) was found to be be -4.49 mv that indicates good stability of the formulation indicates that it was in the acceptable limits. The Average particle size of nanosuspension of optimized Formulations (NS12) was found to be 500.4 nm with the PDI of 0.251 which was in acceptable limit. From the in vitro studies we can say that formulation NS12 shows best drug release of 98.42±1.27% within 30 minutes. Then the optimized drug release of the Nanosuspension was explained by the using mathematical model equations such as zero order, first order, Higuchi model and Korsmeyer-Peppas model methods. Based on the regression values it was concluded that the optimized Formulation NS12 follows first order kinetics with super case-II transport mechanism. The stability investigations were conducted on the optimized trails (NS12). The trials were sealed in an impermeable container and stored in a stability laboratory at 40 ± 2°C and 75 ± 5% RH for the first and third months. The samples were subsequently withdrawn at intervals of 30days and 90days was evaluated for percentage yield, drug content uniformity, entrapment efficiency, and in vitro dissolution. The stability experiments concluded that the optimized nanosuspension was stable for up to 3 months.

ACKNOWLEDGEMENT

I would like to thank my Guide and my VJ's College of Pharmacy for the support and encouragement in every phase and thank to Akrivis Pharma Pvt. Ltd staff for being a good mentors in completing my research work in your company, and finally I would like to thanks my family and friends.

REFERENCES

1. Trojnar MK, Wojtal K, Trojnar MP, Czuczwar SJ. Stiripentol. A novel antiepileptic drug. Pharm Reports. 2005;57:154–160. Trojnar MK, Wojtal K, Trojnar MP, Czuczwar SJ. Stiripentol. A novel antiepileptic drug. Pharm Reports. 2005;57:154–160.
2. Poisson M, Huguet F, Savattier A, Bakri-Logeais F, Narcisse G. A new type of anticonvulsant, stiripentol. Pharmacological profile and neurochemical study. Arnzeimittel-forschung. 1984;34:199–204.
3. Quilichini PP, Chiron C, Ben-Ari Y, Gozlan H. Stiripentol, a putative antiepileptic drug, enhances the duration of opening of GABAA-receptor channels. Epilepsia. 2006;47:704–716.
4. Nagaraju P, Krishnachaithanya K, Srinivas VD, Padma SV. Nanosuspensions: A promising drug delivery systems. Int J Pharm Sci Nano. 2010;2:679–84.
5. Cornelia MK, Muller RH, Drug nanocrystals of poorly soluble drugs produced by high-pressure homogenisation, Eur. J.Pharm.Biopharm, 62,2006,: 3–16.
6. Patravale VB, Abhijit AD, and Kulkarni RM, Nanosuspensions: a promising drug delivery strategy. J. Pharm. Pharmcol, 56, 2004, 827-840.
7. Muller RH, Bohm BHL, Grau J, Nanosuspensions : a formulation approach for poorly soluble and poorly bioavailable drugs. In D.Wise (Ed.) Handbook of pharmaceutical controlled release technology, 2000, 345- 357.

8. Liversidge GG, Cundy CK, Bishop JF, Czekai DA, Surfacemodified drugnanoparticles, US Patent, 5, 1992,145,684.
9. Muller RH, Gohla S, Dingler A, Schneppe T, Large-scale production of solid-lipid nanoparticles and nanosuspension, Handbook of pharmaceutical controlled release technology,2000,359-375.
10. Muller RH, Peters K, Nanosuspensions for the formulation of poorly soluble drugs I: Preparation by a size-reduction technique, Int. J. Pharm, 160, 1998, 229–237.
11. Jahnke S, The theory of high-pressure homogenization. In: Muller RH, Benita S, Bohm BHL, Emulsions and nano suspensions for the formulation of poorly soluble drugs, Medpharm Scientific Publishers, Stuttgart, 1998, 177–200.
12. Bodmeier R, McGinity JM, Solvent selection in the preparation of poly (DL-lactide) microspheres prepared by solvent evaporation method, Int. J. Pharm, 43, 1998, 179–186.
13. Sah H, Microencapsulation technique using ethyl acetate as a dispersed solvent: effects on its extraction rate on the characteristics of PLGA microspheres, J. Control. Release, 47, 1997, 233–245.
14. Trotta M, Gallarate M, Carlotti ME, Morel S, Preparation of griseofulvin nanoparticles from water-dilutable microemulsions, Int. J. Pharm, 254, 2003, 235–242.
15. Ethiraj T, Sujitha R, Ganesan V. Formulation and In-vitro Evaluation of Nanosuspension of Glimepiride. Int. J Pharmacy, 3, 2013, 875-2.
16. Maddukuri S, Srawanthi P, Metta S, Radha GV, Krishna KS M. Screeening of Stabilizers in Azithromycin nanosuspensions. Int. J. Pharm. Sci. Rev. Res., 55, 2019, 77-84.
17. Anjane M, Agrawal S, Khan A. Formulation and evaluation of nanosuspension of Valsartan. Int. J Curr Pharm Res, 10, 2018, 68-74.
18. Patil AM, Patil IN, Mane RU, Randive DS, Bhutkar MA, Bhinge SD. Formulation optimization and evaluation of cefdinir nanosuspension using factorial design. Marmara Pharm J., 22, 2018, 587-98.
19. Remziye Aysun KepekçiBurcu Yener İlçeSibel Demir Kanmazalp, in Studies in Natural Products Chemistry, 2021.
20. Nidhi Raval, ... Rakesh K. Tekade, in Basic Fundamentals of Drug Delivery, 2019.
21. Dr. Yasmin Begum etal, preparation and evaluation of nanosuspension of nifedipine, 9(09), 57091-57098,2017.
22. Hadjiioannou TP, Christian GD, Koupparis MA; Quantitative calculations in pharmaceutical practice and research. VCH Publishers Inc., New York, 1993.
23. Gibaldi M, Perrier D; Statistical methods for population pharmacokinetic modeling. Drugs and the Pharmaceutical Sciences, 2nd ed., Marcel Dekker, Inc, New York and Basel, 1982.
24. Mulye NV, Turco SJ; A simple model based on first order kinetics to explain release of highly water soluble drugs from porous dicalcium phosphate dihydrate matrices. Drug Dev. Ind. Pharm., 1995; 21: 943–53.
25. Zhenzhong Pan, Bo Cui, Zhanghua Zeng, Lambda- Cyhalothrin Nanosuspension Prepared by the Melt Emulsification-High Pressure Homogenization Method, Journal of Nanomaterials, 2015; 16 (1): 1687-4110.
26. Anju M, Abhijit A, Darshana.DH, Celecoxib Nanosuspension: Single Step Fabrication Using modified nanoprecipitation method and invivo evaluation, Drug Deliv and Transl.Res, 2014; 4: 365-376
27. Vikram M, Jay Vadank P, Dhaval JP: Formulation optimization and characterization of simvastatin nanosuspension prepared by nanoprecipitation technique. Der Pharmacia Lettre, 2011; 3(2): 129-140
28. Rupali S, Shashikant ND, Santosh LS: Formulation and evaluation of nanosuspension formulation for drug delivery of simvastatin, Int Jou of Pharm Sci and Nanotechnology, 2014; 7(4): 265-2665.
29. Adeel S, Dongmeri C, Lishun J, Yuanhu P, et.al,Preparation characterization and pharmacokinectics of cyadox nanosuspensions, Scientific Reports of Cyadox Nanosuspension. Scientific Reports, 2017; 7:2289.
30. Krutik S, Mitali HP, Krishna P, Cefdinir nanosuspension for imporved oral bioavailability by media milling technique formulation, characterization and invitro – invivo evaluation, Drug Dev Ind Pharm, 2016, 42(5): 758-768.