Asian Pub. Corp.

www.asianpubs.org

ARTICLE

Formulation Development and Characterization of Rilpivirine Nanosuspension for Improved Solubility by Nanomilling

Satya Sankar Sahoo[1,✉] and Chandu Babu Rao[2]

Asian Journal of Organic & Medicinal Chemistry

Volume: 4 Year: 2019
Issue: 2 Month: April–June
pp: 130–137
DOI: https://doi.org/10.14233/ajomc.2019.AJOMC-P210

Received: 3 June 2019
Accepted: 22 June 2019
Published: 29 June 2019

ABSTRACT

The objective of this study was to formulate and optimize a stable rilpivirine nanosuspension. In the present study, yttrium stabilized zirconium oxide beads being used as the milling media in nanomilling process. The lyophilized nanocrystals were being characterized by particle size distribution (PSD), polydispersity index (PDI), X-ray diffraction (XRD) and FTIR (Fourier transform infrared spectroscopy). Optimized nanosuspension has mean particle diameter of 266 nm, PDI of 0.158, zeta potential of 22.1 mV and spherical in shape with surface oriented stabilizer molecules. Flow properties like sedimentation volume, pourability with the F value of 0.94 and also the redispersability even after 4 weeks of storage was found to be satisfactory for the optimized nanosuspension. Many folds increase in solubility and rate of drug release observed, The lyophilized nanocrystals retains its crystallinity after nanomilling, stable chemically with high drug content, therefore, the developed nanosuspension would be an alternative better formulation than its conventional formulation to address its bioavailability issue. However, this should be further confirmed by appropriate techniques *in vivo* studies.

KEYWORDS

Nanosuspension, Rilpivirine, Nanomilling, Optimization.

INTRODUCTION

About 70 % of New chemical entity (NCE) has the common problem of poor aqueous solubility which in-turn leads to poor bioavailability [1,2]. Therefore, to improve the solubility, dissolution rate and bioavailability of poorly soluble BCS class II drugs is the need of the hour for formulation scientists. The conventional methods like micronization, chemical modification to convert to different salts form, solid dispersion to convert to amorphous form and a few new emerging approaches like cyclodextrin complexation, mucoadhesive microspheres, micro emulsions, self-emulsifying systems, nanoparticles and nanosuspensions are available to improve the solubility and bioavailability of these poorly soluble BCS Class II drugs [3,4]. Nanomilling has become an effective, convenient and popular approach to improve the solubility whereby the particle size of the drug reduced to nano-metric range and maintained as crystals (known as nanocrystals) which are then suspended in a liquid (usually water) to form nanosuspensions [3,5]. The

Author affiliations:

[1]University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nargarjuna Nagar-522510, India
[2]Department of Pharmaceutics, Priyadarshini Institute of Pharmaceutical Education Research, 5th Mile, Pulladigunta, Guntur-522017, India

✉To whom correspondence to be addressed:

E-mail: satyasahoo2003@gmail.com

Available online at: http://ajomc.asianpubs.org

smaller the particles in size, greater the surface to volume ratio which in-turn increases the dissolution rate of active ingredients more effectively by raising the saturation solubility as governed by Noyes Whitney theory [6,7]. As the surface area increases, nanocrystals has a tendency to aggregate and increase in size, therefore selection of stabilizer and optimization of its concentration is very important [8]. Though several nanotechnological approaches available, but nanosuspension has certain advantages like high drug loading as its core composed of pure drug, low incidents of side effects by surfactant/stabilizer and drug retain its crystallinity to ensure no polymorphic conversion throughout its shelf life. In marketed product of Taxol®, anticancer drug paclitaxel is dissolved in the blend of ethanol and Cremophor ®EL (polyoxyethylated castor oil) in 1:1, causes serious hypersensivity reaction [9].

Nanosuspensions can be obtained mainly by two approaches, in Top-down approach, reduction of large crystals to nanosize and in bottom-up approach by the precipitation of dissolved molecules into solid particles [10]. Nanomilling technique is a typical top down approach where the mechanical grinding bymedia milling of yttrium stabilized zirconium beads in water is being used to obtain drug/stabilizer suspensions. The selected nanomilling (wet milling) avoids use of organic solvents and can be easily scaled it up at commercial scale. Rilpivirine, a widely prescribed novel antiretroviral drug comes under BCS class II and exhibit low and variable oral bioavailability due to its poor aqueous solubility [11]. Commercially it is available as film coated tablets of 25 mg, brand name Edurand, manufactured by Janssen Products, USA. It is practically insoluble in water and aqueous fluids. Therefore, its oral absorption is dissolution rate limited and improvement in the solubility and dissolution rate for increasing its oral bioavailability is need of the hour. The maximum plasma concentration of rilpivirine (C_{max}) decreased by 46 % and the area under the rilpivirine plasma concentration curve (AUC) decreased by 43 % under fasting condition [11]. Similarly, its C_{max} and AUC are reduced by 50 % when given with a protein-rich nutritional drink [12]. About 20 nanocrystal products available presently in the market and most of them are made by the wet milling technique [13]. However, the problems with the wet milling still exist. There is no single stabilizer available which is suitable for all the drug compounds. Different drugs require their optimal stabilizers. The understanding on the interaction of stabilizer and drug is inadequate. Though the wet milling technique has been considered as a simple milling process for size reduction, but multidisciplinary knowledge, like knowledge of grinding mechanism, nanocrystal's breakage kinetics, crystal stability, formulation and processing factors that affects the fate of drug *in vivo* are necessary to fully understand the technique [14]. Therefore, using differently behaving stabilizer and process parameters of wet milling, it is a demanding task to get the desired stable particle size of nanosuspension. Therefore, the objectives of the present study were to use nanogrinding technique in the preparation of rilpivirine nanosuspension for oral administration thereby avoiding harmful additives and permissive to enhance solubility, dissolution and oral absorption of rilpivirine. The prepared nanosuspension was studied for different physicochemical parameters and dissolution study compared to pure drug and marketed product. Rilpivirine′s nature of high permeability but practically insoluble in water, makes it an exceptional candidate for nanomilling.

EXPERIMENTAL

Rilpivirine hydrochloride was obtained from Mylan Laboratories Ltd., India. Hydroxypropyl methyl cellulose (Hypromellose 2910, Methocel® E3 LV, Methocel® E5 LV) was gift sample from Dow Chemicals, USA. All other materials used were of pharmaceutical grade and produced from commercial sources.

Preparation of nanosuspension: For nanogrinding rilpivirine, solutions of surfactant (Poloxamer 188, Poloxamer 407, Tween 20) and polymer stabilizers (Methocel E3, Methocel E5, Methocel E15, PVP K30, PEG 10000) in purified water were first prepared. Rilpivirine hydrochloride (d90: 15.2 µm) was then dispersed in the stabilizer solution. The composition of different formulation with polymer stabilizer and surfactant mention in Table-1.

TABLE-1
COMPOSITION OF NANOSUSPENSION OF RILPIVIRINE

Formulation (code)	Polymer 1		Polymer 2		API (% w/w)	Total solid content (% w/w)
	Name & Grade	% w/w	Name & Grade	% w/w		
F1A	HPMC E3	10.0	Poloxamer 188	2.0	8.0	20.00
F1B	HPMC E3	10.0	Poloxamer 188	2.0	8.0	20.00
F2	HPMC E3	12.5	Poloxamer 188	2.5	10.0	25.00
F3	HPMC E3	15.0	Poloxamer 188	3.0	12.0	30.00
F4	HPMC E3	12.0	–	–	8.0	20.00
F5	HPMC E5	12.0	–	–	8.0	20.00
F6	HPMC E15	5.0	–	–	15.0	20.00
F7	PVPK30	12.0	–	–	8.0	20.00
F8	PEG 10000	12.0	–	–	8.0	20.00
F9	Poloxamer 188	12.5	–	–	10.0	22.50
F10	Poloxamer 407	12.5	–	–	10.0	22.50
F11	HPMC E3	3.75	–	–	5.0	8.75
F12	HPMC E3	4.0	–	–	16.0	20.00
F13	HPMC E15	2.6	–	–	17.4	20.00
F14	HPMC E3	10.0	–	–	20.0	30.00
F15	–	–	–	–	20.0	20.00
F16	HPMC E3	7.8	Tween 20	0.15	12.5	20.45
F17	HPMC E3	7.8	–	–	12.5	20.30

The resulting dispersion was comminuted using colloidal mill (Make: Pharmatech) for 30 min with Zero clearance. The colloidal mill passed dispersion was further milled in a high-energy Nanomill (LabStar, Netzsch, Germany) filled (to 70 %, v/v) with yttrium-stabilized zirconium oxide beads (0.4 mm in diameter). Nanomilling was performed in circulation mode using 325 g of drug suspension. The nanomill was refrigerated to control the product temperature below 37 ºC. The details of nanomilling parameters for different trails were mentioned in Table-2.

TABLE-2
MILLING PARAMETERS OF NANOSUSPENSION OF RILPIVIRINE

Formulation code	Pump speed (rpm)	Milling speed (rpm)	Bead volume (mL)	Pressure (bar)
F1A	25	1500	130	0.28
F1B	40	3000	130	0.28
F2	40	3000	130	0.30
F3	40	3000	130	0.32
F4	40	3000	130	0.28
F5	40	3000	130	0.28
F6	40	3000	130	0.34
F7	40	3000	130	0.17
F8	40	3000	130	0.17
F9	40	3000	130	0.17
F10	40	3000	130	0.21
F11	40	3000	130	0.21
F12	40	3000	130	0.18
F13	40	3000	130	0.18
F14	40	3000	130	0.25
F15	40	3000	130	0.17
F16	40	3000	130	0.20
F17	40	3000	57	0.17

Lyophilization: The optimized nanosuspension was lyophilized. The vials of nanosuspension were freeze-dried (FTS Lyostar II freeze drying system, SP Industries Inc., Warminster, USA). The primary drying was operated in -30 ºC for 20 h and secondary drying was completed stepwise from -25 ºC to 45 ºC.

Solubility studies: The study was done before and after nanomilling to study the effect of nano-sizing on the solubility and dissolution rate of the drug. Rilpivirine (25 mg) and rilpivirine nanosuspension containing 25 mg of rilpivirine were weighed and transferred separately into conical flasks containing 100 mL of different dissolution media: water, pH 1.2 (0.1 N HCl), pH 2.0 (0.01 N HCl), pH 4.5, pH 6.8, 0.01 N HCl with 0.5 % Tween 20. These flasks were hermitically sealed and incubated at 37 ºC in an incubator shared rotated at 50 rpm for 48 h. Then, the samples were filtered and subsequently diluted with same media and absorbance was noted at 281 nm.

Characterization of nanosuspension: The characterization of nanosuspension are in similar ways as those used for conventional suspensions like evaluation of physical, chemical and flow properties. Physical evaluation includes appearance of phases, particle size analysis, zeta potential, solubility studies. Assay, dissolution and related substance were checked as part of chemical evaluation. For flow properties, determination of sedimentation volume, pourability and redispersibility were carried out.

Particle size analysis: The size distribution and average particle diameter of the prepared nanosuspensions were measured by laser photon correlation spectroscopy [15,16] using Zetasizer Ver. 7.02 (Malvern Instruments, Worcestershire, UK). Nanosuspensions were appropriately diluted with deionized water as dispersant. Further, sonicated for 2 min to reduce any interparticle aggregation. Then, the samples were analyzed by placing in disposable sizing cuvette. The 50 % and 90 % volume percentiles (d_{50} and d_{90}) were being used to exhibit the particle size of nanosuspension. Samples were analyzed in duplicate per batch, and the measurements were taken in triplicate for each sample. The average (± standard deviation) of the six measurements were given as result. Similarly, particles size also determined after lyophilization of optimized nanosuspension.

Zeta-potential measurement of nanosuspensions: Zetasizer Ver. 7.02 (Malvern Instruments, Worcestershire, UK) was being used to measure zeta-potential of the prepared nanosuspensions. The samples were appropriately diluted with deionized water and analyzed by keeping in disposable zeta cells. The Smoluchowski equation [17] of the electrophoretic mobility was being used to measure the mean zeta potential in mV.

FTIR: The FTIR spectra was recorded for micronized drug of rilpivirine, polymer (HPMC E5) and lyophilized nanocrystal of optimized nanosuspension formulation using KBr pellet technique. The spectrums were scanned over 3600-400 cm^{-1} at ambient temperature with a resolution of 4 cm^{-1}.

Sedimentation volume: Each suspension (50 mL) was being kept in stoppered measuring cylinder and stored undisturbed at room temperature. Further, the separation of clear liquid was noted at an interval of 2 and 4 h. The following equation was being used to calculate the sedimentation volume (F %),

$$F\,(\%) = \frac{V_u}{V_o} \times 100 \qquad (1)$$

where, V_u is the end volume of the sediment, V_o is the initial volume of the suspension.

Pourability: This test assures that the final nanosuspension is pourable and will not encounter any problem during filling and handling by end user.

Redispersability: In calibrated tubes fixed volume of each suspension (50 mL) was stored at room temperature for different intervals (2 and 4 h). One tube was removed at regular interval of 2 h and shaken actively to redistribute the sediment. Further, the presence of deposit if any was recorded. The time taken to redisperse the sedimented suspension was recorded.

Assay: Rilpivirine nanosuspension (10 mL) was dissolved in 200 mL methanol and sonicated for 30 min. The volume was adjusted to 500 mL using 0.01 N HCl and continued the sonication for 5 min. Further, 3 mL of this solution was diluted to 100 mL with 0.01 N HCl. Filtered through a 0.45 µm membrane filter and analyzed by measuring the absorbance at 281 nm against blank using UV spectrophotometer. The readings were taken in triplicate (Shimandzu UV-1700).

Dissolution study: *in vitro* Dissolution study was carried out using USP dissolution test Apparatus-2 (Paddle assembly, Make: Electrolab). The dissolution was performed using 900

mL of 0.01 N HCl with 0.5 % Tween 20 (official FDA listed dissolution media) and 0.01 N HCl, respectively maintained at 37 ± 0.5 ºC and 75 rpm for micronized rilpivirine, lyophilized rilpivirine nanosuspension formulation and marketed product (Edurant® marketed by Janssen). Samples (10 mL) were withdrawn at regular intervals of 10 min for 60 min and replaced with fresh dissolution medium. This solution (5 mL) was diluted to 10 mL with the medium and filtered through 0.22 µm Nylon filters (Millipore) and assayed at the wavelength of 281 nm. Dissolution for each formulation was performed in triplicates.

Related substances: To check the impact of nanogrinding process on the chemical stability of rilpivirine, related substances analysis was performed using HPLC method. Following chromatographic conditions were used to analyze the samples. Separation was done using Ace 5 C18-HL, 250 mm × 4.6 mm column. Detector wavelength was set at 305 nm with the flow rate of 0.8 mL/min. Degassed and filtered pH 4.77, 0.01 M potassium dihydrogen phosphate buffer was used as mobile phase A and mixture of methanol and pH 4.77, 0.01 M potassium dihydrogen phosphate buffer in the ratio of 80:20 v/v was used as mobile phase B. Gradient program was set as 90/0, 47/15, 35/25, 25/40, 20/50, 10/65, 5/75, 90/80 and 90/90 (mobile phase A/min). Methanol and degassed 4 % decaethylene glycol mono dodecyl ether solution was used as diluent for extraction and make up, respectively. Test solutions were prepared at the nominal concentration of 0.2 mg/mL of rilpivirine.

Microscopy test: The samples (before and after nanomilling) were visualized by using optical microscope (LEICA, DFC 395) at 40X zoom. Air-dried samples of nanosuspensions were mounted on the aluminum stubs with the help of carbon double-sided tape (Nisshin EM Co. Ltd., Tokyo) and sputter coated with platinum by using Auto fine coater (JEOL, JFC- 1600) for 90 s under vacuum (3Pa) and observed under the scanning electron microscope (JEOL, JSM-6380) at a magnification of 500X.

Solid state analysis: The pure drug, physical mixture and lyophilized optimized nanocrystals were analyzed by using Bruker D8 Advance X-ray diffractometer (Bruker, Germany). The pattern of spectra was collected in the range of 3º to 45º 2θ. Cu with K = 1.5405Å was used as anode X-ray source, the voltage was kept as 40 kV and tube current as 40 mA. In continuous mode, scanning was performed with time/step of 0.4 s and step size of 0.01.

Stability studies: The optimized rilpivirine nanosuspension was filled in HDPE containers sealed and loaded into stability chamber at 40 ºC ± 2 ºC/75 ± 5 RH (Newtronics stability chamber). The samples were withdrawn after 3 months and analyzed for particle size, assay, dissolution studies, related substances.

RESULTS AND DISCUSSION

Most of the recent new chemical entity coming out through extensive research and development or marketed drug substances are practically water insoluble therefore has negative impacts for their bioavailability. So, it limits or even prevents their therapeutic use. Rilpivirine HCl, a diamino pyrimidine derivative novel NNRTI developed and marketed for the treatment of ARV naive HIV-1 infected individuals with the aim to have a better safety/tolerability profile compared to other NNRTIs is practically insoluble drug across the pH gradient of GIT. For any active ingredient given per oral route needs to be dissolved first before it can permeate the membranes of the gastro intestinal tract to reach systemic circulation. Therefore, poorly water soluble drugs will exhibit dissolution rate limited absorption. So, in this study nanomilling technique has been selected to improve the solubility which in turns increases the rate of drug release.

Optimization of formulation and process: In this study, various formulation and process parameters were evaluated to get the optimized nanosuspension. Formulations F1A and F1B were milled at low and high pump and milling speed to check their effect on milling. It was observed that F1B with a higher milling speed gave more particle size reduction in the same time as would be expected (Table-3, Fig. 1).

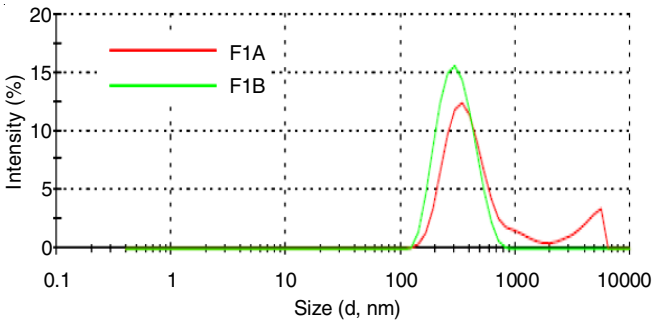

Fig. 1. PSD of nanomilling speed

For optimizing the milling time, F1B was milled for 120 min and F2 for 45 min. Upon characterization, it was found that particle size reduction was significant till 30 min. However, post 30 min, there was a maximum of 20 nm size reduction in the next 90 min (Table-4). Therefore, milling time was optimized as 30 min.

Formulations with increasing solid content of 20, 25 and 30 % w/w for F1B, F2 and F3, respectively, were characterized (Table-5, Fig. 2). It was found to have insignificant difference in the chosen range, especially for F1B and F2. The solid content was optimized as 20-25 % w/w. A very low or a very high viscosity is not favourable for efficient milling, since at low viscosity there is insufficient attrition optimized as 20-25 % w/w. A very low or a very high viscosity is not favourable for efficient

TABLE-3
CHARACTERIZATION FOR MILLING SPEED OPTIMIZATION

Time (min)	F1A			F1B		
	Z-average (nm)	D-90 (nm)	PDI	Z-average	D-90 (nm)	PDI
30	393.9	674	0.162	284.1	483	0.120
45	321.2	519	0.165	273.4	480	0.142

TABLE-4
CHARACTERIZATION FOR MILLING TIME OPTIMIZATION

Time (min)	Z-average (nm)	D-90 (nm)	PDI
F1B			
30	284.1	483	0.12
45	273.4	480	0.142
60	276.9	439	0.101
90	278.4	480	0.153
120	264.7	434	0.104
F2			
15	311.8	536	0.145
30	295.7	503	0.115
45	295.1	486	0.101

TABLE-5
OPTIMIZATION OF SOLID CONTENT

Batch details	Z-average (nm)	D-90 (nm)	PDI
F1B	284.1	483	0.120
F2	295.7	503	0.115
F3	360.2	628	0.182

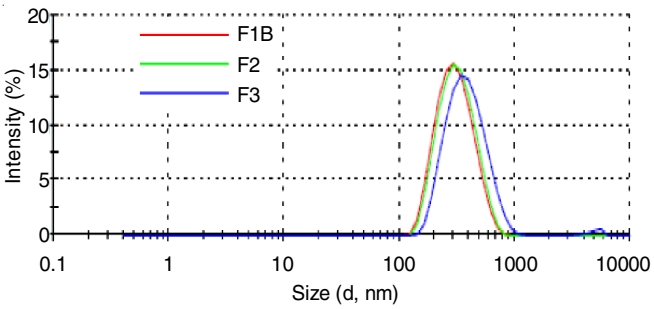

Fig. 2. PSD for solid content optimization

milling, since at low viscosity there is insufficient attrition, whereas high viscosity hinders movement of milling beads and energy transfer.

The total free energy of system along with the surface energy of the particle increases in nanomilling process. The system always tries to reduce the high energy of the system, so nanoparticles has a tendency to agglomerate and/or aggregate as per particle-particle interaction theory established by Derjaguin, Landau, Verwey and Overbeek (DLVO theory). Accordingly, this theory, the strong but short-ranged van der Waals attractions between particles governed the fundamental instability of colloidal dispersions. Further electrostatic repulsions countered to stabilize the system. Addition of non-ionic and ionic excipients can modify such electrostatic repulsion between particles. The short-ranged van der Waals attraction on the other side can be protected by steric barriers given by surface-adsorbed macromolecules. Therefore, stabilizing nanosuspension by selecting proper excipients is very important. Both the processing and safety aspects considering specific route of administration needs to keep in mind.

The effect of different polymers on the milling efficiency was evaluated. Milled drug with poloxamer (F9, F10) were found to have very poor milling efficiency. Polymers PVP (F7) and PEG (F8) also exhibited low milling efficiency (Fig. 3). This could be attributed to two factors. Firstly, their low viscosity leading to inefficient attrition and secondly, their hydrophilic nature, as in the case of PVP and PEG, causing insufficient

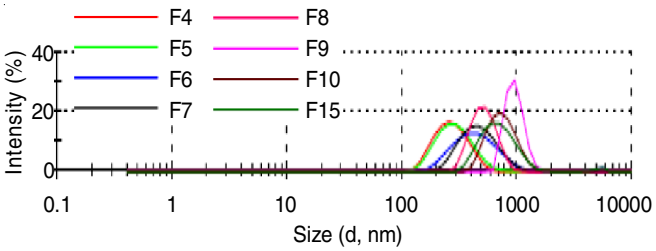

Fig. 3. PSD of optimization of stabilizer type

adsorption of drug particles and thus poor milling efficiency. Formulations with HPMC E3 and E5 were found to have good efficiency.

However, with HPMC E15 (F6, F13) had poor milling. This could be due to significantly higher viscosity of E15 grade. Milling of drug without any polymer (F15) has been indicated as reference for comparison of the effect of polymers on milling. HPMC E3 and E5 were therefore considered for further studies. Formulation F16 was milled with 0.15 % w/w Tween 20 to evaluate its effect on milling. It was found that Tween 20 at the chosen concentration did not increase milling efficiency and on the contrary might have reduced the efficiency.

Formulation F17 was milled with only 30 % bead volume as compared to previous 70 % bead volume and found to have lesser milling efficiency characterized by higher PSD (Table-6).

TABLE-6
PSD AND PDI OF ALL FORMULATIONS

Batch details	30 min		
	Z-average (nm)	D-90 (nm)	PDI
F1A	393.9	674	0.162
F1B	284.1	483	0.120
F2	295.7	503	0.115
F3	360.2	628	0.182
F4	295.5	478	0.215
F5	273.0	441	0.158
F6	416.4	761	0.217
F7	443.8	751	0.160
F8	560.3	708	0.240
F9	932.0	1220	0.440
F10	722.0	1070	0.199
F11	248.7	420	0.127
F12	346.4	560	0.112
F13	634.3	1430	0.267
F14	283.6	449	0.089
F15	684.1	1080	0.204
F16	331.6	492	0.039
F17	325.9	525	0.148

Zeta potential of final formulation was found to be increasing with the time of milling as shown in Table-7. After 30 min, change in zeta potential was negligible. As the zeta potential of rilpivirine nanosuspensions is greater than ± 20 mV, it can be concluded that nanosuspensions are likely to be stable [18]. The optimum formulation exhibited mean particle size of 266 nm (Z average), 441 nm (d_{90}), PDI of 0.158 and zeta potential of 22.1 mV (Figs. 4 and 5). The zeta potential clearly signifies the stability of nanosuspension prepared. The redispersability of all the formulations were found to be in good agreement with the theoretical value, which in turn indicates good sedimentation behaviour of the formulation.

TABLE-7
IMPACT OF ZETA POTENTIAL IN NANOMILLING TIME

Time (min)	Mill speed (rpm)	Pump (rpm)	Zeta potential (mV)
15	3000	40	7.8
30	3000	40	20.6
45	3000	40	22.1

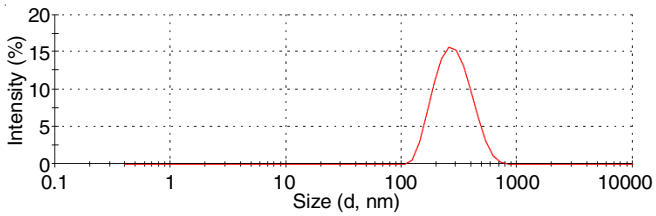

Fig. 4. PSD of optimized rilpivirine nanosuspension (F5)

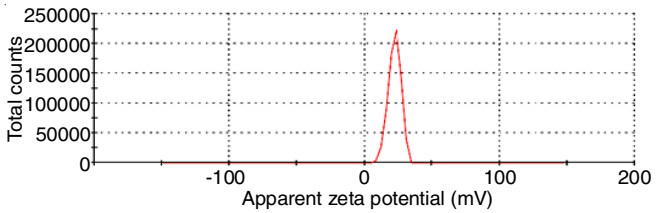

Fig. 5. Zeta potential of optimized rilpivirine nanosuspension (F5)

The saturation solubility study was done before nanomilling using micronized drug and after nanomilling, using the lyophilized nanocrystal of optimized nanosuspension to study the effect of nanomilling on the solubility and dissolution rate of the drug. Rilpivirine HCl is practically insoluble in water and the solubility is pH dependent. The drug has more solubility at lower pH. Many fold increase in solubility observed about 5 to 8 times across the pH gradient when formulated as nanosuspension further process as nanocrystal by lyophilization as shown in Fig. 6. This is due to reduction in particle size which in turn increases the surface area to dissolve the drug.

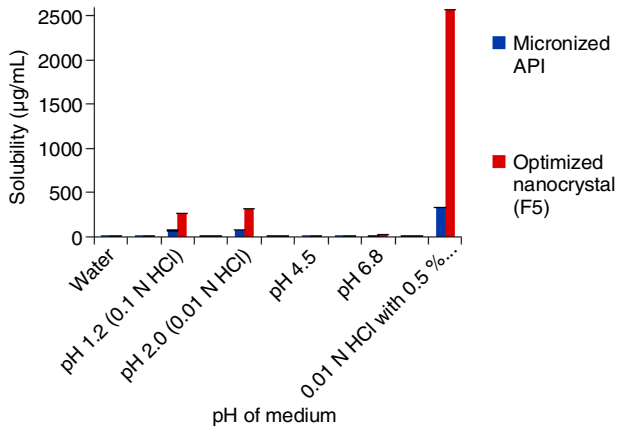

Fig. 6. Solubility enhancement of micronized drug and optimized formulations in different medium

The crystalline form of rilpivirine is characterized by an infrared spectrum comprising of peaks at wavenumbers of 2216 and 1656 cm^{-1}. These peaks were retained in the physical mixture and lyophilized nanocrystal formulation (F5), along with added peaks of HPMC. It can be thus being inferred that the drug and HPMC are chemically compatible for preparation of lyophilized nanocrystals.

Further, the interaction between carrier and guest molecules in the solid state was assessed by X-ray diffraction [19]. Raw rilpivirine and lyophilized nanocrystals exhibited same X-ray spectra (Fig. 7), which confirms that the chemical structure of the drug has not changed after the nanomilling process.

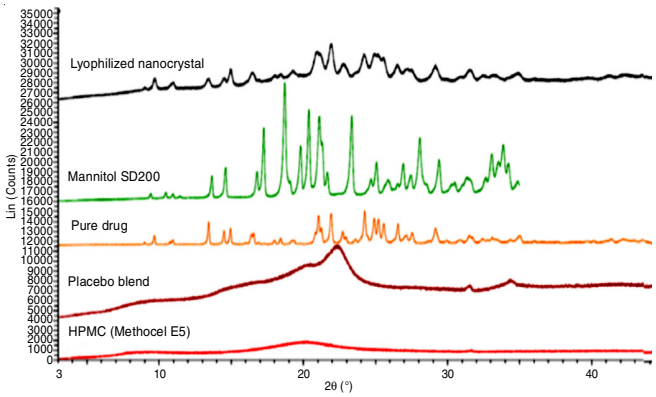

Fig. 7. XRD of pure drug, HPMC (Methocel E5), Mannitol SD200, Placebo blend and lyophilized nanocrystal

Optical microscopic images (Fig. 8) showed great differences between suspension without milling of the pure drug and nanosuspension. In suspension of micronized drug, the particles of rilpivirine were found to be large, irregular. However, after nanogrinding, the particles disappeared and the drug became small and uniform. The same nanomilled formulation was screened through SEM to show the better particulate nature of the drug (Fig. 9).

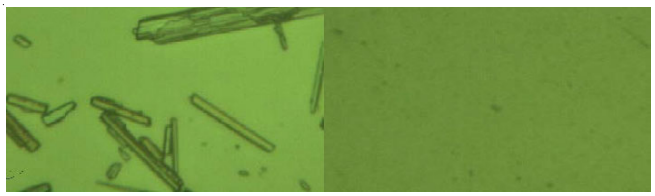

Fig. 8. Optical microscopic image of rilpivirine suspension and nanosuspension at 40X zoom

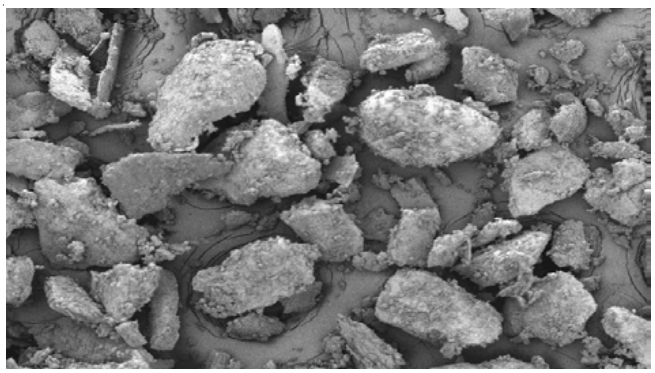

Fig. 9. SEM image of rilpivirine optimized nanocrystals at 500X

Drug release from micronized drug, marketed product (Edurant®) and optimized nanosuspension after lyophilized with mannitol was determined by dissolution study. As seen in Fig. 10, in 0.01 N HCl there was only 15 % drug release for micronized drug and for suspension before nanomilling in 30 min as compared to 45 % drug release for brand product and

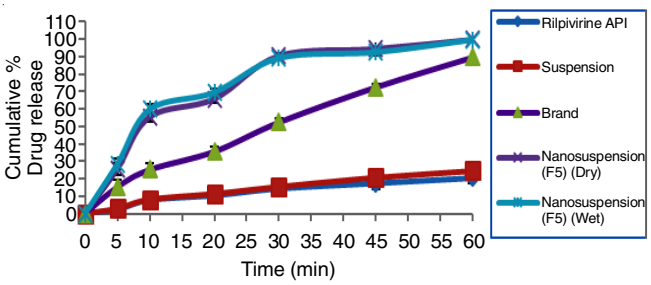

Fig. 10. Dissolution profile of drug and formulation in 0.01 N HCl

90 % drug release for optimized formulation both in dry and wet form respectively in the same time.

In 0.01 N HCl with 0.5 % Tween 20 (official FDA listed dissolution media), it was found that optimized formulation gave 90 % drug release in just 10 min in distinct comparison to 10 % drug release for micronized drug and for suspension before Nanomilling and 45 % drug release for brand product respectively in the same time (Fig. 11). The significant increase in available surface area to the dissolution medium and hydrophilic stabilizer coating on the particle surfaces may be considered as the main reason for about four-fold increase in dissolution rate in optimized formulation.

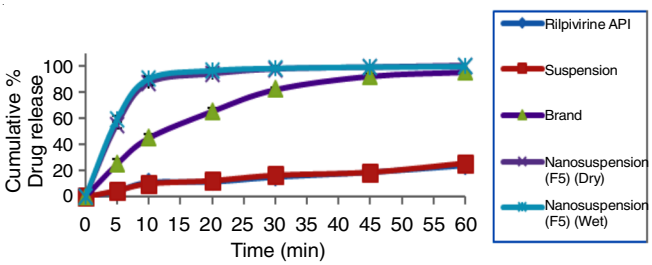

Fig. 11. Dissolution profile of drug and formulation in 0.01 N HCl + 0.5 % Tween 20

The drug release pattern of optimized formulation found to be first-order with correlation coefficient value of 0.958 indicating that the drug release rate is concentration dependent.

Flow properties like sedimentation volume, pourability was found to be satisfactory for optimized nanosuspension with the F value of 0.94 signifies the stability of nanosuspension. It is normally found that the greater the value of F, the more stable the product, when F = 1, no sediment is apparent and caking is absent [20,21]. The redispersability was found to be very good even after 4 weeks of storage (Table-8). The stability studies were performed on optimized nanosuspension dosage form of rilpivirine at 40 ± 2 ºC/75 ± 5 RH for 3 months. The results obtained from stability studies shown that there was no significant change in given parameters evaluated and can be concluded

TABLE-8
FLOW PROPERTIES OF OPTIMIZED NANOSUSPENSIONS (F5)

Particulars	0 day	1 week	2 weeks	4 weeks
Z-average (nm)	266	272	283.4	277.3
PDI	0.158	0.158	0.151	0.168
Redispersability (%)	100	100	100	100
Time taken to redisperse (s)	5	8	8	10
Sedimentation Volume (F)	0.94	0.92	0.90	0.85

that the formulation was found to be stable (Table-9). No significant change in related substances observed after storage of optimized nanosuspensions (F5) at accelerated storage condition for 3 months. The representative chromatogram are presented in Fig. 12.

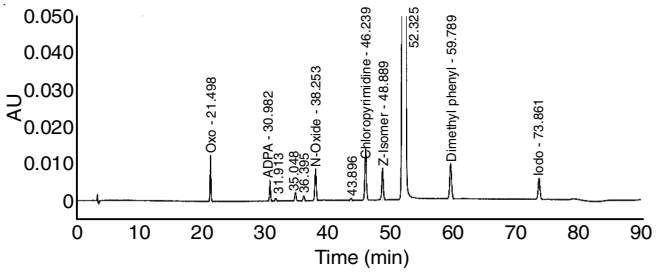

Fig. 12. Chromatogram of rilpivirine related substances

Conclusion

Seven different polymeric stabilizer and two surfactants have been evaluated to get the stable nanosuspension of rilpivirine with minimum particle size and optimum zeta potential. The optimized nanosuspension (F5) formulated using HPMC E5 as stabilizer, solid content of 20 %, and processing milling parameters: milling speed of 3000 rpm, milling time of 30 min and bead volume of 130 mL. Saturated solubility studies performed on rilpivirine in micronized form and nanocrystal of optimized nanosuspension. The study found to exhibit highest solubility in 0.01 N HCl with 0.5 % Tween 20 (for rilpivirine) and selected as dissolution media for further studies. In this study, the optimized nanosuspension and lyophilized nanocrystals have been used as a striking alternative formulation type containing nanomilled drug particles with increased dissolution rate (according to Noyes-Whitney model) and solubility (according to Ostwald-Freundlich and the Kelvin equations). Though, the many fold increase of drug particle surface area upon nanomilling is the main advantage to get higher observed dissolution rate but it may also the cause for major instability problems such as particle agglomeration, aggregation and crystal growth of nanosuspensions which mainly driven by the increased surface energy of the nanosized particles. Therefore, to identify

TABLE-9
STABILITY DATA OF OPTIMIZED RILPIVIRINE NANOSUSPENSIONS (F5) IN ACCELERATED STABILITY CONDITION AS ICH, 40 ± 2 °C, 75 ± 5 % RH

Time point	Assay	Dissolution in 0.01 N HCl with 0.5 % Tween 20, USP II, 900 mL, 50 rpm			Related substances				Particle size (Z-average) (nm)
		10 min	20 min	30 min	N-Oxide	Z-Isomer	Highest unknown	Total impurity	
Initial	100.8	90	99	100	0.08	0.05	0.07	0.45	266
1 Month	100.4	88	98	99	0.1	0.1	0.07	0.55	275
3 Months	100.5	89	99	100	0.12	0.1	0.09	0.8	295

the suitable stabilizer, seven different stabilizer and surfactant being evaluated and hypromellose (HPMC E5) is being considered, as its gives better particle size, narrow PDI and optimum zeta potential. Hypromellose 2910 (HPMC E5) contains high degree of substitution of the methoxy and hydroxypropoxy groups. The optimized formulation being stable when stored at accelerated stability condition up to 3 months. Therefore, the developed optimized nanosuspension would be an alternative better formulation than its conventional formulation to address its bioavailability issue. However, this should be further confirmed by appropriate *in vivo* studies.

REFERENCES

1. R. Shegokar and R.H. Muller, Nanocrystals: Industrially Feasible Multifunctional Formulation Technology For Poorly Soluble Actives., *Int. J. Pharm.*, **399**, 129 (2010); https://doi.org/10.1016/j.ijpharm.2010.07.044.
2. J.M. Irache, I. Esparza, C. Gamazo, M. Agueros and S. Espuelas, Nanomedicine: Novel Approaches in Human and Veterinary Therapeutics, *Vet. Parasitol.*, **180**, 47 (2011); https://doi.org/10.1016/j.vetpar.2011.05.028.
3. J.U.A.H. Junghanns and R.H. Müller, Nanocrystal Technology, Drug Delivery and Clinical Applications, *Int. J. Nanomed.*, **3**, 295 (2008).
4. Y. Kawabata, K. Wada, M. Nakatani, S. Yamada and S. Onoue, Formulation Design for Poorly Water-Soluble Drugs Based on Biopharmaceutics Classification System: Basic Approaches and Practical Applications, *Int. J. Pharm.*, **420**, 1 (2011); https://doi.org/10.1016/j.ijpharm.2011.08.032.
5. B.E. Rabinow, Nanosuspensions in Drug Delivery, *Nat. Rev. Drug Discov.*, **3**, 785 (2004); https://doi.org/10.1038/nrd1494.
6. R.H. Müller, S. Gohla and C.M. Keck, State of the Art of Nanocrystals-Special Features, Production, Nanotoxicology Aspects and Intracellular Delivery, *Eur. J. Pharm. Biopharm.*, **78**, 1 (2011); https://doi.org/10.1016/j.ejpb.2011.01.007.
7. P.P. Ige, R.K. Baria and S.G. Gattani, Fabrication of Fenofibrate Nanocrystals by Probe Sonication Method for Enhancement of Dissolution Rate and Oral Bioavailability, *Colloids Surf.*, **108**, 366 (2013); https://doi.org/10.1016/j.colsurfb.2013.02.043.
8. E. Merisko-Liversidge and G.G. Liversidge, Nanosizing for Oral and Parenteral Drug Delivery: A Perspective on Formulating Poorly-Water Soluble Compounds using Wet Media Milling Technology, *Adv. Drug Deliv. Rev.*, **63**, 427 (2011); https://doi.org/10.1016/j.addr.2010.12.007.
9. F. Danhier, N. Lecouturier, B. Vroman, C. Jérôme, J. Marchand-Brynaert, O. Feron and V. Préat, Paclitaxel-Loaded PEGylated PLGA-Based Nanoparticles: *in vitro* and *in vivo* Evaluation, *J. Control. Rel.*, **133**, 11 (2009); https://doi.org/10.1016/j.jconrel.2008.09.086.
10. T. Picraux, Encyclopedia Britannica Deluxe Edition; Encyclopedia Britannica: Chicago, IL, USA (2010).
11. PIL, Edurand® Tablet, US FDA.
12. H.M. Crauwels, Poster Presented at the Ninth International Workshop on Pharmacology of HIV Therapy, New Orleans, LA. April 7-9 (2008).
13. M. Malamatari, K.M.G. Taylor, S. Malamataris, D. Douroumis and K. Kachrimanis, Pharmaceutical Nanocrystals: Production By Wet Milling and Applications, *Drug Discov. Today*, **23**, 534 (2018); https://doi.org/10.1016/j.drudis.2018.01.016.
14. R.H. Müller and C.M. Keck, *Eur. J. Pharm. Biopharm.*, **80**, 1 (2012); https://doi.org/10.1016/j.ejpb.2011.09.012.
15. R.H. Muller, R. Becker, B. Kross and K. Peters, Pharmaceutical Nanosuspensions for Medicament Administration as Systems with Increased Saturation Solubility and Rate of Solution, US Patent 5858410 (1999).
16. L. Bond, S. Allen, M.C. Davies, C.J. Roberts, A.P. Shivji, S.J.B. Tendler, P.M. Williams and J. Zhang, Differential Scanning Calorimetry and Scanning Thermal Microscopy Analysis of Pharmaceutical Materials, *Int. J. Pharm.*, **243**, 71 (2002); https://doi.org/10.1016/S0378-5173(02)00239-9.
17. Á. Delgado, F. González-Caballero, R.J. Hunter, L.K. Koopal and J. Lyklema, Measurement and Interpretation of Electrokinetic Phenomena, *J. Colloid Interface Sci.*, **309**, 194 (2007); https://doi.org/10.1016/j.jcis.2006.12.075.
18. R.H. Müller and G.E. Hildebrand, Zetapotential und Partikelladung in der Laborpraxis: Einfuhrung in die Theorie praktische Messdurchfuhrung Dateninterpretation: Colloidal Drug Carriers, Paperback APV (1996).
19. S.G. Banker and C.T. Rhodes, Disperse Systems: Modern Pharmaceutics, edn 3, (1988).
20. N.K. Patel, L. Kenon and R.S. Levinson, Pharmaceutical Suspensions: The Theory and Practice of Industrial Pharmacy, 3rd Indian edition, (1986).
21. B. Mishra, J. Sahoo and P.K. Dixit, Formulation and Process Optimization of Naproxen Nanosuspensions Stabilized by Hydroxy Propyl Methyl Cellulose, *Carbohydr. Polym.*, **127**, 300 (2015); https://doi.org/10.1016/j.carbpol.2015.03.077.