Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2013, 5 (6):48-55
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Development and characterization of Ritonavir nanosuspension for oral use

S. Prakash[1,3], S.Vidyadhara[2], RLC.Sasidhar[2], D. Abhijit[1] and D. Akhilesh[1]

[1]Mylan Laboratories Limited, Formulation R and D Centre. Plot No 34A. Anrich Industrial Estate, Bollaram Village, Medak District, A.P.
[2]Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur, A.P.
[3]Department of Biotechnology, Acharya Nagarjuna University, Nagarjunanagar, Guntur, A.P.

ABSTRACT

The purpose of the present study was to prepare an acceptable nanosuspension of ritonavir by increasing the aqueous solubility, dissolution and hence oral bioavailability. Ritonavir is having low aqueous solubility and high permeability which can be considered as drug belonging to class-II according to BCS. In this study nanosuspension technology was tailored to increase solubility and dissolution rate of ritonavir. Ritonavir Nanosuspension was prepared by Pearl milling technique using zirconium oxide beads as milling media, poloxamer 407 as a stabilizer. The prepared nanosuspension was characterized using DSC (Differential Scanning Colorimetry) and XRD (X-Ray Difractometry). The nanosuspension was further evaluated for drug release, redispersability, assay and for stability. The solubility and dissolution studies revealed that there was a considerable increase in the solubility and dissolution rate of ritonavir in nanosuspension formulation as compared to pure drug. Human acceptability studies of prepared placebo nanosuspensions were found to be acceptable when evaluated for organoleptic properties like odour, taste, smell and other characteristics like appearance and pourability.

Keywords: Nanotechnology, Antiretroviral treatment, Solubility enhancement.

INTRODUCTION

The present dosage forms of ritonavir are in soft gelatin capsule and heat stable tablet dosage forms. In soft gelatin capsule approach, a high quantity of oleic acid and alcohol is been used to solubilise the ritonavir drug and hence improve bioavailability. Thus formed capsule is about 21 mm in length and 10mm in width. Where as in tablet dosage form solid dispersion technique by meltrex is been used to incorporate the drug in polymer melt. In both the above strategies the API crystallinity is been changed to amorphous one from crystalline nature. The disadvantage of both marketed dosage forms are like big facility requirement, inability to manufacture smaller batches and relatively more cost. These factors made thought of developing the alternative dosage form as nanosuspension. In present study the crystalline nature of the API remains same.

Solubility is an essential factor for drug effectiveness. A limiting factor for *in vivo* performance of poorly water soluble drugs, following oral administration, is their resistance to being wetted by and dissolved into the fluid in the gastrointestinal tract. Increasing the dissolution rate of poorly water soluble drugs, is thus important for optimizing bioavilability. In this approach poorly water-soluble compounds are formulated as nanometer sized drug particles.

Poor water solubility, insufficient bioavailability, fluctuating plasma levels and high food dependency are the common problems with low soluble drugs [1]. Hence there is a growing need for a unique stratergy that can tackle the formulation related problems associated with the delivery of hydrophobic drugs in order to improve their clinical efficacy and optimize their therapy with respect to pharacoeconomics [2]. Nanosuspensions has shown to be more cost- effective and technically simpler alternative, particularly for poorly soluble drugs [3, 4, 5, 6]. Nanosuspensions are sub-micron colloidal dispersions of pure drug particles in an outer liquid phase. The fineness of dispersed particles causes them to dissolve more quickly owing to their higher dissolution pressure and leads to an increased saturation solubility. Ritonavir is a new class of drug with a mechanism of action that is different from most of the previously available antiretroviral treatments for AIDS. The drug inhibits the HIV viral proteinase enzyme which prevents cleavage of the gag-pol polyprotein, resulting in noninfectious, immature viral particles. The aim of this study was to employ the nanosuspension technique in the preparation of ritonavir nanoparticles for oral administration, therby avoiding harmful additives and enabling to enhance the solubility, dissolution and oral absorption of ritonavir. The prepared nonosuspension was evaluated for different physicaochemical parameters and dissolution study in comparision to the pure drug and marketed formulation.

MATERIALS AND METHODS

Materials:

Ritonavir was obtained from Mylan Laboratories Ltd., Hyderabad, Polaxomer 407 (Lutrol micro 127 MP) was gift sample from BASF (Germany). All other materials used were of pharmacopoeial grade and were produced from commercial sources.

Preparation of Nanosuspension:

Ritonavir (10%w/w) was dispersed in an aqueous solution containing Polaxomer 407 in a container. Ritonavir used in the study has particle size of 52 microns. The resulting dispersion was comminuted using colloidal mill for 2 hrs. The colloid mill passed dispersion was further comminuted using zirconium oxide beads [7] in nanomill (Netzsch) under controlled temperatures. The milling and pump speed was kept at 3000 rpm and 70 rpm respectively. The high energy and shear forces generated as a result of the impaction of the milling media with the drug particles provides the energy input to break the micro particulate of ritonavir into nanosized particles [8].

Solubility studies:

The study was done before and after nano milling to study the effect of nano sizing on the solubility and dissolution rate of the drug. 100mg of ritonavir and ritonavir nanosuspension containing 100mg of ritonavir was weighed and transferred into conical flasks containing 100ml of different dissolution media. These flasks were hermatically sealed and incubated at 37^0c in an incubator shaker rotated at 50rpm for 48hrs. Then the samples were filtered and subsequently diluted with same media and the absorbance was noted at 246nm.

Characterization of Nanosuspensions

Nanosuspensions are characterized in similar ways as those used for conventional suspensions for Physical/chemical/flow properties. Physical evaluations such as appearance of phases, particle size analysis, Zeta potential, DSC, solubility studies, Freeze thaw study. Chemical evaluations such as Assay, Dissolution and Related substance. Flow properties such as Sedimentation volume, pourability and redispersibility.

Particle size analysis:

The particle sizes of the nanosuspensions were determined by photon correlation spectroscopy [9,10], using a Zeta seizer nanoserise (Malvern Instruments, Worcestershire, UK). This technique yields the mean particle diameter and the range of the particle-size distribution (Polydispersity Index, PDI). All the data presented are the mean values of the results on three independent samples produced under identical conditions. To compare the size and the size distribution of the raw API and the nanosuspensions, the samples were dispersed in water, using an ultrasonic bath for 60min. The sonication was used to obtain the size of individual particles. Small volume dispersion unit was added to required amount of super saturated solution of drug for optimization of particle size of true density of API 1.500 g/cm3, 1.5 was used as refractive index for dispersed particles and 1.330 for dispersion medium. Laser diffractometry yields the volume size distribution, with particle measurement in the size range 0.1–2000nm. The reported particle-size distribution typically includes Dv10, Dv50 and Dv90, which are the percentages of particles below the given size. The dilution effect on particle size was also measured and tabulated below. The particle size results of nano suspensions were given in tables 1 & 2.

Differential scanning calorimetry (DSC) analysis:
The thermal characteristics of the pure ritonavir and the nano suspension were determined by differential scanning calorimetry (DSC-60, SHIMADZU) with an intracooler-2 cooling. About 3 to 5 mg of product was placed in perforated aluminum sealed 50-µl pans, and the heat runs for each sample was set from 40°C to 150°C at 10°C/min, under an inert environment using nitrogen [11]. The apparatus was calibrated using pure metals like indium with known melting points and heat of fusion (ΔH fusion). The therrmograms were shown in figure 2.

Freeze thaw study:
The ritonavir suspension so produced was exposed for freeze thaw cycles. The suspension was exposed for 2-8°C for 2 days followed by 40°C±2°C/75±5% for 2 days. The study was done for 5 cycles. After the study the suspension sample was evaluated for Particle size evaluation, Dissolution profile and XRD studies.

Sedimentation volume:
The rate of separation of the suspensions were determined by keeping 50 ml portion of each suspensions in stoppered measuring cylinder and stored undisturbed at room temperature. The separation of clear liquid was noted at an interval of 2W and 4W. The sedimentation volume, F (%), was then calculated using the following equation

$$F = 100Vu/Vo$$

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

Pourability:
This test is carried out on the phases of suspension after mixing to ensure that the final preparation is pourable and will not cause any problem during filling and during handling by end user.

Redispersability:
Fixed volume of each suspension (50 ml) was kept in calibrated tubes which were stored at room temperature for various time intervals (2W, 4W). At regular interval of 2W, one tube was removed and shaken vigorously to redistribute the sediment and the presence of deposit if any was recorded. The time (s) taken to redisperse the sedimented suspension was recorded.

Assay:
Ten ml of suspension was taken and dissolved in 50 ml methanol and sonicated for 30 min, the volume was adjusted to 100 ml using methanol with continuous sonication for 5min. Filtered through a 0.45-µm membrane filter, and analyzed at 246 nm using UV spectrophotometer. The results are shown in table 2.

Dissolution rate studies on ritonavir nanosuspension:
Dissolution rate studies of pure ritonavir and ritonavir nano suspension were performed in USP dissolution testing apparatus (type II) with rotating paddles at 50rpm using 900ml of 0.1N HCl as dissolution medium. The temperature was maintained at 37 ± 0.5^0c throughout the experiment. 10ml of samples were withdrawn at various time intervals and same volume of dissolution medium was replaced for maintaining the constant volume of dissolution medium. Samples were filtered through 0.45µ filter and analyzed by measuring the absorbance at 246 nm. The dissolution profiles were shown in figure 5.

Related Substances:
To check the impact of nanonization process on the chemical stability of Ritonavir, Related substances analysis was performed by using HPLC method. Following chromatographic conditions were used to analyze the samples. Mobile phase consists of phosphate buffer pH 3.5 as mobile phase A and mixture of acetonitrile and water (70:30) as mobile phase B was used (Gradient programme for Mobile A and Mobile phase B was set as 70/30,70/30,20/80,20/80,70/30 and 70/30 respectively at 0,10,50,105,110 and 120 minutes). Separation was done by using C18, 150mm x 4.6mm, 3 µm column. Flow rate was set as 1.2 ml/minute. Detection wave length at 240 nm was used to measure the components response. Injection volume of 10µL was used for both Standard and test. Phosphate buffer pH 3.5 and Acetonitrile were used as diluents for the preparation of both Standard and test solutions. Test solution was prepared at 2000 mg/ml of Ritonavir .Standard solution was prepared at 10 mg/ml concentration. The results are shown in table 2.

Microscopy test:
The samples (before and after nano-nization) were visualized by using Optical Microscope (LEICA, DFC 395) at 40X zoom, the results are shown in figure 6. Air-Dried samples of naosuspensions 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 90sec under vacuum (3Pa) and observed under the Scanning Electron Microscope (JEOL, JSM-6380) at a magnification of 500X. The results are shown in figure 7.

Stability Study:
The ritonavir nanosuspension were filled in HDPE containers sealed and loaded into stability chamber at 40°C±2°C/75±5% RH (Newtronics stability chamber) [12]. The samples were withdrawn after 3 months and analyzed for particle size, Assay, Dissolution studies, Related substances. The stability data were given in table 7.

Acceptability Study:
A placebo formulations (Table 8) with different flavor combinations were prepared and evaluated based on fixed questionnaire set to evaluate the suspension. A scoring system was used to quantify subjective variables listed in the questionnaire. Average score for each formulation was calculated using summation of rating points given by each subject divided by the number of subjects. The formulation was rated as Excellent, Acceptable, Tolerable and Unacceptable based upon the average score obtained by the formulation as shown in Table 4. The study was conducted on 20 healthy human subjects (age between 23 to 35 years) using placebo oral solutions. Each subject was given all the four formulations.

RESULTS AND DISCUSSION

The aim of the present investigation was to improve the solubility and dissolution rate ofritonavir. Ritonavir nanosuspension was prepared by pearl milling technique using zirconium beds s milling media and polaxomer 407 as stabilizer. The solubility study was done before and after nanomilling to study the effect of nanosizing on the solubility and dissolution of the drug. The solubility of ritonavir nanosuspension was found to be significantly higher that is 1.62mg/ml and 3.52mg/ml in water and 0.1 N HCl. This is due to reduction in the particle size facilitates range surface for the medium to dissolve the drug. The optimized nanosuspension showed mean particle size of 385nm with PDI of 0.268. The dilution effect and freeze thaw effect on particle size was found to be negligible. The zeta potentiall of -25.4 MV clearly signifies the stability of the nanosuspension prepared. The redispersability of all the formulations were found to be in good agreement with the theoretical value, indicating good sedimentation behaviour of the formulations.

X-ray diffraction was used to analyze potential changes [13] in the inner structure of ritonavir during nanonisation. The crystallinity remained unchanged even after exposure to Freeze thaw cycle which is shown in fig 1. The figure 2 shows the DSC thermograph of pure drug and nanosuspension. Pure Ritonavir drug showed melting exotherm at 127'C which corresponds to its melting point and its exotherm in formulation was observed at 121'C. From thermograms, it was concluded that the drug and the surfactant do not interact with each other. Optical microscopic images showed great differences between suspension and nanosuspension (fig 3). In suspension the particles of ritonavir were found to be large, irregular. However after nanonisation the particles disappeared and the drug became small and uniform. The same nanonised formulation was screened through SCM to show the better particulate nature of the drug shown in the figure 4. *In vitro* dissolution was performed on pure drug, reference capsules, nanosuspension and freeze thaw exposed nanosuspension. The drug release from nanosuspension was found to be 91% in 60 min when compared to 28% for pure drug. The increase in accessible surface area to the dissolution medium and hydrophilic surfactant coating on the particle surfaces may be the reason for substantial increase in dissolution rate. Poloxamer 407 acts as stabilizer, wetting agent, as polymer helps in enhancement of wettability. The same is been given in table 6. Flow properties like sedimentation volume, pourability was found to be satisfactory with the F value found to be 0.96, signifies the stability of the 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 [14, 15]. The redispersibility was found to be very good even after 4 weeks of storage which is mentioned in table 5. The stability studies was performed on nanosuspension dosage form of the Ritonavir at $40^0C \pm 2^0C$ / 75 ± 5% RH for three months. The results obtained from stability studies shown that there was no significant change in the parameters evaluated and can be concluded that the formulation is found to be stable. The stability data were given in table 2. Human acceptability studies of prepared placebo suspensions were found to be acceptable when evaluated for organoleptic properties like odour, taste, smell and other characteristics like appearance and pourability. The

compositions for placebo formulations for acceptability studies were given in the table 3. The scoring system used for the study is given in table 4.

Table 1: Particle size analysis of Ritonavir nanosuspension

S.No	Time (min)	Mill Speed (rpm)	Pump (rpm)	Z- Average Nanosuspension
1	60	3030	70	864.8
2	120	3030	70	633.7
3	180	3030	70	475.4
4	240	3030	70	384.6
Particle size analysis after freeze thaw cycle				
1	NA	NA	NA	378.2

Table 2: Stability data for Ritonavir Nanosuspensions

S.No	Parameters	Initial	40°C/75% RH (3 month)
1.	**Assay**	100.2	99.5
2.	**Dissolution**		
	Time in min	**% Drug Release**	
	15	73	70
	30	81	79
	45	88	83
	60	91	89
	90	94	92
	120	95	93
3.	**Related substances**		
	Imp A	0.046	0.046
	Imp B	0.021	0.038
	Imp F	0.031	0.029
	Imp I	0.003	0.003
	Imp L	0.028	0.021
	Highest Unknwon	0.114 (0.037)	0.105 (0.037)
	Total Imp	0.920	0.938
4.	**Particle size**	384.6	389

Table 3: Composition of different batches for acceptability studies

		Batch no. of formulations			
S. No.	**Ingredients(gm/batch)**	**A1**	**A2**	**B1**	**B2**
1.	API	-	-	-	-
2.	Poloxamer F 407	10	10	10	10
3.	Strawberry	5	-	5	-
4.	Peppermint	-	5	-	5
5.	Aspartame	15	15	15	15
6.	Iron oxide	-	-	0.1	0.1
7.	Water (qs)	1ml	1ml	1ml	1ml

Table 4: Results of human acceptability studies for API nanosuspension.

Characteristics	A1	A2	B1	B2
Aappearance	2.70	2.80	2.55	2.60
Flavour	2.25	2.70	2.30	2.55
Pourability	3.20	3.15	3.05	3.00
After taste	2.69	2.47	2.11	2.56
Convenience	2.71	2.65	2.41	2.69
Total score	15.84	16.10	14.70	15.81
Average score [a]	2.64	2.68	2.45	2.64

[a] *Average score was calculated by adding sum of ratings given by each subject divided by the number of subjects.*

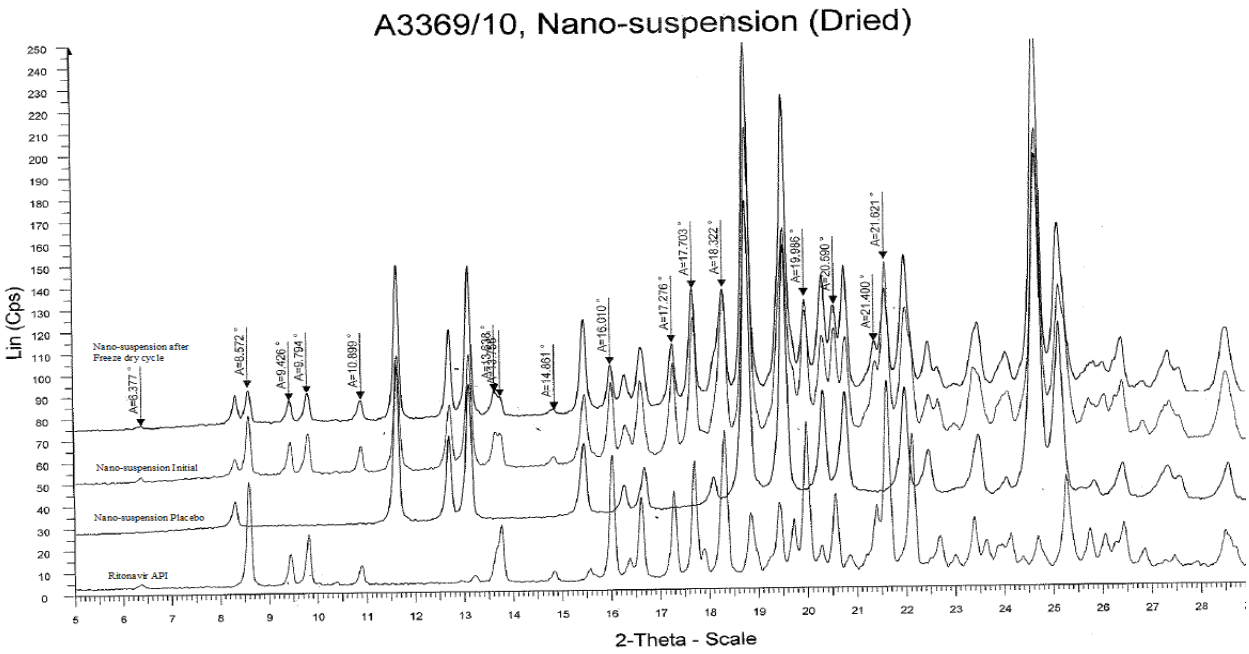

Fig 1: XRD of pure drug, dried nanosuspension (Initial and after freeze thaw cycle) and placebo formulation

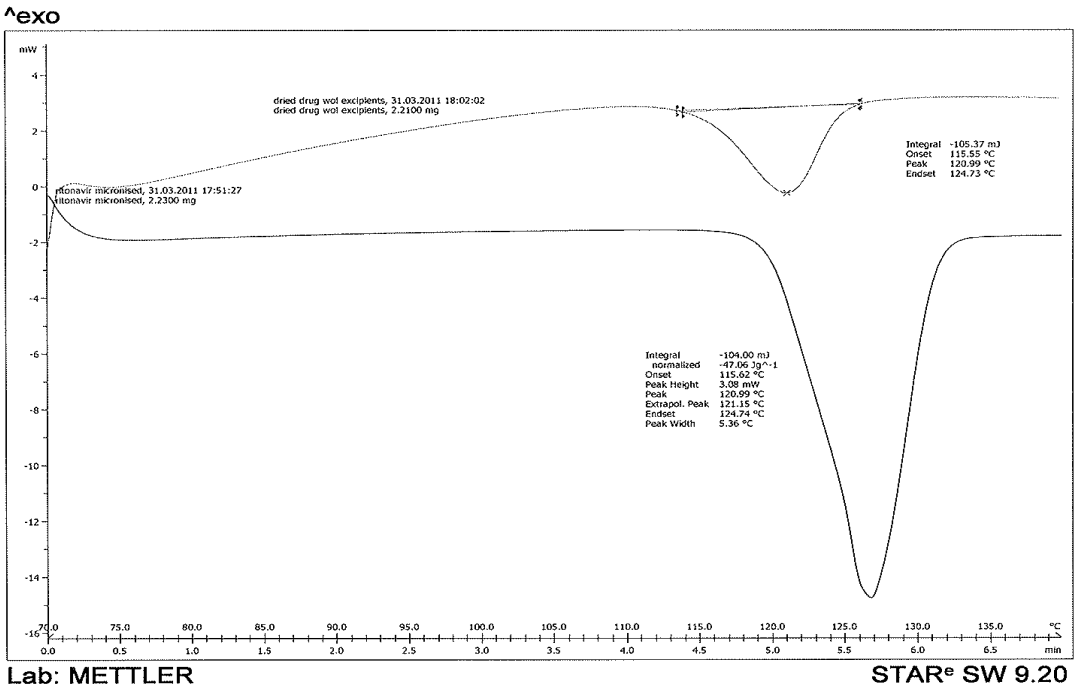

Fig 2: Differential Scanning Calorimetric graph of pure drug and final formulation.

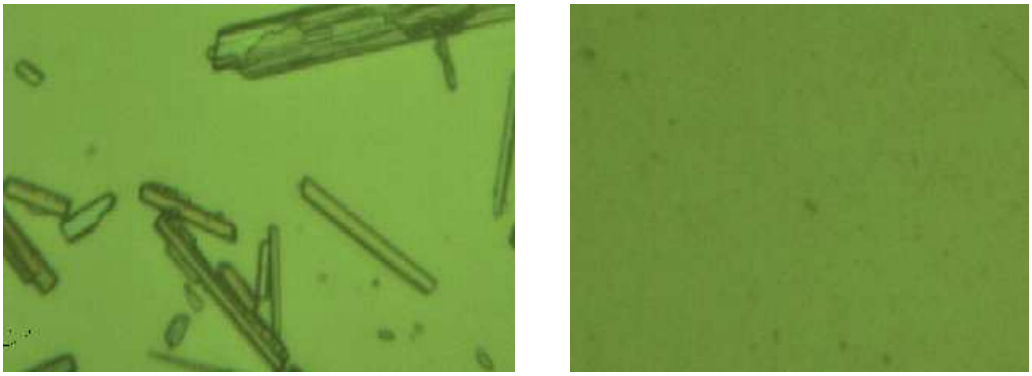

Fig 3: Optical microscopic image of Ritonavir suspension and nanosuspension at 40X zoom

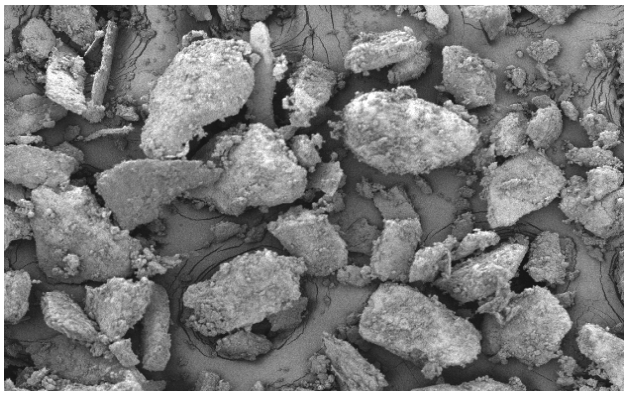

Fig 4: SCM image of Ritonavir nanosuspension (dried) at 500X

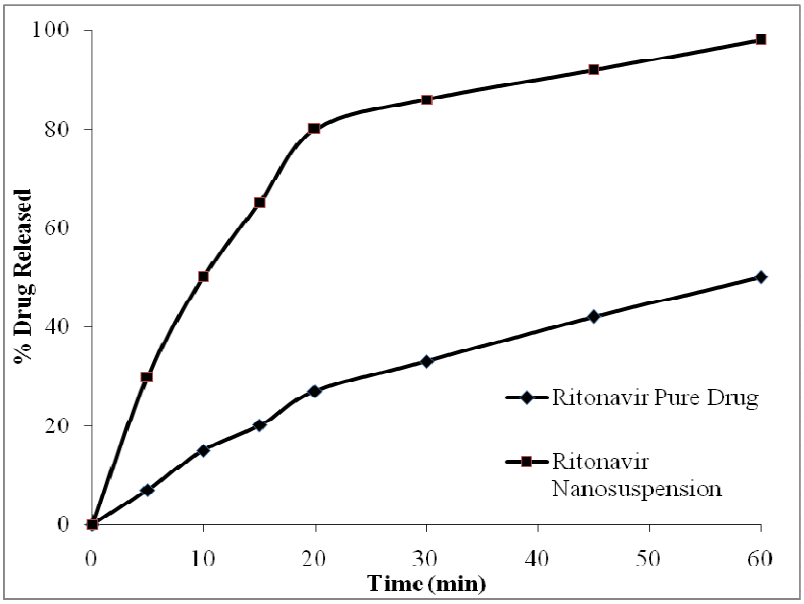

Figure 5: *In vitro* dissolution profiles of Pure drug and Nanosuspension

CONCLUSION

From the results, it may be concluded that nanocrystalline suspensions of poorly soluble drugs such as ritonavir are easy to prepare and represent a promising new drug formulation for oral drug delivery for treatment of AIDS. Nanosuspensions of ritonavir Drug offer a viable ulternative method to formulate poorly soluble drug and enhance the bioavailability. The nanosuspension technique has many advantages, such as simple method of preparation, less requirement of excipients, increased dissolution rate and solubility, increases the bioavailability leading to decrease in the dose and fast-fed variability and ease of large scale manufacturing.

Acknowledgement:
The authors expresses their gratitude to Mylan Labs limited, Hyderabad and Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur for providing the necessary facilities to carry out the research work.

REFERENCES

[1] Ml. Elane, GL.Gary. *Eur J Pharm Sci*, **2003**, 18,113-120.
[2] M.Nakarani, Ak.Mishra, Jk.Patel. *DARU*, **2010**, 18,84-90.
[3] Me.Liversidge, P.Sarpotdar, J.Bruno, S.Hajj, L.Wel. *Pharm Res*, **1996**, 13, 272-278.
[4] Rh.Muller, K.Peters. *Int J Pharm*, **1997**, 160, 229-237.
[5]K.Westesen, B.Siekmann. *Pharm Res*, **1995**, 12, 201-208.
[6]Rh.Muller. Boca Raton, FL, CRC Press inc, **1991**, 211-274.
[7]Gg.Liversidge, Kc.Cundy, Jf.Bishop. US patent 5145684, 1992.
[8]Vb.Patravale, A.Abhijit, Rm.Kulkarni. *J Oharm Pharcol*, **2004**,56, 827-840.
[9]Rh.Muller, R.Becker, B.Kross, K.Peters. United state patent no 5858410, Jan 1999.
[10]B.Laura, A.Stephanie, C.Martyn. *Int. J. Pharm*, **2002**, 243, 71-82.
[11]Bh.Bohm, Rh.Muller. PSTT, **1999**, 2, 336-339.
[12]Rh.Muller, C.Jocobs. *Int. J. Pharm*, **2002**, 237, 151-161.
[13]Rh.Muller, C.Jacobs, O.Kayser. *Adv Drug delivery review*, **2001**, 47, 3-19.
[14]Sg.Bankar, Ct.Rhodes. Disperse Systems: Modern pharmaceutics. **1998**, 3rd addition.
[15]Nk.Patel, L.Kenon, Rs.Levinson. **1986**, Pharmaceutical suspensions" The theory and practice of Industrial pharmacy. 3rd Indian addition.