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Solid Dispersion as an Approach for Dissolution Enhancement of Poorly Water Soluble Drug Ritonavir

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Abstract : Ritonavir is an antiretroviral drug from the protease inhibitor class used to treat Human Immunodeficiency Virus Infection and Acquired Immune Deficiency Syndrome. It is characterized by low solubility and high permeability which corresponds it to BCS class II drug. It exhibits low and variable oral bioavailability due to its poor aqueous solubility and its absorption is dissolution rate limited which makes it a suitable candidate for solid dispersion system. In the current investigation, Ritonavir is selected to improve the solubility and dissolution rate by solid dispersion method. Solid dispersions of Ritonavir were prepared by kneading method by incorporating Arginine and Proline as carriers in molar ratios of 1:0.5, 1:1 and evaluated for solubility studies, drug-carrier compatibility studies and *in vitro* dissolution studies. Based on the results of solubility and dissolution profiles of solid dispersions, Arginine as a carrier was selected to prepare tablets by direct compression method and compared with conventional marketed tablets. Tablets were evaluated for pre compression and post compression parameters and *in vitro* dissolution studies. From the Fourier Transform Infrared Spectrosscopy and Differential Scanning Calorimetry studies, it was confirmed that there was no significant drug and polymer interactions. From the *in vitro* dissolution study, Ritonavir and Arginine 1% tablets shows 76.65 ± 2.5 % drug release within 10 min. The results were much higher compared to conventional marketed tablets containing pure drug (59.19 ± 2.3% in 10 min). The drug release from the formulations is as follows: Arginine 1% tablet>Arginine 0.5% tablet>Marketed tablets. Thus, it is concluded that the formulation of solid dispersions with Arginine as carrier is a suitable approach to improve the solubility and dissolution rate of Ritonavir than pure form of drug.
Keywords: Ritonavir, Arginine, Proline, Solid dispersion.

Introduction

A poorly water soluble drug, more recently, has been defined in general terms to require more time to dissolve in the gastrointestinal fluid than it takes to be absorbed in the gastrointestinal tract. Drugs with low aqueous solubility have low dissolution rates and hence suffer from oral bioavailability problems. The rate and extent of absorption of class II & class IV compounds is highly dependent on the bioavailability which ultimately depends on solubility. Thus, a greater understanding of dissolution and absorption behavior of drugs with low aqueous solubility is required to successfully formulate them into bioavailable drug product[1].

The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. The matrix can be either crystalline or

amorphous. The drug can be dispersed molecularly, in amorphous particles (clusters) or in crystalline particles. Oral bioavailability of a drug depends on its solubility and/or dissolution rate, therefore efforts to increase dissolution of drugs with limited water solubility is often needed. Improvement in the dissolution rate of the poorly soluble drugs after oral administration is one of the most crucial challenges in modern pharmaceutics. Many methods are available to improve these characteristics including salt formation, micronization and addition of solvent or surface-active agents. In this study polyethylene glycol was selected and solid dispersion was prepared by the method of solvent evaporation[2].

Law et al.,[3] prepared the solid dispersion of ritonavir by the solvent evaporation method, vacuum drying using PEG 8000 as a carrier. The main lacuna observed in this study was that 100% release of drug was obtained when the carrier was in the ratio 1:9. As the minimum titrable dose is 100 mg (total dose being 1,200 mg), at the ratio of 1:9 (drug : polymer) the total mass will amount to 1,000 mg with carrier alone and other excipients for tablet and capsule will amount more.

The co-amorphous formulation approach was developed by Löbmann et al., introducing amino acids as low molecular excipients for these systems[4, 5]. The need for suitable low molecular weight excipients was pressing, firstly to enable the formulation of co-amorphous single drug delivery systems and secondly to be able to compete with other amorphous formulation approaches, i.e. solid dispersions.

The main objective of the study was to develop solid dispersion of ritonavir using aminoacidsin order to enhance bioavailability and at the same time not to have bulky formulation so that patient compliance is achieved. Apart from this, the developed formulation was investigated for its performance by in vitro dissolution studies in 0.1 N HCl.

Materials and Methods

Materials

Ritonavir was a gift sample from Aurobindo Pharm Ltd., Arginine, Proline, Calcium-dihydrogen orthophosphate, Anhydrous Crospovidone, Talc, Magnesium stearate, HCl, Methanol were purchased from SD Fine chemicals Ltd. Mumbai.

Analytical Method Development

Determination of λ_{max} of Ritonavir in 0.1N HCl

An accurately weighed quantity of drug was dissolved in suitable volume of 0.1N HCl to prepare the stock solution. An aliquot from this stock solution was diluted with 0.1N HCl to get a final concentration of 10 ug/ml. The solution was prepared in three replicates. The solutions with concentration of 10 ug/ml were scanned in the range of 200-400 nm in 1.0 cm cell against 0.1N HCl and spectra were recorded.

Construction of Calibration Curve of Ritonavir In 0.1 N Hcl

Accurately weighed 10 mg of Ritonavir was dissolved in 100 ml of 0.1N HCl to give 100 µg/ml of stock solution. From the stock solutifon 1, 2, 3, 4, 5 ml of solution was taken and was diluted up to mark in 10 ml volumetric flask to obtain 10, 20, 30, 40, 50 µg/ml concentrated solutions. The absorbance was noted at λ_{max} of 248 nm.

Solubility Analysis

Preformulation solubility analysis was done to select a suitable carrier. The solubility of Ritonavir with different carriers was determined by adding an excess of drug and arginine and proline in different ratios to 25 ml conical flask, containing 10 ml of water and vortexed for half an hour and placed on a rotary shaker for 48 hr at room temperature. The contents were centrifuged at 3000 rpm for 5 minutes. The solubilised drug in the supernatant, was quantified by UV spectroscopy.

Preparation of Solid Dispersions Employing Carriers

Solid Dispersion by Kneading Method

Ritonavir with proline in molar ratios of 1:0.5, 1:1 and ritonavir with arginine in molar ratios of 1:0.5, 1:1 were taken and mixed by trituration in glass pestle–mortar. Methanol was added in small quantity while triturating to get slurry like consistency. The trituration was continued for one hour. The slurry was then air dried at 25 °C for 24 hours, pulverized and passed through sieve number 100 and stored.

Drug–Excipient Compatibility Studies

Fourier Transform Infra Red (FTIR) Study[6]

FT-IR spectroscopy was employed to ascertain the compatibility between drug and the selected excipients. The pure drug and drug with excipient were scanned separately. Potassium bromide was mixed with drug and/or excipient in 9:1 ratio and the spectra were taken. FT-IR spectrum of drug was compared with FT-IR spectra of drug with excipients.

Differential Scanning Calorimetry (DSC)[7]

Thermal transitions were also measured with rapid heat cooled DSC. Pure nitrogen gas, i.e., without water vapor, was purged through the sample cell continuously. The samples, weighing 5–7 mg, were analyzed in pierced-lid aluminum pans which allows for removal of water from sample. Residual moisture was removed from the samples by pre-heating them to a temperature of about 10–20°C below the first glass transition for 30 min. Control experiments revealed that this procedure resulted in complete evaporation of all moisture and completely dried samples. The dried samples were scanned from 50 to 300 °C with a heating rate of 10 °C min−1 under nitrogen atmosphere.

***In Vitro* Dissolution Study of Solid Dispersions[8]**

Dissolution testing was performed in compliance with USP using USP - type II (Paddle) apparatus. The solid dispersion equivalent to 100 mg of drug was taken in empty hard gelatin capsule and evaluated. Dissolution medium, 0.1N HCl 900 ml was chosen. Paddle speed of 50 rpm was selected. The medium was maintained at 37 °C ± 1 °C. The 1-L glass dissolution vessels were covered to minimize evaporation. The samples were drawn at 10, 30, 45, 60 min. Samples were passed through a filter (0.45 μ) at different intervals of time and replaced with same volume of HCl. The dissolution procedure was carried out for four formulations.

Pre-Compression Parameters[9]

Flow Properties of mixture of Tablet composition:

The flow properties were studied by determining various parameters like the angle of repose, Compressibility index and Hausner ratio.

Angle of Repose[10]

Angle of repose was formed on a fixed base with a retaining lip to retain a layer of powder on the base. The base should be free of vibration. The height of the funnel was varied to carefully build up a symmetrical cone of powder. Care should be taken to prevent vibration as the funnel is moved. The funnel height was maintained at 2 cm from the top of the powder pile as it is being formed in order to minimize the impact of falling powder on the tip of the cone. If a symmetrical cone of powder cannot be successfully or reproducibly prepared, this method is not appropriate. Angle of repose was determined by measuring the height of the cone of powder and calculating the angle of repose, from the following equation.

$$\theta = \tan^{-1} (h/r)$$

Where, θ is the angle of repose,
h is the height,
r is the radius in cm

Compressibility Index and Hausner Ratio [11]

The compressibility Index and Hausner ratio are measures of the propensity of a powder to be compressed. As such, they are measures of the relative importance of inter-particulate interactions. In a free-flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poor flowing materials, there are frequently greater inter particle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the Compressibility Index and the Hausner Ratio.

Compressibility Index CI (%) = V_0-Vf/V_o x 100 (OR)

% Compressibility = [(Tapped density –Bulk density) /Tapped density] x 100

Hausner's ratio = Tapped density/Bulk density

Preparation of Ritonavir Tablets by Direct Compression

All the ingredients as shown in table 1 were mixed and passed through # 100 mesh. The drug and other excipients were mixed by adding small portion of each at a time and blending it to get a uniform mixture and kept aside. Then the other ingredients were mixed in geometrical order, magnesium stearate and talc were added last and mixed for further two minutes and the tablets were compressed using 10 mm punch to get tablets of 600 mg weight.

Table 1 Composition of tablets containing Ritonavir and Arginine in 1:0.5 ratio

Ingredients	Quantity
Ritonavir & Arginine (18.7%)	112 mg
Di-calcium phosphate (74.3%)	446 mg
Crospovidone (5%)	30 mg
Talc (1%)	6 mg
Manesium stearate (1%)	6 mg
Tablet weight	600 mg

Post-Compression Parameters

Weight Variation

Twenty tablets were selected at random and average weight was determined. Then individual tablets were weighed and the individual weight was compared with an average weight.

$$\%\text{Weight Variation} = \frac{\text{Average weight - Weight of each tablet}}{\text{Average weight}} \times 100$$

Hardness Test

This is the force required to break a tablet in diametric compression. Hardness of the tablet is determined by Stokes Monsanto hardness tester which consists of a barrel with a compressible spring. The pointer moves along the gauze in the barrel fracture.

Drug Content

To determine drug content, tablets were powdered and accurately weighed quantity of powder containing drug equivalent to 100 mg was transferred to 250 ml volumetric flask and dissolved with appropriate amount of 0.1N HCl with the aid of sonicator. The solution was filtered through Whattman filter paper. The total amount of drug within the tablets was analysed after appropriate dilution of test solution by using UV spectrophotometer at 248 nm.

Friability

Friability of the tablets was checked by using Roche Friabilator. Initial weight of 20 tablets is taken and these are placed in the friabilator, rotating at 25 rpm for 4 min. The difference in the weight is noted and is expressed in percentage. It should be preferably between 0.5 to 1.0%.

% Friability = (W1-W2)/W1 X 100
Where,
W1= weight of tablets before test
W2= weight of tablets after test

Dissolution Study

In vitro dissolution of Ritonavir tablets was studied in USP type-II dissolution apparatus [12] (Electrolab TDT-06P). A dissolution medium of 0.1 N HCl with a volume of 900 ml was used. A paddle speed of 50 rpm was selected. The temperature of dissolution medium was maintained at 37 ± 0.5 ^{o}C throughout the experiment. Samples were withdrawn at 10, 30, 45, 60 min. The volume withdrawn at each time interval was replaced with fresh quantity of dissolution medium to maintain sink conditions throughout the experiment. The collected aliquots were filtered using Whattman filter paper, and further diluted suitably to analyze using UV spectrophotometric method. Cumulative percent Ritonavir released was calculated and plotted against time.

Results and Discussion

Determination of λ_{max} of Ritonavir In 0.1 N HCl:

The λ_{max} of Ritonavir was determined and is shown in the figure 1.

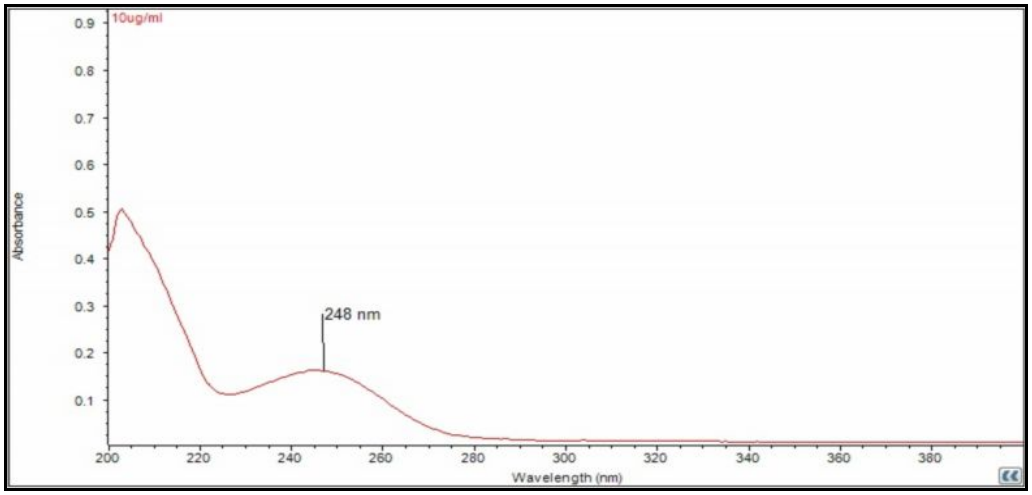

Figure 1: Determination of λ_{max} of Ritonavir in 0.1 N HCl

Linearity of Ritonavir at Different Concentration Levels

The linearity of Ritonavir at different concentration levels was determined and is shown in the figure 2.

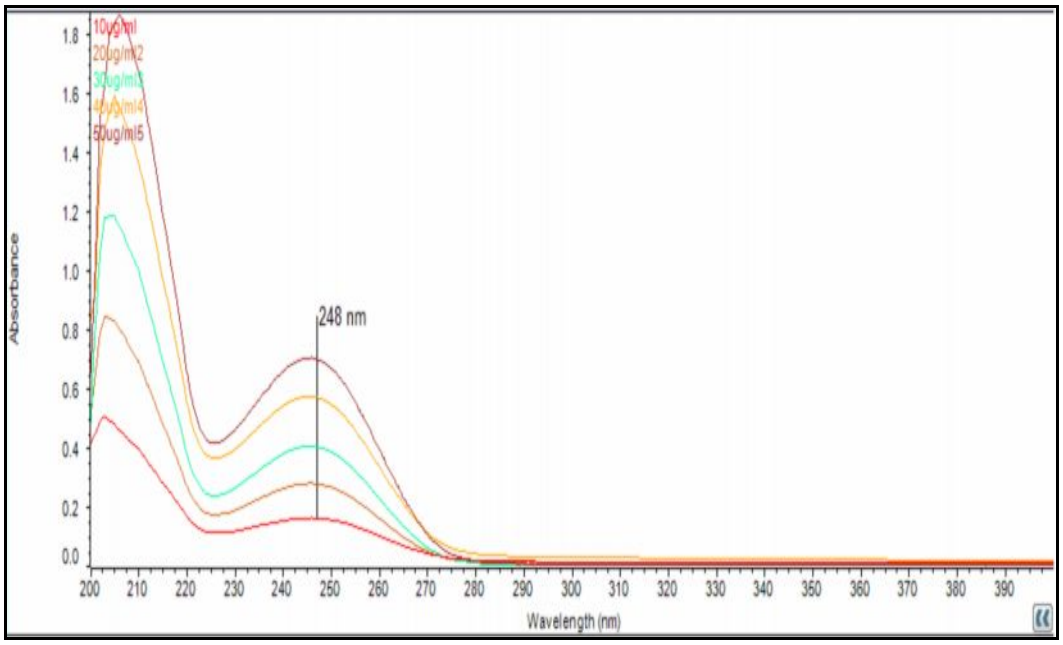

Figure 2: Linearity of Ritonavir at different concentration levels

Construction of Standard Calibration Curve of Ritonavir in 0.1N HCl

The absorbance of the solution was measured at 248 nm, using UV spectrophotometer with 0.1N HCL as blank.A graph of Absorbance vs Concentration was plotted in the concentration range 10-50 µg/ml and was shown in figure. R^2 value was found as 0.997

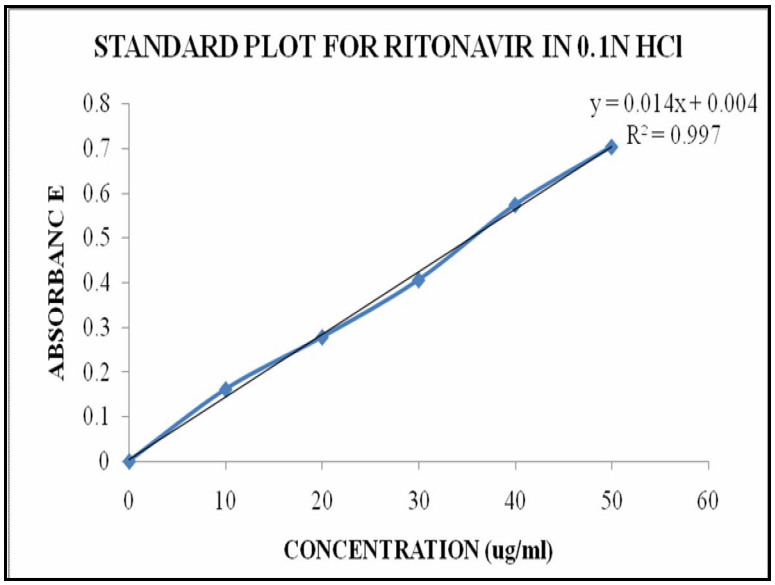

Figure 3: Standard Plot for Ritonavir in 0.1N HCl

Solubility Analysis

Solubility of Ritonavir with different carriers was analysed in distilled water. The solubilised drug in the supernatant was quantified by UV spectroscopy. The solubility was as follows: Arginine 1% > Arginine 0.5% > Proline 1% > Proline 0.5%

Solubility of drug in presence of Arginine 1% was found to be highest.

Drug-Excipient Compatibility Studies

Fourier Transform Infra Red (Ftir) Studies

Ftir Spectra of Proline, Arginine & Ritonavir

In the preparation of tablet formulation, drug and excipient may interact as they are in close contact with each other, which could lead to the instability of drug. Pre-formulation studies regarding the drug-excipient interaction are therefore very critical in selecting appropriate excipients.

FTIR spectra of Proline, Arginine & Ritonavir was obtained and is shown in the figure 4.

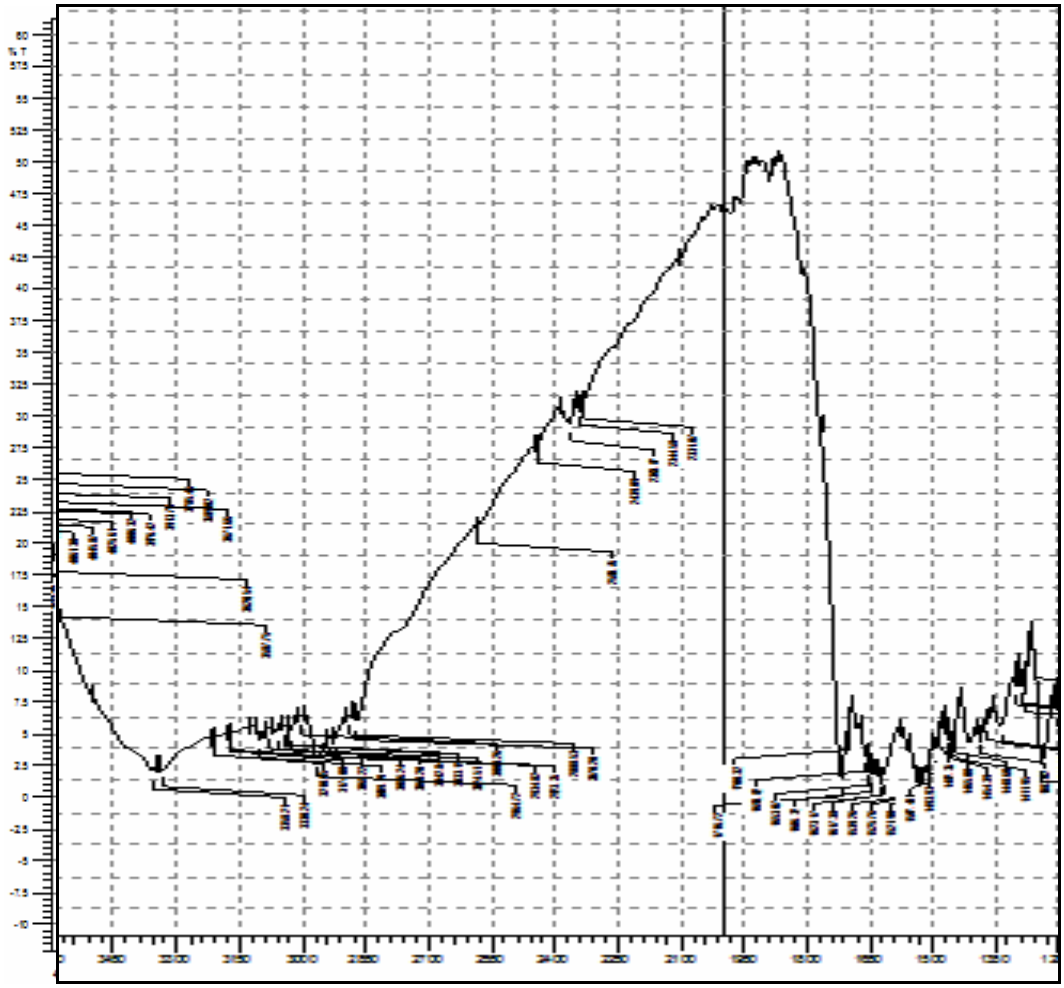

Figure 4: FTIR spectra of Proline Arginine & Ritonavir mixture

When FT-IR was performed, spectrum indicated characteristic peaks.

Table 2 FTIR characteristic peaks of Ritonavir

Characteristic peaks	N–H stretching amide group	Hydrogen-bonded acid within the molecule	Ester linkage	C=C– stretching aromatic carbons
Literature value	3,480 cm−1	2,964 cm−1	1,716 cm−1	1,645, 1,622,& 1,522 cm−1
Experimental value	3,587 cm−1	2,964 cm−1	1,716 cm−1	1,646,1,623,& 1,525 cm−1

Differential Scanning Calorimetry (Dsc)

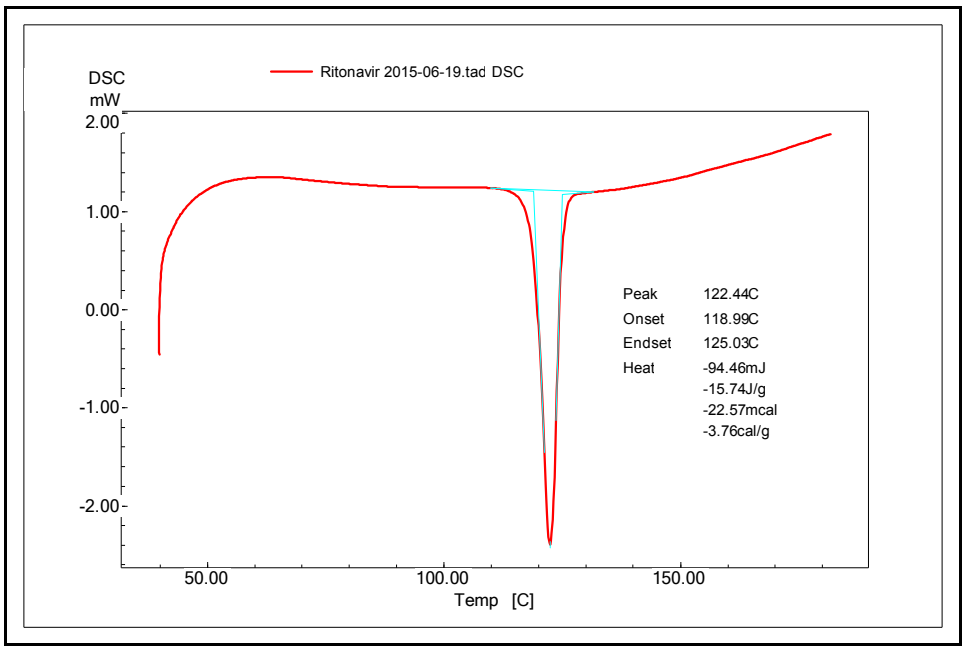

Figure 5: DSC profile of Ritonavir

Dsc Profile of Ritonavir and Arginine (1:1)

DSC of Ritonavir and Arginine was obtained and is shown in figure 6.

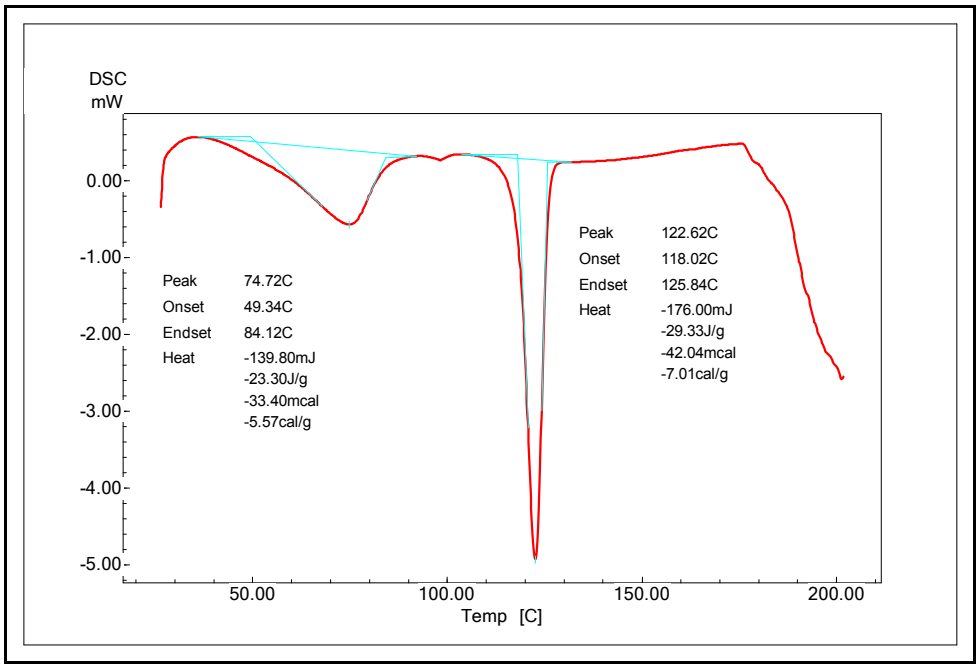

Figure 6: DSC profile of Ritonavir and Arginine 1:1

Dsc Profile of Ritonavir And Proline (1:1)

DSC of Ritonavir and Proline was obtained and is shown in figure 7.

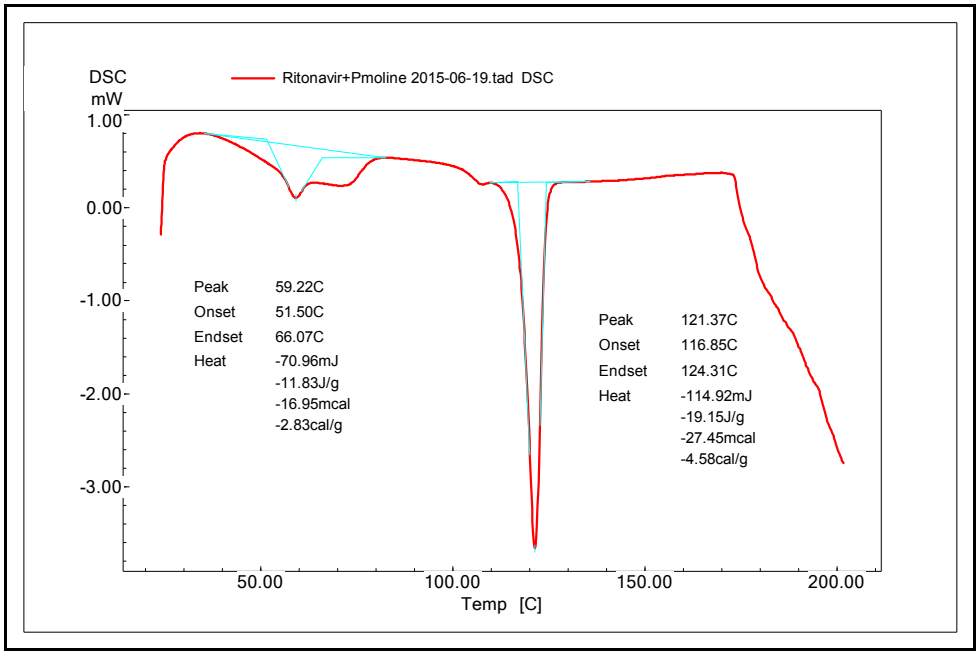

Figure 7: DSC profile of Ritonavir and Proline 1:1

DSC was employed to ascertain the compatibility between the drug and excipients. Melting point of drug was found at 122 °C and it was unaltered by carriers i.e., Arginine and Proline. Thus the drug was compatible with carriers.

Dissolution Profile of Four Formulations

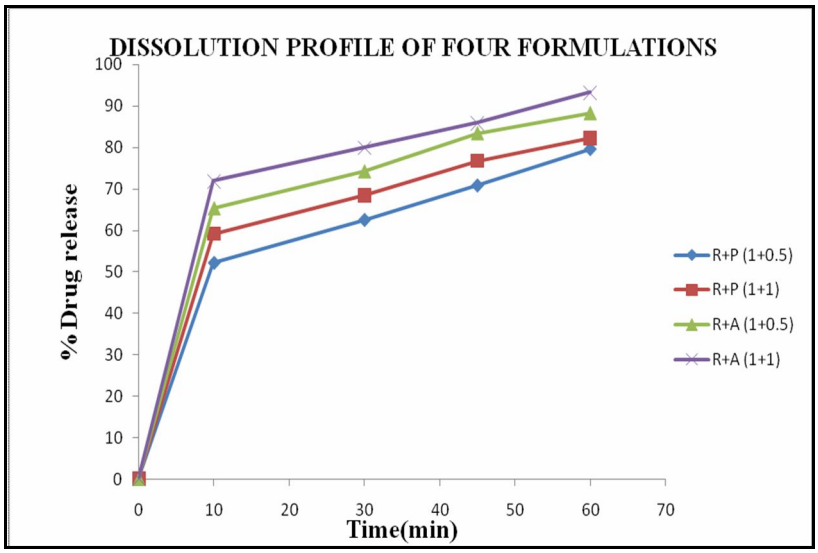

Figure 8: Dissolution profile of four formulations

From the i*n vitro* release studies, Arginine 1% showed maximum release among the solid dispersions formulations. It released 71% drug within 10 minutes. Thus Arginine was selected as a suitable carrier for further study.

Pre Compression Studies

Table 3 Pre-compression studies data

Formulation Batches	Angle of Repose (θ) (Mean±SD)	Bulk Density (g/ml) (Mean ±SD)	Tapped density (g/ml) (Mean±S.D.)	Compressibility (%)	Hausner's ratio
Drug+Arginine(1:1)	31.6 ± 1.7	0.85 ± 0.39	0.96 ± 0.28	11.9 ± 0.5	1.13 ± 0.5
Drug+Arginine(1:0.5)	29 ± 1.5	0.82 ± 0.66	0.88 ± 0.29	6.6 ± 1.4	1.07 ± 0.1

Precompression parameters of all formulations blend were conducted for angle of repose, bulk density, tapped density, compressibility index, and Hausner's ratio. The two most important attributes for the direct compression formula are good flow and good compressibility. The angle of repose for the formulations was found to be 31.6±1.7 and 29±1.5 which indicates good flow property.The bulk density and the tapped density for the formulations were found to be almost similar. The Carr's index and Hausner's ratio were found to be within the range, indicating good flow and compressibility of the blends.

Post Compression Studies of Ritonavir Tablets

The weight variation of tablets was within the pharmacopoeia specifications of USP. The hardness for different formulations was found to be between 3.0 to 3.4 kg/cm^2, indicating satisfactory mechanical strength. The friability was < 1.0% W/W for all the formulations, which is an indication of good mechanical resistance of the tablet. Friability test is performed to evaluate the ability of tablets to withstand abrasion in packing, handling, and transporting. The drug content was found to be within limits.

Table 4 Post-compression studies

Dissolution Data of Marketed & Developed Tablets

Formulation Batch	Hardness kg/sq.in.	% Friability	Disintegration Time (sec)	Weight variation	%Drug Content
Drug+Arginine (1:1)	3.4 ± 0.5	0.44 ± 0.04	66 ± 5	Passed	98.98 ± 2.1
Drug+Proline(1:0.5)	3.3 ± 0.6	0.53 ± 0.03	62 ± 4	Passed	97.35 ± 1.3

From the i*n vitro* release studies, Ritonavir & Arginine 1% tablet showed better and maximum release than other tablets. It released 76% drug within 10 minutes. The drug release from the formulations is as follows: Arginine 1% tablet>Arginine 0.5% tablet>Marketed tablets. Thus the objective of enhancement of dissolution rate of poorly water soluble antiretroviral drug Ritonavir was achieved.

Dissolution Profile of Marketed & Developed Tablets

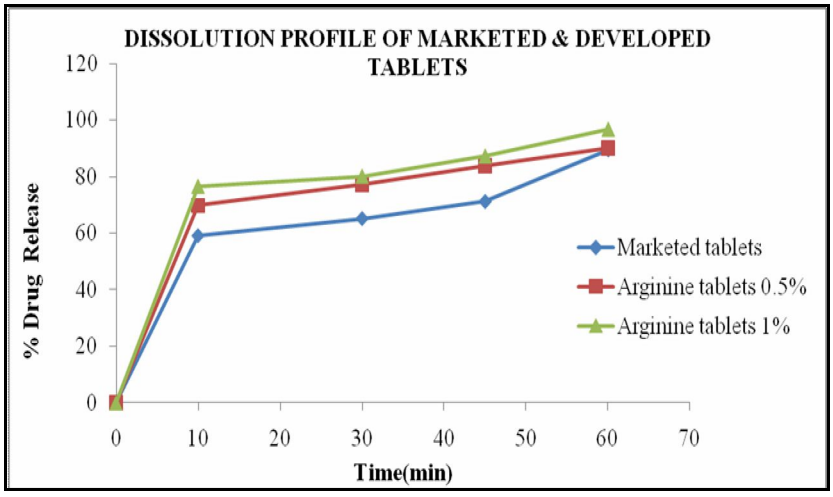

Figure 9: Dissolution profile of Marketed & Developed Tablets

Conclusion

The present study was performed to improve the dissolution of Ritonavir, a poorly soluble drug using amino acids as carriers by solid dispersion technique. Solid dispersion provides a practical means of further enhancing the solubility.Solubility improvement of poorly water-soluble drugs is important to achieve better bioavailability. Thus, to enhance the dissolution, amino acids i.e., Arginine and Proline in various proportions were prepared using kneading method.After comparing the solubility and dissolution profiles it was seen that the solid dispersion with Arginine as carrier was highest. It was observed that the solid dispersion prepared by employing Arginine as carrier showed the fastest dissolution (i.e. more than 70% release within first 10 min); therefore, Arginine was selected as a suitable carrier for further studies. The drug and excipient compatibility studies revealed no interaction between the drug and excipients. The tablets prepared by direct compression method were evaluated for physical properties, *in vitro* dissolution studies. The tablets were found to comply with all the requirements. *In vitro* drug release studies indicated optimum release from tablets containing Arginine & Rironavir in 1:1 ratio showed highest (76.65%) drug release at 10 min. Thus the objective of enhancement of dissolution rate of poorly water soluble antiretroviral drug Ritonavir was achieved by amino acids as carriers by solid dispersion technique.

Acknowledgement

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